EFFECT OF DIETARY FISH ON THE LIPID PROFILE OF CHOLESTEROLSTRESSED RATS DURING COPPER OVERLOAD

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ABSTRACT

The effect of dietary supplementation with Oil sardine (*Sardinella longiceps*) fish at 2/3 fat replacement, on serum lipids in the cholesterol-stressed copper loaded rats was studied. Hypercholesterolemic state observed during copper toxicity was not alleviated by sardines. Copper loading significantly decreased the triglyceride levels and the activity of β -hydroxy β -methyl glutaryl (HMG) CoA reductase. Fish supplementation was further effective in reducing the triglyceride levels in copper loaded rats. Significant increase in the serum phospholipid was observed in the fish supplemented rats during copper overload. The finding suggests that hypolipidemic potential of fish is certainly altered to some extent in copper toxicity since, it is well known that copper enhances the formation of free radicals. Thus it may be concluded that sardine fish is not effective in reducing the cholesterol levels during copper toxicity.

KEY WORDS : Sardine fish, cholesterol-stress, copper

INTRODUCTION

The hypolipidemic effect of marine oils is largely due to the abundant supply of polyunsaturated fatty acids. *Sardinella longiceps* the most abundantly available fish in our country has been shown by Narasimha Rao et. al. (1) to have pronounced hypolipidemic effect in humans. The hypocholesterolemic effect of *Sardinella longiceps* in cholesterol-stressed rats has been reported (2). Recent studies have shown that hypercholesterolemia produces superoxide anion (3). Presence of transition metals enhance the formation of free radicals. Among the transition metal ions which can potentially participate in the activation of oxygen related reactions, copper and iron are the most likely redox active candidates because of their abundance in the biological system. Levels of both these metals are reported to be increased in the atherosclerotic

arterial walls compared to the healthy ones. Epidemiological studies have shown a positive association between serum copper and risk for cardiovascular diseases. High susceptibility of the PUFA to peroxidation prompted us to evaluate the hypolipidemic potential of the n-3 PUFA rich sardine fish in cholesterol-stressed rats in the presence of copper loading. Earlier studies have shown that copper deficiency produces hypercholesterolemia. Information regarding the lipid status in the presence of copper overload is very meagre. The objective of the present study was to assess the influence of *Sardinella longiceps* on the hypolipidemic potential of cholesterol -stressed rats in the presence of copper loading. Fish supplementation was done on isocaloric basis.

MATERIALS AND METHODS

Male albino rats of Wistar strain weighing about 100g were obtained from the Central Animal House of Kasturba Medical College Mangalore. They were housed in individual polypropylene cages

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throughout the experimental study. The animals were fed on basal diets essentially similar to that of Peifer (4). All the animals were put on 'test diets' only after pre-conditioning them with 'basal diets' for a period of three weeks. Test diets were also given for a period of 3 weeks. Water was given *ad libitum*.

Study design : Two groups of rats (A and B) each of seven were fed the basal diet containing 14% hydrogenated vegetable oil & 1% refined groundnut oil for a period of 3 weeks (pre-conditioning). Another two groups of rats (C and D) of seven each were fed basal diet containing 0.5% cholesterol and 0.5% bile salt for a period of 3 weeks. The composition of the basal diet given to group A rats was as follows: Casein 18.0%; sucrose 59%; hydrogenated vegetable oil 13.9%; refined ground nut oil 1.0%; cellulose 5.0%; salt mixture 2.0%; vitamin mixture 1.0%; vitaminized oil 0.1%. The vitaminized oil contained vitamin A (2000 IU), vitamin D (100 IU), and vitamin E (100 IU) / gm of oil. (Vitamin E was not added to groups B and D). It may be noted that the basal diet used in the study was atherogenic since the sucrose content is of the order of 59%. Since the diet given to the cholesterol-stressed rats (groups C and D) was supplemented with 0.5% cholesterol and 0.5% bilesalt, cellulose content was accordingly reduced to 4.0%. At the end of the pre-conditioning period 'test diets' were given to the rats of B and D groups. Test diets were supplemented with sardine fish at two-third fat replacement level, of the dietary lipids isocalorically. Daily requisite quantity of sardine from a canned pack was mixed with the corresponding basal diet as mentioned in our earlier communication (5). 20g of the appropriate basal diet (dry wt basis) was given per day for each rat. This served as the control group (Group I)

To test the hypolipidemic effect of sardine fish in the presence of copper loading another lot of rats (Group II) was maintained on a similar diet. Each rat was administered 3 mg Cu⁺⁺ /Kg body wt (6) in the form of CuSO₄ intraperitoneally for 4 successive days towards the end of the test period. At the end of the test period the animals were sacrificed under light ether anesthesia. They were bled by cardiac puncture and the blood was collected using heparinised syringes. It was centrifuged at 3000 rpm for 10 min. Plasma was

collected. Liver was quickly excised off, and washed in cold saline. It was immediately suspended in saline arsenate solution for the estimation of β -hydroxy β -methyl glutaryl (HMG) CoA reductase activity. Plasma total cholesterol (Tc) and HDL cholesterol (HDL-c) was estimated by the CHOD-PAP method (7-9). Estimation of triglycerides (TG) was done by the GPO-PAP method (10). Method of Connerty (11) was used for the estimation of phospholipids (PL). HMG CoA reductase activity in the liver was estimated by the method of Venugopal Rao and Ramakrishnan (12).

Statistical Analysis: This was performed using Analysis of Variance (ANOVA). The critical value for the statistical significance has been assumed to be 5% level. Dunnet's test was used only in cases where the treatment showed significance in the ANOVA. To test for the difference between the copper treated groups, the unpaired 't' test for difference in means was used.

RESULTS

As given in Table 1, significant decrease in cholesterol (82.96 ± 6.92) was seen in cholesterol stressed fish supplemented animals without metal treatment. Significant increase in cholesterol was seen in copper treated fish fed animals (77.01 ± 4.46) compared to the animals without metal treatment (53.42 ± 7.60). Similarly an increase in cholesterol was also seen in the copper treated cholesterol-stressed animals which were supplemented with fish (138.17 ± 12.54) versus control value of (82.96 ± 6.92). Lowest level of HDL-c was seen in the cholesterol stressed group (C) of both control and copper treated animals (32.45 ± 3.74 and 29.60 ± 4.20). Decrease in TC/HDL-c ratio (1.40) was observed in group D control animals, unlike the test group which was loaded with copper (3.07)

In the control group without metal treatment HMG CoA: mevalonate ratio was highest in the cholesterol-stressed rats (10.79 ± 2.08). Fish feeding to this group resulted in derepression of the enzyme with a decrease in ratio (6.10 ± 0.79).

Table 1. Plasma total cholesterol, HDL cholesterol and liver HMG CoA reductase activity in copper treated rats

Without metal (Group I)				Copper treated (Group II)			
TC mg/dl	HDL-c mg/dl	TC/ HDL-c	HMGCoA: Mevalonate	TC mg/dl	HDL-c mg/dl	TC/ HDL-c	[†] HMGCoA: Mevalonate
A 68.21±7.03	52.90±7.03	1.28	6.68±1.09	75.96±6.42	38.78±6.81	1.95	11.02±3.21 ^a
B 53.42±7.60	42.85±2.57	1.24	7.78±1.40	77.01±4.46 ^{**}	45.72±9.37	1.68	6.51±1.74 [*]
C 126.10±39.70 [*]	32.45±3.74 [*]	3.88	10.79±2.08 [*]	118.45±10.43 [*]	29.60±4.20	3.99	10.65±2.31
D 82.96±6.92 ^b	59.02±8.20 ^c	1.40	6.10±0.79 ^c	138.17±12.54 ^{**ab}	45.01±2.87	3.07	8.74±0.96

[Values are mean ± SE from 7 rats in each group] [†]Ratio is inversely proportional to the activity of the enzyme. ^{**bca} : P< 0.05, A Vs B, C, D: ^{*}, B Vs D : ^b, C Vs D: ^a, Vs corresponding control without metal treatment: ^a

A — Basal diet , B — Basal diet + fish , C — Basal diet + cholesterol, D — Basal diet + cholesterol + fish

Table 2. Plasma triglycerides and phospholipids in copper treated rats.

Without metal (Group I)		Copper treated (Group II)	
Triglyceride (mg/dl)	Phospholipid (mg/dl)	Triglyceride (mg/dl)	Phospholipid (mg/dl)
A 269.45 ± 23.24	42.63 ± 8.95	134.91 ± 12.02 ^a	59.76 ± 9.97
B 132.51 ± 19.50 [*]	44.24 ± 8.03	96.21 ± 18.52 [*]	79.02 ± 12.01 ^a
C 290.80 ± 31.48	82.14 ± 23.20 [*]	244.47 ± 62.28 [*]	107.58 ± 22.83 [*]
D 177.53 ± 12.47 ^{*c}	48.12 ± 6.16	114.40 ± 11.53 ^{ac}	93.83 ± 20.01 ^{ba}

[Values are mean ± SE from 7 rats in each group] ^{* bca} : P< 0.05, A Vs B, C, D: ^{*}, B Vs D : ^b, C Vs D : ^c, Vs corresponding control without metal treatment : ^a

A-Basal diet , B- Basal diet + fish , C - Basal diet + cholesterol, D- Basal diet + cholesterol + fish

In the copper loaded group ratio of HMG CoA: mevalonate was highest in group A. Fish supplementation resulted in derepression of the enzyme with decrease in the ratio (6.51 ± 1.74).

As given in Table 2 highest level of triglycerides (290.80 ± 31.48) is seen in the cholesterol-stressed animals without metal treatment. Copper treated control animals showed a decreased value of 134.91 ± 12.02 against the value of 269.45 ± 23.34 seen in corresponding control group without metal treatment. Fish supplementation resulted in a decrease in the triglyceride levels in both control and copper treated animals (B and D). In the control group (without metal) highest level of phospholipid (82.14 ± 23.20) was observed in the cholesterol-stressed rats (C). Fish supplementation resulted in a significant reduction of PL (48.12 ± 6.16). Fish supplementation in the copper loaded animals resulted in significant increase in PL (79.02 ± 12.01 and 93.83 ± 20.01).

DISCUSSION

From the results (Table 1) it is clear that hypocholesterolemic effect is more when dietary fish supplementation to cholesterol-stressed rats was made. This indicates that fish is more beneficial in relation to hypercholesterolemic state. Copper toxicity resulted in a significant increase in total cholesterol (Tc) in fish fed control animals. This observation is interesting since available literature indicates that hypercholesterolemic state is observed during copper deficiency (13). Hypercholesterolemic state observed in fish fed animals during copper overload may be due to the interference of copper to the action of PUFA. It is possible that esterification of cholesterol with PUFA does not take place in the presence of Cu resulting in hypercholesterolemia. Oxidation of PUFA by copper might have taken place making it unsuitable for esterification. Fish feeding to cholesterol stressed rats increased the HDL-c levels and thus a decrease in TC/HDL-c ratio is seen (Table 1). TC/HDL-c is considered to be an important atherogenic risk factor. Improvement in the TC/HDL-c observed

in cholesterol-stressed, fish supplemented animals (14) is in agreement with another study (15). But this beneficial effect is masked during copper toxicity.

Cholesterol feeding suppressed the HMG CoA reductase activity in the control group. Fish feeding to cholesterol-stressed rats resulted in derepression of the enzyme in the control group where as no significant change occurred in copper loaded animals indicating that the activity of HMG CoA reductase is not inhibited during copper overload. Another important observation is the increased ratio of HMG CoA : mevalonate and thus decreased activity of HMG CoA reductase in copper treated control animal.

Highest level of TG in the cholesterol-stressed groups without metal treatment is consistent with the earlier studies. Fish feeding has profound hypotriglyceridemic effect in the cholesterol-stressed as well as the cholesterol unstressed rats. Significant reduction is seen in the copper treated group A. This reduction may be possibly due to the enhanced activity of lipoprotein lipase. Fish feeding has further reduced the triglyceride levels. The most significant observation made is that, fish supplementation was very effective in bringing a hypotriglyceridemic effect even in the presence of copper overload. There is unequivocal agreement on the remarkable hypotriglyceridemic effect of fish & fish oils rich in n-3 polyunsaturates. The mechanism of triglyceride lowering appears to be the inhibition of secretion of VLDL triglycerides by n-3 PUFA (16). It is opined that n-3 PUFA's might interfere with synthesis of triglycerides in the liver (17). The fact that significant hypotriglyceridemic effect is seen in fish fed cholesterol-stressed animals in the presence of copper overload indicates that beneficial effect of fish is not affected during copper toxicity. It may have implications and opens up scope for an extensive study.

In the control group, while fish feeding does not affect the serum phospholipid level to any significant extent cholesterol feeding does increase its concentration indicating the stimulation of

phospholipid synthesis by cholesterol. However marked decrease was obtained when fish was given to cholesterol-stressed rats. It may be inferred that as with other lipid parameters fish feeding resulted in lowering of serum PL in cholesterol stressed rats. This indicates that rapid turnover of phospholipids is brought about by PUFA of fish when the experimental animals are under cholesterol stress. Fish supplementation to both cholesterol free as well as cholesterol stressed rats resulted in significant increase in phospholipid during copper toxicity. The underlying mechanism is not known but the finding would have relevance in acute copper

poisoning and in women using contraceptives. Establishment of the mechanisms involved in various functions of phospholipids would give a better idea on their turnover rate under various dietary and physiological states.

The isocaloric replacement of fat with sardine fish in the presence of cholesterol- stressed copper overloaded rats shows hypercholesterolemic and hypotriglyceridemic effect indicating disturbance in the overall lipid metabolism. It is concluded that sardine fish is not beneficial in reducing the cholesterol levels during copper toxicity.

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