

## Effect of Aqueous Kola Nut (*Cola nitida rubra*) Extract on Sperm Quality in Male Sprague – Dawley Rats

<sup>1</sup>W.A. Adisa, <sup>3</sup>B.O. Iranloye, <sup>2</sup>C.C. Ezekunie, <sup>4</sup>A.O. Nwaopara, <sup>1</sup>C.U. Osifoh,  
<sup>1</sup>E.N.S. Igbinovia and <sup>1</sup>H.O. Otamere

<sup>1</sup>Department of Physiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria

<sup>2</sup>Department of Physiology, Madonna University, Elele, Rivers State.

<sup>3</sup>Department of Physiology, University of Lagos, Lagos, Lagos State, Nigeria

<sup>4</sup>Department of Anatomy, Ambrose Alli University, Ekpoma, Edo State, Nigeria

**Abstract:** This study evaluated the effects of kola nut extract on sperm quality in male Sprague dawley rats. 30 adult male rats were used. These were divided into three groups: group A served as control, groups B and C received kola nut extract only (8mg/kg body weight), C served as recovery group. All the groups were treated for four weeks. Group C, which served as recovery group, was allowed to recover for another four weeks after the extract administration. Parameters of sperm quality: count, morphology and motility were insignificantly decreased. However, life/dead ratio was significantly decreased ( $p < 0.05$ ). The recovery group showed values that were insignificantly decreased but a bit closer to those of the control animals. This showed that the rats were able to recover to some extent after the extract administration. This indicates that consumption of kola nut has little, if any impact on semen quality.

**Key words:** Kola nut extract, caffeine, sperm quality, semen, Sprague dawley rat, *Cola nitida*

### INTRODUCTION

Kola nuts are the seed-pods of various evergreen trees that are native to Africa. In West Africa and Sudan, 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 kola nut 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 theophylline found in kola nuts are xanthine stimulants. Caffeine was first isolated from green coffee beans in 1820. It was later found in tea mate and kola nuts. Caffeine (1,3,7-trimethylxanthine) is a psychoactive drug, a methylated xanthine that naturally occurs in many foods beverages consumed daily in nearly all countries. Coffee, tea, carbonated beverages and cocoa (Burker and Williams, 1979) are the main sources of caffeine. Moreover, various pharmaceutical products contain caffeine in combination with other drugs (Rall, 1980). Caffeine and some of its metabolites have been suggested as risk factors in birth defect (Jacobson *et al.*, 1981), heart disease (Heyden *et al.*, 1978) and pancreatic cancer.

The popularity of caffeine is due to its stimulant action. Because it occurs naturally in more than 60 plant species, people may have consumed it as long as Paleolithic period.

Epidemiological studies have been advocated as the best approach to exploring any link between caffeine and reproductive outcome (Olsen, 1991). Caffeine has been implicated as a risk factor for delayed conception (Wilcox *et al.*, 1988; Williams *et al.*, 1990; Hatch and Bracken, 1993; Stanton and Gray, 1995; Bolumar *et al.*, 1997; Jensen *et al.*, 1998). In a cohort of 104 healthy women who attempted to become pregnant for 3 months, the high caffeine consumers (i.e. 1 cup of brewed coffee/day) were significantly less likely to become pregnant in each cycle (fecundability ratio: 0.53, 95% confidence interval (CI): 0.35, 0.79) than the lower caffeine consumers (i.e. <1 cup of coffee/day), while adjusting for age, frequency of intercourse, age at menarche, smoking, and a woman's prenatal exposure to her mothers' smoking (Wilcox *et al.*, 1988).

The high caffeine users had an increased risk of infertility (unadjusted relative risk: 4.7,  $p < 0.005$ ); there was a dose-related response to caffeine consumption when women were sub-divided into narrower categories of caffeine consumption, with women in the highest consumption group having a fecundability rate only 26% of that of the lowest consumption group. Only two studies failed to find a correlation between caffeine consumption and delayed conception (Joesoef *et al.*, 1990; Olsen, 1991); both were retrospective and subject to recall bias.

Caffeine consumption has also been implicated as a risk factor for spontaneous abortions (Srisuphan and

Table 1: Effect of Cola nut extract on the weight of organ

	Liver (g)	Kidney (g)	Heart (g)	Testes (g)
Control	7.63±0.24	0.88±0.12	0.65±0.03	2.02±0.09
Cola	8.27±0.028	0.83±0.06	0.89±0.06 <sup>a</sup>	2.35±0.04 <sup>a</sup>
Recovery	7.74±0.10	0.88±0.19	0.74±0.08	2.06±0.08

a = p<0.05, compared with control

Table 2: Effect of Kola nut extract on sperm functions

	Sperm motility%	Sperm count (million)	Sperm morphology (%)	Semen volume (MI)	Life/Dead (%)
Control	74.16±2.6	51.6±1.06	70±2.89	5.16±0.03	95.3±1.72
<i>Cola nitida</i>	69.85±4.05	47.53±2.2	65±2.89	5.14±0.02	80.2±1.45 <sup>a</sup>
Recovery	73.45±2.58	50.56±1.03	68.6±2.83	5.16±0.02	92.52±1.69

a = p<0.05, compare with control

Bracken, 1986; Fenster *et al.*, 1991; Armstrong *et al.*, 1992; Infante-Rivard *et al.*, 1993). Some studies found no relationship (Wilcox *et al.*, 1990; Mills *et al.*, 1993), and it was suggested that nausea (occurring more frequently in the first trimester of pregnancy), might have resulted in decreased caffeine consumption, thereby creating a spurious finding of no association (Stein and Susser, 1991). However, a study, which adjusted for nausea, found a doubled risk for spontaneous abortion in heavy caffeine users (adjusted odds ratio (OR): 2.10, 95% CI: 1.20, 3.70) (Fenster *et al.*, 1991).

There is a relationship between caffeine and birthweight; a combined analysis of mean birthweight from 22 studies resulted in a significant decrease in birthweight of nearly 43 g among newborns of the heaviest-caffeine-consuming mothers (Santos *et al.*, 1998a). In 5476 subjects from an infertility clinic, coffee consumption was associated with increases in sperm concentration, abnormal forms, and motility. The combination of coffee drinking with smoking diminished sperm motility and increased the percentage of dead sperm (Marshburn *et al.*, 1989).

Considering the potentials of caffeine, and the fact that kola nuts are consumed in large quantities by natives of Africa, especially the Hausas in the northern part of Nigeria where its consumption has become a die-hard habit among men and women. This study is therefore designed to investigate its probable effects on reproductive functions in male sprague-dawley rats.

## MATERIALS AND METHODS

**Location and duration of study:** This study was conducted at the animal house of the department of Physiology, University of Lagos, Nigeria. The preliminary studies, animal acclimatization, extraction of Kola nut extract, actual animal experiment, semen analysis and evaluation of results, lasted for a period of eight months (April, 2007 to October, 2007).

**Aqueous extraction:** 8kg of *Cola nitida* were pulverized with an electric blender, the pulverized product was placed inside the Soxhlet apparatus into which water was

added. The soxhlet apparatus was then set up and left for 72 hours. The extracted solution was taken to the oven for concentration to dryness at a regulated temperature of 40<sup>o</sup> and left for 6 days. With this a powdery product was obtained, from which aqueous extract of 8mg/kg body weight was prepared.

**Animals grouping:** 30 male Sprague-dawley rats were divided into 3 groups (A, B and C) containing 10 rats each. Group A, served as control. Group B was given *Cola nitida* extract 8mg/kg body weight. Group C, served as recovery group, was given *Cola nitida* extract 8mg/kg

body Weight. The administration was done orally for four weeks by means of a metal cannula. The animals were weighed daily and average weekly weights were obtained.

**Materials:** Capillary tubes, heparinized bottles, centrifuge.

**Sample collection:** The animals were sacrificed by cervical dislocation. They were cut open from linear alba of the abdominal cavity to the thoracic cavity. The epididymis, testis, and accessory organs were removed for study of sperm quality. Other organs removed were liver, kidney and heart. The organs for each group were weighed immediately after their removal from the animals.

## RESULTS

**Results on weights of organs:** There were no significant difference in the weights of the livers and kidneys (Table 1 and Fig. 1), but there were significant increase (p<0.05) in the weights of the hearts and testes of rats treated with *Cola nitida* extract when compared with control rats (Table 1 and Fig. 1). Weight of organs in the recovery group showed no significant difference when compared the control with the recovery group (Table 1 and Fig. 1).

**Result of semen analysis:** There were no significant differences in sperm motility, count, morphology and

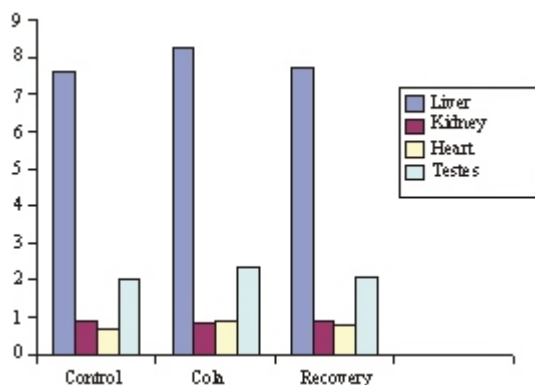


Fig. 1: Showing effect of *Cola nitida* extract on the weights of organs

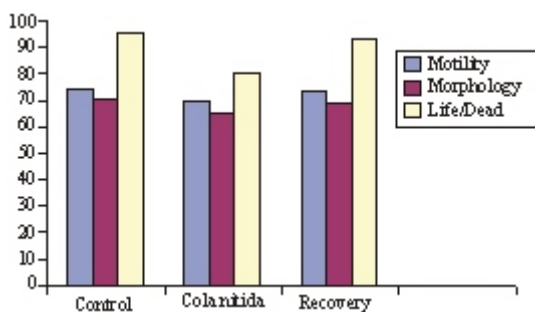


Fig. 2: Showing effect of *Cola nitida* extract on motility, morphology and life/dead ratio

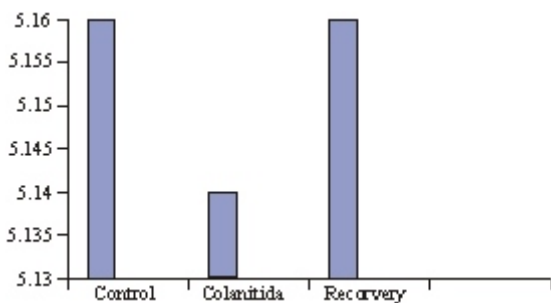


Fig. 3: Showing effect of *Cola nitida* extract on sperm volume

volume, but there was a significant difference ( $p < 0.05$ ) in the life/dead ratio of rats treated with kola nut extract when compare with control rats (Table 2, Fig. 2, 3, 4).

### DISCUSSION

Judging from the findings of this study, it is likely that the significant increase ( $p < 0.05$ ) in hearts' weight may be due to the effect of methylxanthine component of the cola extract (a known inotropic agent), which has the potential to induce cardiac muscle thickening (Gould *et al.*, 1973). Similarly, the significant increase ( $p < 0.05$ ) in the weight of th testes (as observed in the cola

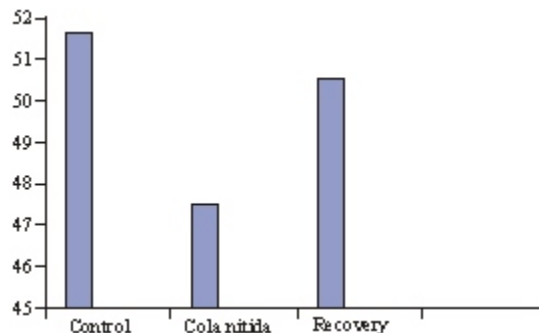


Fig. 4: Showing effect of *Cola nitida* extract on sperm count

treated group) is in line with the report by Ikegwuonu *et al.* (1981), suggesting that the cola extract might have some effect on sex hormones of the rats under study.

Of interest also, is the fact that *Cola nitida* extract did not show any significant effect on semen parameters (i.e. motility, morphology, sperm count and semen volume). This is in agreement with the findings of Lopez and Alvarino (2000), who reported that caffeine, a plant alkaloid, and the main chemical component of kola nut extract, has no effect on sperm motility, sperm morphology and sperm count.

In addition, our finding on semen quality agrees with the report by Nan *et al.* (1992), who reported that that there is no association between sperm quality, smoking habits, coffee drinking, moderate alcohol intake, exposure to heat (sauna, hot baths, type of underwear, sedentary activities) or physical activities in men. Thus, it could be said that *Cola nitida* extract has little, if any, impact on semen quality and therefore may not be associated with infertility problem in males. However, this assertion contradicts the report that caffeine impaired semen quality (Marshburn *et al.*, 1989) and increased motility (Barkay *et al.*, 1977, Harrison 1978, Aitken *et al.*, 1983, Hammit *et al.*, 1989).

Finally, the fact that the recovery group did not show any significant difference in the weight of the livers, kidneys, hearts and testes, as compared to the control group, indicates that the rats were able to recover from any possible effect of cola extract. It is on the basis of this therefore, that we advocate a regulated consumption of *Cola nitida*.

### REFERENCES

- Aitken, R.J., F. Best, D.W. Richardson, R. Schats and G. Simm, 1983. Influence of caffeine on movement characteristics, fertilizing capacity and ability to penetrate cervical mucus of human spermatozoa. *J. Reprod. Fertil.*, 67: 19-27.
- Armstrong, B.G., A.D. McDonald and M. Sloan, 1992. Cigarette, alcohol, and coffee consumption and spontaneous abortion. *Am. J. Publ. Health*, 82: 85-87.

- Barkay, J., H. Zuckerman, D. Sklan and S. Gordon, 1977. Effect of caffeine on increasing the motility of frozen human sperm. *Fertil. Steril.*, 28: 175-177.
- Bolumar, F., J. Olsen, M. Rebagliato and L. Bisanti, 1997. Caffeine intake and delayed conception: a European multicenter study on infertility and subfecundity. *Am. J. Epidemiol.*, 145: 324-334.
- Burker, M.L. and M. Mc Williams, 1979. Caffeine content of common beverages. *J. Am. Diet. Assoc.*, 74: 28-32.
- Fenster, L., B. Eskenazi, G. Windham and S.H. Swan, 1991. Caffeine consumption during pregnancy and spontaneous abortion. *Epidemiology*, 2: 168-174.
- Gould, L., K. Ventakaman, M. Gosuani and R.F. Gomprecht, 1973. The cardiac effects of coffee. *Angiology*, 24: 453.
- Hammit, D.G., E. Bedja, P.R. Rogers, C.H. Syrop, J.F. Donovan and R.A. Williamson, 1989. Comparison of motility stimulants for cryopreserved human semen. *Fertil. Steril.*, 52: 495-502.
- Harrison, R.F., 1978. Insemination of husband's semen with and without the addition of caffeine. *Fertil. Steril.*, 291: 532-534.
- Hatch, E.E. and M.B. Bracken, 1993. Association of delayed conception with caffeine consumption. *Am. J. Epidemiol.*, 138: 1082-1092.
- Heyden, S., H.A. Tyroler, Cr.H. Hciss and C.G. Bartel, 1978. A coffee consumption and mortality total mortality, stroke and coronary heart disease mortality. *Arch. Lutern. Med.*, 138: 1472-1475.
- Ikegwonu, F.I., T.A. Aire and S.O. Ogwuegbu, 1981. Effects of kola nut extract administration on liver, kidney, brain, testis and some serum constituents in rats. *J. Appl. Toxicol.*, 1(6): 292-294.
- Infante-Rivard, C., A. Fernandez, R. Gauthier, M. David and G.E. Rivard, 1993. Fetal loss associated with caffeine intake before and during pregnancy. *J. Am. Med. Assoc.*, 270: 2940-2943.
- Jacobson, M.F., A.S. Goldman and R.H. Syme, 1981. Coffee and birth defects. *Lancet*, 1: 145-146.
- Jensen, T.K., T.B. Henriksen, N.H. Hjollen, T. Scheike, H. Kolstad, A. Giwerzman, E. Ernst, J.P. Bonde, N.E. Skakkbaek and J. Olson, 1998. Caffeine intake and fecundability: a follow-up study among 430 Danish couples planning their first pregnancy. *Reprod. Toxicol.*, 12: 289-295.
- Joesoef, M.F., V. Beral, R.T. Rolfs, S.O. Aral and D.W. Cramer, 1990. Are caffeinated beverages risk factors for delayed conception? *Lancet*, 335: 136-137.
- Lopez and Alvero, 2000. Assessment of motility, acrosomal integrity and viability of giant panda (*Ailuropoda melanoleuca*) sperm following short term storage at 4°C. *Zoo Biol.*, 22(6): 529-544.
- Marshburn, P.B., C.S. Sloan and M.G. Hammond, 1989. Semen quality and association with coffee drinking, cigarette smoking, and ethanol consumption. *Fertil. Steril.*, 52: 162-165.
- Mills, J.L., L.B. Holmes, J.H. Aarons, J.L. Simpson, Z.A. Brown, L.G. Jovanic-Peterson, M.R. Conley, B.I. Craubard, R.H. Knopp and B.E. Metzger, 1993. Moderate caffeine use and the risk of spontaneous abortion and intrauterine growth retardation. *J. Am. Med. Assoc.*, 269: 593-597.
- Nan, B., H.R. Oldereid and P. Keneth, 1992. Life style of men in barren couples and their relationship to sperm quality. *Eur. J. Obstet. Gyn. R. B.*, 43(19): 51-57.
- Olsen, J., 1991. Cigarette smoking, tea and coffee drinking, and subfecundity. *Am. J. Epidemiol.*, 133: 734-739.
- Purgesleve, J.W., 1977. *Tropical crops dicotyledons*. Longmans London, pp: 564-612.
- Rall, T.W., 1980. The Xanthine in Goodman LS. In: Gilman, A., (Ed.), *The Pharmacological Basis of Therapeutics*. New York, Macmillan, Inc., 6: 592-607.
- Russel, T.A., 1955. The kola nut of West Africa. *World Crop.*, 7: 221-225.
- Santos, I.S., C.G. Victora, S. Huttly and S. Morris, 1998a. Caffeine intake and pregnancy outcomes: a meta-analytic review. *Cadernos de Sude Publica*, 14: 523-530.
- Stanton, C.K. and R.H. Gray, 1995. Effects of caffeine consumption on delayed conception. *Am. J. Epidemiol.*, 142: 1322-1329.
- Somarin, O., 1973. Spectrometry determination of caffeine in Nigerian kola nuts. *Sci.*, 381: 911-913.
- Srisuphan, W. and M.B. Bracken, 1986. Caffeine consumption during pregnancy and association with late spontaneous abortion. *Am. J. Obstet. Gynecol.*, 154: 14-20.
- Stein, A. and M. Susser, 1991. Miscarriage, caffeine and the epiphenomena of pregnancy: The causal model. *Epidemiology*, 2: 163-167.
- Wilcox, A., C. Weinberg and D. Baird, 1988. Caffeinated beverages and decreased fertility. *Lancet*, 2: 1453-1456.
- Wilcox, A.J., C.R. Weinberg and D.D. Baird, 1990. Risk factors for early pregnancy loss. *Epidemiology*, 1: 382-385.
- Williams, M.A., R.R. Monson, M.B. Goldman, R. Mittendorf and K.J. Ryan, 1990. Coffee and delayed conception. *Lancet*, 335: 1603.