The influence of Cola Nitida on Testosterone and Progesterone Concentrations in the Underweight Humans Under Resting Condition in Ambrose Alli University.

[1]Igbinovia, ENS [2]Edebiri, O.E., [3]Edwin, E.E., [4]Omodiagbe, O., [5]Blackies, HOT [6]Turay, A.A, [7]Ohiwerei,W.O

[1.2,3,4]*Department of Physiology, Ambrose Alli University, Ekpoma, Edo State,Nigeria*
[5]*Department of Human Anatomy, Ambrose Alli University, Ekpoma, Edo State*
[6]*Department Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State*
[7] *Institute of Research and Training, Ohilux Global, Ekpoma, Edo State*

ABSTRACT

In quest of a stimulant to cope with rather challenging activities, some individuals take to the consumption of Cola nitida. The most active ingredient, caffeine, could be responsible for the physiological or clinical effects of Cola nitida in humans. From some published evidences of the high prevalence of infertility nowadays, the need for this study was thereby necessitated. Here we report the influence of Cola nitida on testosterone and progesterone concentrations in the underweight humans under resting condition in Ambrose Alli University. Twenty (20) underweight volunteers (10 males and 10 females) and non-habitual Cola nitida chewers, aged 18-28 years were used for the study. 0.5g/kg body weight of Cola nitida was administered to each subject to be chewed as a bolus. After ingestion, 50ml of water was given to each volunteer to flush the masticated Cola nitida down the gut. The subject was allowed to rest for 90 minutes. Blood sample was collected from the medial cubital vein using vacutainer syringe. The radio-immunoassay principle was used for the estimation of testosterone and progesterone levels. The results showed that Cola nitida consumption by the underweight female subjects reduced serum levels of Progesterone (from 2.960±0.9631 nmol/L to 2.530±0.912 nmol/L) but increased serum levels of Testosterone (from 2.640±0.3433 to 3.640±0.682 nmol/L). The change, however, was not significant (P>0.05). We show that Cola nitida, at the specific dosage, could reduce the chances of fertility in the female but increases that of the male underweight subjects.

Key words: *Cola nitida, fertility, testosterone, progesterone, radio-immunoassay.*

INTRODUCTION

Cola nuts are the seed pods of various evergreen trees that are native to Africa. In West Africa and Sudan, they are popular masticatory (Russel, 1955). They are important in various social and religious customs and may also be used to counteract hunger and thirst. In Nigeria for instance, the rate of consumption of Cola nuts especially by students is very high as a principal stimulant to keep awake and withstand fatigue (Purgesleve, 1977).

Somorin (1973) reported that caffeine, theobromine and theophiline found in Cola nuts are xanthine stimulants. Ogutuga (1975) suggested that caffeine content of Cola nuts could be as high as 7% and is often considered to be the agent responsible for the physiological or clinical effect of Cola nuts in humans and other mammals (Chukwu *et al*., 2006).

Caffeine products are so widely distributed these days that abuse of the substance may be unnoticed. Aside from occurring organically in tea and coffee, caffeine is now an additive in soft drinks, energy drinks, chocolates, bottled water, chewing gum and medication (Mednick *et al*., 2008).

The mechanisms of action of caffeine includes inhibition of hydrolysis of cyclic 3', 5'- adenosine monophosophate and 3',5'- guanosine monophosphate (Weathersbee and Lodge, 1977) and antagonism of adenosine (Rall, 1990), making it plausible that caffeine might alter hormonal profiles and thereby affect menstrual function. Menstrual function, in turn, may be related to other health outcomes, such as fertility, osteoporosis and breast cancer (Harlow and Ephross, 1995).

Several studies in humans have reported an association between caffeine intake and delayed time to conception (Stanton and Gray, 1995; Boldmar *et al*., 1997). Examination of the relation between caffeine intake and menstrual function may help to elucidate possible biologic mechanisms by which caffeine might alter fecundability.

The present study described how the consumption of *Cola nitida* might influence both the testosterone and progesterone levels in the underweight male and female subjects respectively, and by extension, how such could further impact fertility in the said humans.

MATERIALS AND METHODS

Subjects

A total of twenty (20) subjects were involved in the study (Igbinovia et al., 2020). They were twenty (20) underweight volunteers (10 males and 10 females). They were likewise non-habitual Cola nitida chewers (Chukwu *et al*., 2006), aged 18-28 years were used for the study. Individuals from Ambrose Alli University were used and their health status was assessed with the aid of questionnaire and physical examination (Ugwu and Oyebola, 1996). Informed consent was obtained from each subject before the study.

Inclusion/Exclusion Criteria

Subjects with hypertension (Artfield, 1985), kidney and heart related conditions (Chukwu *et al*., 2006), those with ulcer, diabetes, pregnant women and those allergic to the consumption of caffeine-related substances were excluded from the study. Knowing that the commonly accepted body mass indices (BMI) are: underweight (under 18.5 kg/m^2), normal weight (between 18.5-25.0 kg/m^2), overweight (between 25.0-30.0 kg/m^2) and obese (over 30.0 kg/m^2) (Omorede *et al*., 2016), only the underweight subjects were so categorized and included in the study. Before the study, the subject's age (years), weight (kg), height (m), body mass index (kg/m^2), systolic and diastolic blood pressure (mmhg) and pulse rate (beats/min) were recorded.

0.5g/kg body weight of *Cola nitida* (Obika *et al*., 1995) was administered to each subject to be chewed as a bolus (Igwe *et al*., 2007). After ingestion, 50ml of water was given to each volunteer to flush the masticated *Cola nitida* down the gut. The subject was allowed to rest for 90 minutes (Igwe *et al*., 2007)

Collection of blood sample

Blood sample was collected from medial cubital vein using vacutainer syringe on the same day that the serum sample was collected. Blood sample was transferred into an anticoagulant-free tube. After allowing for about 60 min for spontaneous blood clotting, the serum was separated by centrifugation at 3,000 rpm for 10 minutes at room temperature. Testosterone and Progesterone were measured in serum by EIA kit (Syntron Bioresearch, Inc., CA, USA).

Determination of Testosterone and Progesterone levels

In Testosterone test, the assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The solid phase receptacle (SPR), serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready – to – use and predispensed in the sealed reagent strips

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. The sample is taken and transferred into the well containing alkaline phosphate-labeled anti-Testosterone conjugate.

The sample/conjugate mixture is cycled in and out of the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to the conjugate forming a "sandwich ". Unbound components are eliminated during the washing steps. During the final detection step, the substrate (4-Methyl – Umbelliferyl phosphate) is cycled in and out of the SPR.

The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescence of which is measured at 450nm. The intensity of the fluorescence is proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated by the VIDAS in relation to the calibration curve stored in memory, and then printed out (Butt and Blunt, 1988)

Statistical Analysis

Statistical analyses were conducted using Micro cal origin for windows. Descriptive statistics were reported as Mean ± SEM. A P-value of less than 0.05 was considered to be statistically significant

RESULTS

The study showed that Cola nitida reduced Progesterone levels in the female underweight individuals (P≥0.05), whereas it increased Testosterone levels in the male underweight individuals (P≥0.05) .

Table 1: Showing the mean values of testosterone in male and progesterone in female underweight individuals following the consumption of *Cola nitida*.

Parameter	Control	Test	P-values	Significance status
Progesterone (ng/ml)	2.960 ± 0.9631	2.530 ± 0.912	0.7495	Not Significant
Testosterone (ng/ml)	2.640 ± 0.3433	3.640 ± 0.682	0.2069	Not Significant

P < 0.05 indicates significant different.

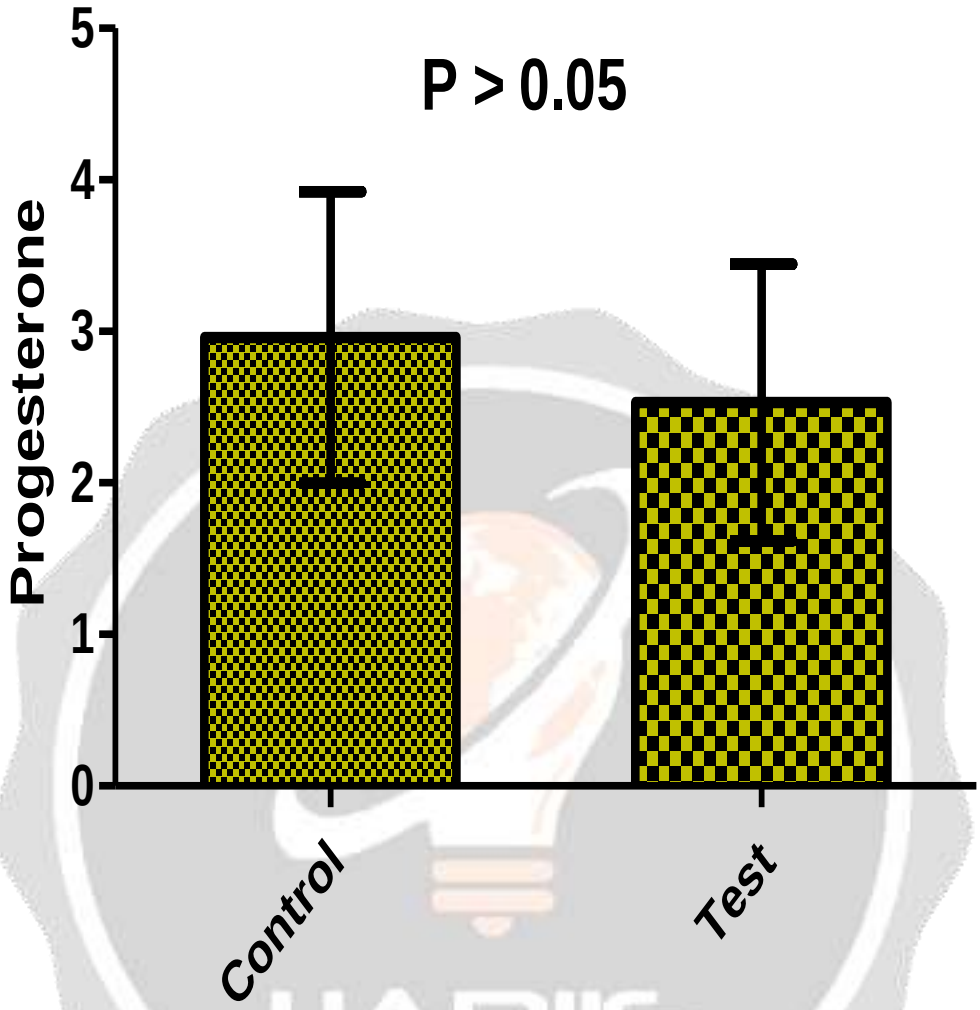

Figure 1: A bar-chart showing the progesterone level in female underweight individuals following the ingestion of *Cola nitida.*

There was no significant difference between control and test subjects (P > 0.05)

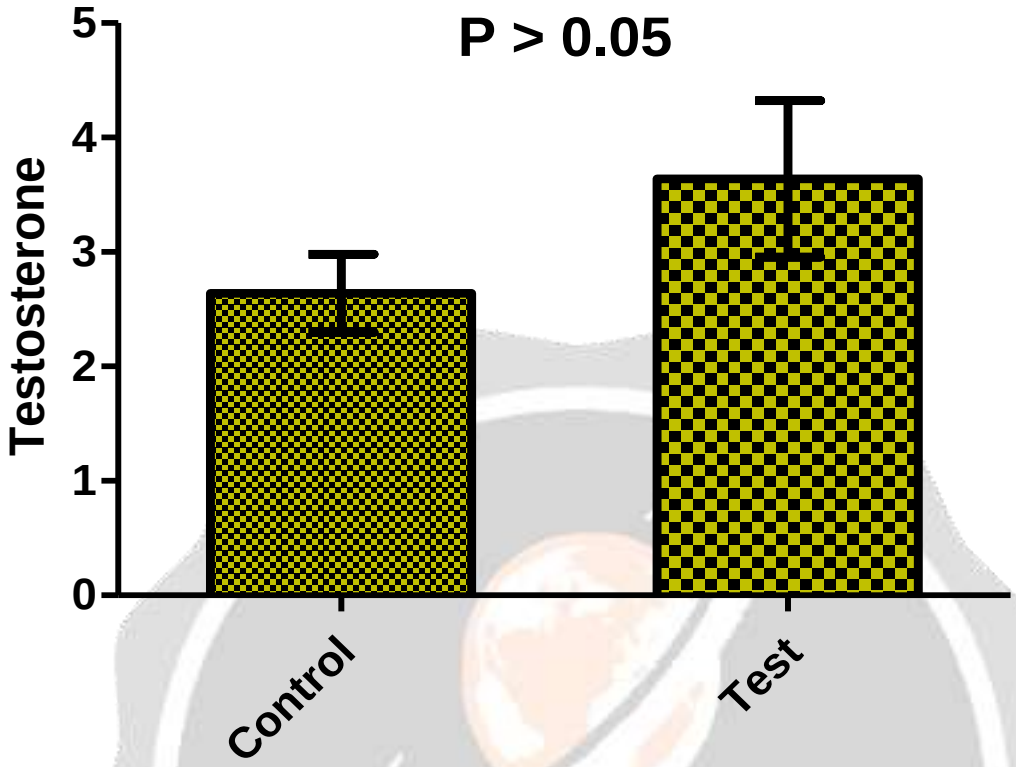

Figure 2: A bar-chart showing the testosterone level in male underweight individuals following the ingestion of *Cola nitida.*

There was no significant difference between control and test subjects (P > 0.05)

DISCUSSION

The present study showed that *Cola nitida* consumption by the underweight female subjects reduced serum levels of Progesterone (from 2.960±0.9631 nmol/L to 2.530±0.912 nmol/L) but increased serum levels of Testosterone (from 2.640±0.3433 to 3.640±0.682 nmol/L). The change, however, was not significant (P>0.05).

The most active chemical constituent of *Cola nitida* is caffeine which is a stimulant (Russel, 1955). It has been found that most of the physiological actions of *Cola nitida* are due to caffeine (Eijnatten, 1973).

While researching on the effects of caffeine on women, Fenster et al. (1999) observed that women who consumed caffeine could likely have alteration in the duration of their menstrual flow.

Recalling the fact that Progesterone plays a major role in the menstrual cycle and from the findings above, it is obvious that caffeine might have inhibited the physiological state of the female reproductive system-due to the decrease in Progesterone level. This could equally be factual as in the present study.

The mechanism of action could be traceable to the fact that caffeine inhibits the action of adenosine which in laboratory studies affects luteinizing hormone and follicle – stimulating hormone (Polan et al., 1983; Picanco et al., 1989), which could in turn affect menstrual cycle length. Gilbert and Rice (1991) found depressed estrogen levels in female monkeys at a dose level of caffeine associated with miscarriages, still births and decreased maternal weight gain.

The data obtained in this present study showed a non-significant decrease in Progesterone level. This could be because caffeine inhibited the action of adenosine which would have in turn inhibited those of LH and FSH. Consequent upon the findings above, there could have also been a decrease in Progesterone level. By implication, the consumption of Cola nitida could be disadvantageous to the female underweight individuals in terms of fertility. This finding could also be due to the fact that the underweight individuals are mainly associated with anabolism, as against catabolism which is characteristic of the overweight subjects (Igbinovia et al., 2020).

Going by the mechanisms of action of caffeine, it becomes plausible that caffeine might alter hormonal profiles and thereby affect menstrual function, which in turn, may be related to other health outcomes, such as fertility (Harlow and Ephross, 1995).

The effect of caffeine on pregnancy was reported by Russell (2007). He observed that consuming more than 300 milligrams of caffeine a day will increase one's chances of a miscarriage and based on studies on animals, high levels of caffeine may also cause birth defects, preterm delivery, reduced fertility and low birth weight. Some constituents in Cola nitida other than caffeine or sugar may cause ovulatory disorder.

Progesterone is predominantly produced by the corpus luteum in the non-pregnant female. The most important function of progesterone is the regulation of endometrial receptivity (de Swiet *et al*., 2002).

On the contrary, male fertility depends on but not limited to serum testosterone concentration, LH concentration, sperm count and sperm quality. Altered levels of male sex hormones are indicative of male reproductive dysfunction.

Parkhurst *et al*. (2000) reported that 50 ml oral dose of methylxanthines appeared to be detrimental to the sperm. Also, caffeine, a major methylxanthine constituent of *Cola nitida* seed extract inhibits androgen binding protein (ABP) resulting in reduced caudal epididymal sperm reserve, seminiferous tubular fluid volume, resulting in low sperm production and infertility (Eteng, 1997). The decreased sperm count observed in their study might also be an implication of the reduced testosterone and LH concentration, which are major regulators of spermatogenesis (Seeley *et al.*, 1997). The present study is in disagreement with that of Parkhurst *et al*. (2000), going by the increase in testosterone levels in the male underweight individuals, though non-significantly.

From the foregoing, it is quite obvious that the test substance (Cola nitida) in the present study might have stimulated the hypothalamo-pituitary-gonadal pathway processes. Hence, the increase observed in the testosterone levels in the underweight individuals, though non-significantly.

Conclusion

In conclusion, the study shows that Cola nitida, at the specific dosage, could reduce the chances of fertility in the female underweight subjects. Therefore, some caution should be taken in its consumption or any caffeine-related substances. But the test substance increased the chances of fertility in the male underweight subjects. However, some caution should still be taken in its consumption or any caffeine-related substances.

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