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The effect of intake of palm wine on body weight and testicular microarchitecture in adult wistar rats

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Abstract:

BACKGROUND: The growing concerns about male reproductive dysfunction as well as obesity have necessitated the present study.

OBJECTIVE: The aim of this research was to investigate the effect of intake of palm wine on body weight, testis weight and microarchitecture of the testis in adult Wistar rats.

MATERIALS AND METHODS: Ten mature male rats weighing between 101 g and 180 g were assigned into two groups of A and B, consisting of five animals each that all had access to rat chow and water ad libitum. Fresh undiluted palm wine gotten as sap (juice) from oil palm trees was collected from a local palm wine tapper at every three days interval and often conserved in a refrigerator after every use before being replaced with a fresh collection on the 4th day. While group A served as control, group B animals were orally administered with 10ml/kg body weight per day of the palm wine for twenty-one days. The G&G(R) Electronic Scale (JJ1000 Capacity) was the instrument used for the assessment of the weight parameters. The analysis of variance was the statistical tool used to determine the weight differences between the two groups and $P < 0.05$ was taken as the statistically significant value; with the data presented as Mean ± SEM.

RESULTS: The outcome of the research on weight parameters showed non significant changes as the means for the initial and final body weights in group A were 108 g and 136 g ± 0.28 while those in group B were 138 g and 165 g ± 0.29 respectively. The testis mean weight of group A animals was 3.3 g ± 0.05 while that of group B was 3.8 g ± 0.07. Following routine histological processing, the micro-anatomic studies of testicular tissue samples from group B revealed focal disordered arrangement of sperm–producing cells.

CONCLUSION: The findings therefore show that regular intake of fermented palm sap popularly known as palm wine may not significantly affect body weight nor testis weight, but can gradually distort testicular tissue architecture with abnormally structured cells.

Keywords:

Intake, microarchitecture, palm wine, testis, weight

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Introduction

Palm wine is a beverage drink and one of the brands of native wines drunk in Nigeria and many other parts of the world. It is gotten (tapped) as sap from the *Raphia* palm trees *Raphia hookeri* or *Raphia vinifera* or oil palm trees *Elaeis guineensis* and is the major brand of wine in some parts of the world like the southern part of Nigeria. Palm wine is commonly known as "Mmanya" in Igbo, "Emu" in Yoruba, "Bammi" in Hausa, "Mmin-efik, and Mmin-ukot," respectively in Efik and Ibibio-speaking tribes in Nigeria. It is popularly known by a variety of names in different countries of the world among which are "Matango," "Fitchuk," or "Mbu" in Cameroon; "Nsafufuo" or "Doka" in Ghana; "Tuba" in Mexico; "Toddy" in India; "Lambong" in the Philistines; and "Panamcullo" in China.

Palm wine is usually consumed in homes and at social events, thus occupying a distinct place in the society. It is often added to

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various herbal medicines or products and is said to be a nourishing drink which promotes lactation and improves eyesight due to its yeast content Agriculture and Consumer protection (2014). The yeast contained in palm wine has been reportedly used for industrial purposes such as baking and production of alcoholic drinks or portable ethanol (Chandrasekhar *et al*., 2012; Nwachukwu *et al*., 2008; Chilaka *et al*., 2010; Mbuagbaw and Noorduyn, 2012). Biological conditions that predispose to deteriorations in testicular structure and function have shown some increase in the recent past, thereby generating widespread concerns among the human population (Creasy, 1997). Many of these biological disorders have been reported in animals exposed to endocrine disrupting chemicals (Reddy and Bhavanarayana, 2013).

Endocrine-disrupting chemicals which are commonly referred to as drugs of which wine, in general, is a class, often act by altering the natural state of the body and may therefore play a strategic role in an organism's cell profile and life span. Palm wine is said to contain varying amounts of alcohol ranging from 2%–8% by volume depending on its state of fermentation (Ogbulie *et al*., 2007; Nakneam *et al*., 2010; and Oladeinde *et al*., 2002). Palm wine has also been reported to have some nutritional components and mineral elements such as carbohydrate, crude protein, lipid, crude fiber, ash, calories, moisture, Vitamins A and C, iron (Fe^{2+}), copper (Cu^{2+}), magnesium (Mg^{2+}), calcium (Ca^{2+}), and zinc (Zn^{2+}) (Ogbonna *et al*., 2013; Okafor, 1972; Agriculture and Consumer Protection, 2014).

While the biological impacts of alcohol-containing products are still being debated, some good attempts have been made to ascertain the changes that could result when organic cells of the liver, kidney brain, and reproductive structures are exposed to alcoholic products (Capsi *et al*., 1996; Clark *et al*., 2001; and Onu *et al*., 2014). Palm wine has been indicated as an alcoholic drink that is widely consumed mostly by males, but little is known about its effect on body weight as well as male reproductive (testicular) profile. The growing cases of reproductive dysfunction and obesity across the globe justify; therefore, this study which used animal models for its investigations.

Palm wine has been reported to cause a decline in testicular function by decreasing testosterone levels, sperm motility, and sperm viability, but without a significant change in the morphology (Oyedeji *et al*., 2012. Similarly, teratogenic studies of the fetal kidney following administration of beer and palm wine from the 7^{th} to 13^{th} day of gestation have indicated these products as nephrotoxic (Eluwa *et al*., 2008). With regard to the effect of alcoholic products on weight, the outcome of cross-sectional studies as well as prospective, cohort and well-powered studies with long period of follow-ups has been contradictory. Findings from short-term experimental trials also did not show any clear trend thereby ruling out any possible association between alcohol intake and weight gain (Sayon-Orea *et al*., 2011).

Materials and Methods

The study was conducted in the Department of Anatomy, College of Medicine and Health Sciences of Abia State University, Uturu, Nigeria, in compliance with all ethical guidelines regarding the use of experimental animals.

Animals

Ten adult Wistar rats weighing between 103 and 180 g were allocated into two Groups of A and B after 3 weeks leave of acclimatization. They were fed with rat chow and received water *ad libitum*. Group A served as the control while Group B animals were subjected to an oral treatment (through orogastric tube) of 10 ml/kg body weight per day of palm wine for 21 days. The dosage was calculated as 10 (ml)/1000 (g) × the body weight of the animal.

Palm wine

Fresh undiluted palm wine from the sap of oil palm trees was collected at every 3 days interval from a local palm wine tapper in Uturu, Abia State, Nigeria. The palm wine on collection was administered on the animals and then conserved in a refrigerator for 3 days following which it was often replaced with a fresh collection.

Tools

The apparatus used for the assessment of the weight parameters was an electronic weighing scale, JJ1000 capacity manufactured in Neuss, Germany by G&G® Company. The materials used for the routine histological processing of the tissue samples included a kit of dissecting tools, 10% buffered formalin, alcohol, xylene, tap water, staining dyes (eosin and hematoxylin), paraffin wax, knife, rotary microtome, embedding mold, wooden blocks, tissue slides, film mounted, coverslips, and a Light microscope with digital camera.

Procedure

The initial and final body weights of the animals were recorded using the weighing balance earlier described. They were then anesthetized by putting them one after another in a container laden with cotton material that has been soaked in chloroform. The animal was brought out after 1 min, and a mid-vertical incision was made along the ventral abdominal wall. The testes were carefully dissected out and weighed on the balance following which they were fixed in 10% buffered formalin.

Histological processing

The tissue samples were passed through routine histological processes, and sections from these tissues

were finally cut and mounted on slides that have been coated with a synthetic resin known as distyrene plasticizer xylene; and encased by cover slips to keep the specimens pressed flat and in place while also protecting the specimen from dust and accidental contact. The tissue slides were then examined under the light microscope and photomicrographs of different magnifications were taken with the aid of a digital camera.

Data analysis

The hypothesis for the weight parameters was tested using the analysis of variance while the resulting data were expressed as mean ± standard error of the mean. P <0.05 was considered statistically significant.

Results

The result [Table 1] shows that $P > 0.05$, which is in support of the null hypothesis (H_0) implying that there is no significant difference between the testis weight of rats treated with palm wine for 21 days and that of the control.

The analysis [Table 2] shows that $P > 0.05$ in support of the null hypothesis (H_0) with the implication of a nonsignificant difference between the initial body weight and the final body weight of rats following treatment with palm wine for 21 days.

Discussion

Palm wine is a beverage drink that plays a strategic role in our society as it is often consumed in homes and at social events. It is also used for industrial purposes following the extraction of its contents such as yeast for baking, and alcohol for the production of portable ethanol. The research method adopted in this study was to practically test the hypothesis on the most common way by which many people used the product. This common method of use often involves its preservation in a cool environment for several days after its initial collection.

The biometric studies that followed the regular treatment of rats with a standard dose of palm wine showed there was a nonsignificant difference in the body weight and the testis weight when compared with the control group that received only water and chow. The analysis of variance for testis weight [Table 1] indicated a probability value of 0.357 (> 0.05), which implies that there is no significant difference between the control group and the experimental group. Likewise, the analysis of variance for body weight [Table 2] also produced a non significant value of 0.058 (> 0.05). The mean values for testis weight in group A and B were 3.3 g +/- 0.05 and 3.8 g +/- 0.07 respectively [Figure 1]; while the family wise confidence interval showed a non significant difference in the mean levels of rats' testes weight [Figure 2]. The values in mean for the initial and final body weights of group A animals were 108 g and 136 g +/- 0.28; while those of group B were 138 g and 165 g +/- 0.29 respectively [Figures 3 and 4]. The family wise confidence level for the body weight between the two groups also showed a non significant difference [Figure 5].

The results of the two biometric (weight) parameters that were assessed support the null hypothesis with an indication that regular intake of palm wine for 21 days did not significantly affect the body weight and testis weight of the animals. This biometric finding agrees with an earlier report which indicates that regular administration of 50% diluted palm wine for 21 days caused nonsignificant changes in both testicular and body weights of albino rats Ogbonna *et al* (2013).

Revised regulatory guidelines for the assessment of drugs on reproduction and fertility have emphasized the

Table 1: Analysis of variance for testes weight

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	Probability distribution	P
Between treatment	1	1.3682	0.45607	1.1754	0.357
Within treatment	5	5.0443	0.38803		
Total (*n*)	10				

Table 2: Analysis of variance for rats' body weight after treatment

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	Probability distribution	P
Between treatment	1	3264.8	1088.25	7.0446	0.058
Within treatment	5	2008.2	154.48		
Total (*n*)	10				

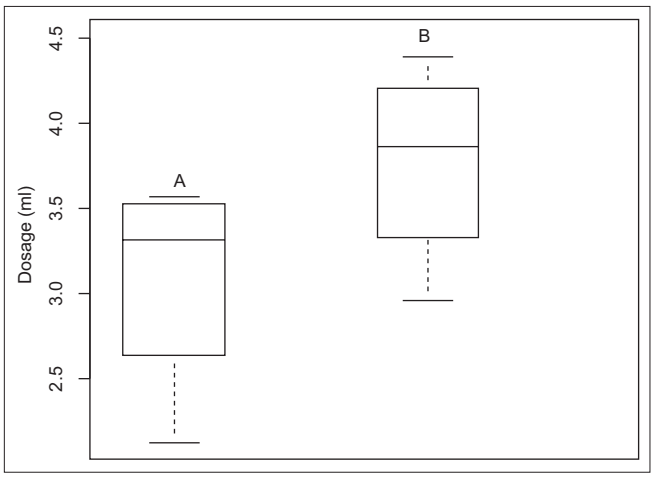

Figure 1: Tukey comparison of means (box plot) for rats' testis weight. The mean levels are marked by the thicker transverse bands; thus group B marked a higher mean level at 3.8 for this biometric parameter

importance of detailed histopathological examination of the testis as a sensitive method for detecting abnormalities in spermatogenesis Chandrasekhar *et al* (2012). The result of the microarchitectural (histological) investigation indicated there was a significant change when tissue samples from the two groups were compared. Figure 6 shows photomicrograph of rat testicular tissue from group A (the control) with normal histological features. However, the evidence deduced from the histological observation of testicular tissue samples from group B following treatment with undiluted palm wine was that of focal (or gradual) disordered arrangement of sperm-producing cells [Figure 7]. The sort of gradual distortion observed in the microarchitectural study is very likely to impair with its function over time since the sperm cells normally follow an orderly pattern of arrangement to allow for their continuous maturation as they propel toward the tubular lumen. The basement membrane which supports differentiating sustentacular and spermatogenic cells also shows some slight distortion at some point marked "F" in Figure 7. These observed histological changes may cause a dysfunction of the seminiferous tubules which of course are the major building blocks (or functional units) of the primary sex organ in males.

The correlation of this observed microstructural disorganization to function agrees with a previous study that reports palm wine caused a significant decrease in testosterone level, sperm count, sperm motility, and sperm viability in male albino rats, however, disagrees

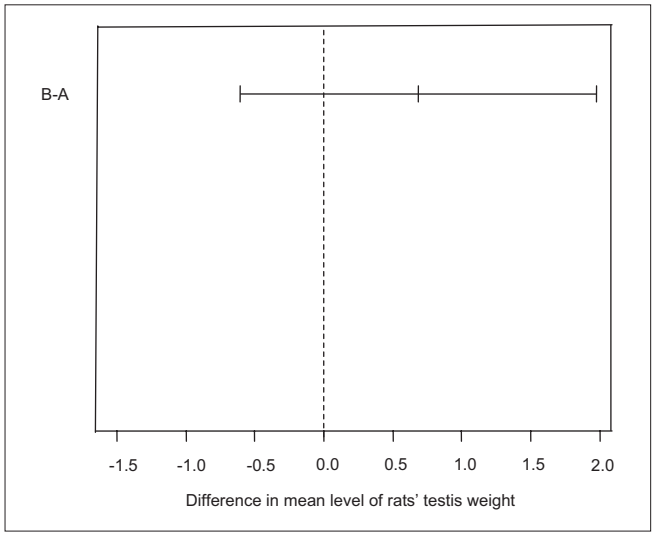

Figure 2: Ninety-five percent of family-wise confidence level showing a nonsignificant difference in the mean level of rats' test is weight as the set (B-A) intersects with the broken line at 0.0 mean level

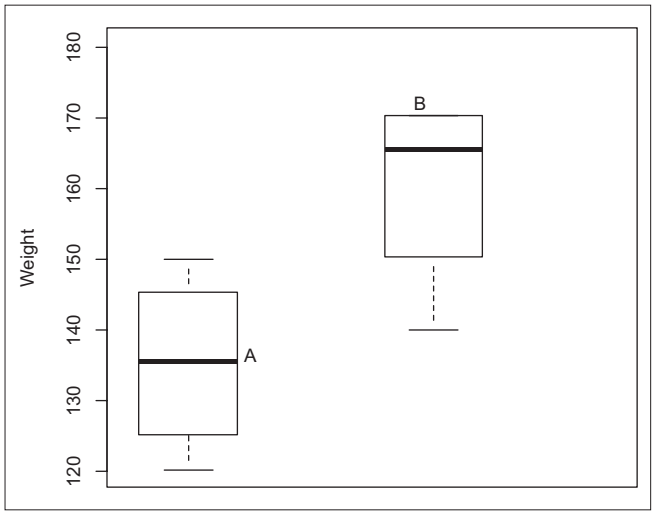

Figure 4: Tukey comparison of means for body weight of rats after treatment indicating Group B marked a higher mean at 165 while Group A marked a mean of 135

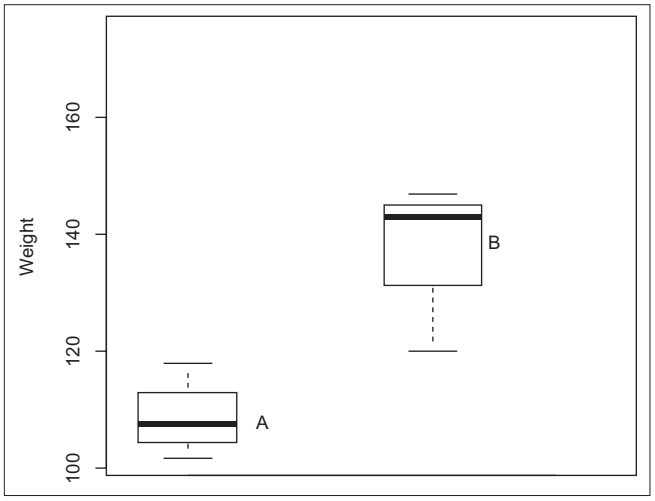

Figure 3: Tukey comparison of means showing the initial body weight of animals in Group A and B before the experiment. The thicker transverse bands mark the mean levels and indicating that the animals in the experimental group were mature enough to undergo the test

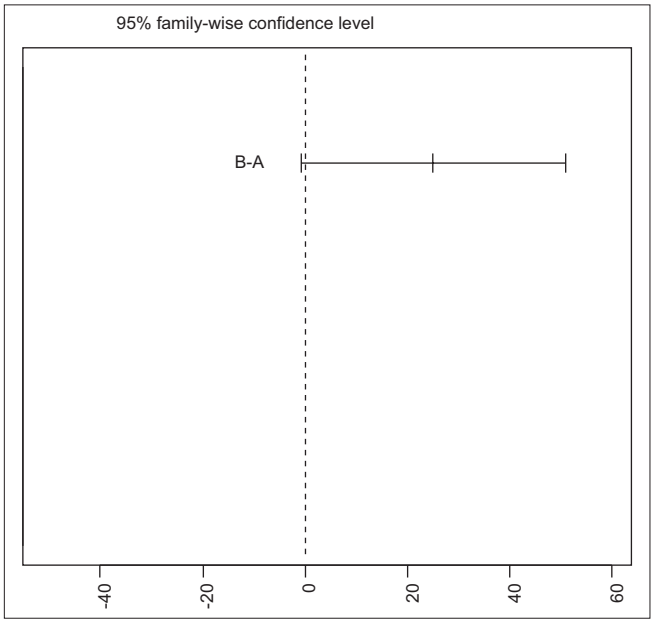

Figure 5: Difference in mean level of rats' body weight after treatment. 95% family-wise confidence level showing a nonsignificant difference between Group B and A as the set (B-A) intersects with the broken line at 0 level

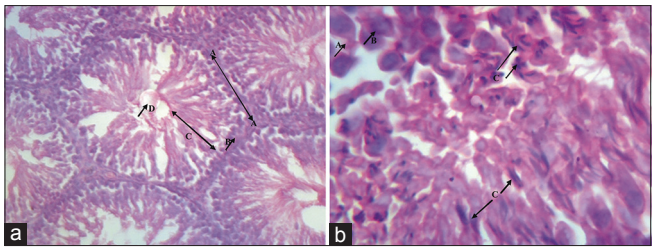

Figure 6: (a) Photomicrograph of rat testicular tissue from Group A, control (H and E, ×150). A - Normal interstitial space; B - Normal geminal epithelium; C - Viable adluminal zone with Sertoli and developing sperm cells; D - Patent tubular lumen. (b) Photomicrograph of a seminiferous tubule of rat from Group A, control (H and E, ×600). A - Normal sustentacular (Sertoli) cell surrounding spermatogenic cell; B - Viable spermatogenic cell; C - Normal sperm cells (at different stages of development)

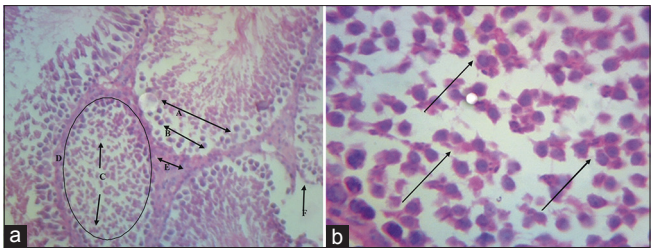

Figure 7: (a) Photomicrograph of rat testicular tissue from Group B treated with 10 ml/kg body weight of palm wine (H and E, ×150). A - Tissue is pale-looking with ruptured basal-adluminal junction; B - Degeneration of germinal epithelium; C - Focal disordered (abnormal) arrangement of sperm-producing cells/ inflammation of tubules; D - Degeneration of epithelium; E - Widened interstitial space; F - Rupture of connective tissue with loss of interstitial cells. (b) Photomicrograph of seminiferous tubule of rat from Group B treated with 10 ml/ kg body weight of palm wine (H and E, ×600). Arrows showing disordered arrangements of sperm-producing cells

with the histological finding (Oyedeji *et al*., 2012). This microstructural disparity may have been borne out of the quality of palm wine that was used in this present study, and this reasonably implies that undiluted palm wine that has been shelved (stored) for longer periods produces a more distortive effect than the diluted one with shorter shelf life.

Similar negative accounts have been given on the teratogenic effect of palm wine on histology of the fetal kidney, fetal cerebral cortex, and the morphometry of fetal femur bone, suggesting the possibility of its interference with the normal process of cell growth (Eluwa *et al*., 2008; Eluwa *et al*., 2010; and Kpela *et al*., 2011). Palm wine is a beverage that contains alcohol as a result of fermentation of the sugary sap from various palm plants (Agu *et al*., 1993). Among its other components are protein carbohydrate, calcium, moisture, ash, magnesium, iron, sodium, potassium, copper, zinc, manganese, phosphorus, and Vitamins A and C (Chandrasekhar *et al*., 2012; Ogbonna *et al*., 2013; Okafor, 1972).

In the analysis of palm wine using tenax gas chromatography and mass spectrometry, 82 components were identified to include 47 esters, 9 alcohols, 5 acids, 6 carbonyls, 2 acetals, 4 terpenes, and 9 hydrocarbons (Ukwuru. Awah, 2013). Palm wine has been reported to be a rich source of nutrition for the growth of microorganisms among which are largely yeast species (Bechem *et al*., 2007). The yeast and other chemical constituents have been said to vary depending on the source and condition of the palm wine. In a study to determine the properties of palm wine yeast and its performance in wine making, the functional activity of the inherent yeast was reported to be dependent on its physical and chemical conditions (Lal *et al*., 1998; Sharma *et al*., 2012). These physicochemical conditions resulting from the quality go further to explain why the longer shelved palm wine could be more debilitating to even the structure. This being the result of a possible increase in the quantity of yeast.

In another study to ascertain the physiological characteristics of ten yeast isolates, some were reported to have tolerance on high sucrose and ethanol concentrations (Bechem *et al*., 2007). While it is the yeast that converts the sugars in the palm wine to alcohol, its effective level may largely depend on the quantity of yeast present in a particular source of palm wine. This understanding may, therefore, be exploited on why the yeast should be implicated first with the negative effects observed in this study. In corroboration of this finding, a related study to determine the effects of coconut palm wine (toddy) and ethanol on liver function and lipid metabolism showed that toddy-treated animals were more severely affected than those exposed to an equivalent quantity of ethanol practically suggesting the role of nonalcoholic components in the negative effect of palm wine (Bechem *et al*., 2007). Another factor that may have contributed to this adverse effect on testicular cytoarchitecture could have been its lack of antioxidant compounds since this deficiency would naturally reduce the product's potential to inhibit oxidations resulting from free radicals.

Conclusion

This study shows that palm wine contains some chemically active compounds and therefore should be regarded as a drug (or product) which regular intake could gradually distort male reproductive (testicular) cytoarchitecture but may not significantly increase testis weight nor body weight. With regard to this observed effect on animal models, palm wine (fermented palm sap or juice) should be consumed with caution, especially by individuals who still hold good procreative or reproductive tendencies.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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