



EFFECT OF FISH OIL AND COCONUT OIL ON LIPID PROFILE AND OXIDATIVE STRESS IN LIVER AND HEART OF RATS*

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Abstract

Effect of fish oil and various preparations of coconut oil viz., copra oil/ Refined, bleached and deodorized (RBD) coconut oil, seasoned coconut oil and virgin coconut oil, was evaluated in liver and heart of rats. The oils were administered @16.4 g/ kg body weight. Administration of copra oil significantly ($P<0.05$) increased the levels of total cholesterol (TC), triacylglycerol (TAG), lipid peroxides (LP) and reduced glutathione (GSH) in both the tissues. Rats fed with seasoned coconut oil showed a significant ($P<0.05$) increase in the level of TC in both the organs and GSH in heart. Levels of TAG and LP did not show any significant variation. Virgin coconut oil significantly ($P<0.05$) decreased the levels of TC and GSH in both the organs whereas, TAG level increased only in heart and LP level did not show any significant variation. There was no significant variation in the level of TAG in both the organs of fish oil fed rats, while TC decreased significantly ($P<0.05$) in liver with no significant variation in heart. However, LP increased ($P<0.05$) in both the organs with increase ($P<0.05$) in the content of GSH only in heart. Significant ($P<0.05$) increase was observed in the weight of both the organs in copra oil and fish oil fed rats whereas, virgin coconut oil did not cause any significant change. Rats fed with seasoned coconut oil showed a reduction ($P<0.05$) in weight of liver with no variation in weight of heart. The study suggested that virgin coconut oil consumption caused least adverse effects on both the

organs followed by seasoned coconut oil. High level of oxidative stress was observed in fish oil while copra oil undesirably affected all parameters except for the level of GSH.

Key words: Fish oil, GSH, Lipid peroxides, RBD coconut oil, Seasoned coconut oil, Virgin coconut oil

Coronary heart disease (CHD) has become a major public health problem in many developing countries. It has been predicted that by 2020, the most common cause of death, globally, would be CHD. The annual global mortality due to CHD is estimated to be 14.3 million and of these, two thirds are occurring in developing countries (Mandal *et al.* 2009). CHD is high among people in the Indian subcontinent and a greater risk appears to be at younger age (Ahmed and Bhopal, 2005). Sedentary life style and increased consumption of calories and saturated fat result in abdominal obesity, insulin resistance and atherogenic dyslipidaemia (Singh and Sen, 2003). High incidence of CHD in Kerala is believed to be due to consumption of coconut kernal and oil that contain high amount of saturated fats (Kumar, 1997). This general belief made the people of Kerala to shift to alternate cooking oils such as, sunflower oil, which is rich in linoleic acid, an essential, ω -6 fatty acid (Sabitha *et al.* 2009). Various preparations of coconut oil are available commercially. Refined, bleached and deodorized (RBD) coconut oil or Copra oil and Virgin coconut oil (VCO) are important among these. Seasoned coconut oil is the one, which

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is used by the people in the western coastal region of India, where heated RBD coconut oil is treated with mustard seeds, onion, curry leaves and turmeric powder which is added in curried dishes to increase its taste and flavour.

It has become a practice to consume fish oil to increase ω -3: ω -6 fatty acid ratio of the diet (Luotola and Luotola, 1985). Although fish oils are good in preventing the incidence of atherosclerosis, it may increase the level of incorporation of long-chain ω -3 fatty acids in membrane phospholipids thereby, potentiate peroxidation of cellular membranes (Iritani and Fuiikawas, 1982). Increased production of hydroperoxides may be deleterious to membrane integrity and can result in the accumulation of degradative products of peroxidized lipids (Hammer and Wills, 1978).

Present study, compares the effect of various preparations of coconut oil and fish oil on lipid profile and oxidative stress in liver and heart of rat.

Materials and Methods

Commercial coconut oil (Copra oil/ RBD coconut oil) was procured from Kerala Agricultural University. Commercial virgin coconut oil (RUBCO Nutri-ko) and commercial shark liver oil were procured from local market. Seasoned coconut oil was prepared in the laboratory as described in our earlier studies (Sreeji and George, 2011). Thiobarbituric acid (TBA), 1, 1, 3, 3 Tetra methoxy propane (TMP), disodium hydrogen phosphate, monosodium dihydrogen phosphate and 5, 5' Dithio bis-2-nitrobenzoic acid (DTNB) were purchased from Himedia Laboratories Pvt Ltd, Mumbai. Sodium dodecyl sulphate (SDS) and Trichloroacetic acid (TCA) were procured from Sigma-Aldrich India, Bangalore and Qualigens Fine Chemicals, Glaxo Smith Kline Pharmaceuticals Ltd, Mumbai, respectively. All other chemicals were procured from Merck India Ltd, Mumbai.

Male Wistar rats weighing 180-220 g were housed in appropriate cages in a well-ventilated experimental animal room under 12: 12 hr LD cycle at 22 to 28°C and 45 to 55% relative humidity with free access to standard rat pellet diet and drinking water. Experiments were conducted with the approval of Institutional Animal Ethics Committee. Rats were randomly divided into 5 groups, G1 to G5, each comprising 6 animals. Except G1, rats under all other groups were administered with various

oils for a period of 90 days as follows:

- G1 – Normal control (NC)
- G2 – Copra oil/ RBD coconut oil (CO)
- G3 – Seasoned coconut oil (SCO)
- G4 – Virgin coconut oil (VCO)
- G5 – Fish oil (FO)

Dose was fixed based on per capita world average oil consumption level (17.8 kg/head/year), consumption level of developed western world (44 to 48 kg/head/year) and the total coconut oil consumption in Kerala (free oil + oil derived from kernel), which comes around 14 kg/head/year (Rajamohan, 2004). A dose of 30 kg/head/year was fixed, which comes to a rat dose of 16.4 g/kg body weight. The dose fixed was an average value of per capita world average consumption and consumption of developed western world. Moreover, it was nearly double the per capita coconut oil consumption in Kerala. Oils were administered every day orally using an orogastric tube in two divided doses, at morning and evening.

On completion of experiment (on day 91) the rats were euthanized and liver and heart were collected. The organs were washed in ice cold saline, dried on a filter paper and weighed. Samples from liver and heart were collected for the preparation of tissue homogenates.

Total cholesterol and Triacylglycerol were estimated by the method of Haug and Hostmark (1987). Level of lipid peroxides in liver and heart tissue homogenates was determined by the method of Ohkawa *et al.* (1979) and that of reduced glutathione (GSH) by the method of Moron *et al.* (1979).

Data obtained were compared by analysis of variance (ANOVA) (Snedecor and Cochran, 1989) followed by Duncan multiple range test to determine the level of significance (Steel and Torrie, 1980).

Results and Discussion

Total cholesterol level increased significantly ($P < 0.05$) in both the organs of G2 and G3 (Table 1). Very high level of TAG was observed in both the tissues of G2 rats while it was maintained to that of control rats in G3. It has been reported that large amounts of lauric (C: 12: O) and myristic (C: 14: O) acids in coconut oil are capable of increasing total cholesterol (Dauqan *et al.*, 2011) and

Table 1. Effect of various preparations of coconut oil and fish oil on total cholesterol and triacylglycerol in liver and heart (mg/g wet tissue) of rats.
(Mean \pm SE, n = 6)

Groups	Total cholesterol		Triacylglycerol	
	Liver	Heart	Liver	Heart
Normal control (G1)	2.01 ^a \pm 0.11	1.30 ^a \pm 0.06	4.41 ^a \pm 0.35	1.91 ^a \pm 0.14
Coconut oil (G2)	2.16 ^{a,b} \pm 0.08	1.83 ^b \pm 0.11	41.61 ^b \pm 2.61	6.44 ^b \pm 0.59
Seasoned coconut oil (G3)	2.28 ^b \pm 0.11	1.80 ^b \pm 0.09	5.35 ^a \pm 0.20	1.90 ^a \pm 0.09
Virgin coconut oil (G4)	1.35 ^c \pm 0.07	0.89 ^c \pm 0.03	4.49 ^a \pm 0.19	3.06 ^c \pm 0.15
Fish oil (G5)	1.70 ^d \pm 0.04	1.10 ^{a,c} \pm 0.06	5.88 ^a \pm 0.41	1.66 ^a \pm 0.19

Level of significance was determined column wise between groups. Values not bearing a common superscript letter (a, b, c and d) in a column differ significantly ($P < 0.05$).

increased HMG CoA reductase activity, can lead to the increased level of tissue cholesterol (Zulet *et al.* 1999). Asai and Miyazawa (2001) showed that supplementation of curcuminoids in the diet significantly increased hepatic acyl-CoA oxidase activity and lowered lipid level in rat liver and the lipid lowering effect of curcuminoids might be due to alterations in fatty acid metabolism.

Significantly ($P < 0.05$) decreased level of cholesterol in both liver and heart was observed in G4 rats. However, TAG level was significantly increased in heart while it was maintained to that of control rats in liver (Table 1) as against the findings of Nevin and Rajamohan (2004). They observed reduced levels of cholesterol and TAG in liver, heart and kidney of rats fed with VCO for 45 days and suggested that this could be due to the reduced activity of HMG CoA reductase and the relative rate of synthesis and oxidation of fatty acids in liver. The reason for the increased level of TAG observed in heart in the present study needs to be explored further.

Rats in group G5 showed decreased ($P < 0.05$) level of liver cholesterol while, that of heart and TAG in both the tissues were similar to that of control rats (Table 1). Similar observations have also been reported by earlier workers (Nalbone *et al.* 1988). Ahmed *et al.* (2006) showed significantly lower level of liver cholesterol in rats fed with FO diet compared to soybean and palm oil diets and suggested that this decrease could be mainly due to an increased rate of excretion of cholesterol and bile acids in faeces.

Significant ($P < 0.05$) increase was observed in the level of LP in both the tissues and GSH in heart of G2 rats. Although, there was an increase in the level of GSH in liver, it was not significant statistically. Rats in G3 did not show any significant variation in the level of LP in both the tissues and GSH in liver whereas, the GSH content increased significantly ($P < 0.05$) in heart (Table 2). This is in agreement with the findings of Nevin and Rajamohan (2006), who suggested that rise in the level of GSH could be a compensatory mechanism to scavenge the free radicals generated. Flavonoids and other polyphenols in SCO have been reported to exert a variety of biological actions such as free radical scavenging, chelation of metal ions, modulation of enzyme activity, signal transduction, activation of transcription factors and gene expression (Rice-evans *et al.*, 1995; Arulselvan and Subramanian, 2007; Benson and Devi, 2009).

Rats in G4 exhibited numerically decreased level of LP in both liver and heart from that of G1, but not significant statistically (Table 2). However, a significant ($P < 0.05$) decrease was observed in the level of GSH in both the organs. Similar observations on the level of LP and related enzymes were reported by earlier workers (Nevin and Rajamohan, 2006 and 2008). They suggested that high content of unsaponifiable components such as, vitamin E and polyphenols might have contributed to the beneficial effect of VCO and the reduction in GSH content of both the tissues might be attributed to the reduced

Table 2. Effect of various preparations of coconut oil and fish oil on lipid peroxides (nM/g) and reduced glutathione (GSH) in liver and heart ($\mu\text{g/g}$ wet tissue) of rats.
(Mean \pm SE, n = 6)

Groups	Lipid peroxides (LP)		Reduced glutathione (GSH)	
	Liver	Heart	Liver	Heart
Normal control (G1)	375.00 ^a \pm 23.13	302.50 ^a \pm 11.76	850.00 ^{a,b} \pm 76.99	695.00 ^a \pm 23.83
Coconut oil (G2)	464.38 ^b \pm 12.08	370.63 ^b \pm 20.08	915.00 ^b \pm 45.63	1125.00 ^b \pm 36.59
Seasoned coconut oil (G3)	385.63 ^a \pm 11.63	290.00 ^a \pm 12.61	765.00 ^a \pm 42.38	865.00 ^c \pm 58.52
Virgin coconut oil (G4)	367.50 ^a \pm 13.56	275.63 ^a \pm 9.13	500.00 ^c \pm 20.00	465.00 ^d \pm 15.00
Fish oil (G5)	749.13 ^c \pm 40.49	435.63 ^c \pm 16.97	745.00 ^a \pm 26.12	1160.00 ^b \pm 52.37

Level of significance was determined column wise between groups. Values not bearing a common superscript letter (a, b, c and d) in a column differ significantly ($P < 0.05$).

oxidative stress in the organs.

Rats in G5 showed significant ($P < 0.05$) increase in LP level in both the organs and GSH in heart whereas, liver GSH did not vary significantly from that of control rats (Table 2) indicating a higher oxidative damage in liver compared to heart. Glutathione functions as a direct free radical scavenger and stabilizes membrane structure through the removal of products of lipid peroxidation (Khajuria *et al.*, 1997). As an adaptive response to increased oxidation, GSH content would have been increased in both the tissues while an insignificant increase in the level of GSH and a significant rise in the level of lipid peroxides in liver, suggest that there might have been a rapid utilization of the newly

synthesized/transported GSH for the elimination of reactive oxygen species (ROS) and quenching the products of lipid peroxidation. Gonzalez *et al.* (1992) reported increased level of tissue lipid peroxides in heart, skeletal muscles and mammary gland of mice fed with fish oil diet containing added synthetic antioxidant while, Sen *et al.* (1997) showed that addition of the antioxidant, vitamin E in FO supplemented diets decreased the levels of lipid peroxides in tissues of rats. Increased lipid peroxidation observed in rats on FO diet could be due to either reduced level of α -tocopherol or its rate of absorption from the intestine (Meydani *et al.*, 1987).

Significant ($P < 0.05$) increase was observed in the weight of liver and heart of G2

Table 3. Effect of various preparations of coconut oil and fish oil on weight of liver and heart (g) of rats

(Mean \pm SE, n = 6)

Groups	Weight	
	Liver	Heart
Normal control (G1)	8.48 ^a \pm 0.42	0.66 ^a \pm 0.03
Coconut oil (G2)	10.34 ^c \pm 0.18	0.79 ^b \pm 0.02
Seasoned coconut oil (G3)	7.23 ^b \pm 0.17	0.67 ^a \pm 0.01
Virgin coconut oil (G4)	8.45 ^a \pm 0.38	0.70 ^a \pm 0.02
Fish oil (G5)	10.61 ^c \pm 0.39	0.83 ^b \pm 0.01

Level of significance was determined column wise between groups. Values not bearing a common superscript letter (a, b, and c) in a column differ significantly ($P < 0.05$).

rats while in G3, SCO administration decreased liver weight and maintained the weight of heart similar to that of control rats (Table 3).

The observations could be correlated to the oxidative stress and TAG content of both the organs. Increased weight of both the organs in rats on CO rich diet could be attributed to oxidative damage and resultant inflammation (Onyema *et al.* 2006), which is evident from the level of lipid peroxides. Very high content of TAG observed in both the tissues also contribute to the rise in weight. Zulet *et al.* (1999) reported similar findings. Rats in group, G4 did not show any significant variation in the weight of both the organs (Table 3), which is supported by the biochemical parameters. Significant ($P < 0.05$) increase in weight of both the vital organs (Table 3) of G5 rats could be attributed to oxidative damage and resultant inflammation of tissues, which is evident from the increased level of lipid peroxidation, which is in accordance with the findings of Parrish *et al.* (1991). They showed that adipose tissue (epididymal and perirenal) was the only tissue whose mass decreased after FO supplementation, whereas mass of liver and spleen increased.

It is evident from the present study that the level of tissue lipids and oxidative stress increased in CO consumption. Though, there was an increase in tissue TC, the TAG level and oxidative status was similar to that of control rats in SCO fed group. VCO administration showed a better lipid profile (low level of TC in both the tissues and liver TAG similar to that of control animals) except for TAG level in heart and a reduced oxidative stress whereas, fish oil exhibited a better lipid profile in both the tissues but with a higher oxidative stress. The study revealed least adverse effects in both the organs on consumption of virgin coconut oil followed by seasoned coconut oil. High level of oxidative stress was observed in fish oil while copra oil undesirably affected all parameters except for the level of GSH.

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