



Virgin coconut oil supplemented diet increases the antioxidant status in rats

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Abstract

Virgin coconut oil (VCO) directly extracted from fresh coconut meat at 50 °C temperature was tested for its effect on the activities of antioxidant enzymes and lipid peroxidation levels in male Sprague–Dawley rats, compared to copra oil (CO) and groundnut oil (GO) as control. Oils were fed to rats for 45 days along with a semi-synthetic diet and after the experimental period various biochemical parameters were done. Individual fatty acid analyses of VCO and CO were done using gas chromatography. Effect of polyphenol fraction isolated from the oils was also tested for the ability to prevent in vitro microsomal lipid peroxidation induced by FeSO₄. The results showed that GO, rich in polyunsaturated fatty acids, reduced the levels of antioxidant enzymes and increased lipid peroxidation, indicated by the very high MDA and conjugate diene content in the tissues. PF fraction from VCO was found to have more inhibitory effect on microsomal lipid peroxidation compared to that from the other two oils. VCO with more unsaponifiable components viz. vitamin E and polyphenols than CO exhibited increased levels of antioxidant enzymes and prevented the peroxidation of lipids in both in vitro and in vivo conditions. These results showed that VCO is superior in antioxidant action than CO and GO. This study has proved that VCO is beneficial as an antioxidant.

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Keywords: Virgin coconut oil; Antioxidants; Lipid peroxidation; PF; TBARS; Fatty acids

1. Introduction

Free radicals that are continuously produced in our body play an important role in the pathogenesis of tissue damage in many different clinical disorders (Halliwell, Gutteridge, & Cross, 1992; Levy et al., 1998; Slater, Cheesman, Davies, Proudfoot, & Xin, 1987). Biological and chemical pro-oxidants are considered to be important for the provocation of free radical mediated diseases in an individual (Tripathi & Upadhyay, 2001). Unsaturated fatty acids easily form peroxides in the presence of oxygen, ultraviolet light, metallic ions and biological catalysts that induce the production of free radicals (Oette, 1965). These free radicals produce breakdown of membrane phospholipids and initiate lipid peroxidation (Ambroso & Chiarello, 1991; Ferrari et al., 1992). The peroxidative effect on membrane lipids and

low density lipoproteins (LDL) is directly implicated to the pathogenesis of atherosclerosis (Eder & Kirchgessner, 1997). Apart from this, free radicals are also capable of damaging enzymes, other proteins and DNA resulting in the wrong genetic information leading to cancer (Parthasarathy, Santanam, & Auye, 1998). Several nutritional factors such as intake of vitamin E as an antioxidant or the nature and amount of dietary fatty acids have been shown to reduce the susceptibility of LDL to lipid peroxidation in humans and laboratory animals (Nicolosi, Wilson, Lawton, & Handelman, 2001; Stephens et al., 1996). Various studies have showed that vegetable oils affect lipid peroxidation and antioxidant parameters, and lead to favorable changes in the plasma lipid status (Scaccini et al., 1992; Visioli, Bellomo, Montedoro, & Galli, 1995). Although fatty acid components and cholesterol in the diet are the primary determinants of diet induced hypo or hypercholesterolemia. However, a review of various studies has also indicated a hypocholesterolemic

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and antioxidant effect of some unsaponifiable components viz vitamins, polyphenols, sterols etc. in reducing the lipid levels and lipid peroxidation (Anderson et al., 2001; Khor, Rajendran, & Gopalakrishnan, 1998; Nicolosi et al., 2001).

Nutritional antioxidants play an important role in cellular antioxidative defense mechanisms (Chow, 1988). Vitamin E and C, β -carotenoids, selenium, copper and zinc are the major dietary factors with the ability to act as antioxidants (Capel, 1988). In addition, antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSH-Red) act to protect tissues from oxidative injury generated by oxygen free radicals [e.g., superoxide anion (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2)] (Bray & Taylor, 1993; Pence, 1991).

Coconut and its extracted oil from copra have served man as important foods for thousands of years. Since it contains mostly saturated fatty acids it is believed to be hypercholesterolemic in action. Presently, virgin coconut oil (VCO) is gaining wide popularity in the scientific field and among the public. It is believed that VCO is more beneficial than usually obtained copra oil since the mode of extraction retains more biologically active components such as vitamin E and polyphenols (Nevin & Rajamohan, 2004).

The purpose of this study was to evaluate the effect of virgin coconut oil on the antioxidant enzyme activities and lipid peroxidation level when fed to normal rats in comparison with copra oil (CO) and groundnut oil (GO) as control.

2. Materials and methods

2.1. Chemicals

α -Tocopherol was purchased from Sigma Chemical Co, USA. All other reagents were of highest analytical grade available.

2.2. Test oils

The solid endosperm of mature coconut (West Coast tall variety) collected from the University campus was used for the extraction of coconut oil.

2.2.1. Virgin coconut oil

Endosperm of mature coconut was made into a viscous slurry and squeezed through cheese cloth to obtain coconut milk which was refrigerated for 48 h. After 48 h, the milk was subjected to mild heating (50 °C) in a thermostat oven. The obtained virgin oil was filtered through cheese-cloth and was used for the present study (Nevin & Rajamohan, 2004).

2.2.2. Copra oil

Endosperm of mature coconut was dried in sunlight for five days to remove the moisture content and the copra obtained was used for the extraction of the oil.

2.2.3. Groundnut oil

Groundnut oil (Postman brand) was purchased from the local market.

2.3. Animal experiments

One month old male Sprague–Dawley rats (100–120 g) bred in our department animal house were used for the study. The animals were housed in polypropylene cages in a room maintained at 25 ± 1 °C with a 12 h light and 12 h dark cycle. The experimental groups were as follows: Group I (Control) – GO (8%), Group II – CO (8%), Group III – VCO (8%). Oils were fed along with a semi synthetic diet for 45 days (Table 1). Gain in body weight was recorded weekly. After 45 days, animals were fasted overnight and sacrificed by sodium pentathone injection, blood and tissues were collected for various estimations.

2.4. Biochemical investigations

Rat liver was washed, minced with scissors and homogenized in KCl solution (11.5 g/L KCl) in ice. The homogenate were diluted 1:4 and centrifuged at 9000g at 4 °C for 20 min. The resultant supernatant was decanted and used for estimating various enzyme activities. SOD (Mc Cord & Fridovich, 1969), Glutathione peroxidase (GPx) (Lawrence & Burk, 1976), Glutathione reductase (GR) (David & Richard, 1983), Catalase (Aebi, 1984), Glutathione content in the blood (Benke, Cheever, Mirer, & Murphy, 1974) and Lipid peroxides (Ohkawa, Ohishi, & Yagi, 1979) were determined. Protein was determined using Folin–Ciocalteu reagent (Lowry, Roseborough, Farr, & Randall, 1951).

2.5. GC analysis of fatty acids

The fatty acid composition of total lipids from VCO and CO were analyzed by gas chromatography. Fats were methylated with trimethylsulfonium hydroxide (Butte, 1983). Fatty acid methyl esters were separated by gas chromatography using a system (HP 5890, Hewlett–Packard GmbH, Waldbronn, Germany) equipped with an automatic on-column injector, a polar capillary column (30 m FFAP, 0.53 mm ID, Macherey and Nagel, Düren, Germany) and

Table 1
Formulation of synthetic diet

Diet g/100 g weight	Group I	Group II	Group III
Corn starch	71	71	71
Casein	16	16	16
Virgin coconut oil	–	–	8
Copra oil	–	8	–
Groundnut oil	8	–	–
Salt mixture	4	4	4
Vitamin mixture	1	1	1

a flame ionisation detector (Eder & Brandsch, 2002). Helium was used as carrier gas at a flow rate of 5.4 mL/min. Fatty acid methyl esters were identified by comparing their retention times with those of individually purified standards.

2.6. Isolation of polyphenol fraction (PF)

PF from the test oils was extracted according to the method described by (Vazquez Roncero, Janer del vall, & Janer del valle, 1973) 10 g oil was dissolved in 50 ml hexane and extracted three times with 20 ml portions of 60% methanol successively. The vacuum dried final residue obtained from the combined extract was dissolved in a known volume of 60% methanol. The total polyphenol content of this solution was estimated using Folin–Ciocalteu reagent (Gutfinger, 1981). PF isolated from CO and VCO was subjected to Thin layer chromatography using BAW (*n*-butanol:acetic acid:H₂O, 4:1:5) (Markham, 1982). UV–vis spectrum of the PF in methanol were also recorded using Shimadzu UV–vis spectrophotometer (Fig. 2).

2.7. In vitro lipid peroxidation

Normal rats were killed and livers excised, rapidly washed and homogenized with 8 vol (w/v) of 5 mM Tris–HCl buffer (pH 7.4) containing 0.25 M sucrose and 0.1 mM EDTA. Sub cellular fractionation was carried out using differential centrifugation and pelleted microsomes were diluted with the same buffer. Reaction mixture contained 50 µl of PF (diluted in 60% methanol) from test oils, 10 µl microsomes, 500 µl Tris–HCl buffer (pH 7.4) containing 12.5 µM FeSO₄ · 7H₂O and 40 µl 2 mM ascorbic acid. The reaction mixture was incubated at 37 °C for 30 min followed by centrifugation. 0.3 ml of TBA was added and kept in a boiling water bath for 5 min. Absorbance was measured at 530 nm. α -Tocopherol was used as positive standard. The result was expressed as percentage inhibition compared to control (Kweon, Hwang, & Sung, 2001).

2.8. Statistical analysis

Statistical differences were determined using one way ANOVA followed by Duncan's post hoc test to identify the differences using SPSS 10. Differences of $p < 0.05$ were considered to be significant. Data are reported as means \pm SD unless otherwise stated.

3. Results and discussion

There were no differences in weight gain pattern in rats between control and treated groups. There was no mortality in any group during the experimental period.

3.1. Activity of antioxidant enzymes

Table 2 represents the activities of antioxidant enzymes in various tissues in GO, VCO and CO treated animals. Activities of catalase (CAT) and superoxide dismutase (SOD) were found to be significantly decreased in GO treated animals. The activities were increased in both VCO and CO treated animals with VCO treated animals showing more beneficial effect. Glutathione peroxidase (GPx) and glutathione reductase (GR) were also significantly decreased in GO fed animals and showed an increase in VCO fed animals compared to GO and CO fed animals.

SOD, CAT, GPx and GR constitute a mutually supportive team of defense against reactive oxygen species (ROS) and preventing lipid peroxidation (Eaton & Bammler, 1999). SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady state of O₂⁻. Decreased activity of SOD leads to increased production of free radicals, e.g., superoxide anion combines with hydrogen peroxide in the presence of copper ion to form powerful hydroxyl radical (Ishikawa, 1993). This radical modifies proteins and DNA, damages cellular membranes of mitochondria, nuclear envelop and endoplasmic reticulum (Hayashi et al., 2005; Kawasaki, Abiru, & Eguchi, 2004; Mimnaugh, Kennedy, Trush, & Sinha, 1985). CAT is a hemoprotein, localized in the peroxisomes and catalyses the decomposition of H₂O₂ to water and

Table 2
Effect on antioxidant enzyme activities in various tissues

Group	Organ	GPx	GR	SOD	CAT
I	Liver	0.13 \pm 0.004	2.36 \pm 0.03	8.24 \pm 0.13	44.95 \pm 0.33
	Heart	0.11 \pm 0.001	11.57 \pm 0.04	7.00 \pm 0.22	7.00 \pm 0.29
	Kidney	0.13 \pm 0.009	15.97 \pm 0.04	10.40 \pm 0.83	20.27 \pm 0.38
II	Liver	0.13 \pm 0.02	2.75 \pm 0.02	11.49 \pm 0.45 ^a	51.47 \pm 1.53 ^a
	Heart	0.19 \pm 0.02 ^a	22.44 \pm 0.03	12.64 \pm 0.19 ^a	10.87 \pm 0.43 ^a
	Kidney	0.18 \pm 0.05	27.45 \pm 0.02	14.47 \pm 0.09	23.50 \pm 1.62
III	Liver	0.16 \pm 0.01 ^{a,b}	2.99 \pm 0.12	11.35 \pm 0.68 ^a	72.49 \pm 1.75 ^{a,b}
	Heart	0.26 \pm 0.001 ^{a,b}	32.74 \pm 0.02 ^a	14.45 \pm 0.08 ^{a,b}	15.29 \pm 1.16 ^{a,b}
	Kidney	0.17 \pm 0.04	38.35 \pm 0.02 ^{a,b}	15.57 \pm 0.31	40.63 \pm 1.58

GPx – glutathione peroxidase (U/mg protein), GR – glutathione reductase ($\times 10^{-2}$ U/mg protein), SOD – superoxide dismutase (U/mg protein), CAT – catalase ($\times 10^{-3}$ U/mg protein), I-GO, II-CO, III-VCO, Results are mean of 6 rats \pm SEM.

^a Statistically significant compared to Group I ($p < 0.05$).

^b Statistically significant compared to Group II ($p < 0.05$).

oxygen. GPx is a selenoenzyme, present predominantly in liver and catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide (Venkumar & Latha, 2002). Increased activity of these antioxidant enzymes results in decreased formation of hydroxyl radical (OH^\cdot) (Gajanan, Chatterji, & Bagga, 1997; Maiorino, Chu, Ursini, Davies, & Duroshow, 1991).

Normal expression of these enzymes in VCO fed animals clearly indicates that they are not under oxidative stress. Even though fatty acid analysis of VCO and CO contains same amount of saturated fatty acids the higher content unsaponifiable components like vitamin E, polyphenols may be contributing to this effect of VCO. Presence of high amount of unsaturated fatty acids may be the reason for the low antioxidant enzyme activities of GO fed animals since polyunsaturated fatty acids (PUFA) deteriorates the antioxidant status due to their liability to become highly oxidized (Noguchi & Niki, 1999; Porter, Caldwell, & Mills, 1995). Feeding oils high in polyunsaturated fatty acids (PUFA) results in their accumulation in cell membranes and increase the oxidative stress since PUFA are highly susceptible to peroxidation than monounsaturated and saturated fatty acids (Beard, Barnard, Robbins, Ordovas, & Schaefer, 1996; Hagve, Lie, & Gronn, 1993; Palozza et al., 1996). VCO with its high saturated fat content and high levels of vitamins and polyphenols may rectify these problems.

3.2. Lipid peroxide contents

The lipid peroxide levels viz MDA and CD were significantly less in the heart, liver and kidney of VCO fed animals compared to other oil fed groups. In vitro microsomal lipid peroxidation using PF from VCO, CO and GO is shown in Fig. 1. α -Tocopherol was used as reference standard. It was found that PF from VCO was more capable of inhibiting invitro lipid peroxidation, indicated

by low TBARS formation as compared to CO and GO PF fractions.

Lipid peroxidation is causally related to chronic and acute events in atherosclerosis and coronary artery disease. The primary products of lipid peroxidation can undergo carbon–carbon bond cleavage via alkoxy radicals in the presence of transition metals, giving rise to the formation of short-chain, unesterified aldehydes (Kawai et al., 2003). Oxidative modification of LDL by these free radicals plays a key role in the pathogenesis of atherosclerosis and antioxidants are protective in the atherogenic process by inhibiting lipid peroxidation (Anderson et al., 1996; Mohr, Bowry, & Stocker, 1992). During the oxidation of LDL, the LDL molecule undergoes a large number of structural changes that alter its metabolism (Kawai et al., 2003). It is generally accepted that the primary generation of lipid hydroperoxides initiates a reaction cascade leading to rapid propagation and to amplification of the number of reactive oxygen species formed; this ultimately leads to extensive fragmentation of the fatty acid chains (Esterbauer, Schaur, & Zollner, 1991) and conversion of the LDL to a more atherogenic form (Quinn, Parthasarathy, Fong, & Steinberg, 1987). Peroxidation of LDL with Cu^{2+} produced cholesteryl ester core aldehydes, such as 9-oxononanoylcholesterol (9-ONC) and 5-oxovaleroylcholesterol (5-OVC), as the major oxidized cholesteryl esters (Kawai et al., 2003).

Polyphenols have been reported to exert a variety of biological actions such as free radicals scavenging, metal chelation, modulation of enzyme activity and more recently to effect signal transduction, activation of transcription factors and gene expression (Bitto, Roy, Sen, & Packer, 2000; Natarajan, Singh, Burke, Grunberger, & Aggarwal, 1996). These compounds are also capable of decreasing

Table 3
Fatty acid composition of virgin coconut oil and copra oil

Fatty acids	Amount (%)	
	VCO	CO
6:0	0	0
8:0	8.05	8.15
10:0	5.42	5.56
12:0	45.51	43.55
14:0	19.74	18.38
16:0	7.83	8.25
18:0	3.14	2.65
18:1, 9c	4.70	6.70
18:2, 9c, 12c	1.88	1.49
20:0	0.086	0.086
20:1, 11c	0.027	0.042
20:2, 11c, 14c	0.037	0.039
20:3, 11c, 14c, 17c	0.011	0.037
20:4, 8c, 11c, 14c, 17c	0.002	0.021
22:0	0.016	0.018
22:1, 13c	0.027	0.040
22:3, 13c, 16c, 19c	0.035	0.031
22:6, 4c, 7c, 10c, 13c, 16c, 19c	0.020	0.017
24:0	0.032	0.065

Values are mean of three estimations.

VCO, virgin coconut oil; CO, copra oil.

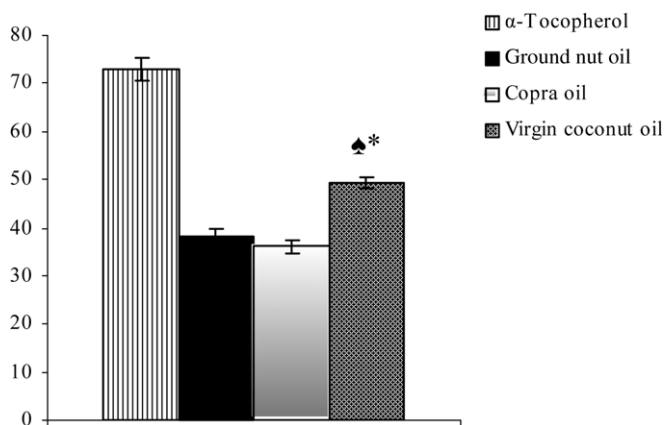


Fig. 1. Effect on in vitro lipid peroxidation. Values are mean of three estimations. * Statistically significant compared to copra oil ($p < 0.05$). \blacktriangle Statistically significant compared to α -tocopherol ($p < 0.05$).

the total and LDL cholesterol in serum and tissues as well as increasing the antioxidant status (Khor et al., 1998).

Higher levels of lipid peroxide products viz: malonaldehyde and conjugate dienes were found in rats fed unsaturated fatty acids since unsaturated fatty acids are more susceptible to peroxidation (Palozza et al., 1996). Previous studies in our laboratory have proved that PF (50 μg) from VCO significantly prevented the in vitro oxidation of LDL by Cu^{2+} than PF from CO and GO (Nevin & Rajamohan, 2004). Experiment using microsomal fraction with the same concentration of PF was found to be capable of preventing the oxidation of lipids.

3.3. Levels of blood GSH

VCO feeding was found to increase the total glutathione content (GSH) in the blood compared to GO and CO fed animals (Table 4). The levels of GSH in the blood is a sensitive indicator of antioxidant status in circulation (Cooper & Kristal, 1997; Piemonte et al., 2001), Since it plays a pivotal defensive role against oxidative insults as an endogenous scavenger of free radicals, which initiate lipid peroxides in the circulation resulting in ongoing lipid peroxidation of other circulating lipids and lipoproteins thus resulting in the disseminated endothelial dysfunction (Goode, Miller, & Heagerty, 1995; Sarandol, Safak, Dirican, & Uncu, 2004). Glutathione is a substrate, scavenging toxic intermediates of incomplete oxidation. Thus the total glutathione

concentration as well as its ability to maintain glutathione in reduced state is an important antioxidant defense (Pricilla, 1995).

3.4. Fatty acid and polyphenol content of oils

Fatty acid analysis of the two oils are shown (Table 3). Analysis showed no major differences among individual fatty acids. Estimation of total polyphenol content showed that VCO (84 mg/100 g oil) contained more polyphenols than CO (64.4 mg/100 g oil) (Nevin & Rajamohan, 2004) (see Table 4). TLC analysis of PF from CO and VCO obtained a single spot which showed a blue fluorescence in UV light (256 nm). Analysis of UV–vis spectrum of PF from both oils showed an absorption maximum (λ_{max} at 275 nm), characteristic for flavanones and dihydroflavonols. VCO also contained more vitamin E and A compared to CO. The free fatty acid and peroxide content are also lower for VCO than CO. Our studies have showed that VCO when exposed to sunlight for several days showed a sequential decrease in vitamin content, which confirms the loss of vitamins from copra when exposed to UV radiation from sunlight (unpublished data) (see Fig. 2).

Previous studies conducted in 258 human volunteers (age 18–65 years) showed that coconut oil consumption (15.40 g/head/day and average contribution of total fats were 16.3%/head/day) was beneficial in reducing LDL cholesterol and thereby raising HDL cholesterol when

Table 4
Effect on blood glutathione content, MDA and conjugate dienes (CD)

Groups	Glutathione ^A	MDA ^B			CD ^B		
		Liver	Heart	Kidney	Liver	Heart	Kidney
I	58.36 \pm 3.67	0.53 \pm 0.02	2.36 \pm 0.07	2.07 \pm 0.05	58.35 \pm 0.81	12.76 \pm 0.59	17.35 \pm 0.07
II	132.03 \pm 9.28	0.56 \pm 0.008	1.92 \pm 0.02 ^a	1.57 \pm 0.04 ^b	61.28 \pm 2.43	8.87 \pm 0.23 ^a	14.75 \pm 0.31
III	165.51 \pm 2.54	0.37 \pm 0.02 ^{a,b}	1.70 \pm 0.07 ^{a,b}	1.30 \pm 0.02 ^{a,b}	34.22 \pm 4.69 ^{a,b}	8.33 \pm 0.32 ^{a,b}	13.17 \pm 0.09 ^{a,b}

^A Glutathione content measured as ^a statistically significant compared to Group I ($p < 0.05$) ^b statistically significant compared to Group II ($p < 0.05$).

^B MDA (malondialdehyde) and conjugate dienes measured as mM/100 g wet tissue.

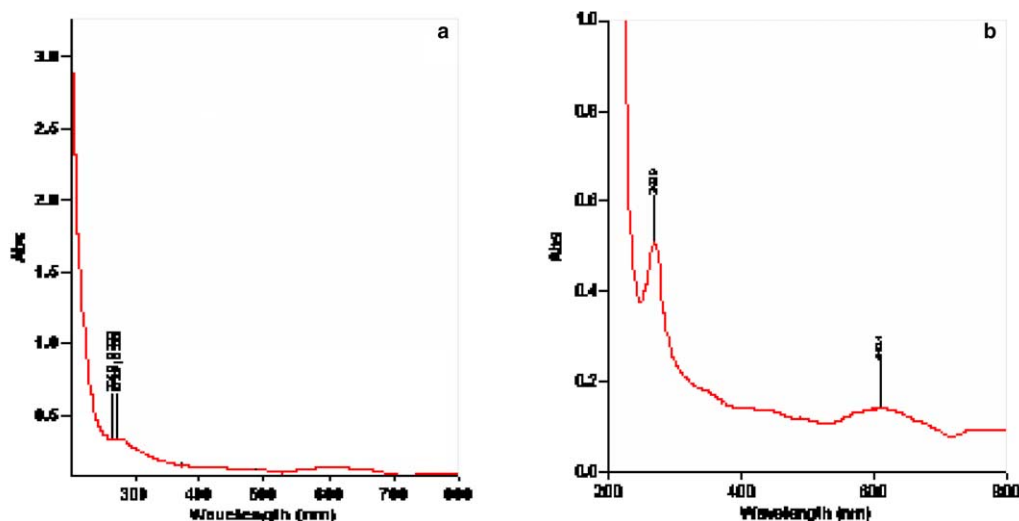


Fig. 2. UV–Vis spectrum of polyphenol fraction from CO and VCO. (A) Spectrum of CO. (B) Spectrum of virgin coconut oil.

compared to groundnut oil (Suma, 1998). Epidemiological observation of Polynesian Puka Pukans (37% calories from fat), whose 75% of the dietary fat come from coconut showed lower cholesterol and lower prevalence of vascular disease (Prior, Davidson, Salmond, & Czochanska, 1981). From the available literature it is evident that coconut oil consumption appears to be neutral in its effect on serum cholesterol (Frantz & Carey, 1961). Due to the world wide marketing of VCO and reports on its beneficial effects over CO, we under took this study. Earlier we have found that it is better in reducing lipid components in rats and capable of reducing the in vitro oxidation of LDL by physiological oxidants (Nevin & Rajamohan, 2004).

4. Conclusion

In conclusion, the study conducted to determine the effect of VCO in comparison with CO and GO on both in vitro and in vivo lipid peroxidation and the levels of antioxidant enzymes in rats showed that VCO administration increased the antioxidant enzymes and reduced the lipid peroxide content. VCO polyphenols are also capable of preventing in vitro lipid peroxidation than polyphenols from CO and GO. Preliminary chemical analysis of PF showed the presence of flavanones/dihydroflavonols like compound. These effect of VCO may be due to the differences in the absorption, transport and catabolism of the consistant fatty acids and also due to higher amount of antioxidant components compared to other oils. These results showed that consumption of VCO extracted from fresh coconut meat, with its high content of biologically active components is superior in antioxidant property than coconut oil extracted by dry process.

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