Indian Journal of Biological Sciences, 18 : 1 – 8, 2012

EFFECT OF OLIVE OIL ADMINISTRATION ON SOME HEMATOLOGIC AND METABOLIC PARAMETERS IN FEMALE RATS

***Moorkath Nandakumaran[1], Majed Al-Shammari[2], Hameed Al-Sarraf[3], Mariam Al-Dossery & Eyad Al-Saleh[4]**

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Kuwait
[2]Obsterics and Gynecology Department, Jahra Hospital, Kuwait
[3]Departments of Physiology, Faculty of Medicine, University of Kuwait
[4]Obstetrics&Gynecology Department, Adan Hospital, Kuwait

ABSTRACT■ Objective: Data on effect of Olive oil intake on various hematologic and metabolic parameters in humans or animals are scanty. Thence we attempted to assess the effect of oral administration of varying doses of this oil on various hematologic and metabolic parameters in female rats. Material and Methods: Groups of adult female Sprague Dawley rats were given oral doses of 1 ml, 2 ml and 4 ml olive oil twice per day respectively. Control group of rats were given tap water. Oral feeding of oil was done continuously for a period of 20 days and at the end of the study period the animals were lightly anaesthetized with ether and sacrificed to collect blood samples for analysis. Various hematologic parameters such as red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), platelets, lymphocytes and mean corpuscular hemoglobin concentration (MCHC) were analyzed by a Hematology Blood Analyzer while metabolic parameters such as cholesterol, triglycerides, urea, uric acid, creatinine and protein were analyzed by specific analytical kits. Activities of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPX) were assessed by specific analytical kits. Statistical analysis of data was performed using a SPSS data analytical package. Results: Oral administration of olive oil for 20 continuous days did not significantly alter any of the hematologic parameters studied, compared to control group even when the oil was administered at a relatively massive dose of 4 ml/day. Administration of olive oil appeared to decrease WBC, Hb, platelet and lymphocyte blood concentrations in treated rats, but the difference was statistically significant (ANOVA Test; $p<0.05$) only in the case of platelet concentration. Olive oil administration did not alter the concentrations of protein, cholesterol, urea, triglycerides, uric acid and creatinine in treated groups of rats significantly (Student t-test, $p<0.05$) compared to those of control rats. SOD level and GPX level in blood of oil- treated animal groups were not significantly different (ANOVA test; $p<0.05$) compared to control group. Conclusion: We conclude that oral administration of olive oil in female rats, even in massive doses does not cause any significant alterations in hematologic and metabolic parameters and may offer potential benefit, by the effect of reducing the platelet levels. More detailed studies however are warranted before extrapolating these results to human situations.

Key words: Olive Oil, Hematologic Parameters, Metabolic Parameters, Oral Feeding, Female Rats

INTRODUCTION

Olive oil is extensively used in Europe, America, Middle East and other parts of the world as a cooking and seasoning medium. Olive oil is established to have a higher content of unsaturated fatty acids and has

* **Corresponding author** : E-mail: moorkath@hsc.edu.kw

been widely recommended to be superior to other oils in maintenance of health, although no detailed comparative study has been reported on its efficacy in humans or animals. Some reports have indicated decreased cardio-vascular dysfunction in persons using olive oil and the relatively decreased incidence of heart related problems in mediterranean countries (Kok and Kromhout, 2004; Keys et al., 1986) compared to others in western Europe has been attributed increased use of olive oil by these populations, in daily diet both as a cooking and a seasoning medium. The beneficial effects of this oil on cardiovascular system (Covas, 2007; Wahle et al., 2004) in humans can be attributed to the presence of phenolic compounds reported by some investigators (Turner et al., 2005; Tripoli et al., 2005). This edible has also been reported to have constituents that provide protection against reactive oxygen species and lipid peroxidation (Fito et al., 2008) Beneficial effects olive oil in reducing lipid peroxidation and in enhancing cardio-protection have been corroborated by other investigators as well (Arrigo et al., 2008). However, no detailed studies have been reported examining the effect of administration of olive oil on hematologic, metabolic and atherogenic parameters in experimental animals or in humans. Hence we have explored the effect of this edible oil administration on hematologic (RBC, WBC, Platelets, Hemoglobin, MCV, MCHC) metabolic (cholesterol, triglycerides, protein, urea, uric acid and creatinine) parameters and on status of anti-oxidant enzymes, namely superoxide dismutase and glutathione peroxidase in female adult rats, after oral gavages of varying volumes of oil for a period of 30 days.

MATERIAL AND METHODS

Adult Female rats (Sprague Dawley Strain) weighing between 220-240 g were used for the study. The animals used for the study were bred in our Medical Faculty animal house and housed in individual polypropylene cages, at room temperature maintained at 25$\pm$1 degree centigrade, with alternating 12 hour light 12 hour dark cycle. Body weights of all rats were assessed before the beginning of the study. 3 groups of 5 rats received 0.5 ml, 1 ml and 2 ml of olive oil (RS Brand, Spain) orally twice per day respectively for a continuous period of 30 days. Control group animals were given tap water during the study period. All animals were allowed diet and water ad libitum during the period of study after oral gavages of oil for the study period, all animals were weighed and anesthetized lightly with ether and sacrificed for collection of blood samples. Hematologic parameters such as RBC, WBC, platelets, Hb, lymphocytes and MCHC were determined in blood samples of all study and control groups, using a Hematology Analyzer(ERMA INC, PCE210, Japan) while the concentrations of metabolic parameters namely, protein, cholesterol, triglycerides, creatinine, urea and uric acid in various blood samples were determined using specific analytical kits (Randox Labs, UK). Activity of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPX) in blood samples of study and control groups was determined spectrometrically, using a widely used and specific analytical method (Randox Labs, UK).

Statistical Analysis

All Data are expressed as Means+SEM and statistical analysis was done using SPSS statistical package. Student t-test or analyses of variance (ANOVA) were used where appropriate. Data were deemed statistically significant, if probability was <0.05.

RESULTS

I n control group, body weight of rats averaged 227 gm while in the 1ml/day olive oil, 2 ml/day olive oil and 4 ml/day olive oil groups, weights of rats averaged 239, 230, and 224 gm before start of experiment After the 30 day study period, the corresponding weights averaged 222, 232, 220 and 218 gm in corresponding groups of rats. Student t-test did not show any significant difference ($p<0.05$) in body weights between control and treated groups. Table I shows details of some hematological parameters, namely RBC, WBC, Platelets, Hb, lymphocytes and MCHC of the control and treated groups of rats after the 30 day period of oil administration. Although WBC and Hb appeared to be lower in rats receiving higher doses of olive oil, Student t-test did not show any significant difference ($p<0.05$) between control and treated groups. However, platelets were significantly lower (Student t-test, $p<0.05$) in rats receiving 1 ml and 2 ml olive oil per day, compared to control although the lower platelet concentration in rats receiving 4 ml oil per day was not significantly different ($p<0.05$) than that of control group. Student t-test showed that lymphocyte concentration in blood of rats receiving 1ml and 2ml olive oil per day was significantly lower ($p<0.05$) while the difference in lymphocyte concentration between control rats and rats receiving 4 ml olive oil per day was not statistically significant ($p<0.05$).

Table I: Haematological parameters in control and olive oil treated Female Rats

	RBC (x 10 6/ul)	WBC (x 10 3/ul)	Platelets (x 10 3/ul)	Hb (g/dl)	MCHC (g/dl)	Lympho (x 10 3/ul)
Control Rats	7.42 ± 0.27	5.40 ± 0.68	861.0 ± 31.49	13.25 ± 0.34	28.7 ± 0.19	3.25 ± 0.08
Phase I	6.76 ± 2.12	2.40 ± 0.81	519.8 ± 84.98	10.08 ± 2.96	24.50 ± 1.8	2.24 ± 0.43
Phase II	6.28 ± 1.52	2.46 ± 0.62	439.0 ± 71.91	10.60 ± 2.15	29.62 ± 2.47	2.46 ± 0.52
Phase III	7.37 ± 0.32	6.20 ± 0.59	721.2 ± 118.7	12.60 ± 0.57	28.90 ± 0.18	3.90 ± 0.34

Values are means ± SEM of 5 rats in each group. Statistical analysis was done by ANOVA or Student t-test where appropriate.
RBC = Red Blood Cell, WBC= White blood cell, Hb= Hemoglobin, MCHC= Mean Corpuscular Hemoglobin Concentration.
Phase I= 1ml dose olive oil/ day
Phase II= 2ml dose olive oil/day
Phase III= 4ml dose olive oil/day

Table II shows values of total protein, urea and uric acid in blood samples obtained from control and oil-treated rats after 30 day study period. Analysis by Student t-test did

Table II: Some Metabolic Parameters in control and olive oil treated Female Rats

	Total protein (g/dl)	Urea (mg/dl)	Uric acid (mg/L)
Control Rats	6.07 ± 0.47	41.09 ± 6.82	22.87 ± 0.67
Phase I	5.44 ± 0.25	24.75 ± 6.44	22.72 ± 0.31
Phase II	5.33 ± 0.23	28.00 ± 9.40	21.78 ± 0.55
Phase III	6.06 ± 0.25	30.02 ± 7.72	22.96 ± 0.21

Values are means ± SEM of 5 rats in each group. Statistical analysis was done by ANOVA or Student t-test where appropriate
Phase I = 1ml dose olive oil/ day
Phase II = 2ml dose olive oil/day
Phase III= 4ml dose olive oil/day

not show any significant difference ($p<0.05$) of total protein, urea and uric acid values between control and olive oil treated rats. Total cholesterol averaged 66 ±9, 72±6, 59 ±7 and 68±8 mg/dl in control, 1ml, 2ml and 4 ml olive oil per day treated groups respectively (Fig.1). Student t test showed no significant difference ($p<0.05$) in cholesterol level in control and treated groups of rats. Triglyceride values averaged 82±12, 79±9, 77 ±11 and 62 ±9 mg/dl in control, 1ml, 2ml, 4 ml olive oil per day treated groups respectively (Fig.2). However the apparently lower triglyceride values in groups receiving the edible oil were not significantly different (Student t-test; $p<0.05$).

Values of creatinine level in blood control and oil treated rat groups are shown in Figure

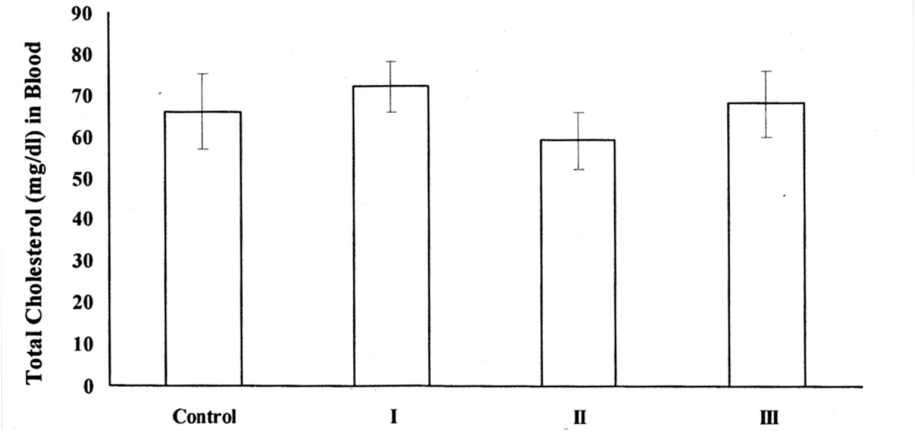

Figure 1. Total cholesterol concentrations in blood of control and olive oil treated rats.
Values are Means± SEM of 5 animals in each group. I=ml/day of olive oil, II=2 ml/day of olive oil ; III=4 ml/day olive oil. Statistical analysis was done by Student t-test. Control vs I $p<0.05$; Control vs II $p<0.05$; Control Vs III $p<0.05$

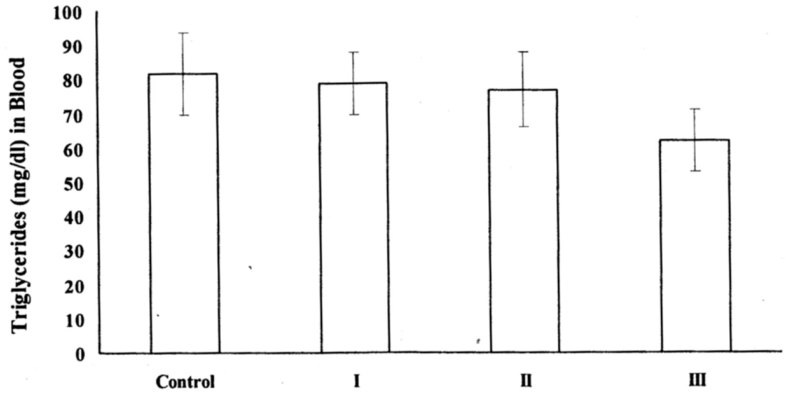

Figure 2. Triglyceride concentrations in blood of control and olive oil treated rats.Values are Means± SEM of 5 animals in each group ; I=ml/day of olive oil, II=2 ml/day of olive oil ; III=4 ml/day olive oil. Statistical analysis was done by Student t-test. Control vs I $p<0.05$; Control vs II $p<0.05$; Control Vs III $p<0.05$

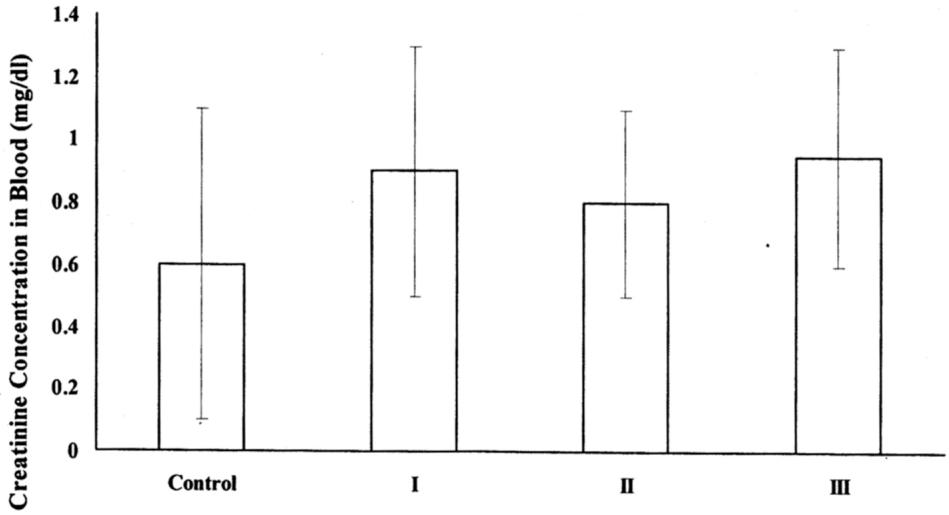

Figure 3. Creatinine concentrations in blood of control and oilve oil treated rats.

Values are Means± SEM of 5 animals in each group ; I=ml/day of olive oil, II=2 ml/day of olive oil ; III=4 ml/day olive oil. Statistical analysis was done by ANOVA test. Control vs I $p<0.05$; Control vs II $p<0.05$; Control Vs III $p<0.05$

3. Creatinine concentration averaged 0.60±0.35, 0.90±0.40, 0.80±0.30 and 0.95±0.35 mg/dl in control, 1ml, 2ml and 4 ml olive oil per day treated groups respectively. However, Student t-test showed no significant difference in creatinine values between control and oil treated rat groups ($p<0.05$).

SOD Enzyme activity values averaged 1.35±0.18, 1.52±0.21, 1.56±0.24 and 1.46 ±0.15 U/ml in control, 1ml, 2ml, 4 ml coconut oil per day treated groups respectively. GPX

concentration in blood averaged 1.35±0.18, 1.52±0.21, 1.56±0.24 and 1.46 ±0.15 Units/L in control, 1ml, 2ml, 4 ml oil treated groups respectively. Although SOD,GPX and TAO values appeared to be higher than control group in all treated animals, ANOVA test showed no significant difference ($p<0.05$) between control and treated groups.

DISCUSSION

Present study did not show any harmful effect on the various hematologic and metabolic parameters in pregnant female rats, despite continuous daily administration of graded moderate to high doses of olive for 30 days. Although olive oil intake appeared to lead to reduction in maternal body weight, compared to control pregnant rats, the difference was not statistically significant. Interestingly, when rats received doses equivalent of about 300 ml, 700 ml and 1200 ml daily of the oil for an average pregnant woman weighing 60 kg for a continuous duration of 20 days, the results indicating absence of any significance in hematological or metabolic parameters were surprising and unexpected. In the present study, since olive oil was administered by oral gavages twice daily and continuously for 30 days, we were able to ensure that the animals received the exact dose of the oil during the full course of pregnancy period.

Our data did not indicate any deleterious effect on either cholesterol or triglyceride levels. However, in a study on adult female rats (Nandakumaran et al., 2009) administration of coconut oil in similar high doses, had in fact resulted in lowered cholesterol levels in treated rats. We are unable to explain the possible reason or reasons for the difference in effects of coconut and olive oil on cholesterol disposition in treated rats. Surprisingly, even after massive administration of olive oil daily for 30 days, triglyceride, urea, uric and creatinine levels did not increase to significant or abnormal levels, implying that olive oil administration per se does not cause any damaging effects on either the liver or the kidney or the heart. Although in rats receiving higher dose of coconut oil, relatively more saturated edible oil, treated animals were found to have significantly lower urea level compared to control group in adult female rats (Nandakumaran et al., 2009) but no such effect of olive oil on urea disposition was noted in this study. Absence of significant difference in uric acid and creatinine levels in blood of control and oilive oil treated rats implies that despite receiving massive amounts of the oil for a period of 30 days, olive oil did not cause any major defect in renal function in treated rats. Cholesterol levels in rats receiving olive oil were not significantly different, compared to the control group. The absence of hypercholesterolemia and triglyceridemia in olive oil treated rats indicate that even after prolonged administration for a period of 30 days, this edible oil does not affect cholesterol and triglyceride metabolism negatively. Interestingly, platelet count in rats receiving massive amounts of olive oil was lower, in two groups of oil-treated rats than control rats receiving no oil and this could be considered as another beneficial parameter preventing formation of thrombus or plaques in lining of coronary or other blood vessels in the body. It is reasonable to assume that study with a larger animal population could clarify the definitive role of olive oil on the status of various hematologic and metabolic parameters .The significant reduction in lymphocyte count in blood of two groups of olive oil treated rats was surprising though in the group receiving higher amount of 4 ml/day of the oil, this difference in lymphocyte

count was not statistically significant. We are unable to attribute the reason or reasons for such an unexpected finding and are unable to ascertain whether such a reduction in lymphocyte count could be associated with altered or diminished immunity in treated animals. More detailed studies are warranted, using a larger animal population and we are currently undertaking such a study. We hasten to add that data from experimental animals cannot be extrapolated to humans and hence the same effect may not be present or replicated in humans.

In this study, olive oil administration was shown to increase activity of antioxidant enzymes SOD and GPX in all three groups of oil-treated rats. Though this increase in anti-oxidant enzyme activity was not statistically significant, it is likely that a statistically significant activity could be demonstrated in a larger sample size of animals. Studies using a larger sample size are currently in progress. The antioxidant function of the above two enzymes, in providing protection from reactive oxygen species is well established. Such a finding of relatively higher anti-oxidant function in animals receiving olive oil is in accord with our earlier findings of increased anti-oxidant activity in rats receiving another edible oil coconut oil in similar experimental conditions (Nandakumaran et al., 2009) as well as in pregnant rats, receiving high amount of coconut oil during pregnancy period (Nandakumaran et al., 2011). We conclude that anti-oxidant activity of phenolic compounds extracted from olive oil (Servili et al., 1996; Visioli et al., 1994; Papadopoulos and Boskou, 1991) can explain the finding of increased anti-oxidant activity of olive oil in treated animals. We speculate that, by reducing oxidation of LDL moiety mediated through reactive oxygen (Giovaninic et al., 1999; Baldioli et al., 1996) olive oil could play a beneficial role in preventing formation of plaques and could explain the lower mortality rates from plaque formations ,etc in populations using olive oil as major edible oil, as reported in earlier studies. Anti-cancerous activity of olive oil has been implied by some investigators (Owen et la., 2004; Menendez and Lupu, 2006) and protective effect of oilive oil against helicobacter pylori infection has also been postulated by another research group (Romero et al., 2007) possible anti-bacterial role of olive oil in treatment of skin infections in adults has also been reported (Verallo-Rowell et al., 2008). We are unable to explain the lower lymphocyte count observed in 2 groups of olive oil treated rats and to speculate whether reduction in lymphocyte count could impact immunity function in treated animals. Further studies in this area are in progress. Although data obtained in animals cannot be extrapolated directly to human, use of olive oil appears to confer a variety of beneficial effects to the consumer. It seems pertinent to emphasize that studies in experimental animals are not directly extrapolatable to human situations and hence we refrain from speculating whether olive oil intake in pregnant women could elicit the same response relating to the hematologic and metabolic parameters investigated in this study. Further studies with a larger animal population and in varying experimental conditions are currently in progress to corroborate our findings.

REFERENCES

Arrigo F.G. Cicero, Simona Nascetti, Maria C. López-Sabater, , et al Changes in LDL Fatty Acid Composition as a Response to Olive Oil Treatment Are Inversely Related to Lipid Oxidative Damage: The EUROLIVE Study. Journal of the American College of Nutrition 2008; 27: 314-320.

Baldioli M, Servili M, Perretti G, Montedoro GF. Antioxidant activity of tocopherols and phenolic

compounds in virgin olive oil. J. Am. Oil chem.Soc 1996; 73: 1589-1593.

Covas MI . "Olive oil and the cardiovascular system". Pharmacol. Res. 2007; 55: 175–86

Fito M, Cladellas M, de la Torre R, et al. Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. Eur J Clin Nutr. 2008; 62: 570-574.

Giovaninic C, Strafce E, Modesti D, et al. Tyrosol, the major olive oil biophenol, protects against oxidized-LDL-induced injury in Caco-2-cells. J.Nutr 1999 ; 129: 1269-1277.

Keys A, Menotti A, Karvonen MJ, *et al* . The diet and 15-year death rate in the seven countries study. Am. J. Epidemiol. 1986; 124: 903–15.

Kok FJ, Kromhout D. Atherosclerosis—epidemiological studies on the health effects of a Mediterranean diet. Eur J Nutr. 2004; 43: 2-5.

Menendez JA, Lupu R. Mediterranean dietary traditions for the molecular treatment of human cancer: anti-oncogenic actions of the main olive oil's monounsaturated fatty acid oleic acid (18:1n-9). Curr Pharm Biotechnol. 2006; 7: 495-502.

Nandakumaran M, Al-Sarraf H, Al-Fadhli R, Al-Shammari M, et al. Effect of oral administration of coconut oil on hematologic and metabolic parameters in female adult rats. Nutr.Ther Metabol 2009 ; 27 : 183-188.

Nandakumaran M, Elisaveth Angelaki , Nasser Al-Azemi, et al . Influence of coconut oil administration on some hematologic and metabolic parameters in pregnant rats. J.Mat-Fet Neonat Med 2011; 24: 1254-1258.

Owen RW, Haubner R, Wurtele G, et al . Olives and olive oil in cancer prevention. Eur J Cancer Prev. 2004; 13: 319-26.

Papadopoulos G, Boskou D. Antioxidant effect of natural phenols in olive oil. J.Am.Oil chem. soc 1991; 68: 669-671.

Romero C, Medina E, Vargas J, et al. In vitro activity of olive oil polyphenols against Helicobacter pylori. J Agric Food Chem. 2007; 7 ; 55 :680-6.

Servili M, Baldioloi M, Miniati E, Montedoro G. Antooxidant activity of new phenolic compounds extracted from virgin oilive oil and their extraction with alpha-tocopherol and beta-carotene. Rv.I.Sost.Grasse 1996 ; 73: 55-59.

Tripoli E, Giammanco M, Tabacchi G D, et al . The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. Nutrition Research Reviews 2005; 18: 98–112.

Turner R, Etienne N, Alonso MG, *et al* . "Antioxidant and anti-atherogenic activities of olive oil phenolics". Int J Vitam Nutr Res 2005; 75: 61–70.

Verallo-Rowell VM, Dillague KM, Syah-Tjundawan BS. Novel anti-bacterial and emollient effects of coconut oil and virgin olive oils in adult atopic dermatitis. Dermatitis 2008; 19: 308-315.

Visioli F, Galli C. Oleuropein protects low density lipoproteins from oxidation. Life Sc 1994; 55: 1965-1971.

Wahle KW, Caruso D, Ochoa JJ, Quiles JL. Olive oil and modulation of cell signaling in disease prevention. Lipids. 2004 Dec; 39(12): 1223-31.