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Serum lipids, lipid peroxidation and glutathione peroxidase activity in rats on long-term feeding with soybean oil or palm oil.

Ima-Nirwana S[a], Z. Merican[a], Jamaluddin M[b] and Khalid BAK[c]

Departments of Pharmacology[a] Pathology[b] and Medicine[c], Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

The atherogenic potential of soybean oil (Sb) and palm oil (PO) was compared by measuring lipid profile, lipid peroxidation (LP) and activity of the antioxidant enzyme glutathione peroxidase (GSHPx) in rat sera and liver and heart homogenates. Male *Rattus norwegicus* rats were fed a basal diet, or basal diet fortified with 20% weight/ weight Sb or PO for 4 or 9 months. There was no difference in high density lipoprotein cholesterol:low density lipoprotein cholesterol ratio between the two groups, but triglyceride concentrations were higher in the PO fed rats compared to the Sb fed rats, although the difference diminished after 9 months. No differences in serum LP and GSHPx activity were seen between the two groups. In the liver and heart, LP was lower in PO after 4 months feeding, but the reverse was seen after 9 months. Liver and heart GSHPx activity was higher in the PO group after both treatment periods. In conclusion, both PO and Sb fed rats appeared comparable in their lipid profile, but the PO food had a temporary beneficial effect on the LP process in liver and heart. GSHPx activity however did not correlate well with LP in liver and heart, suggesting involvement of other antioxidants.

Introduction

Raised levels of low density lipoprotein cholesterol (LDL-chol), as well as reduced high density lipoprotein cholesterol:low density lipoprotein cholesterol (HDL-chol:LDL-chol) ratios are risk factors in atherosclerosis. Lipid peroxides have also been shown to contribute to the development of atherosclerosis, possibly due to oxidation of LDL. Macrophages accumulate malonaldehyde (MDA)-treated low density lipoproteins and form the foam cells of atheroma more so than with native LDL[1-3]. High levels of lipid peroxidation (LP) products have been associated with an atherogenic lipid profile[4,5] while the activity of the antioxidant enzyme glutathione peroxidase (GSHPx) was found to be negatively correlated with LDL-chol levels[5]. Regnstrom et al[6] found an association between susceptibility of LDL to oxidation and severity of coronary atherosclerosis. Thus, LP and hypercholesterolaemia are both risk factors in the promotion and progression of atherosclerosis.

Saturated animal fats rich in cholesterol contribute to the development of atherosclerosis. However, vegetable oils, while being devoid of cholesterol, contain large amounts of polyunsaturated fatty acids (PUFA) that undergo lipid peroxidation when exposed to free radicals in vitro and in vivo. Lipid peroxidation, being an autocatalytic process, generates more free radicals and MDA that can oxidise LDL, therefore worsening atherosclerosis.

Diets rich in the polyunsaturated soybean oil were found to produce higher levels of LP products compared to diets enriched with the more monounsaturated olive oil[7]. Monounsaturated palm oil produced lower levels of serum MDA compared to the more saturated butterfat[8]. These vegetable oils contain varying amounts of vitamin E and carotenoids, which can act as antioxidants to detoxify the hydroxy and peroxy radicals, thus controlling LP(9,11). Therefore, the overall LP process may not depend solely on the fatty acid composition of the oil, but also on the content of antioxidants, such as vitamin E and carotenoids, found naturally in these oils.

Selenium-dependent glutathione peroxidase (GSHPx) is an important antioxidant enzyme found in abundance in organs exposed to high levels of LP, such as the liver, lungs and heart[12]. It catalyses the conversion of lipid hydroperoxides to hydroxy acids in the presence of reduced glutathione. Addition of several types of antioxidants, such as vitamin E and selenium in animal diets has been found to reduce the formation of products of LP[13,14]. In addition to its effects on LP, tocopherols, tocotrienols and selenium were also reported to lower serum cholesterol and reduce atherosclerosis in humans and animals[15-18].

In this study, we compared the atherogenic potential of soybean oil and palm oil by measuring serum lipid profile, LP products and GSHPx activity. The amounts of LP in liver and heart homogenates were also measured and correlated to the tissue GSHPx activity.

Materials and Methods

Animals and diets

Male *Rattus norwegicus* rats weighing between 145-165g (age approximately 2 months) were divided into 3 groups of 8 rats. The control group (K) was fed a basal diet (Gold Coin, Port Klang, Malaysia)[19]. The other 2 groups were fed the basal diet fortified with 20% w/w (weight/weight) either soybean oil (Sb) (Yee Lee Corporation, Ipoh, Malaysia) or palm olein (PO) (Lam Soon, Petaling Jaya, Malaysia). The approximate fatty acid composition of Sb and PO were reported by other authors[20] (Table 1).

The rats were housed 4 per cage at room temperature with a 12 hour light/ dark cycle. Food and tap water was available ad libitum for 4 or 9 months. At the end of 4 or 9 months the rats were fasted overnight, anaesthetised with Pentobarbitone Sodium and sacrificed. Samples of serum, liver and heart were taken for analyses of MDA and CD concentrations, and GSHPx activity. Serum was also taken for lipid profile.

Correspondence address: Dr. Ima Nirwana Soelaiman, Dept. of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jln. Raja Muda Abd. Aziz, 50300 Kuala Lumpur, Malaysia.
Tel: +603-292-3066 ext. 5514; Fax: +603-291-2659.

Table 1. Fatty acid composition of the oils used in the study.

Fatty Acid	Percent of total fatty acids (%)	
	Palm olein	Soybean oil
12:0	0.2	0.1
14:0	1.0	0.1
16:0	38.2	10.5
18:0	4.0	40
18:1	43.2	21.5
18:2	10.8	55.5
18:3	0.2	7.8
20:0	0.4	04
Saturated	43.8	15.1
Monounsaturated	43.2	21.5
Polyunsaturated	11.0	63.3

(adapted from Marzuki et al20).

Sample preparation

Blood was taken from the common carotid artery and allowed to clot at room temperature for 30 minutes. The clotted blood was centrifuged at 3,000 rpm for 25 minutes. The supernatant was divided into aliquots and stored at -700°C until analysed Liver and heart homogenates were prepared as described by Stocks et al[21]. All work was done in ice to minimise peroxidation in vitro.

Assays of MDA and CD concentrations, and GSHPx activity

Measurement of MDA was modified according to Ledwozyw et al[4] and Yagi[22]. Distilled water (0.1 ml) was added to 0.4 ml of the serum or homogenate samples and mixed. 2.5 ml 1.22M trichloroacetic acid 0.6M HCl was added to the mixture, mixed and left to stand at room temperature for 15 minutes 1.5 ml 0.67% thiobarbituric acid 0.05M NaOH was then added to the mixture, mixed and incubated in boiling water for 30 minutes.

The mixture was cooled to room temperature. Then 4 ml n-butanol was added to the mixture and mixed. The top layer consisting of n-buthanol was then taken and the optical density measured using a spectrofluorometer (Shimadzu RF-5000) at excitation and emission wavelengths 515 nm and 553 nm respectively

CD was measured according to Buege and Aust[23]. Measurement of GSHPx activity was as described by Beutler et al[24].

The above measurements were expressed per g of protein. Total protein content of the samples were determined using the computerised autoanalyser, Hitachi 717, based on the Biuret method.

Serum lipid profile assay

The parameters measured were T-chol, TG (triglycerides) and HDL-chol. The analyses was done using kits (Boehringer Mannheim, Germany). All measurements were made using an Hitachi 717 computerised autoanalyser. LDL-chol concentration was obtained by calculation.

Analysis of data

The results obtained were analysed via analysis of variance and Student's t test. A $p<0.05$ was considered significant. This study was approved by the Research and Ethics Committee, Medical Faculty, Universiti Kebangsaan Malaysia, and confirmed by the University's Central Research Committee.

Results

Table 2 shows the serum lipids of the three diet groups after 4 and 9 months of feeding. HDL-chol was lower in both groups given Sb and PO after 4 and 9 months feeding periods. LDL-chol increased with the addition of Sb and PO, and the increase was higher in the group given PO. The ratio of HDL-chol:LDL-chol was lower in both Sb and PO groups, but there was no difference between the two groups. T-chol increased with the addition of Sb or PO for 4 months, the increase being higher in the PO group. However, the differences became insignificant after 9 months. TG concentration declined after 9 months in the groups with added Sb and PO. However, it was highest in the PO group after both feeding periods.

Serum MDA and CD levels, and GSHPx activity are shown in Table 3. Serum MDA and GSHPx did not change with the increased duration of treatment, but serum CD levels declined. No difference was seen between diet groups.

Liver and heart MDA, CD and GSHPx increased after 9 months compared to 4 months (Table 4). After 4 months of feeding, the Sb group had higher MDA and CD levels in the liver and heart, while the PO group had lower levels. However, after 9 months, heart MDA and CD became higher in the PO group, while no significant differences were seen in the liver tissues. The PO group showed highest liver GSHPx activity after both periods of treatment and highest heart GSHPx activity after 9 months.

Table 3. Serum malonaldehyde (MDA) and conjugated diene (CD) concentrations, and glutathione peroxidase (GSHPx) activity in of rats fed soybean oil or palm oil after 4 and 9 months of feeding.

Diet	Duration of feeding (months)	MDA (μmol/g protein)	CD (OD/g protein)	GSHPx (OD/g protein)
Control	4	0.79±0.08	11.0±1.8	30.9±9.7
	9	0.88±0.15	4.7±1.4*	29.4±7.9
Sb	4	0.77±0.12	10.8±1.9	44.7±14.9
	9	0.83±0.04	4.6±1.4*	27.3±7.7
P0	4	0.82±0.12	9.0±2.5	35.8±8.7
	9	0.90±0.15	5.4±1.5*	27.0±7.1

Values marked * are different from values at beginning of feeding (0 months) within the same column at p<O.05. Values bearing the same alphabetical superscript are different at p<O.05 Values are given as mean ± SD (n = 8).

Table 2. Serum lipid profile of rats fed soybean oil or palm oil for 4 or 9 months.

Diet	Duration of feeding (months)	Serum lipids (mmol/L) HDL-chol	LDLchol	HDL-chol / LDL-chol	T-chol	TG
Control	4	^{ab}0.51±0.06	^{ef}0.67±0.12	^{ij}0.76±0.13	^{mn}1.3±0.2	^{p}0.70±0.22
	9	^{cd}0.52±0.07	^{h}0.89±0.17	^{kl}0.60±0.13	1.5±0.2	0.62±0.17
Sb	4	^{a}0.41±0.04	^{eg}0.96±0.12	^{j}0.43±0.06	^{mo}1.5±0.1	^{q}0.71±0.20
	9	^{c}0.40±0.04	1.04±0.12*	^{x}0.39±0.04	1.5±0.1	
PO	4	^{b}0.42±0.07	^{fg}1.14±0.22	^{j}0.39±0.12	^{no}1.8±0.1	^{pq}1.15±0.38
	9	^{d}0.41±0.04	^{h}1.10±0.14	^{l}0.38±0.05	1.7±0.2	^{r}0.73±0.22*

Values marked * are different from values at beginning of feeding (0 months) within the same column at p<0.05. Values bearing the same alphabetical superscript are different at p<0.05. Values are given as mean ± SD (n = 8).

Table 4. Malonaldehyde (MDA) and conjugated diene (CD) concentrations, and glutathione peroxidase (GSHPx) activity in liver and heart of rats fed soybean oil or palm oil after 4 and 9 months of feeding.

Diet	Duration of feeding (months)	MDA (μmol/g protein)	CD (OD/g protein)	GSHPx (OD/g protein)
Liver				
Control	4	^{a}1.4±0.3	69.0±8.3	^{h}592.2±167.9
	9	11.4±3.0*	189.0±26.4*	^{jk}1084.3±133.3*
Sb	4	^{a}2.4±0.6	66.9±109	^{i}833.9±206.3
	9	14.7±4.1*	191.6±36.8*	^{j}1665.4±308.7*
P0	4	1.9±0.3	68.2±14.1	^{hi}1159.0±378.9
	9	10.7±3.5*	166.8±27.8*	^{k}1648.4±535.8*
Heart				
Control	4	^{b}3.6±1.1	^{c}133.8±36.8	2200.4±531.8
	9	^{d}22.8±4.7*	336.4±44.9*	1723.6±88.8*
Sb	4	^{bc}5.5±0.9	^{f}107.1±9.5	2169.7±642.3
	9	29.8±6.1*	^{g}296.7±40.8*	^{m}831.0±150.9*
P0	4	^{c}4.3±0.8	^{ef}75.9±11.8	2054.5±603.1
	9	^{d}33.8±2.6*	^{g}363.6±73.5*	^{lm}1185.7±167.4*

Values marked * are different from values at beginning of feeding (0 months) within the same column at p<0. 05. Values bearing the same alphabetical superscript are different at p<0.05 Values are given as mean ± SD (n = 8).

Discussion

The addition of 20% Sb or PO had an adverse effect on serum lipids as a whole. This difference could be because the basal rat diet contained only 2.5% fat[19]. PO proved to be slightly more hypercholesterolaemic than Sb, but the difference became insignificant when treatment was continued for 9 months. However the HDL-chol concentration and the HDL-chol:LDL-chol ratio, a more useful index of atherogenicity did not differ between the two groups. This finding is in agreement with Marzuki et al[20], but Sundram and co-workers[25] found that PO increased HDL-chol compared to Sb. Zhang et al[26] however, found that PO raised plasma cholesterol significantly compared to Sb. The above studies differed from this study in their much shorter duration of feeding, and Marzuki et al[20] used human volunteers instead of rats. It should be noted that the differences in LDL-chol and T-chol levels between Sb and PO were only significant after 4 months and became insignificant when feeding was continued until 9 months, even though PO contained more saturates than Sb. This could be attributed to the high palmitic acid (16:0) content of PO (Table 1), which was found to enhance HDL production[27]. Also PO is rich in alpha- and gamma-tocotrienol[25], while Sb is rich in gamma- and delta-tocopherol, and this may confer some protection against hypercholesterolaemia in the animals fed these oils.

PO was found to induce hypertriglyceridaemia compared to control and Sb after 4 months. Similar to the observation on serum lipoproteins, the difference became less when feeding was continued to 9 months, where significant difference was only seen between PO and Sb. It may be worthwhile to observe the changes after a longer feeding period. It should be noted that the TG levels declined to that of control values after 9 months in the Sb and PO groups. Sundram et al[25] did not find any significant difference in rat TG after 15 weeks of feeding, while Marzuki et al[20] observed higher TG levels in adolescents fed Sb compared to PO for 5 weeks. Different dietary saturated fats have been shown to have unique effects on TG metabolism[28] and palmitic acid was found to raise serum TG in hamsters[27]. Hypertriglyceridaemia was associated with an increased risk of coronary heart disease in univariate studies, but the association was not seen in multivariate studies. Whether or not TG is a causal factor in development of atherosclerosis still remains to be studied[29,30].

An important observation was that there were no differences in serum MDA, CD and GSHPx between the control, PO and Sb groups after both feeding periods. Oxidation of serum lipoproteins is a major contribution to the overall LP products found in serum. This may indicate that oxidation of serum lipoproteins was not increased by addition of Sb or PO to the low-fat basal diet. Monounsaturates from sunflower oil and olive oil have been shown to protect LDL against oxidative damage[7,31]. Also, the vitamin E in both oils plays an important free-radical scavenging role. Alpha-tocopherol was observed to confer some early protection against peroxidation of LDL) but is not the main antioxidant in LDL[32].

Therefore, adding 20% Sb or PO to a low fat diet was atherogenic in terms of cholesterol levels, but there was not much difference between the two oils, both in terms of serum lipids and serum lipid peroxidation. The only significant difference was seen in TG levels, but the difference tended to diminish with long-term feeding. Further studies could be done to correlate peroxidation of LDL fractions and extent of atherosclerosis in animals fed Sb or PO diets.

In general, LP products were higher in the liver and heart homogenates of the older rats (9 months feeding) compared to the younger group (4 months feeding), and this rise was also seen on addition of Sb and PO. The longer study duration increased the animals exposure to peroxidative stress, a finding which agrees with Wu et al[33]. GSHPx activity increased in accordance with the increased LP in the liver, however, in the heart, the enzyme activity decreased after 9 months, while LP increased more than doublefold. Thus, it appeared that the heart is less able to withstand peroxidative stress as compared to the liver. Other researchers have found conflicting results in fibroblasts[34] and brain tissue[35].

The addition of 20% Sb or PO to the diet did not consistently increase the LP products in liver and heart homogenates. This could be because of the antioxidant effect of vitamin E found in both oils. After 4 months of feeding, the more polyunsaturated Sb had higher liver and heart LP levels, consistent with the fact that PUFA are more prone to peroxidative damage. Huang and Fwu[36], also observed higher LP in rats fed diets with high Sb compared to low Sb for 8 weeks. The more monounsaturated PO did not increase LP products, in fact, heart CD concentration was lower than the control group. Monounsaturated fatty acids are protective against LP[7,31]. Furthermore, PO is rich in tocotrienols.

In animals fed for 9 months, there was an increase in LP in the heart tissue of the PO group. Thus, the protective effect of PO in the heart seemed to diminish with long-term feeding. PO appeared to be a more potent inducer of GSHPx activity in liver and heart homogenates after both periods of treatment, but the enzyme activity did not correlate well with the extent of LP. This could be because there are other endogenous antioxidants, such as catalase and superoxide dismutase.

In conclusion, both PO and Sb appeared comparable in their atherogenic potential. PO may have a temporary beneficial effect on LP process in liver and heart tissues. GSHPx activity did not correlate well with levels of LP in liver and heart and therefore the role of other antioxidants may be important. Long term feeding (9 months) saw a decline in serum TG, CD and heart GSHPx activity. Similar results were seen using saturated fats (unpublished data). Further studies on lipoprotein and TG metabolism, LP and total antioxidant activity after long term feeding with these oils will be useful.

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豆油、棕榈油長期餵養大鼠的血清脂類、脂類過氧化作用和谷胱甘肽過氧化物酶活性

摘要

作者測定了大鼠血清、肝臟和心臟匀漿的脂類、脂類過氧化作用（LP）和抗氧化酶、谷胱甘肽過氧化物酶（GSHPX）活性，從而比較豆油和棕榈油致動脈粥樣硬化的可能性。他們選用 Rattus Norwegicus 大鼠爲對象，喂以基本膳食，或強化20%豆油或棕榈油的基本膳食4或9個月。結果發現，兩組的高密度脂蛋白膽固醇／低密度脂蛋白膽固醇的比值無差異。但棕榈油組大鼠的甘油三酯濃度高於豆油組（喂飼9個月後這些差異減少）。兩組的脂類過氧化作用和谷胱甘肽過氧化物酶活性無差異。喂飼4個月後，棕榈油組大鼠的肝臟和心臟脂類過氧化作用較低，但喂飼9個月後升高。總結：棕榈油組大鼠肝臟和心臟的過氧化作用具暫時的有利影響，但谷胱甘肽過氧化物酶活性與過氧化作用沒有發現有良好關係，作者認爲可能有其它抗氧化劑的參與。

Lipid serum, peroksidasi lipid dan aktiviti glutation peroksidase pada tikus yang diberi makan minyak kacang soya atau minyak kelapa sawit jangkapanjang.

Kajian ini membandingkan potensial aterogenesis minyak kacang soya (Sb) dan minyak kelapa sawit (PO) dengan menentukan profil lipid serum, proses peroksidasi lipid dank aktiviti enzim antioksidan glutation peroksidase (GSHPx) di dalam serum dan homogenat hepar dan jantung tikus. Tikus Rattus norwegicus jantan diberi makan diet asas, ataupun diet asas yang ditambah dengan 20% berat/berat Sb atau PO selama 4 atau 9 bulan. Tiada terdapat perbezaan pada nisbah kolesterol lipoprotein ketumpatan tingi:kolesterol lipoprotein ketumpatan rendah di antara kumpulan Sb dan PO. Kepekatan trigliserid adalah lebih tinggi pada kumpulan PO berbanding kumpulan Sb, tetapi perbezaan ini berkurangan setelah 9 bulan. Proses peroksidasi lipid dan aktiviti GSHPx serum tidak berbeza di antara kumpulan Sb dan PO. Proses peroksidasi lipid di hepar dan jantung adalah lebih rendah pada kumpulan PO berbanding Sb di akhir kajian 4 bulan, tetapi keputusan sebaliknya dicerap sesudah 9 bulan. Aktiviti GSHPx hepar dan jantung adalah lebih tinggi pada kumpulan PO setelah 4 dan 9 bulan kajian. Kajian ini menunjukkan bahawa Sb dan PO tidak banyak berbeza dari segi profil lipid serum, tetapi PO mempunyai kesan menguntungkan yang sementara terhadap proses peroksidasi lipid di hepar dan jantung. Aktiviti GSHPx tidak berkorelasi dengan baik dengan proses peroksidasi lipid di hepar dan jantung, menandakan penglibatan antioksidan-antioksidan yang lain.

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