Altered Serum Lipid Profile in Albino Wistar Rats Following the Consumption of *Cola nitida rubra* (Kola nut)


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**ABSTRACT**

**Background:** Following the increased rate of consumption of kola nut among the male population in Nigeria, this study seeks to determine the effect of its consumption on serum lipid profile, which is an important biochemical indices that checks coronary heart disease. **Methodology:** Eighteen (18) male albino rats weighing 180 - 200 g were used for this research. After 14 days of habituation, the animals were randomly assigned 1 of 3 groups, (n = 6), and were labeled control group, low dose (LD) group and high dose (HD) group respectively. The control group received normal growers' feed. The LD group received a modified feed (15 g *Cola nitida rubra* powder + 85 g growers' feed) while the HD group received 30 g C, *nitida rubra* powder + 70 g growers' feed. All animals had free access to water ab libitum. After 28 days of administration, blood samples were collected via cardiac puncture and lipid profile analyzed. **Results:** The results showed that serum total cholesterol (TC) triglyceride (TG), very low density lipoprotein (VLDL-C) and low density lipoprotein (LDL-C) were significantly (P<0.05; P<0.05; P<0.01 and P<0.05 respectively) higher in the LD group, compared with control. TC, high density lipoprotein (HDL-C) and LDL-C were significantly (P<0.05, P<0.05 and P<0.001 respectively) reduced in the HD group, compared with control. VLDL-C was significantly (P<0.01) higher in the HD group, compared with the LD group, while LDL-C was significantly (P<0.001) lower in the HD group, compared with the LD group. Atherogenic index (AI) was significantly (P<0.05) increased in the LD group, but significantly (P<0.001) reduced in the HD group, compared with control. **Conclusion:** High consumption of kola nut reduces serum cholesterol concentration and may reduce the risk of developing coronary heart disease, while moderate consumption does the direct opposite.

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**INTRODUCTION**

Cholesterol is an essential component of every cell structure in the body. It is necessary for formation and repairs of new and existing cells (Jefcoate *et al.*, 1992). Cholesterol is also utilized during the synthesis of cortisol and testosterone by the adrenal gland and testicles respectively (Jefcoate *et al.*, 1992). Increased levels of cholesterol or triglycerides are most often indications of genetic or inherited disorders of lipid metabolism. These lipids may also be increased by some common medical conditions such as hypothyroidism, diabetes mellitus (DM), kidney and liver disease (Navab *et al.*, 1996; Beckman *et al.*, 2002; Gordon *et al.*, 2010). Fat-rich diets also affect serum cholesterol and TG concentrations negatively (Simon, *et al.*,1995; Romon, *et al.*, 1995).

*Cola* (*Cola nitida*) is the nut of the cola tree, a genus of trees native to the tropical rain forests of Africa, classified in the family Malvaceae, sub family sterculiodeae (Lowor *et al.*, 2010). The cola tree is an evergreen tree, growing up to 20 m tall, with glossy ovoid leaves and star - shaped fruits (Russel, 1995). A number of sub-species within *Cola nitida* have been identified alba, rubra, mixta and pallida, all of which are cultivated in Nigeria (Russel, 1995; Lowor *et al.*, 2010). *Cola nitida rubra* (kola nut) has been a popular consumable in Nigeria, especially among the North and South - Eastern dwellers. In Nigeria today, kola nuts have generally been perceived to be a stimulant and to enable one withstand stress (Lowor *et al.*, 2010). Extracts of *Cola nitida* have been credited with increased gastric acid secretion (Tende *et al.*, 2011), increase in body temperature, blood pressure (BP) and respiratory rate (Fereday *et al.*, 1997). Extract of *C. nitida* has also been credited with the relieve of migraine headaches due to its high caffeine content (Irvine, 1961). Extract of *Cola nitida rubra*
has also been reported to reduce serum reproductive hormone concentrations and sperm count in male wistar rats (Okon et al., 2014).

Following reports on the effects of Cola nitida rubra on various systems of the body, and considering its high consumption rate, it became important to ascertain its effect on serum lipid profile with a view to educate consumers on possible alterations, since there is paucity of scientific literature in this regard.

MATERIALS AND METHODS

Plant Material:
Fresh kola nuts (Cola nitida rubra) were purchased from Watt market in Calabar, Cross River State, Nigeria. The plant material was identified by the Chief Herbarium Officer of Botany Department, University of Calabar, Nigeria. The kola nuts were cut into smaller bits and sundried to remove moisture. The dried nuts were grounded to powder and stored pending usage.

Preparation of Cola nitida rubra Diet:
Cola nitida rubra powder was thoroughly mixed with the palletized growers’ feed in the ratio shown in the table below, and given to the animals in the test groups.

<table>
<thead>
<tr>
<th></th>
<th>Group I Control</th>
<th>Group II LD</th>
<th>Group III HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Rats</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Growers Feed (g)</td>
<td>100</td>
<td>85</td>
<td>70</td>
</tr>
<tr>
<td>Cola nitida rubra Powder (g)</td>
<td>-</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Total feed (g)</td>
<td>100</td>
<td>100</td>
<td>100</td>
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</tbody>
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Experimental Animals and Protocol:
Eighteen (18) male albino wistar rats weighing 180-220 g were obtained from the Animal House of the Department of Physiology, College of Medical Sciences, University of Calabar, Nigeria. The animals were randomly assigned 1 of 3 groups (n = 6), thus, control, low dose (LD) and high dose (HD) group. The animals were allowed 14 days for habituation, after which treatment with Cola nitida rubra begun. The control group received normal growers’ feed, the LD group received 15 g Cola nitida rubra powder + 85 g growers’ feed, while the HD group received 30 g Cola nitida rubra powder + 70 g growers’ feed (Table 1). All animals had access to drinking water ad libitum, and exposed to normal temperature and 12/12 hours dark/light cycle. The feeding regimen lasted for 28 days. Indeed, the principles of laboratory animals’ care as laid down by the ethics committee of the College of Medical Sciences, University of Calabar, was strictly followed.

Sample Collection:
After 28 days of feeding with the modified diet, the animals were anesthetized using chloroform anesthesia. Blood sample was then collected by cardiac puncture for lipid profile analysis. A 5 ml syringe attached to 21 G needle was used to collect blood samples. The samples were collected into plain capped bottles and EDTA-treated bottles, for serum and whole blood samples respectively. The samples were immediately used for the analysis of lipid profile.

Serum Lipid Profile Estimation:
Estimate of Total Cholesterol (TC):
Serum total cholesterol (TC) was estimated using the enzymatic colorimetric test kit method of Sieldel et al., (1985). Cholesterol esterase catalyses the hydrolysis of cholesterol esters into free cholesterol and fatty acid. Cholesterol oxidase then catalysis the oxidation of the free cholesterol to cholestene-3-one and hydrogen peroxide. Phenol and 4-amino-antipyrine then combines with the hydrogen peroxide in the presence of peroxidase to produce a red coloured quinonemine which is read colorimetrically at 540nm. The total cholesterol concentration is directly proportional to the intensity of the colour obtained.

Estimate of Serum Triacylglycerol (TG):
The triglyceride concentration in the samples was determined by method of Negele et al., (1985). TGs are enzymatically hydrolyzed to glycerol and fatty acid by a lipoprotein lipase. Glycerol kinase then phosphorylates the glycerol produced to yield glycerol 3-phosphate, which is then oxidized to dihydroxyacetone phosphate and hydrogen peroxide by glycerol phosphate oxidase. The chromogen comprising of n-ethyl-n-sulpholydroxypropyl-n-foludine is then oxidized. A purple coloured quinoneimine dye formed as a result of these reactions was read colorimetrically at 540nm.
Estimation of HDL-cholesterol (HDL-C):
HDL-cholesterol estimation was done according to the method of Siedel et al., (1985) for total cholesterol estimation previously described.

Estimation of VLDL-cholesterol Concentration (VLDL-C):
The VLDL-C concentration was obtained by dividing the serum TG concentration by 5. This factor of 5 is based on the fact that in fasting subjects with triglyceride concentration of 400 mg/dl, the VLDL to total plasma triglyceride ratio is fixed at 1:5.

VLDL-C (mg/dl) = \frac{\text{Triglyceride (TG)}}{5}

Estimation of LDL-Cholesterol Concentration (LDL-C):
By the Friedewald's (1972) relationship, LDL-cholesterol is derived from the difference between the serum TC and sum of HDL-cholesterol and VLDL-cholesterol.

LDL-C = TC - (HDL-C + VLDL-C).

Atherogenic Index (AI):
Atherogenic index was derived using the formula:

Atherogenic Index (AI) = \frac{\text{LDL-C}}{\text{HDL-C}}

Statistical Analysis:
The data are expressed as the mean ± SEM. One-way analysis of variance (ANOVA) was used for analysis, followed by least square difference (LSD) post hoc multiple comparison, using SPSS software version 15.0. \( P < 0.05 \) was considered significant.

Results:
Total Cholesterol (TC) Concentration:
The total cholesterol (TC) concentration in the control, LD and HD group was 1.16 ± 0.05, 1.36 ± 0.05 and 0.92 ± 0.06 mmol/L for control, LD and HD group respectively. TC concentration was significantly (\( P < 0.05 \)) higher in the LD group, compared with control, but significantly (\( P < 0.01 \)) lower in the HD group, compared with control. TC concentration was significantly (\( P < 0.001 \)) lower in the HD group, compared with the LD group, (Fig. 1).

Triglyceride (TG) Concentration:
The total TG concentration in the LD (0.52 ± 0.04 mmol/L) and HD (0.58 ± 0.04 mmol/L) group was significantly (\( P < 0.05 \) and \( P < 0.01 \) respectively) higher, compared with control (0.36 ± 0.02 mmol/L), (Fig. 2).

Fig. 1: Comparison of total cholesterol (TC) concentration in the different experimental groups. Values are mean ± SEM, n = 6. \*\( P < 0.05 \), \*\*\( P < 0.01 \) vs control; c = \( P < 0.001 \) vs LD.
Fig. 2: Comparison of triglyceride (TG) concentration in the different experimental groups. Values are mean ± SEM, n = 6. *P<0.05, **P<0.01 vs control.

**High Density Lipoprotein (HDL) Concentration:**
The HDL concentration in the control, LD and HD group was 0.38 ± 0.04, 0.34 ± 0.02 and 0.24 ± 0.02 mmol/L respectively. HDL concentration was significantly (P<0.05) lower in the HD group, compared with control and the LD group, (Fig. 3).

**Very Low Density Lipoprotein (VLDL) Concentration:**
The concentration of VLDL in the control, LD and HD group was 0.16 ± 0.01, 0.24 ± 0.02 and 0.30 ± 0.00 mmol/L respectively. VLDL concentration was significantly higher in the LD (P<0.01) and HD (P<0.001) group, compared with control. VLDL concentration was significantly (P<0.01) higher in the HD group, compared with the LD group, (Fig. 4).

Fig. 3: Comparison of high density lipoprotein (HDL) concentration in the different experimental groups. Values are mean ± SEM, n = 6. *P<0.05 vs control; a = P<0.05 vs LD.
Low Density Lipoprotein (LDL) Concentration:

The concentration of LDL cholesterol was 0.64 ± 0.04, 0.78 ± 0.05 and 0.14 ± 0.00 mmol/L for control, LD and HD group respectively. LDL concentration in the LD group was significantly (P<0.05) higher, compared to control, but significantly (P<0.001) reduced in the HD group, compared with control. LDL concentration was also significantly (P<0.001) reduced in the HD group, compared with the LD group, (Fig. 5).

Fig. 4: Comparison of very low density lipoprotein (VLDL) concentration in the different experimental groups. Values are mean ± SEM, n = 6. **P<0.01, ***P<0.001 vs control; b = P<0.01 vs LD.

Fig. 5: Comparison of low density lipoprotein (LDL) concentration in the different experimental groups. Values are mean ± SEM, n = 6. *P<0.05, ***P<0.001 vs control; c = P<0.001 vs LD.
**Atherogenic Index (AI):**

The atherogenic index (AI) in the control, LD and HD group was 1.68 ± 0.01, 2.29 ± 0.01 and 0.58 ± 0.01 respectively. AI in the LD group was significantly (P<0.05) higher, compared with the control. AI was significantly (P<0.001) lower in the HD group, compared with control. It was also significantly (P<0.001) reduced in the HD group, compared to the LD group, (Fig. 6).

![Graph showing comparison of atherogenic index (AI) in different experimental groups.](image)

**Fig. 6:** Comparison of atherogenic index (AI) in the different experimental groups. Values are mean ± SEM, n = 6. *P<0.05, ***P<0.001 vs control; c = P<0.001 vs LD.

**Discussion:**

This study analyzed the effect of consumption of kola nut on serum cholesterol concentration. Cholesterol is an important structural component of the cell, in addition to it being the precursor for synthesis of some hormones, like testosterone, cortisol and estrogen/progesterone by the testicles, adrenal gland and ovaries respectively (Jefcoate et al., 1992). Nonetheless, hyperlipidemia (abnormally increased levels of cholesterol) occurs when there is an abnormal increase in the total plasma concentrations of cholesterol, triglycerides and low density lipoproteins (LDL-cholesterol), with a reduction in the level of high density lipoprotein (HDL-cholesterol). These abnormal levels of the different fractions of cholesterol forms the basis of the development of coronary heart diseases, which by themselves, constitute a major health problem of great concern (Mader, 1994; Poirier, 2006).

In our study, total cholesterol (TC) concentration was significantly increased in the low dose group, compared to control, with the high dose group having a significant decrease, compared with control and the low dose group. This is suggestive of the fact that increased consumption of kola nut maybe beneficial in tackling coronary heart disease (CHD) since it reduces the TC concentration, while a moderate consumption may increase the risk of developing CHD. Triglyceride (TG) levels was found to increase significantly in the LD and HD kola nut - treated groups, with HD group having the highest concentration which was significant, compared with LD group. This is consistent with findings of Boozer et al., (2002) who reported that kola nut increased metabolic rate, provides energy by increasing TGs in muscle tissues that can help during strenuous exercises.

High density lipoprotein (HDL-C) was decreased in a dose - dependent pattern, with the HD group having the lowest concentration which was significantly lower than the LD group. On the other hand, VLDL-C was significantly higher in the LD and HD group, with the decrease being dose - dependent and showed HD group as having the highest concentration compared with control and LD group. Ironically, LDL-C referred to as the bad cholesterol was lowest in the HD group. The effect of kola nut on lipid profile has previously been linked to its caffeine content, however, reports have been inconclusive. Some studies have reported a decrease in TC and LDL-C component of serum lipids in caffeinated beverages (Abrokawah, et al., 2009), while others have reported the reverse (Jee et al., 2001; Ricketts et al., 2007). Caffeine is one of the most widely ingested pharmacologically active substances which is rapidly absorbed in the digestive tract (Matissek, 1997). It is present in many beverages, including kola nut tea. The decrease in TC and LDL in the high dose kola nut -
treated group maybe related to a possible negative feedback mechanism that prevents caffeine uptake, hence, reducing serum lipid composition.

Atherogenic dyslipidemia which is characterized by a combination of an increase in the level of triglycerides, LDL-cholesterol, atherogenic index and a decrease in the level of HDL-cholesterol (Grundy, 2004; Wansi et al., 2009) was observed in the low dose group. This is suggestive of the fact that moderate consumption of kola nut could predispose to atherosclerosis and consequently predispose to cardiovascular diseases.

**Conclusion:**

On the basis of the results obtained from this study, we therefore conclude that high consumption of *Cola nitida rubra* (kola nut) reduces serum cholesterol concentration and may reduce the risk of developing coronary heart disease, while moderate consumption does the direct opposite.

**REFERENCES**


