Effect of Ethanol leave Extract of *Gongronema latifolium* on Female Reproductive System in Albino Wistar Rats.

An Original Research Article

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ABSTRACT

Infertility is a disease of the reproductive system which affects both men and women with almost equal frequency. It is a global phenomenon affecting an average of 10% of human reproductive age population. This study investigated the effect of ethanol leaves extract of *Gongronema latifolium* on the female reproductive system of Albino Wistar rats. Phytochemical screening was conducted using standard methods. The effect of the extract on the estrous cycle and reproductive hormones was evaluated on the experimental rats weighing 140-160g. Twenty-five matured female rats were divided into five groups of five rats each: group 1 and 2 were the normal and positive controls and were given distilled water and standard drugs respectively; while group 3, 4 and 5 were administered the ethanol extract of the plant in graded dosages of 200, 300 and 400mg/kg respectively. The phytochemical screening revealed the presence of alkaloids, tannins, saponins, flavonoid and cardiac glycosides. The hormonal results showed that the low dose and middle dose had a significant decrease ($p<0.05$) in FSH and Progesterone levels, but a significant increase ($p<0.05$) in LH and Estradiol levels ($p<0.05$). The high dose had a significant decrease ($p<0.05$) in FSH and LH but a significant increase ($p<0.05$) in Progesterone and Estradiol. The study further demonstrated that the extract caused irregularity

on the estrous cycle. It could be inferred from the results obtained that the ethanol leaves extract of *Gongronema latifolium* may interfere with the reproductive process in female rats. These effects could be as a result of the secondary metabolites present in the plant.

Keywords: Infertility, *Gongronema latifolium*, Female reproductive system, Phytochemical screening, Estrous cycle, Reproductive hormones.

INTRODUCTION

Evidence from scientific research worldwide has proven that medicinal plants have immense biological and health applicability. Some of the earliest drugs were first discovered from traditionally used plants prior to their availability as synthesized drugs. The use of medicinal plants has found application in several diseases and health conditions and reproductive health is not left out (Enitome, 2017).

Approximately 80 % of the population in Africa use traditional medicinal plants to improve their state of health. Several of these plants are used by women to relieve problems related to their reproductive health, during or after their reproductive life, during pregnancy, or following parturition. The African pharmacopoeia thus provides plants used for preventing and/or treating gynaecological infections, dysmenorrhea, irregular menstruations, oligomenorrhea or protracted menstruation, and infertility. Such plants may then be used as antimicrobials, emmenagogues, or as suppressors of uterine flow. African medicinal plants are also used during pregnancy for prenatal care, against fetal malposition or mal-presentation, retained dead fetus, and against threatened abortion. Some others are used as anti-fertilizing drugs for birth control. Such plants may exert various activities, namely, anti-implantation or early abortifacient, anti-zygotic, blastocytotoxic, and anti-ovulatory effects. Some medicinal plant could also act as sexual drive

suppressors or as a post-coital contraceptive by reducing the fertility index. A number of these plants have already been subject to scientific investigations and many of their properties have been assessed as estrogenic, oxytocic, or anti-implantation (Elumalai *et al.*, 2012).

Infertility is a disease of the reproductive system which affects both men and women with almost equal frequency. It is a global phenomenon affecting an average of 10% of human reproductive age population. Many conditions can be associated to this problem, including intrinsic (anatomic, genetic, hormonal and immunological disorders) and extrinsic factors such as sexually transmitted infections (STIs), infections after parturition or surgery, tuberculosis of the pelvis, and obesity. As an alternative to modern medicine, medicinal plants can also be used to solve part of the reproductive problems. Due to their chemical composition, many plants have showed beneficial properties in the folliculogenesis and steroidogenesis through their antioxidant properties and regulation of some enzyme of the steroidogenesis (Enitome, 2017).

The application of medicinal plant to female reproductive health is gaining interest as reproductive disorders are considered an important public health and social problem. Reproductive health problem is considered the second most prevalent health care problem in Africa (Enitome, 2017).

Medicinal plants can be used for the beneficial effects on many female reproductive processes ranging from ovulation, pregnancy, to labour induction, elimination of retained placenta and management of post-partum haemorrhage. Most often the biological effects elicited by these remedies are due to secondary metabolites that primarily act on the reproductive system. The nature of these actions may involve the modulation of uterine contractions at labour, reproductive processes such as folliculogenesis and reproductive hormone regulation. The

significant effects of these plants on the female reproductive systems are due to their antioxidant capacity, phytochemical constituent and their ability to mimic the effects of steroidogenic enzymes/hormones. Several studies showed that the plant secondary metabolites act either directly on ovarian cells to eliminate the Reactive Oxygen Species (ROS) or through action on several enzymes such as catalase, glutathione, superoxide dismutase and glutathione peroxidase (Nordeng *et al.*, 2013).

Female reproductive health issues have been observed to significantly affect the population apart from its immediate effect as there will be decrease in birth rate and workforce. It is therefore considered as an important public health and social problem.

Gongronema latifolium is a flowering plant of the order Gentianales and the family Apocynaceae, sub family Asclepiadoideae. It is a tropical climbing plant distributed mainly in the tropical and subtropical regions of Africa, Asia and Oceania (Osuagwu *et al.,* 2013). *G. latifolium* is commonly known by the Ikales of Ondo state of Nigeria as Iteji. The Igbos called it Utazi, the Efik/Ibibio called it Utasi while Yorubas called it Arokeke or Madumaw. In Ghana, Senegal and Sierra leone, it is called akan-asantes, gasub and ndondo-polole respectively. The common name for the plant is Amaranth globe while the English name is Bush bock (Bassel *et al.,* 2019). The leaves of *G. latifolium* are used as vegetables and spice to which they add bitter-sweet flavour. The leaves are sometimes used to spice locally brewed beer. The soft stem is used as chewing stick in Sierre Leone (Mosango, 2019). *G. latifolium* is used in small quantities in preparing local soups like Nsala soup, Ugba soup and yam and also in garnishing dish like Abacha, Ncha, Isi Ewu and Nkwobi (Mosango, 2019).

The estrous cycle is the main reproductive cycle of other species females of non-primate vertebrates, for example rats, mice, horses, pig have this form of reproductive cycle. The estrous cycle comprises of the recurring physiologic changes that are induced by reproductive hormones in most mammalian placental females. Humans undergo a menstrual cycle instead. The cycle starts after puberty in sexually mature females and are interrupted at anaestrous phase or pregnancies (Ajayi and Akhigbe, 2020.).

This research work seeks to find solution to female reproductive health issues through the studies on a potential natural plant-based therapy as reproductive performance and processes is definitely influence by food and types of nutrition. Plant based nutrition has been observed to significantly improve female reproductive health. Thus, the study of the effect of *G. Latifolium* on the female reproductive system since this plant is commonly used by most Africans as spice and in the preparation of soup.

MATERIALS AND METHOD

Collection and Identification of Plants

Fresh but matured *G. latifolium* leaves were purchased from a local market at Itam Local Government Area of Akwa-Ibom State, Nigeria in October, 2019. The plant was identified and authenticated by Prof. (Mrs.) Uduak Eshiet of the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. It was deposited at the Faculty of Pharmacy Hebarium with a voucher number UUPH9(a).

Preparation of the Leaf Extract

The wet method of extraction was used for the extraction. The leaves were plucked from the plant stalk, thereafter the leaves were washed and drained to remove the debris and 500g weight of the leaves were cut into pieces and immersed into 2.5L of 50% ethanol and kept in a transparent plastic rubber for 72 hours. It was stirred thrice each day. At the end of the three days the mixture was filtered using a cheese cloth and then with a funnel and No. 2 Wattman filter paper. The filtrate was then put in a beaker and concentrated in a water bath at 31^{o}C; it yielded 25g of extract and was stored in a refrigerator until when needed for analysis.

Phytochemical Screening

The extracts were subjected to phytochemical screening as follows;

Test for Alkaloids

About 0.5g of the extract was stirred with 5ml of 1% aqueous hydrochloric acid on a water bath. 1 ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated similarly with Dragendorff's reagent. Turbidity of precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract being evaluated.

Test for Saponin (Frothing Test)

The plant extract (0.5g) was shaken vigorously with distilled water in a test tube. Frothing on warming and persisting for more than 15 minutes was taken as preliminary evidence for the presence of saponins (Sofowora, 1993).

Test for Tannins (Ferric chloride test)

The plant extract (0.5g) was stirred with 10ml of distilled water and filtered. 5ml of ferric chloride was added to the filtrate. A blue black, green (blue green) precipitate was taken as evidence for the presence of tanins.

Test for Flavonoids

0.5g of the extract was stirred in 10ml of distilled water and filtered. Then 5ml of concentrated hydrochloric acid was added. Crimson coloured precipitate was taken as evidence for the presence of flavonoids (Trease and Evans, 2002). Further test was done for confirmation using Shinoda's reduction test in which few pieces of magnesium metal were added to 5ml solution of the extract and concentrated hydrochloric acid was added.

Test for Cardiac Glycosides (Salkowski's test)

The plant extract of about 0.5g was dissolved in 2ml of chloroform. Concentrated sulfuric acid was carefully added by running down the side of the tube. A reddish-brown colour at the interface indicated the presence of a steroidal ring (that is the aglycone portion of the cardiac glycosides).

Test for Anthraquinones

Borntrager's test was used for the detection of anthraquinones. 5g of the plant extract was shaken with 10ml benzene, filtered and 5ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of free hydroxyl-anthraquinones.

For bound anthraquinones, 5g of the plant extract was boiled with 10ml aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of benzene, the benzene layer separated

and half its own volume of 10% ammonia solution added. A pink, red or violet colouration in the ammonia phase (lower phase) indicated the presence of anthraquinone derivatives in the extract.

Experimental Animals

A total of twenty adult female Albino Wistar rats weighing 140g – 160g were used. The animals were randomly grouped into five (5) groups of five (5) rats each. They were housed in a ventilated room in wooden cages with wire mesh top and maintained under standard conditions of humidity (50±5%) and temperature (28±2^{o}C) and 12 hours light/12 hours dark cycle and acclimatized for two weeks. The animals were fed with grower's pellet feed and water *ad-libitum* throughout the experimental period of fourteen days. Approval for the use of animals was given by the Ethical Committee on Animal Handling of University of Uyo, Nigeria.

Experimental Design

The animals were divided into five (5) groups of five (5) rats each. All administration began at the late pro-estrus phase of estrus cycle in the experimental rats. The animals in group one and two serve as the normal and positive controls and were administered distilled water and 17-B-estradiol orally respectively for fourteen days. Group 3, 4, and 5 were administered three graded doses of *Gongronema latifolium* as follows 200, 300, 400mg/kg respectively for fourteen days via the oral route as shown on table 1.

Table 1: Experimental Design

Groups	No of animals	Treatment	Dosage

1. Normal Control	4	Distilled water	5ml/kg
2. Positive Control	4	Estradiol (E2)	400mg/kg
3. Low Dose	4	Extract of *Gongronema latifolium*	200mg/kg
4. Middle Dose	4	Extract of *Gongronema latifolium*	300mg/kg
5. High Dose	4	Extract of *Gongronema latifolium*	400mg/kg

Administration of the extract was done orally. The vaginal smears were observed daily to monitor the estrous cycle. Vaginal smear was prepared every day at constant interval of 9:00 - 11:00am for 12 days (3 cycles). Dropper pipette containing normal saline was used for collection of the smear from the vaginal lumen by introducing the normal saline into the vagina using the dropper pipette, then dragging out fluid from the vaginal lumen which was used to make an impression smear on a clean microscope slide. Eosin stain was used in staining the cells.

The smeared slides were viewed under the microscope using 40* objective lens. The relative proportions of the recognised cells were used to determine the phases of the estrous cycle.

At the end of the 14 days, feeds were withdrawn from the animals and they were fasted overnight but with free access to water. They were then euthanized under chloroform vapour and sacrificed. Immediately whole blood was collected for sera preparation via cardiac puncture using sterile syringes and needles, emptied into plain tubes and allowed to clot for about two hours. Centrifugation of clotted blood was done using bench top centrifuge (MSE Minor, England), serum was separated with sterile syringes and stored frozen until needed for the female reproductive hormonal analysis.

Biochemical Assay

Hormonal assay was carried out using blood samples obtained after sacrifice of the experimental rat. Hormonal analysis was done using sandwich Elisa test which is based on the principle of a solid phase enzyme- linked immunosorbent assay. The essential reagent required for the immunoenzymometric assay includes high affinity and specific antibodies with different and distinct epitopes recognition in a native antigen. In this analysis, the immobilization takes place during the assay at the surface of the microplate well. Hormonal assay was done for Follicle Stimulating Hormone (FSH), Lutenizing Hormone (LH), progesterone and estrogen.

Statistical Analysis

The results were analysed with one-way ANOVA using SPSS. All data were expressed as mean ± SEM and values were considered significant at values $p<0.05$.

RESULTS AND DISCUSSION

Results

Phytochemical Screening

The phytochemical screening carried out on the ethanol extract of *G. latifolium* showed the presence of alkaloids, saponins, flavonoids, tannins and cardiac glycosides. However, combined and free anthraquinones were absent. The results are shown in table 2.

Table 2: Phytochemical Screening Results

TEST	OBSERVATION	INFERENCE

Akaloids	Creamy precipitate	++
Saponins	Persistent foaming	++
Tanins	Blue black colouration	++
Flavonoids	Yellow colouration	++
Cardiac Glycosides	Ring Formation	++
Free Anthraquinone	No pink colouration	-
Combined Anthraquinones	No pink colouration	-

Key: - Absent, ++ Present

Effect of Extract on the Estrous Cycle of Experimental Rats

The effect of the extract on the estrous cycle is shown in Fig. 1. It was observed that out of the twelve phases of three cycles observed in each group of four animals: 66.7% of the phases were altered for animals in group two (Low dose) while 68.75% and 56.25% of the phases were altered for animals in group three (middle dose) and group four (high dose) respectively. However, no alteration was observed for the animals in group one (control group).

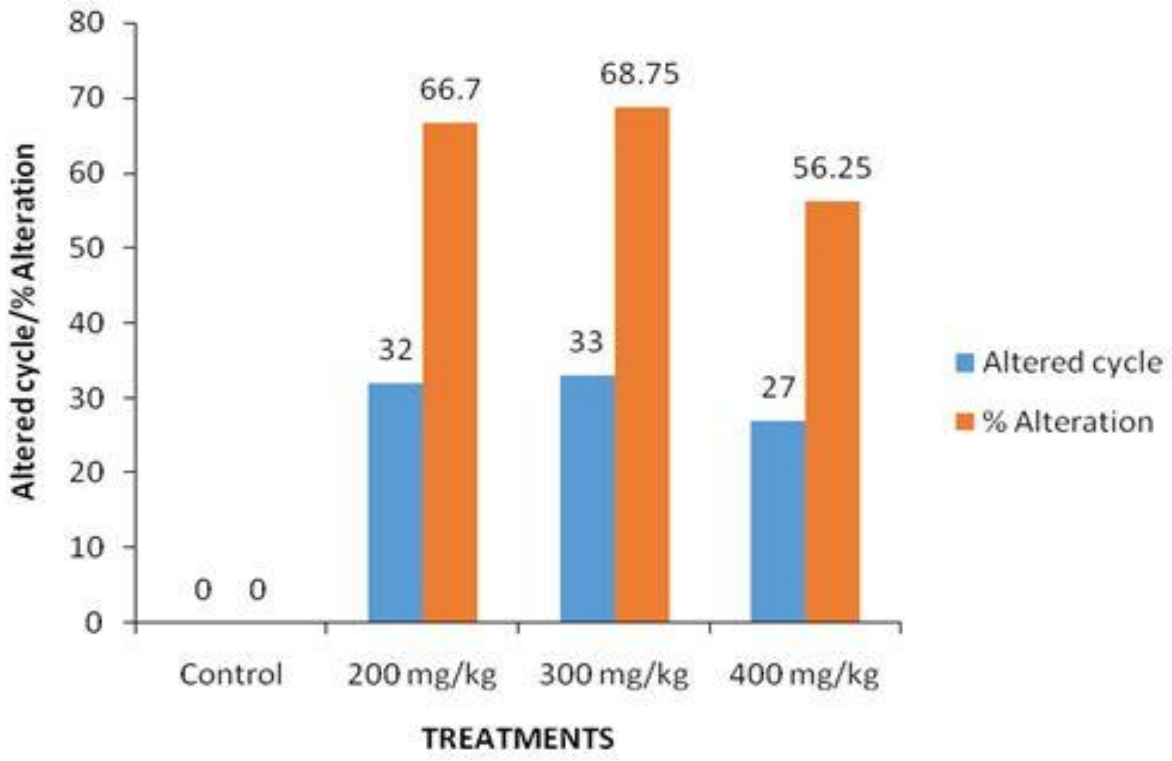

Figure 1: Percentage Alteration of Estrous Cycle against dose of Extract

Effect of Extract on Sex Hormones in Females

The female reproductive hormones were assayed biochemically and the effects of extract on the female sex hormones were as shown in figure 2, 3, 4, 5.

Follicle Stimulating Hormone: The extract showed significant decrease ($p<0.05$) of FSH when compared to controls.

Lutenizing Hormone: Low dose and middle dose had significant increase ($p<0.05$) but high dose had significant decrease ($p<0.05$) when compared to both normal control and positive control.

Progesterone: Low dose, middle dose and standard drugs had significant decrease ($p<0.05$) while the high dose had a significant increase ($p<0.05$) when compared to normal control.

Estradiol: All doses of the extracts showed a significant increase ($p<0.05$) in estradiol when compared to the controls.

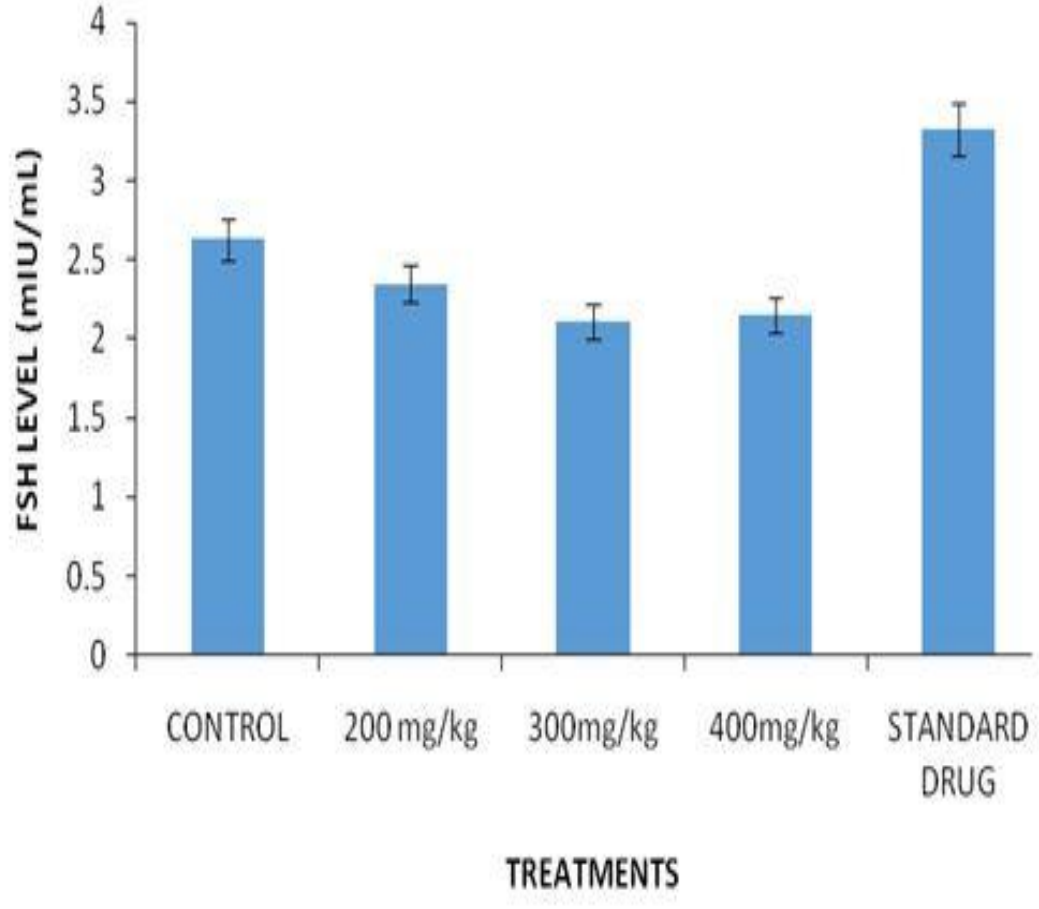

Figure 2: Effect of *G. latifolium* leaves extract on Follicle Stimulating Hormone

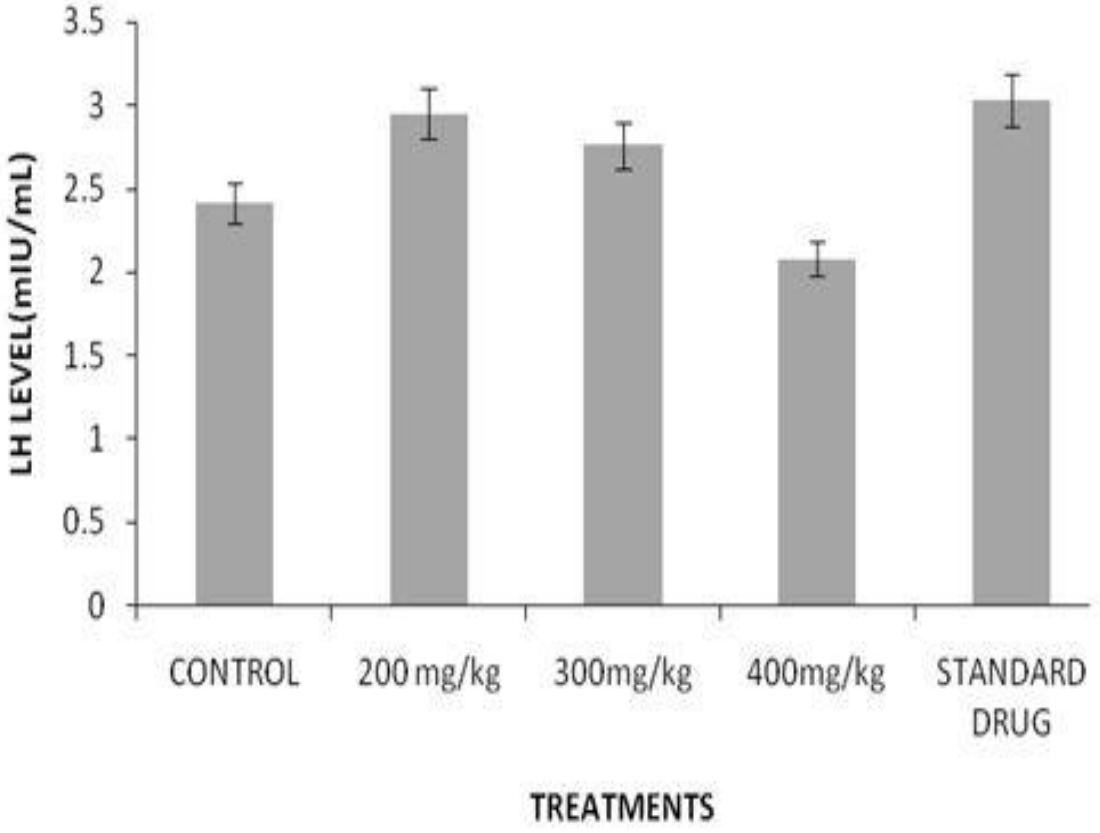

Figure 3: Effect of *G. latifolium* leaves extract on Lutenizing Hormone

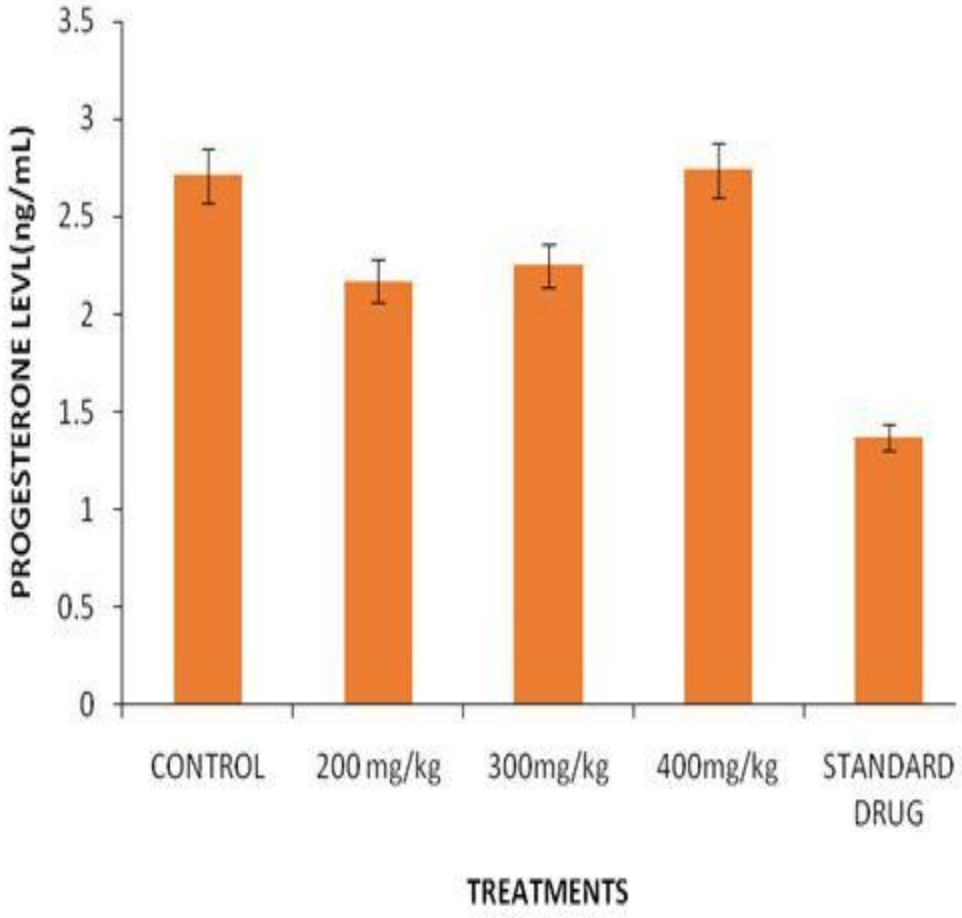

Figure 4: Effect of *G. latifolium* leaves extract on Progesterone

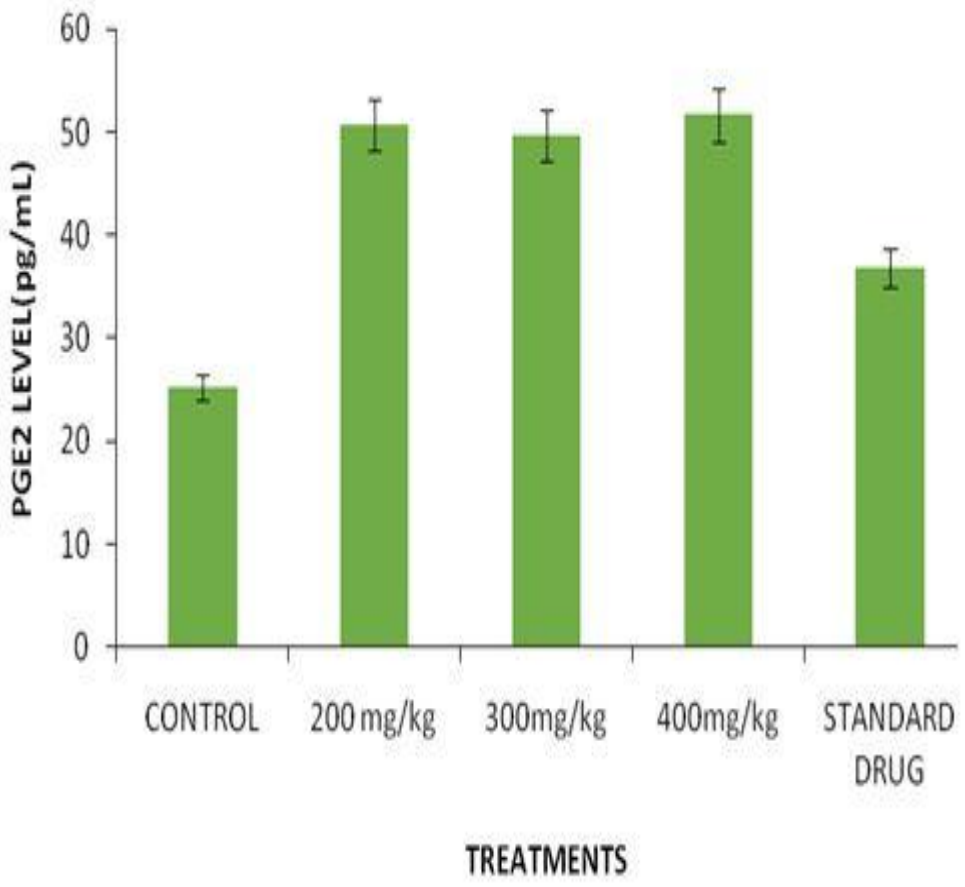

Figure 5: Effect of *G. latifolium* leaves extract on Estradiol

Discussion

The phytochemical screening of the extract revealed the presence of tannins, saponins, alkaloids, flavonoids and cardiac glycosides which was in line with the research report of Nwanjo *et al.,* (2016). Medicinal plants can be used for the beneficial effects on many female reproductive processes ranging from ovulation, pregnancy, to labour induction, elimination of retained placenta and management of post-partum haemorrhage. Most often the biological effects elicited

by these remedies are due to secondary metabolites that primarily act on the reproductive system and their ability to mimic the effects of steroidogenic enzymes/hormones as seen in this research result. The nature of these actions may involve the modulation of uterine contractions at labour, reproductive processes such as folliculogenesis and reproductive hormone regulation. Several studies showed that the plant secondary metabolites act either directly on ovarian cells to eliminate the Reactive Oxygen Species (ROS) or through action on several enzymes such as catalase, glutathione, superoxide dismutase and glutathione peroxidase (Nordeng *et al.*, 2013).

The alteration of estrous cycle observed with the administration of this extract indicated its potential to interfere with fertility. This experimental result showed a high percentage of estrous cycle alteration as compared to the normal control which was similar to the works of (Aritonanga *et al*., 2017; Ajayi and Akhigbe, 2020). The observed increase in estrous cycle and decrease in estrous cycle in some cycles implies impaired fertility. The prolongation of the estrous cycle is an indication of impairement of ovulation. A prolonged proestrus suggests a disturbance in fertility which is a sign of fertility disorder probably caused by the presence of tannin component in the *G. latifolium* extract. Tannin has an anti-inflammatory property which inhibit the COX (Cyclooxgenases) enzymes thereby preventing prostaglandins synthesis hence blocking ovulation (Mustapha *et al.*, 2011).

The effect of the extract on reproductive hormone was observed to be dose dependent. The overall functioning of the reproductive system is largely influenced by physiologic/endocrine hormones. Toxicant that interferes with reproductive function can act directly on the organs of the reproductive system or indirectly through demonstrating its influence at the hypothalamic or/ and pituitary *gland* level.

FSH stimulates the growth and maturation of the ovarian follicles by acting directly on the receptors located on the granulosa cells. The observed reduction in its level which was in tandem with the works of the following researchers (Aritonanga et al., 2017; Dasofunjo *et al*., 2020) may impair the process of folliculogenesis and delay maturation of the follicle.

LH stimulates the secretion of sex steroids from the gonads and it surge in females stimulates ovulation. Reduction of its serum level could disrupt ovulation either by decreasing the number of mature follicles or altering the pattern of estrous cycle. Therefore, the observed reduction in the serum LH levels may be due to the inhibitory effects of the extract on LH release, which then disrupt ovulation process in the treated rats.

The decreased in progesterone level is probably caused by poor synthesis of deficient corpus luteum resulting from lack of ovulation or a direct toxic effect on the corpus luteum.

The increase in estrogen is responsible for the prolonged pro-estrus phase in some cycles which is also an alteration of the cycle, as estrogen is the dominant hormone in the proestrus phase.

These findings agree with result of other work which reported a decrease of release of LH and FSH in rats treated with various dosages of different extracts (Sheeja *et al,* 2012; Essiet *et al.,* 2016).

Conclusion

Vaginal mucosal changes are driven by estradiol which is the main hormone. Progesterone role in vaginal mucosa epithelium is slightly clear although, our data suggest it may have mild consequences on estradiol of vaginal mucosa. Estrogen increases leads to prolonged pro-estrus phasein some cycles thus, the alteration of the cyclebeing that estrogen is dominant hormone in

proestrus phase. It could be inferred from the results obtained that the ethanol leaves extract of *Gongronema latifolium* might interfere with the reproductive process in female Albino Wistar rat. These effects could be as a result of the secondary metabolites present in the plant.

Recommendation

Further research is therefore recommended on the leaves of *Gongronema latifolium* to determine the specific fraction that is most efficacious on the female reproductive system.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Enitome B. (2017). Potentials for use of medicinal plants in female reproductive disorders- the way forward. *African journal of reproductive health.* **24**(4): 3-7.

2. Elumalai A., Eswariah M. and Chinna M. (2012). Herbalism- A review. *International Journal of Phytotherapy.* **2**(2):96-105.

3. Nordeng H., Al-zayadi W., Diallo D., Ballo N. and Paulsen B. (2013). Traditional medicine practitioner's knowledge and views on treatment of pregnant women in three regions of Mali. *Journal of ethnobiology and ethnomedicine.* **9**: 67.

4. Osuagwu A., Ekpo I., Okpako E., Otu P. and Ottoho E. (2013). The biology, utilization and phytochemical composition of fruits and leaves of *Gongronema latifolium* Benth *Agrotechnology Journal.* **2**:3-10.

5. Bassel A., Miriam A., Idris B. and Yusoff N. (2019). Safety assessment of the ethanolic extract of *Gongronema latifolium* Benth. Leaves: a 90 days toxicity study in Sprague dawley rats. *BMC Complementary and Alternative Medicine.* **19**(152):21-27.

6. Mosango D. (2009). *Gongronema latifolium*: Plant Resources of Tropical Africa (PROTA). Retrieved 11th November 2019 18:15pm from http://www.prota4u.org/search.asp

7. Ajayi, A.F., Akhigbe, R.E. (2020). Staging of the estrous cycle and induction of estrus in experimental rodents: an update. *Fertil Res and Pract.*, **6**, 5

8. Sofowora A. (1993). Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd; Screening Plants for Bioactive Agents; pp. 134–156.

9. Trease GE, Evans WC. (2002). Pharmacognosy. 15th Ed. London: Saunders Publishers; pp. 42–44. 221–229, 246–249, 304–306, 331–332, 391–393.

10. Nwanjo H., Okafor M. and Oze G. (2016). Anti-lipid peroxidative activity of *Gongronema latifolium* in streptozotocin- induced diabetic rats. *Nigerian journal of physiological sciences.* **21**(1):61-65.

11. Aritonanga, TR; Rahayob, S; Siraitc, LI; Karod, MB; Simanjuntake, PS; Natzirf, R; Sinrangg, AW; Massih, MN; Hattai , M; Kamelia E. (2017). Role of FSH, LH, Estradiol and Progesterone Hormone on Estrus Cycle of Female Rats. *International Journal of Sciences: Basic and Applied Research (IJSBAR)*, **35**(1): 92-100.

12. Ajayi, A.F., Akhigbe, R.E. (2020). Staging of the estrous cycle and induction of estrus in experimental rodents: an update. *Fertil Res and Pract.*, **6:**5

13. Mustapha A. R., Bawa E. K., Ogwu D., Abdullahi U. and Kaikaba A. (2011). Effects of ethanol extract of *Rhynchoosia sublobata* on estrous cycle in wistar rats. *International Journal of Medicinal Aromatic Plants.* **1**(2):122-123

14. Dasofunjo K , Asuk AA and Nku CI (2020). Evaluating the effect of ethanol leaf extract of

Gongronema latifolium on some reproductive hormones of male Wistar rats, *GSC Biological and Pharmaceutical Sciences*, **12**(03):166–173

15. Sheeja E. J., Ajeet P., Papiya B. and Shilpi S. (2012). Antifertility activity of *Momordica charantia* discount pulp and seed hydroalcohol extract. *Journal of Applied Pharmacy.* **3**(4): 682-696.

16. Essiet G. A., Essien A., Akuodo G., Udoh V. and Essiet A. (2016). Effect of ethanol extract of the root, bark of *Salacia lehmbachi* on male reproductive hormones in male Albino rats. *European Journal of Medical Physics.* **15**: 1-7.