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Effects of Palm Oil Fractions on the Aortic Cell of Wistar Rats: A Pilot Study of the Histochemical Evaluation

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Authors' contributions

This work was carried out in collaboration between all authors. Author SGO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author QOM managed the analyses of the study. Author MSM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the effect (s) of palm oil fractions {Palm Olein (PO) & Palm Stearin (PS)} on the aortic cell of Wistar rats.
Study Design: The histological and cholesterol blood sample examinations were done on the aorta of the Wistar rats, to investigate the effects of the palm oil on the animals.
Methodology: Twelve (12) male Wistar rats were procured from the animal house of the University of Ilorin, four (4) animals in each group A (control), B (PO) and C (PS), and the animals were about

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of the same age. The experiment was carried out within the period of eight (8) weeks with 5ml of respective palm oil and rat pellets given to each animal in their different groups (B and C) three times in a day orally, and group A was fed with rat pellets only. A day after the 8th week, the animals were sacrificed using cervical dislocation under chloroform anaesthesia, after which blood samples were collected from the orbital sinus of the experimental rat, different levels of cholesterol were tested for; Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C) and High Density Lipoprotein Cholesterol (HDL-C) using Randox Lipid kits. The aorta were extracted and fixed in a 10% formalin for 24 hours before preparing for histological examinations.

Results: Histologically, there was no disarrangement in the organization of the three (3) layers of the tissue (tunica intima, tunica media and tunica adventia) in all the three (3) groups, except in the PS group, where a distortion at the adventia layer was observed, but,this could not be really suggested to be a deposition of any kind. The PO group was observed to have higher TC and LDL-C, also, high TC and LDL-C were found in PS group as against the values obtained in group A. This suggested that, PO and PS did not affect the TC, LDLC or HDLC levels of rats in any way in this study. However, PO group had 139.00±7.87 mg/dl of TC and 55.50±3.87 mg/dl of LDL-C results as against 90.25±3.59 mg/dl of TC and 18.50±2.65 mg/dl of LDL-C in group A.

Conclusion: It can be concluded that, Low Density Lipoprotein Cholesterol (LDL-C) in the aortic cell of Palm Olein (PO) treated group could be a factor that brought about the palmitic acids effects and the same effect was observed in Total Cholesterol (TC) of PS-treated group. Both Palm Olein (PO) and Palm Stearin (PS) have little effects on the aortic tissue of the animals, although, the effect has never been reported to be detrimental (histologically and histochemically).

Keywords: Palm oil; palm olein; palm stearin; aorta; TC; HDL-C; LDL-C.

1. INTRODUCTION

Atherosclerosis is a prevalent non-communicable disease of arteries with a multifactorialetiology and long progression time. Although partly genetically determined, its risk is greatly influenced by lifestyle factors such as diet approach during the life course [1]. It is a pathological process of thickening and loss of elasticity of the arterial wall. Injury to the vascular endothelium is the initiating event of the atherogenetic process [2].

The normal endothelium is an important modulator of vascular tone, producing vasoactive substances, and is also involved in the local control of intravascular thrombosis [3]. Alteration in endothelial function precedes the development of morphological atherosclerotic changes and can also contribute to leision development and later clinical complications [4]; Macrophages and lipids, predominantly low-density lipoprotein (LDL) accumulate at the site of injury [5], LDL is oxidized and ingested by macrophages, which produce foam cells, these foam cells aggregate and compose the first lesions of atherosclerosis: the fatty streaks, as a result of high oxidative stress [6]. As the lesion expands, more smooth muscle cells migrate, leading to the transition of the fatty streak into an atherosclerotic plaque, or atheroma which may then undergo a marked increase in fibrous tissue. At this stage, the fibrous plaque leads to the narrowing of the lumen and may manifest as a clinically symptomatic disease [7].

2. ROLES OF PALM OIL IN CHOLESTEROL BUILDING

Oil obtained initially in harvesting the fruit of the oil palm is red due to its content of carotenes, tocopherols and tocotrienols. In the past, the compounds imparting the red colour to the oil have been removed and sold separately under the name of palmvitee. Palmvitee has been shown to lower cholesterol levels in human subjects [8,9]. Tocotrienols have also been shown to lower the cholesterol levels in the human studies, it was reported that, supplementation with palm-derived tocotrienols was associated with a reduction in cholesterol levels in hypercholesterolemic subjects, with gamma-tocotrienols again identified as the most potent cholesterol inhibitor [10]. Palm oil has been stigmatised as a hypercholesterolaemic fat because of its palmitic acid (16:0) content, despite human studies that show it does not raise serum cholesterol levels [11]. It has been shown that the presence of palmitic acid at the SN2 position of a triglyceride renders that triglyceride more atherogenic [12].

In a study where the atherogenic properties of Red Palm Oil were compared with those of

refined bleached deodorized Palm Oil and randomised Palm Oil, fats were incorporated into semipurified diets containing 0.1% cholesterol and fed to rabbits (10/group) for 90 days. It was evident that Red Palm Oil is 21% less atherogenic than randomised, increasing the amount of palmitic acid (16:0) content of palm oil at the SN2 position to 8.3% and trebles the severity of atherosclerosis palm oil and 15% more atherogenic than Red Palm Oil [13].

The findings, while indicative, were not striking. The study was repeated using 0.2% cholesterol and feeding was maintained for 65 days. Red Palm Oil was 25% less atherogenic than randomised Palm Oil and 47% more atherogenic than Red Palm Oil. Red Palm Oil is rich in both carotenoids and vitamin E and this study did not indicate if one or both of the components of Red Palm Oil were responsible for the findings [14].

3. MATERIALS AND METHODS

3.1 Animal Care

Twelve (12) male wistar rats were procured from the animal house of the University of Ilorin, four (4) animals in each group A (control), B (PO) and C (PS), and the animals were about the same age. The experiment was carried out within the period of eight (8) weeks with 5 ml of respective palm oil given to each animal in their different groups (B and C) three times in a day orally. All groups (A, B and C) were fed with rat pellets and given water regularly, and all necessary data were collected using using One Way Anova.

3.2 Tissue Harvesting

A day after the 8th week the experimental animals were sacrificed and the aortic tissue was taken from consistent segments of the ascending aorta for histoological examination. The tissue sections were stained with hematoxylin & eosin (H&E) for light microscopy and also stained with Verhoeff van Gieson for easy identification of different fibres such as elastin, collagen and muscle.

3.3 Blood Collection and Plasma Lipid Profile Test

The experimental animals were fasted for 12 hours on the last day of the experiment. Before sacrificing each animal, 2mls of blood to be analyzed for lipid profile was collected from the orbital sinus with the aid of a capillary tube into Ethylenediaminetetra-acetic acid (EDTA) bottles, tested for Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C) and High Density Lipoprotein (HDL-C). The EDTA bottle with its content was centrifuged at 6000 rpm for 15 min, the supernatant is the plasma. In the case of turbid supernatant caused by elevated triglyceride concentration, the blood sample was diluted with 0.9% NaCl solution of the same volume and the precipitating step was repeated (Randox Lipid profile kit).

3.4 Tissue Staining

3.4.1 Haematoxylin and eosin staining

Sections were re-hydrated by first placing in xylene for 5 minutes to dissolve the paraffin wax. They were then passed through;

i. Two changes of descending grades of 90% and 50% of alcohol for 1 minutes each.
ii. Washing in running tap water and staining with Haematoxylin for 10 minutes.
iii. Differentiation was done in 10% acid alcohol for 4 seconds.
iv. Running in tap water for blueing for 5 minutes.
v. Counterstaining was done with Eosin for 1 minute.
vi. De-hydration through different ascending grades of alcohol starting with 50% alcohol for 2 minutes and changed to 90% alcohol for 2 minutes then two changes of absolute alcohol 1 minute each.

The sections were placed in two changes of xylene for one minute each. Mounting of sections was done using Dimethyl Paraffinate Xylene (DPX) as mountant, after which the sections were ready for microscopic examination.

4. RESULTS

4.1 Animal Body Weight

Initial and final body weights of the animals are given in Table 1 using a descriptive statistic (mean ± SD).

4.2 Blood Sample of Cholesterol

Different levels of cholesterol in the blood sample are shown in Table 2 (mean ± SD) and Table 3 (F-statistic) respectively using One Way ANOVA.

4.3 Histological Observation

The histological observations of the various groups are shown in Fig. 1.

Table 1. Shows initial and final body weights

Group	Initial body weight (g)	Final body weight (g)
A	137.25±9.23	219.25±18.39
B	139.50±9.33	249.00±4.99
C	124.50±6.99	208.75±8.73

Table 2. Shows level of cholesterol in the blood sample

Group/ cholesterol (mg/dl)	Plasma TC	Plasma HDL-C	Plasma LDL-C
A	90.25±3.59	57.35±4.89	18.50±2.65
B	139.00±7.87	68.75±5.38	55.50±3.87
C	133.00±3.37	78.58±4.31	31.25±4.35

Table 3. Shows one way ANOVA of cholesterol in the blood sample

ANOVA/ cholesterol for all groups	Plasma TC	Plasma HDL-C	Plasma LDL-C
F-statistic	98.32	18.95	103.61
P-value	7.67	5.94	6.13

Thus, at P-value ($p<0.05$), the null hypothesis is therefore accepted.
The p value shows that, there is no difference among the studied groups

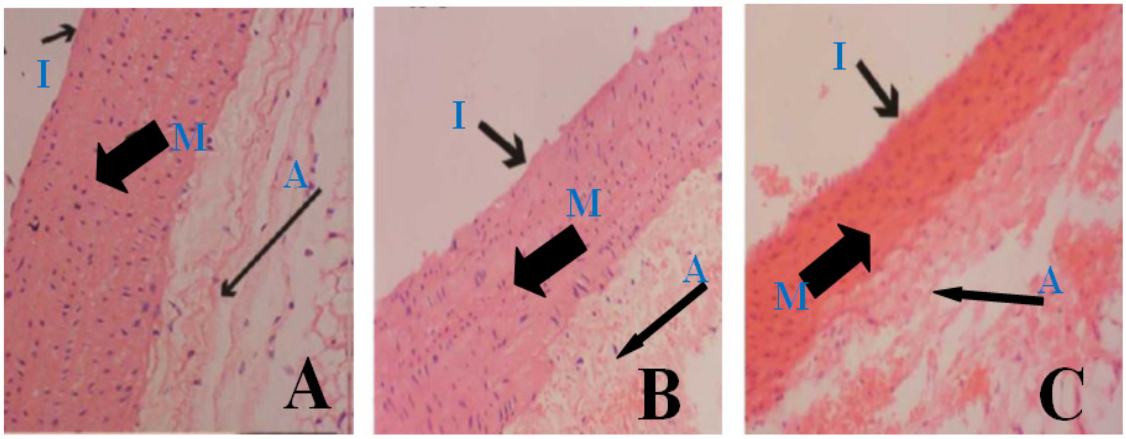

Fig. 1. Shows tunica intima (I), tunica media (M) and tunica adventia (A) in the photomicrographs of group A, B & C stained with H&E X100 respectively

5. DISCUSSION

Histologically, there was no disarrangement in the organization of the three (3) layers of the tissue (tunica intima, tunica media and tunica adventia) in all the three (3) groups, except in the PS-treated group, where a distortion at the adventia layer was observed, and this could not be really suggested to be a deposition of any kind. Thus, the histological findings suggested that, no obvious detrimental effects were observed with both PO and PS groups. Generally, saturated fatty acids increase plasma total cholesterol level and thus increase the risk of coronary heart disease, while unsaturated fatty acids have the opposite effect. But, with the histological observation above, it was shown that, not all palm oil could be associated with this, though PS-treated group was shown to have a bit distortion at the adventia layer of the tissue.

In the palm oil fed group, palm oil, a saturated fatty acid dietary oil that contains 40% of palmitic acid (C16:0) and only 0.2% lauric acid [15]. Palmitic acid is suspected to possess an increasing effect of LDL-C which increases the cholesterol ester transport protein activity, and this is responsible for the transfer of cholesterol

from HDL to LDL, which in turn increases the LDL-C level [16].

An increase in LDL-C and total cholesterol levels that is not statistically significant in any way to the palm oil fractions, further confirms the likely effects of palmitic acid in palm oil [17], which may result to the formation of more oxidization. This is what was observed in PO group with 139.00±7.87 mg/dl of TC and 55.50±3.87 mg/dl of LDL-C, and 90.25±3.59 mg/dl of TC and 18.50±2.65 mg/dl of LDL-C in group A. Considering this, the palmitic acid effects could be as a result of tocotrienols component of palm oil, which is a strong antioxidant [15].

6. CONCLUSION

It can be concluded that, Low Density Lipoprotein Cholesterol level (LDL-C) in the aortic cell of the Palm Olein (PO) treated group could be a factor that brought about the palmitic acids effects, and the same effect was observed in Total Cholesterol level (TC) of the PS-treated group. Thus, both Palm Olein (PO) and Palm Stearin (PS) have little effects on the aortic tissue of the animals, although, the effect has never been reported to be detrimental (histologically and histochemically).

ETHICAL APPROVAL

All authors hereby declare that “Principle of laboratory animal care” (NH publication No. 85-23, revised 1985) was followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethic committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yusuf S, Dagenais G, Pogue J. Vitamin E supplementation and cardiovascular events in high-risk patients. N Engl J Med. 2000;342:154–160.
2. Awtry EH, Loscalzo J. Evaluation of the patient with cardiovascular disease: Cecil's essentials of medicine, 5th edition. Philadelphia: W.B. Saunders; 2000.
3. Ross R. Atherosclerosis - An inflammatory disease. N Engl J Med. 1999;340:115–126.
4. Ross R. The pathogenesis of atherosclerosis: A perspective for the 1990s. Nature. 1993;362:801-809.
5. Targonski P, Jacobsen SJ, Weston SA, Leibson CL, Pfeifer E, Nemetz P, Roger, VL. Effect of antemortem cardiovascular disease: A population-based study in Olmsted County, Minnesota. Ann Epidemiol. 1982;114:264-70.
6. Ceconi C, Antonella B, Anna C, Roberto F. Oxidative stress in cardiovascular disease: Myth or fact? Archives of Biochemistry and Biophysics. 2003;420:217-221.
7. Qureshi AA, Quershi N, Weight JJK, Shen Z, Kramer G, Gapor A, Chong YH, DeWitt G, Ong ASH, Peterson DM, Bradon BA. Lowering of serum cholesterol level in hypercholesterolemic humans by tocotrienolspalmvitee. Am J Clin Nutr. 1991;53:1021S–1026S.
8. Tan DTS, Khor HT, Low WHS, Ali A, Gapor A. Effect of a palm oil-Vitamin E concentrate on the serum and lipoprotein lipids in humans. Am J Clin Nutr. 1991;53: 1027S–1030S.
9. Hood RL. Tocotrienols and their effect on cholesterol metabolism. Malaysian Oil Sci Technol. 1995;4:93–97.
10. Ashaf Qureshi. The most potent cholesterol inhibitor-tecotrienols enriched fraction of palm oil: A pilot study. Am J. Clin Nutr. 53:1021S–1026S.
11. Ng TK, Hassan K, Lim JB, Lye MS, Ishak R. Nonhypercholesterolemic effects of a palm oil diet in Malaysian volunteers. Am J Clin Nutr. 1991;53:1015S–1020S.
12. Kritchevsky D, Tepper SA, Wright S, Kuksis A, Hughes TA. Cholesterol vehicle in experimental atherosclerosis. Cottonseed oil and randomized cottonseed oil. Nutr Res. 1998;18:259–264.
13. Kritchevsky D, Tepper SA, Chen SC, Meijer GW, Krauss RM. Cholesterol vehicle in experimental atherosclerosis. Effects of specific synthetic triglycerides. Lipids. 2000a;35:621–625.
14. Kritchevsky D, Tepper SA, Kuksis A, Wright S, Czarnecki SK. Cholesterol vehicle in experimental atherosclerosis. Refined, bleached, deodorized RBD palm oil, randomized palm oil and red palm oil. Nutr Res. 2000b;20:887–892.
15. Edem DO. Palm oil: Biochemical, physiological, nutritional, hematological, and toxicological aspect: A review. Plant Foods Hum Nutr. 2002;57:319-341.

16. Denkel MA, Grundy SM. Comparison of effects of lauric acid and palmitic acid on plasma lipids and lipoproteins. Am J Clin Nutr. 1992;56:895-898.
17. Schwab US, Maliranta HM, Sarkkinen ES, Savolainen MJ, Kesaniemi YA, Uusitupa MI. Different effects of palmitic and stearic acid-enriched diets on serum lipids and lipoproteins and plasma cholesteryl ester transfer protein activity in healthy young women. Metabolism. 1996; 45:143-149.

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