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PROTECTIVE EFFECT OF MELATONIN AND SOME DIETARY SUBSTANCES ON THE INDUCED HYPERCHOLESTEROLEMIA IN ADULT MALE RATS

(With 3 Tables and 6 Figures)

By

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التأثير الواقي للميلاتونين وبعض المواد الغذائية من زيادة الكولسترول المحدثة فى ذكور الفئران البالغة

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تهدف هذه الدراسة إلى تقييم التأثير الواقي المحتمل لزيت السمك وزيت الزيتون والميلاتونين من زيادة الكولسترول المحدثة فى ذكور الفئران البالغه. استخدم فى هذه الدراسة ٥٠ فارا قسمت إلى ٥ مجاميع، ١٠ فئران فى كل مجموعه. ولقد تم تغذية حيوانات المجموعة الأولي على الغذاء المعتاد والمجموعة الثانية تغذت على الغذاء المعتاد المضاف إليه الكولسترول بنسبة ١% (المجموعة المغذاة بالكولسترول). المجموعات من ٣-٥ تم تغذيتها بغذاء المجموعة الثانية والتي تم معالجتها فى نفس الوقت بزيت السمك فى المجموعة الثالثة (مجموعة زيت السمك) وزيت الزيتون فى المجموعة الرابعة (مجموعة زيت الزيتون) والميلاتونين فى المجموعة الخامسة (مجموعة الميلاتونين). وتم أخذ عينات دم من جميع الحيوانات فى نهاية التجربة التي استمرت ١٠ أسابيع. ثم تم الحصول على شريان الأورطى من جميع الحيوانات بعد الذبح وفحصت هستولوجيا لتقييم وجود تصلب الشرايين. وتم قياس مستوى الكولسترول الكلى بالبلازما ومستوى الكولسترول عالي الكثافة والكولسترول منخفض الكثافة والتراى جليسريد. وتم أيضا قياس إنزيم السوبر أوكسيد ديسميوتيز والثيول الكلى وأكسيد النتريك وفوق أكسيد الدهون. أظهرت النتائج أن التغذية بالكولسترول تحدث زيادة ذات دلالة إحصائية فى مستوى الكولسترول الكلى ومستوى الكولسترول منخفض الكثافة والتراى جليسريد و فوق أكسيد الدهون وانخفاض ذو دلالة إحصائية فى مستوى الكولسترول مرتفع الكثافة و السوبر أوكسيد ديسميوتاز وأكسيد النيتريك والثيول الكلى. وقد أدت المعالجة بزيت السمك وزيت الزيتون والميلاتونين مع التغذية بالكولسترول إلى نقص ذو دلالة إحصائية فى مستوى الكولسترول الكلى والكولسترول منخفض الكثافة والتراى

جليسريد وفوق أكسيد الدهون وزيادة ذات دلالة إحصائية فى مستوى السوبر أو كسيد ديسميوتيز وأكسيد النيتريك والثيول الكلى وزيادة غير معنوية فى مستوى الكولسترول عالي الكثافة . وقد أظهر الفحص الهستولوجى لشريان الأورطى لحيوانات مجموعتي زيت السمك و زيت الزيتون بداية حدوث تصلب شرياني (فى المراحل الأولى) مقارنة بالمجموعة الضابطة التي كان تصلب الشرايين بها مكتمل المراحل. أما مجموعة الميلاتونين فأظهرت مرحلة مبكرة جدا من التصلب الشرياني مقارنة بمجموعتي زيت السمك وزيت الزيتون ويمكن استنتاج أن زيادة الكولسترول تؤدى إلي زيادة التراى جليسريد والكولسترول منخفض الكثافة وفوق أكسيد الدهون والإجهاد التأكسدى وتؤدى إلى نقص فى الكولسترول عالىالكثافة ونضوب الإنزيمات المضادة للأكسدة وقد سبب زيت السمك النقص الأكبر فى التراى جليسريد والزيادة الأكبر فى اكسيد النيتريك. اما زيت الزيتون فكان الأكثر تأثيرا فى زيادة الثيول الكلى وفى خفض فوق أكسيد الدهون وكان الميلاتونين الأفضل تأثيرا فى خفض الكولسترول الكلى و الكولسترول منخفض الكثافة وبالتالي تحسن تصلب الشرايين. وهكذا يوصى باستخدام زيت السمك وزيت الزيتون والميلاتونين امنع حدوث تصلب الشرايين الذي يؤدى إلى نوبات القلب والسكتة الدماغيه وأشكال أخرى من إصابات الجهاز الدورى.

SUMMARY

This study aims to evaluate the possible protective effects of fish oil, olive oil and melatonin on the induced hypercholesterolemia in adult male rats. 50 rats were used in this study and were divided into 5 groups 10 rats each. Rats of group 1 were fed on a standard diet and those of group 2 were fed on a standard diet enriched with 1% cholesterol (cholesterol fed group). Groups 3-5 were fed as in group 2 and treated at the same time with fish oil in group 3 (fish oil group), with olive oil in group 4 (olive oil group) and with melatonin in group 5 (melatonin group). Blood samples were taken from all animals at the end of the experiment after 10 weeks. The aorta of all animals were obtained after slaughtering and examined histologically to assess the presence of atherosclerosis. Parameters of the lipogram [total plasma cholesterol (TPC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG)], superoxide dismutase (SOD), total thiol, nitric oxide (NO) and lipid peroxide (LP) were measured. Feeding cholesterol significantly increased TPC, LDL, TG and LP and significantly decreased HDL, SOD, NO and total thiol. There was a significant decrease in TPC, LDL, TG and LP by using fish oil, olive oil and melatonin while, the level of SOD, NO and total thiol were significantly increased and non significant increase in the level of HDL. Histological examination of the aorta from rats of the fish oil and olive oil groups showed fatty streaks, which are early atheromatous lesions, compared with typical atherosclerotic fibrous plaques in cholesterol fed group. In

melatonin group very early atheromatous lesions were found and they were less pronounced than in fish oil and olive oil group. It was concluded that hypercholesterolemia leads to an increase in TG, LDL, LP and oxidative stress and a decrease in HDL and a depletion of antioxidant enzymes. Fish oil caused the greatest reduction in TG and the greatest increase in NO. Olive oil was the most effective in increasing total thiol and in reducing LP, and melatonin was the best factor reducing TPC, LDL and consequently atherogenesis. Therefore, fish oil, olive oil and melatonin supplementation is recommended to prevent arteriosclerosis that leads to heart attacks, strokes and other forms of cardiovascular damage.

Key words: *Fish oil, olive oil, melatonin, hypercholesterolemia, rat.*

INTRODUCTION

Cholesterol, a very important lipid, has many important functions in the body. It serves as a precursor for plasma membranes, bile salts, steroid hormones and other specialized molecules. On the other hand, high plasma concentrations of cholesterol enhance the development of atherosclerosis, the arterial thickening that leads to heart attacks, strokes and other forms of cardiovascular damage (Vander *et al.,* 1998).

Fish oil as a rich source of omega-3 polyunsaturated fatty acids (PUSFA) has been shown to be a hypolipidemic, anti–inflammatory and anti-platelet agent. So it is a cardioprotectant reducing risk factors for coronary heart disease (Collier *et al.,* 1993, Yilmaz *et al.,* 2002 and Higuchi *et al.,* 2006).

Olive oil with its high oleic acid content and abundant polyphenols guards against atherogenesis. Olive oil increases antioxidant capacity in the liver, heart, aorta, platelets and brain. In addition, olive oil has got nitric oxide (NO) releasing properties (Visioli and Galli, 2001, Puiggros *et al.,* 2002, Faine *et al.,* 2004 and Gonzalez-Santiago *et al.,* 2005).

Melatonin is a potent antioxidant that plays a critical role in free radical scavenging (Reiter *et al.,* 1994 and Ahmed *et al.,* 2005). Melatonin has a hypocholesterolemic effect and thus it has protective effects on the cardiovascular system by reducing risk of atherosclerosis (Bubenik *et al.,* 1998, Sener *et al.,* 2004 and Nishida, 2005).

In this work, evaluation of the possible protective effects of fish oil, olive oil and melatonin on the induced hypercholesterolemia in adult male rats was studied.

MATERIALS and METHODS

This study was carried out on 50 Sprague–Dawley adult male rats weighing between 200-250 grams. The animals were obtained from, and kept in the Animal House Facility of Assiut Faculty of Medicine. Animals were divided into 5 groups 10 rats each. Rats of group 1 (standard diet group) were fed on a standard commercial pellet diet for 10 weeks, animals of group 2 (cholesterol fed group) were fed on the standard diet enriched with 1% cholesterol (Sigma chemical company, USA) dissolved in 0.5% cholic acid for 10 weeks. Groups 3-5 were fed as in group 2 and treated at the same time with fish oil (Menhaden Oil, Sigma) at a dose of 0.5 ml / day administered orally in group 3 (fish oil group), with extravergin olive oil (Wadi food, Egypt) at a dose of 0.5 ml / day administered orally in group 4 (olive oil group) and with melatonin (Sigma) injected subcutaneously at a dose of 75 ug / day 3-2 hours before sunset in group 5 (melatonin group).

Blood samples were taken at the end of the experiment. 3 ml blood sample was collected from each rat after over night fasting in a clean sterile centrifuge tube with anticoagulant (EDTA) by puncture of the retro-orbital sinus. Plasma was separated by centrifugation and divided into small aliquots and frozen at – 20° C until processed.

Lipogram parameters [total plasma cholesterol (TPC) high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG)] were measured by using fluorimetric kits (Boehringer-Mannhim, Germany). TPC was determined by the method of Flegg (1973). HDL was determined according to the method described by Finely (1978). LDL was determined by the method of Friedewald *et al.* (1972). The method for determination of TG was described by Fredrickson *et al.* (1967). Super oxide dismutase (SOD) was estimated according to Misra and Fridovich (1972) using spectrophotometer. Total thiol was determined colorimetrically after Ellman (1959). Nitric oxide (NO) was measured according to Ding et al. (1988) using spectrophotometer. Lipid peroxide (LP) was determined as thiobarbituric acid reactive substances colorimetrically according to the method of Satoh (1978).

The rats were killed by slaughtering and the aorta and its major branches from each animal were obtained, washed in saline and fixed in 10% formalin. Sections were prepared and stained with H&E stain (Carleton and Drury, 1957) for histological examination.

Data were expressed as mean ± standard error (S.E.). t-test was used to compare between groups to determine significance.

RESULTS

As regard the effect of feeding cholesterol on the measured blood parameters, Table (1) showed that TPC levels, LDL, TG and LP levels were significantly increased in the cholesterol fed group (G2) in comparison with the standard diet group (G1). While, HDL, SOD, NO and total thiol levels were significantly decreased in G2 in comparison with G1.

Table (2) and Figure (1) showed that plasma cholesterol level was 593.1 ± 20.90 mg % in the cholesterol fed group and significantly ($P< 0.001$) decreased to 411.3 ± 8.79, 391.6 ± 11.75 and 368.0 ± 10.54 mg% in the fish oil, olive oil and melatonin groups respectively.

The high density lipoprotein level in the cholesterol fed group was 36.09 ± 0.63 mg% and non significantly increased to 37.66 ± 0.69, 38.16 ± 0.62 and 36.53 ± 0:76 mg% in the fish oil, olive oil and melatonin groups respectively.

In the cholesterol fed group the low density lipoprotein was 393.8 ± 10.21.and significantly ($P<0.001$) decreased to 275.3 ± 7.72, 255.7 ± 9.01 and 226.5 ± 5.03 mg% in the fish oil, olive oil and melatonin groups respectively as shown in Table (2) and Figure (1).

Triglycerides level in the cholesterol fed group was 177.9 ± 5.43 and significantly ($P< 0.001$) decreased to 94.7 ± 2.03, 120.9 ± 5.44 and 102.9 ± 2.74mg% in the fish oil, olive oil and melatonin groups respectively.

Table (3) and Figure (2) showed that superoxide dismutase level was 19.00 ± 0. 47 and became 24.0 ± 0.63, 21.70 ± 0.55 and 24.60 ± 0.70 unit/ml in the fish oil, olive oil and melatonin groups respectively. There was a significant increase in the fish oil and melatonin groups ($P<0.001$) and in the olive oil group ($P< 0.01$). Nitric oxide level in the cholesterol fed group was 28.80 ± 0.46 umol/L and significantly ($P< 0.001$) increased to 36.20 ± 0.74, 33.50 ± 0.65 and 32.30 ± 0.42 umol/L in the fish oil, olive oil and melatonin groups respectively. Lipid peroxide level in the cholesterol fed group was 2.37 ± 0.10 and decreased to 0.69 ± 0.02, 0.61 ± 0.01 and 0.96 ± 0.02 nmol/ml in the three groups respectively. This decrease was significant ($P<0.001$) as shown in Table (3) and Figure (2)

In the cholesterol fed group the total thiol level was 161.3 ± 5.10 umol/L and significantly ($P < 0.001$) increased to 268.0 ± 7.42, 321.4, ± 7.58 and 292.9 ± 5.53 umol/L in the fish oil, olive oil and melatonin groups respectively.

Histological examination of the aorta from group1 under a light microscope revealed normal histological features of intima, media and adventitia (Fig. 3) while, in group2, it showed typical atherosclerotic fibrous plaques. An atheromatous plaque consists of lipid rich necrotic core filled with cellular debris and cholesterol clefts covered by a well developed fibrous cap (Fig. 4). Examination of the aortic wall from fish oil and olive oil groups revealed presence of early atheromatous lesions (fatty streaks). The lesions were composed of loosly textured connective tissue with varying amounts of vaculated lipid laden macrophages demonstrated to some extent in all animals of these groups (Fig. 5). In melatonin group, the atheromatous lesion was less pronounced than in olive oil and fish oil groups. The lesion consists of lipid laden macrophages present in between muscle bundles. There is also splitting of elastic lamina due to the presence of foam cells (Fig. 6).

Table 1: Effect of feeding cholesterol on the measured biochemical parameters in adult male rats.

Parameters	Standard diet group	Cholesterol fed group
Cholesterol (mg/dl)	104.5±2.99	593.1±20.90***
HDL (mg/dl)	39.14±0.71	36.09±0.63**
LDL (mg/dl)	53.0±1.85	393.8±10.21***
Triglycerides (mg/dl)	83.5±1.74	177.9±5.43***
Superoxide Dismutase (unit/ml)	27.9±0.64	19.00±0.47***
Nitric oxide (µmol/L)	39.1±0.50	28.80±0.46***
Lipid peroxide (nmol/L)	0.40±0.01	2.37±0.10***
Total thiol (µmol/L)	388.6±13.89	161.3±5.10***

**P<0.01 ***P<0.001

Table 2: Effect of fish oil, olive oil and melatonin on plasma levels of total cholesterol, high density lipoprotein, low density lipoprotein and triglycerides in cholesterol fed rats.

Group	Total Plasma Cholesterol(mg/dl)	High density lipoprotein(mg/dl)	Low density lipoprotein(mg/dl)	Triglycerides (mg/dl)
Cholesterol fed group	593.1±20.90	36.09±0.63	393.8 ±10.21	177.0±5.43
Fish oil group	411.3±8.79***	37.66±0.69 NS	275.3±7.72***	94.7±2.03***
Olive oil group	391.6±11.75***	38.16±0.62 NS	255.7±9.01***	120.9±5.44***
Melatonin group	368.0±10.54***	36.53±0.76 NS	226.5±5.03***	102.9±2.74***

NS: Non significant *** P< 0.001

Table 3: Effect of fish oil, olive oil and melatonin on plasma levels of super oxide dismutase, nitric oxide, lipid peroxide and total thiol in cholesterol fed rats.

Group	Superoxide dismutase μ mol/L	Nitric oxide μmol/L	Lipid peroxide μmol/L	Total thiol μ mol/L
Cholesterol fed group	19.00±0.47	28.80±0.46	2.37±0.11	161.3±5.10
Fish oil group	24.0±0.63***	36.2±0.74***	0.69±0.02***	268.0±7.42***
Olive oil group	21.70±0.55**	33.50±0.65***	0.61±0.01***	321.4±7.58***
Melatonin group	24.60±0.70***	32.30±0.42***	0.96±0.02***	292.9±5.53***

** P<0.01 *** P<0.001

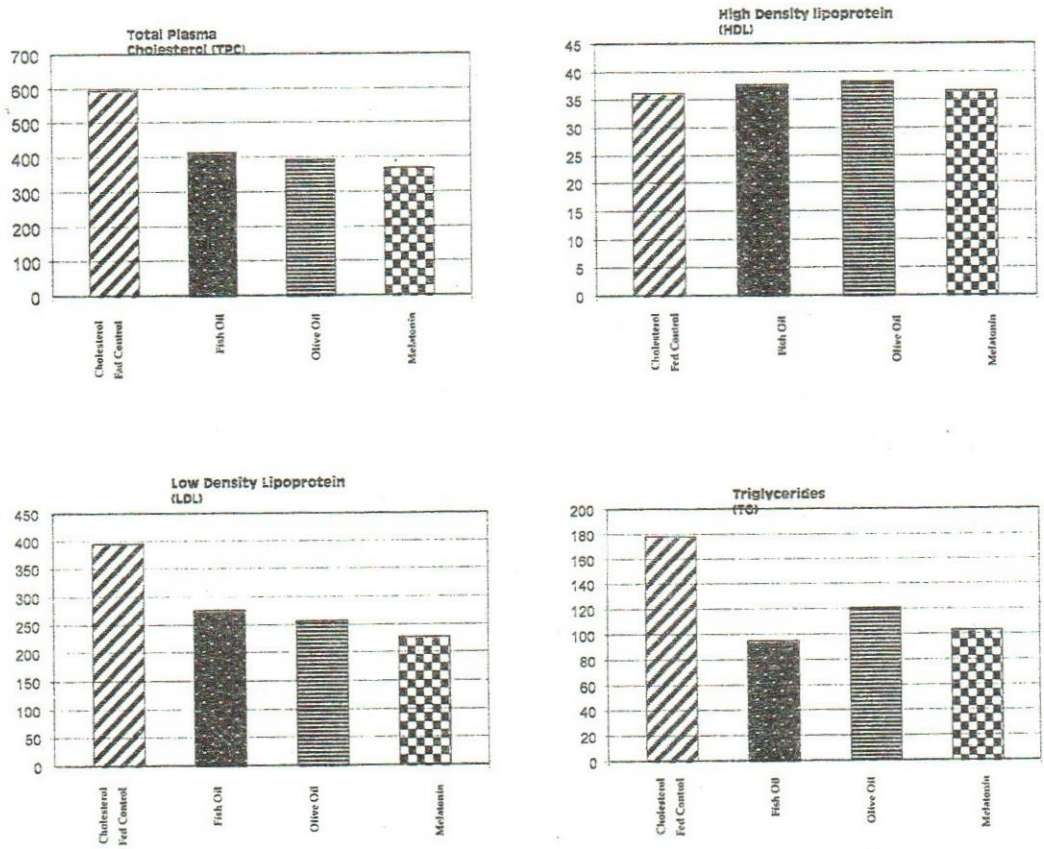

Fig. 1: Effect of fish oil, olive oil and melatonin on plasma levels of total cholesterol, high density lipoprotein, low density lipoprotein and triglycerides in cholesterol fed rats.

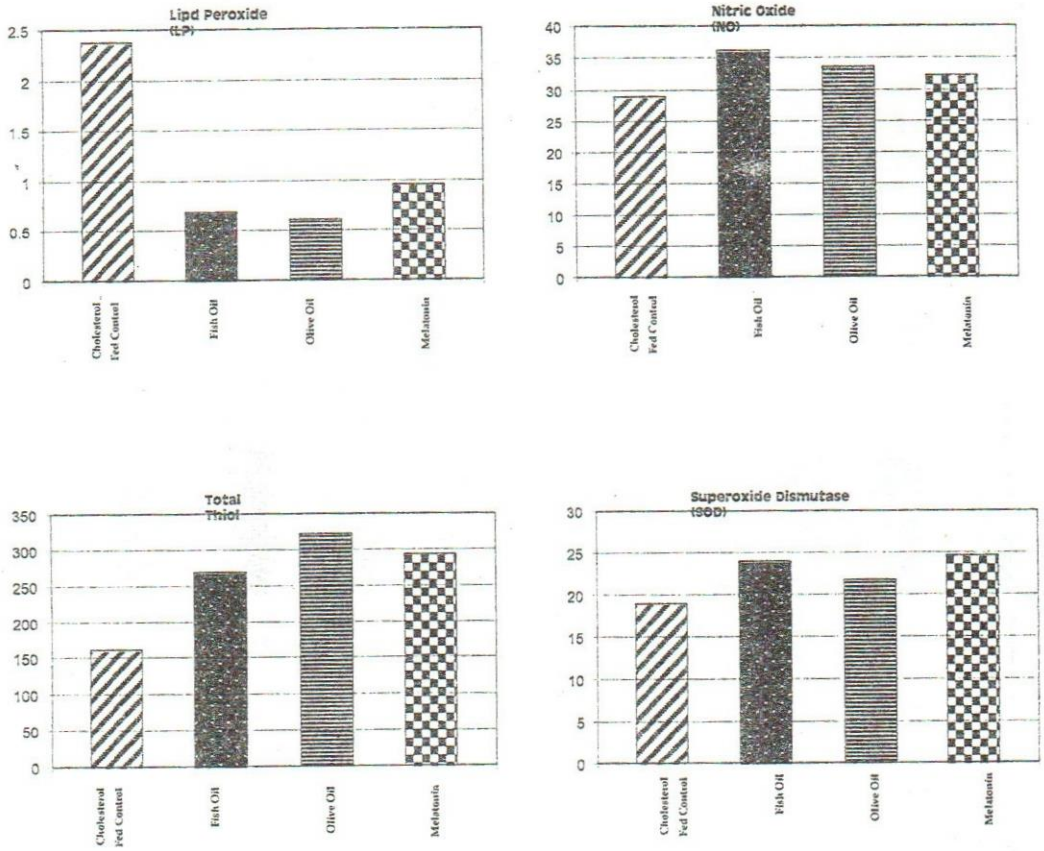

Fig. 2: Effect of fish oil, olive oil and melatonin on plasma levels of superoxide dismutase, nitric oxide, lipid peroxide and total thiol in cholesterol fed rats.

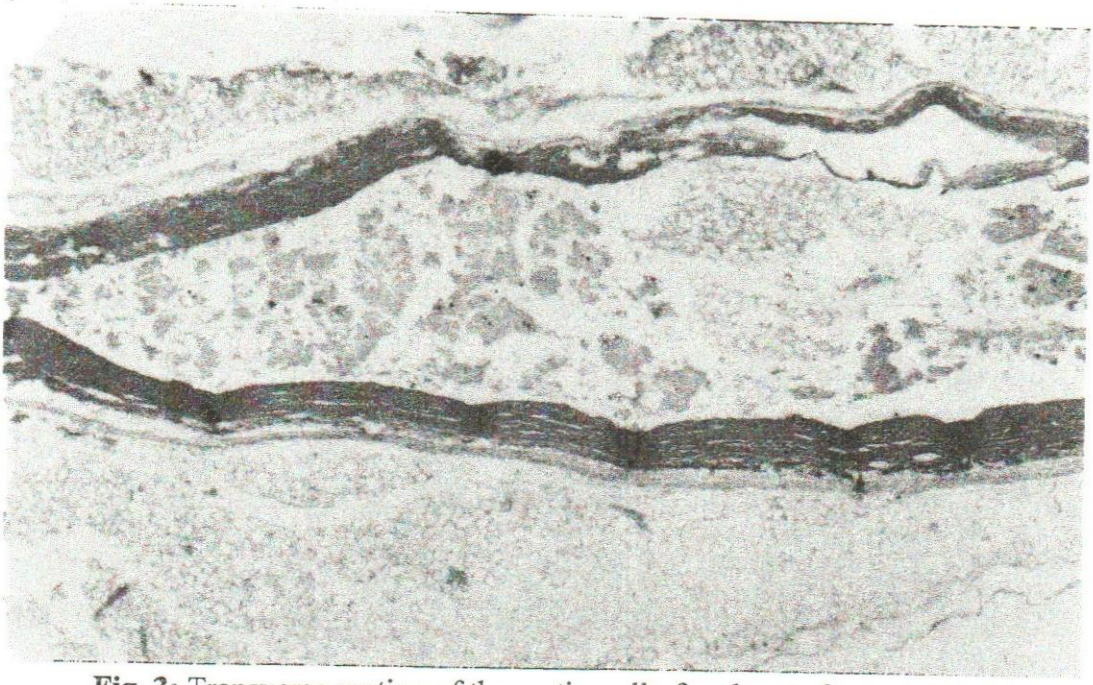

Fig. 3: Transverse section of the aortic wall of male rats from standard diet group showing normal intima, media and adventitia (H&E X 40).

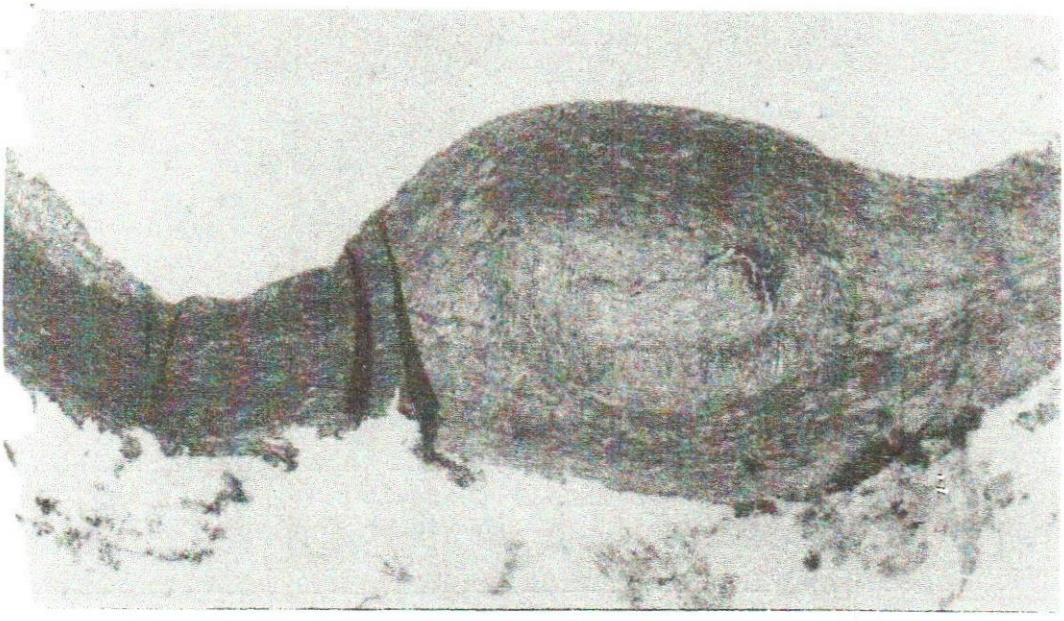

Fig. 4: Transverse section of the aortic wall of male rats from cholesterol fed group showing typical fibrous plaque (H&E X 100).

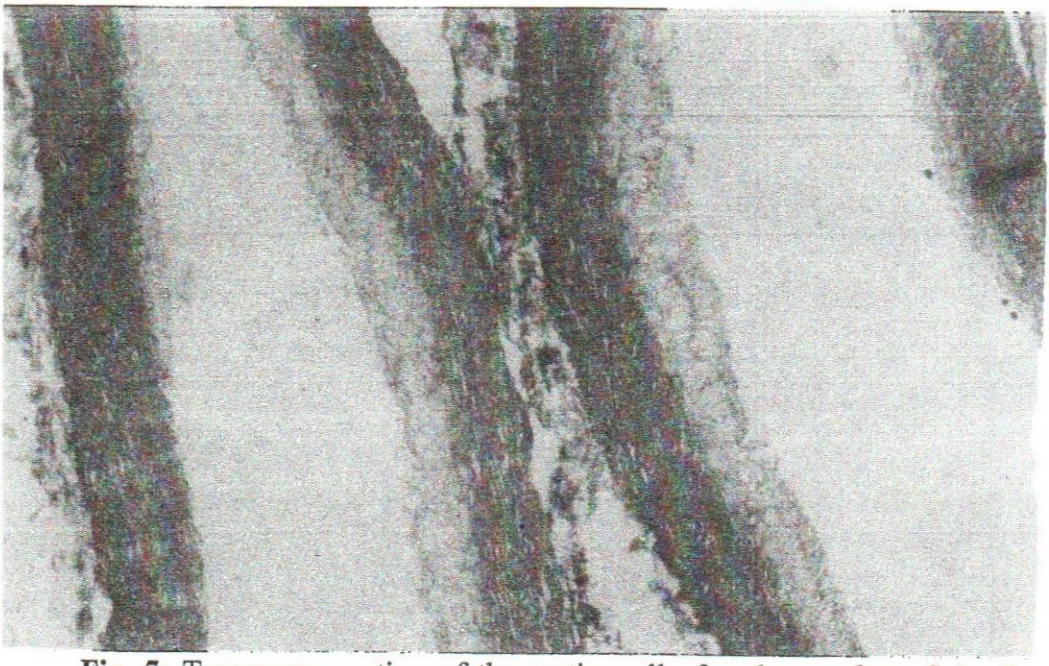

Fig. 5: Transverse section of the aortic wall of male rats from fish oil group illustrating the presence of fatty streak (early atheromatous lesion) (H&E X100).

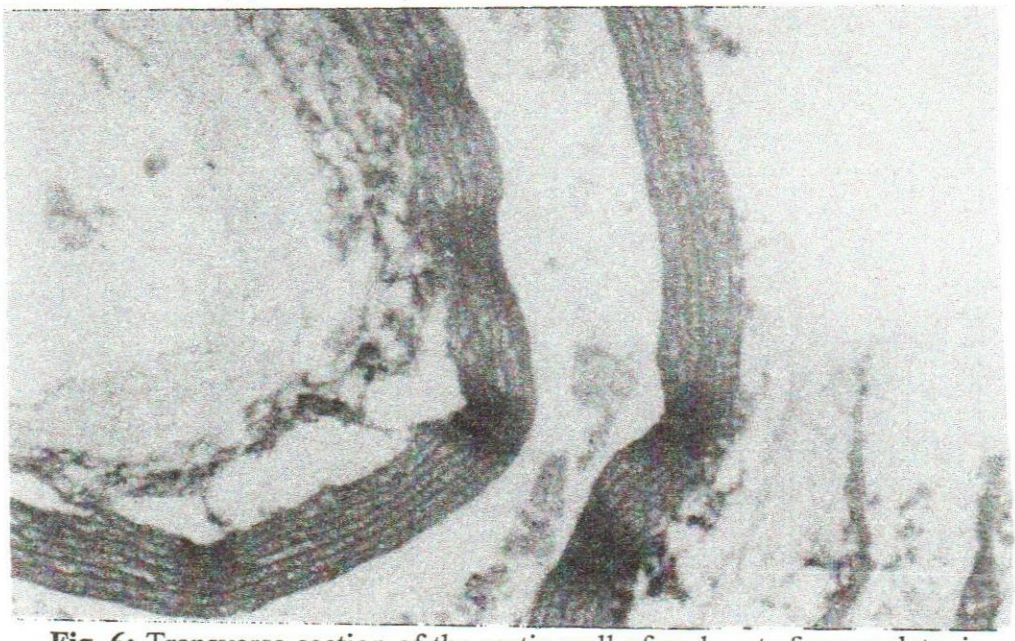

Fig. 6: Transverse section of the aortic wall of male rats from melatonin group showing lipid-laden macrophages between muscle bundles of media (H&E X40).

DISCUSSION

In this work the possible protective effect of fish oil, olive oil and melatonin against hypercholesterolemia was studied. Feeding cholesterol significantly increased (P<0.001) TPC, LDL and TG and significantly decreased (P<0.01) HDL. The marked hypercholesterolemia occurring in this work goes with the results of Nakayama *et al.* (1983) and Leth-Espensen *et al.* (1988) in rabbits, Kunitomo *et al.* (1981) in rats and Sener *et al.* (2004) in mice. Biomarkers of oxidative stress were affected by feeding cholesterol. SOD, NO and total thiol were significantly decreased (P<0.001) and LP was significantly increased (P<0.001) in the cholesterol fed group (G2) in comparison with the standard diet group (G1). Other studies reported that hypercholesterolemia increase lipid peroxidation and oxidative stress and causes depletion of antioxidant enzymes (Bednarek- Tupikowska *et al.,* 2000 and Gonzalez-Santiago, 2005). Histological examination of the aorta from cholesterol fed group showed typical atherosclerotic fibrous plaques. While, Sener *et al.* (2004) reported that no fatty strakes or plaques developed in the aorta of mice following high cholesterol diet containing 1.5% cholesterol and 0.5% cholic acid for 4 months but in some sections derangment of the endothelial layer was detected.

Fish oil significantly decreased (P< 0.001) TPC, LDL and TG and non-significantly increased HDL in comparison with the cholesterol fed group. Many studies reported similar effect of fish oil on TPC (Kris Etherton *et al.,* 1999 and Yilmaz *et al.,* 2002), HDL (Harris *et al.,* 1997) and TG (Flaten *et al.,* 1990). In addition, Baydas *et al.* (2002) found that plasma lipid levels in rats treated with fish oil were significantly lower than those of the control. However, Franzen *et al.* (1993) found that TPC and LDL did not change significantly by fish oil. As regard the effect of fish oil on biomarkers of oxidative stress, it was found that fish oil significantly increased (P<0.001) SOD, NO and total thiol and significantly decreased lipid peroxidation. Harris *et al.* (1997) reported similar effect on NO and Vecera *et al.* (2003) on total thiol. While contradictory effect on LP was reported by Baydas *et al.* (2002) who found non significant increase in LP with fish oil. Histological examination of the aorta from fish oil group showed fatty streaks which are early atherosclerotic lesions compared with typical atherosclerotic fibrous plaques detected in G2. In agreement with our results, studies in swine fed high cholesterol diets with and without cod liver oil showed that there was less coronary atherosclerosis in the cod liver group

(Weiner *et al.*, 1986). Also, dietary polyunsaturated fat protected African green monkey from coronary artery atherosclerosis compared with saturated fat (Rudel *et al.*, 1995).

Olive oil significantly decreased (P< 0.001) TPC, LDL and TG and non significantly increased HDL in comparison with the cholesterol fed group. Olive oil induced better cholesterol reducing results than fish oil. This is in accordance with the results of Kris-Etherton *et al.* (1999) but contradict that of mortensen *et al.* (1998). In addition, Bayindir *et al.* (2002) found that dietary treatment with olive oil improves the lipid profile by lowering TPC in rabbits. While, Gonzalez-Santiago (2005) found that hydroxytyrosol, a phenolic antioxidant present in olive oil reduces TPC by 50% in hyperlipemic rabbits (treating effect) but has no protective effect. In agreement with our results, LDL lowering effect of olive oil was reported by Kiritsakis (1998). Triglycerides lowering effect of olive oil was also reported by Faine *et al.* (2004) in rats and Ahuja *et al.* (2006) in human. The non significant increase in HDL by olive oil in this work contradicts the significant increase reported by Mortensen *et al.* (1992) in rabbits, Faine *et al.* (2004) in rats and Ahuja *et al.* (2006) in human and contradict the significant decrease reported by Ima *et al.* (1979) in rats. This different effect may be due to difference in the dose, duration or species. Concerning the effect on biomarkers of oxidative stress, olive oil significantly increased SOD, NO and total thiol (P<0.01, P<0.001 and P<0.001, respectively) and significantly decreased (P<0.001) LP. Accordant results, were increased myocardial SOD by olive oil in rats (Faine *et al.*, 2004) and a potent antioxidant and anti-inflammatory effect reported by El-Sweidy *et al.* (2005). Histological examination of the aorta from olive oil group showed early atherosclerotic lesions (fatty streaks) compared with typical atherosclerotic fibrous plaques in G2. In agreement with our results, microscopical examination of the aorta of rabbits fed olive oil showed a lower extent of degeneration in tunica intima with better organized endothelium and normal internal elastic membrane compared to corn oil-fed and butter-fed rabbits (Bayindir *et al.*, 2002) indicating that high dietary intake of olive oil may be more effective in the protection of endothelial integrity as evidenced by the lower incidence of atherosclerotic disease in the Mediterranean countries where olive oil is consumed in substantial amounts.

Melatonin significantly decreased (P<0.001) TPC, LDL and TG and non significantly increased HDL in comparison with the cholesterol fed group. Melatonin was more beneficial in lowering TPC than olive oil

and fish oil. The hypocholesterolemic effect of melatonin was reported also by Sewerynek (2002) and Sener *et al.* (2004). The hypocholesterolemic effect of melatonin may work through augmentation of the endogenous cholesterol clearance mechanisms. Melatonin suppressed the formation of cholesterol by 38% and reduce LDL accumulation by 42% (Sewerynek, 2002) and reversed doxorubicin (induce acute cardiac toxicity in rats) induced increase in LDL towards the normal values (Ahmed *et al.* 2005). As regard the effect of melatonin on biomarkers of oxidative stress, melatonin significantly decreased ($P<0.001$) LP. Melatonin and fish oil were about equal in raising levels of SOD more than olive oil, and melatonin restored the level of NO less than fish oil and olive oil and has median effect between olive oil and fish oil on total thiol. Stimulating effect of melatonin on antioxidant enzymes was also reported by Ahmed *et al.* (2005) and Nishida (2005). Melatonin inhibit the elevation of LP but less than olive oil and fish oil. Inhibitory effect of melatonin on plasma LP was found also by Hoyos *et al.* (2000) and Baydas *et al.* (2002). Melatonin inhibited also lipid peroxidation in the heart (Ahmed *et al.*, 2005), in the brain of methionine treated rats (Bouzouf *et al.*, 2005) and high doses of melatonin inhibit lipid peroxidation in vitro (Tailleux *et al.*, 2005).

Histological examination of the aorta from rats injected with melatonin showed that the atheromatous lesions were less pronounced than olive oil and fish oil groups. The lesions were very early in which the lipid laden macrophages were present in between muscle bundles which means that melatonin decreased the development of atherosclerosis. The results of Pita *et al.* (2002) agree with our results while in the study of Sener *et al.* (2004), there were no difference in the aortic histological findings of mice fed on high cholesterol diet with and without melatonin treatment (10mg/L in drinking water for 4 months).

Fish oil was the most effective in reducing TG level and in improving vascular endothelial function as evidenced in a rise of NO level in plasma. Olive oil was the most effective in restoring level of total thiol and in inhibiting LP and melatonin was the best factor reducing TPC, LDL and consequently atherogenesis. It was also the most effective in restoring SOD levels. Fish oil was less effective than olive oil in correcting lipid peroxidation because the highly unsaturated fatty acids of fish oil are the most susceptible to peroxidation (Satio and Nakatsugawa, 1994), while monounsaturated fatty acids in olive oil resist lipid peroxidation and attack by free radicals on account of having a single double bond (Bhadra *et al.*, 1993).

Fish oil supplementation beneficially affect persons with cardiovascular disease by at least three mechanisms. It reduces plasma triglycerides by about 30% (Harris *et al.,* 1997) and reduces blood pressure significantly (Morris *et al.,* 1993). Fish oil also has antithrombotic properties, it reduces platelet aggregation by decreasing thromboxane production (Goodnight *et al.,* 1981). Also, olive oil has been associated with a lower incidence of coronary heart disease and cancer. Olive oil contains a high proportion of monounsaturated oleic acid and high quantities of phenol compounds, hydroxytyrosol and oleuropein with potent biologic activities that may partially account for the cardio protective effects of the Mediterranean diet (Visioli and Galli, 2001). Oleic acid in olive oil is the preferred substrate for acyl-CoA cholesterol acyltransverase (ACAT), thus favouring the formation of cholesterol esters and promoting LDL receptor synthesis. Increased LDL receptor activity results in a higher rate of LDL uptake and clearance from the plasma (Dietschy, 1997).

Melatonin has potent antioxidant properties, so may prevent the development of atherosclerosis, cancer and other consequences of aging (Reiter *et al.,* 1994). In a human study, nocturnal secretion of melatonin was decreased in patients with coronary atherosclerosis (Brugger *et al.,* 1995). Melatonin significantly suppressed the vasospastic effect of oxidized LDL (which has been reported to be the most important risk factor for atherosclerosis) probably because it scavenges hydroxyl radicals arising from oxidized LDL (Okatani *et al.,* 2000).

It can be concluded that hypercholesterolemia lead to an increase in TG, LDL, lipid peroxidation and oxidative stress and a decrease in HDL and depletion of antioxidant enzymes. Fish oil supplementation caused marked reduction in TG and improvement of vascular endothelial function as evidenced in a rise of NO more than with olive oil and melatonin. While olive oil supplementation was the most effective in preventing the marked reduction of total thiol and in reducing LP and melatonin was the best factor reducing TPC, LDL and consequently atherogenesis. So, fish oil, olive oil and melatonin supplementation is recommended for prevention of atherosclerosis that lead to heart attacks, strokes and other froms of cardiorascular damage.

REFERENCES

Ahmed, H.H.; Mannaa, F.; Elmegeed, G.A. and Boss, S.H. (2005): Cardioprotective activity of melatonin and its novel synthetesized derivatives on doxorubicin-induced cardiotoxicity. Bioorg. Chem. Mar,1,13(5): 1847-1857.

Ahuja, K.D.; Pittaway, J.K. and Ball, M.J. (2006): Effect of olive oil and tomato lycopene combination on serum lycopene, lipid profile, and lipid oxidation. Nutrition. Jaan. 12, (Epub ahead of print).

Baydas, G.; Yilmaz, O.; Celik, S.; Yaser, A. and Gursu, M.F. (2002): Effect of certain micronutrients and melatonin on plasma lipid peroxidation, and homocysteine levels in rats . Arch. Med. Res. Nov – Dec 33(6): 515-9.

Bayindir, O.; Ozmen, D.; Mutaf, I.; Turgan, N.; Habif, S.; Gulter, C.; Parildar, Z. and Uysal, A. (2002): Comparison of the effects of dietary saturated, mono-, and 6 polyunsaturated fatty acids on blood lipid profile, oxidant stress, prostanoid synthesis and aortic histology in rabbits. Ann. Nut. Metab. 46 (5): 222-8.

Bednarek-Tupikowska, G.; Gosk, I.; Szuba, A.; Bohanowice-Pawlak, A.; Kosowska, B.; Bidzinska, B. and Millewicz, A. (2000): Influence of dehydroepiandrosterone on platelet aggregation, superoxide dismutase activity and serum lipid peroxide concentration in rabbits with induced hypercholesterolemia. Med. Sci. Monit. Jan-Feb 6 (1) : 40-45.

Bhadra, S.; Banavali, S.D.; Agrawal, M. and Subbiah, M.T. (1993): Cholesterol peroxidation potential as influenced by dietary fat type. Int. J. Vitam. Nutr. Res. 63:223-228.

Bouzouf, M.; Martinez-Cruz, F.; Mdinero, P.; Guerrero, J.M. and Osun, C. (2005): Melatonin prevents hyperhomocysteinemia and neural lipid peroxidation induced by methionin intake. Curr. Neurovasc. Res. April 2(2): 175-8.

Brugger P.; Marktl W. and Herold, M. (1995): Impaired nocturnal secretion of melatonin in coronary heart disease. Lancet ; 345 :1408.

Bubenik, G.A.; Blash, D.E.; Brown, G.M.; Maestroni, G.J.; Pang, S.F.; Reiter, R.J.; Viswanathan, M. and Zisapel, N. (1998): Prospect of the clinical utilization of melatonin. Bio. Signals Recept. Jul-Aug 7(4) :195.

Carleton, H.M. and Drury, R.A.B. (1957): Histological technique from normal and pathological tissues and identification of parasites. 3rd ed London, Oxford University Press New York Toronto.

Collier, P.M.; Vrsell, A.; Zaremba, K.; Payne, C.M.; Staughton R.C. and Sanders, T. (1993): Effect of regular consumption of oily fish compared with white fish on chronic plaque psoriasis. Eur. J. Clin. Nutr. 47 : 251- 4.

Dietschy, J.M. (1997): Reverse cholesterol transport: theoretical considerations of what regulates low-density lipoprotein and high-density lipoprotein cholesterol. Am. J. Clin. Nutr. 65 (suppl.) :8 1581- 89.

Ding, A.H.; Nathan, C.F. and Stuehr, D.J. (1988): Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence of independent production. J. Immun. 141: 1407-2412.

*Ellman, G.L. (1959):*Tissue sulphydryl groups. Arch. Bioch. Biophy. 82 : 70-77.

El Seweidy M.M.; El Swefy, F.E.; Abdallah, F.R. and Hashem, R.M. (2005): Dietary fatty acid unsaturation levels, lipoprotein oxidation and circulating chemokine in experimentally induced atherosclerotic rats. J Pharm. Pharmacol. Nov 57(11): 1467-74.

Faine, L.A.; Diniz, Y.S.; Gatharli, C.M.; Rodrigues, H.G.; Burneiko, R.C.; Santana, L.S.; Cicogna, and Novelli, E.L. (2004): Synergistic action of olive oil supplementation and dietary restriction on serum lipids and cardiac antioxidant defences. Can. J. Physiol. Pharma. Nov, 82(11): 969-75.

Finley, P.R. (1978): Enzymatic determination of HDL-cholesterol. Clin. Chem. 24:931-934.

Flaten, H.; Hostmark, A.T.; Kierulf, P.; Lystad, E.; Trygg, K.; Bjerkedal, T. and Osland, A. (1990): Fish-oil concentrate : effect on variables related to cardiovascular disease. Am. J. Clin. Nutr. 52: 300-6.

Flegg, M.H. (1973): Quantitative-enzymatic-colorimetric determination of total and HDL cholesterol in serum or plasma. Ann. Clin. Biochem. 10 : 79.

Franzen, D.; Geisel, J.; Hopp, H.W.; Oette, K. and Hilger, H.H. (1993): Longterm effects of low dosage fish oil on serum lipid and lipoproteins [in German]. Med. Klin. 134-8.

Fredrickson, D.S.; Levy, R.I. and Leese, R.S. (1967): The quantitative enzymatic-colorimetric determination of triglyceride in serum or plasma. New Eng. J. Med. 276:34.

Friedewald, W.T.; Levy, R.I. and Frederickson, D.S. (1972): Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of preoperative ultracentrifuge. Clin. Chem. 18: 459-500.

Gonzalez-Santiago, M.; Martin-Boutista, E.; Carrero, J.J.; Fonolla, J.; Baro, J.; Bartolome, M.V.; Gill-Loyzaga, P. and Lopez-Huertas, E. (2005): One month adminstration of hydrotyrosol, a phenolic antioxidant present in olive oil, to hyperlipidemic rabbits improve blood lipid profile, antioxidant status and reduces atherosclerosis development. Atherosclerosis. Nov. 17, (Epub ahead of print).

Goodnight, S.H.; J.R., Harris, W.S. and Connor, W.E. (1981): The effect of dietary omega -3-fatty acids upon platelet composition and function in man: a prospective controlled study. Blood. 58: 880-5.

Harris, W.S.; Rambjor, G.S.; Windsor, S.L. and Diederich, D. (1997): N-3 and urinary excretion of nitric oxide metabolites in humans. Am. J. Clin. Nutr. 65: 459-464.

Higuchi, T.; Shirai, N. and Suzuki, H. (2006): Reduction in plasma glucose after lipid changes in mice fed fish oil, Docossahexanoic acid and eicosapentanoic acid diets. Ann. Nutr. Metab. Jan 2, 50 (2): 147-154.

Hoyos, M.; Guerrero, G.M.; Perez-Cano, R.; Olivan, J.; Garci-Pergana, A. and Osuna, C. (2000): Serum cholesterol. and lipid peroxidation are decreased by melatonin in diet-induced hypercholesterolemic rats. J. Pineal Res. Apr. 28(3): 150-5.

Imai, Y.; Shino, A.; Asano, T.; Matswmura, H. and Kakinuma, A. (1979): Increase of serum high density lipoprotein with progression and regresion of aortic lipid deposition in rats. Atherosclerosis. Nov 34(3): 329-38.

Kiritsakis, A. (1998): Food and Nutrition press. Inc Trumbull Connecticut USA; Olive oil-Second Edition, from the tree to the table: 600-611.

Kris-Etherton, P.M.; Pearson, T.A. and Wan, Y. (1999): High monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am. J. Clin. Nutr. 70:1009-10015.

Kunitomo, M.; kinoshita, K. and Bando, Y. (1981): Experimental atherosclerosis in rats fed a vitamin D, cholesterol-rich diet. J. Pharacobiodyn. Sep 4(9): 718-23.

Leth-Espesen, P.; Stender, S.; Ravn, H. and Kjedsen, K. (1988): Antiatherogenic effect of olive oil and corn oils in cholesterol-fed rabbits with the same plasma cholesterol levels. Arteriosclerosis. May-Jun 8(3): 284-7.

Misra, H.L. and Fridovich, I. (1972): The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247(10): 3170-3175.

Morris, M.C.; Sacks, F. and Rosner, B. (1993): Dose Fish oil lower blood pressure? A meta-analysis of controlled trials. Ciraulation. 88: 523-533.

Mortensen, A.; Espensen, P.L.; Hansen, B.F. and Ibsen, P. (1992): The influence of dietary olive oil and margarine on aortic cholesterol accumulation in cholesterol fed rabbits maintained at similar plasma cholesterol level. Atherosclerosis. 96 (2-3): 159-170.

Mortensen, A.; Hansen, B.F.; Hansen, J.F.; Frandsen, H.; Bartnikowska, E.; Andersen, P.S. and Bertelsen, L.S. (1998): Comparison of the effects of the fish oil and olive oil on blood lipids and aortic atherosclerosis in Watanabe heritable hyperlipidemic rabbits. Br. J. Nutr. 80 : 565-73.

Nakayama, S.; Sakashita, M.; Tonooka, M.; Gotoh, H.; Yasuhara, H. and Sakamotok, (1983): Experimental hyperlipidemia and atherosclerosis induced by cholesterol diet in SPE Japanese white rabbits. Jpn. J. Pharmacol. Apr 33(2): 279-89.

Nishida, S. (2005): Metabolic effects of melatonin on oxidative stress and diabetes mellitus. Endocrine. Jul, 27 (2):131-6.

Okatani, Y.; Wakatsuki, A.; Ikenoue, N.; Izumiya, C. and Kaneda, C. (2000): Melatonin inhibits oxidative modification of low-density lipoprotein particles in normolipidemic post-menopausal woman. J. Pineal Res. Apr 28(3): 136-42.

Pita, M.L.; Hogos, M.; Martin-Lacave, L.; Osuna, C.; Fernandez-Santos, J.M. and Guerrero, J.M. (2002): Long term melatonin adminstration increases polyunsaturated fatty acid percentage in plasma lipids of hypercholesterolemic rats. J. Pineal Res. Apr. 32(3): 179-86.

Puiggros, C.; Chacon, P.; Armadans, I.I.; Clapes, J. and Planas, M. (2002): Effect of oleic rich and omega-3-rich diet on serum lipid pattern and lipid peroxidation in mildly hypercholesterolemic patients. Clin. Nutr. Feb, 21(1): 79-87.

Reiter, R.J.; Tan, D.X.; Poeggeler, B.R.; Menendez-Palaez, A.; Chen, L.D. and Saarela, S. (1994): Melatonin as a free radical scavenger : implications for aging and age related diseases. Ann. N. Y. Acad. Sci. 719: 1-12.

Rudel, L.L.; Parks, J.S. and Sawyer, J.K. (1995): Compared with dietary monounsaturated and saturated fat, polyunsaturated fat protects African green monkeys from coronary artery atherosclerosis. Arteriosclerosis Thromb. Vas. Biol. 15 : 2101-10.

Saito, M. and Nakalsugawa, K. (1994): Increased susceptibility of liver to lipid peroxidation after ingestion of a high fish oil diet. Int. J. Vitam. Nutr. Res. 64: 144-51.

Satoh, K. (1978): Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin. Clim. Acta. 90 : 37- 43.

Sener, G.; Balkan, J.; Cevikbas, U.; Keyer-Uyasal, M. and Uyasal, M. (2004): Melatonin reduces cholesterol accumulation and prooxidant state induced by high cholesterol diet in the plasma, the liver and probably in the aorta of C57BL/6J mice. J. Pineal Res. Apr, 36(3): 212-6.

Sewerynek, E. (2002): Melatonin and the cardiovascular system. 23 (Suppl.1): 79-83.

Tailleux, A.; Gozzo, A.; Topier, G. et al. (2005): Increased suscepility of low density lipoprotein to ex vivo oxidation in mice transgenic for human apolipopritein B treated with melatonin-related compound is not associated with atherosclerotic progression. J. Cardiovasc. Pharmacol. Sep, 46(3): 241-9.

Vander, A.; Sherman, J. and Luciano, D. (1998): Human physiology: the mechanisms of body function. Mc Graw Hill Boston USA ; chapters 2 and 18: pp23-24 and pp607-609.

Vecera, R.; Skottova, N.; Vana, P.; Kazdoval, L.; Chmela, Z.; Svagera, Z.; Waltera, D.; Ulrichova, J. and Simanek, V. (2003): Antioxidant status, lipoprotein profile and liver lipids in rats fed on high-cholesterol diet containing currant oil rich in n-3 and n-6 polyunsaturated Fatty acids. Physiol. Res. 52: 177-187.

Visioli, F. and Galli, C. (2001): Antiatherogenic components of olive oil. Curr Atherosclet. Rep. 3: 64 - 67.

Weiner, B.H.; Ockene, I.S.; Levine, P.H.; Cuenoud, H.F.; Fisher, M.; Johnson, B.F.; Daoud, A.S.; Jarmolych, J.; Hosmer, D. and Johnson, M.H. (1986): Inhibition of atherosclerosis by cod liver oil in a hyperlipidemic swine model. N. Eng. J. Med. 315: 841 - 846.

Yilmaz, O.; Ozkam, Y.; Yildirim M.; Ozturk, A.I. and Ersan, Y. (2002): Effect of alpha lipoic acid, ascorbic acid, ascorbic acid-6-palmitate and fish oil on the glutathine, malondialdehyde and fatty acids levels in erythrocytes of streptozotocin induced diabetic male rats. J. Cell Biochem. 36(3): 530-9.