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Contraceptive Potential of Methanol Leaf Extract of *Ananas comosus* In Female Wistar Rats

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Abstract

Background: Traditionally, *Ananas comosus* leaf is used locally to prevent pregnancy among the indians.
Objective: To investigate the effect of *Ananas comosus* methanol leaf extract on oestrus cycle and ovulation
Study Design: The study was conducted using 20 mature female rats with regular oestrus cycle. The three experimental groups of 5 animals each received a stat dose of 485.5mg/kg, 970 mg/kg and 1455.5 mg/kg body weight respectively while the control group received 1ml of normal saline. Doses were gotten from LD_{50}. All administrations were done intraperitoneally at proestrus and vaginal smear were examined for 8 days to investigate the effects of the extract on oestrus cycle and ovulation.
Result: Ovulation was prevented, oestrus cycle disrupted with prolongation of the dioestrus phases and number of mature follicles reduced on ovary histology
Conclusion: The study showed that *Ananas comosus* methanol leaf extract prevented ovulation from occurring and caused irregularity of the oestrus cycle with the disruption of fertility hormones.

Keywords: *Ananas comosus* leaf, ovary, oestrus cycle, contraceptive, pregnancy, ovulation

Introduction

Consistent ovulation inhibition offers effective contraception.[1] The mechanism through which contraception is achieved may affect a woman's choice of contraceptives.[2] More than half of the women in Hong Kong in a survey preferred inhibition of ovulation as a method of contraception.[2] Despite the increase in contraceptive awareness and supply, the use of modern contraceptive is relatively low with an unmet need for contraception in West Africa of 30-37%, while it was 27.8% in Nigeria.[3] The rapid increase in population is an enormous load on the availability of employment, education, housing, health care while worsening sanitation and depleting the environment (energy and raw materials); thus population/fertility control is the most urgent and important of all biosocial and medical problems.[4] Limited choice of contraception and fear of side effects of modern contraceptive, amongst other reasons, are responsible for the high unmet need of contraception.[5,6] Worldwide, more than two hundred million pregnancies occur each year and 50% of them are unintended.[7] Reduction in fertility by one child per woman would increase gross domestic product (GDP) per capita by 13% within 20 years as well as having a positive impact on all the Millennium Development Goals (MDG) especially MDG 5, that is, to improve maternal health.[8,9]

The leaves of *Ananas comosus*, a herbaceous, perennial, tropical plant, commonly known in English as pineapple, 'eyop mbakara' in Ibibio, 'akwuolu' or 'oku ocha' in Igbo, 'opẹ-oyinbo' in Yoruba and 'abarba' in Hausa is used in some parts of Asia for fertility regulating.[10,11,12] It is a herbaceous,

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perennial, tropical plant of the *Liliopsidae* class that can grow up to 1.0 – 1.5 m high and 1 – 2 m wide The leaves are spiny-edged with flowers on the terminal end which are responsible for fruit production. The leaves are about 30 – 100 cm long and arranged in a rosette.[13,14,15]

Medicinal plants are important sources of new chemical substances with potential therapeutic effects. Therefore, research into plants with alleged traditional use as fertility regulating plant is a useful research strategy in the search for new contraceptives.[16]

Materials and methods

Collection and identification of plant material

Fresh leaves of *Ananas comosus* were collected from Joetom Nigeria Limited, a farm located in Ikono Local Government of Akwa Ibom State. The plant was identified and authenticated by a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State, Nigeria. A voucher specimen with number UUH 40013 was deposited at the Herbarium of the same Department.

Preparation of extract

The leaves of *Ananas comosus* was turned over regularly as it was air dried at room temperature (25 ± 4.7ºC) for a period of 2 weeks after being washed under running tap water. Dried samples were pulverized, macerated and extracted by cold percolation using a conical flask with 100% (v/v) methanol at room temperature for 72 hours, and then filtered with Whatman filter paper (No 4). The filtrate was concentrated under reduced pressure at 50ºC using a rotary evaporator. The concentrated extract was stored in the freezer at -4ºC.

Experimental animals

Twenty (20) mature female Wistar rat, with regular oestrus cycle, used for this study were obtained from the Animal House of the Faculty of Basic Medical Sciences, University of Uyo. They were housed in clean and cross ventilated cages at room temperature and humidity with 12 hour dark/light cycle. The animals were fed with growers' pellet feed (Bendel Feeds and Flour Mills Ltd, Nigeria) and water given *ad libitum*. The animals were cared for according to the international recommendations for the use and care of animals by National Research Council.[17]

Experimental design

The selection of animals for use in the study were determined by the presence of at least two consecutive 4-days oestrus cycles before acclimatization. To determine the effect of extract on the oestrus cycle, 20 cycling female rats at the late proestrus stage were randomly divided into four groups of 5 rats each and administration was given stat dose, intraperitoneally.

Group 1 (control) received 1ml of normal saline.

Group 2 (low dose) received 485.5 mg/kg body weight of methanol extract of *Ananas comosus* leaf.

Group 3 (medium dose) received 970.0 mg/kg body weight of methanol extract of *Ananas comosus* leaf.

Group 4 (high dose) received 1455.5 mg/kg body weight of methanol extract of *Ananas comosus* leaf.

All injections were administered at proestrus and the animals were examined every morning between 8.00hr and 9.00hr for changes in the oestrus cycle for eight days.[18] At the end of the experiment all animals were sacrificed at proestrus and the ovaries harvested for tissue processing and staining using Hematoxylin and Eosin according to Bancroft but with some modification. After sacrifice of the animals under anaesthesia, blood was collected via cardiac puncture, kept for 30 minutes for clotting to take place, centrifuged at 5000rpm for 15mins in order to separate the serum. The serum was used to analyze concentrations of follicle stimulating hormones (FSH), luteinizing hormone (LH), progesterone and estrogen using assay kits from Monobind Inc. Methods used were previously described.[19,20,21]

Histological analysis procedures

The histological analysis was carried out as described but with slight modifications. After fixation in 10% buffered formal saline, the tissues were dehydrated through changes of graded alcohols (70-100%) for 15 minutes. it was cleared using xylene, embedded in paraffin wax, sectioned and stained using H/E.[22]

Hormonal assay procedures

The concentration of FSH was determined using serum using assay kits from Monobind as documented.[18] Exactly fifty microliter (50ul) of standards, specimens and control were dispensed into appropriate wells after which FSH enzyme reagent solution (100ul) was dispensed into each well, swirled thoroughly and allowed to mix for 20-

30 seconds. The mixture was allowed to incubate for 60 minutes at room temperature. Working substrate solution (100ul) was dispensed to each well then it was incubated at room temperature for 15 minutes. The reaction was stopped by addition of stop solution (50µl) to each well and gently mixed for 15-20 seconds to ensure a complete colour change. Absorbance at 450nm (using a reference wavelength of 620-630nm to minimize well imperfection) was read within 30 minutes with a microplate reader. The determination of concentration of Luteinizing Hormone were done using the same procedure as above and assay kits from Monobind Inc but LH enzyme reagent solution were used.

To determine concentration of progesterone hormone using assay kits from Monobind Inc,[21] 25ul of standards, specimens and control were dispensed into appropriate wells followed by 50ul of progesterone enzyme reagent solution. After being thoroughly mixed for 20-30 seconds, 50ul of Progesterone Biotin reagent was also dispensed into each well. The mixture was allowed to incubate for 60 minutes at room temperature. The contents of the micro wells were discarded by decantation, then rinsed and flicked 3 times with wash buffer (350µl). Substrate solution (100ul) was dispensed to each well. The mixture was incubated at room temperature for 20 minutes. The reaction was stopped by addition of stop solution (50µl) to each well and gently mixed for 15-20 seconds to ensure a complete colour change. Absorbance at 450nm (using a reference wavelength of 620-630nm to minimize well imperfection) was read within 15 minutes with a microplate reader. A similar procedure was used to determine concentrations of estrogen.

Ethical approval

This was received from the Faculty of Basic Medical Sciences Research and Ethical Committee with number UU_FBMSREC_2024_012

Statistical analysis:

Data were analysed using SSPS Version 19. Results were presented as mean + standard error of mean (S.E.M.). Significance was ascertained using one-way ANOVA followed by Turkey-Kramer multiple comparison post test. Level of significance was pegged at a level of less than 5% ($p< 0.05$).

Results

Effect of *Ananas comosus* methanol leaf extract on oestrus cycle and ovulation in Rats

The frequency of occurrence of oestrus phases were reduced in all the test groups while that of the diaoestrus and metaoestrus phases were increased as shown in Table 1. The medium dose group had the least number of oestrus phases and the highest number of diaoestrus phases, followed by the group that received 1455.5 mg/kg body weight and 970 mg/kg body weight.

Transition of oestrus phases following administration of *Ananas comosus* methanol leaf extract in rats

The transition from one phase to the other were affected in all test groups. The 1455.5mg/Kg body weight dose group had the least number of correct transitions in all phases. While there was a dose dependent decrease in the frequency of transition from proestrus to oestrus and from oestrus to metaestrus; the medium dose group had the highest number of right transitions from metestrus to

Table 1: Number of occurrence of the oestrus phases following administration of *Ananas comosus* methanol leaf extract in rats

Group	N	Proestrus	Oestrus	Metoestrus	Dioestrus
Normal Saline 1ml/kg body weight	5	2.20 ± 0.20	2.40 ± 0.55	2.20 ± 0.20	2.20 ± 0.20
485.5 mg/kg body weight	5	2.00 ± 0.32	1.60± 0.60	3.00 ± 0.84	2.40 ± 1.25
970.0 mg/kg body weight	5	2.80 ± 0.58	0.40 ± 0.25^{abc}	2.80 ± 0.49	3.00 ± 0.71
1455.5 mg/kg body weight	5	2.60 ± 0.60	0.80 ± 0.84^{a}	1.60 ± 0.51	1.60 ± 0.51

Values represent mean ±SEM
Superscript indicate where there is significance @ $p<0.05$

Table 2: Transition of oestrus phases following administration of *Ananas comosus* methanol leaf extract in rats

Group	N	P→E	E→M	M→D	D→P
Normal Saline 1ml/kg body weight	5	2.00 ± 0.0	1.80 ± 0.20	1.80 ± 0.20	1.80 ± 0.20
485.5 mg/kg body weight	5	0.40 ± 0.25^{a}	0.80 ± 0.20^{a}	0.20 ± 0.20^{a}	0.10 ± 0.00^{abc}
970.0 mg/kg body weight	5	0.20 ± 0.20^{a}	0.40 ± 0.25^{a}	0.60 ± 0.25^{a}	0.60 ± 0.25^{bcd}
1455.5 mg/kg body weight	5	0.00 ± 0.00^{a}	0.20 ± 0.20^{a}	0.20 ± 0.20^{a}	0.00 ± 0.00^{cd}

Values represent mean ± SEM
Superscript indicates where there is significance at $p<0.05$

diaestrus and diestrus to Proestrus as in Table 2.

Rate of the scattering of the oestrus order following administration *Ananas comosus* methanol leaf extract in rats.

The four phases of the oestrus cycle must occur in the proper order together for conception to be achieved. Table 3 looked at how many times the sequence of the oestrus cycle was followed as in a normal physiological state. The methanolic extract of *Ananas cosmous* significantly scattered the order of the oestrus cycle in a dose dependent order (Table 3) when compared to control. The phases of oestrus cycle were regular 6.60, 1.40, 1.80 and 0.49 times in the control, low, medium and high doses, respectively. This showed that the extract caused an irregularity of the cycle.

Table 3: Rate of the scattering of the oestrus order following administration of *Ananas comosus* methanol leaf extract in rats.

Group	N	Oestrus Order
Normal Saline 5 ml/kg body weight	5	6.60 ± 0.400
485.5 mg/kg body weight	5	1.40 ± 0.400[a]
970.0 mg/kg body weight	5	1.80 ± 0.490[a]
1455.5 mg/kg body weight	5	0.49 ± 0.245[a]

Values represent mean ±SEM
Superscript indicates where there is significance

Effect of *Ananas comosus* methanol leaf extract on fertility hormones in female Wistar rats

Progesterone was significantly increased in all the test groups. Estrogen was increased in the low dose group but decreased in other groups compared to control. FSH slightly increased in the low dose group (455.5 mg/kg body weight) and medium dose group (970 mg/kg body weight but not significant. LH was decreased in all the test groups though not significant as shown in Table 4.

Table 4: Effect of *Ananas comosus* methanol leaf extract on fertility hormones in rats

Group	N	Estrogen (ng/mL)	Progesterone (ng/mL)	FSH (mIU/mL)	LH (mIU/mL)
Normal Saline 5mL/kg body weight	5	15.6 ± 1.88	0.30 ± 0.37[a]	1.48 ± 0.20	2.60 ± 0.16
485.5 mg/kg body weight	5	20.9 ± 1.56[b]	1.90 ± 0.100[a]	1.80 ± 0.10	2.5 ± 0.15
970.0 mg/kg body weight	5	10.60 ± 2.16[b]	1.56 ± 0.22[a]	1.50 ± 0.21	2.25 ± 0.19
1455.5 mg/kg body weight	5	13.66 ± 1.02[b]	2.05 ± 0.16[a]	1.45 ± 0.18	2.55 ± 0.28

Source: Computed by Researcher.
Values represent mean ±SEM
Letters indicates where there is significance

Effect of *Ananas comosus* methanol leaf extract on the ovary weight and volume

Ovary weight was significantly reduced between control and test groups while the ovary volume was lesser compared to the control only in the medium and high dose groups as shown in Table 5.

Table 5: Effect of *Ananas comosus* methanol leaf extract on the ovary weight and volume

Group	N	Ovarian Weight (g)	Ovarian Volume (mL)
Normal Saline 5mL /kg body weight	5	0.24[a] ± -0.40	0.24 ± 0.40
485.5 mg/kg body weight	5	0.15 ±0.23	0.34 ± 0.40
970.0 mg/kg body weight	5	0.11[a] ±0.01	0.20 ± 0.00
1455.5 mg/kg body weight	5	0.11[a] ±0.02	0.20 ± 0.032

Values represent mean ±SEM
Letters indicates where there is significance

Histological findings on the effect of *Ananas comosus* leaf methanol extract on ovaries

The section of the ovary tissue in the control group showed several follicles at different stages of development in the cortex. The primordial follicles were with a single layer of follicular cells, while the primary follicles had a large eccentric nucleus, prominent nucleolus and multiple layers of granulosa cells (Figure 1). Maturing follicles (graafian follicles), had liquid filled antrum surrounded by the granulosa cells and theca interna cells and the primary oocyte were surrounded by the corona radiata and pressed to one side and several corpora lutea which were made up of the inner granolosa lutein cells and peripheral theca lutein cells. The cortex of the ovary was surrounded by the tunica albugenia and the ovarian surface was covered by an intact germinal epithelium (Figure 1). In the low dose group, lesser number of developing follicles were observed and but with more corpora lutea (Figure 2). This same observation was noted for the medium and high dose groups (Figure 3 and Figure 4). In addition, in the high dose group, some of the blood vessels were eroded and the primary follicles had some eosinophilic collections (Figure 4).

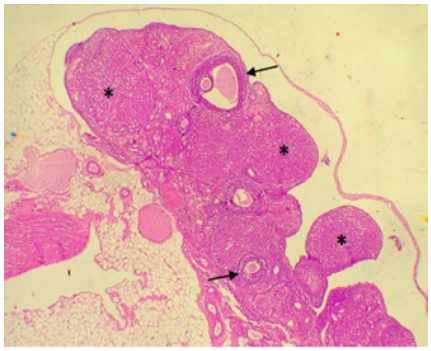

Figure 1: Section of ovary tissue of control group (5 ml/kg body weight of normal saline)
Ovary tissue showing several developing follicles ranging from primary to graaffian follicles (black thin arrow) and corpus luteum (asterisks). (H & E 40×).

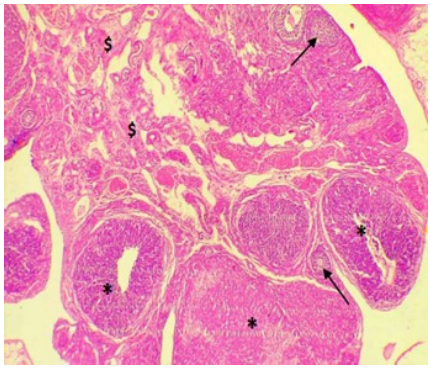

Figure 2: Section of ovary tissue of low dose group (485.5 mg/kg body weight *Ananas comosus* methanol leaf extract)
Ovary tissue showing medullary region ($) with numerous blood vessel and the cortex with few developing primary follicles (black Thin arrow) and several corpus luteum (asterisks). (H & E 40×).

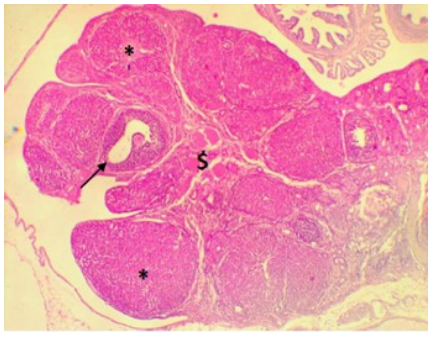

Figure 3: Section of ovarian tissue of medium dose group (970.0 mg/kg body weight *A. comosus* methanol leaf extract)
Ovary tissue showing medullary region ($) with numerous blood vessel and the cortex with few developing follicles and a focal mature (graafian follicle) follicles and several corpus luteum (asterisks). (H & E 40×).

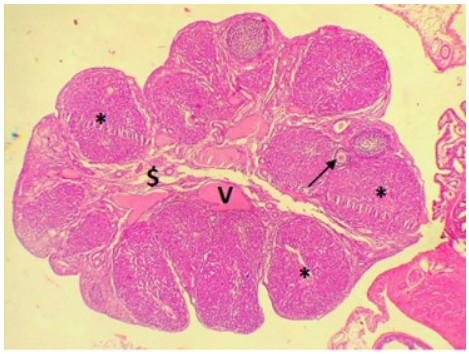

Figure 4: Section of ovary tissue of high dose group (1455.5 mg/kg body weight *A. comosus* methanol leaf extract)
Ovary tissue showing medullary region ($ and V) with numerous blood vessel and the cortex with few developing follicles and a focal mature (Graafian follicle) follicles and several corpus luteum (asterisks). (H & E 40×).

Discussion

The oestrus cycle is a cascade of hormonal and behavioural events which are progressive, highly synchronized and repetitive and usually lasts for 4-5 days.[23] The oestrus cycle is characterized by changes in the physiological, biochemical, morphological and histological aspects of the ovaries. This is regulated by FSH, LH, prolactin, progesterone, estrogen and androgens. Imbalance in these hormones may lead to irregularities in the ovarian function and duration of the oestrus cycle. There is need for the correct transition of the oestrus phases to maintain fertility. The oestrus cycle phases starts from proestrus – oestrus –metaoestrus- diaoestrus. The methanolic extract of *Ananas comosus* significantly disrupted the oestrus cycle and affected the normal transition of the phases. The metoestrus and dioestrus had the highest number of occurrence while the oestrus phase had the least. With the frequency of oestrus and proetrus phases decreased, ovulation would be inhibited. Similar result was derived from the methanolic extract of *Rumex*

steudelii hochst given at a dose of 2.2 mg/kg body weight intragastrically for 21 days. It disrupted the oestrus cycle with an increase in the dioestrus phase. The hydrochloric extract of *Ocimum basilicum* also prolonged the duration of oestrus with dioestrus being the most common phase in the cycle.[24]

The methanolic extract of *Ananas comosus* causes a slight decrease in body weight of animals given the extract compared with control, though not significant. Ovarian weight was significantly reduced between control and test groups, while the volume of the ovary was less compared to the control though not significant. The ethanolic extract of *Ocimum basilicum* has been reported to cause no significant change in body weight but decrease in uterine and ovarian weights.[24] Lilarem and Ahmed (2012) studied the extract of the seeds of *Caesalipinia* at a dose of 200 and 300 kg/ kgb.wt and found that it caused a significant reduction in uterine and ovarian weights.[25]

Ovulation can be inhibited through estrogenication leading to a reduction in the number of follicles as found in the low dose group. Reduction in the concentration of estrogen as seen in the medium and high dose groups can result in insufficiency of estrogen to meet up the steps in the reproductive cycle. Also decreasing concentrations of estrogens indicate the presence of immature follicles which in turn has a negative feedback effect on FSH and LH.[26] The presence of certain phytochemicals like steroids accounts for changes in the levels of reproductive hormones preventing ovulation.[27] Inhibition of ovulation is the primary pathway through which levonogestrel, an emergency contraceptive acts.[28] Untimely increase in Progesterone results in defective oocytes.[29] Increase in progesterone also results in inhibition of pregnancy.[30]

Conclusion: ***Ananas comosus*** methanol leaf extract prevented ovulation from occurring and caused irregularity of the oestrus cycle with the disruption of fertility hormones.

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