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ANTIHYPERLIPIDEMIC ACTIVITY OF OILS EXTRACTED FROM TWO SUDANESE NILE FISHES IN CHOLESTEROL DIET-INDUCED HYPERLIPIDEMIC RATS

Nagwa Adam Abdulrhman Mohammed[1], Hatil Hashim EL-Kamali[2]* and Amna EL-Hassan Hamad[3]

[1]Department of Zoology, Faculty of Science and Technology, Omdurman Islamic University, Omdurman, Sudan.
[2]Department of Botany, Faculty of Science and Technology, Omdurman Islamic University, Omdurman, Sudan.
[3]Department of Pharmacology, Medicinal and Aromatic Plants Research Institute, National Research Centre, Khartoum, Sudan.

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***Corresponding author: Hatil Hashim EL-Kamali**

Department of Botany, Faculty of Science and Technology, Omdurman Islamic University, Omdurman, Sudan.

ABSTRACT

The aim of the present research study was to investigate the possible antihyperlipidemic activity of fish oils obtained from two Nile fishes: *Bagrous bagrous* and *Bagrous docmc* in cholesterol diet-induced hyperlipidemia in albino rats. Treatment with *B.bagrous* "Bayad" fish oil at (1000, 500 and 250mg/kg b. w) showed significant ($p<0.05$) change in low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglyceride (TG) level, in addition , total cholesterol(TC) at (500mg/mg b. w), in 28 days treatment when compared to the control group (GP A).Treatment with *B. docmac* "Kaparose" fish oil at (1000,500 and 250mg/kg b. w) showed significant change ($P<0.05$) in all investigated lipid parameters (except TC at a dose of 250 mg /kg b. w), in 28 days treatment when compared to the control group (GP A). In hyperlipidemic activity rats treated cholesterol diet (GP B), a significant increase in serum cholesterol, LDL-C, VLDL-C and TG was observed on 28th days, when compared to normal rats (GP A). This shows that administration of cholesterol diet induced hyperlipidemia in the present study. .Group C receiving standard drug (Atorvastatin, 0.18 mg/kg b. w) and group D to group K receiving fish oils from "Bayad" and "Kaparose" 1000 mg/kg b. w, 500 mg/kg b. w, respectively showed a significant reduction in TC, LDL, VLDL and TG on 28th days in GP F(rats treated with 250 mg/kg b. w"Bayad" fish oil) , while showed a significant reduction in TC and LDL in GP K(rats treated with 250 mg/kg b. w "Kaparose" fish oil) when compared to cholesterol group(GP B).Group F (rats treated with 250 mg/kg b. w "Bayad " fish oil) showed a significant ($P<0.05$) reduction in TC, VLDL and TG on 28th days , while group K(rats treated with 250 mg/kg b. w "Kaparose" fish oil) showed a significand ($P<0.05$) reduction in TC when compared to standard drug(Atorvastatin).

KEYWORDS: Antihyperlipidemic activity; fish oil; cholesterol diet; hyperlipidemic rats.

INTRODUCTION

Lipids are useful as energy reserves (triglycerides), insulating material to maintain the temperature, constituents of membrane structure (phospholipids and cholesterol), source for fat- soluble vitamins (A, D, E and K), cellular metabolic regulators (steroid hormones and prostaglandins) and many other functions in the body (Jain *et al.*, 2000).[1]

It has been well established that nutrition plays an important role in the etiology of hyperlipidemia. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess the hyperlipidemia related metabolic disturbances in rats (Holmgren and Brown, 1993;[2] Hozumi, 1995[3]). The elevation of lipids in plasma leads to the deposition of lipids especially cholesterol on the arterial walls, subcutaneous tissues, tendons and cornea. The important manifestation of hyperlipidemia is due to the accumulation of lipids on arterial walls and resultant pathological changes leading to atherosclerosis (Vasudevan and Sreekumani, 1995).[4]

High blood cholesterol concentration is the one of the important risk factors for cardiovascular disease. Thus the reduction in serum total cholesterol concentration effected by the fish oil is beneficial and may reduce the risk of cardiovascular disease because agents that have the ability to lower cholesterol concentration in the blood

have been reported to reduce vascular resistance by improving endothelial function (Adebayo *et al.*, 2005).[5] Therefore, the present research study was undertaken to establish scientifically evaluate the antihyperlipidemic activity of the *B.bagrous* "Byad " and *B.docmac* "Kaparose" fish oils in cholesterol diets induced hyperlipidemia model in albino rats.

MATERIALS AND METHODS

Materials

Fish samples

Fish fat samples from Nile fishes (*Bagrous bagrous* and *Bagrous docmac*), were provided from local market (Omdurman-EL-Moarda Fish Market) in March 2015.

Extraction of oil

Oil extraction was carried out according to method described by Sukhdev *et. al.* (2008).[6]

Procedures

100 g of each sample was extracted with n hexane using Soxhelt extractor apparatus. Extraction was carried out for about four hours till the colour of solvent at the last siphoning time returned colorless. Samples were then filtered using filter paper and the solvents was evaporated under reduced pressure using Rotary Evaporator Apparatus. Finally, extracts weighed, stored in dark glass container till used.

Experimental Animals

Albino Wister rats

Male rats weighing (40-70 g) were purchased from Medicinal and Aromatic Plants and Traditional Medicine Research Institute, National Center for Research. The animals were fed on standard diet (Flour 3%: Meet 1%, Salt 1%: oil 0.05%) and clean drinking water was supplied add –libitum. The animals were left 7 days for acclimatization before the beginning of the experiment. Fifty-four Wister Albino rats of either sexes were used in the present study. The animals were housed in standard metal cages at 12-hr light and dark.

Experimental Methods

Animal Treatment

The rats were randomly divided into nine main equal groups, 6 rats per each, placed in individual cages and then classified as follows: Group A(GP): normal control group: received no cholesterol no drugs. Hypercholesterolemia induced to the other groups which nominated group (B, C, D, E, and F) by oral administration of cholesterol powder in thin corn powder solution at a dose 0.5gm/kg, daily for four weeks. Group (B) received cholesterol only without treatment and serve as hypercholesterolemia control. Group (C) treated with Atorvastatin (0.18) gm/kg) orally. Group (D, E, and F) treated with (*Bagrus bagrus*) oils 1000gm/kg, 500mg/ kg and 250mg/kg respectively. Group(G,H, K) treated with (*Bagrus docmac*) oils 1000gm/kg, 500mg/ kg and 250mg/kg respectively concomitantly with cholesterol.

Blood sampling

Blood was withdrawn using the heparinized capillaries from the retro-orbital sinus in the overnight fasted animals. The serum was obtained after centrifuging the blood, which was used to estimate the concentration of biochemical parameters.

Serum Samples

After 4 weeks of the treatment period, blood samples of all groups were attained after 12 hr fasting for serum separation by ocular vein puncture in dry clean and screw capped tubes .Serum were separated by centrifugation at 2500 r.p.m for 15 mins. The clean, clear serum was separated by automatic pipette, received in dry sterile samples tube, and kept in deep freeze at-20 ° C until used for subsequent biochemical analysis. All sera were analyzed for **(**Total cholesterol (TC), high density lipoprotein- cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and triglycerides (TG).

Determination of serum lipoprotein

To determine the cholesterol (CH), high-density cholesterol (HDL) and triglyceride (TG) contents of the blood samples. Serum was separated from the blood and then placed in ebendorve tube in a refrigerator and then rotated or clean samples were analyzed by Jenway 6305.Uvlvis-Spectrophetometer, serial NO2128. SQ4802 UV/VIS Spectrophotometer, 110V–220V UNICO SQ–4802 (Double beam design).

Serum biochemical parameters

The biochemical parameters (HDL, LDL, VLDL, TC and TG) have been investigated in serum after one month of treatment with atorvastatin and fish oil concomitantly with cholesterol as following:

Serum total cholesterol (TC)

Total cholesterol content was determined according to the method described by Young (2000).[7]

Serum high-density lipoprotein (HDL)

Serum high-density lipoprotein content was determined according to the method described by Young (2000).[7]

Serum low-density lipoprotein (LDL)

Serum low-density lipoprotein content was determined according to the method described by Young (2000).[7]

Serum very low-density lipoprotein (VLDL)

VLDL=Triglycerides (TG) mg/dl)/5.

Serum triglyceride (TG)

The triglycerides content was determined according to method describe by Bucolo and David (1973).[8]

Statistical analysis

Data were analyzed by Statistic Package for Social Science (SPPS-Version 16) computer system. The mean and standard error of variables were calculated to obtain t and P values for the test groups, compared with the

control group (P value <0.05) was considered significant of standard.

RESULTS AND DISCUSSION

The effect of cholesterol alone, Atorvastatin, fish oils from *Bagrous bagrous* and *Bagrous dogmac* on lipid profile in albino rats

The effect of cholesterol alone, Atorvastatin, fish oils at (1000 mg/kg b w), (500 mg/kg b w) and (250 mg/kg b w) from *B.bagrous* and *B. dogmac* on lipid profile in albino rats compared to respective control (GP A) when administrated daily for 28 days are reported in Table 1.

The effect of cholesterol alone (GP B) on Lipid profile in Albino rats

The effect of cholesterol alone (GP B) on Lipid profile in Albino rats after treatment for 28 days are shown in Table 1. Treated rats for 28 days (Cholesterol alone) caused significant change at P<0.05 in the TC, LDL, VLDL and TG values relative to their control ,while this rats group showed no significant change at P<0.05 in HDL value relative to respective control.

The effect of Atorvastatin (GP C) on lipid profile in Albino rats

The effect of Atorvastatin (GP C) on Lipid profile in Albino rats after treatment for 28 days are shown in table 1. Treated rats with Atorvastatin (0.18 mg /kg b. w) caused significant change at P<0.05 in the TC, LDL, VLDL and TG values relative to their control. Treatment with the reference standard drug, atorvastatin, effectively prevented the increase in the various lipid levels. Treatment of hyperlipidemic rats with fish oils at different doses (250 mg /kg, 500 mg /kg and 1000 mg /kg) showed a significant increase of serum LDL, VLDL and TG.

The effect of *B.bagrous* fish oils on lipid profile in Albino rats

Treatment of rats (GP D) with *B. bagrous* fish oil (1000 mg /kg b.w) after 28 days caused significant differences at P<0.05 in the LDL, VLDL and TG values relative to their control , while this rats group (GP D) showed no significant change at P<0.05 in TC and HDL values relative to control group. Treatment of rats (GP E) for 28 days with *B.bagrous* fish oil (500 mg /kg b.w) caused significant difference at P<0.05 in all investigated lipid parameters (except HDL)in relative to their control.

The TC and HDL of rats treated with (250 mg /kg b.w)(GP F) of *B.bagrous* fish oil were non significantly different at P<0.05.

The LDL, VLDL and TG of rats treated in (GP F) were significantly different at P<0.05 after the end day of experiment (Table 1).

The effect of *B.docmac* fish oil on lipid profile in Albino rats

Treatment of rats (GP G) with *B.docmac* fish oil (1000 mg /kg b.w) and rats in (GP H) with *B. docmac* fish oil (500 mg /kg b.w) did produce significant difference at P<0.05 in all investigated lipid parameters in relative to their control. Treatment of rats for 28 days with *B.docmac* fish oil (250 mg /kg b.w) caused significant difference at P<0.05 in all investigated lipid parameters (except TC) in relative to their control (Table 1).

The effect of *Bagrous bagrous* fish oil on lipid profile in Albino rats compared to cholesterol (GP B)

Treatment of rats (GP D) with *B.bagrous* fish oil (1000 mg /kg b. w) and rats in (GP E) with (500 mg /kg b. w) did not produce significant difference at P<0.05 in all investigated lipid parameters compared to cholesterol group (GP B), whereas treated rats (GP F),(250 mg /kg b. w) fish oil did produce significant difference at P<0.05 in HDL compared to rats in (GP B).(Table 1).

The effect of *B.docmac* fish oil on lipid profile in Albino rats compared to cholesterol (0.18 mg/kg b.w),(GP B)

Treatment rats group (GP G) with *B.docmac* fish oil (1000 mg/kg b.w) caused significant difference at P<0.05 in TC value in comparison with cholesterol group (GP B). Treated rats for 28 days with *B.docmac* fish oil at (500 mg/kg b.w) and (250 mg/kg b.w) caused significant change at P<0.05 in the HDL value compared to cholesterol group (GP B).

The effect of *Bagrous bagrous* fish oil on lipid profile in Albino rats compared to Atorvastatin group (GP C)

Treatment of rats (GP D) for 28 days with *B.bagrous* fish oil (1000 mg /kg b.w) did not caused significant difference at (P<0.05) in all investigated lipid parameters compared to atorvastatin(GP C). Treated rats for 28 days with *B. bagrous* fish oil (500 mg /kg b.w) caused significant change at (P<0.05) in the TC value compared to atorvastatin (GP C). A significant increase in HDL and decrease in VLDL and TG concentration in rats in group F treated with *B.bagrous* fish oil (250 mg /kg b.w) and non-significant decrease in TC and LDL when compared to atorvastatin (Table 1).

The effect of *B.docmac* fish oil on lipid profile in Albino rats compared to Atorvastatin group (GP C)

No significant change in activity between Atorvastatin group and treated rats (GP G) was occurred in HDL, LDL, VLDL and TG activity, while TC activity showed significant increase in rats group G. The *Bagrous docmac* fish oil (500 mg /kg b. w),(GP H) and 250 mg (GP K) showed no significant changes in TC concentration as LDL,VLDL and TG concentrations when compared to Atorvastatin (Table 1), while it significantly increase at P<0.05 serum HDL cholesterol concentration when compared to Atorvastatin (GP C). Table 1.

The effect of cholesterol alone (GP A) on lipid profile in Albino rats compared to Atorvastatin (GP C)

No significant change at P<0.05 in activity between cholesterol alone rats group (GP B) and Atorvastatin rats group (GP C) was accrued in all investigated lipid parameters. Table 1.

Table 3: The effect of cholesterol (0.05 mg/kg), Atorvastatin (0.18 mg/kg), *B. bayad* Fish oil (1000 mg, 500 mg and 250 mg/kg b w) and *B.docmac* Fish oil (1000 mg, 500 mg and 250 mg/kg b w) on Lipid profile in Albino rats.

Lipid Profile	GP A (Control Normal)	GP B cholesterol (0.05 mg/kg)	GP C Atorvastatin (0.18 mg/kg)	GP D B.bayad (1000 mg/kg)	GP E B.bayad (500 mg/kg)	GP F Bayad (250 mg/kg)	GP G B.docmac (1000 mg/kg)	GP H B.docmac (500 mg/kg)	GP K B.docmac (250 mg/kg)
TC	55.67±4.10	74.67±4.11*	72.50±2.98*	83.5±8.3	85.00±5.89*-***	63.83±5.81*	86.33±5.25*,**,***	79.00±7.03	58.53±11.47
HDL	24.83±2.49	21.83±1.94	23.55±2.34	24.33±2.41	27.03±4.16	32.82±4.63**, ***	26.92±2.74*	38.42±2.19**,***	
LDL	6.90±2.76	23.33±3.72*	16.52±1.56*	23.82±6.02*	17.07±1.93*	16.45±1.59	16.73±1.73*	20.13±3.37	17.92±4.08
VLDL	5.73±0.62	19.53±1.85*	19.03±1.40*	20.97±2.88*	19.6±1.12*	14.23±2.28***	22.4±3.83*	27.45±5.98	19.73±5.76
TG	28.67+3.11	97.66±9.26*	95.17±7.02*	104.83±14.40*	98.00±5.62*	71.17±11.41***	112.00±19.14*	137.33±29.87	98.67±28.82

Values expressed as mean ± SE, N=6 (rats), *Significant compared to control (GP A), ** Significant compared to cholesterol (GP B) and *** Significant compared to Atorvastatin (GP C).

TC=total Cholesterol (mg/kg), HDL=High density Lipoprotein (mg/kg), LDL=Low Density Lipoprotein (mg/kg), VLDL=very Low Density Lipoprotein (mg/kg), TG=Triglycerides (mg/kg).

CONCLUSION

The lipid profile of albino rats was improved by injection of minimum dose of both fish oils. The *Bagrous docmac* oil showed better results in elevating HDL-C level than the *B.bayad* fish oil. The dose 250 mg/Kg b.w in *B.bayad* decreased TC, VLDL and TG and raised HDL which means that it showed a good effect when compared to the *B.domac*. The improve in lipid profile may be attributed to omega fatty acids found in the fish oil. Further studies can be conducted to investigate the effect of small doses of fish oils on parameters of plasma cholesterol in rats. Further pharmacological and toxicological studies should be carried out on fish oils of candidate fish species to assess its safety. The finding of this study could provide satisfactory preclinical evidence of safety to launch clinical trial on standardized formulation of fish oils.

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