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Impact of flaxseed and oil extracted from Nile Tilapia waste on rats feed on high fat diet

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Abstract: The purpose of the present study was investigated the effect of flaxseed oil (FO), and Nile Tilapia waste oil (NTWO) compared with omega 3 oil (OO) on lipid profiles, and liver functions of rats fed with high fat diet. High fat diet resulted significant alterations in plasma lipids profile, and liver functions indicators.
Thirty male albino rats were used over 30 days period. The animals were divided into (5) groups, wherein groups number (1) represent control which were fed basal diet, while group number (2) was received high fat diet to serve as hyperlipidemic group. Other three groups allowed to feed high fat diet supplemented with three oils, group number (3) treated simultaneously with flaxseed oil as (FO) group, fourth group treated with Nile Tilapia waste oil as (NTWO) group while the last group treated with omega 3 oil as (OO) group, then plasma lipid profile and liver functions were detremined.
FO, NTWO and OO groups showed significantly lower levels of total cholesterol, total triglycerides, LDL-C, HDL-C, ALT, AST and total protein in comparison with the hyperlipidlemic group. It could be concluded that FO, NTWO and of course OO under study are useful for the treatment of hypercholesterolemia.
Key-Words: Flaxseed oil – high fat –HDL-cholesterol –LDL-cholesterol – Nile Tilapia waste oil.

1. Introduction

Abnormal lipid metabolism is a main cause of dyslipidemia, which is a major risk factor for cardiovascular disease, obesity and overall mortality[1]. Blood cholesterol is of great importance because blood total cholesterol (TC) and low-density lipoprotein (LDL) correlate strongly with coronary heart disease (CHD). The concentration of plasma cholesterol can be regulated by cholesterol biosynthesis, removal of cholesterol from the circulation, absorption of dietary cholesterol and excretion of cholesterol via bile and feces[2].

In liver, such lipid accumulation initially results in fatty liver that develops fatty infiltration and in chronic stages results in damage of hepatocytes, that causes gross fatty infiltration in parenchyma cells of liver[3].

Cholesterol homeostasis is maintained by a complex mechanism of sterol absorption, anabolism, catabolism and excretion[4,5]. It is well known that diet plays an important role in the control of cholesterol homeostasis.

In animal models diets which contain rich source of saturated fatty acids and cholesterol elevated all blood lipid profiles and also increased liver functions markers in plasma[6, 7, 8].

In general omega 3 fatty acid family known as best way to reduced blood cholesterol especially bad cholesterol (LDL-cholesterol) and also regulates all lipid profiles parameters[9,10].

Flaxseed oil is one of the vegetable sources of alpha-linolenic acid (ALA) and its content ranges from approximately 40% to 60% of the total fatty acids[11]. ALA, found in flaxseed oil desaturates and elongates in the human body to eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) and by itself may have beneficial effects in health and in control of chronic diseases[12].

Fish products for human consumptions include fresh and frozen, whole and fish fillet.[13] Most of the discards composed of head, intestine, skin, bones and etc. These wastes have high content of nutritive compounds like protein which is the substrate of fish meal production[14]. Fish processing by-products contain fish oil, the amount of which depends upon the fat content of the specific fish species, and the distribution of fat in fish parts. Generally, fish contains 2-30% fat, and about 50% of the body weight is generated as waste during the fish processing operation. Therefore, this fish processing by-product could be a great potential source for good quality fish oil that can be used for human consumption[15].

China is the first producer of tilapia and Egypt is the second producer (290000 tones) [16] , so that it is the most important fish waste in Egypt. The aim of this investigation is to evaluate the effect of flaxseed oil and oil extracted from Nile Tilapia fish waste on rats feed on high fat diets and compares its effect with omega 3 fatty acid source.

2. Materials And Methods

2.1. Materials

Flax seeds were purchased from the local market (Menofia, Egypt) while the Nile Tilapia waste was collected from fish stores (Cairo, Egypt), Omega 3 oil purchased from sigma company (capsules contain 400 mg EPA + DHA per gram).

2.2. Methods

2.2.1. Preparation of plant materials

Flax seeds were dried in an air-circulated oven at 40 ℃ and reduced into powder.

2.2.2. Preparation of flaxseed oil

The dried powder of the seeds was placed in a Soxhlet and subjected to extraction using petroleum ether (40–60 °C) to prepare the oil. The solvent was completely removed by evaporation under reduced pressure at a temperature not exceeding 40 °C.

2.2.3. Lipid extraction

The procedure for the lipid extraction was based on modified Kinsella method. About 50 g of fish wastes were selected randomly and homogenized in a warring blender for 2 min with a mixture of 50 ml chloroform and 100 ml methanol. One volume of chloroform (50 ml) and one volume of distilled water (50 ml) were added to the mixture and blended for 30 sec, respectively. The homogenate was filtered through a Whatman No.1 filter paper on a No.3 Buchner funnel with a slight suction and the filterate collected and transferred to a separatory funnel to allow for phase separation. The lower fraction was collected and filtered. It was then transferred to a rotary evaporator for evaporation. The sample was then collected for the fatty acid analysis[17].

2.2.4. Gas chromatography (GC) analysis of fatty acid methyl esters (FAME)

Saturated, unsaturated and total fatty acids were determined in the oil by using methyl esters boron trifluoride method[18]. FAME were identified on a Agilent Technologies 7890A GC equipped with flame ionization detector (PE Auto System XL) with auto sampler and Ezchrom integration system. Carrier gas (He); ca. 25 Psi – air 450 ml/min – Hydrogen 45 ml – split 100 ml/min. Oven temperature 200°C injector and detector 250°C.

2.2.5. Preparation of diets

Balanced and hypercholesterolemic diets were prepared as shown in table (1). The hypercholesterolemic diet was designed as reported by[19], the hypercholesterolemic diet contained 20% coconut oil, 1% cholesterol and 0.25% cholic acid.

Table (1): Composition of different experimental diets (g/100 g)

Ingredients (%)	Balanced diet (Control)	Hypercholesterolemic diet
Casein	12.5	12.5
Corn oil	10	-
Coconut oil	-	20
Sucrose	23.3	20.52
Starch	46.7	41.23
Salt mix.	3.5	3.5
Vit. mix.	1	1
Fiber	3	-
Cholesterol	-	1
Cholic acid	-	0.25

2.2.6. Design of the animal experiment

The work was carried out at the Biochemistry Department, Faculty of Agriculture, Menofia University (Egypt). To study the effect of the FO, NTWO and OO on lipid profiles and liver functions of albino rats, thirty male albino rats (weighting between 120 and 140 g) were used for this investigation. The rats were obtained from The Research Institute of Ophthalmology (Giza, Egypt). The rats were feed *ad libitum* on a basal diet (BD) and water for 15 days as an adaptation period. They were housed individually in stainless steel cages and divided into five groups of six animals.The first was the normal group where the rats received a balanced diet throughout the study period (30 days), all other remaining groups were feed a hypercholesterolemic diet. One served as a hypercholesterolemic control group, whereas the other three groups were feed a hypercholesterolemic diet along with an oral administration of a daily dose of either Flaxseed oil or Nile Tilapia waste oil or omega 3 oil as 100 mg·kg-1 rat body weight throughout the study period.

Their food intake was monitored daily and all the rats fasted before blood sampling. The blood samples were drawn from eye plexuses after 30 days. The rats were anesthetized using diethyl ether. The weight gain of the rats was recorded weekly.

2.2.7. Blood sampling and analysis

Blood samples were collected after 14 and 28 days in tubes containing heparin as an anticoagulant from the eye plexuses under diethyl ether anesthesia and then centrifuged at 3000 rpm for 20 min to obtain plasma, which was kept frozen until analysis.

The total cholesterol was analyzed according to[20]. HDL-C was determined according to[21]. According to [22] . LDL-C was calculated as the difference between total cholesterol and HDL-C. The triglycerides were analyzed according to [23]. Alanine-aminotransferase (ALT) and aspartateaminotransferase (AST) activities were measured according to the method described by [24]. Total protein was determined according to [25]. Albumin was determined according to [26].

2.2.8. Statistical analysis

The results of the animal experiments were expressed as the mean ± SD and they were analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. In all cases $p < 0.01$ was used as the criterion of statistical significance.

3. Results

3.1. Fatty acid profile of FO and NTWO

The fatty acid composition of FO and NTWO is presented in Table 2. According to the result shown, thirteen fatty acids in NTWO and seven fatty acids in FO were identified, while the analysis of FAME gave the proportion of oleic, linoleic, linolenic and palmitic as the major fatty acids. In FO the major fatty acid was linolenic acid (54.7%) while in NTWO the major fatty acid was oleic acid (29.9%). A striking feature of FO was the relatively high level of PUFA, especially linolenic acid.

Table (2): Levels of fatty acids (%) in flaxseed and Nile Tilapia Waste oil

Fatty acids	Flaxseed oil	Nile Tilapia Waste oil
Palmitic acid C16:0	5.13	19.7
Stearic acid C18:0	3.48	4.1
Oleic acid C18: 1 (n-9)	17.6	29.9
Linoleic acid C18:2 (n-6)	15.3	22.5
Linolenic acid C18:3 (n-3)	54.7	1.7
Myristic acid C14:0	nd[a]	2.5
Palmitioleic acid C16:1 (n-7)	nd[a]	4.3
Vaccinic acid C18:1 (n-7)	0.64	2.8
Gamma linoleic acid C18:3 (n-6)	nd[a]	1.4
Gadoleic acid C20:1 (n-9)	nd[a]	1.33
Arachidonic acid C20:4 (n-6)	nd[a]	1.12
Eicosapentanoic acid C20:5 (n-3)	nd[a]	0.9
Docasahexanoic acid C22:6 (n-3)	nd[a]	0.32
Non identified fatty acid	3.15	7.43
U/S[b]	10.25	2.52

[a] Not detected.
[b] Unsaturation ratio = (16:1 + 18:1 + 18:2 + 18:3 + 20:1 + 20:4 + 20:5 + 22:6)/(14:0 + 16:0 + 18:0).

3.2. Impact of FO, NTWA and OO supplementation on the plasma lipid profile

The data in Table 3 show the concentrations of different plasma lipids in all the groups. The results revealed that FO, NTWO and OO groups showed decreases in plasma triglycerides, total cholesterol, HDL-C and LDL–C in comparison with the high fat group, especially OO group appeared high significant effect compared with all treated groups.

Table (3): Effect of flaxseed , Nile Tilapia waste and omega 3 oils on lipid profile in rats feed on high fat diet

Group	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-choleserol (mg/dl)
Control	50.9 e ± 2.4	64.61 e ± 4.12	39.18 d ± 1.88	16.62 e ± 2.54
High fat	237.025 a ± 6.57	217.225 a ± 5.47	53.01 b ± 1.04	116.8 a ± 3.7
Flaxseed oil (FO)	110.34 c ± 4.95	125.85 c ± 4.44	46.455 c ± 1.77	56.97 c ± 2.72
Nile Tilapia waste oil (NTWO)	149.05 b ± 5.72	149.97 b ± 3.14	48.02 c ± 0.71	72.15 b ± 3.97
Omega 3 oil (OO)	84.72 d ± 5.74	106 d ± 4.1	64.125 a ± 1.18	24.96 d ± 2.32

Valus represent means ± S.E obtained from 6 rats

Means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.05$).

3.3. Impact of FO, NTWA and OO supplementation on liver functions

The effect of feeding FO, NTWO and OO is presented in Table 4, which explains the variation between the control and the other treated groups. It can be noticed that the levels of ALT and AST enzyme activity in the high fat group was higher compared with the other groups. The reducing effect of feeding FO, NTWO and OO compared with the high fat group can also be seen at the end of experiment. On the other hand, the high fat group had lower levels of total protein and albumin compared with the control. FO, NTWO and OO groups showed higher levels of total protein and albumin levels compared with the high fat group.

Table (4): Effect of flaxseed , Nile Tilapia waste and omega 3 oils on liver functions in rats feed on high fat diet

Group	Total protein (g/dl)	Albumin (g/dl)	ALT activity (U/L)	AST activity (U/L)
Control	5.96 a ± 0.035	3.82 a ± 0.046	41.02 e ± 1.49	45.6 e ± 4.72
High fat	5.92 a ± 0.038	3.84 a ± 0.029	74.27 a ± 1.06	74.76 a ± 1.5
Flaxseed oil (FO)	5.95 a ± 0.043	3. 855 a ± 0.045	55.6 c ± 0.63	60.52 c ± 0.6
Nile Tilapia waste oil (NTWO)	5.96 a ± 0.039	3.83 a ± 0.0125	69.45 b ± 0.7	66.025 b ± 0.94
Omega 3 oil (OO)	5.95 a ± 0.026	3.857 a ± 0.045	49.35 d ± 0.8	54.7 d ± 0.57

Valus represent means ± S.E obtained from 6 rats

Means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.05$).

4. Discussion

4.1. Fatty acid profile of FO and NTWO

The analysis of FAME in FO (Table 2) gave the proportions of linolenic, Oleic and linoleic as the major fatty acids, together comprising more than 87% of the total identified FAME. In NTWO the major fatty acid was oleic acid (29.9%) followed by linoleic acid (22.5%) and these data are in line with [27 , 28] .

The benefits of oleic and linoleic acids in reducing cholesterol levels were reported by [29] who explained the role of an unsaturated fatty acid balance when selecting food sources to replace saturated fatty acids in the diet. Thus, it can be concluded that high levels of oleic and linoleic acids in NTWO may give high nutritional values for this oil.

NTWO also include two important members from omega 3 fatty acid family, Eicosapentanoic acid (0.9%) and Docasahexanoic acid (0.32%). Many studies suggest that a dietary intake of omega 3 fatty acids may confer a protective effect against atherosclerotic disease and reduce serum triglycerides levels [30,31,32,33]. In FO the major fatty acid was linolenic (57.4%) followed by oleic acid (17.6%) which is very close to the results of [11,34] . The benefits of linolenic acid in reducing cholesterol levels were reported by [35 , 36] .

4.2. Impact of FO, NTWO and OO supplementation on the plasma lipid profile

Hyperlipidemia mainly increased the levels of cholesterol or LDL-C which is an important risk factor in the initiation and progression of atherosclerotic lesions [37]. In our study (Table 3), it can be noted that the high fat group showed high levels of triglycerides (237.025 mg/dl), total cholesterol (217.225 mg/dl), and LDL-C which were greatly increased (116.8 mg/dl) compared with the control group. On the other hand, NTWO group showed lower levels of lipid profile parameters which are in line with [38] who reported that feeding linoleic and

oleic acid (5%) decreased levels of triglycerides, total cholesterol and LDL-C. Also FO group reduced lipid profiles parameters better than NTWO group, we believe because two reasons: first it is contain a high level from linolenic acid (54.7%) this result is very close to the results of [39] who found that linolenic acid was as effective as linoleic acid and oleic acid in lowering plasma total cholesterol and LDL-C, and second it is contain high U/S ratio (10.25) compared with NTWO (2.52) this result is in line with [8] who found that high U/S ratio related with the cholesterol lowering effect in rats feed on high fat diet.

We can notice that OO group showed the best hypolipidemic effect compared with all treated groups, we think because it is contain high amount of both important omega 3 fatty acids EPA and DHA this result is very related with the result of[40 , 41] who found that fish oil reduce both total cholesterol and LDL-C.

There are several explanations about the mechanisms by which dietary fatty acids affect plasma cholesterol concentrations such as changes in lipoprotein composition [42], in LDL production [43], and in very low density cholesterol (VLDL) secretion from the liver and hepatic LDL receptor activity [44,45]. Moreover, PUFA as compared to saturated fatty acids are less efficiently incorporated into triglycerides synthesized by the liver for the export of VLDL [46,47], investigated diets rich in long chain PUFA which stimulate both gene expression and the activation of enzymes involved in beta oxidation.

4.3. Impact of FO, NTWO and OO supplementation on the liver functions.

In Table 4 the hypercholesterolemic control group showed decreases in the levels of total protein and albumin level compared with the control group. A low serum albumin indicates poor liver function. These results agree with [8,48] who reported that the high-fat diet reduced serum albumin. In addition, the hypercholesterolemic group showed elevated ALT and AST activities in plasma. [49] recorded significant increases in the serum AST and ALT activities in rabbits fed a high fat diet (35% palm oil) compared with the control group, fed a standard diet. Increases in serum activities of these enzymes are usually indicative of possible liver damage. FO, NTWO and OO oils have high contents of unsaturated fatty acids. Therefore, FO, NTWO and OO groups showed enhanced levels of ALT and AST in plasma, which may be due to the improving effect of these oils in lipid metabolism. These results agree with [8] who found that the rats in the treated groups Apricot oil and Pumpkin seed oil (which contain high amount of unsaturated fatty acids) showed significantly lower levels of alanine-aminotransferase (ALT) and aspartateaminotransferase (AST) activities as well as high levels of total protein in comparison with the hypercholesterolemic group.

4. Conclusions

FO and NTWO afforded substantial protection to diet induced hyperlipidemic disorders and these effects are mainly mediated by unsaturated fatty acid. Further research, including food nutrition studies are needed to elucidate the best ratio to add these oils to other common vegetable oils

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