Spermatotoxic effects of selected traditional alcoholic beverages from North-Central Nigeria on adult male albino rats

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Abstract

Challenges associated with habitual intake of alcohol including health, social, psychological and especially reproductive health needs urgent attention. This study aimed to determine the spermatotoxic effect of selected traditional alcoholic beverages in rats. A total of 30 normal male *Spaque dawley* strain albino rats weighing 180-220g, divided into 5 groups of 6 rats in each were administered with 10ml/kg p.o each of *pito*, *goskolo* and *ogogo ro*, *goskolo* respectively and 0.5ml/kg normal saline for a period of 21 days.

Sperm samples were harvested from the left caudal portion epididymis assayed for sperm motility, sperm morphology and sperm count after which histological examination was carried out on the testes. Results showed that active, sluggish and dead sperm cells were *goskolo>* *pito>* *burukutu>* control>* ogogoro, ogogoro> burukutu> control>goskolo> pito and control> pito>ogogoro>burukutu>goskolo respectively. For morphology of sperm cells, it was goskolo>ogogoro>burukutu>control> pito (normal) and pito>control> burukutu> ogogoro> goskolo (abnormal). Also, that of sperm count was goskolo>ogogoro>pito>burukutu>control.

Results further showed that *ogogoro* and *goskolo* caused significant negative effects on quantity and quality of sperm cells with alteration of histological parameters marked with altered secondary spermatogonia and spermatid. These effects were however mild with *pito* and *burukutu*.

Traditional alcoholic beverages from North central Nigeria ‘*pito*, *burukutu*, *ogogoro*, and *goskolo*’ have negative on the quantity and quality of sperm cells with marked with infraction of spermatogonia of male albino rats. As a result of the spermatotoxic properties of these locally available and often ingested drinks by males predominantly in the reproductive age brackets will do well to avoid and or minimize its use as it leads to reprotoxicity.

Keywords: Sperm motility; Sperm morphology; Sperm count; Testicular section; Traditional alcoholic beverages; Reprotoxicity
1. Introduction

Challenges associated with habitual intake of alcohol including health, social, psychological and especially reproductive health needs urgent attention. Africans live communal lives and as such drinking of traditional alcoholic beverages is part of their socialization and such a social habit.

The WHO (2018) [1] attributed an approximately 3.3 million deaths every year (or 5.9%) of all death and 5.1% of the global burden of disease to alcohol consumption. The unrecorded alcohol consumption such as the local brews: burukutu, pito, goskolo and ogogoro in Nigeria is said to have been estimated at 3.5 liters pure alcohol per capita for population of people older than 15 for the year after 1995 [2]. Studies have shown that for both men and women there is a sharp upward tick in alcohol consumption during adolescence, that peaks in early adulthood and plateaus at midlife and declines as they get into older ages [3]. It was also found out that 69.1% of alcohol users in certain regions of Nigeria have either moderate or high health risks from the consumption of alcohol [4]. It is also clear that the fertility, reproductive stature age range fall within 15-49 years of age and it peaks within the 20-34 years in different urban settlements for women, with a little difference in rural areas where there is low education and other factors that make people marry at early age [5]. Studies have shown fertility increase within 30-40 years for men [6].

The effect of traditional alcoholic beverages revealed the classical effects of alcoholic drinks by way of significant alteration in the estrous cycle of albino rats with marked alteration of the histological architecture of ovarian tissues. Pito, goskolo and ogogoro and goskolo have a reprotoxic effect on the ovaries and uterus thus a deleterious effect on fertility of female albino rats [7]. Also, ‘pito’, ‘burukutu’, ‘ogogoro’, and ‘goskolo’ has revealed the have classical effects of alcoholic drinks in that, they were able to cause sexual disinhibition in male rats and suppression of sexual motivation in female rats [8]. The toxicological evaluation of traditional alcoholic beverages pito, goskolo and ogogoro go and goskolo revealed significant decrease in the sex hormonal profile of male and female albino rats. This buttressed the toxicological effect by way of decrease in the activity of the sex hormones necessary for fertility and reproduction the rats [9].

The treatment and management of infertility has become a global pressing concern as the need to have children is of great priority in families especially in Africa [10]. It was reported that about 15% of couples of reproductive age are infertile and about 50% of these cases are male related [11]. Alcohol toxicity produce a significant deterioration on sperm concentration output and motility thus resulting in increased damage of spermatozoa [12, 13]. Although male infertility due to endocrine disorders reportedly accounts for below 3% of infertility cases in males [14], hormone assay for males is done to both identify causative factors for endocrine abnormalities and to obtain prognostic information. Agents that interfere with reproduction may do so by affecting spermatogenesis at the testicular level or changing the body’s hormone profile or both.

Global rise in infertility cases amongst male, has been attributed to decline in semen quality [15]. Male infertility among Nigerians has also been reported to be responsible for about 20-50% of all infertility cases in different parts of the country [16]. Understanding the prevalence and nature of male infertility across Africa has been quite difficult because of insufficient data and the fact that African males rarely agree to undergo fertility tests and usually prefer to blame the females for nearly all cases of infertility in the family [17]. A number of factors that contribute to male infertility includes poor semen quality [18], including hypothalamic pituitary disease, testicular disease, disorders of sperm transport and idiopathic male disease [19]. Other known causes are exposure of the testicles to abnormally high temperatures, developmental history such as cryptorchidism, erectile dysfunctions, diseases such as diabetes and respiratory infections, and past surgical and cancer treatments. Other contributors are lifestyle toxins such as cadmium, mercury, arsenic compounds, hydrocarbons, alcohol, cigarette smoking and pesticides [20] could be attributed to the causes of low sperm count, inadequate sperm motility, abnormal morphological structures or a combination of these factors [21].

2. Material and methods

2.1. Procurement of experimental animal and protocol

A total of 30 normal male albino rats (Spaue dawley strain) weighing 180-220g of about 12 weeks were purchased from the Animal House of the University of Jos, Nigeria. The animals were randomly divided five groups (I - V) of six animals per cage, acclimatized for 72 hours prior to commencement of experiment, fed with grower mash (Vital Feed Nigeria) and allowed access to water ad libitum. This was done as reported by Moritiiwon et al., 2021 [8, 9].
2.1.1. Procurement, preparation of alcoholic beverages and administration protocol

The following types of freshly prepared locally brewed alcoholic beverages - goskolo, burukutu, pito and ogogoro were purchased daily from the same commercial brewer in Angwan Rukuba (a settlement in Jos North LGA, North Central Nigeria) for the period of experiment in order to eliminate the errors of fermentation. Following the 72 hours of acclimatization, the animals were administered with various doses of the local alcoholic beverages orally via a canula for a period of 21 days prior to the assays according to the methods of [9, 22], using the following schedule:

Group I received 10ml/kg of pito, Group II received 10ml/kg of burukutu, Group III received 10ml/kg of ogogoro, and Group IV received 10m/kg of goskolo, while Group V received 0.5ml/kg normal saline.

Figure 1 Google map of Angwan Rukuba, Jos Plateau State, Nigeria [23]

2.2. Sperm samples collection and assays

Following various treatments, each rat was euthanized by cervical dislocation and sperm samples were harvested from the left caudal portion epididymis in pre-warmed phosphate-buffered saline at 37 °C and pH = 7.4). The tissue was gently agitated for about 25 minutes to aid smooth dispersion of the sperm cells. The various sperm assays were however carried out as described by El-Sherbiny (1987) [24], Chibundu (2013) [25] and Ukar et al., (2016) [26]. The values were presented in percentages.

2.2.1. Sperm motility assay

The sperm samples collected via epididymal washings from each treatment group (I - V) and evaluated for progressive motile sperm cells immediately after collection. A smear of one drop of the sperm sample was made on a pre-heated glass slide and viewed under light microscopy at X10 and X400 magnifications and subjectively scored in percentage. The following motility scale was also used to score individual sperm motility: 1 (vigorously progressive), 2 (progressive), 3 (cycling movement) and 4 (stationary movement).

Inclusion criteria
These were sperm cells that moved in straight and forward direction.

Exclusion criteria
These included sperm cells moving in backward and circler direction and such that showed pendular movement.

2.2.2. Sperm count and morphology assays

Sperm count was determined by dilution of 1 mL of the sperm suspension with 1 mL of 10% Formaldehyde (FA) fixative. 10mL of mixture was transferred into a hemocytometer and sperm count was evaluated per 250 small squares of a hemocytometer. The morphology in terms of percentage normality and abnormality of the sperm cells were considered and recorded appropriately.
2.3. Histological assay
Following the assays on the sperm, the rats were sacrificed and the testis were harvested, weighed, fixed in Bouin’s fluid for 12 hours and processed, sectioned, stained and mounted according to the methods of Cito et al., (2018) [27], Suvarna et al., (2012) [28] and Ngadjui et al., (2013) [29]. The mounted tissues were observed under the X400 objective of the microscope.

2.4. Statistical analysis
Statistical Package for Social Sciences (SPSS) version 20 was used in analysis of experimental data. While comparisons were done by analysis of variance (ANOVA) and student t-test. The level of significance chosen was p<0.05. The data obtained from all groups were presented percentages, mean ±SEM and charts.

3. Results and discussion
For the sperm motility in terms of percentage active sperm cells, the highest percentage active sperm cell was observed in the shown by the ogogoro administered group (72%) when compared with Control (66.40%) while the least active was that of goskolo (42.4%). There was significant difference for the group that were administered with goskolo 0.005 (p<0.05) thus having the lowest percentage of active sperm cells (42.4%). However, it was different for the other alcoholic drinks - ogogoro, burukutu and pito where there was no significant difference when compared with control. The percentage active sperm cells was higher in the ogogoro group, though not significantly. The order of increase percentage active sperm cell was Goskolo>Pito>Burukutu>Control>Ogogoro [Figure 2].

The percentage sluggish sperm cells were 37.4%, 33.6%, 20% and 18% for pito, goskolo, burukutu and ogogoro respectively compared with the control group (29.6%), leaving the order of increasing sluggish sperm cells as ogogoro>burukutu>control>goskolo>pito. This showed no significant difference in all the groups of alcoholic drinks studied [Figure 2].

As for the percentage of dead sperm cells, the group treated with goskolo was 24% compared with Control (4%) while other groups were pito (8%), ogogoro (12%) and burukutu (16%). There was significant difference in the group fed with goskolo 0.006, burukutu 0.005 and ogogoro 0.010 in each case compared with the control group (p<0.05) except that of the Pito group. The order of increasing dead sperm cell was control>pito>ogogoro>burukutu>goskolo. One could deduce that goskolo reduced the active sperms and caused more sperm cells die [Figure 2].

![Figure 2](image.png) Effect of some traditional alcohol beverages on the sperm motility (%) of albino rats

In terms of the morphology (percentage normal and abnormal sperm cells), the albino rats that were treated with Pito (83%; 17%) compared with the Control (77.60%;22.4%). Both Burukutu and Ogogoro (74%; 26%) and Goskolo (66%; 34%). The result further showed that there was no significant difference between the group fed with Ogogoro, Burukutu, Pito and Goskolo (p>0.05). The alcoholic drinks under this study did not significantly change the structure or morphology of the sperm cells. Similarly, when the test groups were compared with the Control with respect to the
percentage of abnormal sperm cells morphology, all the test groups of Ogogoro, Burukutu, Pito and Goskolo \((p<0.05)\) were not significantly marred. The order of increasing normality of sperm cell from this table was Goskolo > Ogogoro > Burukutu > Control > Pito while the increase abnormality was Pito > Control > Burukutu > Ogogoro > Goskolo [Figure 3].

**Figure 3** Effect of Some Traditional Alcohol Beverages on the Sperm (%) Morphology of Albino Rats

Regarding sperm count, it was observed that there was reduction in the sperm count of the rats. Percentage sperm count in the treated groups compared with the control group were - Burukutu (60.80%), Pito (52%), Ogogoro (49%), Goskolo (41%) and 77.5% for Control respectively. The effect of these alcoholic beverages was not statistically significant for all the drinks except Goskolo 0.011 \((p>0.05)\) when compared with control rats. The order of increasing sperm count was Goskolo>ogogoro>Pito>Burukutu>Control [Figure 4].

**Figure 4** Effect of Some Traditional Alcohol Beverages on Sperm Count (%) of Albino Rats

Findings on the motility of sperm cells suggest a trend that alcohol ingestion have a negative effect on the motility and activeness of sperm cells though, in a moderate manner. The trend is indicative of reduction in the quantity of active sperm cells, increased sluggish sperm cells and significant amount of dead sperm cells, which agreed with the suggestion of Alverez and Struss (1995) [30], that ethanol increases in plasma membrane of spermatozoa which are ready substrate for the free radicals production leading oxidative stress in the testis, explains the gradual reduction in activeness of sperm cells found in the groups that indigested different samples of ethanol found in Pito, goskolo and ogogogo and Goskolo. Mitochondria produces ATP required for movement of flagella of sperm cells, if there is a reduction or impairment in mitochondrial function which was what was reported by Coleman and Cunningham, (1990) [31], for the liver function. This suggests that there are possibilities that the mitochondria of the testicular cells may be affected too, resulting in non-motile, sluggish and dead sperm cell. This ultimately suggest some levels of infertility among the male [32]. On the other hand, Ogogoro displayed an unusual of increase in activity of sperm cell beyond the control rats.
implying that there could be presence of substances in *Ogogoro* that resulted in an increase in the sperm cells activeness. This suggests the need for further research on same.

*Figure 5* Photomicrographs of testicular sections of experimental animals

From the histology of the testis, it was evident that *burukutu, ogogoro* and *goskolo* exerted reprotoxic effects - plates 3, 4 and 5. *Burukutu* caused the formation of clustered sperm cells with no clear distinction of each cell. There was enlargement of spermatids and all spermatogenic cells were clearly altered - plate 3. *Ogogoro* intake also resulted in gross disruption of the testicular architecture with spermatogenic cells hypertrophy and its lumen was empty of matured sperm cells (plate 4) while *goskolo* also caused spermatogenic cells hypertrophy which may be possibly as a result of infarctions, then the lumen was filled with cluster of deformed sperm cells - plate 5. These also may have possibly resulted from the effect of the oxidant that the metabolism of alcohol produces [33, 34]. These results are in line with
studies that suggested that alcohol caused histological abnormalities in testicular tissue of animals such as sloughing and shortening of semiferous, atrophy of semiferous tubules leading to a reduction of seminiferous testicular diameter and cross-sectioned [35, 36, 37].

On the other hand, histological assay of the testis of rats fed with Pito did not show oblivious pathological changes (plate 2). This contradict the study of Dosumu et al., (2014) [37] which suggested that alcohol induced damage on the testis.

4. Conclusion

Traditional alcoholic beverages from North central Nigeria ‘pito’, ‘Burukutu’, ‘Ogogoro’, and Goskolo have negative on the quantity and quality of sperm cells with marked with infraction of spermatogonia of male albino rats. As a result of the spermatoxic property, males in the reproductive age should abstain from habitual intake of ‘pito’, ‘Burukutu’, ‘Ogogoro’, and Goskolo in order to guide against reprotoxicity.

Compliance with ethical standards

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Disclosure of conflict of interest

All the authors - Olusayo Moritiwon, Timothy Olugbenga Ogundeko, David Oyebode, James Bitrus, Adikpe Emmanuel Edugbe, Mamzhil Seljul Crown Ramyil and Amos Paul Bassi hereby disclose no conflict of interest.

Statement of ethical approval

Authors TOO and MSCC are licensed to handle laboratory animals in conformation with ethical standards.

Statement of informed consent

The present research work does not require statement of informed consent.

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