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

5 **Running title:** Nigerian edible oils and atherosclerosis

6

7

8 **ABSTRACT**

9 **BACKGROUND:** Dyslipidemia is partly dependent on consumption of edible oils. We set  
10 out to determine the atherogenic effects of common edible oils in Nigeria, in male Wistar rats  
11 and to find out if administration of oral kolaviron ameliorated these effects.

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

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

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

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

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

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### 34 **INTRODUCTION**

35 One of the modifiable risk factors for atherosclerosis and coronary artery disease is  
36 dyslipidemia,<sup>1</sup> that in part is dependent on the dietary habit of consumption of edible oils.

37 Dietary factors during the life course can influence the pathogenesis and progression of  
38 atherosclerosis.<sup>2</sup> The adoption of western lifestyle and diet has compounded this further in

39 Sub-Saharan Africa. Palm oil and soya bean oil are one of the commonly used cooking oils in  
40 Nigeria and there is paucity of data on their atherogenic propensity in this population.

41 Furthermore, the cooking methods adopted locally in ready to eat and fast foods involve the  
42 use of repeatedly heated oils over several cycles of cooking. Like humans, high fat diets can

43 induce elevated LDL-C<sup>3,4</sup> and atherosclerosis in certain rodent models such as rats, mice,  
44 hamsters, rabbits and guinea pigs. Red palm oil was shown to be significantly less

45 atherogenic than refined, bleached and deodorized (RBD) palm oil in rabbits.<sup>5</sup> They also had  
46 similar effects on serum and liver lipids. Soya oil contains approximately 60% poly-

47 unsaturated fatty acid (PUFA), 24% of mono-unsaturated fatty acid (MUFA) and 16% of  
48 saturated fatty acids.<sup>6</sup> This high level of PUFA dietary intake can improve the blood lipid

49 profile status.<sup>7</sup> In addition, with its high content of tocopherols, soya oil is known to exhibit  
50 various antioxidant actions against lipid peroxidation.<sup>5</sup>

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

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

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

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

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77 **METHODS**78 **Study setting and measurements**

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

- 89 i) Normal chow diet fortified with 15% (w/w) non-heated soyabean oil (NC + SO)  
90 ii) Normal chow diet fortified with 15% (w/w) five times heated soyabean oil (NC + HSO)  
91 iii) Normal chow diet fortified with 15% (w/w) palm olien (NC + PO)  
92 iv) Normal chow diet fortified with 15% (w/w) palm stearic (NC + PS)  
93 v) Normal chow diet fortified with 15% (w/w) five times heated palm oil (NC + HPO)

94 To prepare the heated oils, 2500ml of the test oil was heated for 10 minutes to reach about  
95 180°C.<sup>17,18</sup> This procedure was repeated five times for the five times heated oils, with 5  
96 hours cooling phase between each round. The oil was then cooled to room temperature and  
97 stored before being mixed with the animal chow. Garcinia Kola was purchased from a local  
98 market in Ibadan, Nigeria. A total of 3kg of peeled seeds were sliced, pulverized with electric  
99 blender and then air dried in the laboratory from which coarse powered sample was extracted  
100 usi8Tng the soxhlet apparatus.<sup>15</sup> The extract was concentrated by gentle boiling over water  
101 bath. The concentration of the ethanol extract of kolaviron to be orally administered to  
102 experimental animals was prepared by dissolving 10g in 100ml-distilled water to produce

103 100mg/ml. Rats in Groups CB, E1B, E2B, E3B, E4B and E5B were orally administered  
104 100mg/kg of kolaviron five times a week, for a period of 12 consecutive weeks.<sup>16</sup>

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

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

### 117 **Statistical analysis**

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

### 122 **RESULTS**

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

127 the control (CB) had the highest increase in body weight with a mean weight gain of  $68.75 \pm$   
128  $49.61\text{g}$ , corresponding to  $32.35\%$  increase in body weight. The least percentage increase in  
129 weight gain was found in the non heated and heated soya bean groups and the palm stearin  
130 groups. Using bonferroni multiple comparison test to compare weight gain in all test groups  
131 with the control groups, there was no significant difference in the mean weight gain,  $P =$   
132  $0.9896$ . However, we observed that kolaviron tends to reduce the weight gain within each  
133 group.

134 Table 3 shows the plasma lipid profile for all groups while Figure 1 shows the total  
135 cholesterol (TC) concentrations in controls and all the groups of experimental animals. At the  
136 end of the 12-week study period, the lowest concentration of TC was found in the control  
137 group with no kolaviron CA ( $84.33 \pm 6.35\text{mg/dl}$ ) and control group with oral kolaviron CB  
138 ( $80.99 \pm 11.11\text{mg/dl}$ ) while the highest TC was found in animals in the heated palm oil group  
139 with no kolaviron E5A, with a mean value of ( $173.36 \pm 5.00\text{mg/dl}$ ) and animals in the heated  
140 palm oil group with oral kolaviron E5B, with a mean value of ( $157.56 \pm 7.04\text{mg/dl}$ ),  $p < 0.05$ .

141 High fat diet and kolaviron have influence on the value of TC. High fat diet elevated the  
142 concentration of plasma TC in the test groups compared with the control groups while  
143 kolaviron lowered the plasma TC when compared with the non-kolaviron groups.

144 The lowest mean LDL-C value was observed in control group with no kolaviron ( $16.05 \pm$   
145  $4.70\text{mg/dl}$ ) and control group with oral kolaviron ( $14.84 \pm 3.17\text{mg/dl}$ ), while it was  
146 significantly highest in Palm olein group with no kolaviron E3A ( $50.20 \pm 3.41\text{mg/dl}$ ) and  
147 palm olein group with oral kolaviron E3B ( $42.28 \pm 14.35\text{mg/dl}$ ) at  $P < 0.05$ . There was no  
148 significant difference between the control groups and all the various soyabean oil groups. The  
149 non HDL-C was significantly highest in Palm olein group E3A ( $76.41 \pm 4.50\text{mg/dl}$ ) and five  
150 times heated palm oil group E5B ( $53.32 \pm 1.98\text{mg/dl}$ ) for the non kolaviron and kolaviron  
151 group respectively. This result suggests that the palm olein group is the most atherogenic  
152 while non-heated soyabean oil is the least atherogenic of the treatment groups.

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

## 158 **DISCUSSION**

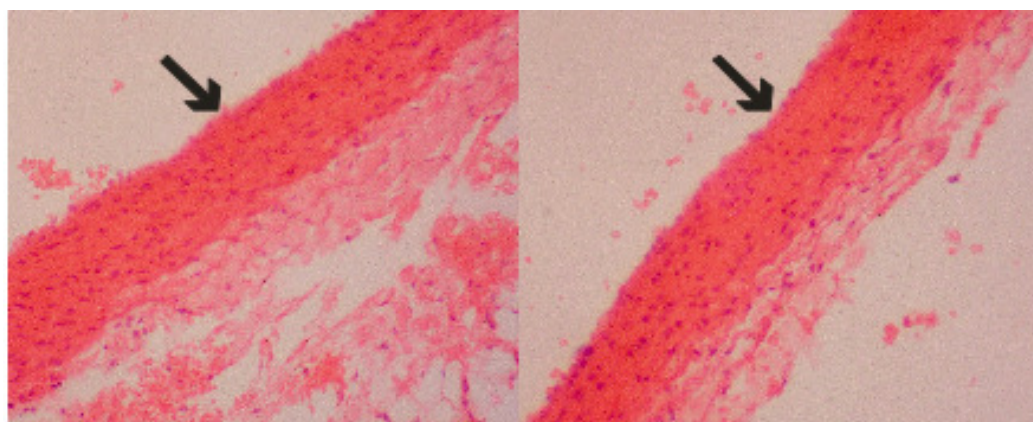
159 We observed an increase in body weight in all the controls and in all groups of experimental  
160 animals. However, within each group, animals on kolaviron gained less weight when  
161 compared with the non-kolaviron groups. This was in spite of the fact that they all fed well  
162 on the same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

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

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

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

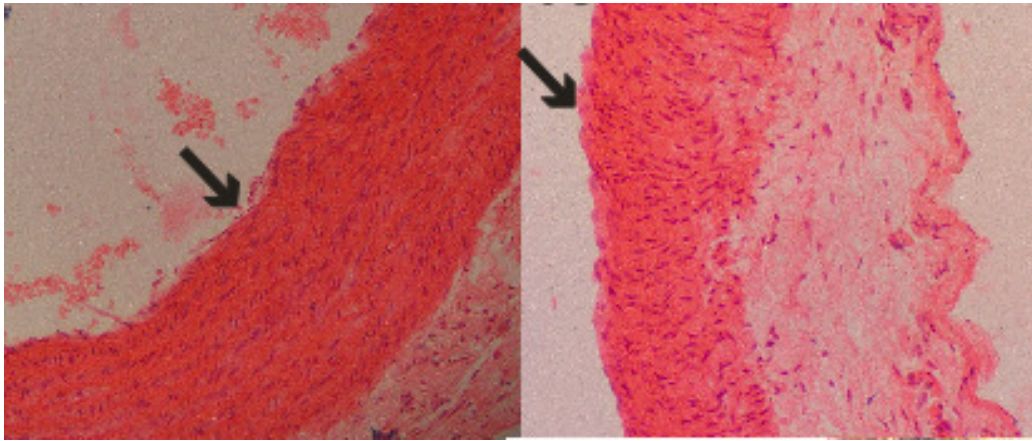
191 Histologically, there was no obvious focal or diffuse atherosclerotic plaque formation seen in  
192 all the control and experimental groups of animals (figure 2), except for the group fed with  
193 five times heated palm oil which showed an isolated single foam cell (figure 3).



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195 Figure 2. Hematoxylin-eosin stain of the aorta x 200 magnifications: control group fed  
196 on normal chow diet with no kolaviron and normal chow diet with oral kolaviron  
197 respectively. The arrows show no disruption of the tunica intimas.  
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200 Figure 3. Hematoxylin-eosin stain of the aorta x 200 magnifications: rats fed on normal  
201 chow diet enriched with 5 times heated palm oil with no kolaviron and with oral  
202 kolaviron respectively. The arrow on the left shows isolated foam cells characteristic of  
203 Type I atherosclerotic lesion. The arrow on the right shows no foam cells in the tunica  
204 intima of the rat treated with kolaviron.  
205

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

214 In conclusion, the findings from this study show that consumption of common edible  
215 Nigerian oils could lead to high TC and elevation of atherogenic lipids in the plasma while  
216 100mg/kg of kolaviron could be used to improve the plasma lipid profile as an alternative or  
217 an adjuvant to drug therapy. Also, early atherosclerosis could result from consumption of  
218 reheated palm oil.

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222 **CONFLICT OF INTEREST**

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

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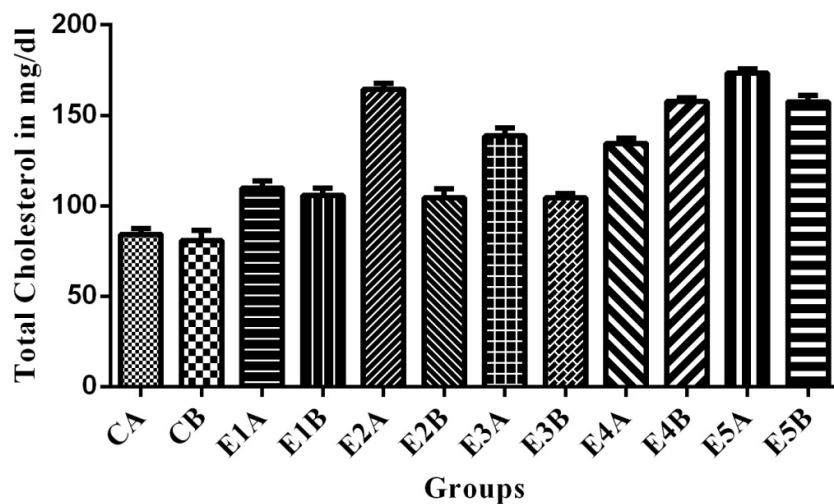
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304 Figure 1: Effect of test diets and kolaviron on plasma total Cholesterol

305 **Key:** CA – Control group with no kolaviron, CB – Control group with oral kolaviron, E1A – Soyabean oil  
 306 group with no kolaviron, E1B - Soyabean oil group with oral kolaviron, E2A – Heated Soyabean oil group with  
 307 no kolaviron, E2B – Heated Soyabean oil group with oral kolaviron, E3A – Palm olein group with no kolaviron,  
 308 E3B – Palm olein group with oral kolaviron, E4A – Palm stearin group with no kolaviron, E4B – Palm stearin  
 309 group with oral kolaviron, E5A – Heated Palm oil group with no kolaviron, E5B – Heated Palm oil group with  
 310 oral kolaviron.

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326 **Table 1: Animal Groupings**

327	<b>Groups</b>	<b>Control</b>	<b>E1</b>	<b>E2</b>	<b>E3</b>	<b>E4</b>	<b>E5</b>
328	<b>No. of Animals</b>	8	8	8	8	8	8
329	<b>Diet</b>	NC	NC + SO	NC + HSO	NC + PO	NC + PS	NC + HPO
330	<b>Animals with</b>	4 (CA)	4 (E1A)	4 (E2B)	4 (E3B)	4 (E4A)	4 (E5A)
331	<b>no Kolaviron</b>						
332	<b>Animals with</b>	4 (CB)	4 (E1B)	4 (E2B)	4 (E3B)	4 (E4B)	4 (E5B)
333	<b>Oral kolaviron</b>						

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 335 **Key 1:** NC – Normal chow diet, SO – non heated soyabean oil, HSO – Five times heated  
 336 soyabean oil, PO – Palm Olein, PS – Palm Stearin, HPO – Five times heated palm oil.  
 337 **Key 2:** CA – Control group with no kolaviron, CB – Control group with oral kolaviron,  
 338 E1A – Soyabean oil group with no kolaviron, E1B - Soyabean oil group with oral kolaviron,  
 339 E2A – Heated Soyabean oil group with no kolaviron, E2B – Heated Soyabean oil group with  
 340 oral kolaviron, E3A – Palm olein group with no kolaviron, E3B – Palm olein group with oral  
 341 kolaviron, E4A – Palm stearin group with no kolaviron, E4B – Palm stearin group with oral  
 342 kolaviron, E1A – Heated Palm oil group with no kolaviron, E1B – Heated Palm oil group  
 343 with oral kolaviron.  
 344  
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346 **Table 2: The effect of high fat diet and kolaviron on body weight**

347	<b>Groups</b>	<b>Body weight (g)</b>				
348		<b>Initial</b>	<b>Final</b>	<b>Weight gain</b>	<b>% Change</b>	<b>p value</b>
350						
351	CA	137.50 ± 17.68	225.00 ± 70.71	87.50 ± 53.03	38.89	
352	CB	143.75 ± 22.53	212.50 ± 33.07	68.75 ± 49.61	32.35	0.713
353						
354	E1A	109.38 ± 11.97	203.13 ± 21.35	93.75 ± 33.07	46.15	
355	E1B	131.25 ± 7.22	183.33 ± 28.87	54.17 ± 26.02	28.41	0.006
356						
357	E2A	114.06 ± 7.86	190.63 ± 15.73	76.56 ± 17.95	40.17	
358	E2B	148.44 ± 24.14	183.33 ± 31.46	31.25 ± 6.25	19.03	0.035
359						
360	E3A	140.63 ± 11.97	206.25 ± 26.02	65.63 ± 21.35	31.82	
361	E3B	123.75 ± 10.51	168.75 ± 36.08	64.17 ± 32.24	26.67	0.549
362						
363	E4A	120.31 ± 5.98	193.75 ± 12.5	73.44 L>± 10.67	37.90	
364	E4B	148.44 ± 13.86	178.13 ± 25.87	62.50 ± 8.84	16.67	0.015
365						
366	E5A	145.25 ± 18.56	193.75 ± 16.14	48.50 ± 28.95	25.03	
367	E5B	156.25 ± 12.5	200.00 ± 22.82	43.75 ± 29.76	21.88	0.686

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

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

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

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

382 Group	TC	TG	HDL-C	LDL-C	nonHDL-C
384 CA	84.33 ± 6.35*	79.45 ± 5.27	52.39 ± 5.07	16.05 ± 4.70*	31.93 ± 5.22
385 CB	80.99 ± 11.11*	68.84 ± 20.59	53.39 ± 4.36	14.84 ± 3.17*	27.61 ± 8.39
386					
387 E1A	109.99 ± 7.73	63.83 ± 12.91	75.07 ± 2.89	22.16 ± 9.67	34.93 ± 8.32
388 E1B	104.34 ± 10.37	70.47 ± 10.43	70.47 ± 5.09	17.50 ± 2.44	35.47 ± 2.98
389					
390 E2A	164.58 ± 6.68*	87.54 ± 4.63	114.36 ± 7.58	32.21±12.51	50.22 ±13.24
391 E2B	105.94 ± 7.56	93.09 ± 5.08	64.48 ± 2.05	22.24±7.23	39.86 ± 8.84
392					
393 E3A	138.73 ± 8.79*	131.08 ± 7.46	62.31 ± 4.37	50.20 ± 3.41*	76.41 ± 4.50
394 E3B	104.54 ± 4.53	77.15 ± 2.62	78.54 ± 7.09	42.28 ± 14.35*	26.00 ± 3.92
395					
396 E4A	134.61 ± 5.56	123.43 ± 1.78	78.96 ± 2.93	30.97 ± 2.57	55.65 ± 2.86
397 E4B	157.75 ± 4.24	104.69 ± 3.86	121.43 ± 6.00	15.38 ± 2.64*	36.32 ± 1.89
398					
399 E5A	173.36 ± 5.00*	97.46 ± 1.21	114.72 ± 3.95	39.15 ± 8.70	58.64 ± 8.88
400 E5B	157.56 ± 7.04*	68.56 ± 3.30	104.24 ± 5.31	39.61 ± 1.47	53.32 ± 1.98

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

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

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

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