

ORIGINAL ARTICLE

Effect of Crude and Decaffeinated Extracts of *Cola nitida* Seeds on Male Reproductive System in Swiss Albino Rats

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Abstract:

Background: Caffeine is present in kola nut and xanthine stimulants which are used as a psychoactive drug. Therefore, the effect of kola nut (*Cola nitida*) extract was carried out on male reproductive system in male albino rats. **Aim and Objectives:** This study was aimed to determine the effect of oral administration of Crude Extract of Kola (CEK) and Decaffeinated Extract of Kola (DEK) on the reproductive function in male Swiss albino rats. **Material and Methods:** Twenty-four adult male albino rats were used for this study, they were assigned into three groups consisting eight rats each. Group 1 (control group) received (8mg/kg bw) of distilled water for six weeks, Group 2 (crude extract group) received (8mg/kg bw) of CEK for six weeks, and Group 3 (decaffeinated extract group) was treated with (8mg/kg bw) of DEK for six weeks. **Result:** CEK showed no significant decrease in the body weight and sperm count when compared with the control group. No significant difference in seminal parameter (motility, morphology, viability), organ weight (testis) and hormonal assay (testosterone, follicle stimulating hormone, luteinizing hormone) when compared with the control group. DEK showed no significant different in body weight, hormonal assay (testosterone and follicle stimulating hormone, seminal parameter (sperm viability, count, morphology and motility), organ weight (testes and epididymis) of the animal; however significant increase was observed in luteinizing hormone when compared with control group. A significant increase in

the sperm count of decaffeinated group was observed ($p = 0.02$) when compared with crude group. **Conclusion:** This study indicates that CEK and DEK have little effects on male reproductive system.

Keywords: Kola Nut Extract (Crude and Decaffeinated), Male Reproductive System, Albino Rat, Hormonal Assay, Sperm Quality

Introduction:

Kola nut a genus of about 125 species of trees native to the tropical rainforest of Africa [1]. It is known as *Cola nitida* belonging to the family Malvaceae, sub family Sterculioideae. It is chewed in many West African cultures [2] is often used ceremonially, presented to tribal chiefs or to guest as a sign of love. [2]. Kola nut is important in various social and religious customs and may also be used to counteract hunger and thirst. [2]. In Nigeria for instance, the rate of consumption of kola nut especially by students is very high as it is being a principle stimulant to keep awake and withstand fatigue [3]. Caffeine is present in kola nut and they are stimulants in nature as Xanthine derivative. Caffeine (1, 3, 7-trimethylxanthine) is a psychoactive drug that is naturally present in many foods, beverages (coffee, tea, carbonated beverages and cocoa) that are consumed daily in nearly all countries [4]. Caffeine has been

reported to have decrease in birth-weight among newborn [5], impaired semen qualities [6]. Despite all these effects, there is a dearth in literature on the effect of a decaffeinated extract of *Cola nitida* (DEK) on male reproduction, hence this study. This study was aimed to investigate the effects of both crude and decaffeinated extract of *Cola nitida* on the male reproduction using some of the markers of reproductive functions.

Material and Methods:

Cola nitida was obtained at Masifa local market Ogbomoso. It was air dried and ground into powdery form by an electric blender.

Crude Extraction of Kola (CEK):

500 g of grinded *Cola nitida* was dissolved in 2 liters of 70% methanol. The mixture was allowed to stand at room temperature for 48 hours to allow more chemical substance in the homogenized *Cola nitida* to diffuse into the solution and filtered. The filtrate poured into round bottom conical flask was fixed with rotary evaporator so as to collect the methanol. Water in the extract dried up gradually by evaporation. The molten brown extract was stored at -4°C . From this, the Extraction Yield Fraction (E.Y.F) was calculated. $\text{E.Y.F} = \text{weight of extract yield} / \text{initial weight of sample} * 100$

$$58.5/500 * 100 = 11.7\% \text{ w/v}$$

Decaffeinated Extraction of Kola (DEK):

Dichloromethane was employed for decaffeination in accordance to Toci *et al* (2006) [7].

501g of *Cola nitida* was soaked in 1.5 liters of 70% dichloromethane (DCM). The mixture was allowed to stand at room temperature for 72 hours to allow caffeine in the cola nitida bind properly to DCM. The solution was filtered leaving a clear light brown filtrate. The residue which contained the other component of *Cola nitida* was open to air

and ensured that no DCM was in the powder. The dried powder was extracted using 70% methanol with the procedure described in crude extraction. The extract was also stored at -4°C . Extraction yield fraction was 12.3%. For the crude extract, 10g of crude extract were dissolved in 1000 milliliters of distilled water ($10\text{g}/1000\text{ml} = 0.01\text{g/ml}$). The same method was used for the decaffeinated extract.

Calculation of Volume:

$$\frac{\text{weight of animal} * \text{dosage}}{1000 * \text{conc in } \frac{\text{mg}}{\text{ml}}} \quad [8]$$

Experimental Design:

Twenty-four adult male Swiss albino rats weighing (150–200) g were used for this study. The animals were obtained from a private breeder in Ibadan; they were allowed to acclimatize to the environment of the animal house (12hr day/ 12hr night) for 4 weeks before the commencement of the study. They were allowed to have access to standard laboratory rat chow and water *ad libitum*. All experimental protocols were in strict compliance with the guidelines for animal research, as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals (National Academy of Sciences and National Institutes of Health Publications, 2011) and approved by local Institutional Research Committee.

The animals were divided into three groups of 8 rats each (n = 24)

Group 1: control group was given distilled water *ad libitum*

Group 2: crude extract group was treated with CEK of (8mg/kg bw) for 6 weeks

Group 3: decaffeinated group was also treated with DEK of (8mg/kg bw) for 6 weeks

After the 6th weeks of administration, the animals

were sacrificed by means of cervical dislocation. The blood samples were collected by cardiac puncture into separate Ethylene Diamine Tetra Acetic Acid (EDTA) bottles. These were centrifuged at 4000 rpm for 15 min at -4°C , using cold centrifuge (Centurium Scientific, Model 8881). Plasma obtained was collected into separate plain bottles for the assessment of hormonal assays such as the testosterone, luteinizing hormone and follicle stimulating hormones. Orchidectomy was performed by open castration method. A midline or pre-scrotal incision was made and the testicles were milked out of the incision site. The testicles were exposed by incising the tunica vaginalis. The spermatic cord was exposed, ligated and incised. Semen samples were thereafter collected from the cauda epididymis [9].

Measurement of Body and Organ Weight:

Weekly body weight of the rats was determined with the aid of a digital weighing balance (Hanson, China) to assess weekly weight gain or loss. The reproductive organs weight: Testes, and epididymis, were also determined.

Statistical Analysis:

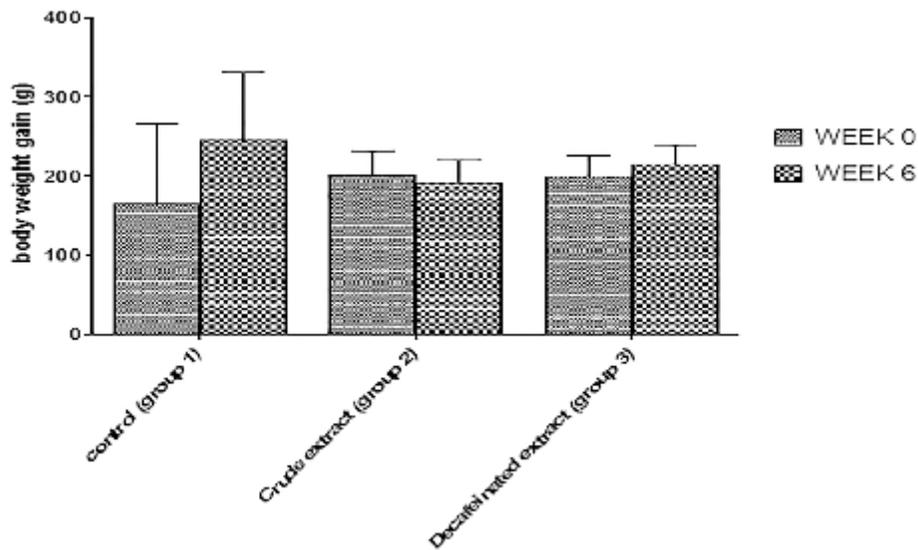
The results obtained were collated and expressed as mean \pm SD and subjected to one-way Analysis of Variance (ANOVA). The data were further subjected to a *post-hoc* test using Student Neumann Keuls' method, and differences with probability values of $P < 0.05$ were considered statistically significant. The statistical analysis was carried out with the aid of Graph Pad Prism 5.03.

Results:

Table 1: Semen Analysis of Control, Crude Extract and Decaffeinated Extract Groups

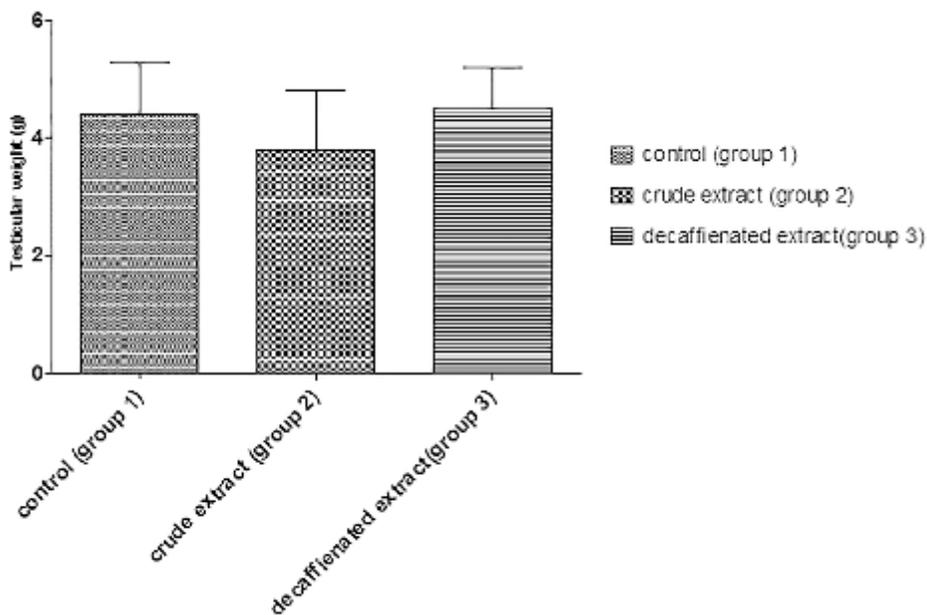
Parameters	Control (Mean \pm SD)	Crude (Mean \pm SD)	Decaffeinated (Mean \pm SD)
Sperm Count (Cells/MI)	57 0,00000 \pm 13.23	377,00000 \pm 2.47*	536,00000 \pm 12.26 ^{α}
Rapid sperm Motility (%)	63.3 \pm 5.16	65 \pm 5.34	65.7 \pm 6.90
Slow Progressive (%)	23.3 \pm 8.16	10 \pm 4.63	15 \pm 8.66
Non Progressive (%)	6.7 \pm 2.58	16.3 \pm 5.18	9.3 \pm 9.3
Dead Sperm (%)	6.0 \pm 2.18	8.8 \pm 2.32	7.1 \pm 2.83
Sperm Morphology (%)	56.7 \pm 5.16	58.8 \pm 8.34	58.6 \pm 3.780
Head Defect (%)	28.3 \pm 7.53	26.3 \pm 7.44	28.6 \pm 6.90
Tail Defect (%)	7.5 \pm 2.74	7.5 \pm 2.67	6.4 \pm 2.44
Sperm Viability (%)	66.7 \pm 5.16	67.5 \pm 7.07	65.7 \pm 7.86
Non Viability (%)	33.3 \pm 5.16	32.5 \pm 7.07	34.3 \pm 7.8
Motility Count Mi (%)	93.3 \pm 2.58	91.2 \pm 2.32	92.9 \pm 2.67

Values expressed in Mean \pm SD where n= 8 in each group



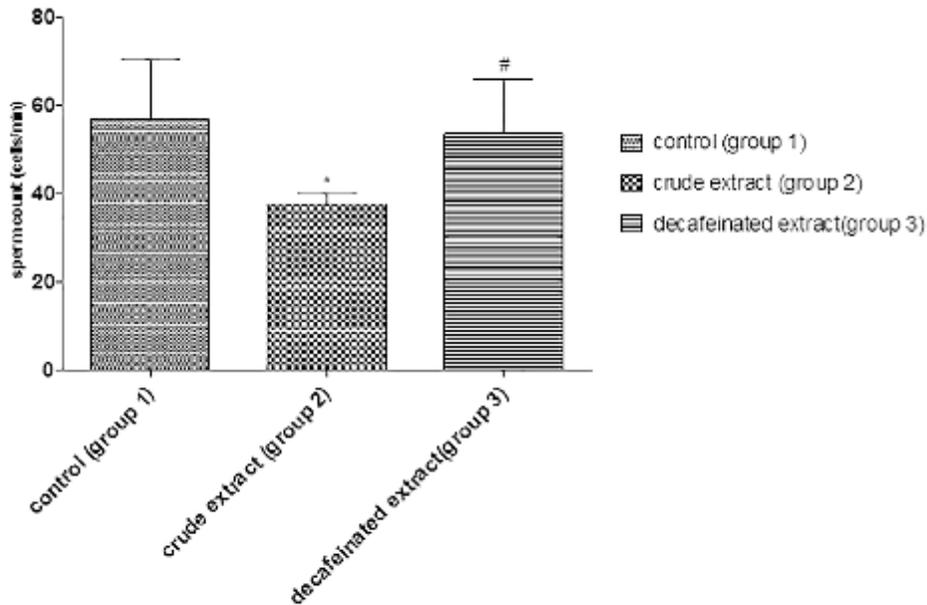
No significant decrease in the final body weight between crude extract group ($p=0.49$), control group ($p=0.25$) and decaffeinated extract group ($p=0.27$) when compared to their initial body weights. Comparison among all groups shows no significant differences ($p=0.23$) $n = 8$, values are expressed as mean \pm SD

Fig. 1: Body Weight Alteration of Control, Crude Extract and Decaffeinated Extract Groups from the Initial to the Final Weights



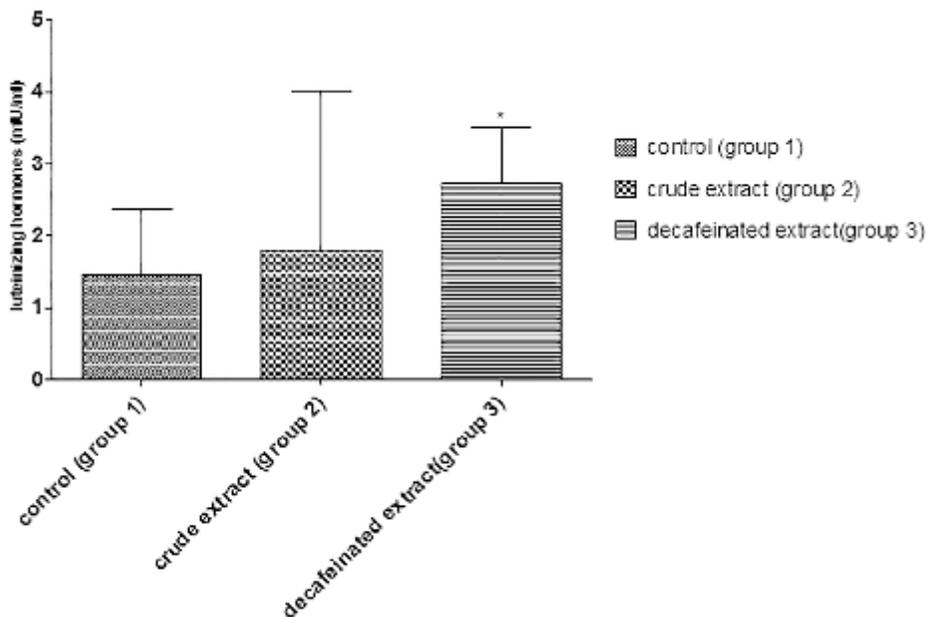
No significant differences in testicular weight between crude extract group ($p=0.27$) and decaffeinated extract group ($p=0.27$) when compared with control group. $n = 8$, values are expressed as mean \pm SD

Fig. 2: Testicular Weight of Control, Crude Extract and Decaffeinated Extract Groups



Significant decrease in sperm count of crude extract group ($p=0.02$) was observed while no significant decrease in sperm count of decaffeinated extract group ($p=0.64$) when both groups compared to control group. Comparison in sperm count between decaffeinated extract and crude extract groups shows significant differences ($p=0.01$). $n = 8$, values are expressed as mean \pm SD * = relative to control, # = relative to crude group at $p<0.05$

Fig. 3: Sperm Count of the Control, Crude Extract and Decaffeinated Extract groups



No significant increase in luteinizing hormone level between crude extract group compared to control group ($p=0.43$) and decaffeinated extract group ($p=0.77$). Significant difference was observed ($p=0.04$) when compared decaffeinated extract group with control group. $n = 8$, values are expressed as mean \pm SD, *is relative to control at $p<0.05$.

Fig. 4: Luteinizing Hormone Level of the Control, Crude Extract and Decaffeinated Extract Groups

Discussion:

From the result, there is no significant decrease in the total body weight of rats treated with CEK as shown in Fig. 1. This finding is not in agreement with the report of Ikegwonu *et al.* (1981) [10] that says, kola nut extract administered to rats for 18 weeks decrease in the total body weight and increases the absolute weight of the liver, kidney, brain and testes. However, the non-significant decrease in the body weight may be due to the duration of administration of the CEK this study (6 weeks). DEK does not decrease the body weight of the experimental rats as observed in the CEK group. This is additional information to support the findings of Myer *et al.* (2003) [11] on his assertion that caffeine consumption decreases body weight and also reduces body deposition of fats. Moreover, the non-significant decrease in the testicular weight of rats treated with CEK as shown in Fig. 2 which was not in agreement with the report of Adisa *et al.* (2010) [12] and Ikegwonu *et al.* (1981) [10] who have reported that there is a significant increase in the testicular weight of rats treated with crude extract of kola nut. This contradiction may be due to the method of extraction used by Adisa *et al.* (2010) [12] whose method is through aqueous extraction.

CEK shows significant decrease in sperm count of crude extract group but no significant effect on its sperm motility, morphology of the same group as shown in Fig. 3 and Table 1. This non-significant effect is in agreement with the findings of Lopez *et al.* (2000) [13] who have reported that caffeine, a plant alkaloid has no effect on sperm motility, morphology but contradicts his findings on sperm count. Also this is also in support with the findings of Adisa *et al.* (2010) [12] reported that, the consumption of kola nut has little or if any impact on semen quality. Furthermore, this finding on

semen quality agreed with the report of Nan *et al.* (1992) [14] stated that there is no association between sperm quality, smoking habits, drinking coffee, moderate alcohol intake exposure to heat or physical activities in men. However, this assertion contradicts the report that caffeine increases sperm motility [15-18]. Decrease in the sperm count of rats treated with CEK was also in agreement with Marshburn *et al.* (1989) [6], reported that caffeine impaired semen quality. Also, increase in sperm count of rats treated with DEK compared to that of crude group treated with CEK confirmed that, caffeine in kola nut causes reduction in sperm count [6]. This decrease in sperm count of rats treated with CEK may be due to toxic effect of caffeine, since it has been reported that caffeine while relatively safe for human is more toxic to other animals like dog, horse and parrots [6].

Increase in Luteinizing Hormone (LH) of rats treated with DEK may responsible due to the other component of kola nut other than caffeine in kola nut as shown in Fig. 4. LH has been reported to cause secretion of testosterone from Leydig cells of the testes when stimulated [6]. Therefore, looking at the level of LH and testosterone of DEK treated group, there was a significant increased in LH when compared to the testosterone, based on this, an inverse relationship between the testosterone and LH was observed. This may be due to the negative feedback mechanism caused by an increase in testosterone level, on the anterior pituitary gland.

It has also been reported that kola nut has aphrodisiac effect (increases libido). Testosterone is responsible for the increase in libido. From the result, there was slightly significant decrease in the testosterone level of CEK and DEK groups

when compared to the control [19]. This assertion contradict the report that kola nut has aphrodisiac effect. This contradiction may be due to the dosage of kola nut extract (both CEK and DEK extract) administered to the rats.

Testosterone and Follicle Stimulating Hormone (FSH) of the CEK group has no significant differences when compared with their control group. The sperm count of crude extract group was significantly decreased when compared with the control group. Follicle stimulating hormone and testosterone are said to initiate the production of sperm cells. Thus, kola nut extract (crude extract) has no significant effect on FSH and testosterone level. The decrease in the sperm count may be due to the depressed in the total protein, DNA and RNA of the testes [10] which may cause initiating impaired functional effects of FSH and testosterone.

From this finding, the epididymal weight of the animal treated with CEK and DEK have no

significant difference when compared with the control group animals. This was not in agreement with the work of Ekaluo *et al.* (2015) [20] who reported that caffeine cause significant decrease in the epididymal weight. The contradiction observed in this study could be due to the other components present in CEK attenuating the effect of caffeine with the evident in DEK.

Conclusion:

Findings in this study shows that CEK causes decrease in the sperm count than the DEK. This could be traceable to the presence of caffeine in the CEK. Despite this observation, the sperm motility, morphology and the hormonal analyses are relatively affected in the group treated with CEK and DEK. Therefore, both CEK and DEK have little or no effect on the male reproductive system. From this finding, care must be taken in consumption of cola nut.

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