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Studies on SFA/TFA (Saturated/Trans Fatty Acid) Rich Dietary Fats on Lipid Profile and Antioxidant Enzymes in Normal and Stressed rats

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ABSTRACT

In many of the preparations instead of saturated fatty acids (SFA), trans fatty acid (TFA) produced by partial hydrogenation of unsaturated oils were advocated as the preferred fatty acid base for solid fats. The present study was designed to investigate the effects of dietary fats rich in SFA and TFA on lipid profile and endogenous antioxidant enzymes in normal and stressed rats. Twenty eight day old male Wistar rats were fed for duration of 45 days with fat enriched special diet (10% fat) prepared with coconut oil (CO), palm oil (PO) (SFA rich), Vanaspathi (VP) (TFA rich) and groundnut oil (control) respectively. Present study claims the protective role CO against atherogenic index and lipid peroxidation under normal and stress conditions. PO though contains SFA possesses less beneficial effect than CO, but safer than VP in causing atherogenicity. Whereas VP by virtue of its hyperlipidemic and oxidative stress generating ability exhibits deleterious effects in causing atherogenicity. Hence the consumption of high content of TFAs may aggravate the occurrence of cardiovascular disease, this effect being worse when accompanied with stress.

KEY WORDS : SFA; TFA; coconut oil; palm oil; vanaspathi; atherogenicity; antioxidant enzymes.

INTRODUCTION

Cardiovascular diseases (CVDs) cause around 17 million deaths, representing approximately one-third of all deaths occurring in the world (1). Nearly 80% of these deaths occur in low and middle income countries (2, 3). With the urbanization and westernization of our society, stress is increasing day by day. Psychological stress induces chronic inflammatory process due to an atherosclerotic lipid profile with oxidation of lipids (4, 5). This in turn plays a significant role in the development of atherosclerotic heart disease (6). The excess burden of CVD in Indians is due to a genetic susceptibility, viz. low concentrations of large high density lipoprotein cholesterol (HDL-C) (7), smaller coronary artery diameter (8), elevated levels of lipoprotein (a) [Lp (a)] (9) etc, which magnifies the adverse effects of lifestyle factors associated with urbanization, affluence and changes in diet. India is now in the middle of a CVD epidemic with urban Indians having CVD rates similar to overseas Indians, which is 4-fold higher than Americans (10). According to World Health Organization, by 2010, Asian Indians will represent 60% of the world's cardiac patients, which amounts to about 100 million patients (11).

Dietary fats have a significant role in the development of dyslipidemias (12) and play an important role in the development of atherosclerosis by modulating serum cholesterol concentration (13 -16) and free radical generation (17 - 19). In general, consumption of saturated oil is considered to be evil and its use is discouraged (20). In many of the preparations instead of saturated fatty acid (SFA), trans fatty acid (TFA) produced by partial hydrogenation of unsaturated oils were advocated as the preferred fatty acid base for solid fats (21-22). TFA were made prominent as a safe alternative over adverse impacts of SFA on CVD risk (22). However, multiple studies have reported the beneficial effects of SFA in CVD and type 2 diabetes mellitus (23, 24). Meanwhile, there are numerous research articles on the adverse affects of TFA in worsening heart diseases (21, 25-28). Ascherio et al (21) have reported that replacement of TFA in the american diet with natural unhydrogenated vegetable oils would prevent approximately 30,000 premature coronary deaths per year, and epidemiologic evidence

suggests that this number is closer to 100,0000 premature deaths annually.

Coconut tree (*Cocos nucifera;* family Arecaceae) in India has been called the 'tree of heaven' or 'kalpavriksha'. Coconut oil (CO) is one of the traditionallty used oil in India. Medicinal uses of CO are mentioned in ayurvedic texts like the "*Kayyadeva Nighantu*", "*Bhavaprakasha Nighantu*", "*Shaligrama Nighantu*" and "*Raja Nighantu*" characterize coconut oil as "hrdyam" or good for heart and cardiovascular system. The "*Bhavaprakasha Nighantu*" mentions that coconut oil can reduce kapha, which actually helps the body to lose than gain weight. CO is said to be useful in management of a wide range of skin diseases including the infectious type. It is said to improve digestion and nutrient absorption and enhance the immunity of the individual. The "*Shaligrama Nighantu*" point out that coconut oil is useful for the management of the disease known as yakshma, a condition that resembles AIDS in many ways (29). Whereas, palm oil (PO) and vanaspathi (VP) had no significance in traditional use in India. Palm oil is obtained from palm tree (*Elaeis guineensis*; family Arecaceae) originated in tropical rain forest region of West Africa (30, 31). VP is a product obtained by partial hydrogenation of various oils predominantly poly unsaturated fatty acid (PUFA) oils (27).

There are so many unanswered questions and highly contradictory reports on dietary vegetable oils of our diet. Some are said to increase the heart risk while others claim to reduce it (23, 24, 28, 32-36). Presently there has been a debate on whether substituting TFA in place of SFA could reduce cardiovascular diseases (28). Hence the present study was carried out to investigate the influence of widely consumed SFA oils (CO and PO) and TFA (VP) on lipid profile and endogenous antioxidant enzymes in rats under normal and stress conditions.

MATERIALS AND METHODS

Experimental animals

In-house laboratory bred male Wistar rats of 4 week old (45±3g) were selected for the study. Animals were housed in polypropylene cage on clean paddy husk bedding and maintained under controlled temperature at 20±2°C with an alternating 12 hour light: dark cycle (light on 6.00-18.00 hrs). Diet and water were provided ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC). Animal ethical guidelines and good laboratory practice guidelines were followed throughout the experimental period. In addition, all the precautions were taken to minimize pain and discomfort to the animals.

Test Dietary Fats

Groundnut oil (GNO), PO, CO and VP - were purchased from the local market.

Test Diets

Diet 1: GNO (Control), Diet 2: PO (SFA), Diet 3: CO (SFA) and Diet 4: VP (TFA). The diets were prepared according to modified American Institute of Nutrition formulae (AIN-76) (37). The dose of the dietary vegetable oil was 10% (w/w). Diets were stored in a refrigerator (2-8°C) and were prepared freshly every week. Individual group (n=6) were fed with respective diet for a duration of 45 days. Animals were provided with fresh diet daily and left over food was discarded.

Experimental Conditions

Normal (N) group: Rats were maintained under standard laboratory conditions and fed with respective diets till the completion of the experiment. Stress (S) group: Rats were subjected to forced immobilization stress using rat restrainer for duration of seven hours/day. Under these conditions rats were fed with respective diets, till the completion of the experiment.

Biochemical analysis

The rats were allowed to feed on fat enriched diets ad libitum. The test diets under normal and stress conditions were tested for their effects on lipid profile and antioxidant enzymes. At the end of 45 days, 2.0 ml of blood was withdrawn from the orbital sinus and the serum was separated from blood by centrifuging at 6000 rpm for 15 min. Lipid profile viz. total cholesterol (TC), (HDL-C) and triglyceride (TG) were estimated by biochemical kits from Ranbaxy using a semiautoanalyser.

Concentration of low density lipoprotein cholesterol LDL-C was calculated using Friedwald equation *(LDL=TC – HDL-1/5 TG)* (38).

Atherogenic index was calculated using the following formula:

Atherogenic index= TC-HDL-C/HDL-C (39).

Activity of endogenous antioxidant enzymes

After the withdrawal of blood, animals were sacrificed by cervical dislocation. Liver of the animals were perfused with normal saline and were dissected out, processed and 10% homogenates were prepared in saline (10%w/v), centrifuged and the supernatant was used for antioxidant enzyme assays (40).

Total protein content

The protein contents of 10% liver homogenates were

determined by using the Lowry's method (41).

Lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) in homogenate were estimated by using standard protocol (42). Briefly, 500 µl of sample (10% liver homogenate) was incubated with 300µl of 15% trichloroacetic acid (TCA), 300µl of 0.375% thiobarbituric acid (TBA) and 300µl of 5N hydrochloric acid (HCl) at 95°C for 15 min, the mixture was cooled, centrifuged and the absorbance of the supernatant was measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using $\varepsilon = 1.56 \times 10^5\ M^{-1}cm^{-1}$ and expressed as nM of (Malionaldehyde) MDA/mg of protein (43).

Catalase activity

The catalase activity was determined spectrophotometrically according to the standard protocol (44). Briefly, to 1.95 ml of 10mM hydrogen peroxide (H_2O_2) in 60mM phosphate buffer (pH =7.0), 0.05 ml of the liver homogenate was added and degradation of H_2O_2 was followed at 240 nm and the rate of decomposition of H_2O_2 was calculated using the formula $k=2.303/\Delta t \times \log (A_1/A_2)\ s^{-1}$ followed by calculation of catalase in terms of units/mg of protein. A unit of catalase is defined as the quantity, which decomposes 1.0 µmole of H_2O_2 per min at pH=7.0 at 25°C, while this H_2O_2 concentration falls from 10.3 to 9.2 mM.

Superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined basing on the ability of SOD to inhibit the auto-oxidation of epinephrine to adrenochrome at alkaline pH (45). Briefly, 25µl of the supernatant obtained from the centrifuged liver homogenate was added to a mixture of 0.1 mM adrenaline in carbonate buffer (pH=10.2) in a total volume of 1 ml and the formation of adrenochrome was measured at 295 nm. The SOD activity (U/mg of protein was calculated by using the standard plot.

Statistical analysis

The results were expressed as mean ± S.E.M (n=6). The statistical analysis involving four groups was performed by means of analysis of variance (ANOVA) followed by Scheffe's test. p value at < 0.05 was considered as statistically significant. All the data were processed with SPSS version 10.0 software.

RESULTS

Serum lipid profile

Compared to N+GNO (normal control), N+PO group has significantly increased levels of TC ($p<0.01$), LDL-C ($p<0.05$) and atherogenic index ($p<0.05$; Table 2). Similarly, N+VP group has significantly increased TC ($p<0.01$), TG ($p<0.001$) and atherogenic index ($p<0.001$; Table 2). However, N+CO group has significantly decreased TG ($p<0.001$) and non-significantly decreased atherogenic index (Table 2). Whereas compared to S+GNO (stress control), S+PO group has shown significant increase in TC ($p<0.01$) and non-significant increase in TG and atherogenic index (Table 2). Similarly S+VP group has shown significant increase in TC ($p<0.001$), LDL-C ($p<0.001$), TG ($p<0.001$) and atherogenic index ($p<0.001$; Table 2). On the other hand, S+CO group has produced significant decrease in TC ($p<0.001$), TG ($p<0.001$) and non-significant decrease in atherogenic index (Table 2).

Compared to the individual normal groups fed with different diets their respective stressed groups have shown increase in the parameters tested. Compared to N+GNO, S+GNO group has shown increase in TC ($p<0.001$), LDL-C ($p<0.05$), TG ($p<0.01$) and atherogenic index ($p<0.05$). Compared to N+PO, S+PO group has shown increase in TC ($p<0.05$), TG ($p<0.001$). Compared to N+CO, S+CO group has shown increase only in TG ($p<0.05$). Whereas Compared to N+VP, S+VP group has shown increase in TC ($p<0.001$), LDL-C ($p<0.01$), TG ($p<0.01$) and atherogenic index ($p<0.05$).

Liver antioxidant enzymes and lipid peroxidation

Compared to N+GNO (normal control), N+VP group has increased lipid peroxidation ($p<0.05$; Fig 3). Similarly compared to S+GNO (stress control), S+VP group has increased catalase ($p<0.05$), lipid peroxidation ($p<0.01$; Fig 3). However, rats fed CO diet under normal and stress conditions have non-significantly increased SOD and catalase and decreased lipid peroxidation compared to their respective controls.

Compared to the individual normal groups fed with different diets their respective stressed groups have shown decrease in SOD, catalase and increase in lipid peroxidation. Compared to N+GNO, S+GNO group has shown decrease in SOD ($p<0.01$) and increase in lipid peroxidation ($p<0.001$). Compared to N+PO, S+PO group has shown increase in lipid peroxidation ($p<0.01$). Compared to N+CO, S+CO group has shown decrease in SOD ($p<0.05$) and increase in lipid peroxidation ($p<0.05$). Whereas compared to N+VP, S+VP group has shown decrease in SOD ($p<0.01$) and increase in lipid peroxidation ($p<0.001$).

DISCUSSION

Due to the alleged involvement of SFAs on CVD many people in India have shifted towards TFA rich VP

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Table1: Diet composition[37]

Diet[a]	GNO Diet	PO Diet	CO Diet	VP Diet
Casein	20	20	20	20
DL-Methionine	0.3	0.3	0.3	0.3
Corn Starch	12.5	12.5	12.5	12.5
Sucrose	47.5	47.5	47.5	47.5
Cellulose	5	5	5	5
Groundnut oil	10	-	-	-
Palm oil	-	10	-	-
Coconut oil	-	-	10	-
Vanaspathi	-	-	-	10
AIN Mineral mix	3.5	3.5	3.5	3.5
AIN Vitamin mix	1	1	1	1
Choline bitartrate	0.2	0.2	0.2	0.2

[a]*g/100g weight. ; GNO- Groundnut oil, PO- Palm oil, CO- Coconut oil and VP- Vanaspathi.*

Table 2- Effect of Groundnut oil (GNO), Palm oil (PO) and Coconut oil (CO) and Vanaspathi (VP) on lipid profile in rats under normal (N) and stress (S) conditions.

	Normal Condition				Stress Condition			
Parameters	**GNO Diet**	**PO Diet**	**CO Diet**	**VP Diet**	**GNO Diet**	**PO Diet**	**CO Diet**	**VP Diet**
TC$^{\Delta}$	62.6±1.6	73.6±1.0**	61.9±3.1	74.8±1.1**	71.4±1.0 ###	77.5±0.8 # $$	64.9±1.3 $$$	85.2±1.3### $$$
HDL-C$^{\Delta}$	22.7±0.6	23.3±0.6	23.2±0.7	21.6±0.6	23.2±0.4	23.6±0.5	23.1±0.4	22.9±0.4 $$$
LDL-C	16.3±1.2	24.58±1.4*	18.89±2.6	22.19±0.9	19.9±0.5#	23.7±1.0	19.4±1.2	27.4±0.1 ##$$$
TG$^{\Delta}$	117.8±2.4	128.1±2.8	98.9±3.1***	155.1±2.8***	141.5±5.9##	151.2±2.0 ###	112.2±4.4# $$$	174±3.1 ## $$$
Atherogenic Index	1.77±0.09	2.17±0.09*	1.66±0.10	2.48±0.09***	2.1±0.06#	2.30±0.09	1.81±0.06	2.71±0.06 # $$$

*Units: $^{\Delta}$mg/dl ; Values show the means ± SEM of 6 rats in each group; p values: *<0.05, **<0.01,***<0.001, as compared with N+GNO Diet; $<0.05, $$<0.01,$$$<0.001, as compared with S+GNO Diet; #<0.05, ##<0.01, ###<0.001 as compared with respective normal groups.*

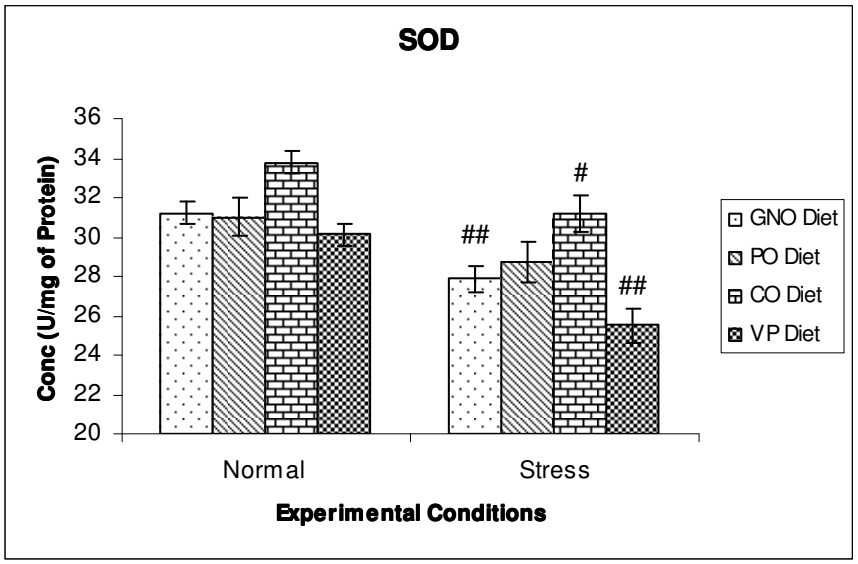

***Figure 1: Effect of Groundnut oil (GNO), Palm oil (PO), Coconut oil (CO) and Vanaspathi (VP) on Superoxide dismutase* activity in rats under normal and stress conditions.**

p values: #<0.05, ##<0.01, ###<0.001 as compared with respective normal group. Bars represent mean values ± SEM of six rats in each group.

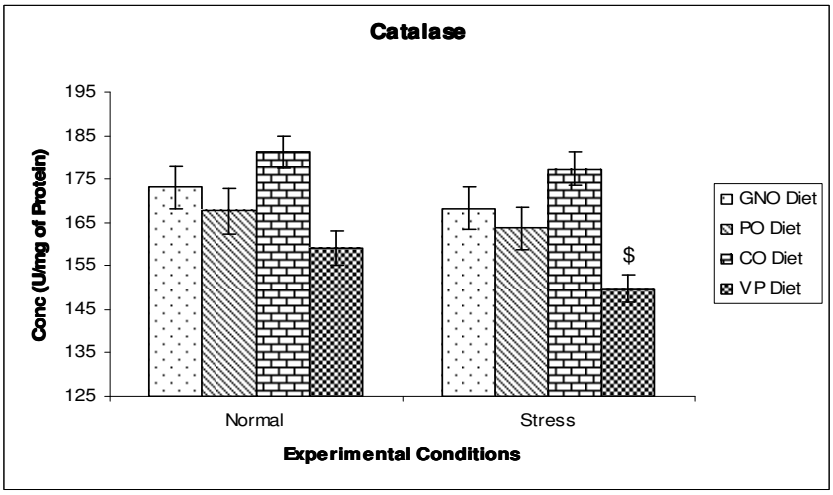

Figure 2: Effect of Groundnut oil (GNO), Palm oil (PO), Coconut oil (CO) and Vanaspathi (VP) on Catalase activity in rats under normal and stress conditions.

p values: $ <0.05, as compared with S+GNO diet;Bars represent mean values ± SEM of six rats in each group.

instead of SFA rich CO, PO and butter (21, 22). On the contrary in the present study compared to control diet, CO diet fed rats under normal conditions have shown significant decrease in TG (Table 2) and significant decrease in TC and TG under stress conditions (Table 2). There are reports available on the hypocholesterolemic and hypolipemic properties of CO in the literature (46-48). Non-significant increase in the SOD, catalase and decrease in the lipid peroxidation were observed in the study by CO diet under normal and stress conditions, signify the potential role of CO diet in reducing atherosclerosis. These beneficial effects observed with CO diet may be due to the fact that, fatty acid content in CO diet must be exerting a beneficial role. SFAs in coconut oil are of short and medium chain length. Nearly 15% of the total fatty acids are composed of short chain fatty acids (caprylic – C8:0 and capric acid-C 10:0), about 48% of fatty acids are of medium chain (lauric acid- C 12:0); and supplies only 2% linoleic acid (ω-6 PUFA) (48). It is well known

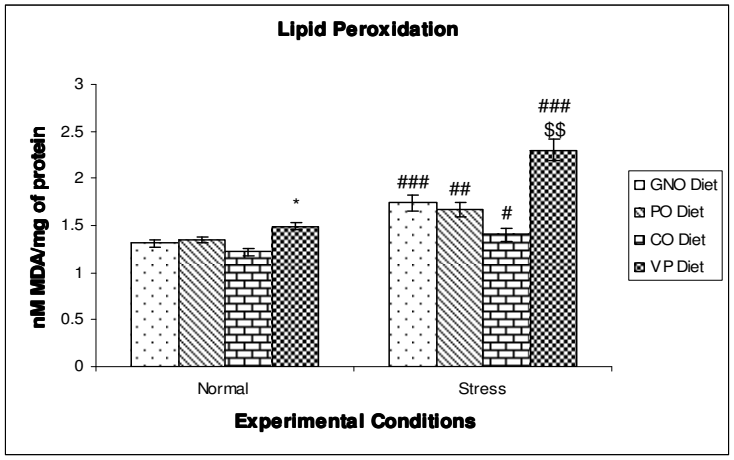

Figure 3: Effect of Groundnut oil (GNO), Palm oil (PO), Coconut oil (CO) and Vanaspathi (VP) on TBARS formation in rats.

*. p values: *<0.05 as compared with N+GNO diet; $$<0.01 as compared with S+GNO diet; #<0.05, ##<0.01, ###<0.001 as compared with respective normal group. Bars represent mean values ± SEM of six rats in each group*

fact that excess linoleic acid increases oxidative stress (17). Because CO contains mostly short and medium chain SFAs it is easily digested, absorbed and utilized by the body. FA are transported to the liver via portal vein and preferentially used for energy production and appeared to have less fat deposition when compared to long chain fatty acids (48). Lauric acid is abundant in CO and is considered responsible for many of its health benefits and the only other abundant source found in nature is in human breast milk (29, 47). Moreover lauric acid is also effective as an antiplaque agent (49). Whereas PO diet when compared to control has significantly increased the TC, LDL-C and atherogenic index under normal conditions (Table 2). There were no significant changes in the SOD, catalase and lipid peroxidation levels under normal and stress condition. It has been also found that PO diet under stress conditions has produced significant increase only in TC level (Table: 2). Increase in lipid profile by PO diet may be due to its low content of lauric acid and increased content of palmitic acid. Palmitic acid is suspected to possess hypercholesterolemic effect (50 – 52).

It has been reported in the literature that an increase in the dietary intake of TFA shifts the physiological state to one that is prothrombotic, proaggregatory, proconstrictive, and proinflammatory state which may increase the risk to develop CVD (27, 28, 53-56). VP by increasing lipid profile (27, 53, 57) possesses deleterious effects in causing and aggravating the CVD complications (27, 58, 59). In the present study normal rats fed VP diet, compared to control, has shown significant increase in TC, TG and atherogenic index (Table 2). Besides, under stress conditions VP diet has further aggravated the lipid profile by producing significant increase in TC, LDL-C, TG and atherogenic index (Table 2). These results clearly indicate the deleterious effects of TFAs in VP diet on lipid profile. Moreover, high consumption of TFA is associated with oxidative stress in humans, which could increase the risk of the development or acceleration of several diseases, such as atherosclerosis, cancer and type 2 diabetes (19). Significant decrease in catalase under stress condition (Fig 2) and significant increase in lipid peroxidation under normal and stress conditions (Fig 3) observed in the study by VP signifies the potential role of TFAs in free radical generation. Meanwhile the exaggerated increase in lipid profile under stress condition supports the hypothesis that oxygen free radicals are involved in the development of hypercholesterolemic atherosclerosis (61).

Overall observation reveals that compared to the individual normal group, their respective stress groups have shown deleterious effects under stress condition in causing alterations in both lipid profile and endogenous antioxidant enzymes in the descending order: S+VP, S+GNO, S+PO and S+CO group (Table 2) (Fig 1-3). S+VP group has shown maximum deleterious effects. Hence it is important to note at this point that, both TFA and stress are having free radical generating nature (19, 62, 63). As such native cholesterol is not atherogenic unless it is modified by

oxidation (64). Excess free radical generation by TFA increases the susceptibility of LDL –C to oxidative modifications that could play an important role in the pathogenesis of several diseases. Oxidized lipoproteins are found to possess diabetogenic and atherogenic property (64). Therefore the increased consumption of TFA under stressful conditions can further enhance the oxidative stress which is mainly due to the depletion of endogenous antioxidants. Likewise both psychological stress and TFAs are known to induce the production of proinflammatory agents (4, 5, 55). Excess intake of TFA in the peripheral blood causes overproduction of proinflammatory cytokines (56). Thus consumption of TFA in the present day stressful conditions can exacerbate CVD conditions.

CONCLUSION

The present study highlights the significance of traditional fat CO in the diet. Our findings are in agreement with previous study reported by other researchers (46, 65, 66). CO though contains SFA, it possesses lauric acid thus exerts beneficial effect. Present study claims the protective role of lauric acid rich CO against atherogenic index and lipid peroxidation under normal and stress conditions. PO though contain SFA due to low content of lauric acid and high content of palmitic acid was found to possess less beneficial effect than CO, but definitely safer than VP in causing atherogenicity. Whereas VP though useful for increasing the shelf life of many eatables; by virtue of its hyperlipidemic and oxidative stress generating ability exhibits deleterious effects in causing atherogenicity. The consumption of high content of TFAs may aggravate the occurrence of CVD, this effect being worse when accompanied with stress. It can be concluded that the quantity of medium chain fatty acid, lauric acid, in the dietary oil must be playing a crucial role in the body against atherogenicity under normal and stress conditions.

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