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 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 reduced testes size, micturition problems, gynecomast-
tia, sleep disturbances, etc.,\[3\] 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.\[2\]

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.

**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; 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**

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 (LD₅₀) 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. 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 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. Sperm viability was examined based on the method reported by Bearden and Fuquay. 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. 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 comparison 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. 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 help in stimulating sperm production such as clomiphene, letrozole, synthetic testosterone pills, bromocriptine, imipramine, etc., yet often these come with various side effects such as breast enlargement, changes in libido, liver problems, high blood pressure, etc. 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. 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

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

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
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. 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>
<thead>
<tr>
<th>Sperm parameters</th>
<th>Group</th>
<th>Mean ±SEM</th>
<th>P-value</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm pH</td>
<td>A</td>
<td>6.16 ±0.16</td>
<td>0.650</td>
<td>1.296</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>6.33 ±0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>6.50 ±2.88</td>
<td>0.650</td>
<td>0.096</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>6.83 ±3.33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4 | The effect of Kunu on sperm pH

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. on their work on 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. 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>
<thead>
<tr>
<th>Hormone</th>
<th>Groups</th>
<th>Mean ±SEM</th>
<th>P-value</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (uIu/L)</td>
<td>A</td>
<td>2.80 ±0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2.73 ±0.08</td>
<td>0.771</td>
<td>0.545</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2.70 ±0.05</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>2.60 ±0.05</td>
<td>0.392</td>
<td></td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>A</td>
<td>4.80 ±0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>4.03 ±0.12</td>
<td>0.001*</td>
<td>16.700</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>4.10 ±0.15</td>
<td>0.002*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>3.80 ±0.05</td>
<td>0.000*</td>
<td></td>
</tr>
</tbody>
</table>

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

Hormone

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Groups</th>
<th>Mean ±SEM</th>
<th>P-value</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Sperm (%)</td>
<td></td>
<td>86.67 ±3.33</td>
<td>1.000</td>
<td>0.667</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>86.66 ±1.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>86.00 ±1.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>90.00 ±0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal Sperm (%)</td>
<td>A</td>
<td>13.37 ±3.33</td>
<td>1.000</td>
<td>0.667</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>13.33 ±1.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>14.00 ±1.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>10.00 ±0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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.
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.\textsuperscript{[15]} on dietary supplementation of ginger and turmeric improves reproductive function in hypertensive male rats and that carried out by Ekaluo et al.\textsuperscript{[14]} the effect of aqueous extract of \textit{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**

**Acknowledgements**

I sincerely acknowledge the Department of Anatomy, Faculty of Basic Medical sciences, Nnamdi Azikiwe University for their support in the course of this research work.

**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

Darlington Cyprain Akukwu contributed to the design and developed the novel theory of the research findings. Godwin Chinedu Uloneme conceived, designed the research work and wrote the paper with input from all the authors. Damian Nnabuihe Ezejindu supervised the findings of this work and contributed to the development of the novel theory. Princewill Sopulu-chukwu Udodi prepared the manuscript for publication, analyzed the anthropometric data collected and interpreted the histological slides. Ifesinachi Ogochukwu Ezejindu obtained the anthropometric data which include; the animal weight and the organ weight. Chukwudi Jesse Nwajagu 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. Benedict Nzube Obiwa sacrificed the animals, identified the organ of study and harvested the organ in all the animals. Ifechukwu Justicia Obiesie processed the tissue in preparation for the histological study. Emeka Christian Okafor contributed to the theoretical formalism and aid in the analytic calculations. Somadina Nnamdi Okeke contributed to the tissue processing and the labeling of the histological slide. Doris Kasarachi Ogbuokiri was part of the team that designed the model and the computational framework. Ambrose Echefulachi Agulanna worked out almost all of the technical details of the research work. Chisom Esther Oguejiofor was majorly saddled with the responsibility of handling the animals and ensure proper acclimatization of the animals. Chizubelu Irene Omile was the scientist that prepared the local beverage and ensure daily availability of the beverage, she was also part of the animal handling. Joshua Izuchukwu Abugu was primarily saddled with the responsibility of ensuring daily administration of the local beverages and also part of the animal handling team.

References

15. Akinyemi, AJ; Adedara, IA; Thome, GR; Morsch, VM; Rovani, MT; Mujica, LK5; Duarte, T; Duarte, M et al. (2015). "Dietary supplementation of ginger and turmeric improves reproductive function in hypertensive male rats". Toxicology Reports 2: 1357–1366. doi:10.1016/j.toxrep.2015.10.001.