

# Dietary Fish and Sesame Oil Effects on Serum Lipid Profile of Rats Fed on Coconut Oil Based High Fat Diet

[Diyette Balık ve Susam Yağının Kokonat Yağı İçeren Yüksek Yağlı Besin ile Beslenmiş Sıçanların Lipid Profiline Etkisi]

<sup>1</sup>Noha Maree,

<sup>1</sup>Iman Al Balouni,

<sup>2</sup>Said S. Moselhy and

<sup>2</sup>Taha Kumosani

<sup>1</sup>Department. of Nutrition, Faculty of Education and Home Economic

<sup>2</sup>Biochemistry Department. Faculty of Science, King Abdul-Aziz University, Jeddah, KSA.

## Yazışma Adresi

[Correspondence Address]

Prof. Said Moselhy

Biochemistry Department, Faculty of Science, King AbdulAziz University. KSA  
Telephone: 00966566991424.  
E-mail: moselhy6@hotmail.com

Registered: 26 June 2009; Accepted: 17 August 2009

[Kayıt tarihi: 26 Haziran 2009; Kabul tarihi: 17 Ağustos 2009]

## ABSTRACT

**Objective:** This study aimed to evaluate the role of dietary fish and sesame oils as protective agents or for treatment of hyperlipidemia.

**Materials and Methods:** There were two experiments addressing protective and treatment effects respectively. First experiment had five groups of ten male albino rats. All groups were fed on respective diets for 12 weeks; Group I (control); standard diet, Group II; standard diet with 20% coconut oil, Group III; group II diet with 10% fish oil, Group IV; group II diet with 10% sesame oil, Group V; group II supplemented with fish/sesame oil (5:1 ratio). The second experiment included 40 rats fed on high fat diet for 12 weeks then divided into four groups to be fed for another 12 weeks. Control group was on standard diet. Second, third and fourth were standard diet supplemented with 10% fish, 10% sesame and fish/sesame oil (5:1) respectively. Fasting lipid profile and atherogenic index were determined.

**Results:** Rats fed on coconut oil showed significant elevation in the levels of serum total cholesterol, LDL-c, atherogenic factor while HDL-c level was significantly decreased. Histological examination revealed large lipid depositions in livers of rats fed high fat diet. Supplementation of diet with fish oil or a mixture of fish/sesame oil lowered blood cholesterol and prevented atherogenesis hence predisposition for coronary heart diseases. We concluded that,  $\omega$ -3 and/or  $\omega$ -6 prevent atherosclerosis and reduces coronary heart disease.

**Key Words:** poly unsaturated fatty acids (PUFA), fish oil, sesame oil, cholesterol, atherogenic, rats

## ÖZET

**Amaç:** Bu çalışma diyette balık ve balık ve susam yağı eklemenin yüksek yağlı diyetle beslenmiş sıçanlarda hiperlipidemi yönünden koruyucu veya iyileştirici etkisinin araştırılmasını amaçlamıştır.

**Materyal ve Metod:** Koruyucu ve iyileştirici etkileri araştırmak üzere iki deney yapıldı. İlk deney 10'ar albino sıçan bulunan beş grup içermektedir. Tüm gruplar kendine has diyetleri ile on iki hafta boyunca beslenmişlerdir. Grup I (kontrol), standart diyet; Grup II, standart diyet ve %20 hindistan cevizi yağı; Grup III, grup iki diyeti ve %10 balık yağı; Grup IV, grup II diyeti ve %10 susam yağı; Grup V, grup II diyetine ek olarak balık/susam yağı (5:1 oranında). İkinci deneyde kırk sıçan on iki hafta süresince yüksek yağlı diyet ile beslenmiş ve daha sonra tekrar on iki hafta kendilerine has diyet ile beslenmek üzere dört gruba ayrılmıştır. Kontrol grubu standart diyet, ikinci üçüncü ve dördüncü gruplar ise standart diyete ek olarak sırasıyla %10 balık, %10 susam ve balık/susam (5:1) yağı ile beslenmiştir. Açlık lipidleri ve aterojenik index belirlenmiştir.

**Sonuçlar:** Hindistan cevizi yağı ile beslenen sıçanların serum total kolesterol, LDL-c, aterojenik faktör değerlerinde belirgin artış, HDL-c düzeyinde ise belirgin düşme gözlemlendi. Histolojik incelemelerde yüksek yağ diyeti ile beslenen sıçanların karaciğerlerinde lipid depozitlerine rastlandı. Supplementation of diet with fish oil or a mixture of fish/sesame oil lowered blood cholesterol and prevented atherogenesis hence predisposition for coronary heart diseases. Diyetin balık yağı veya balık ve susam yağı ile takviyesi kan kolesterolünü düşürmüş, aterojenite engelleyerek kalp hastalığını önlemiştir. Sonuç olarak  $\omega$ -3 ve/veya  $\omega$ -6 ateroskleroza mani olmakta ve koroner kalp hastalık riskini azaltmaktadır.

**Anahtar Kelimeler:** çoklu doymamış yağ asitleri (PUFA), balık yağı, susam yağı, kolesterol, aterojenik, sıçan

## Introduction

The biological and physiological effects of dietary lipids on human health remain a primary focus of nutrition research as consumption recommendations are continually updated in response to new information obtained through epidemiological, clinical, and animal investigations. The role of omega-3 (n-3) polyunsaturated fatty acids (PUFA) in the development of the infant nervous system and retina is clearly established (1). Moreover, implications of a therapeutic effect on reducing cardiovascular disease and cancer risk and actions of their derivatives as biological effectors of human pathologies further drive biochemical and molecular investigations to elucidate the health benefits of dietary fatty acids. In addition to their beneficial impact on cardiovascular pathologies and cancers, n-3 fatty acids are also known to lessen the severity and minimize symptoms of chronic inflammatory diseases, including rheumatoid arthritis and inflammatory bowel disease, and may help correct psychological disorders (2).

Fish oils are sources rich in docosahexaenoic acid (DHA, C22:6  $\omega$ -3) and eicosapentaenoic acid (EPA, C20:5  $\omega$ -3) (3).  $\alpha$ -linolenic acid (ALA), EPA and DHA are known as omega-3 polyunsaturated fatty acids ( $\omega$ -3 PUFA). It was known that DHA taken into the body is mostly delivered to the liver through plasma lipoprotein. Previous studies have shown that DHA has been used for the treatment of several diseases such as glomerulonephritis, rheumatoid arthritis, autoimmune diseases, allergic asthma, hypertension, cardiovascular diseases and as adjuvant in cancer therapy such as mammary and colon tumors (3).

Sesame is an important food source in many parts of the world. Sesame is commonly used for its oil, and is a rich source of cis-unsaturated fatty acid such as linoleic acid (4). Sesame seeds are traditionally believed to have health benefits in cultures such as the Chinese and the Japanese. Recent studies have suggested that sesame may have bioactivities that are beneficial in the setting of cardiovascular disease (CVD), with many of these activities being attributed to its unique lignans, sesamin and sesamol (5). Several animal studies have consistently demonstrated that increased intake of sesame seeds or purified sesamin, has hypocholesterolemic effects in rats (6), and can reduce blood pressure (6).

Long-chain  $\omega$ -3 fatty acids become incorporated into the cell membranes and have anti-inflammatory properties that may be relevant for the prevention of type 1 diabetes, such as decreased expression of HLA class II molecules on activated human monocytes (3).

High levels of saturated fatty acids (SFAs) in the Western diet are associated with the formation of atherosclerotic plaque (4). However, intake of monounsaturated fatty acids (MUFAs)  $\omega$ -9, especially oleic acid (18:1  $\omega$ -9), and PUFAs of the  $\omega$ -3 series, especially EPA and DHA is associated with decreased risk of cardiovascular death (5). Many studies have demonstrated that MUFAs

and PUFAs exert similar effects on lowering blood cholesterol when SFA is substituted in the diet (7).

The aim of the present study is to evaluate the possible protective or treated effect(s) of fish oil (as  $\omega$ -3 fatty acid) or /and sesame oil (as  $\omega$ -6 fatty acid) against atherogenesis and fatty liver in rats fed coconut oil as source of high saturated fatty acids (HFD).

## Materials and Methods

### Animals

This study was carried on a total of 90 male Albino rats weight ranged ( $100 \pm 5$  gm). Animals were obtained from Animal House Unit, King Fahad Center for Medical Research (KAU, Kingdom of Saudi Arabia) Jeddah. They were maintained and handled according to the recommendations of the institutional Ethic Committee. Water and food were given ad libitum with light dark cycle 12 hrs. Two experiments were carried out; the first for protective trail and the second for treatment trial.

The first included 50 rats divided into five groups (each 10) as following

Group (I): Rats fed on standard commercial diet served as control.

Group (II): Rats fed on standard diet supplemented with 20% coconut oil (HFD)

Group (III-Prot.): Rats fed on HFD + 10% fish oil (rich  $\omega$ -3 fatty acids)

Group (IV-Prot.): Rats fed on HFD + 10% sesame oil (rich  $\omega$ -6 fatty acids)

Group (V-Prot.): Rats fed on HFD + mixture of fish oil and sesame oil (5:1). Feeding continues for 12 weeks

The second experiment including 40 rats fed oh HFD for 12 weeks and after that divided into four groups as following

Group (II): Rats fed on standard commercial diet .

Group (III-Treat): Rats fed on standard commercial diet + 10% fish oil Group (IV- Treat): Rats fed on standard commercial diet + 10% sesame oil

Group (V- Treat): Rats fed on standard commercial diet + mixture of fish oil and sesame oil (5:1). Feeding continues for another 12 weeks

Composition of standard diet:

Casein (15.3 gm), sucrose (10 gm), cornstarch (45.26), cellulose (5gm), dextrinated starch (15.5), mineral mix (3.5) vitamin mixture (1gm).

Fish oil was purchased from Fluka-Company (Germany). Coconut and sesame oils were purchased from local market at Jeddah, KSA. The doses of hight fat diet, fish oil, sesame oil and a mixture of oil were given according to the study of Wahrburg (6). After the experiments periods all rats were overnight fasted blood samples were collected from artery, sera were separated and kept at  $-20^{\circ}\text{C}$  till analysis. Liver was removed and kept

in 10% formaldehyde buffer for preparation of slides for histological examination.

## Methods

The biochemical parameters were assayed by using kits purchased from Biosystem. Total serum cholesterol was determined according to (7), triacylglycerol (8), HDL-c (9), LDL-c (10), VLDL-c (11), and atherogenic index (12).

## Liver morphology

Liver slices were dehydrated with ethanol, cleared with xylene and embedded in paraffin wax. After inclusion, material was cut on a microtome (CUT model 445, Olympus) at 4  $\mu$ m. Liver sections were stained with hematoxylin and eosin (Merck) (13).

Pictures were taken with a photomicroscope (Model AX-70, Olympus) using Kodac color Gold 100 as a film at 40 X magnification. Histological analysis was performed as qualitatively.

## Statistical analysis

Collected data were subjected to statistical analysis using SPSS version 8.0. Student's *t* test was used to compare two independent samples (normal and HFD groups). Analysis of variance (ANOVA) was used to compare three or more independent samples (HFD, fish oil, sesame oil and mixture oils).

## Results

Table (1) lists lipid profile of all studied groups in the protective trail against atherogenesis. It was found that in rats fed on high fat diet (20% coconut) serum total cholesterol, LDL-c levels and atherogenic index were statistically significant elevated ( $P < 0.001$ ), and the level of HDL-c was significantly decreased ( $P < 0.05$ ), while the levels of serum triacylglycerol, and VLDL-c were non significant changed as compared to control rats.

Supplementation of HFD with 10% fish oil showed a significant reduction in the levels of T-cholesterol, LDL-c and atherogenic factor (the reduction percentage were

**Table 1.** Serum levels of total-cholesterol, triacylglycerol, HDL-c, LDL-c, VLDL-c and atherogenic factor in the protective trail (Mean  $\pm$ SD)

Groups Parameters	Control	HFD	HFD +10% fish Oil (prot)	HFD+ 10% sesame oil (prot)	HFD+ 1% fish oil, 5% sesame (prot)
T-cholesterol (mmol/L)					
Mean $\pm$ SD	1.85 $\pm$ 0.16	2.78 $\pm$ 0.07	1.08 $\pm$ 0.16	1.49 $\pm$ 0.17	1.31 $\pm$ 0.11
P value	---	<0.001	<0.001	<0.01	<0.001
P*	---	---	<0.001	<0.001	<0.001
Triacylglycerol (mmol/L)					
Mean $\pm$ SD	0.59 $\pm$ 0.14	0.51 $\pm$ 0.04	0.53 $\pm$ 0.1	0.41 $\pm$ 0.05	0.47 $\pm$ 0.10
P value	---	N.S.	N.S.	<0.05	N.S.
P*	---	--	N.S.	<0.001	N.S.
HDL-c (mmol/L)					
Mean $\pm$ SD	0.49 $\pm$ 0.03	0.46 $\pm$ 0.03	0.48 $\pm$ 0.07	0.41 $\pm$ 0.05	0.44 $\pm$ 0.06
P value	---	<0.05	N.S.	<0.05	<0.01
P*	---	--	<0.05	<0.05	N.S.
LDL-c (mmol/L)					
Mean $\pm$ SD	1.48 $\pm$ 0.11	2.41 $\pm$ 0.05	0.77 $\pm$ 0.10	1.16 $\pm$ 0.12	0.69 $\pm$ 0.08
P value	---	<0.001	<0.001	<0.001	<0.011
P*	---	---	<0.001	<0.001	<0.01
VLDL-c (mmol/L)					
Mean $\pm$ SD	0.11 $\pm$ 0.02	0.1 $\pm$ 0.01	0.1 $\pm$ 0.02	0.08 $\pm$ 0.01	0.09 $\pm$ 0.02
P value	---	N.S.	N.S.	<0.05	N.S.
P*	---	--	N.S.	<0.01	N.S.
Atherogenic factor					
Mean $\pm$ SD	3.22 $\pm$ 0.16	5.45 $\pm$ 0.4	2.18 $\pm$ 0.2	3.03 $\pm$ 0.34	2.34 $\pm$ 0.44
P value	---	<0.001	<0.001	N.S.	<0.001
P*	---	--	<0.001	<0.01	>0.001

HFD: High fat diet P: compared with control group  $P < 0.05$  was considered as significant

P\*: compared with HFD group

N.S.: non significant

61%, 68%, 97% respectively) while HDL-c was significantly elevated by 5% as compared to HFD fed rats.

Supplementation of HFD with a mixture of 10% sesame oil and fish oil exert the same effect of fish oil but of lower efficacy (the reduction percentage were 46%, 51%, 44% ,respectively) in contrast HDL-c was significantly decreased by 10% as compared with HFD.

In the second experiment (treatment trail), rats fed on HFD for 12 weeks showed a significant elevation of total cholesterol, triacylglycerol, LDL-c and atherogenic factor. Animals treated with fish oil or a mixture of sesame + fish oil or fish oil alone showed improvement in the studied parameters versus rats fed on HFD fed animals (Table 2) .This is observed well in fig 2, that illustrate the percentage changes in all studied groups.

### Liver Histology

Figure (1) shows lipid deposition in liver slices. The intensity of liver steatosis was classified as more than 30% of hepatocytes affected (+), more than 50% of hepatocytes affected (++), and more than 75% of hepatocytes

affected (+++). Lipid vesicles appeared in livers of all animals. Hepatocytes of the HF group presented a flattened nucleus due to high lipid content.

### Discussion

The high content of  $\alpha$ -linolenic acid may play an important role in decreasing the risk of coronary heart disease (CHD) by lowering levels of low-density lipoprotein cholesterol, as reported in the literature (15). Low levels of saturated fatty acid (SFA) as palmitic acid (C16:0), may be another protective factor against CHD (16).

Fish oil is reported to have a higher content of  $\omega$ -3 fatty acids EPA and DHA in the liver than in the filet (17). The high content of MUFAs is associated with a low incidence of CHD (18), because it decreases total cholesterol (10%) and low-density lipoprotein cholesterol (14%) (19). This effect, however, depends on levels of SFA in the diet (20). Coconut oil, which has a high content of MUFAs (C18:1 and C16:1), presented the highest content of SFAs (C16:0) among the foods evaluated in this study and, hence, low PUFA/SFA ratio. Palmitic

**Table 2.** Serum levels of total-cholesterol, triacylglycerol, HDL-c, LDL-c, VLDL-c and atherogenic factor in the treated trail (Mean $\pm$ SD)

Groups Parameters	Control	HFD	HFD +10% fish Oil(Treat)	HFD+ 10% sesame Oil(Treat)	HFD+ 1% fish oil, 5% sesame Oil(Treat)
T-cholesterol (mmol/L)					
Mean $\pm$ SD	1.85 $\pm$ 0.16	2.95 $\pm$ 0.21	1.52 $\pm$ 0.17	2.1 $\pm$ 0.5	1.76 $\pm$ 0.19
P value	---	<0.001	<0.05	N.S	N.S
P*	---	---	<0.001	N.S	<0.01
Triacylglycerol (mmol/L)					
Mean $\pm$ SD	0.59 $\pm$ 0.14	0.72 $\pm$ 0.05	0.54 $\pm$ 0.1	0.57 $\pm$ 0.12	0.53 $\pm$ 0.48
P value	---	<0.01	N.S.	<0.05	N.S.
P*	---	--	<0.05	<0.001	<0.05.
HDL-c (mmol/L)					
Mean $\pm$ SD	0.49 $\pm$ 0.03	0.44 $\pm$ 0.04	0.48 $\pm$ 0.07	0.51 $\pm$ 0.19	0.41 $\pm$ 0.05
P value	---	N.S	N.S.	N.S	N.S
P*	---	--	<0.05	N.S	<0.05
LDL-c (mmol/L)					
Mean $\pm$ SD	1.48 $\pm$ 0.11	2.13 $\pm$ 0.19	1.11 $\pm$ 0.15	1.71 $\pm$ 0.34	1.42 $\pm$ 0.13
P value	---	<0.05	<0.01	<0.001	N.S
P*	---	---	<0.001	<0.001	<0.001
VLDL-c (mmol/L)					
Mean $\pm$ SD	0.11 $\pm$ 0.02	0.18 $\pm$ 0.01	0.13 $\pm$ 0.02	0.09 $\pm$ 0.02	0.1 $\pm$ 0.01
P value	---	<0.05	N.S.	<0.05	N.S.
P*	---	--	<0.05	<0.01	<0.05
Atherogenic factor					
Mean $\pm$ SD	3.22 $\pm$ 0.16	5.11 $\pm$ 0.42	2.4 $\pm$ 0.34	3.81 $\pm$ 0.6	3.42 $\pm$ 0.26
P value	---	<0.01	<0.01	N.S.	N.S
P*	---	--	<0.001	<0.01	<0.01

HFD: High fat diet P: compared with control group P<0.05 was considered as significant

P\*: compared with HFD group

N.S.: non significant



acid (C16:0) is associated with the risk of CHD because of its effect in increasing blood cholesterol levels and platelet aggregation (21).

The lipid and protein contents of coconut found in this study were similar as reported (22). The coconut diet, however, resulted in a low food efficiency ratio, perhaps due to the presence of limiting amino acid and antinutrient factors.

Animals fed on HFD showed higher levels of blood cholesterol compared with animals fed the normal diet. This unexpected result may be due to an activation of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Moreover, cholesterol deposition in the liver was high in the HFD group, which may explain why cholesterol was high in the bloodstreams of these animals.

It has been shown that the atherogenic effect of these SFAs may be enhanced by a cholesterol-rich diet (23), which leads to high levels of LDL-c and VLDL-c and lower liver lipid deposition. Also, fish oil showed a high content of MUFAs (C18:1), which has been reported to play an important role in decreasing serum total cholesterol and increasing HDL-c.

HDL-c was not significantly changed between the normal and fish oil groups. However, animals fed the sesame oil or mixture of sesame and fish oil diets showed lower HDL-c level ( $P < 0.05$ ) than did animals in the HFD group.

Animals fed the normal diet had higher levels of triacylglycerol than did animals fed the HFD, which may be due to the higher content of carbohydrate in the former diet as a consequence of its lower fat content, as shown in Table 1. Animals supplemented with fish oil had higher triacylglycerol levels than did animals fed HFD while animals fed sesame oil or a mixture of sesame and fish oil showed lower triacylglycerol than HFD rats. This difference may be attributed to the fatty acid profile of these diets because it has been demonstrated that the  $\omega$ -3 fatty acids decrease triacylglycerol levels in hyperlipidemic subjects and in Eskimos from Greenland who consume a diet low in saturated fat (24).

The diet high in saturated fat increased blood cholesterol up to 25%. This seems to be the result of liver lipid deposition, which provides acetyl coenzyme A to liver cells for cholesterol synthesis (25).

The excessive liver lipid deposition leads to steatosis (26), which represents an imbalance between triacylglycerol synthesis in the liver and its secretion (27).

Intake of  $\omega$ -3 fatty acids, either as fish oil or ethyl-ester formulations (selectively enriched in EPA and DHA), is associated with a variety of biochemical changes that might be beneficial for diabetes: reduced triglyceridemia mainly through enhanced triacylglycerol lipolysis, enhanced fatty acid oxidation and raised HDL-c levels; a trend for a more beneficial profile of the LDL particles (28). This is in line with our study that revealed improve-

ment in the lipid profile in rats supplemented with fish oil alone or combined with sesame oil more than sesame oil.

A number of clinical, experimental and epidemiological studies have generally confirmed that intake of  $\omega$ -3 fatty acid exerts beneficial effects on atherosclerosis development and progression (29). These protective effects are attributed to several favorable modifications: changes in plasma lipids, reduction of triglycerides (30), inhibition of the production of arachidonic acid-derived eicosanoids, namely the prothrombotic thromboxane  $A_2$  by activated platelets, and the pro-inflammatory leukotrienes  $B_2$  and  $C_4$  by activated leukocytes (31). Dietary fish oils rich in  $\omega$ -3 fatty acids have been proved to be effective in the lowering of the plasma triacylglycerol and VLDL-c levels in experimental animals, thereby being attributed a role in the prevention of cardiovascular diseases (32). Fish or fish oil incorporated into a diet providing 40% of energy as fat, increased total cholesterol, HDL, HDL<sub>2</sub> and LDL, but decreased triacylglycerols (33). Mori et al (34) reported that EPA and DHA decreased triacylglycerols and increased fasting insulin. Only DHA increased HDL-c, particularly HDL sub-fraction.

## Conclusion

Daily intake of fish oil or mixture of fish and sesame oil were the most efficient PUFA in decreasing total cholesterol and LDL-c and elevating HDL-c level. So, they protect against atherosclerosis and CHD. In addition these oils prevent deposition of lipids in nephrocytes and protecting the liver parenchyma. In addition, the protection is better than treatment.

## References

- [1] Russo GL (2009). Dietary n - 6 and n - 3 polyunsaturated fatty acids: From biochemistry to clinical implications in cardiovascular prevention. *Biochemical.Pharmacology*. 77(6); 937-946.
- [2] Rothstein W G. (2006). Dietary fat, coronary heart disease and cancer: A historical review. *Preventive Medicine*, 43(5): 356-360.
- [3] Ma DW, Seo J, Switzer KC, Fan YY, McMurray DN, Lupton JR, Chapkin RS. (2004).n-3 PUFA and membrane microdomains: a new frontier in bioactive lipid research. *Journal of Nutritional Biochemistry*. 15(11): 700-706.
- [4] Gladine C, Morand C, Rock E, Bauchart D, Durand D. (2007). Plant extracts rich in polyphenols (PERP) are efficient antioxidants to prevent lipoperoxidation in plasma lipids from animals fed n - 3 PUFA supplemented diets. *Animal Feed Science and Technology*. 136(3-4): 281-296.
- [5] Zambon D, Sabate J, Munoz S, Campero B, Casals E, Merlos M, Laguna JC, Ros E. (2000). Substituting walnuts for monounsaturated fat improves the serum lipid profile of hypercholesterolemic men and women: a randomized crossover trial. *Annual Review of Internal Medicine*; 132: 538-46.
- [6] Soha MH, Moselhy SS, Makhlof A, Amira FH (2007). Cod liver oil in chemically induced diabetes mellitus in rats. *Journal of Biological Research* 43: 100-109.

- [7] Wu JH, Hodgson JM, Puddey IB, Belski R, Burke V, Croft KD (2009) Sesame supplementation does not improve cardiovascular disease risk markers in overweight men and women. *Nutr Metab Cardiovasc Dis.* 19(11):774-780
- [8] Tiffany T and Morton J.(1974). Clinical evaluation of kinetic analysis of serum triacylglycerol. *Clin.Chem.* 20: 476-481.
- [9] Grove T. (1979). Effect of pH on determination of HDL-c. *Clin. Chem.*25: 260-265.
- [10] Mayne PD and Zilva PM (1994). *Clinical chemistry in diagnosis and treatment.* Sixth ed.,273-99.
- [11] Sheehan D and Deckelbourn R. (1980). *Practical histotechnology.* Second ed , Ohio, 11-29.
- [12] Jenkins DJA, Kendall CWC, Vidgen E, Agarwal S, Rao AV, Rosenberg RS, Diamandis EP, Novokmet R, Mehling CC, Perera T, Griffin LC, Cunnane SC. (1999). Health aspects of partially defatted flaxseed, including effects on serum lipids, oxidative measures, and ex vivo androgen and progestin activity: controlled crossover trial. *American Journal of Clinical Nutrition.* 69: 395-402.
- [13] Ghafoorunissa, Reddy V, Sesikaran B. (1995). Palmolein and groundnut oil have comparable effects on blood lipids and Platelet aggregation in health Indian subjects. *Lipids.* 30: 1163-9.
- [14] Caballero MJ, Obachb A, Rosenlundb G, Monteroa D, Gisvoldb M, Izquierdo MS. (2002). Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology ofrainbow trout, *Oncorhynchus mykiss.* *Aquaculture.* 214:253-71.
- [15] Almario RU, Vonghavaravat V, Wong R, Kasim-Karakas SE. (2001). Effects of walnut consumption on plasma fatty acids and lipoproteins in combined hyperlipidemia. *American Journal of Clinical Nutrition* 74:71-9.
- [16] Fashing P, Ratheiser K, Waldhausl W, Rohac M, Osterode W, Nowotny P,Vierphapper H. (1991). Metabolic effects of fish-oil in patients with impaired glucose tolerance. *Diabetes.* 40: 583-589.
- [17] O'Keefe JHJ, Nguyen T, Nelson J (1999). Potential beneficial effects of monounsaturated and polyunsaturated fats in elderly patients with or at risk of coronary artery disease. *Cardiology Elderly.* 3:5-10.
- [18] Billet MA, Bruce JS, white DA, Bennett AJ, Salter AM. (2000). Interactive effects of dietary cholesterol and different saturated fatty acids on lipoprotein metabolism in the hamster. *British Journal of Nutrition;* 84:439-47.
- [19] Djousse L, Hunt SC, Arnett DK. (2003). Dietary linoleic acid is inversely associated with plasma triacylglycerol: the National Heart, Lung, and Blood Institute Family Heart study. *American Jjournal of Clinical Nutrition.* 78:1098-102.
- [20] Guyton AC, Hall JE. (1996). *Tratado de fisiologia medica.* Rio de Janeiro: Guanabara Koogan;. Prostaglandine, 30: 205-211.
- [21] Pereira FEL. (2000). Degeneracoes. Morte celular. Alteracose do interstrecio. In: Brasileiro Fiho G, patella JEH, pereira FEL, BAMBIRRA EH, Barbosa AJA, editors. *Bogliolo-patologia.* 5<sup>th</sup> ed. Rio de Janeiro: Guanabara Koogan;, P. 393-4.
- [22] Leclercq I, Horsmans Y, Desager JP. (1998). Reduction in hepatic cytochrome P-450 is correlated to the degree of liver fat content in animal models of steatosis in the absence of inflammation. *Journal of Hepatology;* 28:410-6.
- [23] Snedecor G and Cochram W, (1980). *Statistical methods* 7<sup>th</sup> Ed. Iowa State university.Press.Library of congress31-44.
- [24] Hooper L, Sarmuellerbell CD, Higgins JP, Thompson J, Capps NS, smith GD, Riemersma RA, Ebrahim S. (2001) Dietary fat intake and prevention of cardiovascular disease: systematic review. *British Medical Journal,* 322: 757-763.
- [25] Harris WS. (1997) N-3 fatty acids and serum lipoproteins: human studies. *American Journal of Clinical Nutrition.* 71:121-132.
- [26] Fischer S, Weber PC, Dyeberg J. (1986) The Prostacyclin Thromboxane balance is favourably shifted in Greenland Eskimo. *Prostaglandine.* 32: 235-241.
- [27] Nestle PJ. (1990). Effect of n-3 fatty acids on lipid metabolism. *Annual Review of Nutrition.* 10: 149-167.
- [28] Mori TA, Vandongen R, Beilin LJ, Burke V, Morris J, Ritchie. (1994) Effects of varying dietary fat, fish and fish oils on blood lipids in randomized controlled trial oils on blood lipids in randomized controlled trail in men at risk of heart disease. *American Journal of Clinical Nutrition.* 59: 1060-1068.
- [29] Mori TA, Burke V, Puddey IB, Watts GF, O'Neal DN, Best JD, Beilin LJ. (2000) Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *American Journal of Clinical Nutrition.* 71: 1085- 1094.
- [30] Kris-Etherton PM, Yu-poth s, Sabate J. (1999) Nuts and their bioactive constituents: effects on serum lipids and other factors that affect disease risk. *American Journal of Clinical Nutrition;* 70:504S-5011S.
- [31] Ramesh G, Das UN. (1996). Effects of free fatty acids on two-stage skin carcinogenesis in mice. *Cancer letters.* 100: 199-209.
- [32] Morise A, Serougne C, Gripois D, Blouquit M, Lutton C, Hemier D. (2004). Effects of dietary alpha linolenic acid on cholesterol metabolism in rats. *Metabolism and Nutrition:* 10: 111-123.
- [33] Annuzzi G, Rivellese A, Capaldo B, Di Marino L, Lovine C, Marotta G, Riccardi G. (1991) A controlled study on the effect of n-3 fatty acids on lipid and glucose metabolism in non-insulin-dependent diabetic patients. *Atherosclerosis.* 87: 65-73.