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Protective role of olive oil and /or folic acid on some biochemical biomarkers related to cardiac disease in Rabbits exposed to Methionine overload

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ABSTRACT

The present study was aimed to investigate the protective role of olive oil and/or folic acid against cardiac damage induced by methionine overload rabbits. Forty male rabbits were divided into five groups (8/group), the first group was intubated with tap water and served as control group(GI). Rabbits in the second group were intubated orally with 100 mg/kg B .W of methionine (GII). While animals of the third group were intubated with methionine and 0.51 ml/kg B.W. of olive oil (GIII), in addition to methionine, rabbits in the fourth group (GIV) were intubated orally with 0.07 mg/kg B.W of folic acid . Animals of the last group (GV) were intubated with methionine, folic acid and olive oil. Fasting blood samples were collected at zero,28 and42 days of 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 . Furthermore, aortic and cardiac muscle tissue were assessed for histopathological changes . The results revealed that intubation of male rabbits with 100mg/kg B.W .of methionine daily for four weeks caused a significant ($p<0.01$) increase in serum TC , TAG, LDL-C, VLDL-C and MDA concentration, and a significant decrease ($p<0.01$) in serum GSH and HDL-C concentration, comparing to control ,folic acid and olive oil treated groups. Histological section revealed that methionine initiated aortic atheromatus lesion characterized by a thickness of the intimae, infiltration of inflammatory cells with focal foamy cells in subintimal layer and infiltration of inflammatory cells in cardiac muscles. The result also showed that oral olive oil and /or folic acid caused significant correction of the previous parameters manifested by significant elevation in serum HDL-C , GSH, in addition to significant suppression of serum TC , TAG , LDL-C ,VLDL-C ,MDA concentrations ,as well as complete regression of atheromatous lesion were observed after olive oil intubation. In conclusion, the results of this study confirm the protective role of olive oil against cardiac damage (both structurally and functionally) induced by methionine overload and documented the prevalence of olive oil upon folic acid in all measures issued.

Key word: Olive oil, Folic acid, Lipid profile, Atherosclerosis, MDA.

INTRODUCTION

Methionine is the source of sulfur for numerous compounds in the body .The body used sulfur to influence hair follicles and promote healthy hair, skin and growth. Sulfur also increase the liver production of lecithin (which reduce cholesterol), reduced liver fat (1), protects the kidney, helps the body to excrete heavy metals and reduced bladder irritation by regulating the

formation of ammonia in the urine (2). Thus sulfur containing amino acid deserves special attention because it is converted to S- adenosyl methionine (SAM), the major methyl-group donor in one–carbon metabolism required for many cellular process(1,3).However, excess methionine caused a case of hyperhomocysteinemia (HHCY) which are toxic to human and animal health. HHCY is a condition in which the regulation of intracellular homocysteine (Hcy) level is disrupted and Hcy export to the plasma compartment is accelerated and / or normal plasma clearance is decreased (4). Mild HHCY in fasting conditions can be attributed to any of the following factors : methionine overload, impairment in the methylation pathway like folate or vitamine B_{12} or B6 deficiencies and methylated tetrahydrofolate (MATHR)]. In addition to pathological states like renal insufficiency , breast , ovarian and pancreatic cancers and lymphocytic leukemia (5,6).

Olive oil has the best characteristic as regards digestibility and absorption and has a mild laxative effect (7). Olive oil has beneficial effect on gastritis, ulcers and gall stones also helps the body to absorb calcium (8). Studies in diabetic patient have shown that healthy meals that contained some olive oil had better effect on blood sugar even than healthy meals that were low in fat through a diponectin hormones which is produced and secreted by fat cells (9), its phenols protect DNA from free radicals damage , where the antioxidants properties of olive oil may be one of the key reason for the lowering incidence of cancer in the Mediterranean region (10).

Folic acid (Vitamin B9) with the collaboration of vitamin B_{12} converts Hcy to methionine, therefore reducing blood levels of Hcy and lowering risks of heart disease, it is vital for reproduction of the cells within the fetus (11). The antioxidant effect of folic acid is documented, through its reduction of blood homocysteine levels and then depression of lipid peroxidation and free radicals production (12).The present study was designed to investigate the ameliorative effect of olive oil relative to folic acid in methionine overload rabbits .

MATERIALS AND METHODS

Forty (40) adult rabbits were randomly divided into 5 groups (eight each group) and treated as follows for 4 weeks: Group(GI), animals in this group were intubated orally ordinary tap water , serving as control ,Rabbits in group (GII) were intubated orally 100mg/kg B.W of D-L methionine. In addition to methionine ,animals in group (GIII) were intubated orally effective dose of olive oil obtained from our previous (13) experiment (0.51 ml/kg BW),while animals of group(GIV) were intubated 100 mg/kg BW of methionine plus 0.07mg/kg B.W of folic acid. Rabbits of group (GV) were administered orally same doses of methionine, folic acid and olive oil. Fasting blood samples were collected by cardiac puncture technique at days zero and 30 of experiment for measuring serum concentration of the following: a-Total cholesterol , Triacylglycerol (TAG), high density lipoprotein-cholesterol (HDL-C) using Kit (Biocan , Germany), b- low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C) according to(14), c- Glutathione (GSH) as described by (15) and d- Malon-dialdehyde (MDA) according to (16).

In addition, Histological sections from heart and aorta were taken for histopathological examination (17). Statistical analysis of data for two experiments was performed on the basis of two way analysis of variance (ANOVA) using significant level of ($P<0.01$) and ($P<0.05$) .Differences were determined using least significant differences (LSD)(18).

RESULTS AND DISCUSSION

The mean value of serum TC,TAG,LDL-C and VLDL-C concentrations in different treated and control groups were clarified in Tables 1,2,3 and 4 respectively. Total serum cholesterol concentration,as well as, cholesterol concentration in VLDL and LDL was increased significantly ($p<0.01$) after intubation of methionine (100mg/kg /BW) daily compared to control and other treated groups .While oral intubation of olive oil, folic acid or both in combination with methionine caused significant ($p<0.01$) depression in the previous parameters in groups GIII,GIV and GV comparing to methionine treated group GII and the values in GV tend to normalize that of the control .On the contrary, comparing to GII group , olive oil or folic acid intubation caused significant($p<0.01$) elevation in mean value of serum HDL-C concentration and the values tend to normalize that of the control in group GV (table 5).

Table (1) Effect of daily oral intubation of olive oil and folic acid for four weeks on serum Total Cholesterol (TC) concentration (mg/dl) in methionine treated rabbits.

Group Time	GI control	GII Methionine (M)	GIII Methionine +olive oil (O)	GIV Methionine +Folic acid (F)	GV Methionine +Olive oil +Folic acid
Zero time	130.6±0.67 A a	130.0±0.7 A a	129.8±1.5 A a	133.4±1.94 A a	128.6±0.92 A a
After 4 weeks	130.8±0.58 A a	217.6±10.9 B b	171.25±3.12 C b	193.7±1.27 D b	128.7±0.73 A a

Values are expressed as mean ± SE n=8/ group.
Capital letter denote between groups difference ($p<0.01$) vs. control.
Small letter denote within group difference ($p<0.01$) vs. zero time .
M=100mg/kg B.W; O= 0.51ml/kg B.W; F= 0.07mg/kg B.W.

Table (2) Effect of daily oral intubation of olive oil and folic acid for four weeks on serum triacylglycerol (TAG) concentration (mg/dl) in methionine treated rabbits.

Group / Time	GI control	GII Methionine (M)	GIII Methionine+ olive oil(O)	GIV Methionine+ folic acid(F)	GV Methionine +olive oil + folic acid
Zero time	131.6±1.0 A a	132.2±1.11 A a	132.0±1.37 A a	132.8±0.86 A a	131.6±0.24 A a
After 4 weeks	131.0±0.94 A a	190.6±2.33 B b	143.0±3.0 C b	166.4±1.8 D b	130.3±2.1 A a

Values are expressed as mean ± SE n=8/ group .
Capital letter denote between groups difference ($p<0.01$) vs. control.
Small letter denote within group difference ($p<0.01$) vs. zero time .
M=100mg/kg B.W; O= 0.51ml/kg B.W; F= 0.07mg/kg B.W.

Table (3) Effect of daily oral intubation of olive oil and folic acid for four weeks on serum Low Density Lipoprotein-Cholesterol (LDL-C) concentration (mg/dl) in methionine treated rabbits.

Group / Time	GI control	GII Methionine (M)	GIII Methionine +Olive oil (O)	GIV Methionine +Folic acid (F)	GV Methionine +Olive oil +Folic acid
Zero time	68.2±1.0 A a	68.3±1.7 A a	66.6±1.3 A a	68.4±1.2 A a	65.0±1.5 A a
After 4 weeks	71.01.8 A a	164.6±1.4 B b	110.4±5.2 C b	138.5±3.5 D b	69.8±1.9 A a

Values are expressed as mean ± SE n=8/ group .
Capital letter denote between groups difference ($p<0.01$) vs. control.
Small letter denote within group difference ($p<0.01$) vs. zero time .
M=100mg/kg B.W; O= 0.51ml/kg B.W; F= 0.07mg/kg B.W.

Table (4) Effect of daily oral intubation of olive oil and folic acid for four weeks on serum Very Low Density Lipoprotein-Cholesterol (VLDL-C) concentration(mg/dl) in methionine treated rabbits .

Group Time	GI control	GII Methionine (M)	GIII Methionine +olive oil (O)	GIV Methionine +Folic acid (F)	GV Methionine +olive oil +Folic acid
Zero time	26.3±0.21 A a	26.4±0.22 A a	26.3±0.27 A a	26.9±0.53 A a	26.4±0.6 A a
After 4 weeks	26.2±0.18 A a	38.1±0.47 B b	28.6±0.61 C b	33.2±0.36 D b	25.2±0.59 A a

Values are expressed as mean ± SE n=8/ group .
Capital letter denote between groups difference ($p<0.01$) vs. control.
Small letter denote within group difference ($p<0.01$) vs. zero time .
M=100mg/kg B.W; O= 0.51ml/kg B.W; F= 0.07mg/kg B.W.

Table (5) Effect of daily oral intubation of olive oil and folic acid for four weeks on serum High Density Lipoprotein-Cholesterol (HDL-C) concentration (mg/dl) in methionine treated rabbits.

Group Time	GI control	GII Methionine (M)	GIII Methionine +olive oil (O)	GIV Methionine +Folic acid (F)	GV Methionine +Olive oil +Folic acid
Zero time	34.8±0.73 A a	34.8±1.0 A a	36.8±0.37 A a	36.0±0.83 A a	36.8±1.2 A a
After 4 weeks	33.6±1.3 A a	16.0±1.8 B b	29.6±1.1 C b	19.4±0.6 D b	33.2±1.1 A a

Values are expressed as mean ± SE n=8/ group .
Capital letter denote between groups difference ($p<0.01$) vs. control.
Small letter denote within group difference ($p<0.01$) vs. zero time .
M=100mg/kg B.W; O= 0.51ml/kg B.W; F= 0.07mg/kg B.W.

Serum Glutathione and Malondialdehyde concentrations

Serum GSH concentration (table-6) was significantly ($p<0.01$) decreased and serum MDA concentration (Table 7) was significantly ($p<0.01$) increase after intubation of methionine (Group II) for four weeks as compared to control and other treated groups, at the same time combined intubation of folic acid or olive oil to methionine overload rabbits for four weeks, showed significant decrease ($p<0.01$) in the serum concentrations of MDA and elevation in serum GSH comparing to methionine treated group. As well as, combination of

olive oil and folic acid with methionine (Group V) caused further significant correction in these parameters comparing to other groups and the value seems to normalize the control one.

Table (6) Effect of daily oral intubation of olive oil and folic acid for four weeks on serum Glutathione (GSH) concentration (µmol /l) in methionine treated rabbits.

Group Time	GI control	GII Methionine (M)	GIII Methionine +Olive oil(O)	GIV Methionine +Folic acid (F)	GV Methionine +olive oil +Folic acid
Zero time	15.2±0.89 A a	15.3±0.47 A a	15.5±0.16 A a	14.1±0.13 A a	15.2±0.10 A a
After 4 weeks	15.6±0.11 A a	8.2±0.25 B b	13.0±0.16 C b	9.8±0.13 D b	18.8±0.10 A a

Values are expressed as mean ± SE n=8/ group .
Capital letter denote between groups difference ($p<0.01$) vs. control.
Small letter denote within group difference ($p<0.01$) vs. zero time .
M=100mg/kg B.W; O= 0.51ml/kg B.W; F= 0.07mg/kg B.W.

Table (7) Effect of daily oral intubation of olive oil and folic acid intubation for four weeks on serum Malondialdehyde (MDA) concentration (µmol/d l) in methionine treated rabbits.

Group Time	GI control	GII Methionine (M)	GIII Methionine +Olive oil (O)	GIV Methionine +Folic acid (F)	GV Methionine +olive oil+ Folic acid
Zero time	0.41±0.008 A a	0.42±0.007 A a	0.40±0.05 A a	0.41±0.005 A a	0.42±0.009 A a
After 4 weeks	0.40±0.009 A a	0.96±0.04 B b	0.84±0.03 C b	0.82±.0.35 C b	0.40±0.007 A a

Values are expressed as mean ± SE n=8/ group .
Capital letter denote between groups difference ($p<0.01$) vs. control.
Small letter denote within group difference ($p<0.01$) vs. zero time .
M=100mg/kg B.W; O= 0.51ml/kg B.W; F= 0.07mg/kg B.W.

Histological sections of heart and aorta

Proliferation of the intima with fatty streak and foamy cells (early stage of atherosclerosis) as well as thickness in the intima were clarified in aorta of methionine treated rabbits (Fig. 1), while other sections showed hemorrhage in the media layer and vaculation of endothelial cell lining the intima (Fig. 2), while hyperemia of aorta and vaculation of intima were cleared in another section (Fig. 3). Besides, microscopic examination of sections of heart

in animals treated with methionine showed inflammatory cells infiltration mainly neutrophils between myocardial muscles. Other histological sections showed mononuclear cells infiltration in adipose tissue in atrium with congestion of blood vessels (Figs. 4 and 5). Histological section of heart and aorta in animals intubated with olive oil,folic acid or both showed normal histology of cardiac muscle and aorta with exception mononuclear cells infiltration (Figs. 6,7,8 and 9).

The antioxidants status :

Studying the effect of methionine overload on the antioxidant status of the rabbits in the present study ,showed a significant decrease in serum GSH concentration in methionine treated group. The result of the present study was correlated with(19,20,21). Such changes may be attributed to HHcy induced after methionine overload. Mild HHcy is much more common and is associated with postmethionine loading in water (22) or in diet (23).

The increase of MDA level may be due to an increase in the production of free radicals more than ability of the scavenging system to remove them with resultant depression in serum GSH levels. This findings is in agreement with many laboratory studies which indicated alteration in the antioxidants status of different tissue as a results of an increase in lipid perooxidation (24,25). Oral administration of olive oil and/or folic acid to adult's male rabbits for four weeks exerted a significant increase in serum GSH level and a significant decrease in serum MDA levels. There is a strong correlation between elevation of GSH level and depression of lipid peroxidation after olive leaves (24), or olive oil treatment (9).

GSH is regarded as a major cellular antioxidants, so it is elevation by olive oil indicates augmented cellular protection (26) against free radicals damage after methionine overload and insure the free radicals scavenging properties of olive oil accompanied by subsequent depression in lipid peroxidation and MDA levels (27). Healthy effects of olive oil may be due to high concentration of antioxidants , including chlorophyll , caratenoids and polyphenolic compounds tyrosol , hydroxyl tyresol , all which not only have free radicals scavenging abilities , but protect vitamin E (alpha tocopherol) present in olive oil (28,29).

The antioxidant effect of folic acid is documented, such effect may be due to reduction of blood homocysteine levels and then depression of lipid peroxidation and free radicals production (30,31,32). One other possible pathway is that folic acid may effectively inhibit NADPH oxidase mediated superoxide production leading to reduction of lipid peroxidation and subsequent decrease in MDA level (33).

Serum lipid profile:

This study showed that methionine intubation to male rabbits for four weeks lead to significant changes in lipid profile system .These changes were manifested by increase in TC, TAG, LDL-C, VLDL-C and decrease in serum HDL-C concentrations. Hyperhomocysteinemia are claimed to be the major cause of adverse effect of methionine overload. An association between hyperlipidemia and HHcy has been suggested ,where high plasma Hcy was associated with lower HDL-C level and disturbed plasma lipids or fatty liver (34,35). Elevated Hcy may impaired nitric oxide (NO) through depression in plasma cysteine (36,37) , the most important amino acid for cholesterol excretion , and salt formation (38) , accordingly HHcy after methionine overload may produce hyperlipidemia through this mechanism.The increase in

TAG levels in animals receiving methionine in the present study may be duo to increment in plasma VLDL-c levels(which act as carrier for the TAG in the plasma),partial deficiency of lipoprotein lipase ,associated with increase put out of lipoprotein from liver(39).

Oxidative stress is one mechanism by which HHcy affect lipoprotein particles and their by damage endothelial cells through formation of oxidized (40) or homocysteinylated LDL(41) ,as well as decreased activity of hepatic thiolase and serum LCAT (42),two main enzymes involved in HDL metabolism as well as down regulation of key player in HDL production (apo-A1,LSAT,ABC1) by Hcy (34) could be another possible mechanism.

The present results showed that oral intubation of 0.51 ml of olive oil for 4 weeks, produced pronounced correction of dyslipidemia. The hypolipidemic effect of virgin and extra virgin olive oil are documented by other studies (24) on olive leave and (43,44) on virgin olive oil. .Monounsaturated fatty acids found in olive oil is responsible for its hypolipidemic effect. Oleic acid of olive oil may increase the break down of fats in adipocytes (45) and cause reduction of plasma LDL-C concentration(46Covas2006a).PUFA of olive oil also involved in the treatment of dyslipidemia ,the hypolipidemic role of PUFA may be either through inhibition of hepatic synthesis and secretion of TAG and reduction of chylomicron secretion from intestinal cell ,while their role in limiting VLDL-C secretion can be mediated by a lowering rate of VLDL-C lipoprotein B conversion to LDL-C (47). It is likely that the abundant polyphenolic compounds and vitamin E present in olive oil, rather than it's monounsaturated fatty acids are responsible for its well hypolipidemic effect (48), where it can provide benefits for lowering plasma lipid levels and oxidative damage (49,50).

Oral administration of folic acid 0.07mg/kg B.W/daily for four week caused correction of dyslipidemia. The hypolipidemic effect of folic acid in present study may be result from reduction in the plasma homocysteine concentration .It has been documented that supplementation of folic acid reduced plasma HCY level by approximately 21-33%(51,52)which lead to significant change in lipid profile (53) and protected LDL and VLDL particles from oxidation (54).The antioxidant effect of folate can be related to HCY-lowering which causes lowering of LDL-C and increased HDL-C with slight reduction in total cholesterol and TAG(55). Folic acid may altered lipid metabolism through its action on 5-deoxy adenosylcoblamine which catalysis the conversion of L-methylmalonyl COA to succinyl COA, an important reaction in energy production from fat and protein (56) .Besides ,reduction in lipid peroxidation by folic acid may suppress hepatic cholesterol and TAG concentrations through elevation lipoprotein lipase activity (57).

Histological sections:

The present study pointed that intubation of male rabbits with methionine (100mg/kgB.W/daily)for 28 days , resulted in the appearance of atheromatous lesion in the aorta. Several mechanisms of Hcy-associated atherosclerosis have been proposed. These include endothelial dysfunction, an increase in vascular smooth muscle cell proliferation, stimulation of chemokinase expression, oxidative stress, endoplasmic reticulum stress and disruption of lipid metabolism(37,40). Oxidation of LDL by Hcy transforms these lipoproteins into more atherogenic particles with vasomotor and thrombogenic properties, thereby favoring the onset and progression of atheromatous plaques(41).

The histological examination of aortic and cardiac muscle of rabbits received olive oil and/or folic acid revealed complete regression of aortic, cardiac atheromatus lesion cased by

methionine overloads. Numerous Studies have confirmed a negative correlation between plasma HDL level and atherosclerosis and a positive correlation between HDL and a longer life expectancy (58) .Olive oil, which is rich in monounsaturated and polyunsaturated fats have a positive effect on total cholesterol reduction, and HDL- cholesterol elevation which help to protect against observed atheromatuse lesions (59,60). Furthermore, olive oil supplies of vitamin E and phenols with high antioxidation activity may be responsible for correction of atherosclerotic lesion induced by methionine overload(61) .

Many studies suggest that folic acid can help to reduce risk factors for heart disease and the harm that caused by cholesterol and Homocysteine (62,54). Treatment with folic acid is effective in reducing plasma homocysteine concentrations, and consequently regression of atheromatous lesion. Besides, folic acid may exert either an indirect antioxidant effect by reducing the pro-oxidant action of homocysteine and consequently lowering oxidative stress leading to regression of atheromatus lesion or direct antioxidant effects during restoration of endothelial function, probably by affecting cellular oxidative metabolism (63) . In conclusion, based on the hypolipidemic and antioxidants effect of olive oil correlated with its beneficial effect in the removal of atherosclerotic lesion and cardiac damage produced by methionine overload , olive oil could be taken to prevent deleterious effect of methionine .

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الدور الوقائي والعلاجي لزيت الزيتون وحامض الفوليك في بعض المعايير الكيميوحيوية والنسيجية في ذكور الأرانب المعرضة لفرط الميثيونين

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المستخلص

أجريت هذه الدراسة لمعرفة الدور الوقائي لزيت الزيتون وحامض الفوليك ضد التلف الحاصل في القلب المستحدث بفرط الميثيونين في ذكور الأرانب. تم أستخدام (40) من ذكور الأرانب البالغة والتي قسمت الى خمس مجاميع متساوية. جرعت حيوانات المجموعة الأولى الماء العادي فموياً (السيطرة GI) ،اما ارانب المجموعة الثانية (GII) فقد جرعت 100 ملغم /كغم من وزن الجسم من المثيونين ،بالأضافة الى المثيونين جرعت حيوانات المجموعةالثالثة (GIII) والرابعة (GIV) 0.51 مل/كغم من وزن الجسم من زيت الزيتون و0.07 ملغم/كغم من الجسم من حامض الفوليك على التوالي، في حين جرعت أرانب المجموعة الخامسة(GV) المثيونين ،زيت الزيتون وحامض الفوليك.تم جمع عينات الدم في الفترات 0,28 و42 يوم من التجربه لقياس صورة دهون الدم ،المالوندالديهايد (MDA) والكلوتاثايون المختزل (GSH) في مصل الدم ،فضلاً عن دراسة التغيرات المرضية النسجية للابهر والقلب. أظهرت نتائج هذه التجربة حدوث ارتفاع معنوي في تركيز الكوليستيرول الكلي (TC) ،ثلاثي أسيل الكليسيرول(TAG) ،الكوليستيرول في الشحوم البروتينية واطئة الكثافة (LDL-C) والواطئة جدا(VLDL-C) و المالوندالديهايد في مصل الدم نتيجة اعطاء المثيونين مع حصول انخفاض معنوي في تركيز الكوليستيرول في الشحوم البروتينية عاليةالكثافة (HDL-C)و الكلوتاثايون المختزل مقارنة مع مجموعة السيطرة والمجاميع المعاملة بزيت الزيتون وحامض الفوليك.حيث تسبب التجريع الفموي مع /او حامض الفوليك بعودة المعايير السابقة الى تراكيزها الطبيعية والمتمثلة بحدوث ارتفاع معنوي في تراكيز HDL-CوGSH بالاضافة الى الأنخفاض المعنوي في تراكيز TCوTAGوLDL-CوVLDL-CوMDA .كما أظهر الفحص النسيجي ظهور الأفات الأولية للتصلب العصيدي متميزة بتثخن البطانة وارتشاح الخلايا الألتهابية مع بؤر من الخلايا الدهنية في تحت البطانة وارتشاح الخلايا البطانية في عضلة القلب .في حين لوحظ انكفاء كامل لعلامات التصلب العصيدي والتلف القلبي بعد اعطاء زيت الزيتون .يستنتج من الدراسة الحالية الدور الوقائي لزيت الزيتون من التأثيرات الضارة لفرط الميثيونين في القلب وتؤكد أفضلية زيت الزيتون على حامض الفوليك في المعايير المحسوبة .

Protective role of olive oil and /or folic acid on some biochemical biomarkers related to cardiac disease in Rabbits exposed to Methionine overload

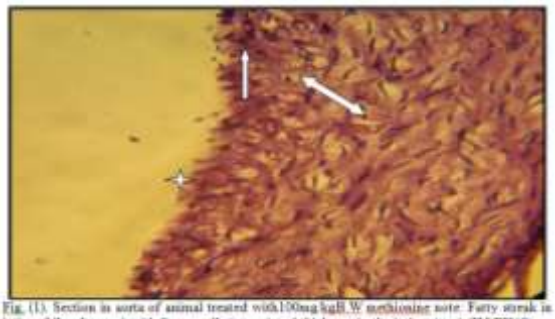

Fig. (1): Section in aorta of animal treated with100mg/kgB.W methionine note: Fatty streak in intima (bihead arrow) with foamy cells (arrow) and thickness in the intima (star) (H&EX40).

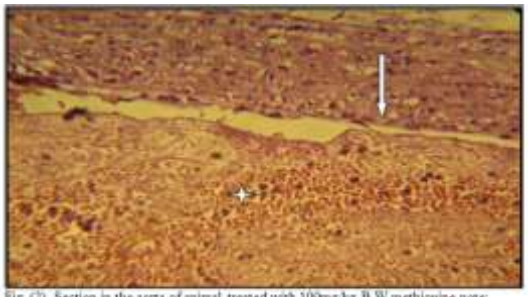

Fig. (2): Section in the aorta of animal treated with 100mg/kg B.W methionine note: Hemorrhage in the media layer (star) with vaculation of intima (arrow) (H&EX40).

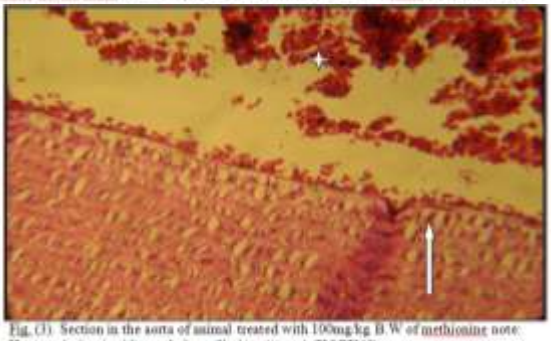

Fig. (3): Section in the aorta of animal treated with 100mg/kg B.W of methionine note: Hyperemia (star) with vaculation of intima (arrow) (H&EX40).

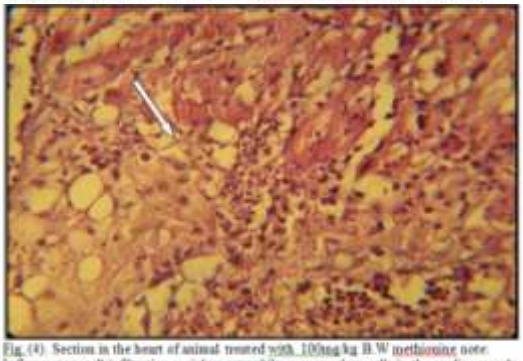

Fig. (4): Section in the heart of animal treated with 100mg/kg B.W methionine note: Inflammatory cell infiltration mainly neutrophils, mononuclear cells in the cardiac muscle fibers (arrow) (H&EX40).

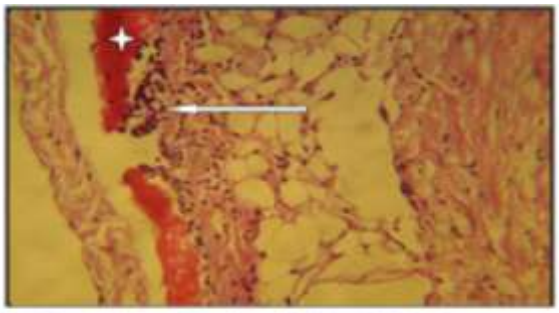

Fig. (5): Section in the heart of animal treated with100mg/kg B.W methionine note: mononuclear cells infiltration in adipose tissue in atrium (arrow) with congestion of blood vessels (star) (H&EX40).

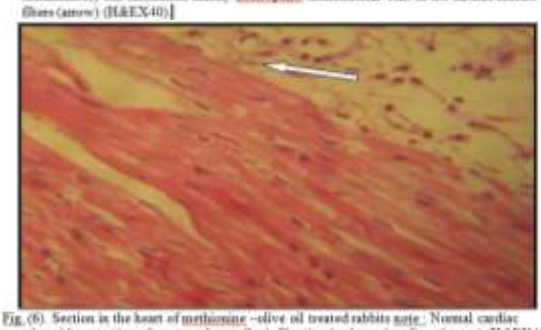

Fig. (6): Section in the heart of methionine –olive oil treated rabbits note : Normal cardiac muscle with exception of mononuclear cells infiltration in the pericardium (arrow) (H&EX40)

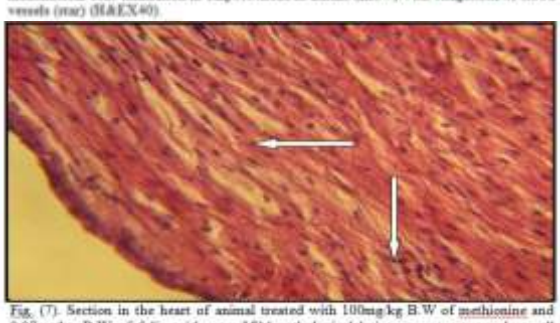

Fig. (7): Section in the heart of animal treated with 100mg/kg B.W of methionine and 0.07mg/kg B.W of folic acid note: Mild pathological lesion except mononuclear cell infiltration between muscle fibers (arrow) (H&EX40).

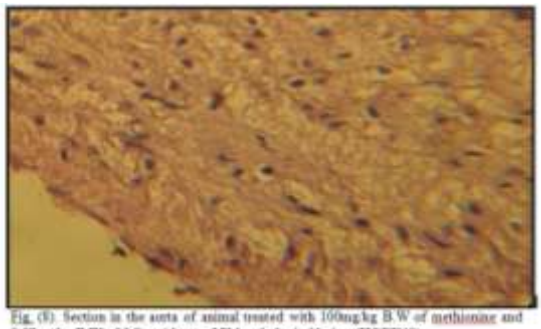

Fig. (8): Section in the aorta of animal treated with 100mg/kg B.W of methionine and 0.07mg/kg B.W of folic acid note: Mild pathological lesion (H&EX40).

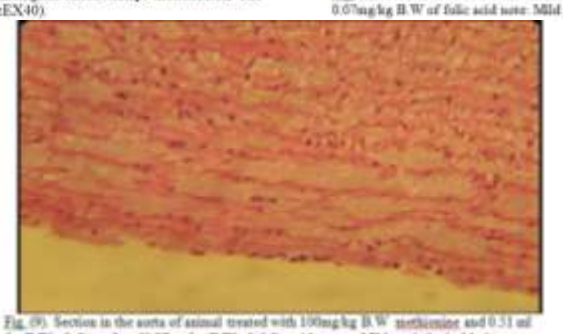

Fig. (9): Section in the aorta of animal treated with 100mg/kg B.W methionine and 0.31 ml /kg B.W of olive oil and0.07mg/kg B.W of folic acid note – Mild pathological lesion (H&EX40).