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IMPROVING EFFECT OF FISH OIL, OLIVE OIL AND MELATONIN ON INDUCED HYPERCHOLESTEROLEMIA IN ADULT MALE RATS

(With 3 Tables and 6 Figures)

By

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تأثير زيت السمك، زيت الزيتون والميلاتونين في تحسن زيادة الكولسترول المحدثة في ذكور الفئران البالغة

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تهدف هذه الدراسة إلى تقييم التأثير المحتمل لزيت السمك وزيت الزيتون والميلاتونين فى علاج زيادة الكولسترول المحدثة فى ذكور الفئران البالغه٠استخدم فى هذه الدراسة ٥٠ فارا قسمت إلى ٥ مجاميع، ١٠ فئران فى كل مجموعه٠ ولقد تم تغذية حيوانات المجموعة الأولي على الغذاء المعتاد والمجموعة الثانية تغذت على الغذاء المعتاد المضاف إليه الكولسترول بنسبة ١% (المجموعة المغذاة بالكولسترول) لمدة ١٠ أسابيع٠ المجاميع من ٣-٥ تم تغذيتها كما في المجموعة الثانية ثم تم إستبدال الغذاء بالغذاء المعتاد والعلاج بزيت السمك في المجموعة الثالثة (مجموعة زيت السمك) وبزيت الزيتون في المجموعة الرابعة (مجموعة زيت الزيتون) وبالميلاتونين في المجموعة الخامسة (مجموعة الميلاتونين) لمدة أسبوعين٠ وتم أخذ عينات دم من جميع الحيوانات فى نهاية التجربة ثم أخذ شريان الأورطى من جميع الحيوانات بعد الذبح وفحصت هستولوجيا لتقييم وجود تصلب الشرايين٠ وتم قياس مستوى الكولسترول الكلى بالبلازما ومستوى الكولسترول عالي الكثافة والكولسترول منخفض الكثافة والترای جليسريد٠ وتم أيضا قياس إنزيم السوبر أوكسيد ديسميوتيز والثيول الكلى وأكسيد النيتريك وفوق أكسيد الدهون٠ أظهرت النتائج أن التغذية بالكولسترول تحدث زيادة ذات دلالة إحصائية فى مستوى الكولسترول الكلى ومستوى الكولسترول عالي الكثافة والترای جليسريد وفوق أكسيد الدهون وإنخفاض ذو دلالة إحصائية فى مستوى الكولسترول مرتفع الكثافة والسوبر أكسيد ديسميوتاز والثيول الكلى وأكسيد النيتريك٠ وقدّ أدت المعالجة بزيت السمك وزيت الزيتون والميلاتونين إلى نقص ذو دلالة إحصائية فى الكولسترول الكلى والكولسترول منخفض الكثافة والترای جليسريد وفوق أكسيد الدهون وزيادة ذات دلالة

إحصائية في مستوى السوبر أكسيد ديسميوتاز وأكسيد النيتريك والثيول الكلي وزيادة غير معنوية في مستوى الكولسترول عالي الكثافة. وأحدث العلاج بزيت السمك أكبر نقص في التراي جليسريد وأكبر زيادة في أكسيد النيتريك مما يدل على تحسن الغشاء المبطن للأوعية الدموية بينما كان زيت الزيتون الأكثر تأثيرًا في خفض الكولسترول الكلي وزيادة الثيول الكلي وكان الميلاتونين الأفضل تأثيرا فى خفض الكولسترول منخفض الكثافة وفوق أكسيد الدهون والأكثر تأثيرا في إستعادة مستوى السوبر أكسيد ديسميوتاز وبالتالي تحسن تصلب الشرايين. وقد أظهر الفحص الهستولوجى لشريان الأورطى لحيوانات مجموعتي زيت السمك وزيت الزيتون والميلاتونين بداية حدوث إنحسار التصلب الشرياني مقارنة بالمجموعة الضابطة التي كان تصلب الشرايين بها مكتمل المراحل. ونستنتج من هذه الدراسة أن لزيت السمك وزيت الزيتون والميلاتونين تأثيرا في تعديل صورة الدهون ودلالات الإجهاد التأكسدي والصورة الهستولوجية لتصلب الشرايين وأن التحسن الطفيف في جدار الأورطي يعزى لقصر مدة العلاج التي إستمرت إسبوعين فقط فكانت غير كافية لإحداث تغيرا واضحا. وهكذا يوصى بإستخدام زيت السمك وزيت الزيتون والميلاتونين في علاج زيادة الكولسترول وتصلب الشرايين وعلى أن يستمر العلاج لفترة أطول.

SUMMARY

This study aims to evaluate the possible improving 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) for 10 weeks. Groups 3-5 were fed as in group 2 then the diet was replaced by standard diet and fish oil in group 3 (fish oil group), standard diet and olive oil in group 4 (olive oil group) and standard diet and melatonin in group 5 (melatonin group) for 2 weeks. Then, blood samples were taken from all animals and the aorta of each animal was 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. Fish oil caused the greatest reduction in TG and the greatest increase in NO denoting improvement of vascular endothelial function. Olive oil was the most effective in reducing TPC and total thiol and melatonin

was the best factor reducing LDL and LP and consequently atherogenesis and was the most effective in restoring SOD. Histological examination of the aorta from rats of the fish oil , olive oil and melatonin groups showed atheromatous fibrous plaques nearly to the same extent in all groups but absence of well developed fibrous cap which was found in the cholesterol fed group denoting slight improvement. It was concluded that diet additives as fish oil, olive oil or melatonin injection have modulating effect on the parameters of the lipogram, oxidative stress markers and histological features of atherosclerotic lesion and that the improvement of the aortic wall was slight due to the short period of treatment (2 weeks only) to produce marked change in the aortic wall.

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

INTRODUCTION

Because atherosclerosis of human being is so prevalent and its clinical importance is so serious, there has been a major research effort to better understand its pathogenesis and thereby provide a more rational approach to prophylaxis and therapy (Clarkson *et al.,* 1974).High plasma concentration of cholesterol enhance the development of atherosclerosis (Vander *et al.,* 1998). Like most other lipids, Cholesterol circulates in the plasma as a part of various lipoprotein complexes. LDL are the main cholesterol carriers and they deliver cholesterol to cells. In contast to LDL, HDL promote the removal of cholesterol from cells and its secretion into the bile by the liver (Boyd *et al.,* 1969).

Fish oil is a rich source of omega-3 polyunsaturated fatty acids (PUSFA). Populations that consume large amounts of marine fish containing omega-3 PUFA have low plasma levels of cholesterol and triglycerides and low incidence of coronary heart disease (Krauss *et al.,* 2002).

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). Considerable evidence supports the hypothesis that LDL oxidation plays an important role in atherosclerosis .Even though high melatonin doses

inhibit LDLoxidation in vitro, the effect of melatonin on atherosclerosis has never been well studied (Tailleux *et al.*, 2005). The aim of this study is to evaluate the possible role of fish oil, olive oil and melatonin in improving induced hypercholesterolemia and atherosclerosis in adult male rats.

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 then the diet was replaced by standard diet and fish oil (Menhaden Oil, Sigma) at a dose of 0.5 ml / day administered orally in group 3 (fish oil group), with standard diet and extravergin olive oil (Wadi food, Egypt) at a dose of 0.5 ml / day administered orally in group 4 (olive oil group) and standard diet and melatonin (Sigma) injected subcutaneously at a dose of 75 ug / day 3-2 hours before sunset in group 5 (melatonin group) for 2weeks.

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 565.5 ± 14.97 mg % in the cholesterol fed group and significantly ($P< 0.001$) decreased to 504.6 ± 14.51, 424.3± 15.61 and 487.9 ± 10.79 mg% in the fish oil, olive oil and melatonin groups respectively.

The high density lipoprotein level in the cholesterol fed group was 34.13 ± 0.62 mg% and non significantly increased to 35.15 ± 0.5, 36.00 ± 0.73 and 34.44 ± 0.76 mg% in the fish oil, olive oil and melatonin groups respectively.

In the cholesterol fed group the low density lipoprotein was 371.5 ± 11.83.and significantly ($P<0.05,<0.001,<0.001$) decreased to 331.7±11.35, 315.3± 7.70and 300.8± 7.06 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 185.3 ± 5.27 and significantly ($P< 0.001,<0.01,<0.001$) decreased to 113.4 ± 2.89, 162.9 ± 4.86 and 155.9 ± 3.27 mg% in the fish oil, olive oil and melatonin groups respectively.

Table (3) and Figure (2) showed that superoxide dismutase level was 19.70 ± 0. 65 and became 23.20 ± 0.78, 21.60 ± 0.54 and 24.00 ± 0.84 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 ($P< 0.05$). Nitric oxide level in the cholesterol fed group was 30.30 ± 0.59 umol/L and significantly ($P< 0.001,< 0.01$) increased to 34.60 ± 0.58, 33.00 ± 0.47 in the fish oil, olive oil groups respectively and non significantly increased to 31.00 ±

0.66 umol/L in the melatonin group. Lipid peroxide level in the cholesterol fed group was 2.56 ± 0.08 and decreased to 0.63 ± 0.02, 0.68 ± 0.02 and 0.42 ± 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 232.0 ± 6.80 umol/L and significantly (P < 0.001,< 0.01,< 0.01) increased to 285.0 ± 8.89, 330.3, ± 9.35 and 304.8 ± 10.12 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). Histological examination of the aorta from rats of the fish oil, olive oil and melatonin groups showed atheromatous fibrous plaques nearly to the same extent in all groups but absence of well developed fibrous cap which was found in the cholesterol fed group (Fig. 5).

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)	95.9±2.99	565.5±14.97***
HDL (mg/dl)	37.1±0.64	34.13±0.62**
LDL (mg/dl)	48.0±1.54	371.5±11.83***
Triglycerides (mg/dl)	82.5±2.01	185.3±5.27***
Superoxide Dismutase (unit/ml)	26.10±0.82	19.70±0.65***
Nitric oxide (µmol/L)	37.5±0.50	30,30±0.59***
Lipid peroxide (nmol/L)	0.37±0.01	2.56±0.08***
Total thiol (µmol/L)	410±10.67	232±6.80***

** 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	565.5±14.97	34.13±0.62	371.5 ±11.83	185.3±5.27
Fish oil group	504.6±14.51***	35.15±0.50 NS	331.7±11.35*	113.4±2.89***
Olive oil group	474.3±15.61***	36.09±0.73 NS	315.3±7.70***	162.9±4.86**
Melatonin group	487.9±10.79***	34.44±0.63 NS	300.8±7.06***	155.9±3.27***

NS: non significant * P<0.05 ** P<0.01 *** 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	19.70±0.65	30.30±0.59	2.56±0.08	232.0±6.80
Fish oil group	23.20±0.78***	34.6±0.58***	0.63±0.02***	285.0±8.89***
Olive oil group	21.60±0.54*	33.00±0.47***	0.68±0.02***	330.3±9.35**
Melatonin group	24.00±0.84***	31.00±0.66***	0.42±0.02***	304.8±10.12***

NS: non significant * P<0.05 ** P<0.01 *** P< 0.001

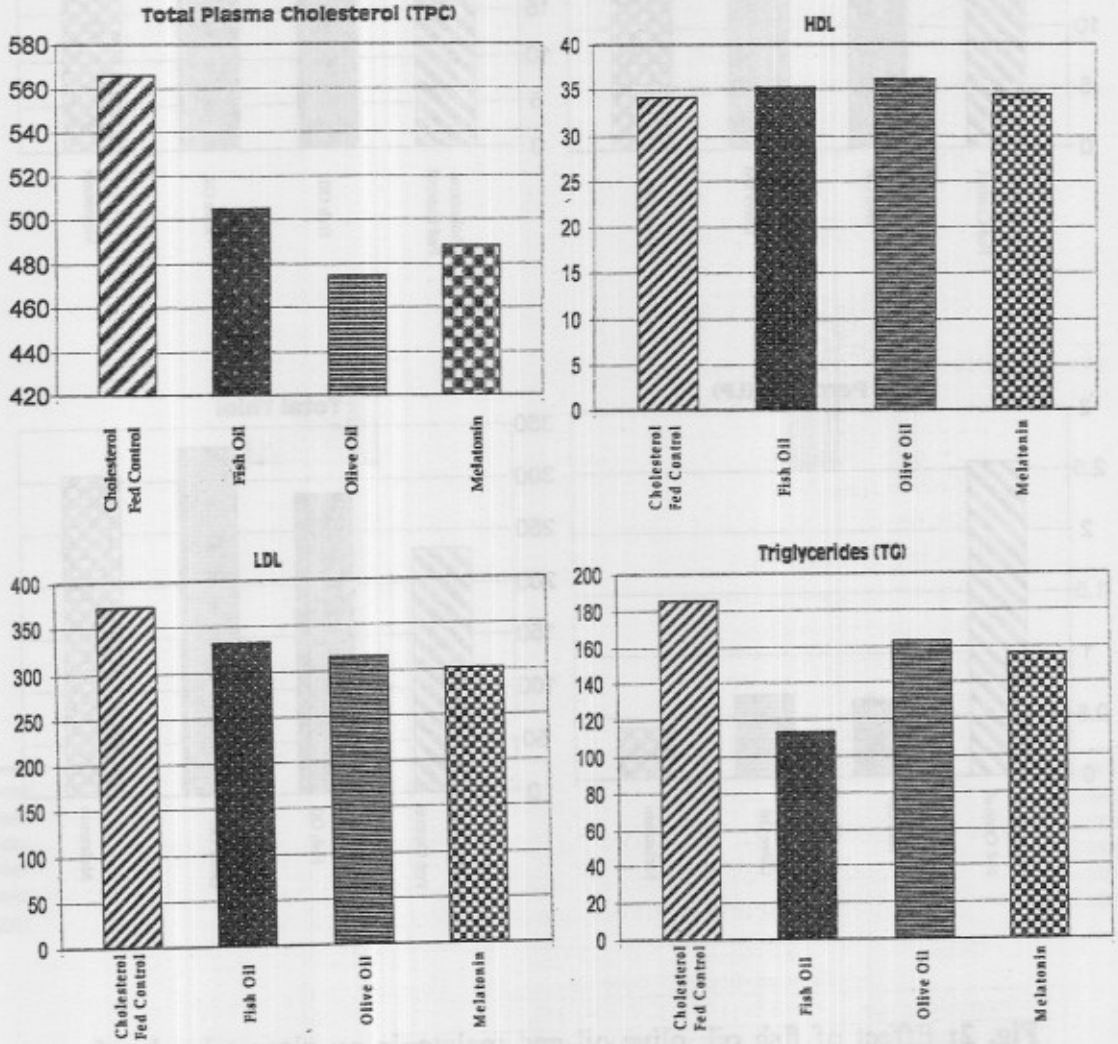

Fig. 1: Effect of fish oil, olive oil and melatonin on plasma levels of total cholesterol, high density lipoprotein, low density lipoprotein and triglycerides in cholesterol fed rats.

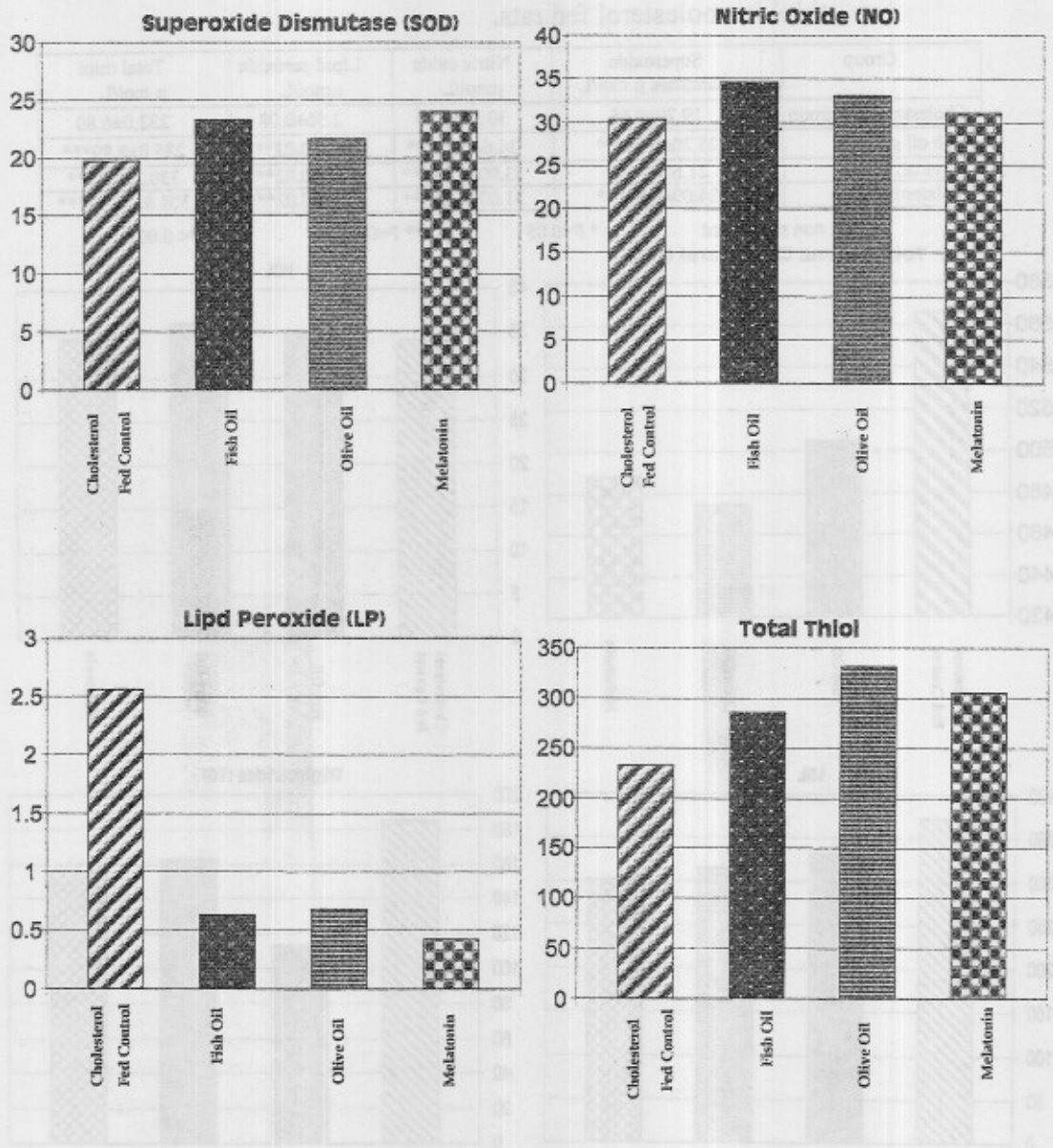

Fig. 2: Effect of fish oil, olive oil and melatonin on plasma levels of superoxide dismutase, nitric oxide, lipid peroxide and total thiol in cholesterol fed rats.

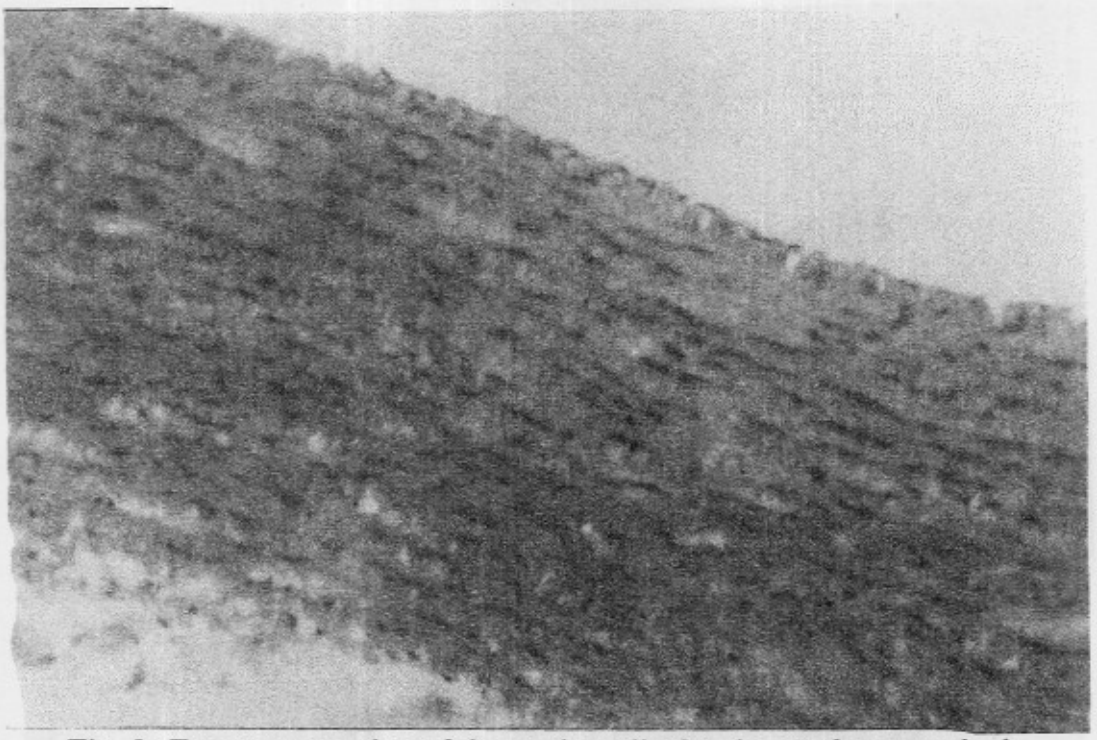

Fig. 3: Transverse section of the aortic wall of male rats from standard diet group showing normal intima, media and adventitia (H&E X 40).

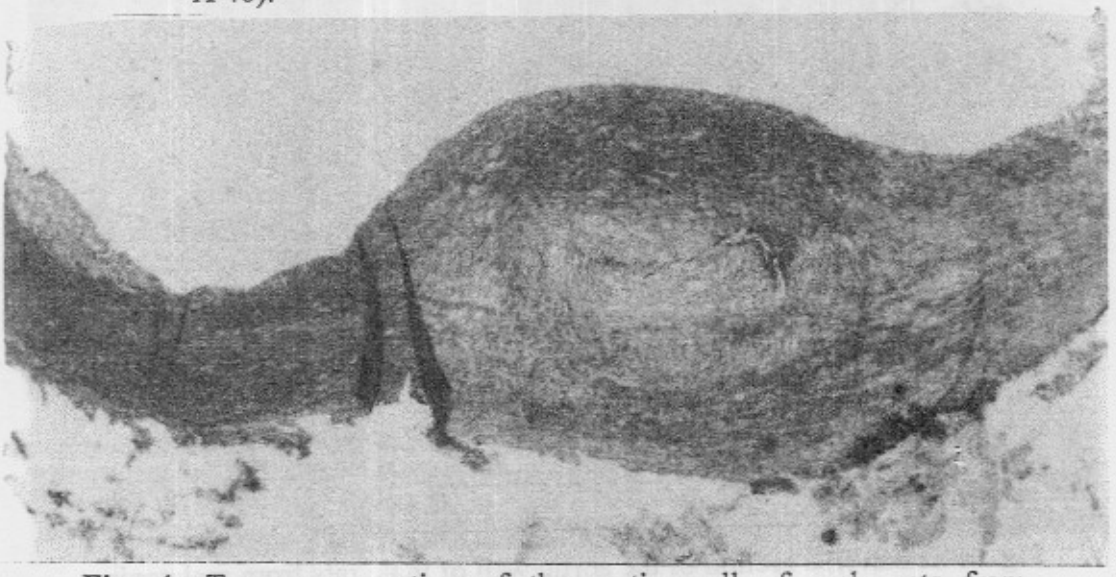

Fig. 4: Transverse section of the aortic wall of male rats from cholesterol fed group showing typical fibrous plaque (H&E X 100).

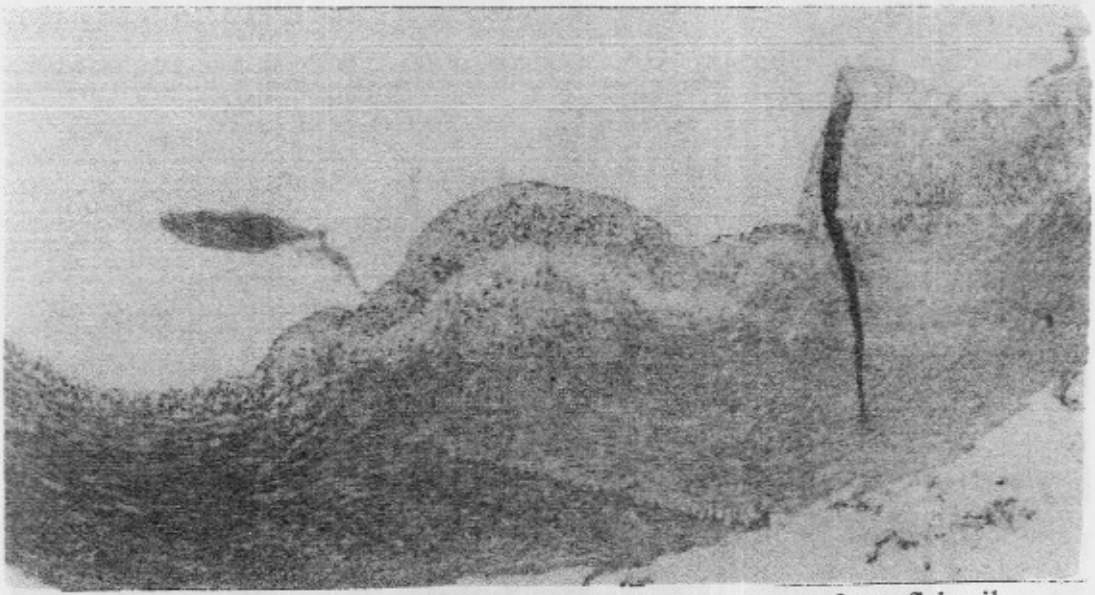

Fig. 5: Transverse section of the aortic wall of male rats from fish oil group showing atheromatous fibrous plaque (H&E X100).

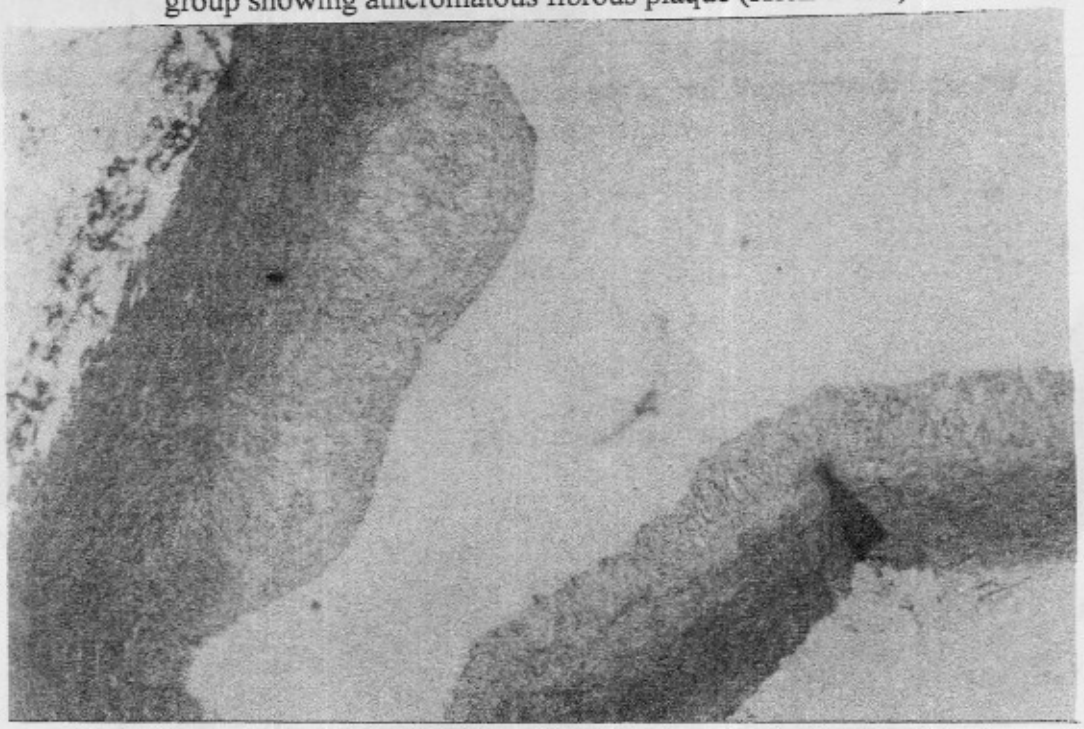

Fig. 6: Transverse section of the aortic wall of male rats from melatonin group showing multilayered foam cells and cholesterol clefts in subendothelial space (H&E X200).

DISCUSSION

In this work the possible treating effect of fish oil, olive oil and melatonin on the induced 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), Leth-Espensen *et al.* (1988) and Hsu *et al.* (2001) in rabbits, Kunitomo *et al.* (1981), El Seweidy *et al.* (2005) and Bastida *et al.*(2006) 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, Hsu *et al.*, 2001 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 streakes 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,<0.05,<0.001) TPC, LDL and TG respectively 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, Castillo *et al.*, 2000 and Yilmaz *et al.*, 2002), HDL (Wilt *et al.*,1989 and Harris *et al.*, 1997) and TG (Wilt *et al.*, 1989, Flaten *et al.*, 1990, Castillo *et al.*, 2000 and Bravo *et al.*, 2006). 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, contradictory results were reported by Demke *et al.*, 1988(significant increase in TPC, LDL, HDL and non significant decrease in TG), Franzen *et al.*, 1993 (non significant change in TPC and LDL), Balesterieri *et al.*, 1996 (non significant change in TPC, LDL, HDL and TG) and Jeyaraj *et al.*, 2005 (increase in LDL). 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. Similar effect was reported by Harris *et al.* (1997) on NO, Vecera *et al.* (2003) on total

thiol and Chen *et al.* (1995) on LP in great arteries. While, contradictory effect on LP was reported by Baydas *et al.* (2002) and Bravo *et al.* (2006) who found non significant increase in LP with fish oil. Histological examination of the aorta from fish oil group showed atheromatous fibrous plaques but absence of well developed fibrous cap which characterize the cholesterol fed group denoting slight improvement. In agreement with our results, antiatherosclerotic effect of fish oil in diet induced hypercholesterolemic rabbits was reported by Chen *et al.* (1995) and a favourable influence of fish oil on the progression of atherosclerosis in hypercholesterolemic patient was reported by Balestrieri *et al.* (1996). While, adverse effect of fish oil on atherosclerosis was reported by Achtani *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.* (1992). TPC lowering effect of olive oil was also reported by Sirtori *et al.* (1992) and puiggros *et al.* (2002) in hypercholesterolemic patients, Hapan *et al.* (2004) in elderly subjects and Gonzalez-Santiago *et al.* (2005) in hyperlipidimic rabbits treated with hydroxytyrosol a phenolic antioxidant present in olive oil. 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. 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 normal rats, Gonzalez-Santiago (2005) in hyperlipidimic rabbits and Ahuja *et al.* (2006) in healthy human. The non significant increase in HDL by olive oil in this work contradicts the significant increase reported by Mortensen *et al.* (1992) and Gonzalez-Santiago (2005) in rabbits, Faine *et al.* (2004) in rats and Ahuja *et al.* (2006) in human and contradict the significant decrease reported by Imai *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). In addition, increased concentration of NO by olive oil was reported by Ruano *et al.* (2005) and decreased LP was reported by Cullinen (2006). Histological examination of the aorta from olive oil group showed atheromatous fibrous plaques but absence of well developed fibrous cap which characterize the cholesterol fed group. 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 LDL 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 and significantly ($P<0.001$) increased SOD and total thiol and non significantly increased NO. Melatonin was more effective than fish oil and olive oil on LP and SOD and has median effect between fish oil and olive oil on total thiol. Inhibitory effect of melatonin on plasma LP was found also by Hoyos *el al.* (2000) in hypercholesterolemic rats and Baydas *et al.* (2002) in normal rats. 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). Stimulating effect of melatonin on antioxidant enzymes was also reported by Ahmed *et al.* (2005) and Nishida (2005).

Histological examination of the aorta from rats injected with melatonin showed that the atheromatous lesions were similar to that of olive oil and fish oil groups denoting slight improvement. 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 reducing TPC and in restoring level of total thiol and melatonin was the best factor reducing LDL and LP and consequently atherogenesis. It was also the most effective in restoring SOD levels.

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). 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 diet additives as fish oil, olive oil or melatonin injection have modulating effect on the parameters of the lipogram, oxidative stress markers and histological features of atherosclerotic lesion and that the improvement of the aortic wall was slight due to the short period of treatment (2 weeks only) to produce marked change in the aortic wall. So, fish oil, olive oil and melatonin supplementation for longer period is recommended for treatment of

hypercholesterolemia and atherosclerosis that lead to heart attacks, strokes and other froms of cardiovascular damage.

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