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# Comparative Study of Artherogenic Effects of Common Nigerian Edible Oils in Male Rabbits

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***Authors' contributions***

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

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## ABSTRACT

**Aims:** To compare the atherogenicity of edible oils namely, palm, groundnut and *egusi* melon oils in male rabbits by determining their lipid profile and presence of atherosclerosis in their aortas.
**Methodology:** Twenty-five 8 weeks old male rabbits divided into five groups were used. Group A served as control and was fed on normal chow. The remaining four groups were fed on normal chow fortified with cholesterol, palm oil, groundnut oil or *egusi* melon oil respectively. At the end of 12-weeks, animals were fasted overnight and their blood samples were assayed for total cholesterol (TC), high- density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels. Atherosclerotic lesions of the vascular walls were classified according to American Heart Association (AHA) classification.
**Results:** The TC was significantly lower in *egusi* melon oil group (63 ± 19 mg/dl) than in controls (103 ± 8.8 mg/dl) with p-value < 0.001. They also had the lowest non-HDL-C (38 ± 19 mg/dl), significantly lower than that of controls (87 ± 9.7 mg/dl), p-value< 0.001. The palm oil fed rabbits

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had the highest plasma HDL-C (33 ± 2.0 mg/dl) that was significantly higher than that of controls (17 ± 0.55 mg/dl) but not significantly different for animals fed on groundnut oil (27 ± 5.1 mg/dl) or *egusi* melon oil (25 ± 2.3 mg/dl). Rabbits fed on *egusi* melon oil had the lowest AI (1.5 ± 0.79).
Rabbits fed on palm oil had lower tunica intima (TI)/tunica media (TM) ratio than other groups p <0.001. Atherosclerotic changes in keeping with AHA type II lesions were found in aorta of rabbits on groundnut oil and *egusi* melon oil.
**Conclusion:** E*gusi* melon oil produced better plasma cholesterol lowering effect but still produced atherosclerotic lesion. No lesion was observed with palm oil supplemented diet possibly because the group had the highest level of HDL-C that is considered to reduce cardiovascular risk. Further evaluation of the test oils is needed to identify factors responsible for their beneficial and atherogenic effects.

*Keywords: Atherogenicity; lipid profile; palm; groundnut; egusi melon oils; rabbits.*

## 1. INTRODUCTION

Atherosclerosis is the leading cause of cardiovascular disease death and disability worldwide. An increase in the frequency and severity of atherosclerosis in the past four decades has been reported in an autopsy study in a Nigerian population [1]. Risk factors such as hypertension, diabetes and high-fat diets are prevalent among Nigerians [2]. Atherosclerosis in rabbit models develops due to factors such as dietary fats comparable to those existing in man [3]. Atherosclerosis can be ameliorated by the type of fat consumed in the diet. Saturated fatty-acids (SFA) increase low-density lipoprotein cholesterol (LDL-C) levels, whereas polyunsaturated fatty-acid (PUFA) and monounsaturated fatty-acid (MUFA) reduce it, with PUFA being more potent in this regard than MUFA. The dietary modification of substituting SFAs with PUFA and MUFA in the United States of America coincided with a decline in the rate of atherosclerosis [4]. In Nigeria, palm oil and refined bleached deodorized palm olien oil are important dietary sources of SFA while soyabean oil and groundnut oil are important dietary sources of PUFA and MUFA respectively. The primary dietary source of PUFA that have been studied are fish oil, sunflower oil, linseed oil, canola oil, olive oil, corn oil and soyabean oil and sources of MUFA that has been studied is olive oil. Little is known about how other food sources of PUFA, *egusi* melon oil and source of MUFAs such as groundnut oil, might affect the plasma lipid response to a low cholesterol diet. *Egusi* melon oil and groundnut oil are common edible oils in Nigeria and exploring their role in the process of atherosclerosis is of utmost importance.

We compared the atherogenic effects of palm oil, groundnut oil and *egusi* melon oil on the plasma lipid profile and the development of atherosclerosis in male rabbits fed on low cholesterol diet.

## 2. METHODS

Twenty-five 8 weeks old male rabbits were purchased from the local rabbit farm in Ibadan. The animals were divided into five groups each containing five animals. They were housed in well-ventilated wooden cages with wire mesh floors and fed with rabbit chow prepared at University of Ibadan feed mill project. Animals had feeds at 50grams/kg/animal/day and free access to drinking water. Animals were handled in compliance with the ethical guidelines on animal studies of the University of Ibadan. The edible oils used in this study were Palm oil, Groundnut oil and *Egusi* melon oil were purchased from a local market in Ibadan. The oils were stored away from light in tightly capped containers at room temperature. The physicochemical properties of the test oils including percentage FFA, saponification, acid, iodine and peroxide values were analyzed [5] at the Department of Human Nutrition, University of Ibadan.

The control animals were assigned to group A and they were fed on normal rabbit chow diet. Four different test diets were prepared for the animals in the experimental groups. The test diets were prepared by suspending cholesterol in the test oils and adding the suspension, with continuous mixing, to the powdered standard rabbit chow [6]. Animals in group B were fed normal rabbit chow enriched with 0.25% cholesterol and 0.3% methionine diet; group C normal rabbit chow enriched with 0.25% cholesterol, 0.3% methionine and 15% palm oil; group D normal rabbit chow enriched with 0.25% cholesterol, 0.3% methionine and 15% groundnut

oil; group E normal rabbit chow enriched 0.25% cholesterol, 0.3% methionine and 15% *egusi* melon oil. The 97% extra- pure cholesterol used was from Oxford Laboratory Chemicals, Maharashtra, India (Batch No. 4642). The DL-methionine used is manufactured by Evonik Degussa Antwerpen N.V Belgium (LOT 140706A6). The diets were prepared freshly every week, and protected away from heat and sunlight to prevent peroxidation.

At the end of 12 weeks feeding period, all animals were fasted overnight for 12 hours.

Animals were anaesthetized intraperitoneally with ketamine (100 mg/kg) and xylazil (5 mg/kg), following which midline incision was made on the anterior abdominal and chest wall. Five milliliters of blood was drawn by cardiac puncture into ethylene diamine tetraacetic acid (EDTA) bottles for determination of lipid profile. The assay kits for total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG), were produced by Randox Laboratories Ltd, 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom. The aortas were harvested and processed for light microscopy. The vascular wall of each section was carefully examined with an Olympus light microscope (CH model) for the presence of atherosclerotic lesions that were classified based on the AHA method of classification. Photomicrographs capturing images of the vessel wall were taken, using the attached Olympus camera model CX41. The thickness of the tunical layers was measured directly using a stage micrometer.

The results were expressed as mean ± standard deviation (SD). The statistical analysis was performed using one-way analysis of variance (ANOVA). P-value < 0.05 was considered as statistically significant. All the data were processed with Graph Pad Prism version 6.00 software.

## 3. RESULTS

All the animals tolerated their diets well. One mortality each was recorded in animals in group A on normal diet and in group B on cholesterol diet at week nine and four respectively. The physicochemical properties of palm oil, groundnut oil and egusi melon oil are shown in Table 1.

Table 2 shows the percentage composition of fatty acids in palm oil, groundnut oil and *egusi* melon oil. Palm oil had the highest SFA (51.56%); *egusi* melon oil had the highest PUFA (49.42%); while groundnut oil had the highest MUFAs (47.77%).

The lipid profile of the control and the experimental groups of animals are shown in Table 3. The mean plasma TC was significantly lower in the *egusi* melon oil group than in the controls with p-value < 0.001 while there was no significant difference between groundnut and palm oil groups when compared with the controls. Rabbits fed on *egusi* melon oil had significantly lower TC when compared with those fed on palm oil with p-value<0.001, there was however no significant difference when compared with those fed on groundnut oil. The rabbits fed with the *egusi* melon oil had the lowest value of Non-HDL-C (38 ± 19 mg/dl), which was significantly lower than that of controls with p-value< 0.001. The plasma HDL-C was significantly higher in rabbits fed on palm oil, groundnut oil and *egusi* melon oil than in controls with p-value<0.001. The palm oil fed rabbits had the highest plasma HDL-C (33 ± 2.0 mg/dl). There was no significant difference in plasma TG among the control rabbits and the other experimental animal groups.

The atherogenic index (AI) of the control and experimental groups of animals are shown in Table 4. Rabbits fed on palm oil, groundnut oil and *egusi* melon oil had lower AI than the control group with p-value<0.001. Rabbits fed on *egusi* melon oil had the lowest AI (1.5 ± 0.79).

Table 5 shows the mean and standard deviation values of the tunica intima (TI)/tunica media (TM) ratio of the animal groups. The mean TI/TM ratio of the control group was significantly lower than that of all the experimental groups at p –value of 0.0011. The group of rabbits on palm oil diet had significantly lower TI/TM ratio than animals in the cholesterol, groundnut oil and *egusi* melon oil groups at p < 0.001.

No atherosclerotic lesions were found in the aorta of control rabbits fed on normal chow diet (Plates 1a and 1b) and rabbits fed on cholesterol diet enriched with palm oil (Plates 2a and 2b). The endothelium linings of the tunica intima were intact.

Atherosclerotic changes in keeping with AHA type II lesions were found in sections of the aorta of rabbits fed on cholesterol diet (Plates 3a and 3b); rabbits fed on cholesterol diet enriched with

**Table 1. Physicochemical properties of palm oil, groundnut oil and *egusi* melon oil**

| | FFA % | Acid (MgKOH/g) | Iodine (g/100g) | Peroxide (mEq/kg) | Saponification (MgKOH/g) |
|---|---|---|---|---|---|
| Palm oil | 18 ± 0.29* | 0.477 | 9.14 | 8.6 | 232 ± 0.45* |
| Groundnut oil | 2.7 ± 0.21* | 0.196 | 9.65 | 16.2 | 246 ± 4.9* |
| *Egusi* melon oil | 2.3 ± 0.89* | 0.168 | 12.19 | 3.8 | 240 ±11* |

*KEY: FFA- free fatty-acid, KOH-Potassium hydroxide*
** values are mean and standard deviation of triplicate determination*

**Table 2. The composition of fatty acids in palm oil, groundnut oil and egusi melon oil**

| | % Composition | | |
|---|---|---|---|
| **FA type** | **SFA** | **MUFA** | **PUFA** |
| Palm oil | 51.56 | 39.77 | 8.66 |
| Groundnut oil | 21.67 | 47.77 | 31.01 |
| *Egusi* melon oil | 27.53 | 23.06 | 49.42 |

*Key: FA – fatty acid, SFA- Saturated fatty-acid, MUFA- monounsaturated fatty-acid, PUFA- Polyunsaturated fatty-acid*

groundnut oil (Plates 4a and 4b); and rabbits fed on cholesterol diet enriched with *egusi* melon oil (Plates 5a and 5b).

## 4. DISCUSSION

In our study, all the test oils had low acid values < 0.5 MgKOH/g indicating that they are good edible oils. However, the peroxide value of groundnut oil was significantly higher, $p<0.0001$, compared with that of others. This may be because PUFA easily undergoes oxidation [7], though the oils used in this study were kept away from light. The peroxide value of the *egusi* melon oil was 3.8 mEq/kg, which is about a half (8.3 ± 4.6 mEq/kg) of what was reported in a previous study. [8] Specie variation may account for this difference. The three test oils used in our study have high saponification values indicating their high proportion of short and medium chain fatty-acids. [9] The *egusi* melon oil is rich in PUFA, 49.42% and its fatty-acid composition is comparable with that of cottonseed, sunflower and soyabean oils.

The mean weight gains in all the experimental groups were not significantly different from that of the control, however rabbits in the cholesterol only diet group had the highest weight increase while rabbits on diet supplemented with *egusi* melon oil gained the least weight. Cholesterol and high fat diets provide high-energy value that has positive impact on weight gain. The TC 207 ± 25, and Non-HDL-C 185 ± 26, were significantly higher in cholesterol diet rabbit group compared with the control rabbits, 103 ± 8.8. and 87 ± 9.7 respectively ($p<0.05$). This result is similar to previous observation [10] that dietary cholesterol has a modest plasma cholesterol-raising effect. The significantly lower values of plasma TC 105 ± 3.4 and non-HDL-C 72 ± 1.4 of rabbits fed diet enriched with palm oil, compared to rabbits on cholesterol diet alone is similar to the report that different palm oil preparations reduce plasma cholesterol concentrations and aortic cholesterol accumulation in hypercholesterolaemic hamsters [11]. The low percentage of myristic acid compared to palmitic acid in the palm oil may contribute to this observation because palmitic acid is less hypercholesterolaemic than myristic acid.

**Table 3. Mean and standard deviation values of TC, HDL-C and Non-HDL-C in the experimental animal groups**

| Groups | TC(MG/DL) | HDL-C (MG/DL) | NON-HDL-C (MG/DL) | TG (MG/DL) |
|---|---|---|---|---|
| Control | $103 \pm 8.8^{B}$ | 17 ± 0.55 | $87 \pm 9.7^{B}$ | 163 ± 3.3 |
| Cholesterol | 207 ± 25 | 22 ± 4.5 | 185 ± 26 | 239 ± 93 |
| Palm oil | $105 \pm 3.4^{B}$ | $33 \pm 2.0^{A}$ | $72 \pm 1.4^{B}$ | 158 ± 12 |
| Groundnut oil | $92 \pm 4.7^{B}$ | $27 \pm 5.1^{A}$ | $65 \pm 7.1^{B}$ | $112 \pm 5.7^{B}$ |
| *Egusi* melon oil | $63 \pm 19^{ABC}$ | $25 \pm 2.3^{A}$ | $38 \pm 19^{AB}$ | 160 ± 28 |

*Key: TC- total cholesterol, HDL-C- high-density lipoprotein cholesterol. TG-triglyceride.*
*[a] =statistically significant values in comparison with the control group.*
*[b]= statistically significant values in comparison with the cholesterol-diet group*
*[c] = statistically significant values in comparison within the test oil groups*

**Table 4. Atherogenic index of the control and the experimental animal groups**

| Groups | AI (NON HDL-C / HDL-C) |
|---|---|
| Control | $5.3 \pm 0.76^{B}$ |
| Cholesterol | $8.8 \pm 2.3$ |
| Palm oil | $2.2 \pm 0.12^{AB}$ |
| Groundnut oil | $2.5 \pm 0.71^{AB}$ |
| *Egusi* melon oil | $1.5 \pm 0.79^{AB}$ |

*Key: HDL-C- high-density lipoprotein cholesterol*
*[a] =statistically significant values in comparison with the control group.*
*[b]= statistically significant values in comparison with the cholesterol-diet group*

**Table 5. Mean and standard deviation values TI/TM ratio in the experimental animal groups**

| Experimental groups | TI/TM ratio |
|---|---|
| Control | $0.04511 \pm 0.01625^{B}$ |
| Cholesterol | $1.033 \pm 0.2478^{A}$ |
| Palm oil | $0.05853 \pm 0.03676^{BC}$ |
| Groundnut oil | $0.9369 \pm 0.07248^{A}$ |
| *Egusi* melon oil | $1.272 \pm 0.2105^{A}$ |

*Key: TI – Tunica Intima, TM- Tunica media*
*[A] =statistically significant values in comparison with the control group.*
*[B]= statistically significant values in comparison with the cholesterol-diet group*
*[C] = statistically significant values among test oil groups*

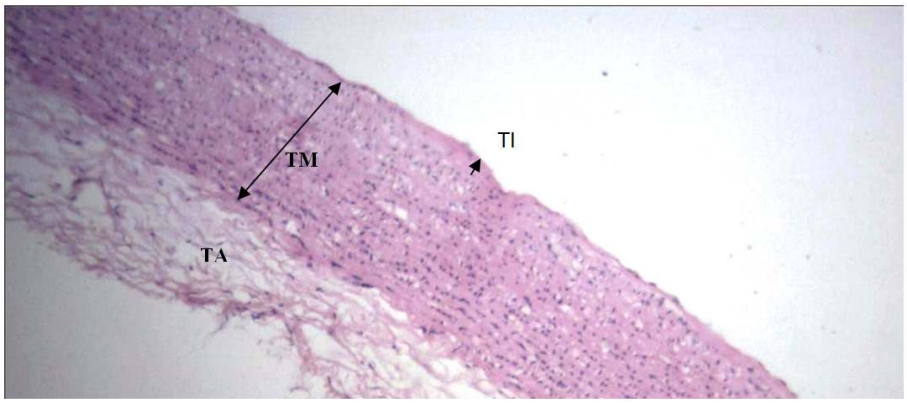


**Plate 1a. Photomicrograph showing section of the aorta of rabbit fed on normal chow. (H& E stain. Mag x100): The tunica intima closely overlies the tunica media. TI- tunica intima, TM – tunica media, TA- tunica adventitia**

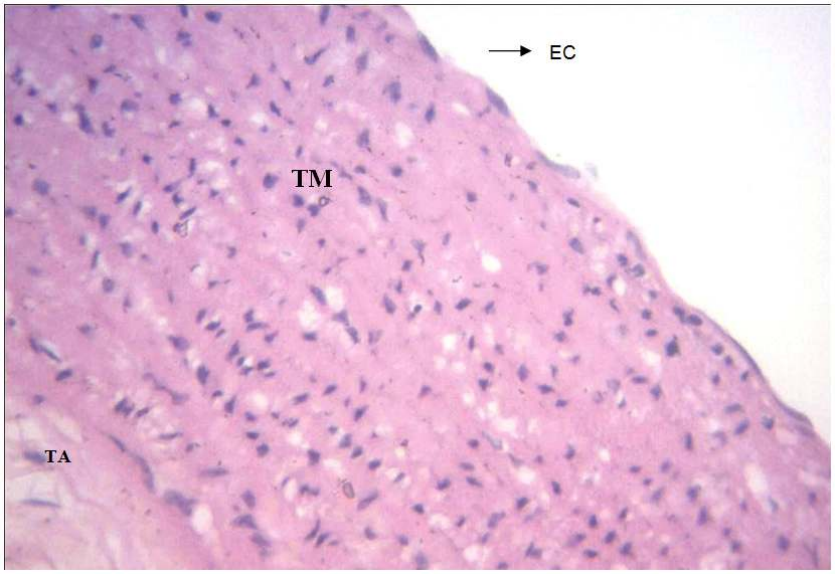


**Plate 1b. Photomicrograph showing section of the aorta of rabbit fed on normal chow. (H&E stain.Mag.X400): The endothelium (EC) is intact and forms a continuous single layer of cell. TM –tunica media, TA- tunica adventitia**

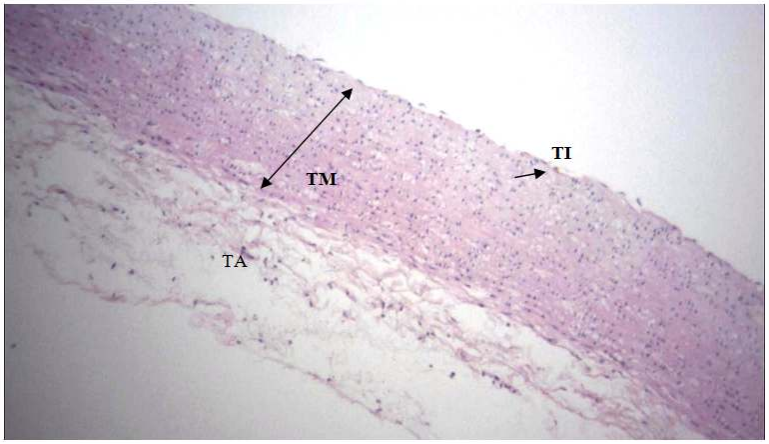


**Plate 2a. Photomicrograph showing section of the aorta of rabbit fed on cholesterol diet enriched with Palm oil. (H&E stain. Mag.x100). The tunica intima (TI) closely overlies the tunica media(TM).TA- tunica adventitia**

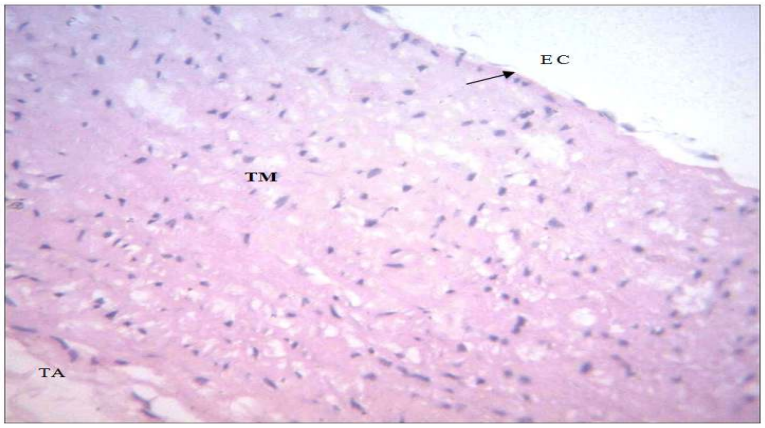


**Plate 2b. Photomicrograph showing section of the aorta of rabbit fed on cholesterol diet enriched with palm oil. (H&E stain.Mag.X400): The endothelium (EC) is intact and forms a continuous single layer of cell. TM –tunica media, TA- tunica adventitia**

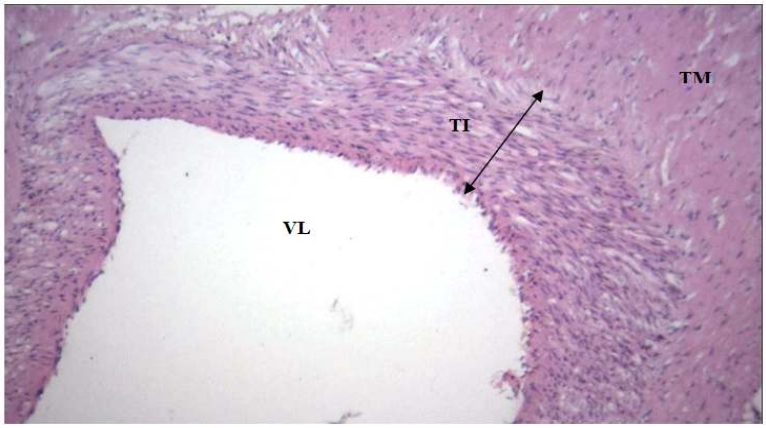


**Plate 3a. Photomicrograph showing section of the aorta of rabbit fed on cholesterol diet (H&E stain. Magnification x100): The tunica intima(TI) is thickened. The vessel lumen (VL) is narrowed by the atherosclerotic lesion**

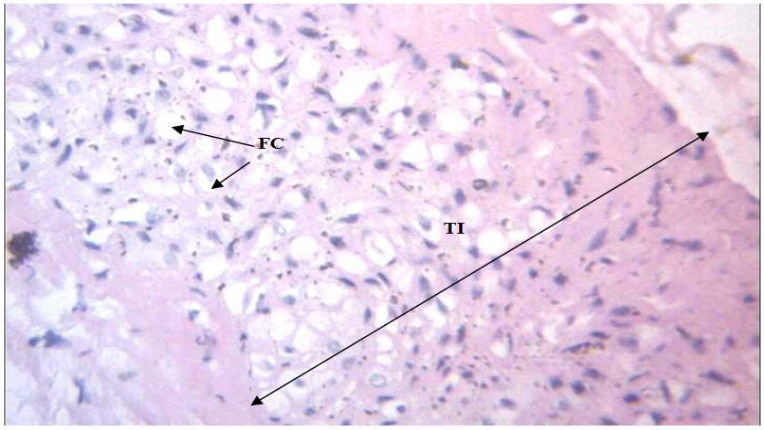


**Plate 3b. Photomicrograph showing section of the aorta of rabbit fed on cholesterol diet (H&E stain. Magnification x400): several layers of foam cells (FC) are seen in the tunica intima(TI)**

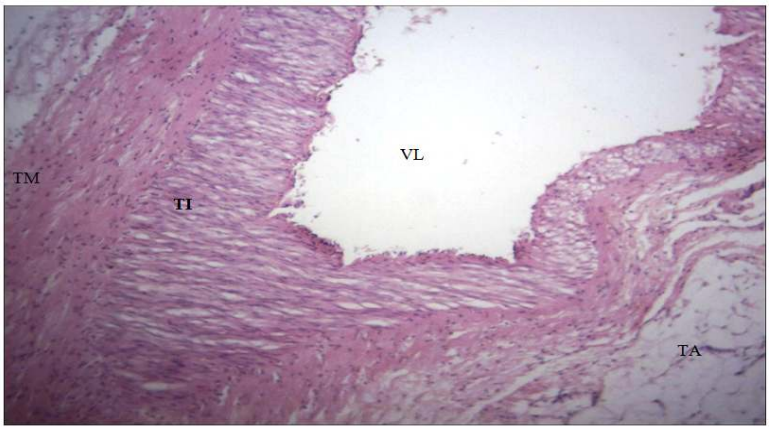


**Plate 4a. Photomicrograph showing section of the aorta of rabbit fed on cholesterol diet enriched with groundnut oil (H&E stain, Mag.x100). Varying thickness of the tunica intima (TI) along the vessel length is seen. The vessel lumen (VL) is narrowed. TM- tunica media, TA- tunica adventitia**

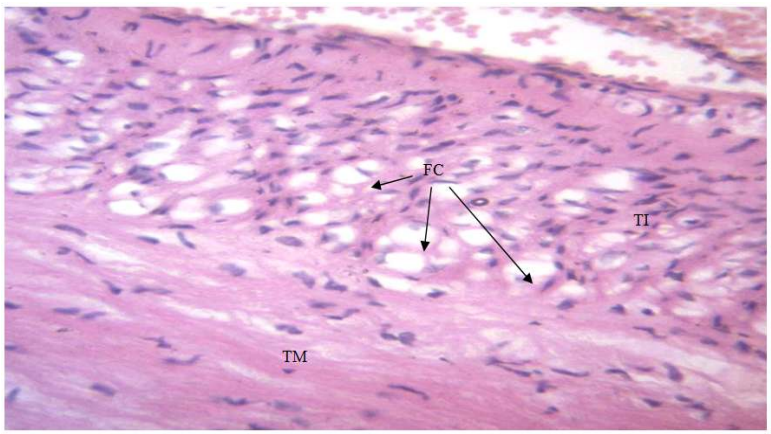


**Plate 4b. Photomicrograph showing section of the aorta of rabbit fed on cholesterol diet enriched with groundnut oil (H&E stain. Magnification x400): several layers of foam cells (FC) are seen in the tunica intima (TI). TM-tunica media**

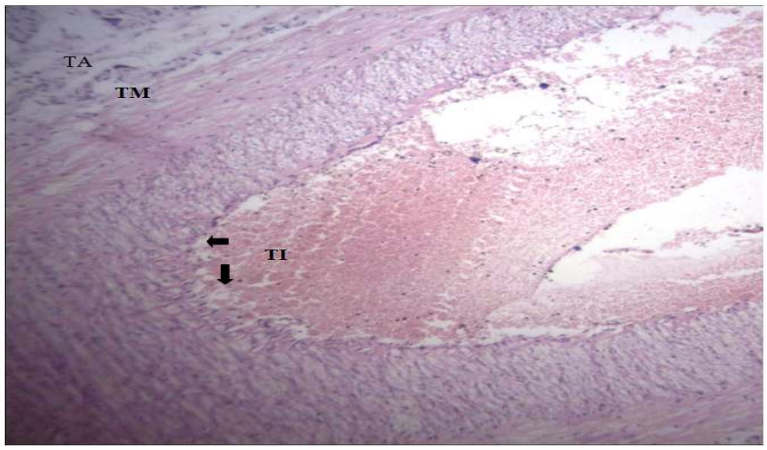


**Plate 5a. Photomicrograph showing section of the aorta of rabbit fed on cholesterol diet enriched with *Egusi* melon oil. (H&E stain. Magx100): black arrows shows thickening of the tunica intima with a well circumscribed atherosclerotic lesion**

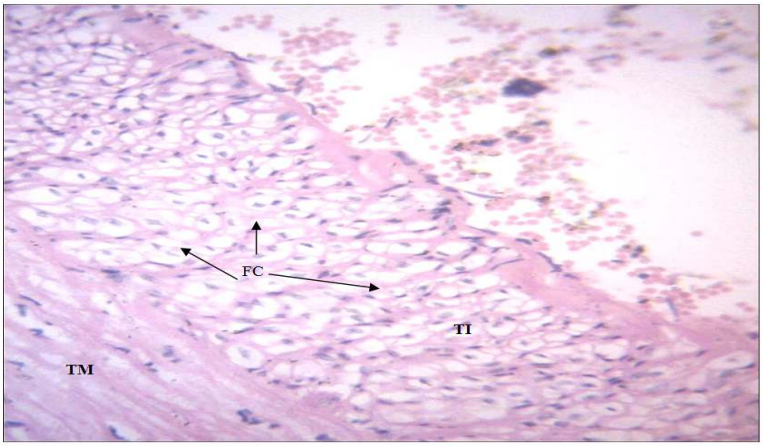


**Plate 5b. Photomicrograph showing section of the aorta of rabbit fed on cholesterol diet enriched with *egusi* melon oil. (H&E stain. Magx400): Shows multiple layers of foam cells (FC) in the tunica intima (TI)**

Rabbits fed diets enriched with groundnut oil had significantly lower plasma TC 92 ± 4.7 and non-HDL-C 65 ± 7.1 compared to rabbits fed on cholesterol diet alone. This is similar to the findings in the study on experimental atherosclerosis in rabbits fed on cholesterol-free diets [12,13]. Groundnut oil also reduced the plasma TG and this may be due to its high MUFA content [14]. *Egus*i melon oil significantly lowered TC 63 ± 19 and non-HDL-C 38 ± 19 than palm oil at 105 ± 3.4 and 72 ± 1.4 respectively. Our observation is in accordance with a study where Palm and *Egusi* melon oils lowered serum and liver lipid profile and improve antioxidant activity in rats fed a high fat diet. [15] The effects of *Egusi* melon oil observed in this study may be accounted for by the rich content of linoleic acid, a PUFA.

The three test oils used in this study increased HDL-C. This observation is similar to the report of a randomized clinical trial of the effects of dietary fats on serum HDL-C [16]. In addition, they significantly reduced the AI compared to controls and rabbits fed on cholesterol diets only, $p< 0.05$. Based on the favourable reduction of AI, it appears that palm oil; groundnut oil and *egusi* melon oils may reduce the risk of atherosclerosis. However, the rabbits fed on cholesterol - only diet, diet supplemented with groundnut oil and *egusi* melon oil developed Type II atherosclerotic lesions in their aortas. This may imply the presence of other risk factors for atherosclerosis in these experimental animals. A previous study associated unexpected atherogenic effect of groundnut oil to arachidic and behenic fatty-acids [17,18]. The

inability of diet rich in PUFAs such as *egusi* melon oil, to prevent atherosclerosis in proportion to its plasma cholesterol lowering effect may be linked to the propensity of PUFA-enriched lipoproteins to undergo oxidative modification [19]. High levels of malondialdehyde, a product of lipid peroxidation was observed in the *egusi* melon oil. Oxidized lipoprotein exhibit rapid and unregulated uptake by macrophages forming foam cells seen in the arthesclerotic lesions in this animal group.

## 5. CONCLUSIONS

This study of the effect of palm, groundnut and *egusi* melon oils on the development of atherosclerosis in male rabbits fed on low cholesterol diet revealed that all three oils reduce plasma TC and Non-HDL-C. E*gusi* melon oil produced a better plasma cholesterol lowering effect. Groundnut and *egusi* melon oils produced atherosclerotic lesion in male rabbits fed on low cholesterol diet while no lesion was observed with palm oil supplemented diet. Further evaluation of components of the test oils should be carried out to identify factors responsible for their beneficial and artherogenic effects.

## 6. LIMITATIONS OF THE STUDY

Exposure of the rabbits to the test oils for12 weeks may not be directly extrapolated to human subjects, as the oils may not mimic the same effects in humans.

## CONSENT

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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