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Effects of Heated Vegetable Oils on Serum Lipids and Aorta of Ovariectomized Rats

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Abstract: This study examined the effects of heated vegetable oils in estrogen deficient rats. Eighty female Sprague-Dawley rats were divided equally into eight groups and given treatment as follows: (I) intact (non-ovariectomised), basal diet (control group); (II) ovariectomised, basal diet; (III) ovariectomised, basal diet fortified with 15% weight/weight (w/w) fresh soya bean oil (FSO); (IV) ovariectomised, basal diet fortified with 15% weight/weight (w/w) soya bean oil heated once (1H-SO); (V) ovariectomised, basal diet fortified with 15% weight/weight (w/w) soya bean oil heated five times (5H-SO); (VI) ovariectomised, basal diet fortified with 15% weight/weight (w/w) fresh palm oil (FPO); (VII) ovariectomised, basal diet fortified with 15% weight/weight (w/w) palm oil heated once (1H-PO); (VIII) ovariectomised, basal diet fortified with 15% weight/weight (w/w) palm oil heated five times (5H-PO). Duration of treatment was 6 months. Blood was taken at baseline and monthly interval for 6 months for determination of serum lipid profiles and malondialdehyde (MDA) levels. Serum homocystein and interleukin-6 were assayed at baseline and after 6 months of study. At the end of the study the rats were killed and consistent segments of the ascending aorta were taken for histopathological examination. The specimens were sectioned transversely and stained with haematoxylin-eosin and Verhoeff van Gieson for light microscopy. Measurements of the intimal thickness and the ratio between tunica intima / tunica media were calculated using computerised image analyser. Heated and fresh palm oil cause transient changes in lipid profiles, whereas soya oil; fresh, heated once and heated five times as well as heated once palm oil caused an increase in serum LDL-cholesterol. Fresh and heated vegetable oils diet did not alter the ratio between tunica intima and tunica media, serum MDA and homocystein level. Histological study showed no obvious focal or diffuse atherosclerotic plaque formation with an intact internal elastic lamina and no evidence of smooth muscle cell migration.

Key words: Heated palm oil, soya oil, atherosclerosis, ovariectomized rats

Introduction

The association between diet, plasma lipid concentrations and atherosclerosis has been well-documented and reviewed (Steinberg and Witztum, 1990). Atherosclerotic lesions in man and in animals appear to be related to elevated plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), decreased high-density lipoprotein cholesterol (HDL-C) and excess fat consumption. The mechanisms by which hypercholesterolemia (almost always associated with increased LDL-cholesterol) caused atherosclerosis is not clear. There is considerable evidence to suggest that oxidative damage to LDL significantly increased LDL atherogenicity. Oxidised LDL (oLDL) is believed to have several mechanisms of promoting atherogenicity. Oxidised LDL may directly alter both the structure and function of the endothelial cells. Secondly it may chemotactically attract monocytes or macrophages to the endothelium which develop then into lipid laden foam cells of an atheromatous plaque. Oxidised LDL is

also taken up by macrophages more rapidly than unoxidised LDL. Inflammatory and immune processes have been shown to be implicated in the pathogenesis of atherosclerosis. The mediators of immune and inflammatory response, such as cytokines IL-1 and TNF may influence their development (Antonio and Rodolfo 1990; Hegsted, 1981; Golden and Ramdath, 1987; Prasad and Kalra, 1989).

Hyperhomocysteinemia has been identified as one of the risk factors for cardiovascular disease. Hyperhomocysteinemia can damage blood vessels, cell structures, blood lipids and eventually lead to the development of atherosclerosis and other forms of heart disease.

Soya and palm oil are the leading source of the world supply of oils and fats (Yeong, 2001).

Studies have reported that soya and palm oils reduce both total and LDL cholesterol concentrations and increase HDL-C concentration compared to coconut oil, which is rich in lauric and myristic acids (Marzuki *et al.*,

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1991; Flider, 2001; Jackson *et al.*, 1978; Kamsiah and Nafeeza, 1997; Shepherd *et al.*, 1980; Vega *et al.*, 1982; Ima-Nirwana *et al.*, 1996).

Much of the fat consumed in our diet has been exposed to heat during processing and in the preparation of food during cooking. In deep-frying, often the fat is kept hot for a long period of time at 180°C and both moisture and air are mixed into the hot oil. This heating process generates free radicals. The fried food absorbs this heated oil and free radicals thus it becomes part of our diet. The common practice of reusing these heated oils for frying may generate more free radicals that are harmful to tissues (Hill *et al.*, 1982; Isong *et al.*, 1992; Narasimhamurthy and Raina, 1999; Corcos *et al.*, 1990; Friedewald *et al.*, 1972). Several studies have also demonstrated the adverse effect of oxidized dietary fats on humans and experimental animals. These include hemolytic anemia, increased blood clotting time and hepatomegaly (Owu *et al.*, 1998). Reproductive toxicity, elevation of total cholesterol and free fatty acid levels of various tissues, thrombocytopenia and enhanced platelet aggregation levels have also been documented (Isong *et al.*, 1992; Narasimhamurthy and Raina, 1999). Other known effects of oxidized fats include essential fatty acid deficiency, nucleic acid deficiency and micronutrient malnutrition resulting in deactivation of key metabolic enzymes (Hill *et al.*, 1982; Isong *et al.*, 1992). In many parts of the world including Malaysia, there is a tendency for the oil to be used repeatedly in frying and cooking. Such practice appears to cut the cost of cooking without due consideration of its effect on health. The harmful effect of the thermally oxidized oil on lipid profiles and its relation with the development of atherosclerosis has not been much explored. This aspect is also important, as studies have reported that lipoprotein oxidation is a necessary step in the development of fatty streaks into atherosclerotic plaques (Antonio and Rodolfo, 1990; Hegsted, 1981; Golden and Ramdath, 1987; Prasad and Kalra, 1989).

Estrogen deficiency occurring in post-menopausal women predisposes these women to atherosclerosis. Estrogen has antioxidant properties and can protect the body from the effects of lipid peroxidation. In view of the potential hazardous effect of heated oils on health, we undertake this study to see whether thermally oxidized soya and palm oils have any effect on the factors affecting atherosclerosis in rats made estrogen-deficient by ovariectomy. Estrogen-deficient female rats are used to intensify the effects of heated oils on lipid peroxidation and free-radical production. This will give us some information on the effects of repeated intake of reheated vegetable oils on menopausal women in particular, and the population in general.

Materials and Methods

Eighty female rats of Sprague-Dawley species (200-250g) were randomly divided equally into eight groups. All the rats were ovariectomized except for rats in group I. The rats were given the following prescribed course of food: (Group I) basal diet as the control (without oil) non ovariectomized, or (Group II) basal diet as the control (without oil), or (Group III) basal diet fortified with 15% weight/weight (w/w) fresh soya bean oil (FSO), or (Group IV) heated once soya bean oil (1H-SO), or (Group V) heated five times soya bean oil (5H-SO), or (Group VI) fresh palm oil (FPO), or (Group VII) heated once palm oil (1H-PO), or (Group VIII) heated five times palm oil (5H-PO) for 24 weeks. The animals were housed in stainless steel cages at room temperature of $27 \pm 2^\circ\text{C}$ and were quarantined for a two-week period prior to introduction of the test diets. All the test and control animals had free access to food and tap water for 24 weeks. The fasting serum lipid and MDA were taken at baseline and at intervals of 4 weeks for 24 weeks, whilst serum homocystein was done at baseline and end of 6 months. The rats were killed and aortic tissue was taken from consistent segment of the ascending aorta for histopathological examination 24 weeks after feeding with the heated vegetable oils. The specimens were sectioned transversely and stained with hematoxylin-eosin for light microscopy. Tissue sections of aorta were stained with Verhoeff van Gieson for easy identification of different fibres such as elastin, collagen and muscle. Measurement of the intimal thickness were calculated using computerised image analyser. The image of each slide was captured and analysed with an image analyser. The mean intimal thickness will be calculated from lumen to the internal elastic lamina and the ratio between tunica intima / tunica media was measured.

Source and preparation of heated oil diets: The vegetable oils used were palm oil (Lam Soon Edible Oils, Malaysia) and soya oil (Yee Lee edible oils, Malaysia). The oils were used fresh, heated once or heated five times (as described by Owu *et al.*, 1998). The heating process involves using 2500 ml of the vegetable oil to fry 1 kg of 'keropok lekor' (fish-flavored chips) in a metal wok. The temperature of the heated oil reached about 180°C, and the cooking process lasted about 10 min. To heat the oil 5 times, the oil was cooled for 5 hours, then the whole frying process repeated with a fresh batch of 'keropok lekor'. Standard rat pellets were obtained from Gold Coin (Malaysia). 15% weight/weight of the respective oils were mixed with ground rat pellets. The pellets were reformed, dried in an oven at 70-90°C and used.

Female Sprague-Dawley rats aged 3 months and

weighing between 180-250 g were obtained from the university's animal facility. The animals were ovariectomised and divided into the following groups:

	Fresh oil	Heated 1X	Heated 5X
PALM OIL	n=8 (FPO)	n=8 (1HPO)	n=8 (5HPO)
SOYA OIL	n=8 (FSO)	n=8 (1HSO)	n=8 (5HSO)

A group of 8 rats were used as normal (non-ovariectomised) controls, NC. This group was fed the standard rat pellets without any addition of oils. Another group of 8 rats were ovariectomised and fed the standard pellets. This is the ovariectomised control group (OVX C). Dietary treatment was started 2 weeks post-ovariectomy and continued for 4 weeks.

Determination of serum lipids and lipoprotein: Blood was extracted from the orbital vein after 12 hours of fasting. Serum total cholesterol (TC), HDL-cholesterol and triacylglycerols were analysed by the enzymatic method using kits (Boehringer Mannheim). The addition of phosphotungstic acid and magnesium ion to the sample led to the precipitation of various lipoproteins on centrifugation. The HDL contained in the supernatant portion can then be separated and measured using enzymatic methods. The LDL cholesterol was calculated by using the Friedwald formula (Friedewald *et al.*, 1972).

Determination of serum malondialdehyde: The malondialdehyde (MDA) content in the serum was determined using a method described by Ledwozyw *et al.*, 1986 with some modifications. A sample of 0.5 ml was acidified with 2.5 ml of 1.22 M trichloroacetic acid /0.6 M HCl and left to stand at room temperature for 15 min after which 1.5 ml of 0.67% thiobarbituric acid/0.05 M NaOH was added. The samples were incubated in a 100°C water bath for 30-min. Subsequently they were left to cool at room temperature before the addition of 4 ml of n-butanol. After thorough mixing, the mixture was centrifuged for 10 min. at 1500xg. The absorbency of the upper phase was read at 535 nm.

Homocysteine assay: The sample was analysed by Fluorescence polarization immunoassay technique Using Abbott AxSYM System. The homocysteine reagents and sample are pipetted in the following sequence:

Sample & controls volume, 210 uL each are loaded into sample cups

Sample and all AxSYM homocysteine reagents required for one test are pipetted by the sampling probe into various wells of a reaction vessel (RV).The sample,

pretreatment solution, solution 4 (Line Diluent) and S-adenosyl-homocystein hydrolase are pipetted into one well of the reaction vessel to make up the predilution mixture. Subsequently the reaction vessel is immediately transferred into the processing center. At the processing center an aliquot of the predilution mixture, antibody & solution 4 are transferred to the cuvette of the RV. Later tracer, solution 4, and a second aliquot of the predilution mixture are transferred to the cuvette. After SAH and labeled fluorescein tracer compete for the site on the monoclonal antibody molecule.

The FPIA optical assembly measures the intensity of polarized fluorescent light. Automated dilution protocol was provided to assist in quantitating test results greater than 50umol/l up to 500umol/l The AxSYM system performs a 1:10 dilution of the specimen using one reaction vessel. The AxSYM system automatically calculates the concentration of the diluted specimen and reports the result.

Sensitivity of test : < 0.8 u mol / L : It is the concentration at 2 SD from the mean of the AxSYM Calibration and represent the lowest measurable concentration of homocystiene that can be distinguished from zero.

Microscopic appearance of aortic wall: Aortic tissue was taken from a consistent segment of the ascending aorta for histopathological examination 24 weeks after feeding with heated vegetable oils. The specimens were sectioned transversely and stained with haematoxylin and eosin for light microscopy. Tissue sections of aorta were also stained with Verhoeff van Gieson for easy of identification of different fibres such as elastin, collagen and muscle.

Aortic histomorphometric study: Measurements of the intimal thickness were calculated using computerised image analyser. The image of each slide was captured and analysed with an image analyser. The mean intimal thickness will be calculated from lumen to the internal elastic lamina.

Statistics: The data was presented as the mean ± S.E.M. Normally distributed data were analysed using parametric tests, i.e. Student's t-test and ANOVA. Data which were not normally distributed were analysed using non-parametric test, i.e. the Kruskal-Wallis, Mann-Whitney and Wilcoxon Signed Rank tests. A value of p<0.05 was considered significant. All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) software.

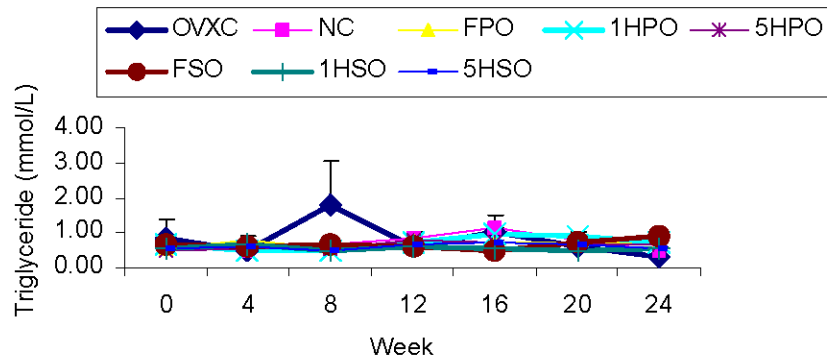


Fig. 1: Effect of heated vegetable oils on serum triglyceride (TG)

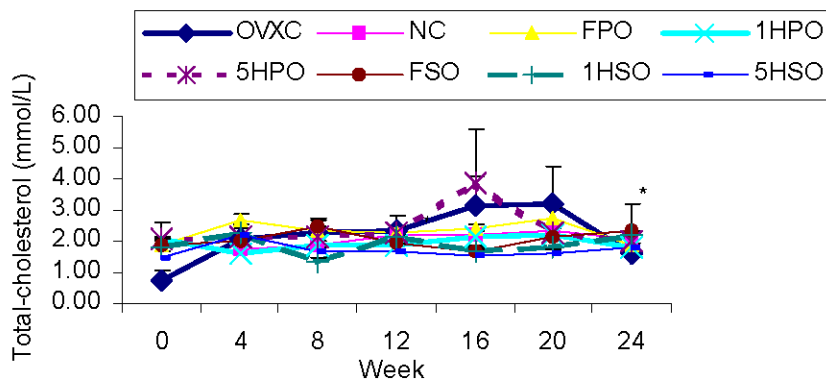


Fig. 2: Effect of heated vegetable oils on serum total cholesterol (TC)

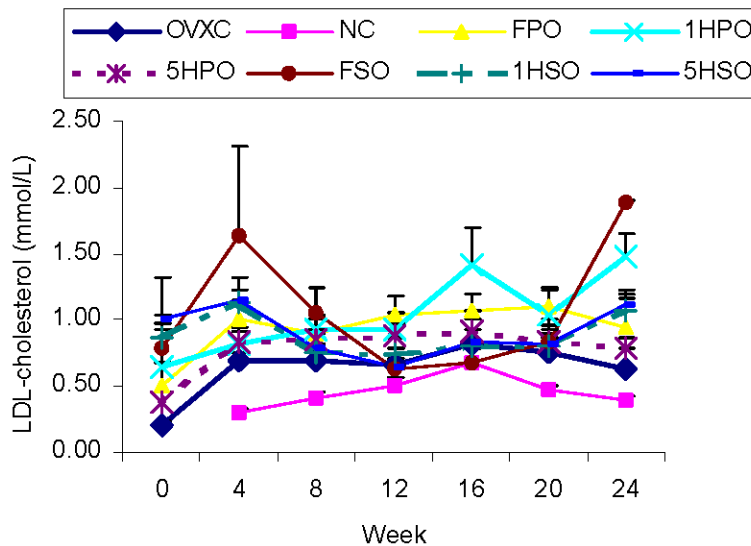


Fig. 3: Effect of heated vegetable oils on serum LDL-cholesterol(LDL-C)

Results

Effect of heated vegetable oils on serum triglyceride (TG) (Fig. 1): There were no significant changes in serum TG in all the groups compared to their respective baseline values. However, the serum TG was

significantly higher in FSO group at the end of the study compared to the rest of the groups ($p < 0.05$)

Effect of heated vegetable oils on serum total cholesterol (TC) (Fig. 2): The serum TC in-group fed

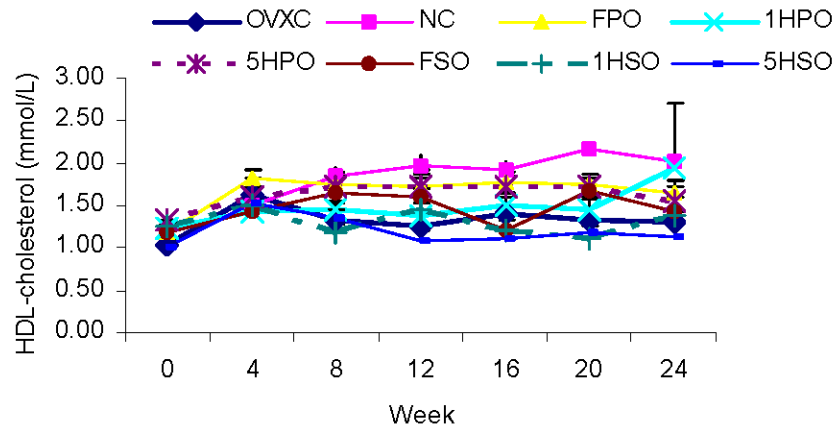


Fig. 4: Effect of heated vegetable oils on serum HDL-cholesterol (HDL-C)

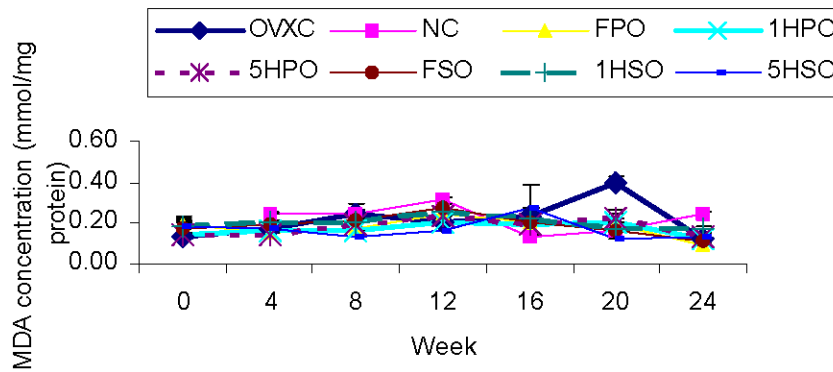


Fig. 5: Effect of heated vegetable oils on serum malondialdehyde (MDA)

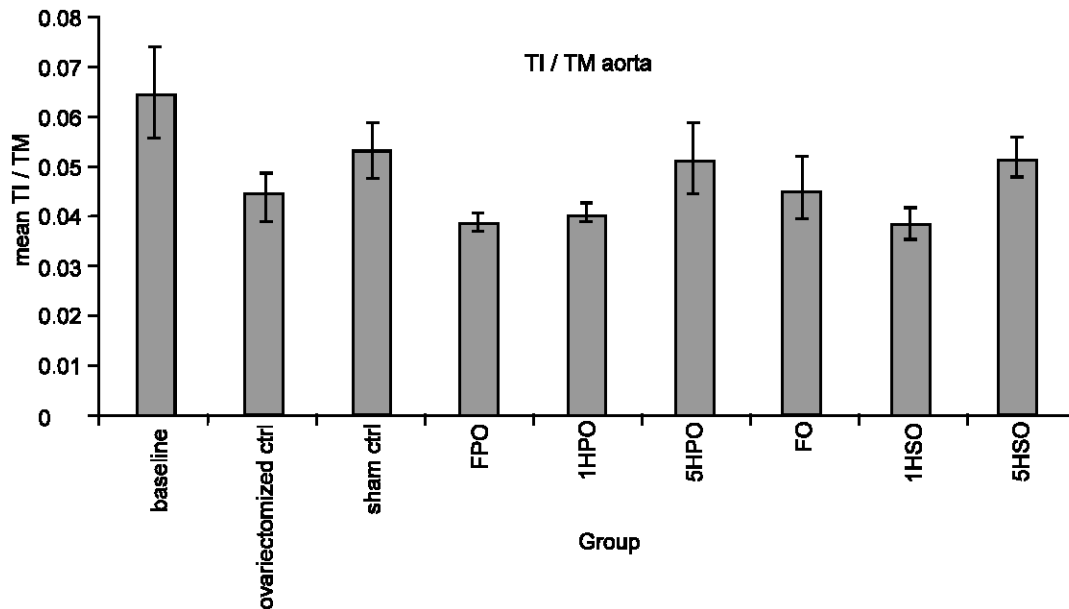


Fig. 6: Effect of heated vegetable oils on the ratio between tunica intima/ tunica media (TI/TM)

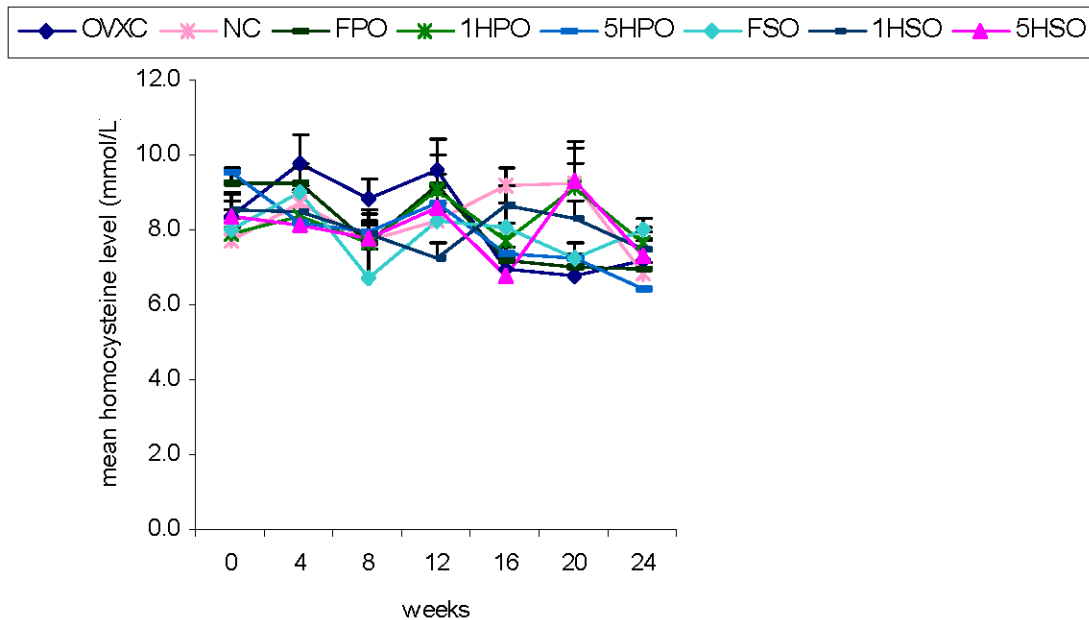


Fig. 7: Effect of heated vegetable oils on serum homocystine

with 5HPO and ovariectomized control transiently increased at 16 weeks and 20 weeks of feeding respectively. However, Serum TC came back to baseline level at the end of study. There was no between group differences in TC among the groups at the end of the study period.

Effect of heated vegetable oils on LDL Cholesterol (LDL-C) (Fig. 3): There was a significant increase in LDL cholesterol in all groups at 4 weeks of feeding. However, the concentration of LDL cholesterol in this group came down almost to baseline values after 24 weeks of feeding in NC and ovariectomized control except for FSO, IHSO, 5HSO and IHPO where the serum LDL-C were significantly higher compared to control. Again, there was no between group differences in LDL-C at the end of the study period

Effect of heated vegetable oils on serum HDL Cholesterol (HDL-C) (Fig. 4): There were no significant changes in serum HDL-C in all the groups compared to their respective baseline values.

Effect of heated vegetable oils on serum MDA (Fig. 5): There was a significant increase in serum MDA in the ovariectomized group at 20 weeks of study. However, this reverted back to baseline values at 24 weeks. Again there was no between group differences in HDL-C throughout the study period.

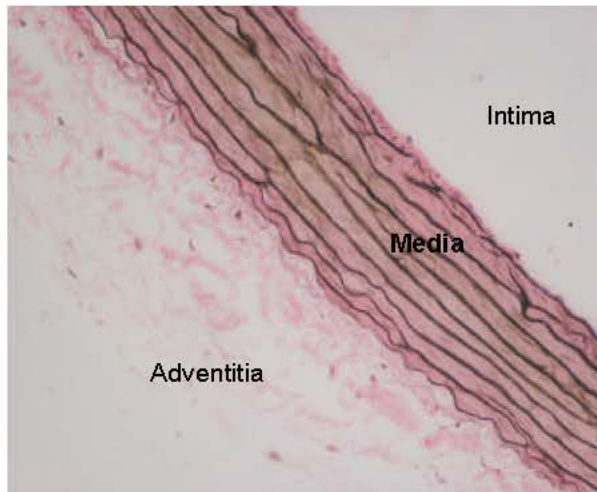
Effect of heated vegetable oils on ratio between tunica intima / tunica media (TI / TM) (Fig. 6): There were no significant changes in TI/TM ratio all the groups compared to control.

Effect of heated vegetable oils on serum homocystein (Fig. 7): There were no significant changes in serum homocystein in all the groups compared to their respective baseline values and control

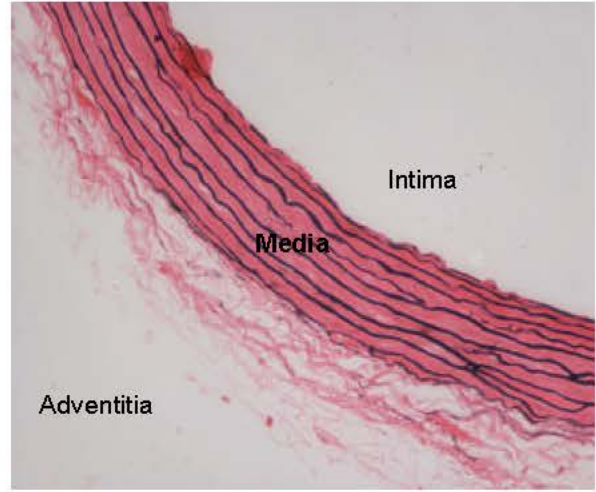
Microscopic appearance of aortic wall: Cross section of aorta stained with Verhoeff van Gieson (VVG) and hematoxilline-Eosin (H&E) 200X magnification

For baseline, Shame operated and normal control (Fig. 8 and Fig. 11): Tunica intima is seen as a thin layer of endothelial cells lining the lumen. The internal elastic lamina can be seen as a continuous layer separating the tunica intima and media. Tunica media is composed of thick wavy elastic fibers (stained brown-black with VVG) intersperse with collagen fibers (stained pink with VVG). Nucleus of the smooth muscles cells (darkly stained with H&E) located in between the thick elastic layers of tunica media. The collagenous tunica adventitia can be seen forming the outer layer of the aorta.

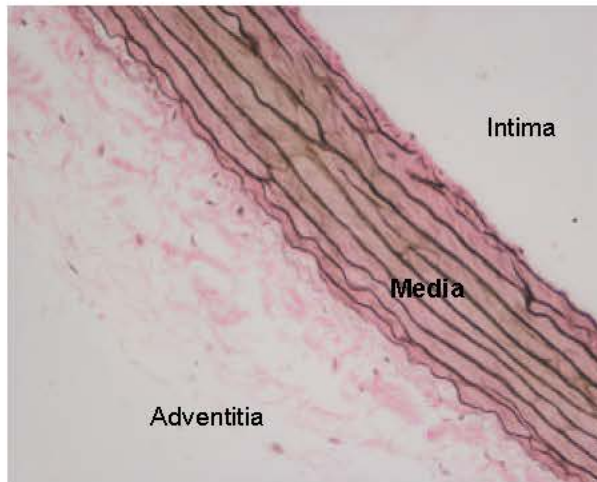
For the treatment Group; FPO, IHPO, 5HPO (Fig. 9 and Fig. 12), FSO, 1 HSO and 5 HSO (Fig. 10 and Fig. 13): No thickening and absence of foam cells formation in the tunica intima of the aorta. No obvious disjointed or fragmentation of internal elastic lamina seen as this would indicate the migration of lipid-laden smooth muscle cells (foam cells) to the intima layers a histological finding in atherosclerosis. No obvious straightening of the elastic fibers of the tunica media, as this would indicate the weakening of the elastic nature of the aorta.



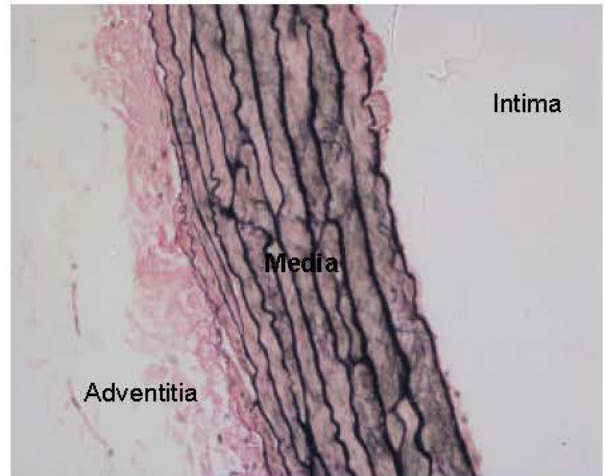
baseline



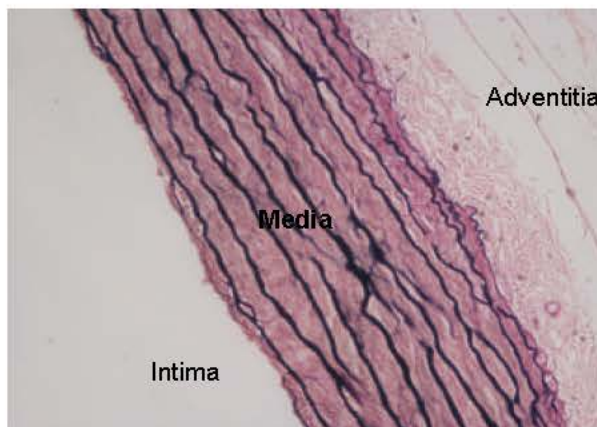
FPO



Normal control



1HPO



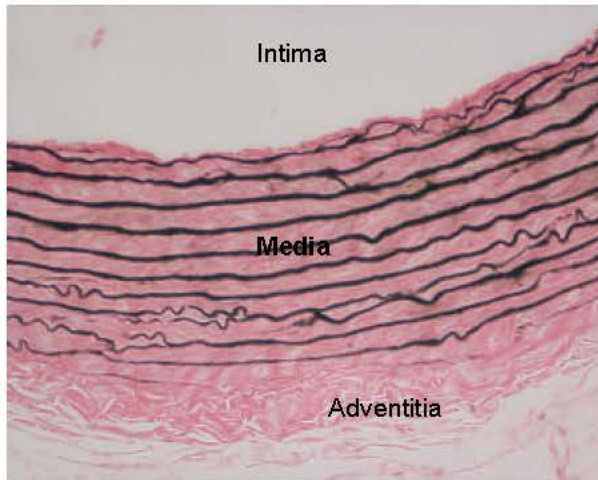
Sham control



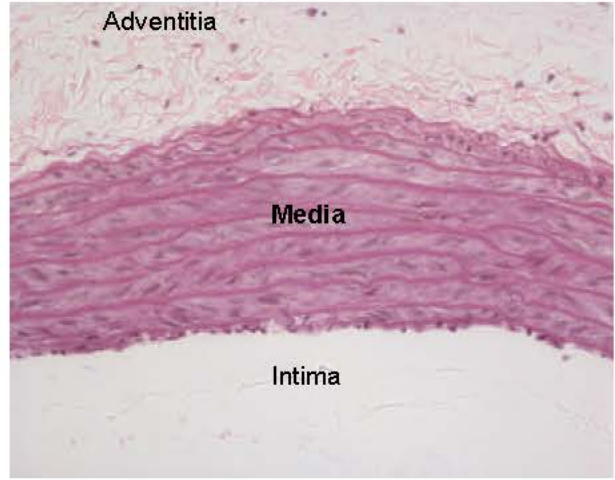
5HPO

Fig 8: Cross section of the aorta stained with Verhoeff-van Gieson (control and baseline)

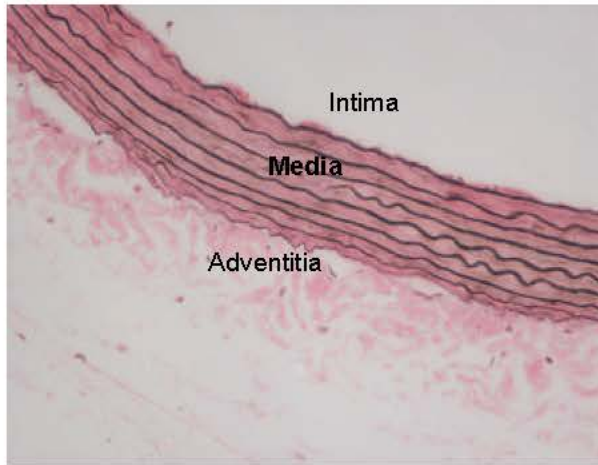
Fig 9: Cross section of the aorta stained with Verhoeff van Gieson [fresh soya oil (FPO), heated once (1HPO) and heated 5 times palm oil (5HPO)]



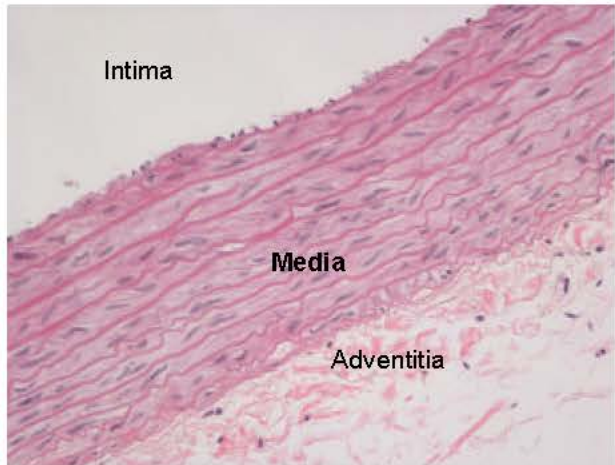
Fso



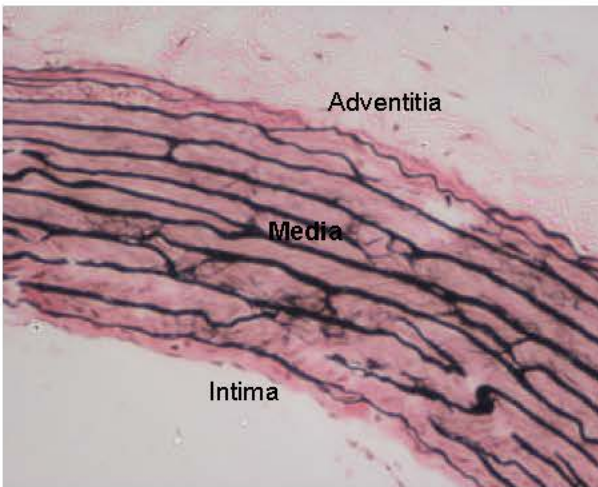
Baseline



1HSO



Normal control



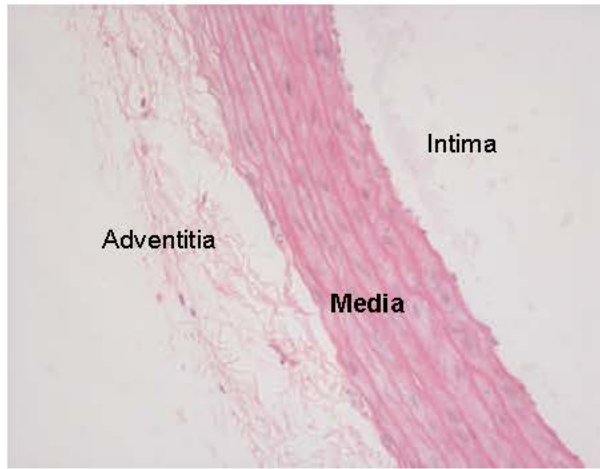
5HSO



Sham control

Fig 10: Cross section of the aorta stained with Verhoeff van Gieson [fresh palm oil (FSO), heated once (1HSO) and heated 5 times soya oil (5HSO)]

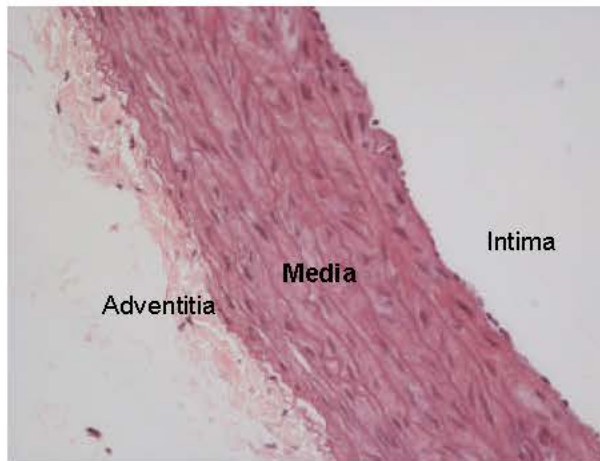
Fig 11: Cross section of the aorta stained with hematoxylin-eosin (control and baseline)



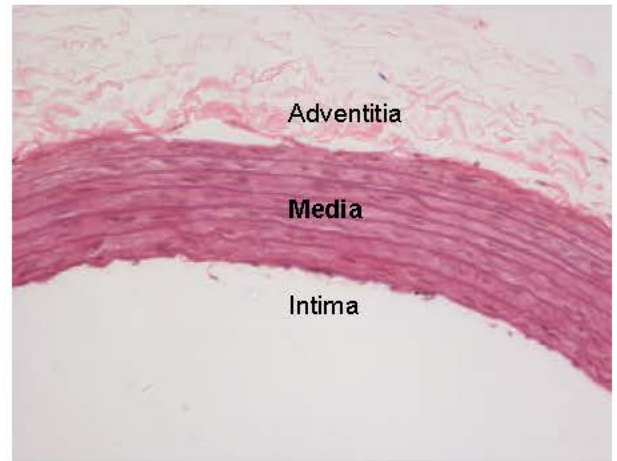
FPO



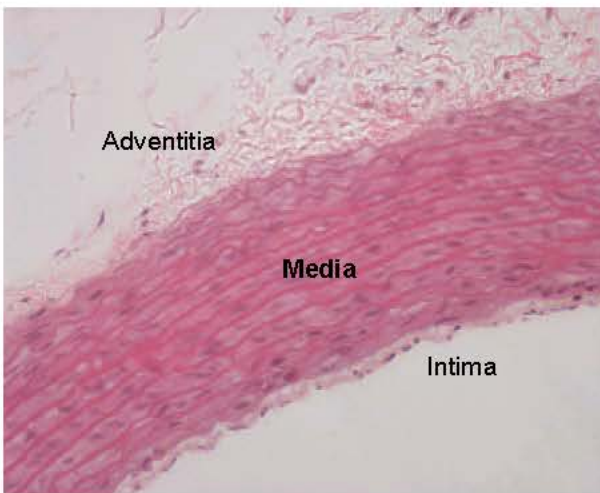
FSO



1HPO



1HSO



5HPS



5HSO

Fig 12: Cross section of the aorta stained with hematoxylin-eosin [fresh Soya oil (FPO), heated once (1HPO) and heated 5 times palm oil (5HPO)]

Fig 13: Cross section of the aorta stained with hematoxylin-eosin [fresh Palm Oil (FSO), heated once (1HSO) and heated 5 times Soya oil (5HSO)]

Discussion

The addition of 15% palm oil (PO) caused a temporary increase in total-cholesterol (TC) and LDL-C. However, after prolonged feeding, the LDL-C was significantly higher in the group fed with fresh, heated once soya, heated 5 times soya and heated once palm oil compared to the controls. This finding suggests that prolonged feeding with heated soya oil and heated once palm oil increased serum LDL-cholesterol. Heated 5 times PO did not appear to be more hypercholesterolemic than heated once PO. The effect of fresh soya oil (SO) in this study was in contrast with Kamsiah *et al.*, 2004 who reported that both fresh and heated SO did not interfere with serum TC, TG, LDL-C but reduced HDL-C.

The detrimental effect of SO and 1HPO on lipid profiles in this study could be attributed to the estrogen deficiency state of the rats secondary to ovariectomy. This factor was not present in our earlier work.

Fresh and heated vegetable oils did not have any significant effects on serum MDA. This again was in contrast with our earlier work that reported fresh and heated soya oil decrease serum MDA. The reason for the discrepancy in the finding was not clear. We presume that ovariectomy may be the compounding factor that attribute to the difference in the result.

Fresh and heated vegetable oils did not have any significant effects on serum homocystein and on the ratio between tunica intima and tunica media. There was no obvious focal or diffuse atherosclerotic plaque formation seen in all the tested groups. The tunica intima was seen as a thin layer of endothelial cells with no foam cells formation. The intact and continuous internal elastic lamina did not support the smooth muscle migration as seen in atherosclerosis. There was no obvious thickening or swelling of the tunica media indicating that there was no formation of the lipid-laden foam cells.

These histological findings suggested that no obvious detrimental effects were seen using repeatedly heated soya and palm oil. Although plasma lipid profile of the soya oil showed some unfavorable results, these changes were not significant enough to be manifested in the aortic tissue. The short duration of treatment and the type of animal used in this study may contribute the negative findings. Rats are known to be quite resistant to atherosclerosis compared to rabbits. Additional studies on rabbits are required to ascertain whether consumption of heated vegetables oils is detrimental to vascular structure. Electron microscopic study (EM) is needed to see ultra structural changes in the aorta.

In conclusion, fresh and heated soya and palm oils appear comparable in their effects on serum MDA, homocystein and have no obvious detrimental effect on the aorta of rats in spite of some unfavorable effect of heated soya oil on serum lipid profile. Heated soya and

palm oil did not render it more atherogenic in ovariectomized rats.

Acknowledgement

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References

- Antonio, M.G. and P. Rodolfo, 1990. Atherosclerosis reviews 21, 53-89. Raven Press, Ltd, New York.
- Corcos, B.P., M. Di Felice, V. Gentili, B. Tangliamonte and G. Tomassi, 1990. Influence of dietary thermally oxidized soybean oil on oxidative status of rats of different ages. *Ann. Nutr. Metab.*, 34: 221-231.
- Flider, F.J., 2001. Soybean oil emerges from the pack <http://www.prepaidfoods.com/archives/1999/9911/9911soy.html>
- Friedewald, W.T., L.I. Robert and D.S. Fredrickson, 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin. Chem.*, 18: 499-502.
- Golden, M.H.N. and D. Ramdath, 1987. Nutritional aspects of free radicals. *Free Rad. Biol. Med.*, 46: 55-68.
- Hegsted, D.M., 1981. Dietary fatty acids, serum cholesterol and coronary heart disease. In: Nelson G.J. Ed. Health effects of dietary fatty acids. Champaign, IL: Am. Oil Chem. Soc., 50-68.
- Hill, E.G., S.B. Johnson, L.D. Lawson, M.M. Manfouz and R.T. Holman, 1982. Perturbing of the metabolism of essential fatty acids by dietary partially hydrogenated vegetable oil. *Proc. Natl. Acad. Sci. USA*, 79: 953-957.
- Ima-Nirwana, S., Z. Merican, M. Jamaluddin and B.A.K. Khalid, 1996. Serum lipids, lipid peroxidation and glutathione peroxidase activity in rats on long term feeding with soybean oil or palm oil. *Asia Pacific J. Clin. Nutr.*, 5: 100-104.
- Isong, E.U., E.T. Ikon, E.U. Eka, E.U. Essien and I.B. Umoh, 1992. Effect of intermittently thermoxidized Palm oil on normal and malnourished rats. *Trop. J. Appl. Sci.*, 2: 118-121.
- Jackson, R.L., O.D. Taunton, J.D. Morrisett and A.M. Gotto, 1978. The role of dietary polyunsaturated fat in lowering blood cholesterol in man. *Circ. Res.*, 42: 447-453.
- Kamsiah, J., S. Nik Aziz, M.A. Imran, O. Azizah and J. Buddy, 2004. Changes in serum lipid profile and malondialdehyde following consumption of fresh or heated soya oil. *Malaysian J. Biochem. Molec. Biol.*, 9: 1-6.
- Kamsiah, J. and M.I. Nafeeza, 1997. Evaluation of the effects of red palm oil on serum lipid profiles, lipid peroxidation and atherogenesis. *Malaysian J. Biochem. and molecular biology*, 1: 32-35.

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- Ledwozyw, A., J. Michalak, A. Stepień and A. Kadziolka, 1986. The relationship between plasma triacylglycerols, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. *Clinica Chimica Acta*, 155: 275-284.
- Marzuki, A., F. Arshad, T.A. Razak and K. Jaarin, 1991. Influence of dietary fat on plasma lipid profiles of Malaysian adolescents. *Am. J. Clin. Nutr.*, 53: 1010S-1014S.
- Narasimhamurthy, K. and P.L. Raina, 1999. Long term feeding effects on heated and fried oils on lipids and lipoproteins in rats. *Molecular and Cellular Biochem.*, 195: 143-153.
- Owu, D.U., E.E. Osim and P.E. Ebong, 1998. Serum liver enzymes profile of Wistar rats following chronic consumption of fresh or oxidized palm oil diets. *Acta Tropica*, 69: 65-73.
- Prasad, K. and J. Kalra, 1989. Experimental atherosclerosis and oxygen free radicals *Angiology*, 40: 835-843.
- Shepherd, J., C.J. Packard, S.M. Grundy, D. Yesurun, A.M. Gotto and O.D. Taunton, 1980. Effects of saturated and polyunsaturated fat diets on chemical composition and metabolism of low density lipoproteins in man. *J. lipid Res.*, 21: 91-98.
- Steinberg, D. and J.L. Witztum, 1990. Lipoprotein and atherogenesis: current concept *JAMA* 264, 3047-3052.
- Vega, G.L., E. Groszek, R. Wolf and S.M. Grundy, 1982. Influence of polyunsaturated fats on composition of plasma lipoprotein and apolipoprotein. *J. lipid Res.*, 23: 811-822.
- Yeong, B.Y., 2001. An update and review of soybean oil in health and medical Research, http://www.asasea.com/technical/hn23_1996.html