



## Effect of Ethanol Leave Extract of *Gongronema latifolium* on Female Reproductive System in Albino Wistar Rats

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### ABSTRACT

Reproductive system has infertility as a disease that affect both sex at equal frequency. A ten percent population of human reproductive age are affected with the phenomenon globally. This study investigated how ethanol crude leaves of *Gongronema latifolium* affect the reproductive system of female Albino Wistar rats. Phytochemical screening was conducted using standard methods. The extract effect on reproductive hormones and estrous cycle were ascertained on experimental rats weighing 140-160g. Matured females; twenty-five in numbers were equally shared into five groups: The normal and positive controls were groups 1 and 2 which were administered distilled water and standard drug; On the other hand crude ethanol leaves of *Gongronema latifolium* were given to the last three groups in a 200, 300 and 400 in mg/body weight i.e mg/kg. Phytochemical profile revealed saponins, alkaloids, tannins, flavonoids including cardiac glycosides. Hormonal results indicated that the low dose and middle dose were decreased significantly ( $P = .05$ ) in both FSH and Progesterone levels, but increased significantly ( $P = .05$ ) in LH and Estradiol levels. The high dose had a significantly decreased ( $P = .05$ ) FSH and LH but a significantly increased ( $P = .05$ ) Progesterone and Estradiol. The study further demonstrated irregularities of estrous cycle as an effect of the ethanol crude *Gongronema latifolium* leaves. Also, the crude leaves might distort the process of reproduction of female Wistar rats. The secondary metabolites in *Gongronema latifolium* leaves could have caused these effects.

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## 1. INTRODUCTION

Proofs by the evidence from worldwide scientific research has it that immense health and biological application are found in plants. The availability of traditionally used plants was initially discovered by earliest drugs as synthesized drugs. Medicinal plants usage are found in several applications of health and disease conditions including reproductive health [1].

Eighty percent of the African population improves their health state by using medicinal plants. Women relieves reproductive related problems using most plants in their period of reproductive life, during pregnancy or parturition. African pharmacopoeia thereby gives plants as gynaecological dysmenorrhea, infections, oligomenorrhea, infertility, irregular menstruations and protracted menstruation.

Plants thus could be utilized as antimicrobials, suppressors or emmenagogues of uterine flow. Medicinal plants from Africa could be used to prevent fetal malposition or mal-presentation, care for prenatal or against threatened abortion and retained dead fetus. These medicinal plants serve as birth control via anti-fertilizing drugs exerting various activities like; early abortifacient or anti-implantation, blastocytotoxicanti, anti-ovulatory and anti-zygotic effects. The fertility index can be reduced by some medicinal plants as they may exert post-coital contraceptive or sexual drive suppressors activities. Most of these plants' properties had been ascertained as oxytocic, anti-implantation or estrogenic as they were investigated scientifically [2].

Infertility is almost affecting women and men at an equal frequency as a disease of the reproductive system; an average of 10% of human reproductive age are affected as a global phenomenon. Intrinsic (hormonal, anatomic, immunological and genetic disorders) and extrinsic factors like infections after surgery or parturition, sexually transmitted infections (STIs), obesity and tuberculosis of the pelvis are the many conditions that could be associated with this problem. As an alternative to modern medicine, an application to solving reproduction problems is the use of medicinal plants, achieved by their chemical content, beneficial properties of most plants shown in steroidogenesis and folliculogenesis via their ability to regulate some enzymes and antioxidant properties [1].

The consideration of reproductive disorders as a social problem and public health has gained interest as female reproductive health is solved using medicinal plant. Reproductive health problem is Africa 's most prevalent health care problem considered as second [1].

Many female reproductive processes gained beneficial effects from the use of medicinal plants which range from ovulation, labour induction to post-partum haemorrhage management. Biological effects most oftenly elicited by these remedies act on the reproductive system due to the primary action of secondary metabolites. These actions's nature could involve uterine contractions modulation at labour reproductive process like the regulation of reproductive hormone and folliculogenesis.

The significant effects of these plants on the female reproductive systems are because of their capacity of antioxidant, phytochemical constituent including mimic effect's ability on steroidogenic enzymes/hormones. Plant secondary metabolites in many studies indicate direct act on cells of ovary in eliminating the Reactive Oxygen Species of several enzymes action such as superoxide dismutase, glutathione peroxidase, glutathione and catalase [3].

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 mostly found in the Asia, Oceanic and Africa's tropical and subtropical region [4]. *G. latifolium* is commonly known by the Ikales of Ondo state of Nigeria as Iteji. The Igbo called it Utazi, the Efik/Ibibio called it Utasi while Yorubas called it Arokeke or Madumaw. Senegal calls it gasub, Ghana calls it akan-asantes while Sierra leone call it ndondo-polole.

Amaranth globe is a common name for *G. latifolium* and Bush bock is its English name [5]. The plants' leaves with bitter-sweet taste flavour are eaten as vegetables and spice.

The leaves are sometimes used to spice locally brewed beer. The soft stem is used as chewing stick in Sierra Leone [6]. *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 [6].

Females in other species of non-primate vertebrates has their main reproductive cycle as estrous cycle with typical examples like mice, pigs, horses and rats. Physiological changes reoccur in estrous cycle which are induced usually in most mammalian placental females by reproductive hormones. Whereas, menstrual cycle is undergone by human females; where puberty is the onset of the cycle in females that are matured usually interrupted by pregnancy or at anaestrous phase. [7].

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.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Plants

Matured leaves of *G.latifolium* that were fresh were gotten from the main market in Itam, in Akwa Ibom state, Nigeria in October, 2019. Prof. Uduak Eshiet (Department of Botany and Ecological Studies, University of Uyo, Nigeria.), identified and authenticated the plant. A voucher number of UUPH9 (a) was given to the plant and it was deposited at Faculty of Pharmacy Herbarium.

### 2.2 Preparation of the Leaf Extract

Extraction was done using the wet method. Plucked leaves from plant stuck were washed, drained to remove dirt and 500g of the leaves were cut into pieces and poured into a 2.5L transparent plastic container filled with 50% ethanol and kept for 72 hours. It was stirred thrice a day. The mixture was filtered after 72

hours using first a cheese cloth and later using a funnel and a Number 2 Wattman filter paper. Filtrate were decanted into a 50cl beaker, concentrated at 31°C to yield 25g of extract which was stored in a refrigerator until when needed for administration to the experimental animals.

### Analysis of phytochemicals

Phytochemical screening for terpenoids, anthraquinones, alkaloids, saponins, tannins, flavonoids, and steroids were conducted in accordance with standard method were conducted as follows;

### 2.3 Test for Alkaloids

About 0.5g of extract was stirred with 5ml of 1% HCL on a steam bath and then filtered. A few drops of Mayer's reagent were used to treat 1ml of filtrate, also a few drops of Dragendorff's reagent were used to treat a second 1ml of filtrate. An orange turbidity precipitate indicated the presence of alkaloids.

### 2.4 Test for Saponin (Frothing Test)

For saponins, the frothing test was used. About 0.5g of extract was mixed with 15ml of distilled water and shaken vigorously for 5 minutes. The formation of a stable froth persisting for more than 15 minutes indicated the presence of saponins [8].

### 2.5 Tannins (Ferric chloride) Test

For tannins, 0.5g of extract was dissolved in 10ml of distilled water and filtered. 5ml of 1% ferric chloride solution were added to the filtrate. Formation of a blue-black or blue-green colour indicated the presence of tannins.

### 2.6 Flavonoids Test

Trease and Evans, 2002 [9] method was used to determine the presence of flavonoids; 10ml of distilled water was poured into a beaker containing 0.5g extract stirred and filtered. Concentrated hydrochloric acid of 5ml added and a precipitate of crimson colour indicate the presence of flavonoids. confirmation test was done using Shinoda's reduction test in which magnesium metal of few pieces and concentrated hydrochloric acid were added to 5ml solution of extract.

## 2.7 Cardiac Glycosides test (Salkowski's)

2ml of chloroform was used to dissolve .5g extract, concentrated sulfuric acid was added carefully running down the tube's side. At interface, a reddish – brown colour indicate the presence of Cardiac Glycosides as aglycone portion in steroid ring.

## 2.8 Anthraquinones Test

Detection of anthraquinones was done using Borntrager's test; 10ml benzene used to shake 5g of the extract and filtered. To the filtrate, 5ml of 10% ammonia solution was added and the mixture shaken. The presence of free hydroxyl-anthraquinones was indicated by the presence of a red, violet or pink colour in the ammoniacal (lower) phase. 5ml of benzene used to shaken 5ml of extract, half the volume of benzene in 10% ammonia solution was added as the benzene layer was separated. The presence of anthraquinone derivatives in the extract was indicated as a 10ml aqueous sulphuric acid was used to boil 5g of the plant extract and filtered while hot to determine for the presence of bound anthraquinones as a red, violet or pink colour in the ammoniacal (lower) phase.

## 2.9 Animals for the Experiment

Twenty-five female adult Albino rats used weighed 140g – 160g. They were grouped randomly in five of five animals each. Rats were caged in wooden materials with wire mesh top and kept in a well-ventilated room while maintaining standard conditions of humidity ( $50 \pm 5\%$ ) and temperature ( $28 \pm 2\%$ ) and also 12 hours light /dark cycle. Animals were acclimatized for two weeks before commencement of experiment. They were fed with feeds for grower pellet and water *ad libitum* throughout the fourteen days experimental period.

## 2.10 Design of Experiment

Five (5) groups of rats with five (5) rats each were all administered substances according to their groups with the extract, distilled water and standard drug only at beginning of the late pro-estrus phase of estrus cycle in the experimental rats. The animals in group one and two serve as the normal control and positive control, administered distilled water and 17- $\beta$ -estradiol orally respectively for fourteen days. The remaining three groups were administered leaf extract of *Gongronema latifolium* at graded doses of 200, 300, 400mg/kg respectively also for fourteen days through oral route. This is as shown on Table 1.

Daily observant of vaginal smears was done for estrous cycle monitor, with a daily fresh prepared vaginal smear usually at a constant interval time of 9 – 11am for 3cycles of 12days. Smear from the vaginal lumen was collected using normal saline in a dropper pipette by introducing the normal saline into the vagina and then drags out fluid from the lumen of vagina and was used to make an impression smear on a clean microscope slide. Eosin stain was used in staining the cells.

Objective lens of 40\* microscope was used to view the smeared slides while determining the estrous cycle phase using relative proportions of recognized cells. Animals were fasted overnight on the last day of administration but water was given *ad libitum*, by 7am the animals were euthanized under chloroform vapour and sacrificed. For the preparation of sera, whole blood was immediately collected through cardiac puncture using sterile syringes and needles, and emptied into plain tubes. The clot was allowed to stand for two hours and then centrifuged using a bench top centrifuge (MSE Minor, England), sterile syringes were used to separate serum which was frozen stored until needed for the female reproductive hormonal analysis.

**Table 1. Experimental Design**

| Groups              | No of animals | Treatment                               | Dosage   |
|---------------------|---------------|---|----------|
| 1. Normal Control   | 5             | Distilled water                         | 5ml/kg   |
| 2. Positive Control | 5             | Estradiol (E2)                          | 400mg/kg |
| 3. Low Dose         | 5             | Extract of <i>Gongronema latifolium</i> | 200mg/kg |
| 4. Middle Dose      | 5             | Extract of <i>Gongronema latifolium</i> | 300mg/kg |
| 5. High Dose        | 5             | Extract of <i>Gongronema latifolium</i> | 400mg/kg |

### 2.11 Biochemical Assay

Hormonal assay was carried out using blood samples obtained after sacrifice of the experimental rat. Hormonal analysis was done using the principle of a solid phase enzyme-linked immunosorbent assay which is the method for sandwich Elisa test. Immunoenzymometric assay required essential reagent includes specific antibodies and high affinity having distinct and different epitopes recognition in a native antigen. Here, during assay at the surface of the microplate well is when immobilization takes place. Hormonal assay was done for LH, FSH, estrogen and progesterone.

### 2.12 Statistical Analysis

One-way ANOVA using SPSS were used for data analysis of results. Mean ± SEM used to express all data with  $P \leq .05$  considered significant.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

#### 3.1.1 Phytochemical screening

Results of phytochemical analysis on ethanol leaf extract of *G. latifolium* indicate the presence of

saponins, tannins, alkaloids, cardiac glycosides and flavonoids. However, combined and free anthraquinones were absent. The results are as represented on Table 2.

#### 3.1.2 Leaf extract effect on estrous cycle of experimental animals

Leaf extract effect on estrous cycle of experimental rats is presented on Fig. 1. Observation of the three cycles of twelve phases showed that each group of five rats had; 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).

#### 3.1.3 Extract effect on female hormones

Female reproductive hormones were assayed biochemically on extract effect in rats and the results is shown on Figure 2, 3, 4, 5:

Follicle Stimulating Hormone: There was a significant decrease ( $P \leq .05$ ) of extract treated groups in FSH compared with controls.

Table 2. Results of Phytochemical Screening

| 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

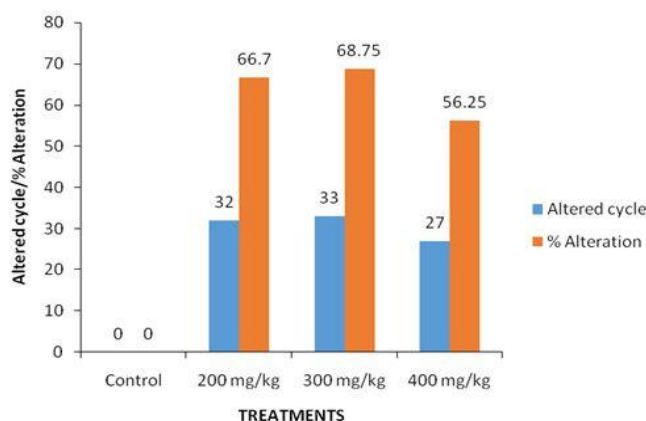


Fig. 1. Percentage Alteration of Estrous Cycle against dose of Extract

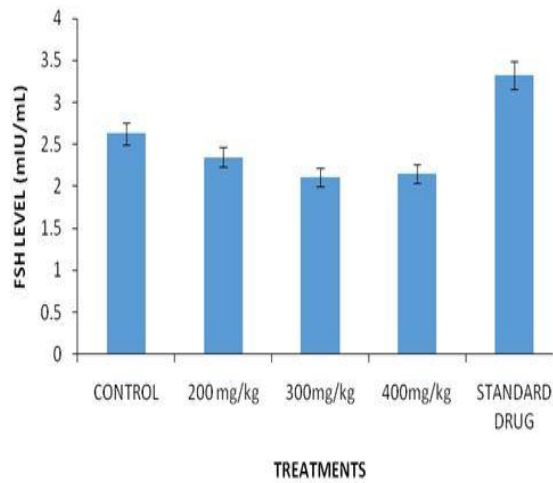


Fig. 2. *G. latifolium* leaves extract Effect on Follicle Stimulating Hormone

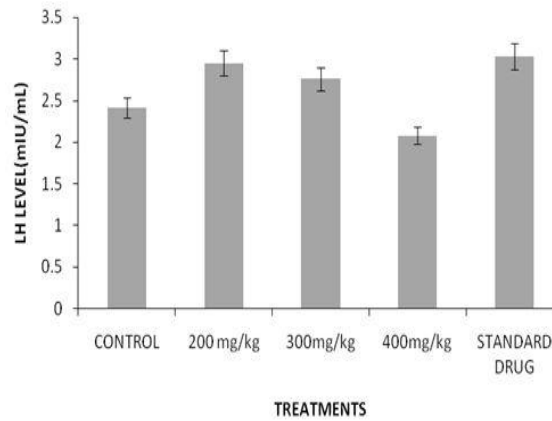


Fig. 3. *G. latifolium* leaves extract Effect on Lutenizing Hormone

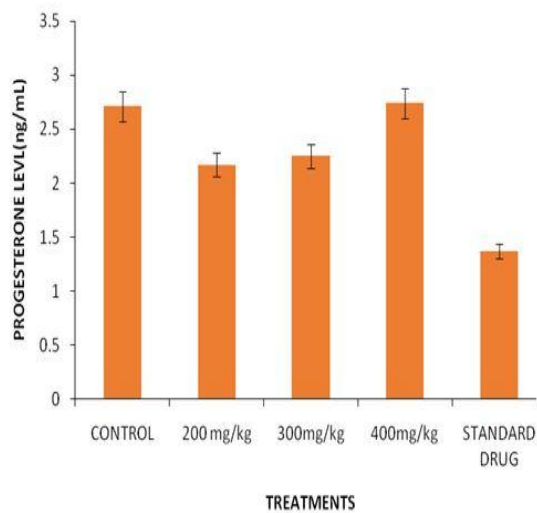
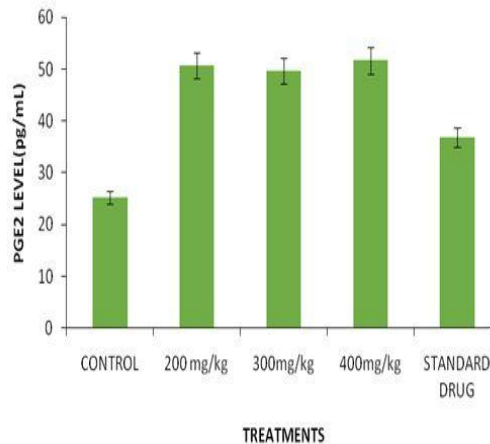


Fig. 4. *G. latifolium* leaves extract Effect on Progesterone



**Fig. 5. *G. latifolium* leaves extract Effect on Estradiol**

Lutenizing Hormone: 200 and 300mg/kg extract treated groups had significant increase ( $P \leq 0.05$ ) while groups treated with 400mg/kg rather had a significant decrease ( $P \leq 0.05$ ) in comparison with both normal and positive controls.

Progesterone: Low dose, middle dose and standard drugs treated groups had a significant decrease ( $P \leq 0.05$ ), while high dose group had an increase significantly ( $P \leq 0.05$ ) compared to the normal control.

Estradiol: All doses of the extracts treated group had a significant increase ( $P \leq 0.05$ ) estradiol when in comparison with controls.

#### 4. DISCUSSION

The extract phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, cardiac glycosides and saponins, which was in tandem with Nwanjo *et al.*, [10] research work. The beneficial effects of medicinal plants on female reproductive processes ranges from ovulation, pregnancy, to labour induction, elimination of retained placenta and management of post-partum haemorrhage. Biological effects often elicited by these remedies because of secondary metabolites that primarily act on the reproductive system, along with mimic ability effects of steroidogenic hormones/enzymes as seen in this research result. Actions involving the modulation of uterine contractions at labour, reproductive processes such as folliculogenesis and reproductive hormone regulation are natural. Studies showed that the plant secondary metabolites act either through

action on several enzymes such as glutathione peroxidase, superoxide dismutase, glutathione, and catalase or directly on ovarian cells to eliminate the Reactive Oxygen Species [3].

Administration of this extract showed that estrous cycle alteration interferes with fertility with the extract treated groups indicating a high percentage of estrous cycle alteration in comparison with the normal control. This result was similar to the works of [11,12]. 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 impairment 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 inhibited enzymes of the COX (Cyclooxygenases) thereby blocking ovulation [13].

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.

The growth and maturation of the ovarian follicles are stimulated by FSH via direct action on the receptors located on the granulosa cells. Reduced level of FSH observed in this work

which was in tandem with the following works [11,14] could impair the process of folliculogenesis and also delay maturation of the follicle. The secretion of sex steroids from the gonads may be stimulated by LH and its release in females stimulates ovulation. LH level reduction in serum could disrupt ovulation either by altering the pattern of estrous cycle or by decreasing the number of mature follicles. Thus, the reduced serum LH levels may be because of the inhibitory effects of the extract on LH release, thereby disrupting ovulation process in the treated rats.

Progesterone reduced level is probably due to poor synthesis of deficient corpus luteum resulting from a direct toxic effect on the corpus luteum or lack of ovulation.

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 results of other works by [15,16] which reported a decrease of LH and FSH in the leaf extract treated groups.

## 5. CONCLUSION

Vaginal mucosal changes are driven by estradiol which is the main hormone. Progesterone role in epithelia of vaginal mucosa may be slightly cleared but our result suggests a mild consequence of estradiol on the vaginal mucosa. Increased estrogen leads to prolonged pro-estrus phase in some cycles thus, the alteration of the cycle as estrogen is the dominant hormone in proestrus phase.

Conclusively, the ethanol leaf extract of *Gongronema latifolium* might alter the reproductive process in female Albino rat. These could be the effects of secondary metabolites present in the plant.

## ETHICAL APPROVAL

Ethical Committee on Animal Handling of University of Uyo, Nigeria gave the approval for use of animals.

## 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.

## DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used 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.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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