The influence of *Cola nitida* on Testosterone and Progesterone Concentrations in the Overweight Humans under Resting Condition

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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. Such a practice could culminate in the abuse of the said substance. Caffeine might alter hormonal profiles and thereby affect menstrual function, which might have a direct bearing on fertility. Amongst other factors, testosterone and progesterone are important to the chances of fertility in the humans. Besides, it has been well documented that the probability of pregnancy is reduced by 5% per unit of body mass index exceeding 29 kg/m^2. 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 overweight humans under resting condition in Ambrose Alli University. Twenty (20) overweight 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 overweight male and female subjects significantly ($P<0.05$) increased serum level of progesterone in the females (from 0.730 ± 0.065 ng/ml to 2.880 ± 0.083 ng/ml) and decreased serum testosterone level in the males (from 5.600 ± 0.382 ng/ml to 2.340 ± 0.157 ng/ml). We show that Cola nitida, at the specific dosage, could increase the chances of fertility in females but not in the male overweight subjects.

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

INTRODUCTION

Cola nuts, the seed pods of various evergreen trees native to Africa, are widely chewed in regions like West Africa and Sudan (1). They hold significant cultural and religious value and are also used to alleviate hunger and thirst. In Nigeria, for example, students frequently consume Cola nuts as a primary stimulant to stay awake and combat fatigue (2).

Somorin (1973) identified caffeine, theobromine, and theophylline in Cola nuts as xanthine stimulants. Ogutuga (1975) suggested that Cola nuts could contain up to 7% caffeine, which is

believed to be responsible for their physiological or clinical effects on humans and other mammals (3).

Today, caffeine is so prevalent that its overuse might go unnoticed. Beyond naturally occurring in tea and coffee, caffeine is now added to soft drinks, energy drinks, chocolates, bottled water, chewing gum, and medications (4).

Caffeine works by inhibiting the breakdown of cyclic 3', 5'-adenosine monophosphate and 3', 5'-guanosine monophosphate (5) and blocking adenosine receptors (6). This suggests that caffeine could influence hormonal balance and, consequently, menstrual function. Menstrual function is linked to various health outcomes, such as fertility, osteoporosis, and breast cancer (7).

Research on the impact of coffee and caffeinated beverage consumption on fertility has produced mixed results. Some studies have found a connection between caffeine intake and delayed conception (8; 9), while others have found no association (10; 11) or only a link at very high consumption levels (12). Investigating the relationship between caffeine consumption and menstrual function could shed light on the biological mechanisms by which caffeine might affect fecundability. Alcohol, tobacco, and caffeinated beverages are common exposures and have been extensively studied for their effects on the female endocrine system (13). The present study described how the consumption of *Cola nitida* might influence both the testosterone and progesterone levels in the normal weight male and female subjects respectively, and by extension, how such could further impact fertility in the said humans.

MATERIALS AND METHODS

Subjects

The study involved twenty participants (14), all of whom were overweight volunteers—10 males and 10 females. None of them habitually chewed *Cola nitida* (15). The participants, aged 18-28 years, were selected from Ambrose Alli University. Their health status was evaluated through questionnaires and physical examinations. Informed consent was obtained from each participant prior to the study.

Inclusion/Exclusion Criteria

Subjects with hypertension (16), kidney and heart conditions (3), ulcers, diabetes, pregnancy, and caffeine allergies were excluded from the study. According to commonly accepted body mass index (BMI) categories—underweight (under 18.5 kg/m^2), normal weight (18.5-25.0 kg/m^2), overweight (25.0-30.0 kg/m^2), and obese (over 30.0 kg/m^2) (17)—only overweight individuals were included. Prior to the study, each participant's age, weight, height, BMI, systolic and diastolic blood pressure, and pulse rate were recorded.

Each subject was given 0.5g/kg body weight of Cola nitida to chew, based on a preliminary study where the intake ranged from 0.39g/kg to 0.57g/kg body weight (18). After chewing, participants drank 50ml of water to swallow the masticated Cola nitida (19). They then rested for 90 minutes, as preliminary experiments indicated that the effects of the nut were observable in body tissue after this period

Collection of blood sample

Blood samples were drawn from the medial cubital vein using a vacutainer syringe on the same day as the serum sample collection. The blood was then placed into a tube without an anticoagulant. After approximately 60 minutes to allow for spontaneous clotting, the serum was separated by centrifuging at 3,000 rpm for 10 minutes at room temperature. Serum testosterone and progesterone levels were measured using an EIA kit from 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 result of this present study shows that Table 1 shows the comparison of the mean values of testosterone level of overweight male individuals following the *Cola nitida* consumption in which the testosterone control was 5.600 ± 0.382 while that of the test were 2.340 ± 0.157. Table 1 shows the comparison of the mean values of progesterone level of female overweight individuals following the *Cola nitida* consumption in which the control was 0.730 ± 0.065 while that of test was 2.880 ± 0.083. However, both were significant.

Table 1: Comparing the mean values of testosterone level of overweight male individuals following the *Cola nitida* consumption.

Parameter	Control	Test	P-value	Significance

Testosterone level (ng/ml)	5.600 ± 0.382	2.340 ± 0.157	< 0.0001	Significant

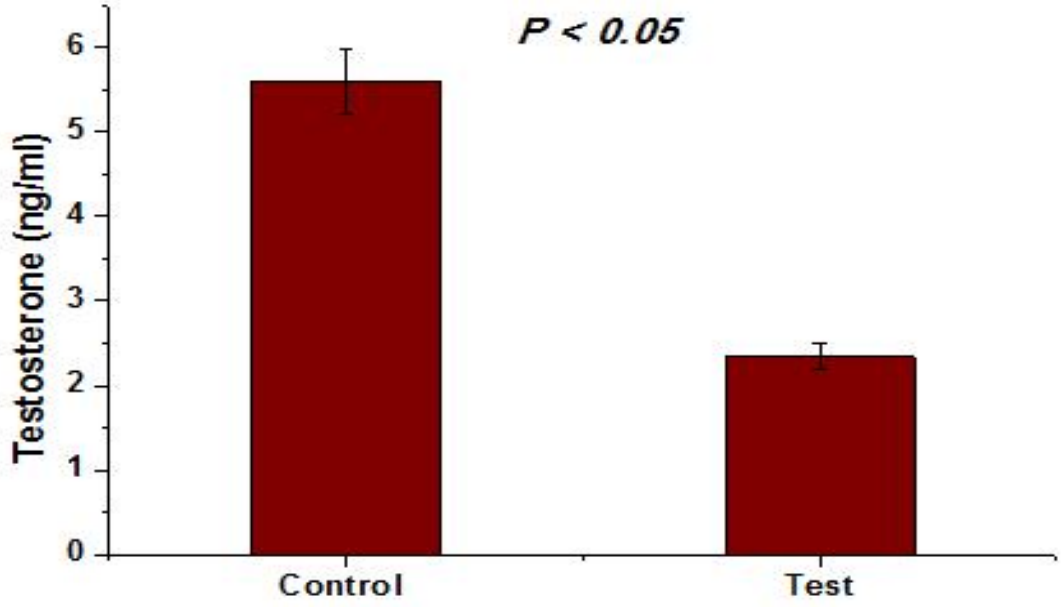

Fig I: Showing testosterone level of male overweight individuals following consumption of *Cola nitida.*

There was a significant decrease in the testosterone level of the overweight male individuals following the consumption of *Cola nitida* compared to before (P< 0.0001)

Table 2: Comparing the mean values of progesterone level of female overweight individuals following the *Cola nitida*consumption.

Parameter	**Control**	**Test**	**P-value**	**Significance**
Progesterone level (ng/ml)	0.730 ± 0.065	2.880 ± 0.083	< 0.0001	Significant

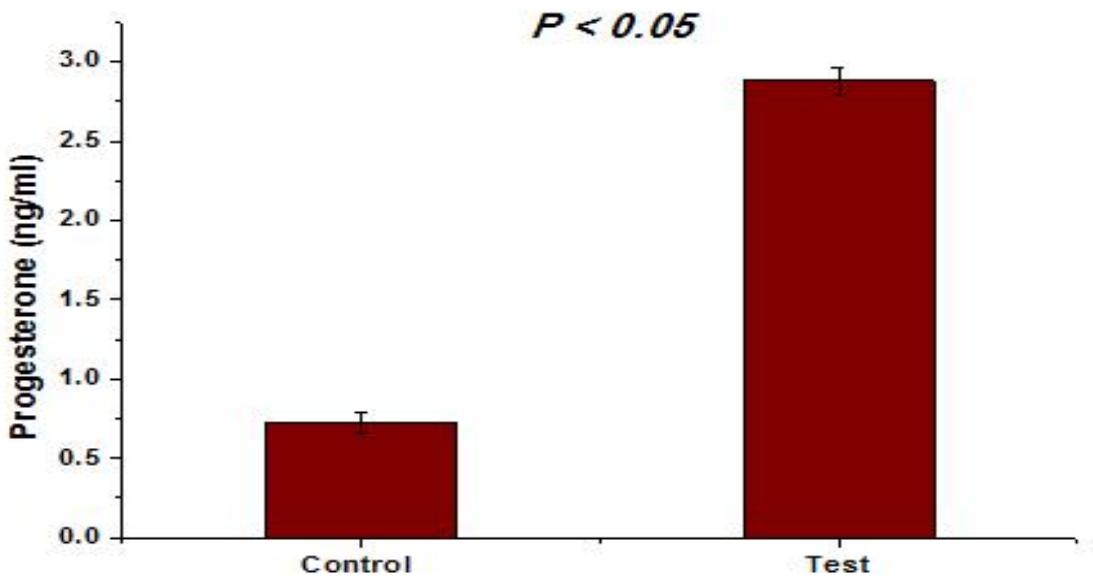

Fig II: Showing progesterone level of female overweight individuals following consumption of *Cola nitida.*

There was a significant increase in the progesterone level of the overweight female individuals following the consumption of *Cola nitida* compared to before (P< 0.0001)

DISCUSSION

The current study found that Cola nitida consumption significantly (P<0.05) increased serum progesterone levels in overweight female participants (from 0.730±0.065 to 2.880±0.083 nmol/L) but significantly (P<0.05) decreased serum testosterone levels in overweight male participants (from 5.600±0.382 nmol/L to 2.340±0.157 nmol/L).

Umoh et al. (2014) (20) discovered that phytochemical screening of aqueous seed extract of Cola nitida revealed several active chemical constituents such as caffeine, glucoside, theobromine, and kolatin, which are known stimulants (21; 22). Additional compounds identified include methylxanthines, theophylline, d-catechin, epicatechin, kolanin, glucose, starch, fatty matter, tannins, anthocyanin pigment, betaine, and protein (23; 24).

The primary active compound in Cola nitida is caffeine, a known stimulant (1). Most of the physiological effects of Cola nitida are attributed to caffeine (25). In a study on caffeine's impact on women, Fenster et al. (1999)(26) observed that women who consumed caffeine were less likely to experience prolonged menstrual periods. This is biologically plausible, as caffeine is a vasoconstrictor (27). Constriction of uterine blood vessels is expected to reduce uterine blood flow, potentially decreasing menstrual bleeding and shortening the duration of menses. Research in both pregnant animals (28) and humans (29) has shown that caffeine increases uterine vascular resistance and reduces uterine blood flow.

Considering that progesterone plays a significant role in the menstrual cycle, and based on the findings above, it is evident that caffeine may have improved the physiological state of the female reproductive system by increasing progesterone levels. This aligns with the current study's results.

Fenster et al. (1999)(26) also noted that heavy caffeine consumers are twice as likely to have shorter menstrual cycles compared to non-consumers. The exact mechanism by which caffeine affects menstrual cycle duration is unclear, but it might involve caffeine's influence on sex hormone receptors. Kitts (1987) (27) provided evidence suggesting that coffee constituents are weakly estrogenic. Caffeine inhibits adenosine, which in laboratory studies affects luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (28; 29), potentially influencing menstrual cycle length. Gilbert and Rice (1991) (30) observed decreased estrogen levels in female monkeys at caffeine doses associated with miscarriages, stillbirths, and reduced maternal weight gain. Associations between caffeine intake and estradiol and/or estrone levels have been found in three human studies (31; 32), but not in two others (33; 34).

In the present study, data showed a significant increase in progesterone levels, possibly because caffeine stimulated adenosine action, which in turn may have stimulated LH and FSH. Based on these findings, the consumption of Cola nitida could potentially benefit overweight females in terms of fertility, provided the appropriate dose is considered.

Further evidence supports these claims, with several studies in humans reporting an association between caffeine intake and delayed conception (35; 9). Considering caffeine's mechanisms of action, it is plausible that caffeine might alter hormonal profiles and thereby affect menstrual function. Menstrual function, in turn, may influence other health outcomes, such as fertility (7).
Russell (2007)(1) reported on the effects of caffeine on pregnancy, observing that consuming more than 300 milligrams of caffeine per day increases the risk of miscarriage. Animal studies suggest that high caffeine levels may also cause birth defects, preterm delivery, reduced fertility, and low birth weight. Caffeine can impact multiple body systems, including the cardiovascular, digestive, reproductive, and neurological systems. It may alter menstrual cycle duration by affecting sex hormones like progesterone or their receptors.

Some components in Cola nitida, aside from caffeine or sugar, might cause ovulatory disorders. In non-pregnant females, progesterone is mainly produced by the corpus luteum, with small amounts from the developing follicle and adrenals (36). Progesterone's key function is regulating endometrial receptivity (37).

Male fertility relies on serum testosterone concentration, LH concentration, sperm count, and sperm quality. Altered levels of male sex hormones indicate reproductive dysfunction.

Testosterone is a steroid hormone, sharing its chemical classification with compounds such as cholesterol, bile acids, vitamin D, and hormones produced by the adrenal glands and ovaries. The systematic chemical name for testosterone is 17-beta-hydroxy-4-androsten-3-one. In men, most circulating testosterone is produced by the interstitial cells of Leydig in the testicles, with smaller amounts produced by the zonas reticularis and fasciculata of the adrenal gland. Testosterone plays a crucial role in the development and function of male accessory sex glands (prostate, seminal vesicles, and epididymis), which are essential for sperm development, function, and the act of copulation.

Parkhurst et al. (2000)(37) found that a 50 ml oral dose of methylxanthines negatively affected sperm. Additionally, caffeine, a primary methylxanthine in Cola nitida seed extract, inhibits

androgen-binding protein (ABP), leading to reduced caudal epididymal sperm reserves, decreased seminiferous tubular fluid volume, low sperm production, and infertility (Eteng, 1997). The reduced sperm count in their study might be due to decreased testosterone and LH concentrations, which are key regulators of spermatogenesis (38). The current study aligns with Parkhurst et al. (2000) (37), showing a significant reduction in testosterone levels in overweight males.

The regulation of testosterone production in the testicles involves a negative feedback loop between the hypothalamus, anterior pituitary, and testicles (39). The hypothalamus periodically releases pulses of gonadotrophin-releasing hormone (GnRH) into the hypophyseal circulation, which supplies the hypothalamus and anterior pituitary. GnRH stimulates the anterior pituitary to produce and release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). This pulsatile release of GnRH causes LH and FSH to be released into the systemic circulation in a similar manner.

In healthy males, approximately 2 to 4 LH and FSH pulses occur over a 6- to 8-hour period, with LH pulse amplitudes being much greater than those of FSH. LH and FSH interact with their primary target tissue receptors (LH with Leydig cells; FSH with Sertoli cells) located on the respective cell membranes in the testicles. Once a hormone-receptor complex forms, an adenyl cyclase-mediated increase in cyclic AMP occurs, leading to the phosphorylation of intracellular proteins via a protein kinase mechanism.

In Leydig cells, the activation of protein kinase leads to the mobilization of steroid precursors, particularly the synthesis of pregnenolone from cholesterol. Pregnenolone is the precursor from which testosterone is produced. Synthesized testosterone then diffuses from the Leydig cells into the testicular vascular system or adjacent compartments containing Sertoli cells. In Sertoli cells, testosterone plays a crucial role in facilitating spermatogenesis, with the FSH-receptor-hormone complex initiating this process (40).

From this understanding, it is evident that Cola nitida in the present study may have inhibited the hypothalamo-pituitary-gonadal pathway processes, resulting in a significant reduction in testosterone levels in overweight individuals.

Testosterone's major reproductive role involves sperm cell development. In the Sertoli cells of the testicles, testosterone induces a nuclear activation process that stimulates and catalyzes the maturation and development of spermatozoa during spermatogenesis. Maintaining adequate testosterone levels in Sertoli cells is essential for developing sufficient numbers of mature and viable sperm necessary for male fertility.

In conclusion, Cola nitida significantly increased progesterone levels in overweight female subjects ($P<0.05$) but significantly reduced testosterone levels in overweight male subjects ($P<0.05$). Since these hormones are related to fertility, Cola nitida might enhance fertility in overweight females when consumed in appropriate dosages. However, caution should be exercised with its consumption or any caffeine-related substances such as soft drinks, energy drinks, chocolates, bottled water, chewing gum, and medications. Conversely, Cola nitida might reduce fertility chances in overweight males.

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