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Research Article

The Influence of Cola Nitida on Testosterone and Progesterone Concentrations in the Normal Weight Humans under Resting Condition in Ambrose Alli University

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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 normal weight humans under resting condition in Ambrose Alli University. Twenty (20) normal weight 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 normal weight male and female subjects significantly ($P<0.05$) reduced serum level of progesterone in the females (from 16.47±039 ng/ml to 4.690±0.592 ng/ml) and serum testosterone level in the males (from 7.392±0.479 to 4.727±0.466 ng/ml). We show that Cola nitida, at the specific dosage, could reduce the chances of fertility in both the male and female normal weight subjects.

Keywords: 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).

The results of studies of coffee and caffeinated beverage consumption in relation to fecundity are inconsistent. Several

studies in humans have reported an association between caffeine intake and delayed time to conception (Stanton and Gray, 1995; Chavarro et al., 2009). In contrast, others have shown either no association (Alderette *et al*., 1995; Joesoef *et al*., 1990) or a relation only at very high levels of intake (Olsen, 1991; Florack *et al*., 1994). Examination of the relation between caffeine intake and menstrual function may help to elucidate possible biologic mechanisms by which caffeine might alter fecundability. Alcohol, tobacco and caffeinated beverages are common exposures that have been subjects to a number of studies related to their effects on the female endocrine system (Lucero *et al*., 2001).

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.

Infertility is the lack of pregnancy despite regular unprotected sexual intercourse after a year or therapeutic donor insemination in women less than 35 years of age and after 6 months in women 35 years and older (Gemmill, 2018). It is one of the most frequent disorders of the reproductive system in developing countries. Vahrati and Smith have found that a larger portion of women who are seeking medical help to get pregnant are obese (Parihar, 2003).

Obesity causes infertility through various pathways, including impaired ovarian follicular development, qualitative and quantitative development of the oocyte, fertilization, embryo development and implantation. Autonomic changes such as increased body temperature have been reported to be associated with the consumption of a diet comprising cola nuts for 7 days of normal rats (Osim and Udia, 1993). In Mali, macerated powdered nut or bark of *Cola nitida* is used for amenorrhea (Togola *et al*., 2008). Animal experiment showed that chronic consumption of cola nut and caffeine diets caused decrease in food intake and body weight (Umoren *et al*., 2009).

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. Caffeine can also affect multiple body systems, including the cardiovascular, digestive, reproductive and neurological systems. Caffeine may alter the duration of menstrual cycle via the effect of caffeine on sex hormones or the hormone receptors. Some constituents in Cola nitida other than caffeine or sugar may cause ovulatory disorder. It has been discovered that sons of mothers drinking 4-7 cups/day of coffee had lower testosterone levels than sons of mothers drinking 0-3 cups/day.

Umoh *et al*. (2014) reported that phytochemical screening of aqueous seed extract of *Cola nitida* revealed that it contained some active chemical constituents like caffeine, glucoside, theobromine and kolatin, which are stimulants (Armstrong *et al*., 1992; Infante-Rivard *et al*., 1993). Others include methylxanthines, theopilline, d-catechin, lepicatechin, kolanin, glucose, starch, fatty matter, tannins, anthocyanin pigment, betaine and protein (Tende *et al*., 2011; Smith, 2002).

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 (Van Eijnatten, 1973).

Testosterone is a hormone that belongs to the discrete chemical classification known as steroids. Other members of this classification are such compounds as cholesterol, bile acids, vitamin D and hormones of the adrenal glands and ovary. The systematic chemical name for testosterone is 17-beta-hydroxy-4-androsten-3-one. The majority of the circulating testosterone in men comes from production in the interstitial cells of Leydig at the testicles. Additionally, the zonas reticularis and fasciculata of the adrenal gland produce small amounts. Testosterone also assists in the development and functioning of the male accessory sex glands (prostate, seminal vesicles and epididymis) which aid in the sperm development and function, as well as in the act of copulation.

Regulation of testicular production of testosterone occurs via a negative feedback loop system involving the hypothalamus, anterior pituitary and testicles (Ferin *et al*., 1993). Periodically the hypothalamus releases pulses of gonadotrophin-releasing hormone (GnRH) into the hypophyseal circulation, which supplies the hypothalamus and anterior pituitary. The GnRH stimulates the anterior pituitary to produce and release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The pulsatile release of GnRH results in LH and FSH also being released into the systemic circulation in a similar pulsatile manner.

Progesterone is predominantly produced by the corpus luteum in the non-pregnant female. Small amounts are produced by the developing follicle and adrenals (Meyer *et al*., 1997). The most important function of progesterone is the regulation of endometrial receptivity (Gorelik et al., 2002). Other functions of progesterone are that it stimulates the secretory activity of the uterine tubes, uterus and vagina; Its responsible for the pregestational changes in the endometrium, and together with oestrogen is responsible for the cyclic changes that occur in the cervix and the vagina; Increases the membrane potential of the myometrial muscle fibres, thus decreasing their sensitivity and excitability to oxytocin and prostaglandin. This explains why progesterone therapy is sometimes so effective in threatening abortion. Other function of progesterone includes decreasing the number of oestrogen receptors in the endometrial muscle fibres, promoting protein anabolism, stimulating alveolar formation in the breasts during pregnancy and antagonizing the action of aldosterone on the kidney. Oestradiol and progesterone also act on the endometrium – the lining tissue of the uterine cavity. Oestradiol stimulates growth of all elements of the endometrium. Under the influence of progesterone from the corpus luteum during the second half of the cycle, the endometrium is converted from a proliferative pattern to a secretory pattern, as the endometrial glands become tortuous and convoluted. As progesterone and

oestradiol levels fall towards the end of the cycle, the endometrium can no longer be sustained, so it breaks up and is cast off in the process of menstruation (Willocks and Neilson, 1991). Oestradiol secretion remains low during the early follicular phase period, but increases 1 week prior to the mid-cycle gonadotrophin surge; first at a slow, then at a very rapid, quasi-exponential rate to reach a peak at the time of the onset of the LH surge: the late follicular phase oestradiol peak. Within a few hours after the initiation of the mid-cycle gonadotrophin surge, oestradiol concentrations fall abruptly. They rise again with the appearance of the corpus luteum.

Materials and Methods

3.1 Subjects

A total of twenty (20) subjects were involved in the study (Igbinovia et al., 2020). They were twenty (20) normal weight 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.

3.2 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) (Schmiegelow et al., 2014), only the underweight, normal weight and overweight subjects were so categorized and included in the study. Before the study, the subject 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* (refers to preliminary study in which the dose of Cola nitiida taken in the study was worked out by allowing an ad-libitum intake until the subject was satisfied. The range of the intake was between 0.39g/kg and 0.57g/kg body weight (Obika *et al*., 1996) was administered to each subject to be chewed as a bolus (Igwe & Onyegbado, 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 (preliminary experiment had suggested that the effect of the nut were observable in body tissue 90 minutes after ingestion).

3.3 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).

3.4 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).

3.5 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

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

Parameter	Control	Test	P-values	Significance status
Progesterone (ng/ml)	16.47 ± 1.039	4.690 ± 0.592	< 0.0001	Significant
Testosterone (ng/ml)	7.392 ± 0.479	4.727 ± 0.466	0.0009	Significant

P < 0.05 indicates significant different.

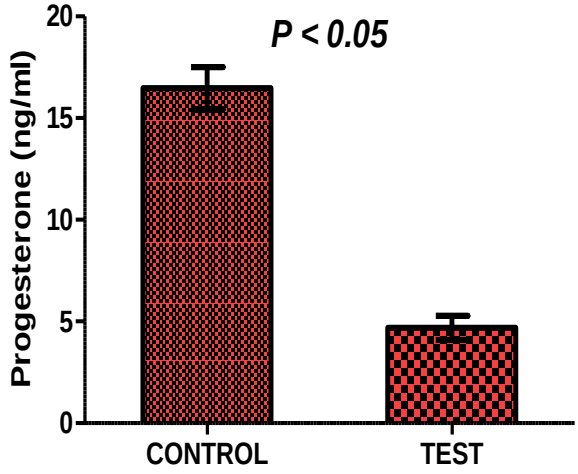

Fig i: A bar-chart showing the progesterone level in female individuals following the ingestion of *Cola nitida.*

There was a significant decrease in test compared with control subjects (P < 0.05).

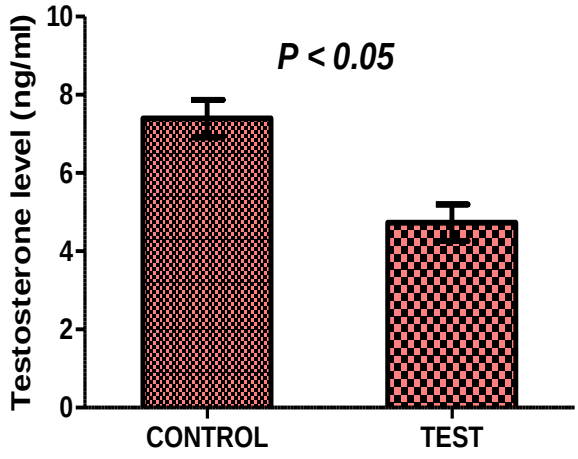

Fig ii: A bar-chart showing the testosterone level in male individuals following the ingestion of *Cola nitida.*

There was a significant decrease in test compared with control subjects (P < 0.05).

Discussion

The present study showed that *Cola nitida* consumption by both the normal weight female and male subjects significantly reduced serum levels of both Progesterone (from 16.47±1.039 nmol/L to 4.690±0.592 nmol/L) (P<0.05) and Testosterone levels (from 7.392±0.479 to 4.727±0.466 nmol/L) (P<0.05).

Umoh *et al*. (2014) reported that phytochemical screening of aqueous seed extract of *Cola nitida* revealed that it contained some active chemical constituents like caffeine, glucoside, theobromine and kolatin, which are stimulants (Armstrong *et al*., 1992; Infante-Rivard *et al.,* 1993). Others include methylxanthines, theopilline, d-catechin, lepicatechin, kolanin, glucose, starch, fatty matter, tannins, anthocyanin pigment, betaine and protein (Tende *et al.*, 2011; Smith, 2002).

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 (Van Eijnatten, 1973).

While researching on the effects of caffeine on women, (Fenster et al., 1999) observed that women who consumed caffeine are less likely to have long menses. This finding is biologically plausible because caffeine is a known vasoconstrictor (Benowitz, 1990). Constriction of uterine blood vessel would be expected to reduce uterine blood flow, which could reduce menstrual bleeding and shorten the duration of menses. Research in pregnant animals (Wilson et al., 1983) and humans (Miller et al., 1994) indicates that caffeine increase uterine vascular resistance and reduces uterine blood 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 distorted the physiological state of the female reproductive system-due to its effects on Progesterone level. This could equally be factual as in the present study.

Fenster et al. (1999) also observed that those whose caffeine consumption is heavy are twice as likely to have a short menstrual cycle compared with non-consumers. The mechanism by which caffeine may alter the duration of the menstrual cycle is not clear but such an effect could occur via the effect of caffeine on sex hormones on receptors. (Kitts, 1987) found evidence to suggest that constituents of coffee are weakly estrogenic. Caffeine inhibits the action of adenosine which in laboratory studies affects luteinizing hormone and follicle – stimulating hormone (Polan et al., 1983; Domingos,1990),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. Associations between caffeine intake and estradiol and/or estrone levels have been found in three studies of humans (Fenster et al., 1999) but not in two other (Cooper et al., 1992).

The data obtained in this present study showed a 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 normal weight individuals in terms of fertility, even in the appropriate dose.

As further clues to the above assertions, several studies in humans have reported an association between caffeine intake and delayed time to conception (Stanton and Gray, 1995).

Going by the mechanisms of action of caffeine, it becomes 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 (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. Caffeine can also affect multiple body systems, including the cardiovascular, digestive, reproductive and neurological systems. Caffeine may alter the duration of menstrual cycle via the effect of caffeine on sex hormones (such as Progesterone) or the hormone receptors. 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. Small amounts are produced by the developing follicle and adrenals (Meyer *et al*., 1997). The most important function of progesterone is the regulation of endometrial receptivity (Gorelik et al., 2002).

On the other hand, 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.

Testosterone is a hormone that belongs to the discrete chemical classification known as steroids. Other members of this classification are such compounds as cholesterol, bile acids, vitamin D and hormones of the adrenal glands and ovary. The systematic chemical name for testosterone is 17-beta-hydroxy-4-androsten-3-one. The majority of the circulating testosterone in men comes from production in the interstitial cells of Leydig at the testicles. Additionally, the zonas reticularis and fasciculata of the adrenal gland produce small amounts. Testosterone also assists in the development and functioning of the male accessory sex glands (prostate, seminal vesicles and epididymis) which aid in the sperm development and function, as well as in the act of copulation.

(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 agreement with that of (Parkhurst *et al*. 2000), going by the significant reduction in testosterone levels in the male normal weight individuals.

Regulation of testicular production of testosterone occurs via a negative feedback loop system involving the hypothalamus, anterior pituitary and testicles (Ferin *et al*., 1993). Periodically the hypothalamus releases pulses of gonadotrophin-releasing hormone (GnRH) into the hypophyseal circulation, which supplies the hypothalamus and anterior pituitary. The GnRH stimulates the anterior pituitary to produce and release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The pulsatile release of GnRH results in LH and FSH also being released into the systemic circulation in a similar pulsatile manner.

For normal, healthy males approximately 2 to 4 LH and FSH pulses are observed over a 6- to 8- hour period; however the amplitudes of the LH pulses are much greater than those observed for FSH. At the testicles, LH and FSH interact with their primary target tissue receptors (LH, Leydig cells; FSH, Sertoli cells) located on the respective cell membranes. Once a hormone-receptor complex is formed, there is an adenyl cyclase-mediated increase of cyclic AMP, which produces a phosphorylation of intracellular proteins by activation of a protein kinase mechanism.

In the Leydig cells this protein kinase activation leads to a mobilization of steroid precursors, in particular the activation of pregnenolone synthesis from cholesterol. Pregnenolone serves as the parent compound from which testosterone is derived. Synthesized testosterone diffuses from the Leydig cells into the testicular vascular system and/or into adjacent testicle compartments containing the Sertoli cells. In the Sertoli cells, testosterone plays an essential role in the facilitation of the spermatogenesis process (the FSH-receptor-hormone formation at the Sertoli cell results in the initiation of the spermatogenesis process) (Hackney, 1996). From the foregoing, it is quite obvious that the test substance (Cola nitida) in the present study might have inhibited the hypothalamo-pituitary-gonadal pathway processes. Hence, the significant reduction observed in the testosterone levels in the normal weight individuals.

A major reproductive role of testosterone involves the development of the sperm cell. At the Sertoli cells of the testicles, testosterone induces a nuclear activation process which stimulates and catalyses the maturation and development of the spermatozoa (i.e., developing sperm) during the process of spermatogenesis. Maintenance of testosterone levels within the Sertoli cells is essential for the development of adequate numbers of mature and viable sperm that are necessary for a male to be fertile.

In conclusion, Cola nitida significantly reduced both the progesterone and testosterone levels in the female and male normal weight subjects ($P<0.05$). As these hormones are related to fertility, there could therefore be the tendency for the occurrence of human female and male infertility if abused. In the light of the above study, some caution should therefore be taken in the consumption of Cola nitida or any caffeine-related substances (such as soft drinks, energy drinks, chocolates, bottled water, chewing gum and medications) by both the female and male human subjects.

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