Original Research Article

Title: The effects of Kolaviron on the atherogenic propensity of Nigeria local edible oils in male Wistar rats

Running title: Nigerian edible oils and atherosclerosis

ABSTRACT

BACKGROUND: Dyslipidemia is partly dependent on consumption of edible oils. We set out to determine the atherogenic effects of commonedible oils in Nigeria, in male Wistar rats and to find out if administration of oral kolaviron amelioratedthese effects.

METHODS: Forty-eight male Wistar rats were randomly divided into six groups of two replicates. One replicate was fed test diet while second replicate was administered 100mg/ml of kolaviron four times weekly in addition to the test diet. Group one served as control and was fed on normal chow (NC) diet. The remaining five groups were fed different diets added to the NC as follows: non heated soya oil, heated soya oil, palm olein, palm stearin, heated palm oil respectively for a period of 12 weeks.Plasma lipids were determined at the end of the experimental period and their aortas were examined histopathologically.

RESULTS: Compared with controls, experimental groups had higher values of Total Cholesterol (TC). There was a significant increase in TC in five times heated palm oil and five times heated soya oil groups compared with non heated soya oil, palm olein and palm stearin groups ($P < 0.05$).Palm olein group with no oral kolaviron had the highest percentage proportion of Low Density Lipoprotein Cholesterol, while the lowest was found in the palm stearin group with oral kolaviron. From our study, palm olein was the most atherogenic oil thanfive times heated palm oil, palm stearin, five times heated soya oil and non-heated

soyaoil respectively. An early stage of atherosclerosis was found in the group fed on five times heated palm oil with no kolaviron.

CONCLUSION: Consumption of edible oils commonly used locally, especially when repeatedly heated during frying, could lead to high levels of atherogenic lipids in the plasma while 100mg/kg of kolaviron could be beneficial.

Key words: Atherosclerosis, kolaviron, atherogenic lipids, soya bean oil, palm oil, palm olein, palm stearin, heated oils.

INTRODUCTION

One of the modifiable risk factors for atherosclerosis and coronary artery disease is dyslipidemia, [1] that in part is dependent on the dietary habit of consumption of edible oils. Dietary factors during the life course can influence the pathogenesis and progression of atherosclerosis.[2]The adoption of western lifestyle and diet has compounded this further in Sub-Saharan Africa. Palm oil and soya bean oil are one of the commonly used cooking oils in Nigeria and there is paucity of data on their atherogenic propensity in this population. Furthermore, the cooking methods adopted locally in ready to eat and fast foods suggeststhe use of repeatedly heated oils over several cycles of cooking. Like humans, high fat diets can induce elevated LDL-C [3,4] and atherosclerosis in certain rodent models such as rats, mice, hamsters, rabbits and guinea pigs. Red palm oil was shown to be significantly less atherogenic than refined, bleached and deodorized (RBD) palm oil in rabbits.[5] They also had similar effects on serum and liver lipids. Soya oil contains approximately 60% poly-unsaturated fatty acid (PUFA), 24% of mono-unsaturated fatty acid (MUFA) and 16% of saturated fatty acids. [6] This high level of PUFA dietary intake can improve the blood lipid profile status.[7] In addition, with its high content of tocopherols, soya oil is known to exhibit various antioxidant actions against lipid peroxidation.[5]

Degradation of the quality of oils occurs during deep-frying. The hydrolysis produces free fatty acids that oxidize and generate peroxide, hydroxyl peroxide compounds, and secondary lipid oxidation products, such as, aldehydes, ketones, and alcohols.[8, 9]Oxidation has been implicated in the promotion of atherosclerosis. According to the oxidation hypothesis of atherosclerosis [10]oxidized LDL-C play a role in the initiation of the atherosclerotic lesion, and oxidized LDL-C appear to affect almost every step of the atherogenic process. [11] This suggests that dietary oxidized lipids, if incorporated into LDL-C, could be proatherogenic.

Therefore, the use of such diets for promoting atherosclerosis in these models has been a valuable tool for both gaining more understanding of this disease and testing therapies that can potentially reverse it. There is no known study comparing the atherogenic propensity of different fractions of palm oil, that is, palm olien/liquid fraction and palm stearin/thick fraction, repeatedly heated palm oil, soya bean oil and repeatedly heated soya bean oil; hence the need for this study.

The seed of Garcinia kola tree is used locally in Southern Nigeria and some parts of West Africa as alternative medicine in the treatment of cough, oral infections, and liver diseases amongst others.[12] The active component is kolaviron, a biflavinoid fraction of the defatted alcohol extract of the seed.[13]Kolaviron has been demonstrated to exhibit many pharmacological effects. These include: anti inflammatory, anti oxidant[14], anti diabetic, [15]and anti hepatotoxic[12]effects. It has been suggested that it may have cholesterol-lowering potentials. [16]

We set out to determine the atherogenic effects of the two most commonly used cooking oils in Nigeria, that is, palm oil and soya bean oil in male Wistar rats. We also determined if administration of oral kolaviron reduces these atherogenic effects..

METHODS

Study setting and measurements

Forty-eight male Wistar rats weighing 120 – 150g were obtained from the Veterinary Anatomy Department Animal House, University of Ibadan. The animals were fed on normal chow with access to drinking water *ad libertum*. They were acclimatized for one week before the commencement of the experiment. They were then distributed randomly into six groups of eight animals each as shown in table 1. Of these, four animals from each group were randomly selected and marked on the body as animals to be orally administered 100mg/kg of kolaviron during the period of experiment.[16] All the animals in group one served as control and were fed on normal chow (NC) diet. Animals in the remaining five groups served as the experimental animals, with each group assigned to one of the five different test diets prepared and added to the normal chow diet as follows:

i) Normal chow diet fortified with 15% (w/w) non-heated soyabean oil (NC + SO)

ii) Normal chow diet fortified with 15% (w/w) five times heated soyabean oil (NC + HSO)

iii) Normal chow diet fortified with 15% (w/w) palm olien (NC + PO)

iv) Normal chow diet fortified with 15% (w/w) palm stearic (NC + PS)

v) Normal chow diet fortified with 15% (w/w) five times heated palm oil (NC + HPO)

To prepare the heated oils, 2500ml of the test oil was heated for 10 minutes to reach about 180°C. [17,18] This procedure was repeated five times for the five times heated oils, with 5 hours cooling phase between each round. The oil was then cooled to room temperature and stored before being mixed with the animal chow.Garcinia Kola was purchased from a local market in Ibadan, Nigeria. A total of 3kg of peeled seeds were sliced, pulverized with electric blender and then air dried in the laboratory from which coarse powered sample was extracted using the soxhlet apparatus.[15] The extract was concentrated by gentle boiling over water bath. The concentration of the ethanol extract of kolaviron to be orally administered to experimental animals was prepared by dissolving 10g in 100ml-distilled water to produce

100mg/ml.Rats in Groups CB, E1B, E2B, E3B, E4B and E5B were orally administered 100mg/kg of kolaviron five times a week, for a period of 12 consecutive weeks.[16]

On the last day of the experiment, the animals were fasted overnight. Before sacrificing each animal, 2mls of blood for lipid profile analysis was collected from the orbital sinus with the aid of a capillary tubeinto Ethylenediaminetetra-acetic acid (EDTA) bottles.Plasma lipid profile was determined enzymatically using commercially available kits from Randox Laboratories Limited, United Kingdom, following the manufacturer's instructions. The absorbance of the sample and standard randox reagent was measured in spectrum lab S23A spectrophotometer (Gulfex Medical and Scientific England). The handling of the animals adhered to the guidelines of ethical conduct of animal research of the University of Ibadan.

After sacrificing the animals, their aorta was dissected out and the ascending aorta was processed for histopathological examination. Photomicrographs of the tissue slides were taken with a Sony digital camera (M340). A computerized image analyzer, Motic Image Plus, version 2.0 was utilized for measuring the intimal and tunica media thickness of the aorta.

Statisticalanalysis

The results were expressed as mean ± SD (n=4). The statistical analysis involving the six groups was performed by one – way analysis of variance (ANOVA) followed by Dunnett's test. P – value < 0.05 was considered statistically significant. All the data were processed with GraphPad Software, Inc La Jolla, CA, USA, prism version 5.00.

RESULTS

Table 2 shows the percentage weight gain at the end of the 12 weeks of experiment in the controls and various groups of experimental animals. The increase in body weight of rats was highest in the soyabean oil group without kolaviron (E1A), with a mean weight gain of 93.75 ± 33.07g, corresponding to 46.15% increase in body weight. For the oral kolaviron groups,

the control (CB) had the highest increase in body weight with a mean weight gain of 68.75 ± 49.61g, corresponding to 32.35% increase in body weight. The least percentage increase in weight gain was found in the non heated and heated soya bean groups and the palm stearin groups. Using bonferroni multiple comparison test to compare weight gain in all test groups with the control groups, there was no significant difference in the mean weight gain, P = 0.9896. However, we observed that kolaviron tends to reduce the weight gain within each group.

Table 3 shows the plasma lipid profile for all groups while Figure 1 shows the total cholesterol (TC) concentrations in controls and all the groups of experimental animals. At the end of the 12-week study period,the lowest concentration of TC was found in the control group with no kolaviron CA (84.33 ± 6.35mg/dl) and control group with oral kolaviron CB (80.99 ± 11.11mg/dl) while the highest TC was found in animals in the heated palm oil group with no kolaviron E5A, with a mean value of (173.36 ± 5.00mg/dl) and animals in the heated palm oil group with oral kolaviron E5B, with a mean value of (157.56 ± 7.04mg/dl), $p < 0.05$. High fat diet and kolaviron have influence on the value of TC. High fat diet elevated the concentration of plasma TC in the test groups compared with the control groups while kolaviron lowered the plasma TC when compared with the non-kolaviron groups.

The lowest mean LDL-C value was observed in control group with no kolaviron (16.05 ± 4.70mg/dl) and control group with oral kolaviron (14.84 ± 3.17mg/dl), while it was significantly highest in Palm olein group with no kolaviron E3A (50.20 ± 3.41mg/dl) and palm olein group with oral kolaviron E3B (42.28 ± 14.35mg/dl) at $P < 0.05$. There was no significant difference between the control groups and all the various soyabean oil groups. The non HDL–C was significantly highest in Palm olein group E3A (76.41 ± 4.50mg/dl) and five times heated palm oil group E5B (53.32 ± 1.98mg/dl) for the non kolaviron and kolaviron group respectively. This result suggests that the palm olein group is the most atherogenic while non-heated soyabean oil is the least atherogenic of the treatment groups.

No atherosclerotic lesions were observed in the histology slides of the aorta of animals in the control groups. Experimental animals fed with heated palm oil diet showed isolated foam cells in the tunica intima that was classified as type I atherosclerotic lesion. All other treatment groups showed no thickening of thetunica intima of their aortas and there was absence of foam cells formation.

DISCUSSION

We observed an increase in body weight in all the controls and in all groups of experimental animals. However, within each group, animals on kolaviron gained less weight when compared with the non-kolaviron groups. This was in spite of the fact that they all fed well on the same quantity of food and it cannot be adduced to poor feed intake resulting from the bitter taste. Likewise, the heating process, which causes physical changes in the oils, did not have any significant effect on food intake of the rats. From this study, it appears that kolaviron might have weight reducing properties independent of food intake but further studies are needed to verify this.

The amount of dietary fat intake positively correlates with the value of TC and morbidity from coronary artery disease.[19] In our study, the addition of 15% w/w high fat diet to the chow caused an increase in TC and LDL-C in the control and experimental animals.TC value was lowest in the control groups fed with normal chow. The highest levels of atherogenic cholesterol [20], that is, LDL-C and non-HDL-C were found in animals in the palm olein and 5 times heated palm oil groups whilst the lowest levels were found in the soya bean oil group. An increase in non-HDL-C levels further confirms the harmful effects of palmitic acid – rich palm oil on health which may result in the formation of more oxidized LDL-C, accelerating the atherosclerosis process. This is not surprising as soya bean oil contains unsaturated fatty acid whereas, palm oil contains saturated fatty acid as 40% palmitic acid and only 0.2% lauric acid [21].

Heating soya bean oil 5 times altered the lipid profile by increasing the levels of TC, TG, LDL-C and non-HDL-C. This is in contrast to a previous report that fresh and heated soya oil did not interfere with serum TC, TG, and LDL-C but reduced HDL-C levels [22]. The duration and temperature to which the oils are heated may be responsible for these differences. A comparison between the five times heated oils and the non-heated oils suggest that repeated heating of oils might generate free radical formation which derange the lipid profile [23].

All animals in the treatment groups administered kolaviron orally, had lower TC values when compared with animals that were not administered kolaviron. Biflavonoids such as kolaflavonone, garcinia biflavonones (GB-1 and GB-2) xanthones and benzophenones have been reported as the constituents of *G.kola*[15]. These antioxidants in G.kola appear to be effective at 100mg/kg in lowering the TC as reported in earlier studies [16]. A trend similar to the levels of TC was observed in the values of LDL-C for both the non-kolaviron and kolaviron groups.

Histologically, there was no obvious focal or diffuse atherosclerotic plaqueformation seen in all the control and experimental groups of animals (figure 2), except for the group fed with five times heated palm oil which showed an isolated single foam cell (figure 3).

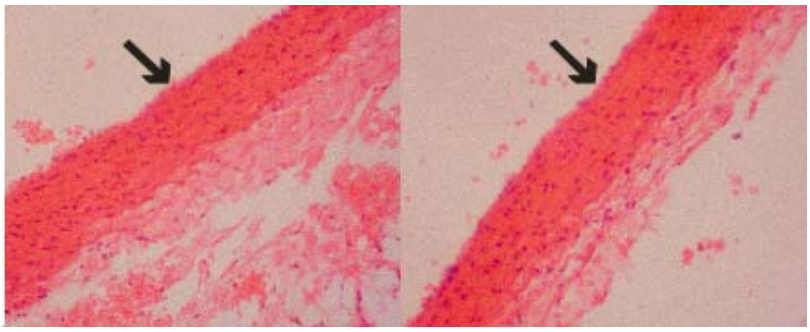

Figure 2. Hematoxylin-eosin stain of the aorta x 200 magnifications: control group fed on normal chow diet with no kolaviron (left plate)and normal chow diet with oral kolaviron(right plate) respectively. The arrows show no disruption of the tunica intimas.

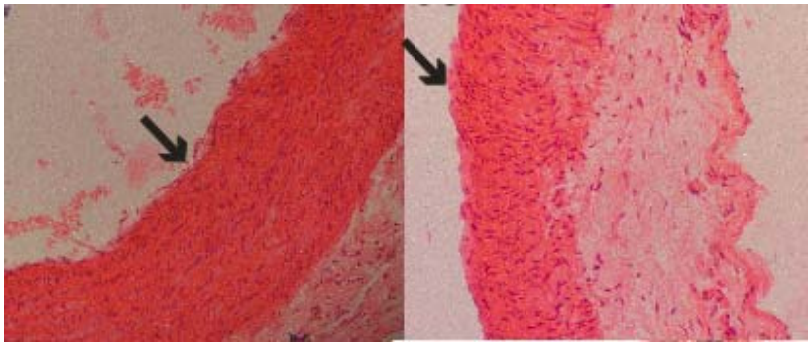

Figure 3. Hematoxylin-eosin stain of the aorta x 200 magnifications: rats fed on normal chow diet enriched with 5 times heated palm oil with no kolaviron(left plate) and with oral kolaviron(right plate) respectively. The arrow on the left shows isolated foam cells characteristic of Type I atherosclerotic lesion. The arrow on the right shows no foam cells in the tunica intima of the rat treated with kolaviron.

Therewas no obvious thickening or swelling of the tunica media indicating that there was no formation of lipidladenfoam cells. These findings suggest that repeatedly heated soyabean and palm oils cause no obvious detrimental effects on blood vessels at least on short to medium term basis even when they affect the lipid profile adversely. This may be because the changes in the lipid profile are not significant enough to manifest as structural lesions in the lining of the aorta. The relatively short duration of exposure during the experiment may also be contributory. Furthermore, the unexpected anti-atherosclerotic characteristic of palm oil may be due to its rich content of tocotrienols, which inhibit cholesterol synthesis *in vivo*[24].

In conclusion, the findings from this study show that consumption of common edible Nigerian oils could lead to high TC and elevation of atherogenic lipids in the plasma while 100mg/kg of kolaviron could be used to improve the plasma lipid profile as an alternative or an adjuvant to drug therapy. Also, early atherosclerosis could result from consumption of reheated palm oil.

CONFLICT OF INTEREST

The authors hereby declare that there are no conflicts of interest.

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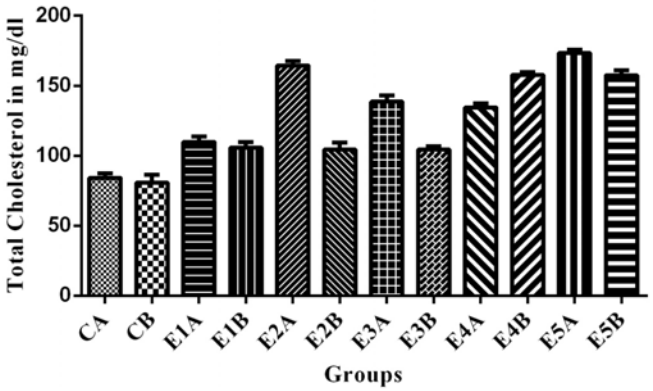

Figure 1: Effect of test diets and kolaviron on plasma total Cholesterol

Key: CA – Control group with no kolaviron, CB – Control group with oral kolaviron, E1A – Soyabean oil group with no kolaviron, E1B - Soyabean oil group with oral kolaviron, E2A – Heated Soyabean oil group with no kolaviron, E2B – Heated Soyabean oil group with oral kolaviron, E3A – Palm olein group with no kolaviron, E3B – Palm olein group with oral kolaviron, E4A – Palm stearin group with no kolaviron, E4B – Palm stearin group with oral kolaviron, E5A – Heated Palm oil group with no kolaviron, E5B – Heated Palm oil group with oralkolaviron.

Table 1: Animal Groupings

Groups	Control	E1	E2	E3	E4	E5
No. of Animals	8	8	8	8	8	8
Diet	NC	NC + SO	NC + HSO	NC + PO	NC + PS	NC + HPO
Animals with no Kolaviron	4 (CA)	4 (E1A)	4 (E2B)	4 (E3B)	4 (E4A)	4 (E5A)
Animals with Oral kolaviron	4 (CB)	4 (E1B)	4 (E2B)	4 (E3B)	4 (E4B)	4 (E5B)

Key 1: NC – Normal chow diet, SO – non heated soyabean oil, HSO – Five times heated soyabean oil, PO – Palm Olein, PS – Palm Stearin, HPO – Five times heated palm oil.
Key 2: CA – Control group with no kolaviron, CB – Control group with oral kolaviron, E1A – Soyabean oil group with no kolaviron, E1B - Soyabean oil group with oral kolaviron, E2A – Heated Soyabean oil group with no kolaviron, E2B – Heated Soyabean oil group with oral kolaviron, E3A – Palm olein group with no kolaviron, E3B – Palm olein group with oral kolaviron, E4A – Palm stearin group with no kolaviron, E4B – Palm stearin group with oral kolaviron, E1A – Heated Palm oil group with no kolaviron, E1B – Heated Palm oil group with oral kolaviron.

Table 2: The effect of high fat diet and kolaviron on body weight

Groups	Body weight (g)				
	Initial	**Final**	**Weight gain**	**% Change**	**p value**
CA	137.50 ± 17.68	225.00 ± 70.71	87.50 ± 53.03	38.89	
CB	143.75 ± 22.53	212.50 ± 33.07	68.75 ± 49.61	32.35	0.713
E1A	109.38 ± 11.97	203.13 ± 21.35	93.75 ± 33.07	46.15	
E1B	131.25 ± 7.22	183.33 ± 28.87	54.17 ± 26.02	28.41	0.006
E2A	114.06 ± 7.86	190.63 ± 15.73	76.56 ± 17.95	40.17	
E2B	148.44 ± 24.14	183.33 ± 31.46	31.25 ± 6.25	19.03	0.035
E3A	140.63 ± 11.97	206.25 ± 26.02	65.63 ± 21.35	31.82	
E3B	123.75 ± 10.51	168.75 ± 36.08	64.17 ± 32.24	26.67	0.549
E4A	120.31 ± 5.98	193.75 ± 12.5	73.44 L>± 10.67	37.90	
E4B	148.44 ± 13.86	178.13 ± 25.87	62.50 ± 8.84	16.67	0.015
E5A	145.25 ± 18.56	193.75 ± 16.14	48.50 ± 28.95	25.03	
E5B	156.25 ± 12.5	200.00 ± 22.82	43.75 ± 29.76	21.88	0.686

Values expressed are mean ± SD of four animals per subgroup, statistical significance at P<0.05

Key: CA – Control group with no kolaviron, CB – Control group with oral kolaviron, E1A – Soyabean oil group with no kolaviron, E1B - Soyabean oil group with oral kolaviron, E2A – Heated Soyabean oil group with no kolaviron, E2B – Heated Soyabean oil group with oral kolaviron., E3A – Palm olein group with no

kolaviron, E3B – Palm olein group with oral kolaviron., E4A – Palm stearin group with no kolaviron, E4B – Palm stearin group with oral kolaviron, E5A – Heated Palm oil group with no kolaviron, E5B – Heated Palm oil group with oral kolaviron.

Table 3: Plasma Lipid Profile (mg/dl) of experimental male wistar rats at the end of experiment

Group	TC	TG	HDL-C	LDL-C	nonHDL-C
CA	84.33 ± 6.35*	79.45 ± 5.27	52.39 ± 5.07	16.05 ± 4.70*	31.93 ± 5.22
CB	80.99 ± 11.11*	68.84 ± 20.59	53.39 ± 4.36	14.84 ± 3.17*	27.61 ± 8.39
E1A	109.99 ± 7.73	63.83 ± 12.91	75.07 ± 2.89	22.16 ± 9.67	34.93 ± 8.32
E1B	104.34 ± 10.37	70.47 ± 10.43	70.47 ± 5.09	17.50 ± 2.44	35.47 ± 2.98
E2A	164.58 ± 6.68*	87.54 ± 4.63	114.36 ± 7.58	32.21±12.51	50.22 ±13.24
E2B	105.94 ± 7.56	93.09 ± 5.08	64.48 ± 2.05	22.24-±7.23	39.86 ± 8.84
E3A	138.73 ± 8.79*	131.08 ± 7.46	62.31 ± 4.37	50.20 ± 3.41*	76.41 ± 4.50
E3B	104.54 ± 4.53	77.15 ± 2.62	78.54 ± 7.09	42.28 ± 14.35*	26.00 ± 3.92
E4A	134.61 ± 5.56	123.43 ± 1.78	78.96 ± 2.93	30.97 ± 2.57	55.65 ± 2.86
E4B	157.75 ± 4.24	104.69 ± 3.86	121.43 ± 6.00	15.38 ± 2.64*	36.32 ± 1.89
E5A	173.36 ± 5.00*	97.46 ± 1.21	114.72 ± 3.95	39.15 ± 8.70	58.64 ± 8.88
E5B	157.56 ± 7.04*	68.56 ± 3.30	104.24 ± 5.31	39.61 ± 1.47	53.32 ± 1.98

Values expressed are mean ± SD of four animals per subgroup, *statistical significance at P<0.05

Key1: CA – Control group with no kolaviron, CB – Control group with oral kolaviron, E1A – Soyabean oil group with no kolaviron, E1B - Soyabean oil group with oral kolaviron, E2A – Heated Soyabean oil group with no kolaviron, E2B – Heated Soyabean oil group with oral kolaviron, E3A – Palm olein group with no kolaviron, E3B – Palm olein group with oral kolaviron, E4A – Palm stearin group with no kolaviron, E4B – Palm stearin group with oral kolaviron, E5A – Heated Palm oil group with no kolaviron, E5B – Heated Palm oil group with oral kolaviron.

Key2: TC is Total Cholesterol, TG is Triglyceride, HDL-C is High Density Lipoprotein Cholesterol, LDL-C is Low Density Lipoprotein Cholesterol, non HDL-C is High Density Lipoprotein Cholesterol.