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

[1]AKUKWU Darlington Cyprain, [2]ULONEME Godwin Chinedu, [3]EZEJINDU Damian Nnabuihe, [4]UDODI Princewill Sopuluchukwu, [5]EZEJINDU Ifesinachi Ogochukwu, [6]NWAJAGU Chukwudi Jesse, [7]OBINWA Benedict Nzube, [8]OBIESIE Ifechukwu Justicia, [9]OKAFOR Emeka Christian, [10]OKEKE Somadina Nnamdi, [11]OGBUOKIRI Doris Kasarachi, [12]AGULANNA Ambrose Echefulachi, [13]Chisom Esther OGUEJIOFOR, [14]Chizubelu Irene OMILE, [15]Joshua Izuchukwu ABUGU

[1-2]Department of Human Anatomy, Imo State University. Owerri, Imo State.

[3-12][15]Department of Anatomy, Nnamdi Azikiwe University. Anambra State, Nigeria.

Correspondence email address: udodip@gmail.com
Correspondence phone number: +2347032865098
Correspondence Orcid ID: 0000-0002-2156-5268

ABSTRACT

Background:
Kunu is a local beverage drink that finds its origin in the northern part of Nigeria. ~~This~~The study was aimed at determining the effect of the liquid drink on the ~~epididymes~~epididymis, testes, sperm parameters, and hormonal ~~assay~~assays.

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~~Then the animals were ~~then~~ sacrificed and the testes and ~~epididymes~~ epididymis were harvested and fixed in 10 % formal saline. Group A ~~were~~was fed only rat feed and water. Groups B, C, and D were orally fed 0.~~2ml~~2 ml, 0.~~9ml~~9 ml and 2.~~5ml~~5 ml of Kunu ~~respectively orally~~ using a metal cannula for ~~a period of~~ 21 days, respectively.

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 follicular stimulating hormone (FSH) in groups B, C, and D when compared to group A. The histopathological findings revealed that ~~the~~ group B rats of 0.~~2ml~~2 ml and group C rats of 0.~~9ml~~9 ml showed epididymal tissue with ~~moderate~~moderately accumulation of spermatozoa and testicular tubules with moderate enhanced spermatogenesis. ~~The group~~Group D rats showed well accumulated spermatozoa in the epididymal lumen and improved spermatogenesis in the testis as did group A.

Conclusion:
Kunu ~~beverage~~(local beverages) 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.

KEY WORDS: Kunu, Epididymis, Testes, Sperm count, Sperm motility

INTRODUCTION

In a sexual world ~~as~~like ours, there is ~~the~~an urgency for people across the globe to meet their sexual needs ~~on a~~ daily ~~basis~~. 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~~is: the use of synthetic steroids and natural boosters (1).

The use of synthetic steroids such as synthetic testosterone and gonadotropins has ~~its own~~ adverse effects such as reduced testes size, micturition problems, ~~gynaecomastia~~gynecomastia, sleep disturbances, *etc* (1); which is why a better approach to the problem of male factor infertility (due to ~~azospermia~~azoospermia, oligospermia or any other related spermatic problem) is the use of natural boosters of which the local northern drink kunu is a prominent example.

Kunu is a popular drink consumed throughout Nigeria but mostly in the North. It can be made from grains such as millet, sorghum, maize, and rice. The variety of ~~drink~~drinks made from sorghum is a milky light-brown ~~colour~~color while that of maize or millet is whitish ~~in color~~. 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~~Zaki (2).

Testes, also called testicle in animals is the organ that produces sperm, ~~the male reproductive cell and androgens, the male reproductive hormone.~~and androgen. In humans, the testes occur as a pair of oval ~~-~~shaped organs. ~~They are contained within the scrotal sac which is located directly behind the penis and in front of the anus.~~ 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. ~~The both~~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 can be increased by over 500% as compared to the pre-pubertal size (5).

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

METHODS

Ethical Clearance: 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.

Materials: The following materials were used in this experiment: Sixteen male ~~wistar~~Wistar Rats, oral cannula, kunu (local beverage~~, Four~~), four standard cages, ~~Distilled~~distilled water, ~~Cotton~~cotton wool and hand gloves, ~~Beakers~~beakers and measuring cylinder, ~~Animal~~animal weighing balance (CAMRY LB11), ~~Electronic~~electronic weighing balance (NAPCO Precision Instruments JA-410), ~~Diethyl~~diethyl Ether, ~~Vital~~vital top feed (Jos, Nigeria), ~~Dissecting Kits~~dissecting kit, EDTA container and ~~Plain~~plain container, ~~Micro~~micro-hematocrit centrifuge

SH120, ~~Capillary Tube,5ml~~capillary tube, 5 ml hypodermic syringe, Deep and flat feeding plates, Plastic bottles, 10% buffered formalin, ~~Haemocytometer, Filter~~hemocytometer, filter paper (Whatman qualitative filter paper n. 1, sigma ~~aldrich~~Aldrich WHA1001042), ~~Thermostat~~thermostat oven (DHG-9023A, PEC MEDICAL USA), and ~~Spectrophotometer~~spectrophotometer (Model 721).

Preparation of Kunu ~~Beverage:~~(local beverage): 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; the 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: ~~16~~Sixteen male ~~wistar~~Wistar rats weighing between 170-~~200g~~200 g were used for this study. The animals were allowed to acclimatize for ~~a period of~~ 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 ~~100mg~~100 mg/kg or 0.~~2ml~~2 ml of ~~the Kunu~~kunu (local beverage)
Group C received ~~400mg~~400 mg/kg or 0.~~9ml~~9 ml of ~~the Kunu~~kunu (local beverage)
Group D received ~~1200mg~~1200 mg/kg or 2.~~5ml~~5 ml of ~~the Kunu~~kunu (local beverage~~.~~).
The administration lasted for ~~a period of~~ 21 days, taking place between ~~7am~~7 to 10 am daily. The animals were then sacrificed after the aforementioned period, semen and blood collected for seminal analysis and ~~Hormonal Assay~~hormonal assay test, while the testes and ~~epididymes~~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 (6). No toxic effect was observed on the treatment of Kunu drink up to the effective dose of ~~5000mg~~5000 mg/kg body weight of adult ~~wistar~~Wistar rats. The behavior of the treated rats appeared normal, and no ~~death~~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 (7) ~~by~~ 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 (8). Sperm viability was examined based on the method reported by Bearden and Fuquay (9). 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 ~~the~~ 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.* (10) based on the slides used for the calculation of sperm viability.

PROCEDURE FOR HORMONAL ASSAY

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

Determination of Follicle Stimulating Hormone by Radioimmunoassay Technique: Serum levels of Follicle~~-~~stimulating hormone (FSH) ~~was~~were assayed

by RIA using reagents supplied by Rat Pituitary Distribution and NIDDK (~~Bathesda~~Bethesda, MD, USA)

Statistical Analysis: The statistical analysis of this research was done using ANOVA and student's t-test of SPSS version 23 software package and P~~<~~ < 0.5~~0~~5 was considered as the level of ~~signify~~signifying.

RESULT

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		
	Final	176.66 ±14.52	0.588	-0.640
Group B	Initial	186.67 ±8.81		
	Final	213.33 ±12.01	0.208	-1.835
Group C	Initial	173.33 ±8.81		
	Final	203.33 ±8.81	0.225	-1.732
Group D	Initial	183.33 ±6.66		
	Final	193.33 ±6.66	0.221	-1.075

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

Table 1: 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 ~~was~~were analyzed using One-way ~~Anova~~ANOVA, followed by ~~multiple~~LSD comparison ~~using LSD~~, and data were considered significant at P~~<~~ < 0.05. ~~*P<0.05 means significant*~~ and P > 0.05 means not significant~~.~~, it is also significant at the level of 0.01 and less

Table 3: ~~Shows the~~The effect of Kunu on ~~Sperm~~sperm motility and ~~Total~~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 ~~was~~were analyzed using One-way ~~Anova~~ANOVA, followed by ~~multiple comparism using~~ LSD comparison, and data were considered significant at P~~<~~ < 0.05~~. *P<0.05 means significant*~~ and P > 0.05 means not significant~~.~~, it is also significant at the level of 0.01 and less

Table 4: ~~Shows the~~The effect of Kunu on ~~Sperm~~sperm pH

Sperm parameters	groups	Mean ~~±sem~~ 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 ~~was~~were analyzed using One-way ~~Anova~~ANOVA, followed by ~~multiple comparism using~~ LSD comparison, and data were considered significant at P~~<~~ < 0.05~~. *P<0.05 means significant*~~ and P > 0.05 means not significant~~.~~, it is also significant at the level of 0.01 and less

Table 5: ~~Shows the Effect~~The effect of Kunu on FSH and ~~Testosterone Level~~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*	

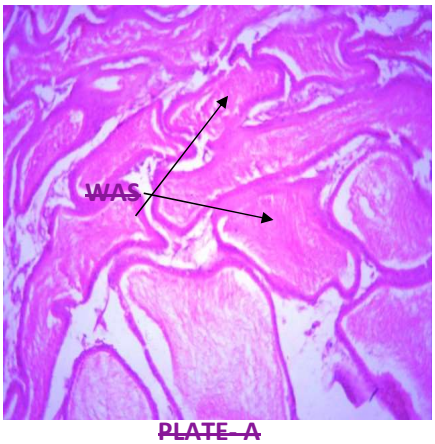
Data ~~was~~were analyzed using One-way ~~Anova~~ANOVA, followed by ~~multiple comparism using~~ LSD comparison, and data were considered significant at P~~<~~ <

0.05~~. *P<0.05 means significant*~~ and P > 0.05 means not significant~~.~~, it is also significant at the level of 0.01 and less.

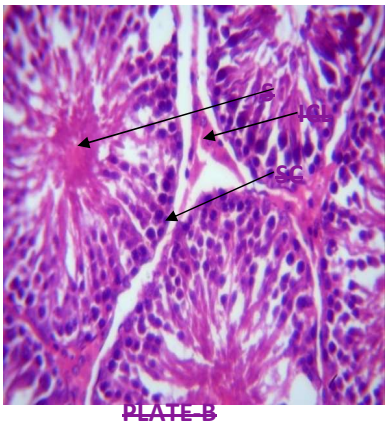
Table 6: Shows the effect of Kunu on ~~Normal Sperm~~normal sperm and ~~Abnormal~~abnormal sperm

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	

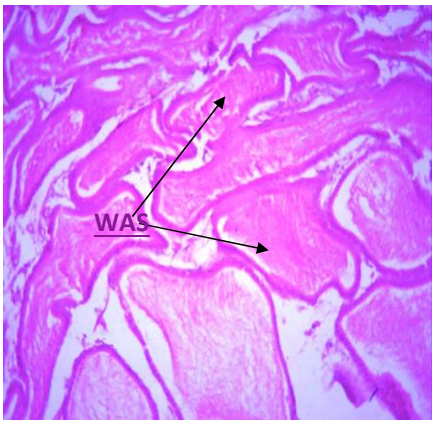
Data ~~was~~were analyzed using One-way ~~Anova~~ANOVA, followed by ~~multiple comparism using~~ LSD comparison, and data were considered significant at P~~<~~ < 0.05~~. *P<0.05 means significant*~~ and P > 0.05 means not significant~~.~~, it is also significant at the level of 0.01 and less

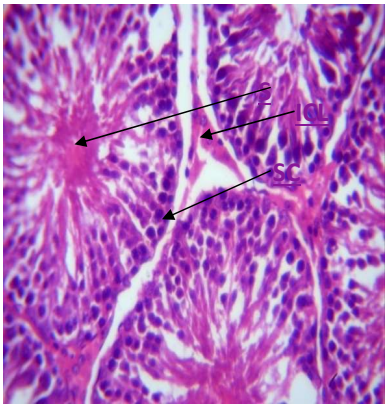


~~PLATE A~~

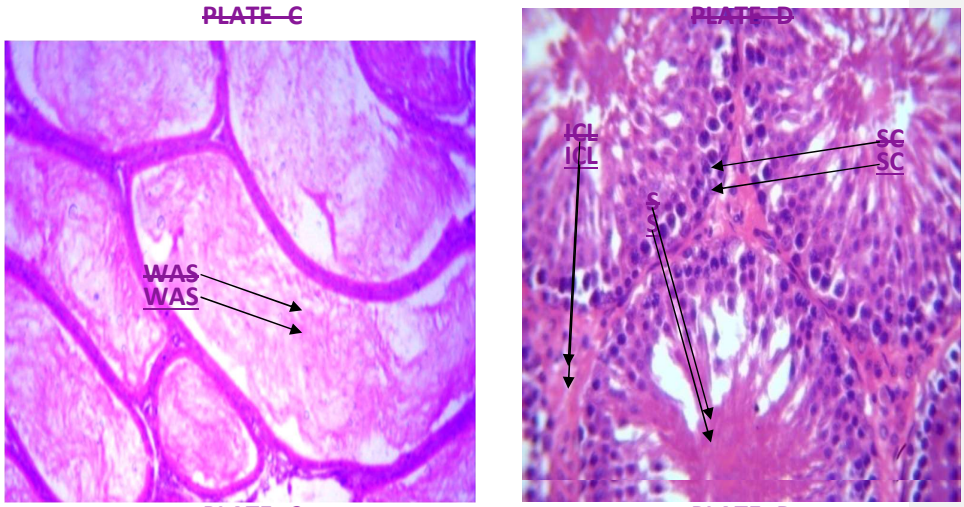


~~PLATE B~~





Photomicrograph section of normal control of epididymis (PLATE- - A) and testes (PLATE- - B). WAS: well accumulated spermatozoa, S: spermatogenesis, ICL: interstitial cells of Leydig, SC: Sertoli cells



Photomicrograph section of epididymis (PLATE- - C) and testes (PLATE- - D) 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

DISCUSSION

Men take fertility drugs to increase their sperm count and motility. The maleMale hormones should be adequate to produce healthy sperms. The anterior pituitary is responsible for controlling the male hormones, hence, sperm production. Around 2% of men with infertility experience secondary hypogonadism (pituitary gland disease). ItThis happens when the gland fails to function properly preventing sperm and testosterone production. Men havingwith this condition will either have no or low sperm count (11). This condition is treatable by either pharmaceutical or natural means. There are very few drugs, approved by the USU.S. Food and Drug Administration (FDA) which), may help in stimulating sperm production such as Clomiphene, Letrozole, and Synthetic testosterone pills, Bromocriptine, Imipramine, *etc*. (12) yet often times they come with various side effects such as breast enlargement, changes in libido, liver problems, high blood pressure, etc. (13). Hence, there is a growing call, despite the cheapness and commonness of these drugs, to use natural remedies such as the beverage (Kunu) this study chose to investigate to lessen the ample evidencesevidence indicating a steady decline in human sperm count and quality (14) without the backlog of any adverse effects.

The results of this study showsshowed that there was no significant change in weight of the experimental rat groups B, C, and D just as that of the control. This could be attributed to the low fat and protein content of the beverage. This study differs withfrom the report made by Abolfazl *et al.* (15) who reported that *Zingiber officinale* Roscoe (ginger), a condiment of Kunu, increasesincreased the body weight significantly in wistarWistar rats at 1g1 g/kg of body weight.

There was a significant increase ($P \leq 0.05$) in the relative testicular weight in the other groups when compared with the control group. This is in consonanceagrees with the discovery of (16) 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. ~~Result~~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.* (16) who reported increasing weight of ~~epididymes~~epididymis of the rats given an aqueous extract of *Cyperus esculentus* 1.8~~g~~8 g/kg body weight.

There was a significant decrease (P~~<~~ < 0.05) in sperm motility in the experimental groups when compared with the control. This does not agree with the findings of Abolfazl *et al.* (15) who states increased levels of sperm viability and motility of the ~~wistar~~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 ~~group~~groups C, and D when ~~compare~~compared to the control. This contradicts the findings of Hafez (7) who reported a significant increase in sperm quality and quantity of ~~wistar~~Wistar rats to feed with ~~2g~~2 g/kg body weight ginger roots and cinnamon bark.

Sperm pH in ~~group~~groups B, C, and D slightly increased when compared to the control group A. This is in concordance with the work of (16) on the effects of ~~acqueous~~aqueous extract of *Cyperus esculentus* on male albino rats at 1.8~~g~~8 g/kg per ~~bw.t~~body weight which ~~revealsr~~evealed a concomitant improvement in semen pH. This is due to higher sperm production as a result of an increase in testosterone stimulation of the spermatogenic cells to undergo successful spermatogenesis, sperm maturation in the ~~epididymes~~epididymis and the secretory activity of the accessory sex glands as a result of the acidic pH environment provided by Kunu.

The tabular results also evidence a significant (P<0.05) decrease in testosterone levels in the test groups when compared with the control group. This sharply contrasts with the report of Ayodele *et al.* (17) on their work on ginger and ~~Cinnamon~~cinnamon on male albino rats at 10mg/kg body weight.

~~Insignificant~~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 as compared with group A and this counters (18) 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 (16) 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.

Histopathological results of photomicrographs ~~shows~~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.* (18) who reported that ~~100mg~~100 mg/kg ~~Ginger~~ginger and ~~Cinnamon~~cinnamon fed rats showed increased spermatogenesis and testicular architecture. Dissimilar results were also found by the administration of *Cyperus esculentus* (Kunu aya) by (16) in male albino rats. Also, 2.5ml 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 by Ayodele *et al.* (17) on ~~Dietary Supplementation~~dietary supplementation of ~~Ginger~~ginger and ~~Turmeric~~turmeric improves reproductive function in hypertensive male rats and that carried out by Ekaluo *et al.* (16), the effect of ~~acqueous~~aqueous extract of *Cyperus esculentus L.* who reported improved spermatogenesis and testicular tissue enhancement ~~at 180mg~~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. Kunu, instead attempts to maintain or slightly reduce normal levels of these parameters and the testicular and epididymal architectures as such may not be used as a possible natural fertility booster in males.

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Competing interests

No conflict of interest.

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THE AUTHORS CONTRIBUTION

AKUKWU Darlington Cyprain: He contributed to the design and developed the novel theory of the research findings.

ULONEME Godwin Chinedu: He conceived, designed the research work and wrote the paper with input from all the authors.

EZEJINDU Damian Nnabuihe: He supervised the findings of this work and contributed to the development of the novel theory.

UDODI Princewill Sopuluchukwu: He prepared the manuscript for publication, analyzed the anthropometric data collected and interpreted the histological slides.

EZEJINDU Ifesinachi Ogochukwu: She obtained the anthropometric data which include; the animal weight and the organ weight.

NWAJAGU Chukwudi Jesse: He acquired the animals and kept them under his care for the period of acclimatization and also administered the test substance in the entire test group except the control group.

OBINWA Benedict Nzube: He sacrificed the animals, identified the organ of study and harvested the organ in all the animals.

OBIESIE Ifechukwu Justicia: She processed the tissue in preparation for the histological study.

OKAFOR Emeka Christian: He ontributed to the theoretical formalism and aid in the analytic calculations.

OKEKE Somadina Nnamdi: He contributed to the tissue processing and the labeling of the histological slide.

OGBUOKIRI Doris Kasarachi: She was part of the team that designed the model and the computational framework.

AGULANNA Ambrose Echefulachi: He worked out almost all of the technical details of the research work.

OGUEJIOFOR Chisom Esther: She was majorly saddled with the responsibility of handling the animals and ensure proper acclimatization of the animals.

OMILE Chizubelu Irene: She was the scientist that prepared the local beverage and ensure daily availability of the beverage, she was also part of the animal handling

ABUGU Joshua Izuchukwu: He was primarily saddled with the responsibility of ensuring daily administration of the local beverages. He was also part of the animal handling team.

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