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RESEARCH ARTICLE

The Effects of Varieties Sources of Omega- 3 Fatty Acids on Diabetic Male Rats

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Abstract

The aim of the present study was to investigate the effects of varieties sources of omega-3 on blood parameters, lipid profile and liver enzymes in diabetic male rats. Fish oil and flaxseed oil were used and the animals were maintained with (0.5ml/animal/day). The results indicated that the treatment of diabetic male rats with fish oil and flaxseed oil caused a significant increase ($P<0.05$) in Hb and PCV and caused a significant decrease ($P<0.05$) in WBC in compared with diabetic male rats. The results showed a significant decrease in cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and liver enzymes, whereas it explained a significant increase in high density lipoprotein (HDL) of diabetic male rats treated with fish oil and flaxseed oil when compared with diabetic male rats. The study suggested that fish oil and flaxseed oil especially may be as antidiabetic.

Keywords: *Diabetic male rats; Omega-3; Fish Oil; Flaxseed Oil; Blood parameters; Lipid profile; Liver enzyme.*

Introduction

Diabetes mellitus is considered to be a syndrome associated with disorders in the metabolism of carbohydrates, proteins and lipids caused by the absolute or relative lack of insulin [1].In the physiologic state pancreatic ß-cells respond to hyperglycemia by producing insulin. This insulin then acts via receptors at various end organs to reduce the elevated glucose levels by converting glucose to glycogen, fats and other storage forms. In patients with DM, this normal response to elevated glucose level is somehow interrupted.

This may be secondary to an inability of the pancreas to produce an adequate amount of insulin. It is also possible that the insulin that is produced is not functioning properly as a consequence of receptor malfunction or intrinsic protein alteration [2]. Omega-3 fatty acids are important for normal metabolism. Human are unable to synthesize omega-3 fatty acids, but can obtain the shorter-chain omega−3 fatty acid ALA (18 carbons and 3 double bonds) through diet and use it to form the more important long-chain omega−3 fatty acids, EPA (20 carbons and 5 double bonds) and then from EPA, the most crucial, DHA (22 carbons and 6 double bonds).

Fish and fish oil is major sources of EPA and DHA. British National Foundation recommends females and males have an intake of DHA/ EPA of 1.1 and 1.4 g/day, respectively [3].An in-creased intake of omega-3 fatty acids might reduce the risk of developing diabetes has been tested in mice, where supplementation with fish oil inhibited hyperglycemia and pancreatic insulitis in streptoztocin-induced diabetes. It is usually recommended that the long-chain Omega-3 fatty acids should come from fish.

For those who do not eat enough fish, Omega-3 supplements or functional foods with added Omega-3 fatty acids could be an alternative [4].Flaxseed, or Linseed (*Linum Usitatissimmum*) is a blue flowering rabi crop and a member of family Linaceae. Annual production of flax was 3.06 million tons and Canada is the world's largest producer of flax (about 38% of total production) [5]. Flax contains a mixture of fatty acids.

It is rich in polyunsaturated fatty acids, particularly ALA, the essential omega-3 fatty acid, and linoleic acid (LA), the essential omega-6 fatty acid. Flaxseed is alternative to plant source of omega-3 fatty acid.

It is one of the richest plant sources of omega-3 fatty acid alpha-linolenic (ALA, C18:3n-3) [6]. ALA is considered to be nutritionally essential because of its specific function as precursor of the long-chain n-3 PUFA [7]. The higher consumption of ALA was associated with higher plasma insulin and improves glucose used and efficiency [8]. The present study aimed to investigate the effects of fish oil and flaxseed oil as the different sources of omega-3 fatty acids on some helatological and biochemical parameters of diabetes male rats.

Materials and Methods

Experimental Design

The study was carried out on twenty four mature male rats (*Rattus norvegicus*), aged as 10-12 weeks and weighing between 180 - 200 gm were procured from Department of Biology, College of Science, University of Thi Qar, Iraq. The animals were housed in a well ventilated 12 hrs light and 12 hrs dark cycles. The animals were divided into four equal groups, each group consists of [6] rats:

- The first group (the negative control group) was injected with (0.5ml/animal/day) from normal physiological saline (0.9% NaCl) for 30 days.
- The second group was injected with (0.5ml/animal/day) of alloxan (125mg/kg) as a positive control.
- The third group was injected with (0.5ml/animal/day) of alloxan (125mg/kg), after week, this group was treated orally with (0.5ml/animal/day) of fish oil for 30 days.
- The fourth group was injected with (0.5ml/anima/day) of alloxan (125mg/kg), after week, this group was treated orally with (0.5ml/animal/day) of flaxseed oil for 30 days.

Induction of Diabetes Mellitus

The animals were fasted for 12 hr and diabetes was induced by a single intraperitoneal (IP) injection of alloxan monohydrated (BDH, England) dissolved in D.W at a dose of 125 mg/kg body weight in a volume of 0.5 ml. The diabetic state was confirmed 7 day after alloxan injection by the blood serum. Sugar value was greater than 200 mg/dl (hyperglycemia).

Survived rats with a fasting blood glucose level higher than 200 ml /dl were included in the study [9].

Blood Collection

After 30 days of treatment, the animals were sacrificed. Blood samples were collected by cardiac puncture, 5 ml of blood were drawn from each animal of experimental groups, 1 ml put in tubes with EDTA for hematological parameters, while 4 ml put in tubes without EDTA, then centrifuged at 3000 rpm for 15 minutes for separated of serum and kept in the refrigerator at -20^{o}C until the time of assay. The blood parameters were determined by Hematology analyzer Corporation.

Measurement of Serum Lipid Profile

The used reagents were supplied by Biolabo (France), and serum total cholesterol was measured according to Allan and Dawson, [10], and Serum TG was measured according to Tietz et *al* [11]. While serum HDL was measured according to Lopes-Virella, [12]. And measurement of LDL and VLDL according to Friedwald *et al.,* [13], LDL and VLDL concentration was measured as follows:

LDL = total cholesterol-(HDL + VLDL)

VLDL = serum TG /5

Measurement of Serum Liver Enzymes

Serum alanine transaminase (ALT) and serum aspartate transaminase (AST) were determined by enzymatic colorimetric methods using Atlas Medical (UK).

Statistical Analysis

Statistical analyses were done utilizing the computer data processing (SPSS, version 14). A probability value ($P<0.05$) was considered to be statistically significant and used to calculate least significant difference (LSD) values for the comparison of means following.

Results and Discussion

The results showed a significant decrease ($p<0.05$) in Hb and PCV, but there was a

significant increase ($p<0.05$) in WBC of diabetic male rats compared with the control group. While, the diabetic male rats treated with fish oil and flaxseed oil showed a significant increase ($p<0.05$) in Hb and PCV and a significant decrease ($p<0.05$) in WBC of the diabetic male rats compared with diabetic male rats (Table 1). These results are compatible with another study by Abbas *et al* [14]. Who found that administration of omega-3 was associated with an increase in the levels of Hb and RBC in sucrose treated rats. The results obtained in this study indicated that Omega-3 successfully maintained normal hematological values in diabetic male rats. results which reported that supplementing rats with Omega-3 showed appreciable improvement in the hematological indices as evidenced by significant increase in Hb, PCV, and RBC counts and decrease in WBC counts [15]. They have been demonstrated to be beneficial in prevention and management of many conditions such as autoimmune and inflammatory [16]. Omega 3 could be excellent adjuvant support in the therapy of diabetic metals and prevent its complications.

Table 1: Effect of fish oil & flaxseed oil on blood parameters of diabetic male rats

Animal groups	Hb (gr/L)	PCV (L/L)	Total WBC (10^3/mm^3)
First group	15.00 ±0.31^a	41.67± 1.78^a	4.58±0.45^c
Second group	10.57± 0.86^c	35.83± 0.30^b	9.75±0.51^a
Third group	13.55± 0.47^b	43.83± 1.44^a	6.83±0.60^b
Fourth group	12.67 ±0.67^b	44.50± 0.34^a	6.76±0.29^b
LSD	1.53	3.49	1.37

-Values are means ± S.E
- Different letters refer to significant differences at ($p<0.05$)
- Same letters refer to no significant differences at ($p<0.05$)

The results showed a significant increase ($p<0.05$) in the level of cholesterol and TG of the diabetic male rats compared with the control group. While, the diabetic male rats treated with fish oil and flaxseed oil showed a significant decrease ($p<0.05$) in cholesterol and TG when compared with the diabetic rats (Table 2).The results showed a significant decrease ($p<0.05$) in the serum level of HDL of the diabetic male rats compared with the control group, while the diabetic male rats treated with fish oil and flaxseed oil showed a significant increase in plasma HDL when compared with the diabetic rats. Diabetic male rats showed a significant increase in plasma LDL, VLDL when compared with control group.

Treatment of diabetic male rats with fish oil and flaxseed oil caused a significant decrease ($p<0.05$) in plasma LDL, VLDL when compared with diabetic rats (Table2). Similar results obtained by Soltan [17].Who reported that omega-3 fatty acid especially long chain polyunsaturated fatty acid (EPA, DHA) in fish oil have antidiabetic effect. Omega-3 fatty acids have potential effect to protect pancreas from up normality changes which induced in diabetic disease. The data suggested that, omega-3 fatty acid specifically long chain (EPA and DHA in fish oil) may be beneficial in diabetic disease. From these results it could be noticed that long chain polyunsaturated (fish oil) and short chain polyunsaturated fatty acid (flaxseed oil) was beneficial for diabetic disease. The different oil and EPA, DHA in fish oil decrease glucose. Observed that a higher ALA intake decrease cholesterol content in liver, the reduced hepatic cholesterol content was accounted for higher cholesterol secretion into bile thus leading to a depletion of into intrahepatic pool of cholesterol [18].The triglyceride-lowering effect of omega-3 fatty acids has been mainly ascribed to reduce hepatic synthetic of VLDL. Omega-3 fatty acid suppressed hepatic lipogenesis and reduced circulating TG level [19]. The present study, exhibited increase in HDL of different source of omega-3. The vast majority of studies conducted of treated with n-3 fatty acids have demonstrated 5% - 10% increases in HDL cholesterol levels [20].

The mechanisms for this effect are not known. Should further studies confirm this finding, the increase in HDL during treatment with fatty acids might be another antiatherogenic mechanism of these compounds. The mechanisms for the effect of omega-3 fatty acid in increase HDL are not known. That decrease in serum triacylglycerol, LDL and VLDL of all treatments. The triglyceride-lowering effect

of omega-3 fatty acids has been mainly ascribed to reduce hepatic synthetic of VLDL. Omega-3 fatty acid suppressed hepatic lipogenesis and reduced circulating TG level [19].

Table 2: Effect of fish oil & flaxseed oil **on lipid profile of diabetic male rats**

Animal groups	Cholesterol Mg/dl	T.G Mg/dl	HDL Mg/dl	LDL Mg/dl	VLDL Mg/dl
First group	59.66±0.42 ^{d}	46.16±0.42 ^{b}	52.60±0.88^{a}	38.00±0.77^{d}	12.00±0.33^{c}
Second group	80.00 ±0.67^{a}	51.83±0.35 ^{a}	34.53±0.64^{c}	46.42±0.23^{a}	20.00±0.66^{a}
Third group	67.50±0.61 ^{c}	47.33±0.54 ^{b}	42.64±0.54^{b}	40.50±0.23^{b}	16.16±1.55^{b}
Fourth group	73.37±1.65 ^{b}	46.16±0.27 ^{b}	43.42±0.54^{b}	40.64±0.20^{b}	16.00±0.65^{b}
LSD	6.0	4.0	2.0	1.22	2.18

-Values are means ± S.E
- Different letters refer to significant differences at ($p<0.05$)
- Same letters refer to no significant differences at ($p<0.05$)

The results showed a significant increase ($p<0.05$) in the level of liver enzymes of diabetic male rats compared with the control group. While, the diabetic male rats treated with fish oil and flaxseed oil showed a significant decrease ($p<0.05$) in liver enzymes when compared with the diabetic rats (Table 3). ALT and AST are elevated following tissue damage in which cellular enzymes are released from cells into the bloodstream.

AST is found in high constitutive levels in the heart and liver whereas ALT is most active in the liver. Elevations are seen following eating and osteoblastic activity, impairment of liver function and obstruction of bile flow, depressions are seen in malnutrition [21]. The protective effect of Omega-3 on liver tissue was confirmed by the attenuation of the activities of serum ALT and AST (Table 2). These results are consistent with the results of Attaia *et al* [22]. The mode of action of Omega-3 can be intercepted pharmacologically at different levels with agents that scavenge free reactive oxygen, block their generation, or enhance endogenous antioxidant capabilities.

The antioxidant and anti-inflammatory effects of Omega-3 through scavenging of free radicals and inhibiting lipid peroxidation have been reported previously by Pauwels and Kostkiewicz [23]. Omega-3 fatty acids have potential effect to protect liver from up normality changes which induced in diabetic disease.

Table 3: Effect of fish oil & flaxseed oil on liver enzyme in diabetic male rats

Animal groups	ALT(UL)	AST(UL)
First group	23.92± 1.42 ^{c}	44.00± 2.31 ^{c}
Second group	50.00± 0.57^{a}	93.21± 1.16^{a}
Third group	33.65± 0.45^{b}	66.34± 2.34 ^{b}
Fourth group	38.33± 0.45^{b}	74.50± 0.40^{b}
LSD	0.9	12

-Values are means ± S.E
- Different letters refer to significant differences at ($p<0.05$)
- Same letters refer to no significant differences at ($p<0.05$)

Conclusion

Fish oil and flaxseed oil as the different sources of omega-3 fatty acids have potential effect to protect pancreas from up normality changes which induced in diabetic disease. The data suggested that omega-3 fatty acid especially may be effective in the prevention of diabetes mellitus.

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