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THE EFFECT OF METHANOL EXTRACT OF *ZINGIBER OFFICINALE* ROSCOE (ZINGIBERACEAE) RHIZOMES ON OVULATION AND ESTRUS CYCLE OF VIRGIN FEMALE WISTAR RATS.

[1]*Essien Grace Emmanuel, [2]Tologbonse Adedayo Adedoyin and [3]Okoli Ebere Angela

[1,2,3]Department of Pharmacology and Toxicology, University of Uyo, Uyo-Akwaibom State, Nigeria.

***Corresponding Author: Dr. Essien Grace Emmanuel**
Department of Pharmacology and Toxicology, University of Uyo, Uyo-Akwaibom State, Nigeria.

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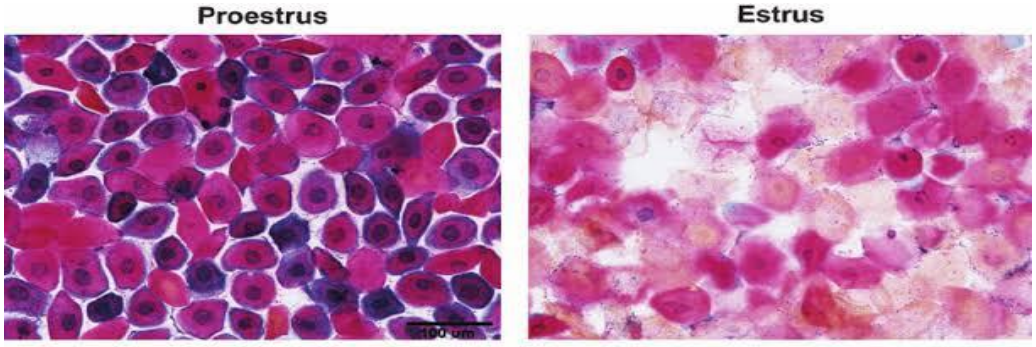
ABSTRACT
This study investigated the effects of the methanol extract of the rhizome of ginger (*Zingiber officinale*) on the estrous cycle and ovulation of sexually mature virgin female wistar rats. The effect on the estrous cycle was studied using sixteen (16) sexually matured virgin female Wister rats (140 -160g). The extract was further investigated on the effect on ovulation using sixteen (16) sexually mature virgin female rats, using standard procedures. Data obtained from the study were presented on bar chart and pie chart. The results of this study revealed that the extract caused irregularity on the estrous cycle and significantly interfered with ovulation ($p<0.05$)..It could be inferred from the results obtained that methanol extract of *Zinigiber offincinale* would interfere with the reproductive process in female wistar rats. Consequently, excessive consumption of *Zingiber officinale* could affect fertility in females. These effects could be as a result of the secondary metabolites present in the plant.

KEYWORDS: *Zingiber officinale*, oestrous cycle, ovulation, cyclicity, Wistar rats.

1. INTRODUCTION
Reproductive health is a state of physical, emotional, mental and social well-being in relation to reproduction and sexuality and not necessarily the absence of reproductive disease or infirmity.[1] It addresses the reproductive processes, functions and systems at all stages of life. Reproductive health has suddenly become a popular area in the field of research; this is because recent studies have shown that 8 to 12% of women worldwide have difficulty in conceiving, partly (8 to 22%) due to male infertility while 25 to 37% is related to female infertility problems.[2] Reproductive well-being, therefore implies that people are able to have well regulated reproductive systems, the capability to reproduce and freedom to decide if, when, and how often, to do so.).It is well known that fertility results from coordinate functioning of components of the hypothalamic- pituitary- ovarian-genital axis. In normal women, sex steroid hormones(estrogen, progesterone and Follicle Stimulating hormone, etc.) act as the mediators of these interactions.

The estrous cycle comprises the recurring physiologic changes that are induced by reproductive hormones in most mammalian placental females. Estrous cycle starts after puberty in sexually mature females and are interrupted by anestrous phase or pregnancies. Typically, estrous cycles continue until death. Some animals may display bloody vaginal discharge, often mistaken for menstruation, also called a "period".[3]



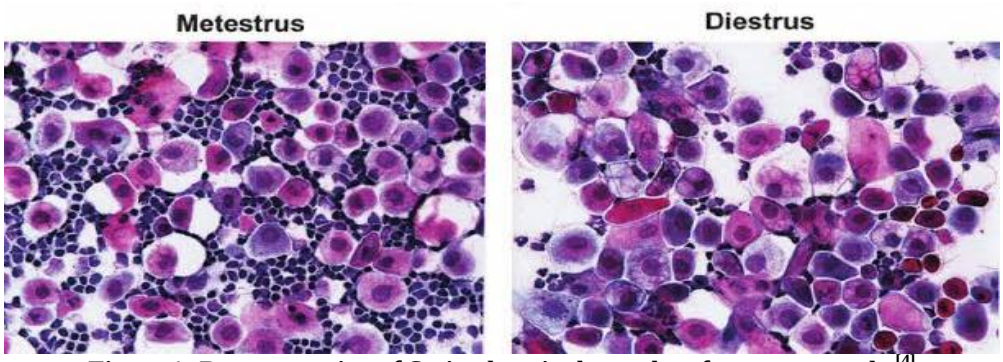


Figure 1: Representation of Stained vaginal samples of rat estrus cycle.[4]

The estrous cycle comprises four phases; in proestrus the vaginal smear when observed under a microscope is characterized by mostly large, nucleated epithelial cells[5]: in estrous (in sexual heat) phase, the vaginal smear shows predominantly cornified, squamous epithelial cells. Metestrus shows declining estrogen levels and the vaginal smear shows predominantly leukocytes[6]; whereas, diestrus is characterized by the activity of the corpus luteum that produces progesterone. In the absence of pregnancy, the diestrus phase (also termed pseudo pregnancy) terminates with the regression of corpus luteum, here the microscopic view of vaginal smear shows predominantly leukocytes.[7]

A variety of plants are claimed to have fertility promoting or inhibiting properties and a few have been tested for such effects.[8] Examples of such plants include *Fiscus asperifolia, Aspillia africana, Momordica charantia and Garcinia kola.*

Zingiber officinale

Figure 2: *Zingiber officinale* rhizome (Wikipedia).

Ginger (*Zingiber officinale* Roscoe) is a perennial herb belonging to the family Zingiberaceae, primarily grown in Asia and tropical regions, and is one of the most important and widely consumed herbs worldwide. Cultivated for its edible under-ground stem (rhizome), ginger has been used since antiquity both as a spice and as a herbal medicine to treat a variety of primarily gastrointestinal ailments, such as nausea, vomiting (emesis), diarrhea, and dyspepsia, and also diverse ailments, including arthritis, muscular aches, and fever.[9]

The rhizome of ginger contains a wide variety of biologically active secondary metabolites. The major pharmacological activity of ginger appears to be attributed to gingerols and shogaols, which are the dehydrated products of gingerols. Consequently, gingerols are the major components in the fresh ginger rhizome, whereas shogaols, especially 6-shogaol, are the most abundant polyphenolic constituents of dried ginger.[10] The Gingko (*Ginkgo biloba*), particularly standardized extract known as Gb761, appears to produce improvement in awareness, judgment and social functions in people with Alzheimer's disease and dementia.[11] Over the last few years interest in ginger or its various components as a valid preventive or therapeutic agent has increased markedly and scientific studies focusing on verification of gingers pharmacologic and physiologic actions have likewise increased.[12]

Aqueous extract of ***Zingiber officinale*** was found to cause a significant increase in weight of testis and epididymis of male rats. Dose and duration of administration dependent increase in sperm count and motility also occurred, as well as increase in serum testosterone.[13]

Administration of methanol and aqueous extract of ginger on alcoholic male rats, showed induced increase in serum luteinizing hormone, follicle stimulating hormone and testosterone levels, thus showing potential increase in mounting, mating ability and fertility.[14]

Oral administration of 250mg of ginger capsules, twice daily to 100 infertile patients for three months produced a significant reduction in semen DNA fragmentation (SDF) but no significant effect was produced in the semen parameters (semen concentration and semen motility).Showing that ginger is effective in decreasing SDF in infertile men.[15]

2. MATERIALS ND METHODS

Plant collection and identification

Fresh rhizomes of *Zingiber officinale* were bought from Akpan Andem Market, in Uyo local government area of Akwa Ibom State, Nigeria. The plants were identified and authenticated by Prof.(Mrs.) Margaret Bassey, the Taxonomist at the Department of Botany and Ecological

Studies, University of Uyo, as *Zingiber officinale* Roscoe (Zingiberaceae) by its morphological characteristics and a specimen of the plant which already existed in the University of Uyo Pharmacy Herbarium in Nigeria with voucher no: UUPH80(C).

Extraction of Plant Material

The rhizomes were washed, cut into smaller pieces, air-dried and crushed to powder using mortar and pestle. The powder weighed 486.7 g. 300 g of the powder sample was weighed and cold macerated with 1500 ml methanol for 72 hours and allowed for proper extraction. The crude liquid filtrate extract was concentrated to dryness using a water bath. The percentage yield of the methanol extract was about 39.4 %. The extract was stored at -4^{o}C until when used for the experiments.

Animals

A total of thirty-two (32) female wistar rats(140- 160g) and twenty (20) Swiss mice (20g -28g) were purchased from the animal house of the department of Pharmacology and Toxicology, University of Uyo. The animals were fed with standard rodent feeds for laboratory animals, andall the animals were allowed free access to drinking water. The animals were well acclimatized for two (2) weeks prior to the experimental administration of the extract/ drugs. They were all kept in standard condition of temperature (22^{0} C), with good ventilation. The "Principles of laboratory animal care[16] guidelines and procedures were followed in this study. The Ethical Committee of the Department of Pharmacology and Toxicology, University of Uyo, also approved this study.

The extract of *Zingiberofficinale*was subjected to phytochemical analysis for the identification of its constituents using the methods of Sofowora.[17]

The median lethal dose (LD_{50}) of the extract was estimated using the Lorks method.

Three pre-treatment estrous cycles were established by vaginal smears as described by Marcondes, to determine the rats with regular estrous cycle. Vaginal smear was prepared everyday at a constant interval of 9:00-10:00am for 8 days. Briefly, 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 is used to make an impression smear on a clean microscope slide.

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. The female rats that had undergone 4 or 5 days successful cycles were selected for this study.

For the estrous cycle, the 16 animals were divided into four groups of four animals each (each group contained virgin female rats) and treated as follows; group 1 served as control and received 10mg/kg of normal saline. Group 2 received 35.36 ml/kg of *Zingiber officinale* extract, while group 3 and group 4 received 70.71 mg/kg and 106 mg/kg of the same extract respectively for 12 days. Administration of the extract was done orally. The vaginal smears were observed daily in all the groups. Rats exhibiting a 4 or 4-5 days estrous cycle of proestrus-estrus-metestrus –diestrus were described as normal while any deviation from the pattern in terms of duration and sequence was categorized as abnormal. At the end of the treatment the animals were weighed and their weights were compared to the previous weight of the animals.

For ovulation, sixteen (16) female cycling rats were randomized and divided into four groups of four rats per group. Group 1 received normal saline (10 ml/kg) as the control; while groups 2-4 received the extract in different doses (35.36 mg/kg, 70.72 mg/kg and 106.08 mg/kg respectively). All administration occurred at the late proestrus phase. At the end of the estrous, the animals were laparectomized and the ovaries were examined with a hand lens to observe if there was any interference with the ovulation as described by Telleria.[18]

Results were presented as Mean of normal and abnormal cycles, Mean percentages(%) of altered estrous cycle and ovulations. Differences between mean values were assessed using one way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison posttest. Values of ($p< 0.05$) were considered as significant.

3. RESULTS

The phytochemical screening carried out on the methanol extract of *Zinger officinale* showed the presence of alkaloids, saponins, flavonoids, terpenes, cardiac glycosides and trace amount of anthraquinones and tannins as shown in the table below.

Table 1: Phytochemical Screening Results.

S/N	TEST	INFERENCE
1	ALKALOIDS	+++
2	SAPONINS	+++
3	TANNINS	+
4	ANTHRAQUINONES (Borntrager's test for combined anthraquinones)	++
5	FLAVONOIDS	+++
6	TERPENES	+++
7	CARDIAC GLYCOSIDES (Salkowski's test)	+++

KEY
+ TRACE
++ POSITIVE
+++ STRONGLY POSITIVE
-NEGATIVE

The median lethal dose (LD_{50}) was calculated to be 353.55 mg/kg via oral route (Lorke's method)

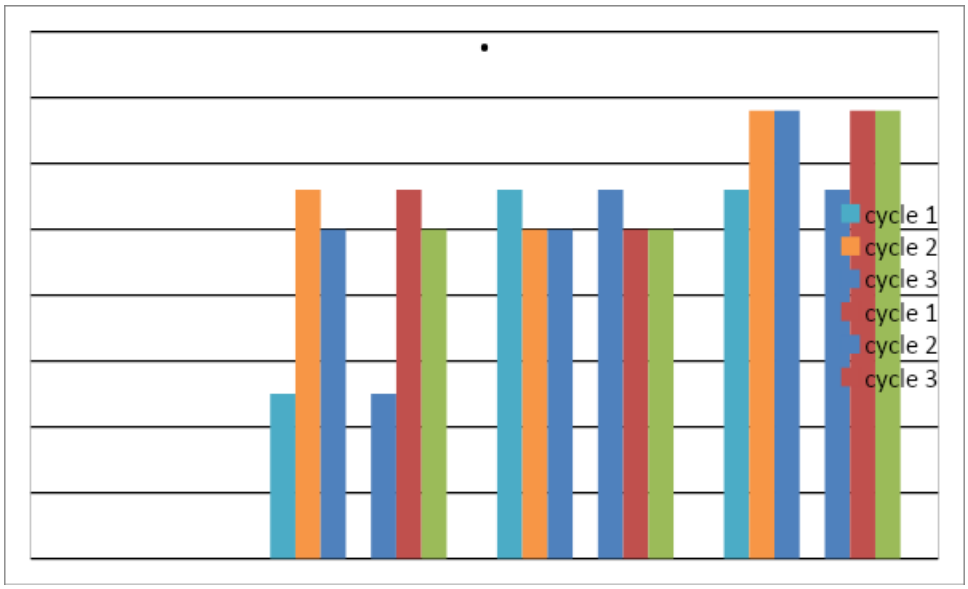


Figure 3: Effect of extract on estrus cycle.

Effect of treatment with 35.36 mg/kg, 70.72 mg/kg and 106.08 mg/kg of methanol extract of *Zingiberofficinale* on the phases of estrous cycle in adult virgin female Wistar rats.

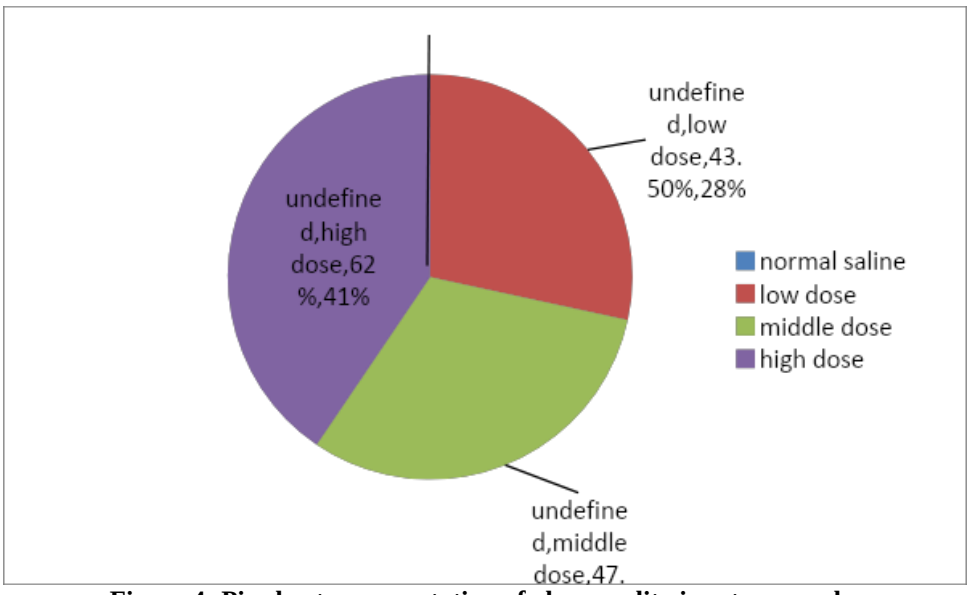


Figure 4: Pie chart representation of abnormality in estrous cycle.

Effect of treatment with 35.36 mg/kg, 70.72[a]mg/kg and 106.08[ab] mg/kg of methanol extract of *Zingiberofficinale* on the phases of estrous cycle in adult virgin female Wister rats . Values are expressed as means; significance relative to control [a]$p< 0.05$, [b]$p<0.01$.

THE EFFECT OF EXTRACT ON OVULATION OF RATS

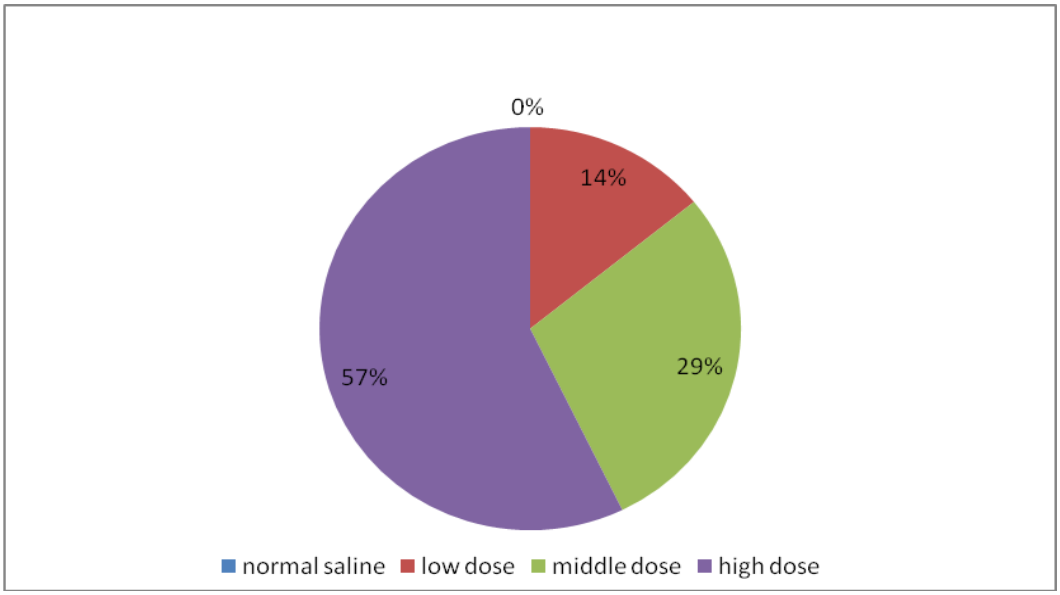


Figure 5: Pie chart showing effect of extract on ovulation. Values are expressed as means; significance relative to control [a]p< 0.05, [b]p<0.01.

Table 2: The Effect of Extract on Ovulation of Rats.

DOSE	D0	D1	INTERFERENCE
Normal saline 5ml/kg	At late estrous stage	ROS	Consistent with ovulation
	At late estrous stage	ROS	Consistent with ovulation
	At late estrous stage	ROS	Consistent with ovulation
	At late estrous stage	ROS	Consistent With ovulation(0%)
Low dose 35.36ml/kg	At late estrous stage	ROS	Consistent with ovulation
	At late estrous stage	ROS	Consistent with ovulation
	At late estrous stage	NROS	No ovulation (25%)
	At late estrous stage	ROS	Consistent with ovulation
Middle dose 70.72 ml/kg	At late estrous stage	RNOS	Evidence of ovulation
	At late estrous stage	NROS	No ovulation
	At late estrous stage	NROS	No ovulation (50%)[a]
	At late estrous stage	RNOS	Evidence of ovulation
High dose 106.08 ml/kg	At late estrous stage	NROS	No ovulation (100%)[a,b]
	At late estrous stage	NROS	No ovulation
	At late estrous stage	NROS	No ovulation
	At late estrous stage	NROS	No ovulation

Values are expressed as means; significance relative to control [a]p< 0.05, [b]p<0.01.

Table 3: Showing Percentage Alteration in Estrous Cycle of Rats

S/N	GROUP	WEIGHT RANGE (mg)	ESTROUS CYCLE	ALTERED CYCLE	% OF ALTERATION
1	CONTROL	144 – 154	Normal	0	0%
2	Low Dose (35.36 mg/kg)	150 – 155	Abnormal	20	43.7%
3	Middle Dose (70.72 mg/kg)	143 – 150	Abnormal	24	52%[a]
4	High Dose (106.08 mg/kg)	143 – 156	Abnormal	33	64%[a,b]

Values are expressed as means; significance relative to control [a]p< 0.05, [b] p<0.01.

4. DISCUSSION

The phytochemical screening of **the** extract reveals the presence of flavonoids, polyphenols, saponins, alkaloids and minute trace of anthraquinones and tannins (Table .1). Flavonoids have been reported to possess anti-inflammatory properties.[19] Ovulation is a type of inflammatory reaction and anti-inflammatory drugs were used to block ovulation[20], which might explain the reductions in the number of eggs released. Flavonoids exert their anti-inflammatory action by inhibiting cyclooxygenase-2. More so, this action had also been reported on 6-gingerol which is a major poly phenolic compound found in *Zingiber officinale.*[21]

Zingiber officinale might be blocking ovulation as reported in this study, by suppressing activity or formation of cyclooxygenase-2, hence preventing follicular rupture. It is a documented fact that rats with cyclooxygenase-2 deficiency suffer defective reproductive function as cyclooxygenase-2 plays an important role in follicular rupture.[22]

It was also observed that out of the twelve (12) phases of three cycles in each group of four animals in this study; 43.7% of the phases were altered for rats pretreated with 35.36mg/kg of extract, while increasing higher doses (70.72 mg/ kg and 106.08 mg/ kg altered 52% and 64% of the phases in groups three and four animals respectively). These effects were statistically significant when compared to the control group. The effects also occurred in a dose dependent manner ($p<0.05$) .The alteration of estrous cycle and inhibition of ovulation observed with administration of increasing doses of the extract, (70.72 mg/ kg and 106.08 mg/ kg) altered 75% and 100% of the ovulation in groups three and four animals respectively .Similarly, the interference of the extract with ovulation was dose- dependent, with the highest dose 106.08 mg/ kg exhibiting 100% inhibition (Table 2), which revealed its great potential to interfere with fertility.

The results obtained showed that methanol extract of *Zingiber officinale* altered the estrous cycle and blocked ovulation and this conclusion is supported by the fact that the estrus cycle of pretreated rats were altered and pretreated animals failed to ovulate the day following their proestrusphase. Steroidal saponins have been found to inhibit estrus cycle and reduce fertility in animals upon continuous administration.[23] The interference in estrus cycle and ovulation observed with the administration of the extract indicated its great potential in interfering with fertilization and ovum implantation.

5. CONCLUSION

This study revealed that the methanol extract of *Zingiber officinale* caused dose dependant alteration of estrus cycle and inhibition of ovulation in female wistar rats. This suggests its potential to interfere with fertility and corroborates the use of this plant by the locals for treatment of dysmenorrhea and inflammation.

Compliance with ethical standards

ACKNOWLEDGMENTS

The technical advice of the Laboratory technologist Ms. Sifon Akpan is highly appreciated.

Ethical approval

Approval for the use of the animals for the study was obtained from The Animal Ethics Committee of the Faculty of Pharmacy, University of Uyo Nigeria. All Animal experiments were conducted in accordance with Internationally accepted Laboratory Animal use and care of Laboratory Animals (1966) as adopted and promulgated by the National Institute of Health (NIH) Publication Number 85 23 Revised 1996 based on the Helsinki Convention and Guidelines and Rules of the Faculty of Pharmacy, University of Uyo for Animal Experimentation.

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