Effect of some Nigerian traditional alcoholic beverages on the estrous cycle and histological assessment of the ovaries and uterus of albino rats

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Abstract

Alcohol related health challenges have lingered over the years. Such associated with locally brewed alcoholic beverages have received little attention. The urgency to explore this becomes imperative. This study aimed at evaluating the effect of regular consumption of local alcoholic beverages on the estrous cycle and histological image of the ovaries and uterus thus fertility of female albino rats. Thirty screened female albino rats weighing 180-220g were divided into five groups and daily administered each with 10ml/kg of pito, burukutu, ogogoro, goskolo and 0.5ml/kg normal saline respectively for 21 days. Effect of the beverages on the estrous cycle as well as histopathological evaluation was carried out on the isolated ovaries and uterus. Results showed significant increase proestrous phase of the estrous cycle with ingestion of pito, burukutu, ogogoro and goskolo. Also the histology of the ovary was basically without obvious pathological changes with pito, while there was alteration of histological parameters by burukutu, marked with formation of fibrosis, corpus luteum cyst resulting immature ovarian follicle, ogogoro marked with formation of fibrosis, corpus luteum cyst resulting immature ovarian follicle and goskolo marked with formation of multiple follicular cyst resulting in complete collapse of the ovarian section. 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, burukutu, ogogoro and goskolo have a reprotoxic effect on the ovaries and uterus thus a deleterious effect on fertility of female albino rats.

Keywords: Pito; Burukutu; Ogogoro; Goskolo; Estrous cycle; Reprotoxicity

1. Introduction

Alcohol related health challenges have lingered over the years. Such associated with locally brewed alcoholic beverages have received little attention. The urgency to explore this becomes imperative. An alcoholic beverage is a drink which contain substantial amount of psychoactive drug called ethanol [1]. Alcohols are hydroxyl derivatives with straight or branched chain hydrocarbons. Alcoholic beverages contain different alcoholic contents depending on the source and the fermentation process. It ranges from ethanol content of 2.5% - 55% [2]. They are usually from grains, juice, fruits and honey [3]. Consuming alcoholic drinks has long been a part of human life. There is no certainty as to when humans
first produced alcoholic drinks [4]. Considering the good use of alcoholic beverage has not been without grave cost on mankind, WHO (2018) [5] 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.

Given the above mentioned morbidity and mortality from alcoholic drink consumption and Nigeria being among the 30 countries of the world that have the highest per capita of alcohol consumption and alcohol related problems [6].

_Ogogoro also known as Sapele water’, ‘kai-kai’, ‘Sun gbala’, ‘Egun inu igo’ (meaning ‘The Masquerade in the bottle) in Yoruba language’, ‘push-me-push-you’ among many other local names is a West African alcoholic drink, usually brewed locally and quite popular in Nigeria as one of the country’s home brew [7]. The active ingredient in ogogoro is ethanol whose concentration within the drink is very high; the alcohol content of local ogogoro ranges between 30-60% while pito and burukutu are traditional Nigerian alcoholic beverages brewed with red or white sorghum malt and/or maize. The brewing process for pito and burukutu [8]. Preclinical studies in female rats suggest that the rewarding effects of alcohol are stronger in females than in males while several studies are consistent in highlighting the importance of ovarian hormones as mediators of the rewarding effects of alcohol in females and in proving the major vulnerability of female sex to the neurological effect [9]. The unrecorded alcohol consumption such as the local brews: burukutu, pito, goskolo and ogogore in Nigeria is said to have been estimated at 3.5 litres pure alcohol per capita for population of people older than 15 for the year after 1995 [10]. 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 [11]. 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 [12]. 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 [13]. Studies show fertility increase within 30-40 years for men [14].

WHO (2000) [15] defined infertility as the inability of a couple to conceive after a year of regular unprotected sexual intercourse. 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 [16]. It was reported that about 15% of couples of reproductive age are infertile and about 50% of these cases are male related [17].

Reproductive toxicity is usually defined practically, to include several different effects which are unrelated to each other except in their outcome of lowered effective fertility and as such, a hazard associated with some chemical substances, which interfere in some way with normal reproduction; such substances are called reprotoxic as they may adversely affect sexual function and fertility in adult males and females, as well as causing developmental toxicity in the offspring [18, 19, 20]. Many drugs have effects on the human reproductive system: these may be desired (hormonal contraceptives), a minor unwanted side effect (many antidepressants) or a major public health problem (Thalidomide). However, most studies of reproductive toxicity have focused on occupational or environmental exposure to chemical and their effects on reproduction [21]. It may be noted that consumption of alcohol and tobacco smoking are known to be toxic for reproduction in the sense that the term reprotoxic is used (WHO, 2001) [22]. A study of female rats, fed alcohol or control diet for 17 weeks starting at young adulthood found that alcohol did not lead to anovulation but rather to irregular ovulation [23, 24]. Other investigations however reported that ovaries of alcohol exposed female rats were infantile, showing no evidence of ovulation at all and uteri appeared completely estrogen deprived [25]. Alcohol toxicity produce a significant deterioration on sperm concentration output and motility [26, 27] resulting in increased damage of spermatozoa [27].

2. Material and methods

2.1. Experimental animal protocol

A total of 50 female normal albino rats (spaque Dawley strain) weighing 180-220g of about 12 weeks were purchased from the Animal House of the University ofJos, Nigeria. The animals were fed with compressed grower mash (Vital Feed Nigeria) and allowed water _ad libitum_ and housed in 12 standard plastic cages of 5 animals each for aclimatization for 72 hours prior to commencement of experiment. This was done as reported by Moritiwon et al., 2020 [28].

2.2. 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. This was done to eliminate the errors of fermentation while administration of
various alcoholic beverages was with a canula via the oral route. The animals were randomly divided into five groups of six animals per cage and were later administered with various doses of the local alcoholic beverages orally for a period of 21 days prior to the assays also according to the methods of [29, 30] as reported by [28] with the following schedule:

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

2.3. Estrous Cycle Study

2.3.1. First 21 Days Estrous Cycle Determination (Untreated/Control)
Sexually mature female albino rats were screened via daily vaginal smear. Vaginal smear of each rat was taken almost at same time between 12:00 noon and 1:00 pm for 21 days with untreated animals in order to ascertain regular estrous cycles. The vagina of each rat was briefly flushed two to three times with normal saline with the aid of a micro pipette and the fluid placed on a glass slide, smeared and stained in 0.1% crystal violet and the stained slide examined directly under the light microscope with x40 objective [31, 32, 33, 30]. A total of 30 rats were that passed the screening of regular estrous cycle were selected for the second 21 days.

2.3.2. The second 21 days for estrous cycle study (treated)
Thirty screened female albino rats were divided into five groups and daily doses of their group beverage was administered as earlier stated. Same processes as stated in the first 21 days were repeated for each rat and the estrous phase for each recorded.

The animals whose vaginal smears contained predominately leukocytes, primarily nucleated epithelial cells and few leukocytes, primarily cornified cells and primarily cornified cells with a significant number of leukocytes and nucleated epithelial cells were classified as diestrus, proestrus, estrus and metestrus respectively according to the methods of [34].

2.4. Histopathological evaluations

2.4.1. Preparation of samples and relative organ weights
The experimental animals were sacrificed under formalin as the anesthetic agent while the ovaries and uterus were isolated, cleaned, weighed and fixed in Bouin’s fluid for 12 hours. The tissues were then subjected to tissue processing, paraffin wax embedding, section cutting, and staining according to the methods of [35]. Relative organ weight of the isolated organs was each calculated using the method of Ngadjui et al., 2013 [36] as stated below:

Relative organ weight (mg/100 g body weight) = Absolute organ weight (g) × 100 / Body weight of rat on sacrifice day (g).

2.5. Statistical analysis
Statistical package for social sciences (SPSS) version 20 was used in analysis of experimental data [37], while statistical comparisons were 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 expressed as mean ±SEM.

3. Results and discussion

3.1. Burukutu and estrous cycle of female albino rats
There was reduction in the number of days at the diestrous and estrous phases. This reduction was not significant (at p<0.05) when compared with control. However, there was a significant increase in number of days at the metestrous phase (p<0.05). Same increase but not significantly (p<0.063) was also observed at the proestrous phase This, therefore suggest a reduction in the number of fertile days of the rats fed with burukutu - Figure 1.
3.1.1. *Ogogoro and estrous cycle*

A statistically insignificant reduction ($p<0.05$) was observed during the diestrous and estrous phases, while the metastrous ($p<0.05$) and proestrous ($p=0.058$) phases experienced a slight increase but not significant and nearly significant different respectively compared with the control – Figure 2.

3.1.2. *Goskolo and estrous cycle*

Figure 1 effect of 21 day treatment with burukutu on phases of estrous cycle rats

Figure 2 effect of 21 days treatment with ogogoro on phases of estrous cycle in female rate

Figure 3 effect of 21 days treatment with ogogoro on phases of estrous cycle in female rate
Results showed insignificant decrease at diestrous ($p=0.856$) and estrous ($p<0.056$) phases. However, there was a significant increase in the number of days at the proestrous phase ($p<0.056$) while the increase observed at the metastrous phase was without significant difference ($p=0.921$) - Figure 3

3.1.3. Pito and estrous cycle

There was reduction in the number of days at the diestrous and metastrous phases, but the difference was not statistically significant ($p<0.05$). However, the proestrous phase displayed a significant increase in the number of days ($p<0.05$) when compared with control, while the estrous phase though with increase, did not show any significant difference when compared with the control – Figure 4.

![Figure 4](image)

**Figure 4** effect of 21 days treatment with pito on phases of estrous cycle in female rate

3.2. Alcoholic beverages and estrous cycle of female rats

The estrous cycle study of the female albino rats revealed that *Pito* and *Goskolo* consumption caused changes in duration of some phases of the estrous cycle. The change was particularly significant for proestrous phase (the preovulation phase) of the estrous cycle. This finding is in agreement with the work of [30], on the effect of 20% (10ml/kg) of alcohol on the estrous cycle of female albino rats. This suggests that *Pito and Goskolo* consumption caused an imbalance of the ovarian and extra ovarian hormone responsible for each phase of the estrous. Circostal *et al.*, [38] reported that an imbalance in hormones usually leads to irregularity in ovarian function and duration of estrous cycle.

Treatment of rats with *Pito* and *Goskolo* induced significantly increase in proestrous phase (pre ovulatory phase) of the estrous cycle which probably might be an indication that maturation of the follicles in the preovulatory phase was delayed leading to non-maturation of the Graafian follicles as it is required. Or a prolongation of the duration of maturation of follicle which may possible leads to atratic follicle, which also will result in non-availability of matured Graafian follicle. Treatment of rats with *pito* and *goskolo* also caused slight reduction in the metestrous of the estrous cycle which indicative of an alteration of processes that lead to production of matured Graafian follicles. However, there was non-significant increase in duration of the estrous phase in the rat treated with *pito*, which probably indicates that ovulation was not compromised. This agrees with the report of [30] in *Portulaca oleracea* extract treating rats. On the contrary *goskolo* caused a nearly significant reduction in the estrous phase of the estrous cycle which probably indicated that ovulation was compromised suggesting the non-availability of matured Graafian follicles or less occur ovulation in the albino rats.

The *ogogoro* fed rats has very similar trend with *pito* and *goskolo*. The only difference includes: that the proestrous phase of estrous cycle in the group treated with *ogogoro* is slightly non-significant but the trends were absolutely the same, suggesting the non-maturation Graafian follicles as mentioned in rat treated with *pito*. The additional difference includes a slight non-significant increase in the metestrous phase of the estrous of rat treated *ogogoro* which may suggest an indication of availability of matured Graafian follicles. Similar trend of results was reported by [39] in *Achgranthes aspera* extracts treated rats. Which did not compromise the ovulatory phase as seen in *ogogoro* treated rat.
Plate 1: Ovarian section of female rat treated with 0.5 ml normal saline showing normal ovarian architecture.

Plate 2: Ovarian section of female rat treated with 100 mg/kg of pito showing normal ovarian architecture.

Plate 3: Ovarian section of female rat treated with 10ml/kg of burukutu showing altered ovarian architecture.

Plate 4: Ovarian section of female rat treated with 10ml/kg of ogogoro showing collapsing ovarian architecture.

Plate 5: Ovarian section of female rat treated with 10ml/kg of goskolo showing collapsed ovarian architecture.

Figure 5 Photomicrographs ovarian sections of female albino rat treated with normal saline goskolo, burukutu, pito and ogogoro at X400

Burukutu consumption caused change in the duration of the phases of the estrous cycle. This was significant for metaestrous phase of the cycle. This result is in agreement with the work of [30] on effect of alcohol 20% (10ml/kg) in the estrous cycle of albino rats. This suggests that compromised ovulatory phase of estrous cycle and tampered with maturation process of the follicle thereby suggesting the non-availability of matured Graafian follicles.

In view of the histological evaluation, plate1 shows photomicrograph of a section of the ovary from experimental rats of the group of the Control. The observable features are a developing ovarian follicle and mature ova, ready for fertilization. Plate2 shows photomicrograph of a section of the ovary from experimental rats of the pito group. The
observable features include normal mature ovarian follicle with no observable pathologic features. Plate 3 shows photomicrograph of a section of the ovary from experimental rats of the group of burukutu. The observable features include atratic follicle: a follicle that degenerates before coming to maturing. There is also immature ovarian follicle which is a clear sign of pathologic changes. Plate 4 shows a photomicrograph of a section of the ovary from experimental rats of the ogogoro group. The observable pathologic changes showing formation of fibrosis and the formation of corpus luteum cyst resulting in completely immature ovarian follicle. Plate 5 shows photomicrograph of a section of the ovary from experimental rats of the group treated with goskolo. The following are the observable pathologic changes: the formation of fibrosis within the cortex, and formation of multiple follicular cyst resulting in complete collapse of the ovarian section.

3.3. Alcoholic beverages and histopathology and weight of isolated rat's reproductive organs

From the histology of ovaries, it is evident that burukutu, ogogoro and goskolo possess an anti-ovulationary properties which give them a reprotoxic effect on the ovary. Specifically, the ingested burukutu resulted in formation atratic follicles as well immatured follicle marked with signs of pathological changes (plate 3). Ogogoro also resulted in disruption of the architectural framework of the ovary of rats, showing formation of fibrosis and of corpus luteum cyst and gave rise to what we possible suggest as an ovarian failure (plate 4). Goskolo completely destroyed the ovarian architecture by the formation of multiple fibrosis within the cortex with multiple follicular cyst producing a complete collapse of the ovarian system (plate 5). These results may be as result of the present of very reactive ionic substrate that are product of these traditional alcoholic beverages metabolism. There was no visible pathologic damage cause on the pito-fed rats (Plate 2). The present study showed that there was a significant reduction in wet weight of the ovaries and uterus of the pito, burukutu, ogogoro and goskolo administered groups compared to the control. This weight reduction was more in the ovaries followed by the uterus – Table 1.

Table 1 Effect of alcoholic beverages on weight of rat ovaries and uterus of albino rats.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mean±SD</th>
<th>t-test</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td><strong>Weight of rat (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>178.48±15.16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ogogoro</td>
<td>191.76±18.48</td>
<td>1.24</td>
<td>0.249</td>
</tr>
<tr>
<td>Burukutu</td>
<td>176.50±24.26</td>
<td>0.15</td>
<td>0.881</td>
</tr>
<tr>
<td>Pito</td>
<td>187.24±17.79</td>
<td>0.84</td>
<td>0.426</td>
</tr>
<tr>
<td>Goskolo</td>
<td>186.98±12.11</td>
<td>0.98</td>
<td>0.356</td>
</tr>
<tr>
<td><strong>Weight of ovaries of rats (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.21±0.15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ogogoro</td>
<td>0.12±0.02</td>
<td>1.33</td>
<td>0.220</td>
</tr>
<tr>
<td>Burukutu</td>
<td>0.13±0.03</td>
<td>1.17</td>
<td>0.276</td>
</tr>
<tr>
<td>Pito</td>
<td>0.10±0.02</td>
<td>1.63</td>
<td>0.143</td>
</tr>
<tr>
<td>Goskolo</td>
<td>0.12±0.03</td>
<td>1.32</td>
<td>0.225</td>
</tr>
<tr>
<td><strong>Weight of uterus (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.57±0.28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ogogoro</td>
<td>0.56±0.37</td>
<td>0.05</td>
<td>0.963</td>
</tr>
<tr>
<td>Burukutu</td>
<td>0.30±0.11</td>
<td>2.01</td>
<td>0.080</td>
</tr>
<tr>
<td>Pito</td>
<td>0.32±0.08</td>
<td>1.92</td>
<td>0.091</td>
</tr>
<tr>
<td>Goskolo</td>
<td>0.36±0.18</td>
<td>1.41</td>
<td>0.196</td>
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Summarily, significant increase proestrous phase of the estrous cycle with ingestion of pito, burukutu, ogogoro and goskolo suggests the alteration in fertility status of the albino rats. Also the histology of the ovary was basically without obvious pathological changes with pito, while there was alteration of histological parameters by burukutu, marked with formation of fibrosis, corpus luteum cyst resulting immature ovarian follicle, ogogoro marked with formation of fibrosis, corpus luteum cyst resulting immature ovarian follicle and goskolo marked with formation of multiple follicular cyst resulting in complete collapse of the ovarian section. Thus significant reprotoxic effect on female albino rats.
4. Conclusion

The effect of traditional alcoholic beverages - *pito*, *burukutu*, *ogogoro*, and *goskolo* has 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*, *burukutu*, *ogogoro*, and *goskolo* have a reprotoxic effect on the ovaries and uterus thus a deleterious effect on fertility of female albino rats. Women with fertility problem are advised to abstain from the traditional alcoholic beverages.

Compliance with ethical standards

Disclosure of conflict of interest

All the authors declare no conflict of interest/ Competing Interest

Statement of ethical approval

The animals were maintained throughout of experiment in accordance with the recommendations of the guide for the care and use of laboratory animals as reported by [40]. One of the authors TOO is however licensed to handle laboratory animals.

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