Preventive Role of Olive Oil On Serum Lipid Profile and Antioxidant Status of Methionine Overload Rabbits (Part II)

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Abstract

This study was designed to show the possible preventive role of olive oil on serum lipid profile and antioxidant status of methionine overload rabbits. Thirty adult male rabbits were divided into three equal groups (10/group) and treated as follows for eight weeks: first group (GI) was intubated orally with tap water and served as control group, animals in the second group were intubated daily with 100mg/kg B.W. of methionine (GII), while rabbits in the third group were intubated daily with 100mg /kg B.W of methionine for four weeks and then after intubated orally with 0.51 ml /kg B.W/ of olive oil daily for further four weeks (GIII). Fasting blood samples were collected at zero, four and eight weeks of the experiment for measuring serum total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein(LDL-C),very low density lipoprotein(VLDL-C), high density lipoprotein (HDL-C), glutathione (GSH) and Malondialdehyde (MDA) concentrations .The results of this experiment showed the preventive role of olive oil in alleviation of hyperlipidemia and oxidative stress induced by methionine overload.

Key word: Methionine over load, Lipid profile, GSH, MDA, olive oil.

الدور المانع لزيت الزيتون في صورة دهون الدم وحالة مضادات الأكسدة في ذكور الأرانب المعرضة لفرط الميثيونين (الجزء الثاني)

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المستخلص

أجريت هذه الدراسة لمعرفة تأثير زيت الزيتون على الصورة الدموية للدهون وحالة مضادات الأكسدة في ذكور الأرانب المستحدث بها فرط الميثيونين . تم أستخدام(30) من ذكور الأرانب البالغة والتي قسمت الى ثلاثة مجاميع متساوية وعوملت لمدة ثمانية اسابيع كالأتي : جرعت حيوانات المجموعة الأولى (السيطرة GI) الماء العادي فموياً ،اما ارانب المجموعة الثانية (GII) فقد جرعت 100 ملغم /كغم من وزن الجسم من المثيونين ، جرعت حيوانات المجموعةالثالثة (GIII) نفس الجرعة اعلاه من المثيونين لمدة اربع اسابيع ثم جرعت بعد

ذلك 0.51 مل/كغم من وزن الجسم من زيت الزيتون لمدة اربع اسابيع اخرى . تم جمع عينات الدم في الفترات 0 و 4 و8 أسبوع من التجربه لقياس صورة دهون الدم : تركيز الكوليستيرول الكلي (TC) ،ثلاثي أسيل الكليسيرول (TAG) ،الكوليستيرول في الشحوم البروتينية واطئة الكثافة (LDL–C) والواطئة جدا(VLDL–C) و تركيز الكوليستيرول في الشحوم البروتينية عاليةالكثافة (HDL–C) ، بالأضافة الى تركيز المالوندالديهايد (MDA) والكلوتاثايون المختزل (GSH) في مصل الدم. أظهرت نتائج هذه التجربة الدور المانع لزيت الزيتون في تحسين حالة فرط دهون الدم والأجهاد التأكسدي المستحدث بأعطاء فرط المثيونين .

الكلمات المفتاحيه : فرط المثيونين ،صورة الدهون ،الكلوتاثايون ،مالونداياالديهايد ، الأرانب .

Introduction

Methionine is non -polar essential amino acid coded by initiation codon AUG which indicates mRNA's coding region where translation in protein begins. Together with cysteine, methionine is one of two sulfur-containing proteinogenic amino acids (19). Its derivative *S*-adenosyl methionine (SAM) serves as amethyl donor required for many cellular process, as it act an intermediate in the biosynthesis of cysteine, carnitine, taurine, lecithin, phosphatidylcholine,and other phospholipids(29,34) . Inspite of being nutritionally essential , increased concentration of methionine inside the body can be toxic for poultry , human and animal tissue (12), and Methionine overload is one of many factors responsible for causing disturbance in homocysteine metabolism resulting in accumulation of homoysteine with subsequent development of hyperhomocysteinemia (HHcy) (31,41). In addition to methionine overload, HHcy could be caused by deficiency of Co-factor (folic acid, B6 and B12) (35,42), or by some genetic enzyme defect (9,16).

Observations in many clinical and epidemiologic studies have suggested that Hyperhomocysteinemia (HHcy) is an independent risk factor for cerebrovascular disease (11) coronary artery disease including coronary, carotid, aortic, peripheral vessels and deep venous thrombosis (24,17). The effect of HHCY has been seem to be similar to that observed in elevation cholesterol levels, associated with pulmonary hypertension (28). Homocysteine is readily auto-oxidized, leading to the formation of metabolites and oxygen free radicals generation ($O_2^{\cdot-}$ and H_2O_2) which are implicated in vascular toxicity and initiation of lipid peroxidation (10).Early studies with high Homocysteine level showed a variety of deleterious effects on endothelial or smooth muscle cells in cultures (6,40), where , ROS induced by HHcy , importing oxidative insult by damaging structural and functional components of cellular system and then cell death (22).

Material and Methods

Thirty adult male rabbits were randomly divided into three equal groups (10/group), first group (GI) were intubated orally for eight weeks with tap water and served as control group. Animals in the second group were intubated daily with 100mg/kg B.W. of methionine for eight weeks (GII), while rabbits in the third group were intubated daily with 100mg /kg B.W of methionine for four weeks and then af-

ter that intubated orally 0.51 ml /kg B.W/ of olive oil daily for further four weeks (GIII). Fasting blood samples were collected by cardiac puncture technique at days zero, four and eight weeks of the experiment for measuring serum concentration of the following: a- Total cholesterol(TC) , Triacylglycerol (TAG) and high density lipoprotein-cholesterol (HDL-C) using enzymatic Kit (Biocon, Germany),b- low density lipoprotein-cholesterol (LDL-C)and very low density lipoprotein-cholesterol (VLDL-C) according to(14), c- glutathione (GSH) according to (7) .

Malondialdehyde (MDA) concentration was measured by thiobarbituric acid (TBA) assay as described by (20): one ml`of serum was added to 3 ml of 1% phosphoric acid, 1 ml of 0.6% TBA and 0.15ml of 0.2% hydroxyl butylated toluene (BHT) in 95% methanol. The samples were heated in a boiling water bath for 45 minutes; MDA was reacted with TBA under high temperature (90-100°C) and acidic condition .The reaction yielded MDA –TBA adducts (Pink color). The solution was left to cool and 4 ml of butanol was added .The butanol phase was separated by centrifugation at 3000rpm for 10 minutes and absorbance was measured at 532nm with an extinction coefficient of absorbance **532 =** 1.53 x10 M cm. Statistical analysis of data for two experiments was performed on the basis of two way analysis of variance (ANOVA) using significant level of ($P<0.01$).Differences were determined using least significant differences (LSD) according to(33).

Results and Discussion

Effect of olive oil on Serum lipid profile of methionine overload rabbits

The mean value of total cholesterol concentration (TC) and TAG in male rabbits was illustrated in table -1 and 2 respectively. Serum TC concentrations were close in all groups in zero time. The results showed a general trend for the TC and TAG mean values to significantly ($P<0.01$) increased after four weeks of treatment in GII and GIII groups as compared to the control group. However, this increment was continued in methionine (GII)treated group after eight weeks of experiment. Whereas , TC concentration was observed to be significantly ($p<0.01$) decreased in GIII group comparing to the values in GII group. Within the time significant ($p<0.01$) decrease in TC concentration was observed in GIII at week eight comparing to week four. A significant($P<0.01$) decrease was observed in serum HDL-C in GII and GIII groups after 4 weeks of experiment comparing with control (GI) group .Such decrement was continued till the end of the experiment in group GII.While the value of HDL-C tended to significantly ($p<0.01$) increase after 8 week of experimental period in GIII comparing with GII (table -3). After 4week of treatment with methionine, significant ($p<0.05$) increase in concentration of LDL-C (table-4) and VLDL-C (table-5) was observed in GII and GIII comparing to control group. The results here pointed to the positive effect of intubation of olive oil for four week in methionine overload rabbits in the reduction of both parameters in group GIII comparing to group GII.

Table (1) Effect of olive oil on serum Total cholesterol (TC) concentration (mg/dl) in methionine over load rabbits .

Group Time	GI	GII	GIII
Zero time	130.9±0.29 A a	131.0±0.33 A a	130.6±0.25 A a
After 4 weeks	131.2±0.29 A a	208.9±3.1 B b	203.4±5.0 C b
After 8 weeks	130.1±0.23 A a	211.0±4.3 B c	131.5±0.34 A a

Values are expressed as mean ± SE. n=10/ group .GI= considered as control group.
GII= rabbits received (100mg/kg B.W/day) of methionine for eight weeks .GIII= rabbits received (100 mg/kg B.W/day) of methionine for four week after that received 0.51ml /kg BW/day) of olive oil for four weeks only.
Capital letter denote difference between groups ($p<0.01$) vs. control. Small letter denote within group difference ($p<0.01$) vs. zero time.

Table (2) Effect of olive oil on serum Triacylglycerol (TAG) concentration (mg/dl) in methionine overload rabbits.

Group Time	GI	GII	GIII
Zero time	128.8±0.38 A a	127.9±0.47 A a	128.3±0.61 A a
After 4 weeks	129.3±0.33 A a	194.2±0.35 B b	190.5±0.44 C b
After 8 weeks	129.4±2.33 A a	198.9±1.2 B c	128.9±0.37 A a

Values are expressed as mean ± SE. n=10/ group .GI= considered as control group.
GII= rabbits received (100mg/kg B.W/day) of methionine for eight weeks .GIII= rabbits received (100 mg/kg B.W/day) of methionine for four week after that received 0.51ml /kg BW/day) of olive oil for four weeks only.
Capital letter denote difference between groups ($p<0.01$) vs. control. Small letter denote within group difference ($p<0.01$) vs. zero time.

Table (3) Effect of olive oil on serum High Density Lipoprotein – Cholesterol (HDL-C) concentration (mg/dl) in methionine overload rabbits.

Group / Time	GI	GII	GIII
Zero time	36.6±0.32 A a	36.9±0.37 A a	36.3±0.91 A a
After 4 weeks	36.8±0.31 A a	24.6±0.33 B b	22.9±0.82 B b
After 8 weeks	35.8±0.44 A a	20.8±1.2 B c	35.3±0.21 A a

Values are expressed as mean ± SE. n=10/ group .GI= considered as control group.
GII= rabbits received (100mg/kg B.W/day) of methionine for eight weeks .GIII= rabbits received (100 mg/kg B.W/day) of methionine for four week after that received 0.51ml /kg BW/day) of olive oil for four weeks only.
Capital letter denote difference between groups ($p<0.01$) vs. control. Small letter denote within group difference ($p<0.01$) vs. zero time.

Table (4) Effect of olive oil on serum Low Density Lipoprotein-Cholesterol (LDL-C) concentration (mg/dl) in methionine over load rabbits.

Group / Time	GI	GII	GIII
Zero time	68.7±.36 A a	68.5±0.48 A a	67.3±0.55 A a
After 4 weeks	68.4±0.44 A a	135.9±3.4 B b	136.3±1.4 B b
After 8 weeks	68.5±0.53 A a	139.5±2.9 B c	66.44±0.37 A a

Values are expressed as mean ± SE. n=10/ group .GI= considered as control group.
GII= rabbits received (100mg/kg B.W/day) of methionine for eight weeks .GIII= rabbits received (100 mg/kg B.W/day) of methionine for four week after that received 0.51ml /kg BW/day) of olive oil for four weeks only.
Capital letter denote difference between groups ($p<0.01$) vs. control. Small letter denote within group difference ($p<0.01$) vs. zero time.

Table (5) Effect of olive oil on serum very Low Density Lipoprotein-Cholesterol (VLDL-C) concentration (mg/dl) in methionine overloads rabbits.

Group / Time	GI	GII	GIII
Zero time	25.7±0.07 A a	25.5±0.1 A a	25.80±0.21 A a
After 4 weeks	25.8±0.07 A a	38.8±0.7 B b	37.9±0.41 B b
After 8 weeks	25.8±0.06 A a	40.1±1.6 B c	25.4±0.12 A a

Values are expressed as mean ± SE. n=10/ group .GI= considered as control group.
GII= rabbits received (100mg/kg B.W/day) of methionine for eight weeks .GIII= rabbits received (100 mg/kg B.W/day) of methionine for four week after that received 0.51ml /kg BW/day) of olive oil for four weeks only.
Capital letter denote difference between groups ($p<0.01$) vs. control. Small letter denote within group difference ($p<0.01$) vs. zero time.

An association between hyperlipidemia and hyperhomocysteinemia (HHCY) has been suggested (26). The expected hyperhomocysteinemia induced after methionine over load plays an important role in cholesterol biosynthesis by inducing transcription as well as translation of 3- hydroxy-3- methylglutaryl coenzyme A(HMG-CoA) reductase **(**Limiting enzyme in the cholesterol biosynthesis). It also increased cholesterol synthesis, intestinal absorption and suppressed excretion of cholesterol (15). Furthermore, Hypercholesterolemia under effect of methionine overload may cause mutation of LDL receptors adaptor protein, leading to defective LDL receptors and reduction in plasma LDL clearance (25). There is a negative correlation between plasma Hcy and HDL –cholesterol(23,43). Hypolipidemic effect of olive oil is documented (8,21), due to its content of polyunsaturated fatty acids (PUFA) (1,18,39). These essential fatty acid have a noticeable effect on lowering plasma homocysteine (3) and hence reducing cholesterol concentration. Besides, Omega 3 and 6 fatty acids of olive oil may be responsible for elevation of HDL-C (good cholesterol) concentration through elevation hepatic HDL-C receptor activity and facilitating transition of fatty acids from VLDL-C to HDL-C (1). Furthermore , high sequalene content in olive oil may exert hypolipidimic effect through inhibition HMG-CoA reductase activity and then farnesylation (13).

Effect of olive oil on antioxidant status of methionine overload rabbits

A significant ($p<0.01$) decrease in serum GSH and significant increase in serum MDA concentration were detected at four week in methionine treated group (GII and GIII) comparing to the control (table -6 and -7 respectively).The protective effect of olive oil intubation on the antioxidant status of the animals were clarified was observed in GIII after four weeks of olive oil treatment (after 8weeks of the experiment). Significant elevation in serum GSH and depletion in serum MDA concentra-

tion were reported comparing to GII group. Herein the value of MDA in GIII become below that of control group at the end of the experiment .

Table (6) Effect of olive oil on serum Reduced Glutathione (GSH) concentration in methionine overload rabbits.

Group / Time	GI	GII	GIII
Zero time	16.4±0.45 A a	16.4±0.33 A a	16.2±0.21 A a
After 4 weeks	15.7±0.36 A a	7.1±0.23 B b	8.0±0.41 C b
After 8 weeks	15.8±0.24 A a	6.1±0.51 B c	15.7±0.26 A a

Values are expressed as mean ± SE. n=10/ group .GI= considered as control group.
GII= rabbits received (100mg/kg B.W/day) of methionine for eight weeks .GIII= rabbits received (100 mg/kg B.W/day) of methionine for four week after that received 0.51ml /kg BW/day) of olive oil for four weeks only.
Capital letter denote difference between groups ($p<0.01$) vs. control. Small letter denote within group difference ($p<0.01$) vs. zero time.

Table (7) Effect of olive oil on serum Malondialdehyde (MDA) concentration (µmol/dl) in methionine overloads rabbits.

Group / Time	GI	GII	GIII
Zero time	0.40±0.005 A a	0.43±0.007 A a	0.37±0.005 A a
After 4 weeks	0.39±0.004 A a	0.87±0.023 B b	0.9±0.031 B b
After 8 weeks	0.41±0.008 A a	1.0±0.08 B c	0.35±0.05 C a

Values are expressed as mean ± SE. n=10/ group .GI= considered as control group.
GII= rabbits received (100mg/kg B.W/day) of methionine for eight weeks .GIII= rabbits received (100 mg/kg B.W/day) of methionine for four week after that received 0.51ml /kg BW/day) of olive oil for four weeks only.
Capital letter denote difference between groups ($p<0.01$) vs. control. Small letter denote within group difference ($p<0.01$) vs. zero time.

The results found in GII group manifested by significant decrease in serum GSH and elevation in MDA concentration provide an inductor for oxidative stress (38). Many authors demonstrated that mild HHcy is much more common and associated with postmethionine loading in water (4) or in diet (2) . It can be hypothesized that a de-pression in scavenging activity of metalothionine after methionine overload (5) may lead to production of superoxide and decrease in the antioxidant production including GSH in this study. In addition , such predicted HHcy after methionine overload may decrease the ability of the cells to detoxify H_2O_2 and other lipid peroxide (29, 36),

and it might indirectly participate in reduction in the activity of intracellular antioxidant. An elevation in generation of lipid peroxidation (MDA level) was postulated to cause a gradual cell injury via liberating lipoxygenase enzymes which oxidize unsaturated membrane fatty acids and subsequent production of MDA (32) .

Healthy effects of olive oil have been suggested to reflect its content of high concentration of antioxidants , including chlorophyll , caratenoids , polyphenolic compounds tyrosol , hydroxyl tyresol (30,37). The protective effects of olive oil is also exerted by its ingredient (biophenol namely protoacahuic acid oleuorpein) via inhibiting LDL oxidation (27). On conclusion, olive oil should be included in our food attributed to its capacity in alleviation oxidative stress and dyslipidemia accompanied many disease condition.

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