EFFECT OF VARIOUS VEGETABLE OILS ON THE LIPID PROFILE AND ANTIOXIDANT STATUS IN HYPERCHOLESTEROLAEMIC WISTAR RATS- A COMPARATIVE STUDY

Prasad Ravindra Manjeshwar[1], Urban D'Souza[2], Shankar Bhat[3], Kumar Megur Ramakrishna Bhat[4], Nayanatara Arunkumar[5]

[1]Assistant Professor, Department of Biochemistry, A. J. Institute of Medical Sciences, Mangalore.
[2]Professor, Department of Physiology, KVG Medical College, Sullia.
[3]Professor, Department of Physiology, Yenepoya Medical College, Deralakatte, Mangalore.
[4]Professor, Department of Anatomy, Ras al-Khaimah College of Medical Sciences, Ras al-Khaimah, UAE.
[5]Associate Professor, Department of Physiology, Kasturba Medical College, Mangalore.

ABSTRACT

BACKGROUND
Various vegetable oils are used for cooking foods in India. Controversies have been created that consumption of certain vegetable oils cause atherogenesis. A little is known about the effect of vegetable oils in hypercholesterolaemic conditions. Hypercholesterolaemia, mainly the increased plasma Low-Density Lipoprotein (LDL) cholesterol levels and Reactive Oxygen Species (ROS) has been implicated in the early development and progression of atherosclerosis and Coronary Heart Diseases (CHD). Current study is designed to assess the effect of various vegetable oils such as coconut, sunflower, palm, olive oil and vanaspati on lipid profile and oxidative stress parameters in rats fed on a high-cholesterol diet.

MATERIALS AND METHODS
Hypercholesterolaemia is induced by supplementing cholesterol with the basal diet. Reference dose of various vegetable oils were administered once daily for 90 days. After the treatment period of 90 days, triacylglycerol, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol and oxidative stress parameters are estimated and analysed.

RESULTS
In the present study, we observed the lipid-lowering effect of various vegetable oils in rats fed with high-cholesterol diet. Administration of cholesterol showed increased level of lipid profile. Concurrent administration of various vegetable oils with high-cholesterol diet caused a significant decrease in serum total cholesterol, LDL and VLDL cholesterol. This conclusion is made based on the observation that the vegetable oils were able to restore, at least partially, the lipid profile of hypercholesterolaemic rats. A decline of antioxidant status associated with an increase in lipid peroxidation was observed in all the vegetable oil treated groups. Among the oils, coconut oil showed a mild increase in High-Density Lipoprotein (HDL) and least increase in lipid peroxidation compared to other vegetable oils treated groups.

CONCLUSION
Results suggest that the addition of vegetable oils showed a positive influence on lipid metabolism in hypercholesterolaemic rats. Among the vegetable oils, coconut oil was found to be beneficial as it has more antioxidant property. These findings can provide useful information regarding the choice of vegetable oils in hypercholesterolaemic patients in future.

KEYWORDS
Hypercholesterolaemia, Atherosclerosis, Lipid Profile, Antioxidants, Oxidative Stress, Vegetable Oils.

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BACKGROUND
Coronary Heart Diseases (CHD) is the cause of majority of the deaths (about 25-30%) in most industrial countries.[1] Hypercholesterolaemia, mainly the increased Low-Density Lipoprotein (LDL) cholesterol is the predominant risk factors in Coronary Heart Diseases (CHD).[2] Coconut oil has been used as a cooking medium in coastal parts of south India for a long time. Sunflower oil, palm oil, olive oils are being the other vegetable oils used. Saturated fats are known to cause hypercholesterolaemia,[3] a predominant risk factor in atherogenesis and consequent Coronary heart diseases (CHD). Coconut oil contains approximately 90% saturated fats.[3] So, a panic has been developed in the public that consumption of coconut oil causes atherogenesis. However, most of the saturated fats in coconut oil are mainly short chain fatty acids (lauric acid - 43-51%) and medium chain fatty acids (myristic acid - 16-21% and capric acid - 6-8%).[4] These are mainly transported through the portal venous

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Corresponding Author:
Prasad Ravindra Manjeshwar,
Assistant Professor, Department of Biochemistry,
A. J. Institute of Medical Sciences,
Mangalore - 575004, Karnataka, India.
E-mail: prasadrm@yahoo.com
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system to the liver. In hepatic cells, these medium chain fatty acids do not require carnitine acyltransferase for their transport into the mitochondria for their oxidation. So, these medium chain fatty acids are more available for beta-oxidation and are considered to be less implicated in accumulation in body lipids. In addition, analysis shows that none of the saturated fatty acids that found in atheroma were reported to be lauric acid and myristic acid.[5] Low-Density Lipoprotein (LDL) cholesterol, more importantly, oxidised LDL play an important role in atherogenesis. Conversion of LDL to oxidised LDL depends both on concentration of pro-oxidant stimuli and the concentration of antioxidants.[6] Present work is designed to study the role of coconut and other vegetable oils on lipid profile and oxidative stress parameters in hypercholesterolaemic male Wistar rats.

AIM AND OBJECTIVE
The aim and objective of the present study is to compare the effect of different vegetable oils in lipid profile, oxidative stress and the development of atherogenesis on hypercholesterolaemic male Wistar rats.

MATERIALS AND METHODS
Animals
48 male Wistar rats weighing about 120-150 grams were obtained from Central Animal Facility of Kasturba Medical College (Manipal University), Manipal. They were housed in Institutional Experimental Animal Laboratory. The rats were kept in cages at room temperature. They had free access to food and water. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and approved by Institutional Animal Ethics Committee.

Experimental Design
Animals were divided into 7 groups (control group n=12 and n=6 in all other groups).
Group 1- Rats fed with normal chow diet (control).
Group 2- Rats fed with High-Cholesterol Diet (HCD).
Group 3- Rats fed with HCD + coconut oil (2 mL/day).
Group 4- Rats fed with HCD with olive oil (2 mL/day).
Group 5- Rats fed with HCD with palm oil (2 mL/day).
Group 6- Rats fed with HCD with sunflower oil (2 mg/day).
Group 7- Rats fed with HCD with vanaspati (2 mg/day).

Induction of Hypercholesterolaemia[7,8]
Cholesterol powder was purchased from HiMedia Laboratories and supplemented to the basal diet (1%) of rats to induce hypercholesterolaemia.[7,8]

All the oils were administered orally using gavage once daily for ninety days. At the end of the experiment, the animals were euthanized after 12 hrs. of fasting. Blood were collected by cardiac puncture in a Vacutainer. The serum was separated by centrifugation at 3000 rpm for 10 minutes and lipid profile was estimated. Triacylglycerol (TAG), Total Cholesterol (TC) and High-Density Lipoprotein (HDL) cholesterol were determined in serum samples using commercial kit (Randox, India); Very Low-Density Lipoprotein (VLDL) cholesterol was calculated as TAG/5 and Low-Density Lipoprotein (LDL) cholesterol was estimated using Friedewald's formula.[9] Serum vitamin C was determined by the method of Tietz.[10] In the erythrocytes, the activity of superoxide dismutase was determined by the method of Marklund and Marklund.[11] Glutathione (GSH) level was determined using the method of Beutler et al.[12] Catalase activity was determined by the method of Aebi et al.[13] MDA level was determined using the method of Ohkawa et al.[14]

Statistical Analysis
All data were represented as mean±SD. The mean values were statistically analysed using one-way Analysis of Variance (ANOVA). The significant differences between the groups were further analysed by Student's t-test. P values less than 0.05 were considered as significant.

RESULTS
Table 1 compares the serum lipid profiles of control rat (rats fed with normal diet) with rats fed with HCD and rats fed with HCD and different vegetable oils.

When the rats were fed with high-cholesterol diet, there was a significant increase in the ($P<0.001$) in the serum total cholesterol level and LDL cholesterol levels (both) in all groups.

VLDL level increased in the oil treated groups (coconut oil ($P<0.001$), olive oil, palm oil ($P<0.05$ for both), vanaspati ($P<0.001$) and cholesterol fed rats ($P<0.001$)). Further, triacylglycerol level showed a significant rise in the cholesterol fed rat group ($P<0.001$) whereas a significant change was observed in coconut oil ($P<0.001$), olive oil, palm oil ($P<0.05$ for both), vanaspati ($P<0.001$). HDL level increased in the oil treated groups (coconut oil ($P<0.001$), palm oil ($P<0.05$) and sunflower oil ($P<0.01$)).

Table 2 compares the antioxidant parameters of control rat (rats fed with normal diet) with rats fed with HCD and rats fed with High-Cholesterol Diet (HCD) and different vegetable oils.

MDA level increased significantly in High-Cholesterol Diet group and all the oil treated groups ($P<0.001$ for all the groups). There was a significant decrease in the GSH level in palm oil and vanaspati ($P<0.05$ for both), sunflower oil ($P<0.001$). Similarly, SOD level decreased significantly in palm oil ($P<0.01$), olive oil, sunflower oil, vanaspati ($P<0.001$ for all 3). Further, catalase level decreased significantly in all the groups - olive oil, coconut oil, palm oil, sunflower oil and vanaspati ($P<0.001$ for all the groups). Vitamin C level decreased significantly ($P<0.001$) in all the groups.

Values are expressed as Mean±SD; n=12 for control, n=6 for all other groups.

Groups (Control n=12, Other Groups n=6)	TAG (mg/dL)	Total Cholesterol (mg/dL)	HDL Cholesterol (mg/dL)	VLDL Cholesterol (mg/dL)	LDL Cholesterol (mg/dL)
Group 1: Control (Normal diet)	75.41±6.23	77.08±6.43	42.41±5.58	15.08±1.26	19.58±1.70
Group 2: High cholesterol diet	*** 126.33±9.24	*** 253.16±25.8	48.33±5.50	*** 25.26±1.84	*** 179.56±19.09
Group 3: High cholesterol diet + coconut oil	*** 96.0±5.89	*** 150.5±12.17	*** 55.83±5.87	*** 19.2±1.17	*** 75.46±5.63
Group 4: High cholesterol diet + olive oil	* 87.33±6.65	*** 142.66±8.35	49.83±6.14	* 17.46±1.33	*** 75.36±2.31
Group 5: High cholesterol diet + palm oil	* 86.66±7.44	*** 142.66±11.28	* 52.16±4.59	* 17.33±1.19	*** 73.16±3.20
Group 6: High cholesterol diet + sunflower oil	83.16±5.98	*** 137.16±8.91	** 54.16±4.99	16.63±1.02	*** 66.7±3.06
Group 7: High cholesterol diet + vanaspati	*** 118.16±5.11	*** 173.16±8.84	48.5±5.23	*** 23.63±1.48	*** 101.03±6.51

Table 1. Effect of Different Vegetable Oils on Lipid Profile in Normal and Hypercholesterolaemic Rats

*P<0.05, **P<0.001, ***P<0.0001; control (rats fed with normal chow diet versus rats fed with normal chow diet + different oils).

Values are expressed as Mean±SD; n=12 for control, n=6 for all other groups.

Groups (Control n=12, Other Groups n=6)	MDA (nmol/g Hb)	GSH (µmol/g Hb)	SOD (U/g Hb)	Catalase (U/g Hb)	Vitamin C (mg/dL)
Group 1 Control (normal diet)	30.50±2.72	10.24±0.70	5567.22±342.70	25102.25±3740.82	1.12±0.06
Group 2 High cholesterol diet	***72.42±3.75	9.82±0.45	5426.44±361.39	25636.94±1238.85	***0.92±0.03
Group 3 High cholesterol diet + coconut oil	***74.66±3.18	9.60±0.73	5246.32±294.61	***18211.68±1363.47	***0.88±0.04
Group 4 High cholesterol diet + olive oil	***78.32±2.61	*8.96±0.96	***4436.42±248.46	***18680.86±1196.08	***0.87±0.03
Group 5 High cholesterol diet + palm oil	***76.82±3.28	9.36±0.89	**4908.62±312.96	***18322.24±1275.20	***0.86±0.03
Group 6 High cholesterol diet + sunflower oil	***84.48±3.22	***8.26±0.91	***3838.80±228.71	***17266.61±2246.70	***0.83±0.03
Group 7 High cholesterol diet + vanaspati	***80.42±2.67	*8.62±1.04	***4286.67±261.76	***16286.62±1276.95	***0.77±0.04

Table 2. Effect of Different Vegetable Oils on Oxidative Stress Parameters in Normal and Hypercholesterolaemic Rats

*P<0.05, **P<0.01, ***P<0.001; control (rats fed with normal chow diet versus rats fed with normal chow diet + different oils.

DISCUSSION

Hyperlipidaemia is one of the predominant risk factors for development of atherogenesis and coronary heart diseases.[15] Reactive Oxygen Species (ROS) play a major role in development and progression of atherogenesis causing the conversion of LDL to oxidised LDL. Oxidised LDL is considered as one of the most important factor in the development of atherogenesis. Reactive oxygen species oxidise normal LDL to oxidised LDL, which is taken up by macrophages. Excessive uptake of modified macrophages causes the transformation of these cells into foam cells, which participate in the formation of atherosclerotic plaques.[16] Vulnerability of LDL to oxidation depends both on concentration of pro-oxidant stimuli and the entity and the concentration of antioxidants.[6]

Administration of vegetable oils in rat daily for 90 days found a significant change in the antioxidant status as evident from the results of this study.

GSH is a major non-protein antioxidant compound. Administration of coconut oil decreases the GSH level when compared to that of the normal group. However, this is found to be statistically nonsignificant. A statistically decreased value of GSH was found in the sunflower oil and vanaspati-treated group. The decrease of GSH among the vegetable oil treated group was sunflower oil> vanaspati> olive oil> palm oil> coconut oil. The decrease of SOD among the vegetable oil treated group was sunflower oil> vanaspati> olive oil> palm oil> coconut oil. The decrease of catalase among the vegetable oil treated group was vanaspati> sunflower oil> coconut oil> palm oil> olive oil. The decrease in vitamin C in the vegetable oil treated group was vanaspati> sunflower oil> palm oil> olive oil> coconut oil. The decline in levels of GSH, SOD and vitamin C was lowest in the coconut oil treated group (refer table no. 2).

Sunflower oil treated groups show maximum increase in lipid peroxidation compared to other vegetable oil treated groups. This can be attributed to the low GSH level in this group. Compared to other vegetable oil treated animals, coconut oil showed the least increase in the MDA level. This demonstrates that the saturated medium chain fatty acids of coconut oil are least susceptible to the peroxidation by free radicals. There is a considerable increase in MDA level in all other vegetable oil treated groups, maximum in sunflower oil indicating PUFA in sunflower oil are more susceptible to the peroxidation.

Rats fed on high cholesterol diet and vegetable oils show a significant increase in lipid profile compared to the control. There's a significant increase in serum total cholesterol, LDL and VLDL cholesterol levels in all groups. Coconut oil increased the HDL cholesterol significantly more than other oil treated groups. Sunflower oil and palm oil also increased the HDL significantly; vanaspati and olive oil did not change its level. Furthermore, total cholesterol level and LDL cholesterol found to be increased in all the vegetable oil treated groups. The increase in TAG found least in the sunflower treated group.

Addition of vegetable oils to rats fed with high-cholesterol diets has a positive influence on the lipid metabolism. In the present study, we observed the lipid-lowering effect of various vegetable oils in rats fed with high-cholesterol diet. Increased level of lipid profile was found in hypercholesterolaemic rats. Simultaneous administration of various vegetable oils with high-cholesterol diet caused a significant decrease in serum total cholesterol, LDL and VLDL cholesterol. Vegetable oils are able to restore at least partially the lipid profile of hypercholesterolaemic rats. The underlying mechanism of this activity is not elucidated by the present study. However, possible reasons for cholesterol reduction property of oils in rats fed with high-cholesterol diet can be attributed due to increased fecal excretion of bile acids, cholesterol metabolism rearrangement and for a small extent, inhibition of cholesterol absorption by naturally occurring plant sterols present in vegetable oils.

Major pathway for disposal of cholesterol from body is via the synthesis and excretion of bile acids and faecal sterols. Vegetable oils increase the synthesis and excretion of faecal bile acids and sterols in hypercholesterolaemic diets as elucidated in the study by A Arunima et al.[17] Dietary oils have a role in modulating hepatic lipid metabolism and turnover. Decrease in serum lipid profile maybe due to the reduction in the hepatic biosynthesis of cholesterol, stimulation of receptor-mediated catabolism of LDL-cholesterol and increase in the uptake of LDL from blood by liver.[18] Naturally-occurring plant sterols present in vegetable oils inhibit cholesterol absorption.[19] There is an inverse relationship between dietary intake of naturally-occurring sterols and serum cholesterol levels.[20] Sunflower oil has more sterols than other vegetable oils as given by a study.[21]

CONCLUSION

Evidence from this study confirms the lipid-lowering effects of vegetable oils in rats fed with high-cholesterol diet. Among the various oils used, coconut oil was found to be comparatively more beneficial. This conclusion is made based on the benefit of significant HDL increase and non-significant mild induction of peroxidation in coconut oil treated groups compared to other vegetable oil treated groups. Further detailed studies are required to elucidate the exact mechanism of vegetable oils on lipid metabolism. However, our findings can serve as a possible indicator for the choice of vegetable oils in hypercholesterolaemic patients in future.

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