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# Effect of Chronic Consumption of *Cola nitida rubra* (Kola Nut) Diet on Biliary Secretion and Composition in Albino Wistar Rats.

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**ABSTRACT**

The consumption of *Cola nitida rubra* (kola nut) in West Africa and in Nigeria specifically is on the rise. Previous studies had reported an increase in gastric acid secretion following the consumption of kola nut and kola nut beverages. This study therefore seeks to ascertain the effect of kola nut consumption on bile secretion and composition, which is an important indices if the duodenum is to escape ulceration by acidic chyme emptied into it by the stomach. Eighteen (18) albino wistar rats weighing 180 - 200 g were used for this study. The animals were randomly assigned 1 of 3 groups (n = 6), thus, control group, low dose (LD) and high dose (HD) group. The control group received normal growers feed, the LD group received growers feed and kola nut powder in the ratio 85:15, while the HD group received growers feed and kola nut powder in the ratio 70:30. All animals had access to water *ad libitum*. Normal room temperature and 12/12 hours light/cycle was maintained. After 28 days of feeding, bile was collected and analyzed. Rate of bile secretion in the low dose and high dose group was significantly ($P<0.05$, $P<0.01$ respectively) lower, compared with control. Biliary cholesterol concentration in the LD and HD group was significantly ($P<0.01$; $P<0.001$, respectively) reduced, compared with control. Biliary total bilirubin (BTB), biliary conjugated bilirubin (BCB) and biliary unconjugated bilirubin (BUB) concentration in the HD group was significantly ($P<0.001$) higher, compared to control. BTB and BCB concentrations in the LD group were significantly ($P<0.001$) lower, compared with the HD group. Biliary $Na^+$ and $Cl^-$ concentration in the LD group was significantly ($P<0.001$; $P<0.05$) lower, compared with control. Biliary $K^+$ concentration was significantly ($P<0.001$) higher in the high dose group, compared with control. Following the reduction in the rate of bile secretion observed in this study, the duodenum is at risk of ulceration since it receives acidic chyme from the stomach. Kola nut consumption therefore should be moderated in normal individuals, and abolished completely in gastrointestinal ulcer - prone individuals.

**Keywords:** Bile secretion, bilirubin, *Cola nitida rubra*, liver, kola nut

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## INTRODUCTION

Bile is greenish-yellow compound produced by the liver cells, concentrated and stored in the gall bladder [1]. Bile is instrumental in the digestion of fats, neutralization of acidic chyme that enters the duodenum and in the excretion of the product of haemoglobin metabolism (bilirubin). Bile appears to be the only medium of bilirubin excretion, after conjugation by the liver. Alterations in the composition of bile could serve as a useful marker in assessment of liver functions and the fate of the body in general.

In Nigeria today, and in the world in general, plant derived materials are becoming a celebrated source of medications. Some plant materials are used based on mere speculations, rather than a concrete scientific research. Although some plant materials have been widely researched, some are still ignorantly consumed. *Cola* is a large tree grown in tropical countries, belonging to family *Sterculiaceae*. There are over 120 species of *Cola*. *Cola nitida* is one of the over 120 species of *Cola* that is widely in use as consumables. A number of sub-species within *Cola nitida* are; *pallida, alba, rubra* and *mixta,* all of which are cultivated and available in Nigeria [2]. Recently, kola nuts have become popular consumables in west Africa, especially among the Muslim population [3]. In Nigeria, for instance, the rate of cola nut consumption especially, among students is very high, as it has been credited with the ability to withstand fatigue, keep awake, and ease migraine headaches. This feature of *Cola nitida* has been linked to its high caffeine content [4]. There is also increasing demand for *Cola* usage in pharmaceutical industries and for production of soft drinks, wines, and beverages [5,6]. Extracts of *Cola nitida* have been reported to increase gastric acid secretion, thus, making it potentially ulcerogenic [7]. 2011). Okon et al, [8] had also reported that extract of *Cola nitida rubra* reduced serum reproductive hormone concentrations and sperm count in male wistar rats.

Following the various reported negative effects of the consumption of cola nut, and considering the importance of bile in digestion of fats, neutralization of acidic chyme in the duodenum and excretion of bilirubin, it became important to ascertain the effect of kola nut consumption on rate of bile secretion and composition, with a view to determine the state of health of the liver.

## MATERIALS AND METHODS

### Plant Material and Preparation of Extract

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.

*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.

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**Table 1: Diet Formulation**

| | Group I Control | Group II Low Dose | Group III High Dose |
|---|---|---|---|
| Number of Rats | 6 | 6 | 6 |
| Growers' Feed (g) | 100 | 85 | 70 |
| *Cola nitida rubra* Powder (g) | - | 15 | 30 |
| **Total feed (g)** | **100** | **100** | **100** |

## Experimental Animals and Protocol

Eighteen (18) male albino wistar rats weighing 180 - 200 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.

## Determination of Biliary Secretory Rate

Biliary secretion was collected by the method of Vickers et al., [9]. The animals were starved for 12 hours prior to the experiment. They were weighed and anaesthesized by intraperitoneal administration of sodium thiopentone (6mg/100g body weight), and were quickly pinned to a dissecting board for a tracheostomy performed to clear the airway for easy breathing. The stomach was opened along the linea alba to minimize bleeding. A laparotomy was performed and the liver lobes deflected anterolaterally to expose the common bile duct. The common bile duct was then cannulated with a portex Cannula (0.5mm in diameter) after a small incision was made. A thread was used to tie round the common bile duct to hold the cannula in place. The bile content was collected at 3 hours interval for each group.

## Estimation of Biliary Cholesterol

## Determination of Biliary Bilirubin

Biliary bilirubin was measured by colorimetric method as described by Jendrassik and Grof, [10]. Serum bilirubin was measured by the method described by Sherlock, [11].

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**Estimation of Percentage Biliary Conjugated Bilirubin (BCB) Concentration**

Percentage biliary conjugated bilirubin concentration was determined mathematically by method of Nna et al., [12] as follows;

$$\text{Percentage BCB} = \frac{\text{Biliary conjugated bilirubin concentration}}{\text{Biliary total bilirubin concentration}} \times 100$$

**Statistical Analysis**

All results are presented as mean ± standard error of mean (SEM). The One – way Analysis of Variance (ANOVA) was used to determine the differences between means, followed by post hoc multiple comparisons (LSD procedure). $P < 0.05$ was considered significant. Computer software SPSS version 17.0 and Microsoft Excel (2007 version) Analyzer were used for the analysis.

**RESULTS**

Table 2 shows that the rate of bile secretion in the control, low dose and high dose group was 0.44 ± 0.03, 0.42 ± 0.04 and 0.28 ± 0.03 ml/hr respectively. Rate of bile secretion in the low dose and high dose group was significantly (P<0.05, P<0.01 respectively) lower, compared with control.

Biliary cholesterol concentration was significantly (P<0.01, P<0.001 respectively) reduced in the low dose group (0.77 ± 0.03 mmol/L) and high dose group (0.67 ± 0.01 mmol/L), compared with control (0.94 ± 0.00 mmol/L).

Biliary total bilirubin (BTB) concentration in the control, low dose and high dose group was 11.80 ± 0.42, 14.50 ± 0.07 and 31.80 ± 0.21 µmol/L. BTB concentration was significantly (P<0.001) higher in the high dose group, compared with control. BTB concentration was significantly (P<0.001) lower in the low dose group, compared with the high dose group.

Biliary conjugated bilirubin (BCB) concentration in the different experimental groups was 6.88 ± 1.81, 8.20 ± 0.90 and 21.88 ± 2.21 µmol/L for control, low dose and high dose group respectively. BCB concentration was significantly (P<0.001) higher in the high dose group, compared with control. It was also significantly (P<0.001) lower in the low dose group, compared with the high dose group.

The biliary unconjugated bilirubin (BUB) concentration recorded was 4.96 ± 1.26, 6.32 ± 1.09 and 9.94 ± 1.67 µmol/L for control, low dose and high dose respectively. BUB concentration was significantly (P<0.05) higher in the high dose group, compared with control. Consequently, the percentage bilirubin conjugation obtained for the control, low dose and high dose group was 58.34 ± 0.09, 56.57 ± 0.16 and 68.82 ± 0.13% respectively. Percentage conjugation was significantly (P<0.05) higher in the high dose group, compared with control and low dose group.

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Table 3 shows the biliary electrolyte composition. Biliary $Na^+$ concentration for control, low dose and high dose group was 138.0 ± 0.55, 134.0 ± 0.55 and 136.4 ± 0.68 mmol/L. $Na^+$ concentration was significantly (P<0.001) lower in the low dose group, compared with control, but significantly (P<0.05) higher in the high dose group, compared with the low dose group.

Biliary $K^+$ concentration for control, low dose and high dose group was 4.92 ± 0.05, 5.04 ± 0.15 and 5.30 ± 0.05 mmol/L respectively. $K^+$ concentration was significantly (P<0.001) higher in the high dose group, compared with control.

Biliary $Cl^-$ concentration in the low dose (100.4 ± 0.75 mmol/L) and high dose group (98.0 ± 0.89 mmol/L) was significantly (P<0.05; P<0.01 respectively) lower, compared with control.

Biliary $HCO_3^-$ concentration recorded was 23.8 ± 0.37, 24.2 ± 0.37 and 24.4 ± 0.37 mmol/L for control, low dose and high dose group respectively. There was no significant difference in the $HCO_3^-$ concentration in the groups studied.

**Table 2: Comparison of Biliary Secretory Rate, Cholesterol and Bilirubin Concentration**

| Parameter | Control | Low Dose | High Dose |
|---|---|---|---|
| Rate of Bile Secretion (ml/hr) | 0.44 ± 0.03 | 0.42 ± 0.04* | 0.28 ± 0.03** |
| Biliary Cholesterol Conc. (mmol/L) | 0.94 ± 0.00 | 0.77 ± 0.03** | 0.67 ± 0.01 [a, b] |
| BTB (µmol/L) | 11.80 ± 0.42 | 14.50 ± 0.07[c] | 31.80 ± 0.21[a] |
| BCB (µmol/L) | 6.88 ± 1.81 | 8.20 ± 0.90[c] | 21.88 ± 2.21[a] |
| BUB (µmol/L) | 4.96 ± 1.26 | 6.32 ± 1.09 | 9.94 ± 1.67* |
| Percentage Bilirubin Conjugation (%) | 58.34 ± 0.09 | 56.57 ± 0.16 | 68.82 ± 0.13* |

*P<0.05, **P<0.01, a = P<0.001 vs control; b = P<0.05 vs Low dose, c = P<0.001 vs High dose

**Table 3: Comparison of Biliary Electrolytes Composition**

| Parameter | Control | Low Dose | High Dose |
|---|---|---|---|
| Biliary $Na^+$ Conc (mmol/L) | 138.0 ± 0.55 | 134.0 ± 0.55[a] | 136.4 ± 0.68[c] |
| Biliary $K^+$ Conc (mmol/L) | 4.92 ± 0.05 | 5.04 ± 0.15 | 5.30 ± 0.05[a] |
| Biliary $Cl^-$ Conc (mmol/L) | 103.6 ± 0.75 | 100.4 ± 0.75* | 98.0 ± 0.89** |
| Biliary $HCO_3^-$ Conc (mmol/L) | 23.8 ± 0.37 | 24.2 ± 0.37 | 24.4 ± 0.37 |

*P<0.05, **P<0.01, a = P<0.001 vs control; c = P<0.05 vs low dose;

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## DISCUSSION

Plant based materials have been widely depended upon for various medical conditions, most of which have been trusted based on speculations rather than a concrete scientific investigation. Some negative effects like increase in gastric acid secretion [7], reduced sperm count and serum male reproductive hormone concentration [8], etc, have been linked to kola nut (*C. nitida*) consumption.

In our study, *C. nitida* was found to reduce the rate of bile secretion in both the low and high dose groups. Reduced rate of biliary secretion may have been achieved by decreased bile synthesis by the liver (anti-choleretic effect) or by reduced contraction of the gall bladder to release stored bile (anti-cholagogue effect). The anti-choleretic and anti-cholagogue effect may have also been achieved by inhibition of secretin and cholecystokinin (CCK) respectively. Secretin and CCK are grastrointestinal hormones responsible for enhancing bile secretion and flow. Considering previous reports that *C. nitida* increased gastric acid secretion [7,13,14], our study therefore brings to light the fact that *C. nitida* may cause duodenal ulcers. This may occur since acidic chyme entering the duodenum may be inadequately neutralized by bile whose rate of secretion have become low.

Cholesterol is eliminated from the body directly through bile or after conversion to bile acids. Thus, bile constitutes a major channel of cholesterol excretion. Cholesterol was significantly reduced in bile collected from the low dose and high dose *C. nitida* - fed groups, compared with control group. The decrease maybe attributed to decreased cholesterol concentration in the blood. It may also suggest that kola nut consumption may be detrimental to the coronary artery and the heart by extension, since cholesterol accumulation in the blood is indicative of a possible episode of athereosclerosis.

Bilirubin is a product of haemoglobin degradation. About 80 % of the daily bilirubin production is derived from haemoglobin, while the other 20 % is obtained from the breakdown of myoglobin, cytochromes, peroxidase, catalase, and tryptophan pyrrolase [15,16,17,18] (Iyanagi et al., 1998; Drummond et al., 1996; Westwood et al., 1991; Muraca et al., 1988). A dose - dependent increase was observed in total bilirubin, conjugated and unconjugated bilirubin concentration, which was significantly higher in the low dose and high dose *C. nitida* fed groups, compared with control group. Caffeine contained in kola nut has been linked with the interference of the body's absorption of iron, which is necessary for red blood cell production. Drinking caffeine at the same time as an iron source can reduce absorption of iron by up to 80%, according to the Nutrition Desk Reference [19]. This development will probably lead to excess availability of immature red blood cells in circulation, whose membranes are most often easily ruptured. Ruptured RBC membranes exposes haemoglobin which is metabolized to produce bilirubin [15,18]. This probably accounts for the increased biliary bilirubin concentration in bile (Table 2). Normally, bile in concentrated as it passes through the duct where reabsorption of $Na^+$ and $Cl^-$ is done in exchange for $K^+$ and $HCO_3^-$ respectively. This effect was seen in our study as rate of biliary secretion was significantly reduced in the low dose and high dose kola nut - fed groups, compared with control, thus, allowing more time for

reabsorption of $Na^+$ and $Cl^-$ in exchange for $K^+$ and $HCO_3^-$ respectively. The resultant effect as recorded in our study was a reduction in biliary $Na^+$ and $Cl^-$ concentrations in the low dose and high dose kola nut - fed groups which was significant, compared with control, and, a significant increase in biliary $K^+$ and $HCO_3^-$ concentrations in the low dose and high dose kola nut - fed groups, compared with control.

## CONCLUSION

Following previous reports that *Cola nitida rubra* (kola nut) increases gastric acid secretion, the reduced bile flow rate observed in our study is a matter of great concern as the duodenum being the first part of the small intestine is at risk of ulceration. Kola nut consumption therefore should be moderated in normal individuals, and abolished completely in gastrointestinal ulcer - prone individuals. Also, further studies on RBC indices in required to state clearly whether or not the increased biliary bilirubin concentration observed in this study can be linked to RBC destruction.

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