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EFFECT OF FISH OIL AND OMEGA-3 FATTY ACIDS ON BONE MINERALS, BLOOD CONSTIUENTS AND HISTOPATHOLOGICAL CHANGES IN HYPERCHOLESTEROLEMIC AND DIABETIC RATS

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ABSTRACT

Effects of fish oil, corn oil, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and cod liver oil were investigated in hypercholesterolemic rats. Also, the effect of fish oil and corn oil were studied in diabetic rats. Sixty five male rats weighing 130±10g were divided into two major groups. The first group was hypercholesterolemic rats (50 rats) and divided into 10 sub groups, each group was 5 rats. The second group was diabetic rats (10 rats) and divided into two sub groups, each group is formed of 5 rats. The first group (contains 10 sub groups) was fed on different concentrations of fish oil or corn oil, mixed with different concentration of animal fat, cholesterol and bile salt. The last three groups were fed on corn oil supplemented with low dose of (DHA) or EPA or cod liver oil.

The biochemical parameters (serum total cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterol) were performed at the initial times, after 30 days and after 60 days. The second group (contains 2 sub groups) was fed on 10% fish oil and 10% corn oil and the biochemical parameters (serum total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol and blood glucose) were determined at the initial time and after 30 days. At the end of the experiment, animals were sacrificed and the rat livers were removed

for histopathological examination. Also femur bone was isolated for determining its content of calcium (Ca), phosphorous (P) and magnesium (Mg) in hypercholesterolaemic groups. The obtained results showed that, in hypercholesterolaemic groups, the reduction in serum lipid (total cholesterol, triglycerides, LDL-cholesterol) was associated with increase the fish oil especially in group fed on 7.5% and 10% followed by group fed on corn oil supplemented with DHA, EPA and cod liver oil, while HDL-cholesterol was increased in the same groups. Moreover, the histopathological finding showed the improvement in rat liver in the same groups as compared with normal control and other groups. Also Ca, P and Mg were increased especially in groups that received 7.5 and 10% fish oil.

In diabetic groups, reduction in blood glucose, total cholesterol, triglycerides, LDL-cholesterol was seen in group fed on 10% fish oil as compared with group fed on corn oil while HDL-cholesterol was increased in the fish oil group. Moreover, histopathological examination of the liver showed improvement of liver tissues in group fed 10% fish oil. As a conclusion, fish oil could be successfully used as hypolipidaemic agent whether alone or in combination with low percentage of hypolipidaemic factors and enhancement of bone minerals (Ca, P and Mg).

Also fish oil has a beneficial effect on hyperglycaemia and can be used in combination with oral antidiabetic drugs in treatment of diabetic patients.

Key words: Fish oil, docosahexaenoic acid, eicosapentaenoic acid, cod liver oil, hypercholesterolemia, hyperglycaemia, diabetes.

INTRODUCTION

Recent studies have shown that postprandial hypertriglyceridemia may be associated with the risk of coronary heart disease (Roche and Gibney 1999, Bergeron, Havel; 1977 and Karpe and Hamstern 1995). It has been established that the feeding of fish oil suppressed plasma triglycerides concentration in both postprandial and fasting states in humans and experimental animals (Roche and Gibney 1999, Williams 1996). Although the reduction in plasma triglycerides concentration in the fasting state is ascribed to suppression of hepatic fatty acid and triglycerides synthesis (Wong *et al.*, 1984, Surett *et al.*, 1992, Ikeda *et al.*, 1998). However, the

addition of 5% fish oil to high carbohydrate diet lowered plasma triacylglycerol levels while stimulating fatty acid synthase activity and liver lipid accumulation (Delzenne *et al.,* 1998). In contrast, addition of fish oil ameliorated the hepatic steatosis induced by 0.1 cholesterol diet, whereas the ω-6 rich safflower oil did not show the same effect (Yeh *et al.,* 1996). Reported hepatic effects contributing to the ω-3 PUFA induced hypolipidemia, also included increased uptake of chylomicron remnants (Lambert *et al.,* 2001) and diminished synthesis of apoliporotein B-48 and very low-denisty lipoprotein secretion (Brown *et al.,* 1997) potentially leading to liver fat deposition.

Long chain (ω-3) polyunsaturated fatty acids (PUFA) have attracted considerable attention for the last few years, as dietary fish oil rich in eicosapeutaemoic acid (DHA) (20:6 ω-3).Fatty acids play important role in reducing hypertriglyceridemia and seems to lower mortality from coronary heart disease. The ω-3 PUFA have received considerable interest because their consumption has been associated with beneficial health effects.

Epidemiologic studies have shown an inverse relation between the incidence of cardiovascular disease and consumption of fish oil (Mori and Beilin 2001). Another reports by Hanna *et al.*, (1970) revealed that inclusion of high ratio of unsaturated to saturated fatty acids in formula has beneficial effects on Ca absorption. Decreased faecal Ca levels (indicating a positive Ca balance) after increasing dietary linoleic acid (AL), the parent omega-6 fatty acid (Van Dokkum *et al.*, 1983). Repletion with dietary (n-3) fatty acids restored the ratio of n-6/n-3 PUFA in bone compartments and reversed compromised bone modeling in n-3 deficient rats (Reinwald *et al.*,2004).

Diabetes mellitus is a metabolic disorder caused by an absolute or relative lack of insulin. The metabolic abnormalities include hyperglycaemia, hypertriglyceridemia, and other lipoprotein disorder. The effect of fish oil and soybean oil were compared on plasma lipids and glucose concentration in diabetic rats (Yeh *et al.,* 1998). The results demonstrated no difference in concentration of plasma glucose, triglycerides, non-esterified fatty acids and insulin between the two diabetic groups. However Hamazaki *et al.,* (1993) reported that intravenous administration of DHA, soybean oil emulsion, or saline to diabetic rats through the tail vein resulted in significantly lower blood glucose concentration in the DHA group than in soybean oil and

saline group. Deborah *et al.,* (1999), stated that long chain polyunsaturated fatty acids (LCPUFA), especially docosahexaenoic acid is need for optimal retinal and neural.

The present study was divided into two sections the first section aimed to evaluate the effect of fish oil, corn oil, DHA, EPA and cod liver oil on hypercholesterolaemic rats under hypercholesterolaemic factors for 60 days. The biochemical parameters (total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) were determined at the initial time, after 30 days and after 60 days. The second section aimed to evaluate the effect of fish oil and corn oil on diabetic rats and the biochemical parameters (total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol and serum glucose were determined at the initial time and after 30 days. At the end of the experiment, rats liver were removed for histopathological examination in both major groups. But bone mineral analysis (Ca, P and Mg) was determined in the first groups.

MATERIALS AND METHODS

Materials

1- Fish oil was obtained from Agricultural Research Center Giza, Egypt.

2- Cod liver oil, Docosahexaenic (DHA), and eicosapentaenoic acid (EPA) were obtained from sigma company, USA.

Induction of atherosclerosis:

Rats were fed tell becoming hypercholesterolemic The hypercholesterolaemic diet was made up as reported by Kiriyama et al., (1968) and consisted of casein 20%, starch 48.75%, 20% animal fat, 1% cholesterol, 0.25% bile salt, 5% cellulose, 4% mineral mixture and 1% vitamin mixture.. The standard diets were used after hyperchelsterolaemia of rats (Lane-Petter and Pearson 1971) and consisted of 20% casein, 60% starch, 5% cellulose, 4 mineral mixtures, 1% vitamin mixture and 10% fat.

Induction of diabetes:

By intraperitoneal injection of streptzotocin (200 mg/kg) as recently reported by (Alison and Peter, 1998).

Study design

Sixty male albino rats weighing 130±10g were randomly divided

into (five animals each)
The fat was changed between groups as follow:
Group 1 (G1): Fed on basal diet (normal control) at the initial time to the end of experiment.
Group 2 (G2): Fed on hypercholesterolemic diet (10% fat tail, 1% cholesterol and 0.25% bile salt).
Group 3 (G3): Fed on 5% corn oil, 5% fat tail, 0.5% cholesterol and 0.125% bile salt.
Group 4 (G4): Fed on 5% fish oil, 5% fat tail, 0.5% cholesterol and 0.125% bile salt.
Group 5 (G5): Fed on 7.5% corn oil, 2.5% fat tail, 0.25% cholesterol and 0.0625% bile salt.
Group 6 (G6): Fed on 7.5% fish oil, 2.5% fat tail, 0.25% cholesterol and 0.0625% bile salt.
Group 7 (G7): Fed on 10% corn oil.
Group 8 (G8): Fed on 10% fish oil.
Group 9 (G9): Fed on 10% corn oil + DHA (Docosahexaenoic acid) through oral administration injection.
Group 10 (G10): Fed on 10% corn oil + EPA (Eicosapentaenoic acid) through oral administration injection.
Group 11 (G11): Fed on 10% corn oil + cod liver oil through oral administration injection.

The hyperglycemic diet was basal diet supplemented with 10% corn oil (G12) and 10% fish oil (G13)

Biochemical data

Blood samples (1ml) were taken at the initial time, after 30 days and 60 days from the retro-orbital plexus vein of the rat eyes using capillary tube. The blood sample portions allowed to stand at room temperature (20-25°C) for 10 min. for blood coagulation and were centrifuged at 4000 rpm for 3 min. and then the colorless upper layers (heamolysis-free serum) were carefully separated and transferred into sterilized tubes for analysis.

Serum glucose level , total cholesterol, LDL-cholesterol and HDL-cholesterol, triglycerides were determined according to the method of Tridner (1969), Allain et al., (1974), Levy (1981), Burstein (1970), Fossati and Prencipe (1982), respectively.

Analysis of minerals:

Calcium (Ca), phosphorous (P) and magnesium (Mg) were analyzed in rats bone by ICP according to A.O.A.C. (2006).

Histopathological examination

At the end of experiment, rats were scarified. The liver was removed immediately for histopathological examination according to Banchroft *et al.,* (1996). Fatty acids composition of fish oil and corn oil were determined by gas chromatography FID detector according to (A.O.A.C, 2000).

Statistical analysis of the data

Data were subjected to statistical analysis according to Duncan (1955) multiple range test.

RESULTS AND DISCUSSION

The fatty acid composition of fish oil and corn oil are presented in table (1). Data in table (1) shows that the major polyunsaturated fatty acids in fish oil are docosahexaenoic acid (DHA) 9.1% and eicosapentaenoic acid (EPA) 7.46%.

Also table (1) shows that the major polyunsaturated fatty acid is linoleic acid 62.6%.Similar results were given by Kromann and Green (1980) who mentioned that, fish oils are rich sources of ω-3 fatty acids especially EPA and DHA and Connor (1997) suggested that, the intake of oily fish which is high in n-3 polyunsaturated fatty acid EPA and DHA has a beneficial effect on the incidence of heart disease. In hypercholesterolemic groups (G1 to G11) total cholesterol, triglycerides, LDL-cholesterol were determined in serum blood of rat sat the initial time, after 30 and 60 days. The obtained results are given in table (2, 3, 4 and 5). At the initial time, the levels of total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol for normal control was about 87.38, 55.35, 36.00 and 59.00 mg/dl, respectively. While hypercholesterolaemic rats ranged between 247-263, 169.4-189.7, 67.5-73.75 and 44.00-50.00 mg/dl, respectively. However after 60 days the total cholesterol, triglycerides and LDL-cholesterol were significantly decreased especially in groups 6 and 8 which fed on 7.5 and 10% fish oil. Meanwhile HDL-cholesterol increased in the same groups. Similar results were obtained by Moore *et al.,* (2006) who mentioned that two portions of oily fish per week led to significant reductions in triacylglycerol relative to consumption of two portions of white fish per week. The plasma concentration of

triacylglycerols decreased progressively with the decrease in the ω-6: ω-3 ration of the diet (Jeffery *et al.*, 1996). The addition of 5% fish oil to a high carbohydrate diet resulting in lowered plasma triacylglycerol levels in rats (Delzenne *et al.,* 1998).

Table (1): Fatty acid compositions (g/100g) of fish and corn oils:

Identified fatty acids	Oils	
	Fish oil	Corn oil
G12:0 Lauric acid	0.05	-
G14:0 Myristic acid	5.12	-
G15:0 (13-menthyl tetradecanoic)	0.44	-
G15:0 pentadecanoic acid	0.58	-
G16:0 palmitic acid	15.6	7.5
G16:1 ω 7 palmitolic acid	5.87	-
G16:1 ω 5	0.76	-
G17:0 Margeric acid	1.19	-
G16:3 [9-hexadecatrienuic acid chexagonic acid]	0.94	-
G18:0 iso stearic acid	0.24	-
G16:4 ω1	0.18	-
G18:0 stearic acid	1.78	3.6
G18:1 ω 9 oleic acid	16.1	23.6
G18:1 ω 7 vaccinic acid	2.4	0.2
G18:2 ω 5 6-actadecosaenoic acid	0.41	0
G18:2 ω 6 linoleic acid	3.2	62.6
G18:3 ω 3 linolenic acid	1.8	0.3
G18:4 ω 3 steradonic acid	2.55	-
G20:0 arachidic acid	0.75	0.2
G20:1 ω 9 Gondoic acid	6.52	-
G20:1 ω 7 cis-9-eicosaenoic acid	0.27	-
G20:2 ω 6 [cis-8,11,14-eicosatrienoic acid]	0.42	-
G20:3 ω 6 cis-11,14,17-eicosatrienoic acid	0.47	-
G20:3 ω 3	0.13	-
G20:4 ω 3	0.54	-
G20:5 ω 3 Eicosapentaenoic acid (EPA)	7.46	-
G22:6 ω 3 Docosahexaenoic acid (DHA)	9.1	-
G18:3 ω 3	-	1.6
G22:1 ω 9 Eruic acid	0.94	-
Σ saturated	25.75	11.3
Σ monounsaturated	33.24	23.8
Σ polyunsaturated	26.79	64.5
4/5 Ratio	2.33	7.81
Σ ω-6	4.09	62.6
Σ ω-3	21.58	0.3
ω-6/ω-3 Ratio	0.189	208.66

Table (2): Serum total cholesterol levels (mg/dl) in normal and hypercholesterolaemic rats fed different concentrations of experimental diet.

Time / Groups	Before treatment	After 30 days	After 60 days
G1	h 87.38±0.51	j 112.0±0.60	j 102.0±0.58
G2	f 247.9±0.51	a 268.0±0.58	a 295.6±0.65
G3	c 255.0±0.58	b 261.3±0.43	b 269.5±0.29
G4	b 259.0±0.58	g 226.3±0.72	f 201.3±0.72
G5	g 237.3±0.66	e 236.3±0.72	d 219.3±0.43
G6	f 247.4±0.51	h 184.3±0.43	h 147.0±0.60
G7	a 263.3±0.62	c 243.0±0.58	c 224.0±0.60
G8	d 253.3±0.30	i 176.3±0.72	i 110.0±0.58
G9	b 259.0±0.58	d 239.5±0.29	e 209.0±0.58
G10	e 251.3±0.72	f 234.0±0.60	e 209.3±0.66
G11	de 252.0±0.60	g 225.0±0.60	g 199.3±0.66

Table (3): Serum triglyceride levels (mg/dl) in normal and hypercholesterolemic rats fed different concentrations of experimental diet.

Time / Groups	Before treatment	After 30 days	After 60 days
G1	h 55.35±0.72	i 63.15±0.55	i 62.35±0.09
G2	c 183.0±0.56	a 201.8±0.62	a 266.3±0.74
G3	g 169.4±0.46	b 174.6±0.15	b 183.0±0.56
G4	a 189.7±0.96	f 150.4±0.61	d 149.6±0.87
G5	g 170.9±0.64	g 148.0±0.24	f 139.9±0.38
G6	b 185.3±0.72	h 133.2±0.64	g 91.45±0.55
G7	a 188.0±0.77	c 165.9±0.62	c 153.4±0.39
G8	e 175.8±0.72	fg 149.0±0.17	h 77.10±0.52
G9	bc 183.5±0.29	b 175.1±0.38	c 153.0±0.01
G10	f 173.0±0.58	d 163.5±0.53	c 153.6±0.29
G11	d 180.0±0.58	e 153.6±0.29	e 142.6±0.23

Table (4): Serum LDL-cholesterol levels (mg/dl) in normal and hypercholesterolemic rats fed different concentrations of experimental diet.

Time / Groups	Before treatment	After 30 days	After 60 days
G1	h 36.00±0.58	g 40.50±0.29	i 41.00±0.58
G2	efg 68.50±0.29	a 80.50±0.87	a 93.50±0.30
G3	bc 71.00±0.60	b 76.00±0.58	b 83.50±0.29
G4	de 69.50±0.30	b 75.50±0.29	c 78.50±0.29
G5	a 73.75±0.14	c 73.50±0.30	d 75.50±0.30
G6	cd 70.00±0.58	e 64.50±0.30	g 51.50±0.29
G7	g 67.50±0.29	d 70.00±0.60	e 72.00±0.58
G8	def 69.00±0.01	f 60.00±0.01	h 49.00
G9	cd 70.00±0.01	f 61.00±0.60	±0.29 f 58.50±0.60
G10	b 72.00±0.01	e 64.50±0.29	f 59.00±0.60
G11	±0.60 fg 68.00±0.58	e 65.00±0.58	f 59.50±0.30

Table (5): Serum HDL- cholesterol levels (mg/dl) in normal and hypercholesterolemic rats fed different concentrations of experimental diet.

Time / Groups	Before treatment	After 30 days	After 60 days
G1	a 59.00±0.58	a 61.50±0.29	a 60.50±0.29
G2	ef 44.50±0.30	g 38.00±0.01	g 36.00±0.58
G3	cd 46.50±0.29	e 42.50±0.30	f 39.33±0.33
G4	c 47.00±0.60	f 41.00±0.58	e 43.00±0.60
G5	b 49.50±0.29	d 44.50±0.29	e 42.00±0.60
G6	de 45.50±0.30	c 48.00±0.60	c 51.00±0.58
G7	b 50.00±0.60	c 47.50±0.29	e 43.00±0.58
G8	f 44.00±0.58	b 53.00±0.58	b 55.50±0.30
G9	b 50.50±0.29	b 51±0.30	c 51.50±0.29
G10	c 47.50±0.30	c 48.50±0.30	c 50.00±0.60
G11	ef 45.00±0.58	c 48.00±0.58	d 49±0.30

Table (6): Bone minerals (Ca, P and Mg) (mg/100g) in normal and hypercholesterolemic rats fed different concentrations of experimental diet.

Con. / Groups	Ca	P	Mg
G1	c 117.5±0.13	b 53.19±0.22	±0.05 b 2.171
G2	c 116.5±0.40	c 51.12±0.59	±0.01 c 1.934
G3	f 106.9±0.55	de 48.08±0.44	±0.05 c 2.008
G4	d 113.9±0.66	c 50.47±0.59	±0.07 b 2.147
G5	c 116.8±0.78	b 53.28±0.33	±0.03 b 2.259
G6	b 120.6±0.65	ab 54.21±0.55	±0.02 a 2.453
A	g 102.7±0.77	e 46.97±0.55	±0.05 d 1.760
G8	a 125.1±0.38	a 55.38±0.65	±0.01 a 2.433
G9	b 119.6±0.06	b 53.40±0.36	±0.05 b 2.148
G10	h 99.86±0.23	f 44.70±0.42	±0.03 c 1.983
G11	e 108.6±0.48	d 48.63±0.75	±0.02 b 2.151

Table (6) illustrated the concentration of calcium, phosphorus and magnesium in femur bone of rats fed an experimental diet at the end of experiment. The concentration of calcium in femur bone of normal and hyperchlesterolemic control were 117.5 and 116.5 mg/100g respectively, while the other treated groups G3-G11 showed different values for calcium concentration. The highest increase in calcium as recorded in G8,G6 and G9 were 125.1, 120.6 and 119.6 mg/100g respectively, followed by G5, G4 and G11 which were 116.8, 113.9 and 108.6 mg/100g respectively, then G3, G7 in the same range (106.9 and 102.7 mg/100g) respectively, and finally G10 (99.86 mg/100/g).

The same trend was found in phosphorus and magnesium contents in femur bone of treated animal as shown in table (6). Such increase in calcium levels caused by omega-3 fatty acid are in agreement with the results obtained by Hanna *et al.* (1970) who found that inclusion of high ratio of unsaturated to saturated fatty acids in formula has beneficial effect on Ca absorption. Also, Van Dokkum *et al.* (1983) observed decrease of faucal Ca levels (indicating positive Ca balance) after increasing dietary linoleic acid (LA, the parent omega-6 fatty acid.

On the other hand Haag *et al.* (2003) found that dietary supplementation with fish oil that contains omega-3 polyunsaturatd fatty acids has been shown to enhance bone density as well as duodenal calcium uptake in rats. The increase in calcium level attributed to Ca ATPase in calmodulin stripped membranes was activated in a biphasic manner by docasahexenoic acid (DHA) but not eicosapentaeonic acid (EPA). DHA inhibited Na, K-ATPase.

Table (7): Serum glucose levels (mg/dl) in normal and streptozotocin – induced diabetic rats fed on different oil sources

Time / Groups	Before treatment	After 30 days
G1	b 104.5±0.29	c 99.25±0.37
G12	a 246.7±0.62	a 289.3±0.40
G13	a 247.4±0.60	b 169.1±0.51

Table (8): Serum total cholesterol levels (mg/dl) in normal and streptozotocin – induced diabetic rats fed on different oil sources

Time / Groups	Before treatment	After 30 days
G1	c 89.25±0.49	c 94.80±0.23
G12	a 121.2±0.58	a 129.7±0.29
G13	b 114.4±0.69	b 120.7±0.55

Table (9): Serum triglyceride levels (mg/dl) in normal and streptozotocin – induced diabetic rats fed on different oil sources

Time / Groups	Before treatment	After 30 days
G1	c 89.15±0.43	c 93.16±0.50
G12	a 131.7±0.35	a 150.1±0.54
G13	b 130.1±0.05	b 119.1±0.61

Table (10): Serum LDL-cholesterol levels (mg/dl) in normal and streptozotocin – induced diabetic rats fed on different oil sources

Time / Groups	Before treatment	After 30 days
G1	b 35.50±0.29	c 37.33±0.34
G12	a 59.50±0.30	a 67.00±0.58
G13	a 59.43±0.98	b 52.50±0.29

Table (11): Serum HDL-cholesterol levels (mg/dl) in normal and streptozotocin – induced diabetic rats fed on different oil sources

Time / Groups	Before treatment	After 30 days
G1	a 59.33±0.88	a 63.00±0.58
G12	b 44.50±0.29	C 34.50±0.29
G13	b 42.00±0.58	b 48.00±0.60

In diabetic rats, serum glucose, total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol were determined at the initial time and after 30 days. The obtained data are given in table (7, 8,9,10 and 11).

The average of serum glucose, total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol were 104.5, 89.25, 89.15, 35.5 and 59.33 mg/dI, respectively. While in diabetic rats, it were (264.7 and 247), (121.2 and 114.4), (131.7 and 130.1), (59.5 and 59.43), (44.5 and 42.00) mg/dI, respectively. After 30 days, the normal control (G1) values were 99.25, 94.8, 93.16, 37.33 and 63.00 mg/dI for serum glucose, total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol respectively. While these parameters of hyperglycaemic control (G12) were significantly stimulated to 289.3, 129.7, 150.1, 67.00 and 34.50 mg/dI, respectively.

After rats were fed on experimental diet supplemented with 10% fish oil, the results showed, a significant decrease in serum glucose to 169.1 mg/dI while total cholesterol, triglycerides, LDL-

cholesterol and HDL-cholesterol were slightly stimulated to 120.7, 119.1, 52.50 and 48.00 mg/dI, respectively. Buettner *et al.,* (2007) concluded that animal fats and omega-6/omega-9 containing plant oils can be used to generate obesity and insulin resistant phenotype in rodents; whereas fish oil fed animals do not develop these disorders.

Dietary intake of omega-3 fatty acids is associated with reduced risk of islet autoimmunity in children at increased genetic risk for diabetes. Norris *et al.,* (2007). Hamazaki *et al.,* (1993) reported that intravenous administration of DHA, soybean oil emulsion or saline to diabetic rats through the tail vein resulted in significantly lower blood glucose concentration in the DHA group than in soybean oil and saline group. There was no histopathological findings observed and the normal structure was recorded in (Fig.1) for liver in central group. Liver in group 2 showed severe congestion of the central veins associated with diffuse proliferation of kupffer cells in between the hepatocytes (Fig.2).

Focal area in the hepatic tissue showed fatty change in the hepatocytes (Fig.3). There was severe dilatation in liver group 3 and congestion of the central viens (Fig. 4), while the hepatocytes showed karyomegalic nuclei associated with diffuse proliferation of the kupffer cells (Fig.5). Fatty change was noticed in the diffuse manner all over the hepatocytes and mainly surrounding the dilated and congested central veins (Fig.6) in association with focal areas of pyknotic nuclei of the hepatic tissue (Fig.7) in group 4. Liver of rat in group 5 showing fatty change in the cytoplasm of the hepatocytes (Fig. 8).

There was no histopathological finding observed in groups 6 and 8 as recorded in (Fig.9). In contrast, liver in group 7 showed dilatation and congestion in the central vein associated with diffuse proliferation of the kupffer cells in between the hepatocytes (Fig.10). While group 9 showed severe congestion in the central veins (Fig.11). Also, there was diffuse proliferation of the kupffer cells in between the degenerated hepatocytes for group 10 (Fig.12). Liver in group 11 showed dilation in the central vein (Fig.13), associated with inflammatory cells infiltration in the portal area and proliferation of the kupffer cells in diffuse manner between the hepatocytes (Fig.14).

In diabetic groups, group 12 the central veins and sinnsoids were dilated and congested with blood associated with diffuse proliferation of kupffer cells and diffuse infiltration of mononuclear leucocytes

inflammatory cells in between the hepatocytes which had pyknosis in some of their nuclei (Figs.15 and 16). Mononuclear leucocytes inflammatory cells infiltrations were also observed in portal area (Fig.17).

Cytomegally was noticed in most of hepatocytes (Fig.18), with double nuclei in some of them and pyknosis in other (Fig.19). In group 13 showed liver congestion in the central veins associated with diffuse proliferation of kupffer cells in between the hepatocytes (Fig.20). From the biochemical and histological results in hypercholestrolemic and hyperglycemic groups, it was found that, the increased levels of serum lipid (cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) and liver tissues caused by hypercholesterolmic diet were significantly alleviated by high concentration of fish oil in groups G6 and G8 followed by G8, G9 and G10. In hyperglycaemic groups, marked improvement was noticed in biochemical parameter in the group that received 10% fish oil as compared with the group that received 10% corn oil, were proved histopathologically as it was noticed improvement in liver.

The observed alleviation effect of high concentration of fish oil and DHA, EPA, cod liver oil on histopathological change caused by hypercholesterolemic diet and streptozotocin induced diabetic rats were in agreement with (Harris *et al.* 2003) who found that omega-3 fatty acid (1mg/day for 4 month) supplementation reduced the risk of sudden cardiac death and death from any cause in post-myocardial infarction patients.

Some positive effect of fish oil rich in long chain polyunsaturated fatty acids (n-3) on pathology recorded by (Kasiske *et al.*, 1991) and in the prevention of hypertensive renal damage. In these conditions, dietary polyunsaturated fatty acid supplementation increases their incorporation in glomeruli and cortical tissue, improving chronic renal injury (Hobbs *et al.*, 1996). However, Baybutt *et al.*, 2002 found that feeding rats with fish oil (15% w/w) diet protects against inflammation and fibrosis in lung and liver, and against hepatocyte vacuole formation. Also, Du *et al.*, 2004 reported that polyunsaturated fatty acids suppress the development of acute hepatitis and prolong survival in females.

The hypolipidemia caused by ω-3 fatty acids is well established and has been associated with various hepatic mechanisms such as increased fatty acid oxidation (Niot *et al.*, 1994) and inhibition of de

novo fatty acid synthesis secondary to decreased gene expression (Clark and Jump, 1994).

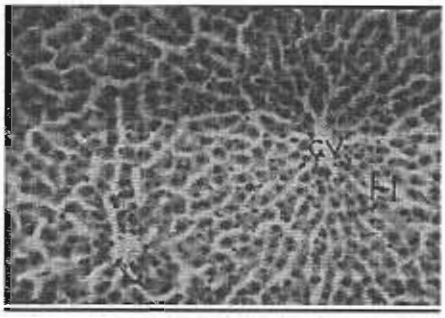

Fig. (1): Liver of rat in group (1) control group showing normal histological structure of central vein (CV) with surrounding hepatocytes (H). H&E X 64.

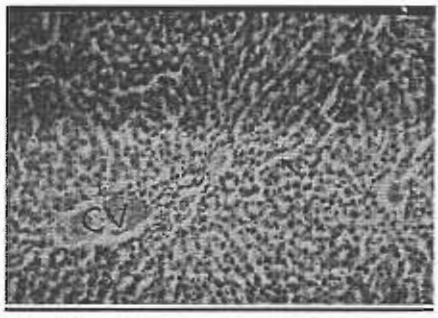

Fig. (2): Liver of rat in group (2) showing severe congestion of the central veins (CV) and diffuse proliferation of kupffer cells in between the hepatocytes. H&E X40.

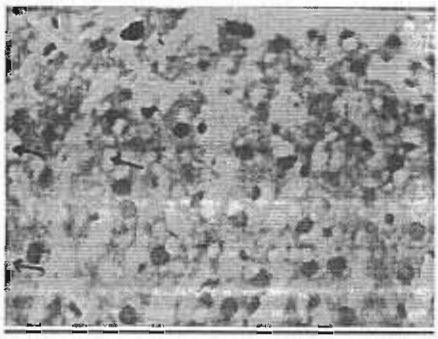

Fig. (3): Liver of rat in group (2) showing fatty change in the hepatocytes in focal manner (arrow). H&E X160.

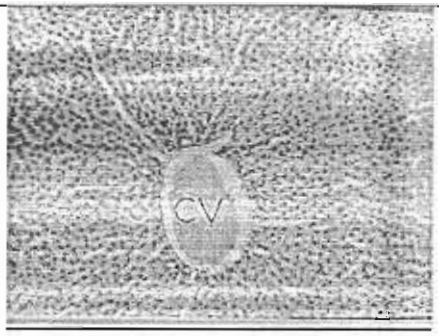

Fig. (4): Liver of rat in group (3) showing severe dilatation and congestion of the central veins (CV). H&E X40.

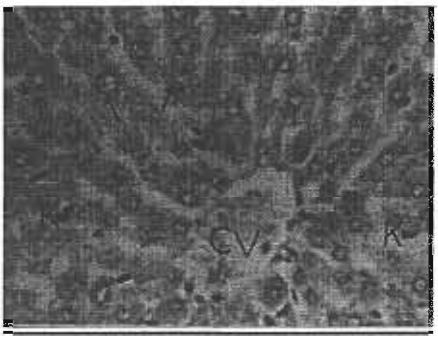

Fig.(5): Liver of rat in group (3) showing karyomegalic nuclei of hepatocytes (arrow) with kupffer Cells proliferation (K) in between. H&E X160.

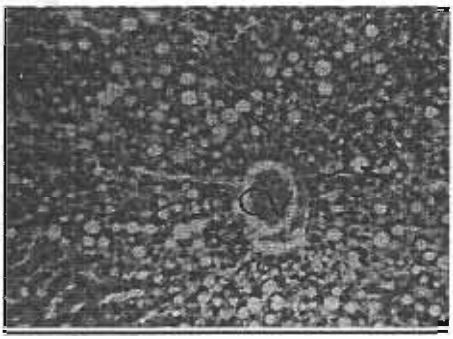

Fig. (6): Liver of rat in group (4) showing Fatty change in the hepatocytes (arrow) with severe congestion in central veins (CV). H&E X64.

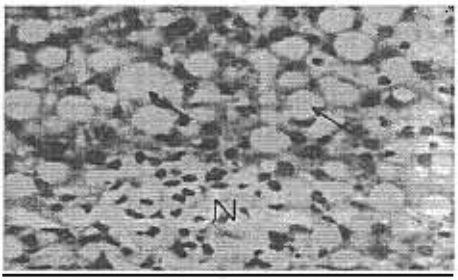

Fig. (7): Liver of rat in group (4) showing focal areas of pyknotic nuclei of the hepatocytes (N) while other had fatty change (arrow). H&E X160.

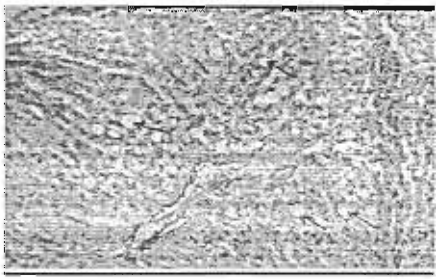

Fig. (8): Liver of rats in group (5) showing fatty change in the cytoplasm of the hepatocytes (arrows). H&E X40.

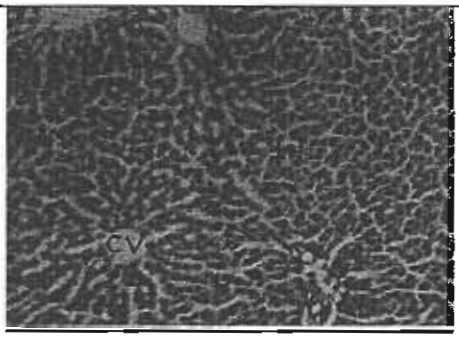

Fig. (9): Liver of rat in group (6) showing normal histological structure of the central vein (CV), portal area (P) and surrounding hepatocytes (H). H&E X40.

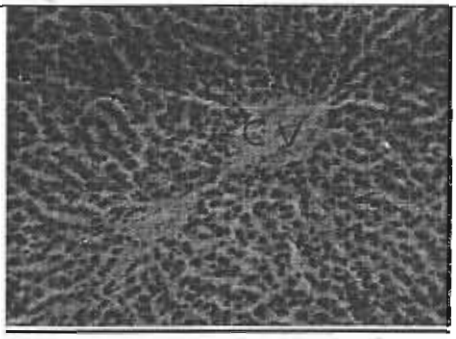

Fig. (10): Liver of rat in group (7) showing dilatation and congestion of central vein (CV) associated with diffuse proliferation of the kupffer cells (arrows) in between the hepatocytes. H&E X40.

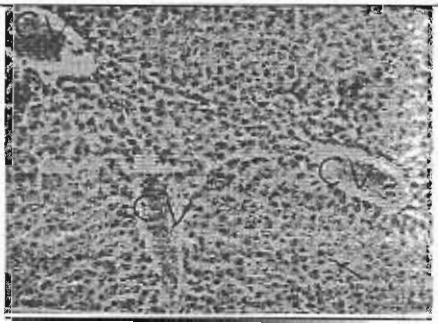

Fig. (11): Liver of rat in group (9) showing dilatation and congestion of central vein (CV) associated with diffuse proliferation of the kupffer cells (arrows) in between the hepatocytes. H&E X40.

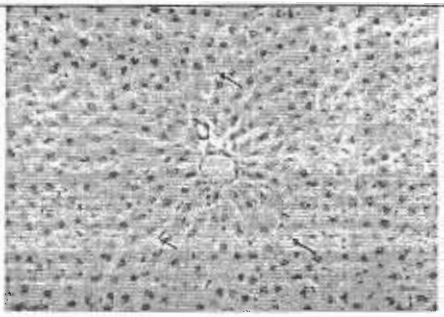

Fig. (12): Liver of rat in group (10) showing diffuse kupffer cells proliferation (arrows) in between the degenerated hepatocytes. H&E X40.

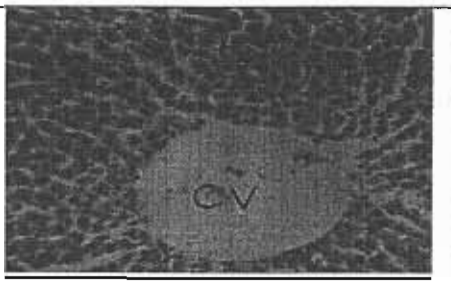

Fig. (13): Liver of rats in group (11) showing dilatation and congestion of central vein (CV). H&E X64.

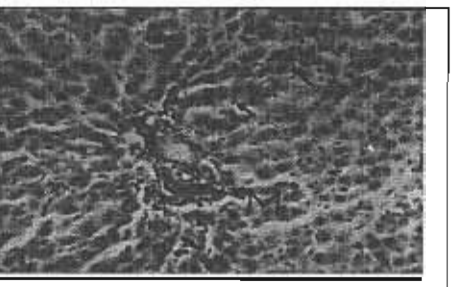

Fig. (14): Liver of rat in group (11) showing inflammatory cells infiltration in the portal area (M) and diffuse kupffer cells proliferation in between the hepatocytes. H&E X64.

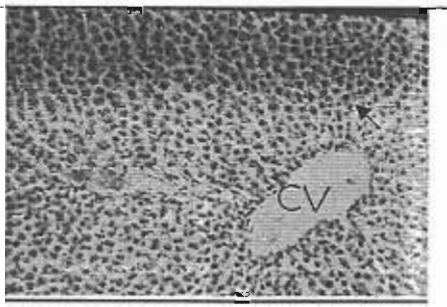

Fig. (15): Liver of rats in group (12) showing dilatation and congestion of central vein (CV) with diffuse kupffer cells proliferation in between the hepatocytes . H&E X40.

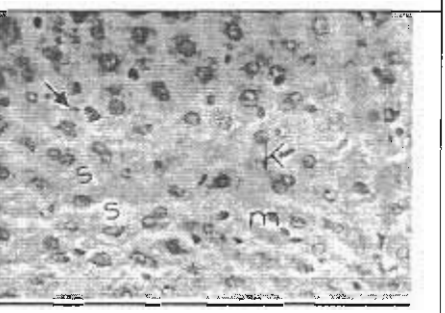

Fig. (16): Liver of rat in group (12) showing severe dilation and congestion of sinusoids (S), pyknosis of the nuclei of some hepatocytes (arrow), kupffer cells proliferation(K) and mononuclear leucocytic inflammatory cells infiltration (M) in between hepatocytes. H&E X160.

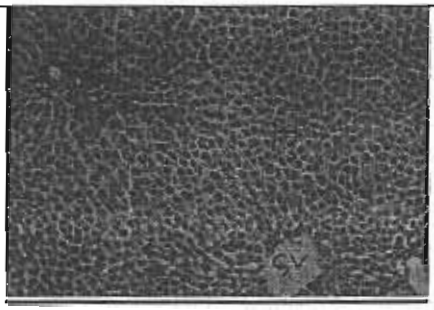

Fig. (17): Liver of rat in group (12) showing mononuclear leucocytic inflammatory cells infiltration in portal area (arrow) .H&E X40

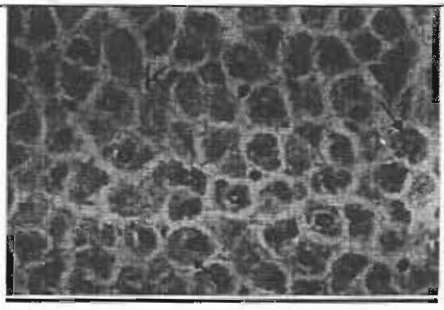

Fig. (18): Liver of rat in group (12) showing cytomegally in most of hepatocytes (arrow). H&E X160.

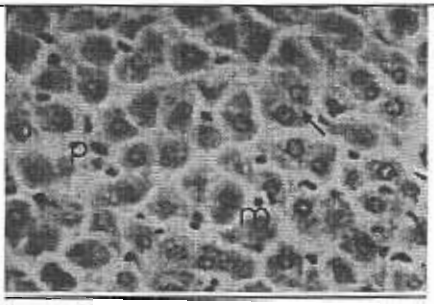

Fig. (19): Liver of rat in group (12) showing double nuclei in the cytomegalic hepatocytes (arrow) with pyknosis in other nuclei (P). H&E X160.

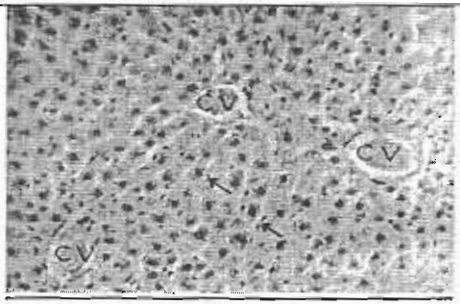

Fig. (20): Liver of rat in group (13) showing severe congestion of central vein (CV) with diffuse kupffer cells proliferation (arrows) in between the hepatocytes. H&E X64.

It can be concluded that the basal diet containing 7.5% fish oil, 2.5% animal fat, 0.25 cholesterol and 0.0625 bile salt and 10% fish oil have the beneficial effect on hyperlipidaemic patients followed by G9, G10 and G11. We can expect that if the dose of DHA or EPA or cod liver oil was increased, the improvement for these treatments will increase. Moreover, fish oil has a vital role for hyperglycaemic patients with improvement of the blood parameters and histopathological picture of the liver.

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تأثير زيت السمك والأحماض الدهنية أوميجا-3 على أملاح العظام ومكونات الدم والتغيرات الهستوباثولوجية فى الفئران المصابة بأرتفاع نسبة الكوليسترول والمصابة بالبول السكرى

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أستهدف البحث دراسة تأثير كلا من زيت السمك وزيت الذرة وحمض الديكوزاهكسانويك وحمض الأيكوزابنتانويك وزيت كبد الحوت على الفئران المصابة بأرتفاع نسبة الكوليسترول كذلك تم دراسة تأثير زيت السمك وزيت الذرة على الفئران المصابة بالبول السكرى.

تم أستخدام 65 من ذكور الفئران تتراوح أوزانها من 130±10 جرام وقد تم تقسيمها الى مجموعتين رئيسيتين:

- المجموعة الرئيسية الأولى: عبارة عن فئران مرتفعة فى نسبة الكوليسترول وعددها 50 فأر قسمت الى 10 مجموعات كل منها يتكون من 5 فئران وتم تحديد مجموعة أخرى تتكون من 5 فئران وهى المجموعة الضابطة (control).

- المجموعة الرئيسية الثانية: عبارة عن فئران مصابة بالبول السكرى وقد قسمت الى مجموعتين كل منها أحتوت على 5 فئران.

بالنسبة للمجموعة الرئيسية الأولى تم تغذيتها على تركيزات مختلفة من زيت السمك وزيت الذرة والتى كانت مدعمة بتركيزات مختلفة من الدهون الحيوانية والكوليسترول الخام وأملاح الصفراء وهى من العوامل المسئولة عن أرتفاع الكوليسترول فى العليقة.

بالنسبة للمجاميع الثلاثة الأخير- تم تغذيتها على زيت ذرة مدعم بحمض الديكوزاهكسانويك أو حمض الأيكوزابنتانويك أو زيت كبد الحوت على التوالى.

فى المجموعة الرئيسية الأولى تم اجراء التحاليل البيوكيميائية وشملت تحليل الكوليسترول الكلى والجليسريدات الثلاثية وLDL و HDL وذلك فى بداية التجربة وبعد 30 يوم وبعد 60 يوم بينما تم تحليل أنسجة الكبد وأملاح عظام الفخذ (الكالسيوم ، الفسفور والماغنسيوم) فى نهاية التجربة. فى المجموعة الرئيسية الثانية تم قياس الكوليسترول الكلى والجليسريدات الثلاثية و

LDL و HDL والجلوكوز فى الدم وذلك فى بداية التجربة وبعد 30 يوم وفى نهاية التجربة تم نزع الكبد لتحليل أنسجته.

النتائج:

أنخفاض نسبة الكوليسترول الكلى والجليسريدات الثلاثية وLDL و HDL وكذلك سلامة أنسجة الكبد فى المجموعة الرئيسية الأولى كان تام الوضوح فى المجموعة التى تغذت على 7.5% زيت سمك مضافا اليها 2.5% دهون حيوانية و 0.25% كوليسترول خام و 0.0625% أملاح الصفراء وكذلك فى المجموعة التى تغذت على 10% زيت سمك، وقد أظهرت الثلاثة مجاميع الأخيرة والتى تغذت على حمض الديكوزاهكسانويك وحمض الأيكوزابنتانويك وزيت كبد الحوت تأثير أقل من المجموعتين السابقتين وأظهرت نتائج تحليل أملاح العظام أرتفاع كلا من نسبة الكالسيوم والفسفور والماغنسيوم فى المجموعة التى تغذت على 7.5% زيت سمك والمجموعة التى تغذت على 10% زيت سمك.

فى المجموعة الرئيسية الثانية وجد ان أنخفاض سكر الدم والكوليسترول الكلى والجليسريدات الثلاثية و LDL وأرتفاع HDL وتحسن أنسجة الكبد قد أرتبط بالمجموعة التى تغذت على 10% زيت سمك وذلك بالمقارنة بالمجموعة التى تغذت على 10% زيت ذرة.

وقد تم أستنتاج الأتى:

ينصح بأستخدام زيت السمك مع المرضى المصابين بأرتفاع نسبة الدهون فى الدم أو فى الأنسجة سواء كان زيت السمك هو المصدر الوحيد للدهون فى الغذاء أو مختلط بنسبة من العوامل المسئولة عن أرتفاع نسبة الكوليسترول فى الجسم، كذلك نتوقع أنه لو أرتفعت جرعة الأحماض الدهنية مثل حمض الديكوزاهكسانويك وحمض الأيكوزابنتانويك وزيت كبد الحوت سوف يكون لها تأثير فعال أكثر، وأيضا وجد أن زيت السمك له فعال للمرضى المصابين بالبول السكرى.