GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

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

Olusayo Moritiwon [1], Timothy Olugbenga Ogundeko [2,*], James Bitrus [3], David Oyebode [4] and Olufunmilayo Ibiyemi Abobarin [5]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Science, University of Jos Nigeria.
[2] Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Bingham University, Jos Campus Nigeria.
[3] Department of Obstetrics and Gynaecology, College of Medicine and Health Sciences, Bingham University, Jos Campus Nigeria.
[4] Department of Hematology, Federal School of Medical Laboratory Science, Jos University Teaching Hospital, Nigeria.
[5] Department of Morbid Anatomy Histopathology and Morbid Anatomy, College of Medicine and Health Sciences, Bingham University, Jos Campus Nigeria.

GSC Biological and Pharmaceutical Sciences, 2021, 14(01), 027–036

Publication history**:** Received on 01 January 2021; revised on 06 January 2021; accepted on 07 January 2021

Article DOI: https://doi.org/10.30574/gscbps.2021.14.1.0001

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*, *ogogorogo* 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

* Corresponding author: Timothy Olugbenga Ogundeko; Phone: +2348033268286; Email: tim_ogundeko@yahoo.com
Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Bingham University, Jos Campus Nigeria.

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 *gbalaja'*, '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 *ogorogo* 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 of Jos, 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 acclimatization 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 10m/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 anesthestic 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

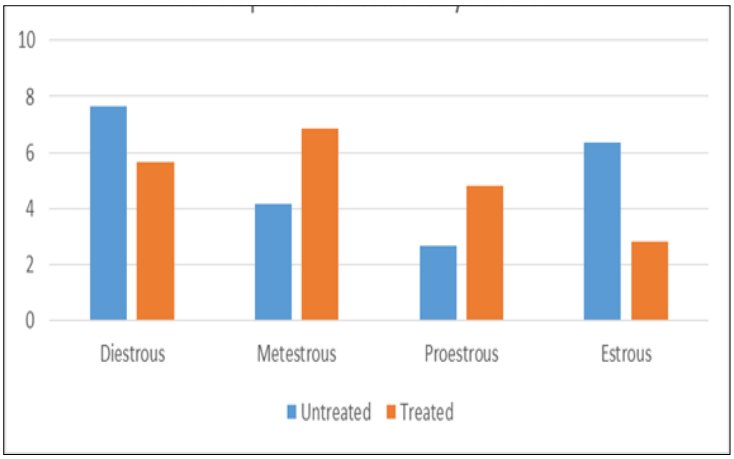

Figure 1 effect of 21 day treatment with burukutu on phases of estrous cycle rats

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.

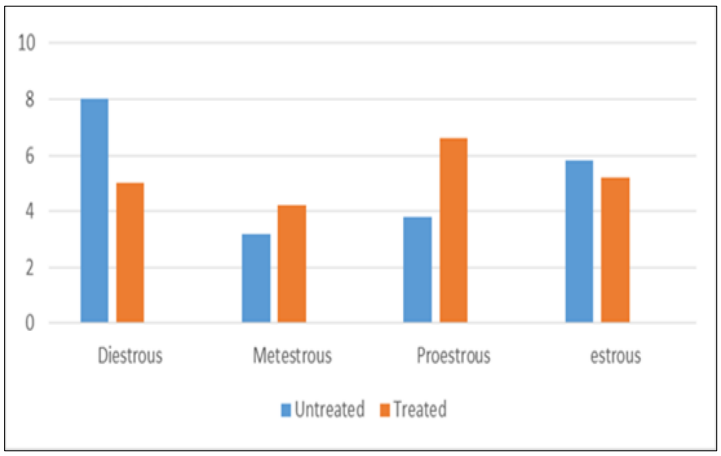

Figure 2 effect of 21 days treatment with ogogoro on phases of estrous cycle in female rate

3.1.2. Goskolo and estrous cycle

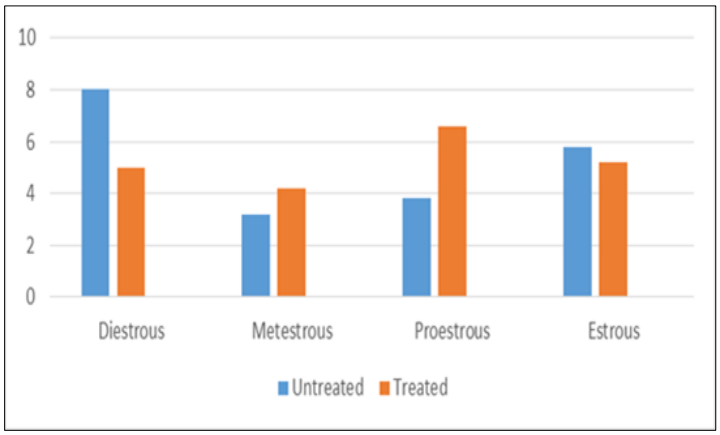

Figure 3 effect of 21 days treatment with ogogoro on phases of estrous cycle in female rate

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<0.05) 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 distrous 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.

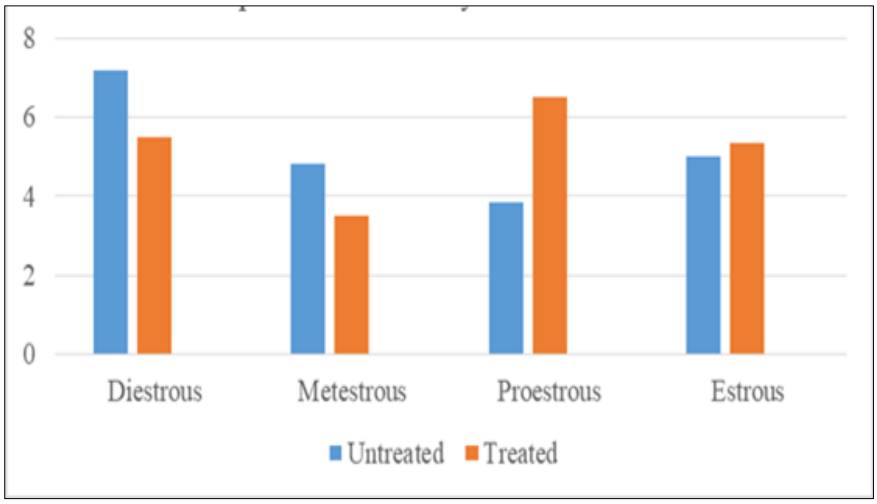

Figure 4 effect of 21 days treatment with pito on phases of estrous cycle in female rate

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 o*gogoro* treated rat.

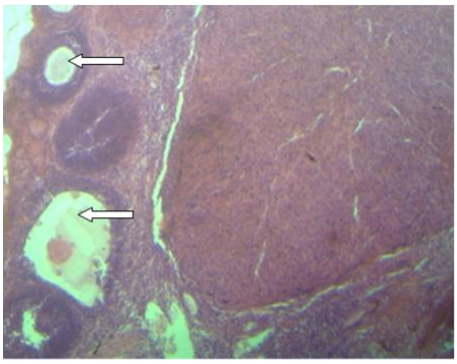

Plate 1: Ovarian section of female rat treated with 0.5 ml normal saline showing normal ovarian architecture.

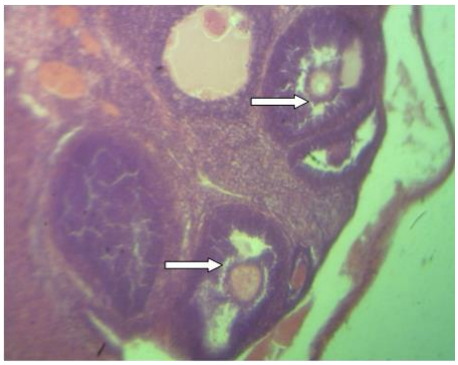

Plate 2: Ovarian section of female rat treated with 100 mg/kg of *pito* showing normal ovarian architecture.

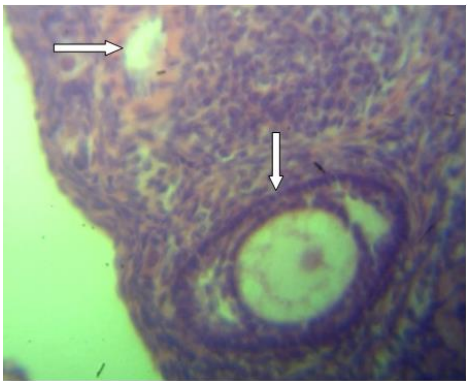

Plate 3: Ovarian section of female rat treated with 10ml/kg of *burukutu* showing altered ovarian architecture.

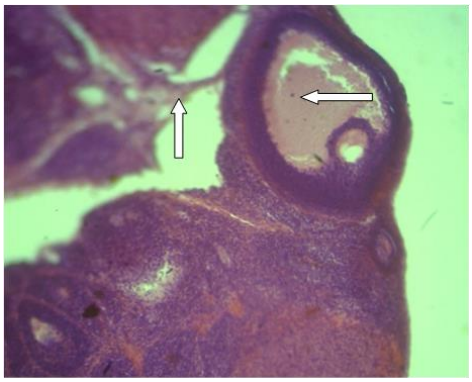

Plate 4: Ovarian section of female rat treated with 10ml/kg of ogogoro showing collapsing ovarian architecture.

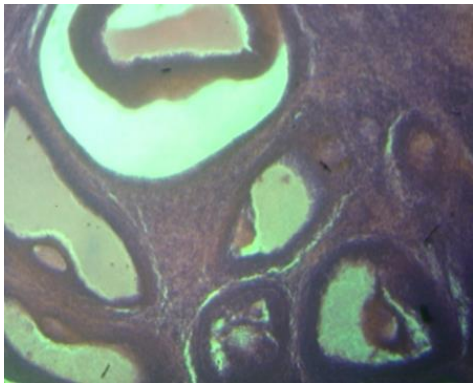

Plate 5: Ovarian section of female rat treated with 10ml/kg of goskolo showing collapsed ovarian architecture.

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 Graafin 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 in 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*, *ogogorogo* 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.

Substance	**Mean±SD**	**t-test**	***P***
Weight of rat (g)			
Control	178.48±15.16	-	-
Ogogoro	191.76±18.48	1.24	0.249
Burukutu	176.50±24.26	0.15	0.881
Pito	187.24±17.79	0.84	0.426
Goskolo	186.98±12.11	0.98	0.356
Weight of ovaries of rats (g)			
Control	0.21±0.15	-	-
Ogogoro	0.12±0.02	1.33	0.220
Burukutu	0.13±0.03	1.17	0.276
Pito	0.10±0.02	1.63	0.143
Goskolo	0.12±0.03	1.32	0.225
Weight of uterus (g)			
Control	0.57±0.28	-	-
Ogogoro	0.56±0.37	0.05	0.963
Burukutu	0.30±0.11	2.01	0.080
Pito	0.32±0.08	1.92	0.091
Goskolo	0.36±0.18	1.41	0.196

Summarily, significant increase proestrous phase of the estrous cycle with ingestion of *pito, burukutu*, *ogogorogo* 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.

References

[1] Hobbs WR, Tall TW, Verdoorin TA. Hypnotics and Sedatives; Ethanol. In: Goodman LS, Brunton LL, Chabner B, Knollmann BC (ED). Goodman and Gillman Pharmacological Basis of Therapeutics 9th Edn, New York: McGraw-Hall. 2001; 386-390.

[2] Rang HP, Dale MM, Ritter JM, Flower RJ. Drug addition, dependence and abuse. In: H.P. Rang & M.M. Dale (ED.). Pharmacology 8th Edn, Philadelphia, USA. Elsevier. 2007; 628-33.

[3] World Health Organization. Types of Alcoholic. 1995.

[4] David JH. Alcohol Science, Policy and Public Health, Historical Evolution of alcohol consumption in the society. Oxford, Oxford University Press. 1-40.

[5] WHO. Global status report on alcohol and health. 2018.

[6] Dumbili E. Changing Patterns of Alcohol Consumption in Nigeria: An Exploration of Responsible factors and Consequences. Medical Sociology online. 2013; 7(1): 21-33.

[7] Heap S. Those that are cooking the gins. The business of Ogogoro in Nigeria, contemporary drug problems. Academia. 2008; 35(4): 573-610.

[8] Ekundayo JA. The production of 'Pito'; a Nigerian fermented beverage. Journal of Food Technology. 1969; 4: 217-225.

[9] Torres OV, Walker EM, Beas BS, O'Dell LE. Female rats display enhanced rewarding effects of ethanol that are hormone dependent. Alcohol Clin Exp Res. 2014; 38: 108-115.

[10] World Health Organization. International programme on chemical safety. Principle of for evaluating health risk to reproduction associated with exposure to chemicals environmental health criteria. Geneva: WHO Press. 2001; 225.

[11] Geggels L. Alcohol intake peaks at age 25 but Continuous into old Age. Live Science. 2015.

[12] Lasebikan VO, Ola BA. Prevalence and Correlates of Alcohol Use among Sample of Nigeria Semirural Community Dwellers in Nigeria. Journal of Addition. 2016; 6.

[13] Nigerian Urban Reproductive Health Initiative. Fertility. www.dhsprogram.com 2003.

[14] Harris ID, Fronezale C, Roth L, Muschan R. Fertility and the Aging Male. Review in Urology. 2011; 13(4): e184-e190.

[15] World Health Organization. Manual for the standardized investigation and diagnosis for the infertile couple. Cambridge: Cambridge University Press. 2000.

[16] Shukla K, Mahdi AA, Ahmad MK, Jaiswar MP, Shankwar SC. Tiwari Mucuna pruriens reduces stress and improves the quality of semen in infertile men. Evidence-Based Complementary and Alternative Medicine. 2010; 7: 137–144.

[17] McLachlan R, Krester D. Male infertility: The case for continued research. The Medical Journal Australia. 2001; 174: 116–117.

[18] International Programme on Chemical Safety. "Principles For Evaluating Health Risks To Reproduction Associated With Exposure To Chemicals". Environmental Health Criteria. Geneva: World Health Organization. 2001; 225.

[19] Annex I, section 3.7, Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJEC, L353, 31.12.2008 1–1355, at pp. 107–14.

[20] Part 3, chapter 3.7, Globally Harmonized System of Classification and Labelling of Chemicals (Second revised ed.), New York and Geneva: United Nations, 2007, pp. 175–86, ISBN 978-92-1-116957-7, ST/SG/AC.10/30/Rev.2.

[21] Kim JH, Scialli AR. Thalidomide: The tragedy of both defects, effective treatment of disease. Toxicology Science. 2012; 139 (1): 25-32.

[22] World Health Organization. International programme on chemical safety. Principle of for evaluating health risk to reproduction associated with exposure to chemicals environmental health criteria. Geneva: WHO Press. 2001; 225.

[23] Krueger WA, Bo WJ, Rudeen PK Estrous cyclicity in rats fed an ethanol diet for four months. Pharmacology Biochemistry and Behavior. 1983; 19: 583–585.

[24] Emanuele NV, Lapaglia N, Steiner J. Effect of chronic ethanol exposure on female rat reproductive cyclicity and hormone secretion. Alcoholism: Clinical and Experimental Research. 2001; 25: 1025–1029.

[25] Gavaler JS, Deal SR, Van Thiel DH Arria A, Allan MJ. Alcohol and estrogen levels in postmenopausal women: The spectrum of the effect. Alcoholism: Clinical and Experimental Research. 1993; 17: 786–790.

[26] Kucheria K, Saxena R, Mohan D. Semen analysis in alcohol dependent Syndrome. Andrologia.1985; 17: 558-563.

[27] Brzek A. Alcohol and male fertility (Preliminary report). Andrologia. 1987; 19: 32-36.

[28] Moritiwon et al. Effects of some traditional alcoholic beverages on some reproductive profiles in albino rats. MSc. Thesis. 2017.

[29] Wannang NN, Gyang SS, Banwat SB, Goncin HY, Mador ES, Oni IA. Effect of Locally Consumed Alcoholic Beverages ('Burukutu', 'Pito', and 'Goskolo') on the weight of and Anatomy of Testicular Tissues. African Journal of Pharmaceutical Research and Development. 2011; 3(1): 57-60.

[30] Oyedeji KO, Bolarinwa AF, Afolabi OA. Effect of tetracycline on heamatological and reproductive parameter in female albino rats. Asian Journal of pharmaceutical and clinical research. 2013; 3: 230-232.

[31] Goldman JM, Murr ÃAS, Cooper RL. The Rodent Estrous Cycle: Characterization of Vaginal Cytology and Its Utility in Toxicological Studies. 2007; 97: 84–97.

[32] Lemuel AM, Muhammad B, Sodiq L, Echoru I, Ssempija F, Owembabazi E, Bukenya E, Mario EFD, Archibong VB, Kokas I. The gender and side asymmetry of length of the styloid process. Anatomy Journal of Africa. 2019; 8(1): 1385-1394.

[33] Mclean AC, Valenzuela N, Fai S, Bennett SAL. Performing Vaginal Lavage, Crystal Violet Staining, and Vaginal Cytological Evaluation for Mouse Estrous Cycle Staging Identification, (September). 2012; 4–9.

[34] Tropp J, Markus E J. Effects of mild food deprivation on the estrous cycle of rats. Physiology & Behavior. 2001; 553–559.

[35] Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. Seventh Edition. London: Churchill Livingstone. 2012.

[36] Ngadjui E, Watcho P, Nguelefack TB, Kamanyi A. Effects of Ficus asperifolia on normal rat estrus cyclicity. Asian Pac J Trop Biomed 2013; 3: 53-57.

[37] Yahaya T, Okpuzor J, Ajayi T. The prophylactic Effiacy of Roselle (H. Sadariffa), Moringa (Moringa oleifera), Ginger (Z Officinale), and Ugwu (T occidentalis) on haematology and serum protein of Albino Rats (Rattus norvegicus) exposed to Cement Dust. Research Journal of Medicinal Plants. 2012; 6(2): 189-196.

[38] Circosta C, Sanogo R, Occhiuto F. Effects of Calotropis procera on estrous cycle and on estrogenic functionality in rats. Farmac. 2001; 56: 373-8.

[39] Shibeshi W, Meatonnen E, Debella E, Zerithum L. Phytochemical contraceptive efficiency and safety evolution of the methanilie hearun extract of Acthyranthes aspera in rats. Pharmacology online, 2006; 3: 217-224.

[40] Tuhin RH, Begum M, Rahman M. et al. Wound healing effect of Euphorbia hirta linn. (Euphorbiaceae) in alloxan induced diabetic rats. BMC Complement Altern Med. 2017; 17: 423.