**Evaluation of selected locally brewed alcoholic beverages on the serum hormonal profile of albino rats**

Olusayo Moritiwon 1, Timothy Olugbenga Ogundeko 2, *, David Oyebode 3, James Bitrus 4 and Amos Paul Bassi 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 Hematology, Federal School of Medical Laboratory Science, Jos University Teaching Hospital, Nigeria.
4 Department of Obstetrics and Gynaecology, College of Medicine and Health Sciences, Bingham University, Jos Campus Nigeria.
5 Department of Community Medicine and Primary Health Care, College of Medicine and Health Sciences, Bingham University, Jos Campus Nigeria.

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**Abstract**

Locally brewed alcoholic beverages have become part of the socio-economic life of the Nigerian and West African communities. The negative effect of the use of the beverages on both health, economic and social life is worth exploring amidst the increasing challenges of poverty, inadequate health facilities, lack of basic social amenities amongst many others.

This study aimed at evaluating the effect of some selected locally brewed Nigerian alcoholic beverage on the hormonal profile of male and female albino rats.

A total of 60 screened rats (spaque Dawley strain) of body weight 180-200g and comprising 30 males and females each were randomly divided into five groups of six animals of same sex per cage and administered with various doses of local alcoholic beverages - goskolo, burukutu, pito and ogogoro per oral for a period of 21 days. Serum hormonal assays were carried out with the use of the respective EIA Kit, ELISA microwells and microplate immunoassay. Results revealed significant decrease (p < 0.05) in the sex hormones (estrogen, progesterone, and testosterone) in all the male treated with these alcoholic beverages, while LH and FSH were not significantly affected.

The toxicological evaluation of traditional alcoholic beverages pito, burukutu, ogogoro 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.

**Keywords:** Sex hormones; Goskolo; Burukutu; Pito; Ogogoro; Angwan rukuba

1. **Introduction**

Epidemiology studies conducted in the United States and other countries demonstrate huge disparity in the amount and pattern of alcohol use between men and women [1, 2]. Sex hormones play an important role in the brain structural and functional variations that could contribute to the sex differences in alcohol consumption behavior [3]. Three main classes of sex steroids are known as androgens, estrogens and progestogens (or progestins), of which the most
important human derivatives are testosterone, estradiol and progesterone, respectively [4]. Estrogen and progesterone are able to interact with neurotransmitters such as dopamine and γ-aminobutyric acid [5], which are thought to be important in mediating the effects of ethanol [6, 7]. Therefore, it is possible that circulating ovarian hormones may influence not only the acute effects of alcohol but also the long-term alcohol consumption behavior.

Alcohol interferes with sex hormone activity by promoting the aromatization of androgens during estrogen biosynthesis [8]. It therefore influences the androgen/estrogen ratio. However, there are several other mechanisms that mediate the relationship between alcohol intake and sex hormones. After alcohol is swallowed, it is absorbed primarily from the small intestine into the veins that collect blood from the stomach and blood and from the portal vein which leads to the liver from there to the liver where it is exposed to enzymatic effects [9]. Alcohol elimination rate varies widely among individuals and is influenced by factors such as chronic alcohol consumption, diet, age, smoking and time of day [10, 11]. Alcohol readily diffuses across membranes and distributes through all cells and tissues and at such concentrations even low in moderate consumption, it can acutely affect cell function by interacting with certain proteins and cell membranes. Alcohol is also metabolized in non-liver (i.e. extrahepatic) tissues that do not contain ADH, such as the brain by the enzyme cytochrome P450 and catalase. In general, alcohol metabolism is achieved by both oxidative pathway – either add oxygen or remove hydrogen (through pathways involving ADH, cytochrome P450 and catalase enzymes) and non-oxidative pathway.

Ogogoro also known as Sapele water’, ‘kparaga’, ‘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 [12]. 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 [13]. 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 [14]. There is also a positive relationship between alcohol intake and estrogen levels in animals [15, 16, 17]. This study aimed at evaluating the effect of some selected locally brewed Nigerian alcoholic beverage on the hormonal profile of male and female albino rats.

2. Material and Methods

2.1. Purchase preparation and animals protocol

A total of 60 screened rats (spaque Dawley strain) of body weight 180-200g and comprising 30 males and females each were purchased from the Animal House of the University of Jos, Nigeria. The animals were fed with compressed grower mash and allowed water ad libitum. They were separated according to gender into various cages and were maintained throughout of experiment in accordance with the recommendations of the guide for the care and use of laboratory animals according to Tuhin et al., [18].

2.2. Preparation of alcoholic beverages and administration protocol

Four 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 Nigeria) for the period of experiment. This was done to eliminate the errors of fermentation, while administration of various alcoholic beverages was done according to the methods of [19] and [20].

The animals were randomly divided into five groups of six animals of same sex per cage. They were later administered with various doses of local alcoholic beverages orally via a canula for a period of 21 days prior to the hormonal assays preparation for both males (a) and females (b) also according to the methods of [19] and [20].

Group I (a) and (b) received 10mL/kg of pito, Group II (a) and (b) received 10mL/kg of burukutu, Group III (a) and (b) received 10mL/kg of ogogoro, Group IV(a) and (b) received 10mL/kg of goskolo, Group V(a) and (b) received 0.5mL/kg normal saline

2.3. Procurement of chemicals and reagents

Follicle Stimulating Hormone (FSH), Leutenising Hormone (LH), testosterone, estrogen, and progesterone, enzyme-linked immunosorbent assay (ELISA) enzyme immuno assay (EIA) kit ways obtained from Monobind Inc. Lake forest
USA. Nicotine hydrogen tartrate salt (C_{10}H_{14}N_{2}C_{2}H_{6}O_{6}) purchased from sigma Aldrich with catalog number N5260-25G.

2.4. Sample preparation

Each animal was anesthetized in a desiccator containing a piece of cotton wool soaked with formalin. They were then removed and dissected to open the heart after which needles and syringes were used to collect blood sample from the retro-orbital plexus of the heart, the blood was transferred into sample bottle, which was left for a while to settle, and centrifuged at 3,500 rpm for 15 minutes after which the collected serum was stored in the refrigerator at -20°C for hormonal assay.

2.5. Serum hormonal assays for male and female albino rats

This was carried out with the use of the respective EIA Kit, ELISA microwells and microplate immunoassay using Statfax-2100 microplate reader [21].

The serum gotten from centrifuged blood is used for the assay of testosterone, the assay was based on the competition between testosterone and a testosterone AChE conjugate (testosterone tracer) for a limited amount of testosterone antisera. The concentration of the testosterone tracer is held constant while the concentration of testosterone varies; the amount of testosterone that is able to bind to the testosterone antisera will be inversely proportional to the concentration of testosterone in the well. This antisera-testosterone complex binds to mono-clonal anti-rabbit immunoglobulin G that has been previously attached to the well. The plate is washed to remove any unbound reagent and then Ellman’s reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of the testosterone tracer bound to the well, which is inversely proportional to the amount of free testosterone present in the well during the incubation; or absorbance α (bound testosterone tracer) α 1/(testosterone). The statfax-2100 utilizes the standards made available with the kits to generate a curve from which the value of the hormone concentration is extrapolated.

Same procedure was carried out for FSH, LH and prolactin based on the same EIA principle using a prolactin anticholinesterase tracer for prolactin, an FSH tracer for FSH and a LH anticholinesterase tracer for LH [21].

2.6. Statistical analysis

Statistical package for social sciences (SPSS) version 20 was used in analysis of experimental data in [22]. All statistical comparisons were by analysis of variance (ANOVA) and student t-test used where needed. The level of significance chosen was p<0.05. The data obtained from all groups were compiled and statistically analyzed and expressed as mean±SEM. Since we had parametric data differences between groups were compared using One-Way ANOVA and t-test with p<0.05 considered significance.

3. Results and discussion

Table 1 depicts the effect of the traditional alcoholic beverages on the hormonal levels and order of effects in the male rats.

Leutenising Hormone (LH) showed goskolo group (0.15 ng/ml); control (1.0ng/ml). The result showed significant decrease for the burukutu and goskolo groups (p<0.05) with order of increasing effect as goskolo>burukutu>pito>ogogoro>control.

Follicle Stimulating Hormone (FSH), results showed only ogogoro group (0.12ng/ml) to be significantly different (p<0.05) compared to the control (3.20ng/ml) with the order of increasing effect on FSH as ogogoro>goskolo>burukutu>pito>control.

Testosterone and Progesterone showed similar results where all the beverage groups were statistically significant when compared with the control (p<0.05) with order of increasing effect as ogogoro>goskolo>burukutu>pito>control and pito>ogogoro>burukutu>goskolo>control respectively.
### Table 1 Effect of some traditional alcohol beverages on male sex hormone of albino rats

| Hormone/Group | Mean±SD | t-test | P     |
|---------------|---------|--------|-------|
| **LH (mIU /ml)** |         |        |       |
| Control       | 1.00±0.36 | -      | -     |
| Ogogoro       | 0.76±0.56 | 0.81   | 0.443 |
| Burukutu      | 0.25±0.14 | 4.34   | 0.002*|
| Pito          | 0.66±0.54 | 0.06   | 0.956 |
| Goskolo       | 0.15±0.03 | 5.26   | 0.001*|
| **FSH (mIU/ml)** |         |        |       |
| Control       | 3.20±1.05 | -      | -     |
| Ogogoro       | 0.12±0.02 | 6.56   | 0.001*|
| Burukutu      | 2.09±0.71 | 1.96   | 0.086 |
| Pito          | 2.19±0.99 | 0.94   | 0.375 |
| Goskolo       | 2.02±0.50 | 2.27   | 0.053 |
| **Testosterone (ng/ml)** |         |        |       |
| Control       | 12.22±0.95 | -      | -     |
| Ogogoro       | 2.13±0.03 | 23.74  | 0.001*|
| Burukutu      | 2.16±0.02 | 23.67  | 0.001*|
| Pito          | 2.26±0.07 | 23.38  | 0.001*|
| Goskolo       | 2.16±0.02 | 23.67  | 0.001*|
| **Progesterone (ng/ml)** |         |        |       |
| Control       | 22.02±6.66 | -      | -     |
| Ogogoro       | 7.85±2.62 | 4.43   | 0.002*|
| Burukutu      | 8.33±0.04 | 4.60   | 0.002*|
| Pito          | 2.32±0.03 | 6.61   | 0.001*|
| Goskolo       | 11.18±2.70 | 3.37   | 0.010*|
| **Oestrogen (pg/ml)** |         |        |       |
| Control       | 29.05±8.13 | -      | -     |
| Ogogoro       | 2.25±0.03 | 7.37   | 0.001*|
| Burukutu      | 2.28±0.34 | 7.36   | 0.001*|
| Pito          | 10.25±3.84 | 4.68   | 0.002*|
| Goskolo       | 2.45±0.12 | 7.32   | 0.001*|

*Significantly different from control

Estrogen showed similar results as those of testosterone and progesterone. The order of increasing effect was *ogogoro > burukutu > goskolo > pito > control*. Table 1 also shows that oestrogen, testosterone and progesterone was shut down by these alcoholic beverages. *Ogogoro* reduced both LH and FSH while *goskolo* has effect significantly on the LH.

Table 2 shows the effect of the traditional alcoholic beverages on the hormonal levels and order of effects in the female rats.
Table 2 Effect of some traditional alcohol beverages on female sex hormone of albino rats

| Hormone/Group | Mean±SD | t-test | P     |
|---------------|---------|--------|-------|
| LH (mIU/ml)   |         |        |       |
| Control       | 2.58±0.54 | -      | -     |
| Ogogoro       | 0.30±0.15 | 9.10   | 0.001*|
| Burukutu      | 0.35±0.02 | 8.66   | 0.001*|
| Pito          | 0.36±0.11 | 9.01   | 0.001*|
| Goskolo       | 0.40±0.10 | 8.88   | 0.001*|
| FSH (mIU/ml)  |         |        |       |
| Control       | 4.03±0.97 | -      | -     |
| Ogogoro       | 2.07±0.42 | 4.15   | 0.003*|
| Burukutu      | 2.30±0.61 | 3.38   | 0.010*|
| Pito          | 1.61±0.52 | 4.92   | 0.001*|
| Goskolo       | 1.82±0.15 | 5.03   | 0.001*|
| Prolactin (ng/ml) | | | |
| Control       | 2.50±1.09 | -      | -     |
| Ogogoro       | 1.70±0.75 | 1.35   | 0.213 |
| Burukutu      | 0.53±0.34 | 3.86   | 0.005*|
| Pito          | 0.55±0.21 | 3.93   | 0.004*|
| Goskolo       | 1.46±0.42 | 1.99   | 0.082 |
| Progesterone (ng/ml) | | | |
| Control       | 8.40±5.88 | -      | -     |
| Ogogoro       | 5.69±1.66 | 0.99   | 0.350 |
| Burukutu      | 1.84±0.42 | 2.49   | 0.038*|
| Pito          | 3.24±0.57 | 1.95   | 0.087 |
| Goskolo       | 4.51±1.17 | 1.45   | 0.186 |
| Oestrogen (pg/ml) | | | |
| Control       | 15.53±4.47 | -      | -     |
| Ogogoro       | 2.36±0.14 | 6.58   | 0.001*|
| Burukutu      | 1.62±0.53 | 6.91   | 0.001*|
| Pito          | 3.24±1.14 | 5.96   | 0.001*|
| Goskolo       | 2.27±0.02 | 6.63   | 0.001*|

*Significantly different from control

LH, FSH and oestrogen levels was significantly reduced (p<0.05) in all the alcoholic beverages studied. The order of the increasing effect for same was ogogoro > pito > burukutu > goskolo > control; pito > goskolo > ogogoro > control and burukutu > goskolo > ogogoro > pito > control respectively.

Prolactin, The Burukutu, and Pito, groups had a significant reduction in concentration of prolactin in the serum (p<0.05). However, no significant difference was seen in the ogogoro and goskolo groups. The order of the increasing effect for prolactin was pito > burukutu > goskolo > ogogoro > control.

Progesterone showed the ogogoro was statistically significantly different (p<0.05) when compared with control, unlike the burukutu, pito, and goskolo groups with no significant difference. The order of the increasing effect was burukutu > Pito > goskolo > ogogoro > control.
3.1. Alcoholic beverages and sex hormones levels in male rats

Alcohol has been reported to reduce serum/plasma testosterone level in experimental animals [23, 24, 25, 26]. In men, low androgen level has also been reportedly associated with both moderate consumptions with chronic alcohol consumption [27, 28, 29, 30]. This study corroborates these reports as testosterone level were significantly reduced with the consumption pito, burukutu, ogogoro and goskolo in rats. The possible mechanisms of reduction level testosterone may be via opioids - Testicular opioids are messenger molecules similar to morphine that when produced within testes suppress testosterone synthesis. Examples of such opioids is Beta-endophine, Nitric oxide (NO) – a ubiquitous gas that results in dilatation of blood vessels. It is synthesized in the testes by Nitric Oxide Synthase (NOS) and inhibition of this enzymes by a variety of NOS inhibitors successfully prevent the decrease in testosterone associated with alcohol consumption (Adams et al., 1992) [31]. Oxidation - reactions that remove hydrogen or add oxygen or both is known as oxidation. For alcohol, oxidation is a process that occurs as part of alcohol metabolism, generating by-products called oxidants, that contributes to cell damages and may play a role in alcohol induced tissue damages in testes. An imbalance in between oxidants and antioxidant can create oxidative stress- a state marked by continual production of oxidizing agents and escalatory cell damage. Increase oxidative stress is a well and accepted as mechanism of alcohol induced tissue injury especially in the liver [32, 33], in heart and central nervous system and also in testes (Emanule et al., 2001 [34]). Metabolism of alcohol and acetaldehyde produced highly toxic ROS. Cell Damage - testicular membrane are rich in molecule known as fatty acid, the prime oxidative injury, it is reasonable to consider lipid peroxidation many contribute to the gonadal dysfunction that occur as a result of acute or chronic alcohol use [34].

Regarding the LH and FSH levels in the male albino rats, LH levels was not altered significantly for ogogoro, and pito groups. These were reduced significantly in the burukutu and goskolo groups FSH level was only altered significantly for ogogoro fed rats, others did not have significant changes. While it was expected that the inhibitory effect of alcohol on the biosynthesis of testosterone should lead to compensatory increase in LH and FSH secretion, so that normal serum concentration of testosterone be restored, it was rather the opposite. Studies on the effect of alcohol on gonadotropin levels have produced varying results. Some studies have found alcohol to depress gonadotropin hormones significantly [35, 36, 25]. The present study is in line with the unchanged and depress of gonadotropin hormone. Ogogoro and pito did not change LH while burukutu and goskolo depressed LH significantly. For FSH, ogogoro caused it depression while burukutu, goskolo and pito did not change the level of FSH. Studies in animals and humans have shown that when testosterone levels decrease LH levels do not increase as would be expected. This inability of the pituitary gland to respond appropriately to a decline in testosterone suggest that alcohol has a central effect in interaction between nervous system and endocrine system [37, 38]. Alcohol do not have much effect on FSH in male rats [39].

Our findings also suggest that all the alcoholic beverages studied caused significant decreased in estrogen level in the male albino rats. This may be contrary to studies that suggested that alcohol consumption causes the aromatization of testosterone to produce more estrogen [40]. We may possible, suggest that these drinks may have a central effect on HPA-axis such that there is a total inhibitory of its reproductive activities or even the conversion of testosterone to estrogen through the aromatization testosterone may not be yielding so much, since there is a low level of testosterone itself.

3.2. Alcoholic beverages and sex hormones in female rats

There was a significant decrease in estradiol levels of the female albino rats treated with samples of some traditional alcoholic beverages. This is contrary to studies that reported that alcohol-induced a rise in oestrogen [41]. This, they attributed to a side effect of alcohol metabolism on the oxidation of oestriadiol and increased aromatization of testosterone to oestradiol [42]. Our result suggest that alcohol has a central effect on interaction between numerous system and endocrine system [37]. There was therefore a downward levels of estrogen in this study. All the alcoholic beverages significantly depressed the level of LH in female albino rats. This agrees with some study that alcohol caused an increase in hypothalamic level of LHRH and a decrease in LH levels in the blood stream [43, 44]. Our result suggests that, these alcoholic beverages may elicit their effects by inducing a decreasing in hypothalamic LHRH secretion leading to the increased hypothalamic content and may account for the decrease for the decrease in LH. All the alcoholic beverages in this study significantly depressed the FSH levels in the female rats. This is in concert with the fact that alcohol appears to prevent the synthetic LHRH stimulation of FSH during the follicular phase of the menstrual cycle in normal female rhesus monkey but not in female humans [45]. These effect can result irregularities in menstrual cycle or estrous as our results have shown [46]. Further work will be required to clarify the inhibitory role of LHRH on FSH in human.

Our findings from the study suggest that prolactin level in female albino rats were inhibited by burukutu and pito significantly. While the level of reduction for ogogoro and goskolo were not significant. This is contrary to studies that reported that ethanol causes hyperprolactinemia by eliciting prolactin release form lactotropes and it increase by
number lactotropes in the pituitary gland (De et al., 2002 [44, 47]. This study suggest that a further research may be needed to clarify the effect of these beverages on the prolactin level as it may possible that there are constituents of these drinks that may have an antagonism effect on the ethanol effect so expected. In this study, progesterone levels in female albino rats were not significantly altered in all the alcoholic beverages except for burukutu fed albino rats, this result agrees with Mendelson et al., [40]. “No change in the baseline progesterone level has been reported in pre-menopausal women administered LHRH and alcohol at both the follicles and mid initial phases of the cycles.” This suggest that, since primary function of progesterone involves pregnancy protection, and safety, it presence may not be significantly obvious in the absence of pregnancy. It therefore suggests that further research is needed to establish the effect of these alcoholic beverages on the progesterone level in pregnant and non-pregnant animals.

4. Conclusion
The toxicological evaluation of traditional alcoholic beverages pito, burukutu, ogogorogo 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 of the rats.

Compliance with ethical standards

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Disclosure of conflict of interest
All the authors hereby disclose no conflicts of interest/ competing Interests

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. One of the authors – Timothy Olugbenga Ogundeko is however licensed to handle laboratory animals.

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