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Research Article

The effect of local millet drink (Kunu) on the testis and epididymis of adult male wistar rats

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Abstract

Background:
Kunu is a local beverage drink that finds its origin in the northern part of Nigeria. This study was aimed at determining the effect of the liquid drink on the epididymis, testes, sperm parameters, and hormonal assay.
Methods: A total of sixteen rats were used for this study and the animals were separated into four groups of four rats each (A-D). The animals were then sacrificed and the testes and epididymis were harvested and fixed in 10% formal saline. Group A was fed only rat feed and water. Groups B, C, and D were fed 0.2 ml, 0.9 ml, and 2.5 ml of Kunu respectively orally using a metal cannula for 21 days.
Findings: There was a significant increase ($P<0.05$) in the relative testicular weights of groups B, C, and D as compared with those of group A. There was a significant decrease ($P<0.05$) in sperm count in groups B, C, and D when compared to group A. There was an insignificant increase ($P>0.05$) in FSH in groups B, C, and D when compared to group A. The histopathological findings revealed that the group B rats of 0.2ml and group C rats of 0.9ml showed epididymal tissue with moderate accumulation of spermatozoa and testicular tubules with moderately enhanced spermatogenesis. The group D rats showed well-accumulated spermatozoa in the epididymal lumen and improved spermatogenesis in the testis as did group A.
Conclusion: Kunu beverage may not be used as a natural male fertility booster since it does little to improve sperm count, motility, morphology, pH, and hormonal levels of FSH and testosterone.

Non-technical summary
To test if the Kunu drink from Nigeria may be useful for fertility in men, we measured its effects in Wistar rats by feeding them different amounts of Kunu over 21 days. Although Kunu caused a small increase in testicle weight, it slightly lowered sperm count and caused no other major changes in sperm when viewed under a microscope or in the rats' male hormones. These results in rats mean it's unlikely that Kunu improves fertility in people.

Introduction

In a sexual world like ours, there is an urgency for people across the globe to meet their sexual needs daily. The problem of infertility touches on several factors which affect both males and females. In cases of male factor infertility which concentrates on testicular activity and sperm production as well as libido, there are two options for raising testosterone production and enhancing sperm production which are: the use of synthetic steroids and natural boosters.[1]

The use of synthetic steroids such as synthetic testosterone and gonadotropins has adverse effects such as

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reduced testes size, micturition problems, gynecomastia, sleep disturbances, *etc.*,[1] which is why a better approach to the problem of male factor infertility (due to azoospermia, oligospermia or any other related spermatic problem) may be the use of natural boosters of which the local northern drink Kunu is a prominent example.

Kunu is a popular drink (Figure 1) consumed throughout Nigeria but mostly in the North. It can be made from grains such as millet, sorghum, maize, and rice. The variety of drinks made from sorghum is a milky light-brown color while that of maize or millet is whitish. Generally, consumption cuts across all age groups and social status with the peak of consumption being the hot season of the year (February – June) when it is served chilled, particularly Kunun Zaki.[2]

Figure 1 | Kunu drink. *Peter Agan, CC-BY-SA 4.0*

Testes, also called testicles in animals, are the organ that produces sperm and androgen. In humans, the testes occur as a pair of oval-shaped organs. Both functions of the testes are influenced by gonadotropic hormones produced by the anterior pituitary gland. Luteinizing hormone (LH) is also produced but the anterior pituitary gland results in testosterone release. Both hormones are needed to support the process of spermatogenesis.[3] There are two phases in which the testes grow substantially: namely in embryonic and pubertal stages.[4] After puberty, the volume of the testes is increased compared to the pre-pubertal size.

Hence, this work set out to assess the effects of Kunu on histomorphology of the testes and epididymis, and parameters of sperm count, sperm motility, and sperm viability using the short-term *in vivo* assays in adult male Wistar rats.

Methods

Materials

The following materials were used in this experiment: Sixteen male Wistar rats, an oral cannula, Kunu (local beverage), four standard cages, distilled water, cotton wool, and hand gloves, beakers and measuring cylinder, animal weighing balance (CAMRY LB11), electronic weighing balance (NAPCO Precision Instruments JA-410), diethyl Ether, vital top feed (Jos, Nigeria), dissecting kit, EDTA container, and plain container, micro-haematocrits centrifuge SH120, capillary tube, 5 ml hypodermic syringe, Deep and flat feeding plates, Plastic bottles, 10% buffered formalin, hemocytometer, filter paper (Whatman qualitative filter paper n. 1, sigma Aldrich WHA1001042), thermostat oven (DHG-9023A, PEC MEDICAL USA), and spectrophotometer (Model 721).

Preparation of Kunu

Millet grains were soaked in a bowl of water and left overnight. The soaked millet was mixed with chops of dried sweet potatoes and ginger and blended into a paste. The paste mixture was divided into two equal parts; one part was stirred with boiling water and left to cool. The other part was then poured into this mixture, and the new mixture was then stirred to achieve thickness and then sieved to remove the chaff.

Experimental Animal

Sixteen male Wistar rats weighing between 170-200 g were used for this study. The animals were allowed to acclimatize for two weeks, after which they were randomly selected into 4 groups of 4 animals each. Group A served as a control (the animals received only water and feed). Group B received 100 mg/kg or 0.2 ml of Kunu. Group C received 400 mg/kg or 0.9 ml of Kunu. Group D received 1200 mg/kg or 2.5 ml of Kunu. The administration of drinks lasted for 21 days, taking place between 7 to 10 am daily. The animals were then

sacrificed after the aforementioned period, semen and blood were collected for seminal analysis and hormonal assay test, while the testes and epididymis were harvested for histopathological findings.

Acute Toxicity Test (LD_{50}) of Kunu

The acute toxicity test of Kunu (local beverage) was carried out in the Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nnewi, Anambra State according to the method employed by Lorke.[5] No toxic effect was observed in the treatment of Kunu drink up to the effective dose of 5000 mg/kg body weight of adult Wistar rats. The behaviour of the treated rats appeared normal, and no deaths occurred.

Procedure for Semen Collection

The caudal epididymis was isolated from the testes and lacerated in a warm physiological solution to collect semen for sperm characteristics studies. A sperm count was conducted according to the method described by Hafez[6] using a microscope with an improved Neubauer hemocytometer. Sperm motility (%) was determined through a light microscope within 5 minutes of isolation of sperm from the epididymis.[7] Sperm viability was examined based on the method reported by Bearden and Fuquay.[8] Eosin and Fast Green were used to distinguish motile (live) sperm from non-motile groups (dead) sperm. These sperm cells were counted under 40× magnification. The average count of motile and non-motile groups was recorded, from which the viability percentage was calculated. The number and percentage of normal sperm were determined according to the method proposed by Chemineau *et al.*[9] based on the slides used for the calculation of sperm viability.

Procedure for Hormonal Assay

Testosterone Test

Testosterone levels were determined in the serum of male rats by Elecsys Analyzer, D-Vi-S, using kits from Roche Diagnostics GmbH, D-68298, Mannheim, Germany.

Determination of Follicle Stimulating Hormone by Radioimmunoassay Technique

Serum levels of follicle-stimulating hormone (FSH) were assayed by RIA using reagents supplied by Rat Pituitary Distribution and NIDDK (Bethesda, MD, USA)

Statistical Analysis

The statistical analysis of this research was done using Analysis of Variance (ANOVA) followed by multiple comparisons using least statistical difference (LSD) and Student's t-test in the SPSS version 23 software package and $P < 0.05$ was considered as the level of statistical significance.

Results and Discussion

The male hormones are typically adequate to produce healthy sperm, however, when this is not the case, many men take fertility drugs to increase their sperm count and motility. Indeed, there is ample evidence indicating a steady decline in human sperm count and quality.[10] The anterior pituitary is responsible for controlling the male hormones from the testes, hence, sperm production. Around 2% of men with infertility experience secondary hypogonadism (pituitary gland disease). This condition is treatable by either pharmaceutical or natural means. There are very few drugs, approved by the U.S. Food and Drug Administration (FDA), that may help in stimulating sperm production such as clomiphene, letrozole, synthetic testosterone pills, bromocriptine, imipramine, etc.,[11] yet often these come with various side effects such as breast enlargement, changes in libido, liver problems, high blood pressure, etc.[12] Hence, there is a growing call, despite the low cost and commonness of these drugs, to use natural remedies (such as the beverage, Kunu, which this study chose to investigate) to address low fertility whilst aiming to avoid adverse effects.

The results of this study showed that there was no significant change in the weight of the experimental rat groups B, C, and D just as that of the control (Table 1). This could be attributed to the low fat and protein content of the beverage. This study differs from the report made by Abolfazl *et al.*[13] who reported that *Zingiber officinale* (ginger), a condiment of Kunu, increased the body weight significantly in Wistar rats at 1 g/kg of body weight.

Table 1 | Effects of Kunu on the body weight

Groups	Body weight (g)	Mean ± SEM	P - Value	T- Value
Group A	Initial	160.00 ± 20.00	0.588	-0.640
	Final	176.66 ±14.52		
Group B	Initial	186.67 ± 8.81	0.208	-1.835
	Final	213.33 ±12.01		
Group C	Initial	173.33 ± 8.81	0.225	-1.732
	Final	203.33 ± 8.81		
Group D	Initial	183.33 ± 6.66	0.221	-1.075
	Final	193.33 ± 6.66		

Data were analyzed using One-way ANOVA, and data were considered significant at $P < 0.05$* and $P > 0.05$ means not significant.

There was a significant increase ($P < 0.05$) in the relative testicular weight in the other groups when compared with the control group (Table 2). This agrees with the discovery of Ekaluo *et al.*[14] who reported a significant increase in the weight of the testis of albino rats administered with *Cyperus esculentus* (used in making Kunu aya) 1.8g/kg body weight which is due to the availability of the antioxidant vitamin C in Kunu and its protective role against oxidative stress and morphological changes of the testicular tissues. Results also revealed a significant decrease ($P < 0.05$) in relative epididymal weight group B compared to the control. The mechanism of this discrepancy is not understood, more so it disagrees with the work of Ekaluo *et al.*[14] who reported increasing weight of epididymis of the rats given an aqueous extract of *Cyperus esculentus* 1.8 g/kg body weight.

Table 2 | Effect of Kunu on relative testicular weight and epididymis weight

Organ weight	Group	Mean ±SEM	P-value	F-value
Relative testicular weight (g)	Group A	0.60 ±0.00		
	Group B	0.77 ±0.00	0.000*	39.661
	Group C	0.78 ±0.01	0.000*	
	Group D	0.71 ±0.02	0.000*	
Relative epididymis weight (g)	Group A	0.50 ±0.00		
	Group B	0.37 ±0.05	0.014*	6.606
	Group C	0.34 ±0.01	0.005*	
	Group D	0.46 ±0.02	0.444	

Data were analyzed using One-way ANOVA, followed by LSD comparison, and data were considered significant at $P < 0.05$ and $P > 0.05$ means not significant, it is also significant at the level of 0.01 and less.

There was a significant decrease ($P < 0.05$) in sperm motility in the experimental groups when compared with the control (Table 3). This does not agree with the findings of Abolfazl *et al.*[13] who state increased levels of sperm viability and motility of the Wistar rats given *Zingiber officinale*, found in Kunu, at 1g/kg body weight. There was also a significant ($P > 0.05$) decrease in the total sperm count in group B and an insignificant ($P < 0.05$) decrease in groups C, and D when compared to the control. This contradicts the findings of Hafez[6] who reported a significant increase in sperm quality and quantity of Wistar rats fed with 2 g/kg body weight of ginger roots and cinnamon bark.

Table 3 | The effect of Kunu on sperm motility and total sperm count

Sperm parameters	Groups	Mean ±SEM	P-value	F-value
Sperm Motility (%)	Group A	90.00 ±2.88		
	Group B	83.33 ±1.67	0.047*	13.888
	Group C	76.67 ±1.66	0.002*	
	Group D	73.00 ±1.52	0.000*	
Total Sperm Count (x10^6/L)	Group A	6.80 ±0.05		
	Group B	3.81 ±0.07	0.459	13.636
	Group C	6.38 ±0.27	0.001*	
	Group D	6.58 ±0.69	0.701	

Data were analyzed using One-way ANOVA, followed by LSD comparison, and data were considered significant at P < 0.05 and P > 0.05 means not significant, it is also significant at the level of 0.01 and less.

Sperm pH in groups B, C, and D slightly increased when compared to the control group A (Table 4). This is in concordance with the work of Ekaluo *et al.*[14] on the effects of aqueous extract of *Cyperus esculentus* on male albino rats at 1.8 g/kg per body weight which revealed a concomitant improvement in semen pH. This is due to higher sperm production as a result of an increase in testosterone stimulation of the spermatogonia cells to undergo successful spermatogenesis, sperm maturation in the epididymis and the secretory activity of the accessory sex glands as a result of the acidic pH environment provided by Kunu.

Table 4 | The effect of Kunu on sperm pH

Sperm parameters	Group	Mean SEM	P-value	F-value
Sperm pH	Group A	6.16 ±0.16		
	Group B	6.33 ±0.16	0.650	1.296
	Group C	6.50 ±2.88	0.650	
	Group D	6.83 ±0.33	0.096	

Data were analyzed using One-way ANOVA, followed by LSD comparison and data were considered significant at P < 0.05 and P > 0.05 means not significant, it is also significant at the level of 0.01 and less.

The tabular results also evidence a significant (P < 0.05) decrease in testosterone levels in the test groups when compared with the control group (Table 5). This sharply contrasts with the report of Ayodele *et al.*[15] on their work on ginger and cinnamon on male albino rats at 10 mg/kg body weight.

Table 5 | The effect of Kunu on FSH and testosterone level

Hormone	Groups	Mean ±SEM	P-value	F-value
Follicular Stimulating Hormone (uIu/L)	Group A	2.80 ±0.10		
	Group B	2.73 ±0.08	0.771	0.545
	Group C	2.70 ±0.05	1.000	
	Group D	2.60 ±0.05	0.392	
Testosterone (ng/mL)	Group A	4.80 ±0.05		
	Group B	4.03 ±0.12	0.001*	16.700
	Group C	4.10 ±0.15	0.002*	
	Group D	3.80 ±0.05	0.000*	

Data were analyzed using One-way ANOVA followed by LSD comparison and data were considered significant at P < 0.05 and P > 0.05 means not significant, it is also significant at the level of 0.01 and less.

An insignificant decrease (P > 0.05) in normal sperm in group B and C and an insignificant increase (P > 0.05) in group D was recorded (Table 6) as compared with group A and this counters Khaki *et al.*[16] who worked on the Anti-oxidant effect of Ginger and Cinnamon on Spermatogenesis Dys-function of Diabetes Rats. There was an insignificant (P > 0.05) decrease in abnormal sperm in group B and D and an insignificant increase (P > 0.05) in group C when compared to group A. This is in agreement with the work of Ekaluo *et al.*[14] that states that there was no significant (P > 0.05) effect of aqueous extract of *Cyperus esculentus* on sperm head abnormality but slight increases in a dose-dependent manner.

Table 6 | The effect of Kunu on normal sperm and abnormal sperm

Hormone	Groups	Mean ±SEM	P-value	F-value
Normal Sperm (%)	Group A	86.67 ±3.33		
	Group B	86.66 ±1.67	1.000	0.667
	Group C	86.00 ±1.67	1.000	
	Group D	90.00 ±0.00	0.282	
Abnormal Sperm (%)	Group A	13.37 ± 3.33		
	Group B	13.33 ±1.67	1.000	0.667
	Group C	14.00 ±1.67	1.000	
	Group D	10.00 ±0.00	0.282	

Data were analyzed using One-way ANOVA followed by LSD comparison, and data were considered significant at P < 0.05 and P > 0.05 means not significant, it is also significant at the level of 0.01 and less.

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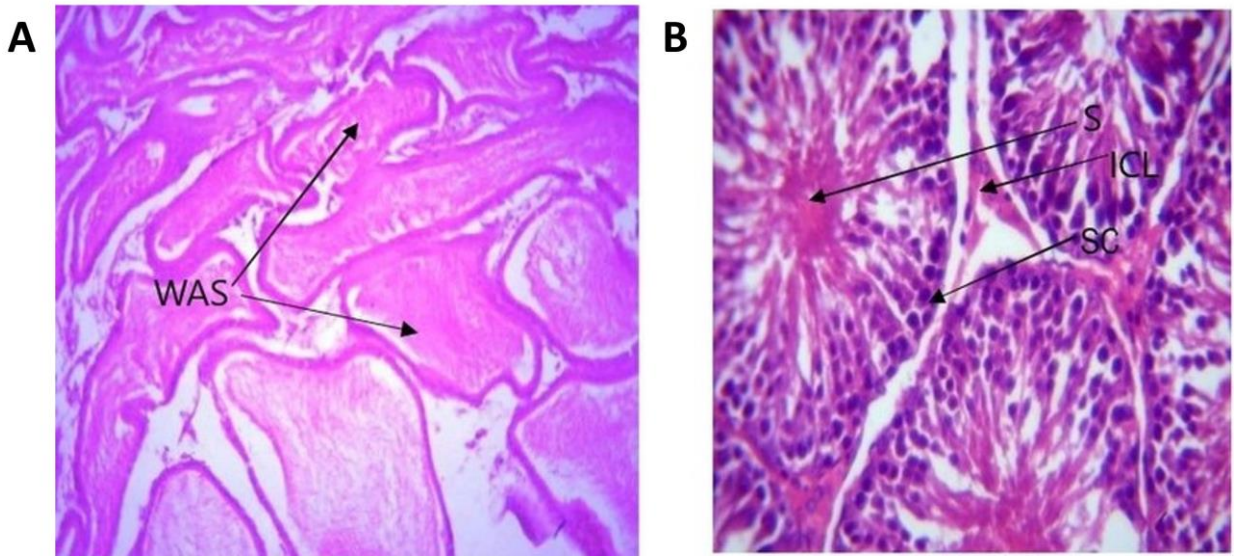

Figure 2 | Photomicrograph sections of normal control of **A**) epididymis and **B**) testes. WAS: well accumulated spermatozoa, S: spermatogenesis, ICL: interstitial cells of Leydig, SC: Sertoli cells

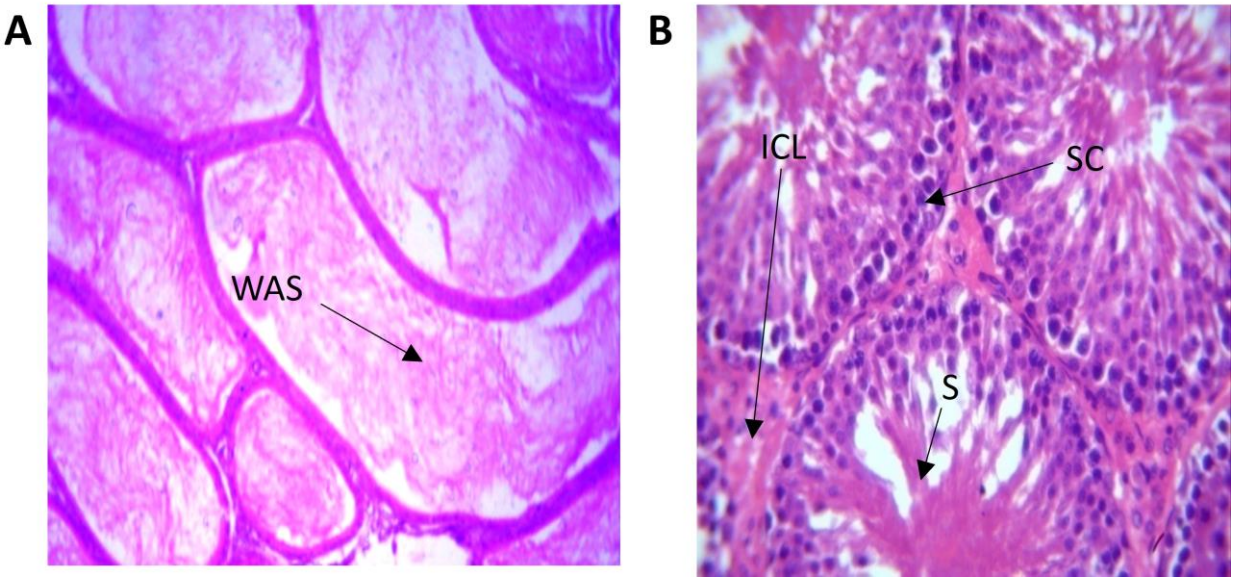

Figure 3 | Photomicrograph section of **A**) epididymis and **B**) testes administered with high dose 2.5ml of local millet drink Kunu (x100) (H/E) showing enhancement of all histoarchitectural structures. WAS: well accumulated spermatozoa, S: spermatogenesis, ICL: interstitial cells of Leydig, SC: Sertoli cells

Histopathological results of photomicrographs (Figures 2 and 3) showed moderate epididymal accumulation of spermatozoa and testicular tissue with slightly enhanced seminiferous tubules and mildly improved spermatogenesis. This opposes the work of Arash *et al*.[16] who reported that 100 mg/kg ginger and cinnamon fed rats showed increased spermatogenesis and testicular architecture. Dissimilar results were also found by the administration of *Cyperus esculentus* (Kunu aya) by Ekaluo *et al.*[14] in male albino rats. Also, 2.5 ml of Kunu shows well enhanced epididymal architecture as well as accumulated luminal spermatozoa with a corresponding enhanced testicular tissue and well improved spermatogenesis. This hardly corresponds with the study carried out by Ayodele *et al*.[15] on dietary supplementation of ginger and turmeric improves reproductive function in hypertensive male rats and that carried out by Ekaluo *et al*.,[14] the effect of aqueous extract of *Cyperus esculentus* who reported improved spermatogenesis and testicular tissue enhancement in the 180 mg/kg administration of ginger, a major condiment of Kunu.

Conclusion

In conclusion, this scientific study shows that local millet drink, Kunu (Kunu-Zaki) even though a product of ginger (which has antioxidant and androgenic properties with the capacity of increasing sperm parameters) does little to improve sperm count, motility, morphology, pH, and hormonal levels of FSH and testosterone in Wistar rat. Kunu instead attempts to maintain or slightly reduce normal levels of these parameters and the testicular and epididymal architectures. These negative results in the Wistar rat animal model indicate that Kunu is unlikely to act as a natural fertility booster in males.

Additional Information

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Competing interests

No conflict of interest.

Ethics statement

Ethical approval with the ethical number; NAU/FBMS/ETH-123 was obtained from the ethical committee, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University.

Location of Study

This study was carried out at the Animal House of the Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State. Ethical approval was also obtained from the ethical committee.

Author contributions

Damian Nnabuihe Ezejindu supervised the findings of this work and contributed to the development of the novel theory. Princewill Sopuluchukwu Udodi prepared the manuscript for publication, analyzed the anthropometric data collected and interpreted the histological slides. Enemuo Chidili Ijeoma sourced for the literatures. Okafor Emeka Christian and Okeke Somadina Nnamdi took part in breeding the animals used for the study, Agulanna Ambrose Echefulachi, Ifeanacho Ezetaonu Abireh, Idorenyin Umoh and Kingsley Akaninyene Okon interpreted the micrographs while Okara Andy-Davis Chidi assisted in tissue processing.

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