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Effect of *Ananas comosus* methanol leaf extract on immature, non-pregnant and pregnant uterus of adult female albino wistar rat.

*Ekemini I. JOHNSON[1], Akpan U. EKANEM[1], Enoobong BASSEY[1], Paul NWAFOR[2]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria.
[2]Department of Pharmacology and Therapeutics, Rivers State University, Rivers State, Nigeria.

Abstract

Background: The leaves of *Ananas comosus* is used traditionally as an abortifacient among the Indians.
Objective(s): The objective of this study is to investigate the anti-conceptive and anti-implantation, estrogenic and anti-estrogenic effect of *Ananas comosus* methanol leaf extract on a uterus of female albino Wistar rat.
Study Design: Mature female wistar rats with regular consecutive oestrus cycle were divided into 5 groups of 5 animals each. The experimental groups received 485.5 mg/kg, 970 mg/kg and 1455.5 mg/kg body weight respectively with the control group receiving 1 ml/rat/day body weight of normal saline. Mating was done before and after administration of the extract for the anti-conceptive and anti-implantation experiments respectively. The antiestrogenic and estrogenic effect was done in immature ovariectomized rats using 17β estradiol as reference while the experimental groups were given same dose as above.
Result: Pregnancy was significantly prevented in a dose dependent manner as the low dose group had a reduced number of pups littered while the medium and high dose groups had no litters. The medium and high dose groups had no implantation sites. There was a very weak estrogenic but strong anti-estrogenic effect of the extract.
Conclusion: The study showed that *A. comosus* leaf extract prevented conception in rats through its anti-implantation and anti-oestrogenic effects.

Key Words: Anti-conceptive, Anti-implantation, Estrogenic and anti-estrogenic, Pregnancy, *Ananas cosmosus*

Introduction

Contraceptives are chemical substances that can impede sperm production or motility, hinder the formation of ovum and decrease the sensitivity of the endometrium for implantation.[1] Population explosion, high rate of prevalent maternal and infant mortality has promoted the use of modern contraceptives as a method of controlling the population growth and bringing a reduction in birth related deaths.[2] As Nigeria's population exceeds 170 million, more than 400,000 Nigerian women's lives are lost to childbirth and associated problems from unintended pregnancies every year.[3,4] Africa in particular would account for 40% of the increase in the world's population and nearly 1/5 of the world population as her population is projected to double by 2050 with a growth rate of 99%.[5,6] Uncurbed population growth continues to be a pressing global concern because a population that is growing rapidly not only has a high incidence of poverty at the time but this poverty transcend generations.[7]

Corresponding Author:
Dr. Ekemini I. JOHNSON
Department of Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

ekeminijohnson@uniuyo.edu.ng

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Despite the increase in contraceptive use awareness and supply, the use of modern contraceptive is relatively low with a high un-met need for family planning. This is due to lack of tactical communication for change in attitude; lack of accurate knowledge on contraceptives; personal and community cynicism on contraceptive use such as fear of return of fertility; cost of contraceptives and side effects such as headaches, hemorhagia; limited choice of methods and religious beliefs. Other limitations include illiteracy, poor coordination of family planning programmes and poor access to contraceptives.[8,9,10] Contraceptive use, to a large extent can curb the menace posed by overpopulation through the control of high fertility rate.[11] The benefits of effective contraceptive ranges from reduction of maternal deaths, abortions, disabilities associated with pregnancy to increasing educational opportunities for women, better child health, greater family savings and strong national economies.[12,13]

With the apathy for modern contraceptives, some women resort to plants as a method of contraception. In some parts of Asia, the leaves of *Ananas comosus* are used as an abortifacient.[1,14,15]

Materials and methods

Collection and identification of plant material

Leaves of *Ananas comosus* were freshly plucked from a farm, Joetom Nigeria Limited, Ikono Local Government Area Akwa Ibom State. Identification and authentication was done by a certified Taxonomist from Botany and Ecological Studies Department of University of Uyo, Akwa Ibom State, Nigeria. UUH 40013 was assigned to the specimen modelled at the herbarium of the same Department.

Preparation of extract

Within 2 weeks, the leaves of *Ananas comosus* were air dried at room temperature (27.0 ± 4.7ºC). After being dried, pulverization, maceration and extraction by cold percolation with 100% (v/v) methanol at room temperature for 72 hours was done. Concentration of the filtrate was done using a rotary evaporator. Storage of the concentrated extract was in the freezer at -4ºC.

Animal handling

A total of eighty (80) female and twenty (20) male Wistar rats were obtained from the Animal House of the Faculty of Basic Medical Sciences, University of Uyo; housed in clean and cross ventilated cages at room temperature and humidity with 12-hour dark/light cycle. Forty (40) immature female Wistar rats were used for the oestrogenic and anti-oestrogenic experiments, while forty (40) mature female Wistar rats were used for the anti-conceptive and anti-implantation experiments. 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.[16]

Study design

Determination of oestrogenic and anti-oestrogenic effects of Ananas cosmosus methanol leaf extract

The oestrogenic and anti-oestrogenic activities of the extract was assessed in 40 bilaterally ovariectomized immature female Wistar rats according to the method of Edgren and Calhoun (1957).[17] Uterine wet weight, degree of vaginal cornification and quantal vaginal opening were used to determine the extent of estrogenic effects. The rats were randomized into eight (8) experimental groups of five (5) rats each one week after bilateral ovariectomy. Bilateral ovariectomy was done under light anaesthesia and sterile conditions. Abdominal incisions were sutured in layers and the incision site cleaned daily with antiseptic solution and methylated spirit. Animals were sacrificed 24 h after the last treatment. Tissues for histology were harvested and fixed accordingly. Administration was done intraperitoneally for 4 days as follows:

Reference group received 17β estradiol dissolved in corn oil, 0.1 mg/rat/day, subcutaneously.

Control rats were administered 1 ml/rat/day of normal saline. Group 1, 2 and 3 were administered 485.5 mg/kg, 970.0 mg/kg, 1455.5 mg/kg body weight of methanol extract of *Ananas cosmosus* leaf. Group 4, 5 and 6 had 17β estradiol 0.1mg/rat/day in addition to the fore-mentioned doses of methanol extract of *Ananas comosus*.

Determination of anti-implantation activity of Ananas cosmosus methanol leaf extract

Animals with at least two-consecutive 4-days estrous cycles were randomly divided into 4 groups

of 5 animals each and left overnight with males of proven fertility in the female: male ratio of 2:1 at proestrus. With confirmation of copulation (day 1 of pregnancy), administration of the extract was done intraperitoneally in doses as follows; Group A (Control) received normal saline (0.15 ml for four (4) days; group B, C and D received 485.5 mg/kg, 970.0 mg/kg, 1455.5 mg/kg body weight of methanol extract of *Ananas cosmosus* leaf.[18]
Laparotomy of all rats under light anesthesia and sterile conditions were done on day 10 of pregnancy and the number of implantation sites on both uterine horns were recorded and the percentage anti-implantation activity was calculated as in Equation 1. The wounds were sutured layer by layer and the animals were allowed to go to term. The number of pups delivered were counted and observed for three weeks for any evidence of teratogenicity.

Equation 1:
% anti-implantation activity = No of implant in control- No of implant in test ×100 / No of implant in control×1

Determination of anti-conceptive effect of Ananas cosmosus methanol leaf extract
Twenty (20) animals with at least two-consecutive 4-days oestrous cycles were used. The same doses of extract used in the determination of anti-implantation activity were given intraperitoneally for 4 days after which they were mated in the female : male ratio of 2:1 on the 5th day and remained till conception.[18] All animals were maintained with normal feed and distilled water *ad libitum*.

Ethical approval
The Faculty of Basic Medical Sciences Research and Ethical Committee gave the ethical approval with number UU_FBMSREC_2024_012

Histology and microscopy
The uterus were dissected and processed for histopathology. Briefly, the tissues were fixed in 10% buffered formalin and later dehydrated in different concentrations of ethanol (70%, 95%, 100%), cleared in two changes of xylene for one hour and infiltrated in paraffin wax using mould, then cooled to make blocks. Sectioning and trimming were done, de-waxed, for fifteen minutes, processed, stained with Haematoxylin and Eosin and viewed under microscope.[19]

Data Analysis
SSPS Version 19 was used to explore and interpret the data generated. Results were presented as Mean + standard error of mean (S.E.M.) was the format used to present the result of data analysis. Significance was ascertained using one-way ANOVA followed by Turkey-Kramer multiple comparison post test. Level of significance was taken at a level of less than 5% ($p< 0.05$).

Results

Oestrogenic and anti-oestrogenic effects of Ananas comosus methanol leaf extract in rats
The extract has a weak oestrogenic effect compared to control but a strong anti-oestrogenic effect. The relative uterine weight of the immature ovariectomised rats of the low dose, medium dose and high dose gradually increased compared to the control group. There was a dose dependent decrease in uterine weight when the extract was administered with 17β estradiol in all the groups (Table 1). The group that received 1455.5 mg/kg body weight of extract and 17β estradiol 0.1mg/rat/day simultaneously had same relative uterine weight as control showing the strong anti-oestrogenic activity of the extract.

Table 1: Oestrogenic and anti-oestrogenic effects of Ananas comosus methanol leaf extract in rats

Group/Treatment	N	Initial body weight (g)	Final body Weight (g)	Uterine weight (g)	Relative uterine weight
Ref/ 17β estradiol (0.1 mg/rat/day, subcutaneously, dissolved in corn oil	5	128.20±4.25	142.20±17.89	2.64±0.80	21.4±0.40
Control (1 ml/rat/day)	5	127.60±5.58	17.89±5.58	0.13±0.10	1.00±0.60
485.5 mg/kg body weight	5	105.20±4.94	133.67±6.39	0.13±0.10	0.73±0.20[a]
970.0 mg/kg body weight	5	130.80±2.13	130.60±3.97	0.15±0.20	1.20±0.20[a]
1455.5 mg/kg body weight	5	119.20±3.26	121.20±3.31	0.18±0.05[a]	1.40±0.25[a]
485.5 mg/kg body weight + 17β estradiol 0.1 mg/rat/day, subcutaneously	5	115.00±4.14	113.80±2.65	1.60±0.25[a]	13.60±1.72[a]
970.0 mg/kg body weight+17β estradiol 0.1 mg/rat/day, subcutaneously	5	107.80±5.13	112.80±2.35	1.46±0.25[a]	4.20±97[a]
1455.5 mg/kg body weight + 17β estradiol 0.1 mg/rat/day, subcutaneously	5	119.20±1.19	117.00±3.81	2.00±0.00[a]	1.00±0.00[a]

Values represent mean ± SEM
Superscript indicate significance at $p<0.05$.

Table 2: Anti-implantation activity of Ananas comosus methanol leaf extract in rats

Group	No of rats pregnant on Day 10	No of implantation sites	No of rats that delivered at term	No of litters delivered at term	Percentage anti-implantation activity (%)
1ml/rat/day, normal saline (Control)	5/5	8.4 ± 0.51*	5/5	8.4 ± 0.51*	
485.5 mg/kg body weight	5/5	2.2 ± 0.58*	4/5	2.2 ± 0.58*	73.8%
970.0 mg/kg body weight	0/5	0.0 ± 0.0*	0/5	0.0 ± 0.0*	100.0%
1455.5 mg/kg body weight	0/5	0.0 ± 0.0*	0/5	0.0 ± 0.0*	100.0%

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

Table 3: Anti-conceptive effect of Ananas comosus methanol leaf extract in rats

Group	No. of Pups	Degree of protection over gestational periods
1 ml/rat/day, normal saline (Control)	8.0 ± 0.31*	Nil
485.5 mg/kg body weight methanol extract of *Ananas cosmosus* leaf	2.4 ± 0.60*	2.0
970.0 mg/kg body weight methanol extract of *Ananas cosmosus* leaf	0.0 ± 0.0*	3.0
1455.5 mg/kg body weight methanol extract of *Ananas cosmosus* leaf	0.0 ± 0.0*	3.0

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

Table 4: Mean weight and length of pups littered

	Weight (mg)			Length (cm)		
	Day 0	Day 10	Day 20	Day 0	Day 10	Day 20
1 ml/kg body weight Normal Saline	4.99 ± 0.02	19.63 ± 0.11	28.99 ± 0.68	5.12 ± 0.10	10.15 ± 0.13	15.12 ± 0.17
485.5 mg/kg body weight methanol extract of *Ananas cosmosus* leaf	5.48 ± 0.12*	20.57 ± 0.14*	29.86 ± 0.13*	5.70 ± 0.07*	10.87 ± 0.18*	16.55 ±0.28*

*p<0.05, Values represent Mean ± SEM

Anti-implantation effect of Ananas comosus methanol leaf extract in rats

There was significant impairment of fertility with a 100% anti-implantation effect in the medium and high doses, however the number of implantation sites counted were carried to term and delivered. Anti-implantation activity of 73.8% was exhibited in the low dose group (Table 2).

Anticonceptive effect of Ananas comosus methanol leaf extract in rats

The extract at 970.0 mg/Kg body weight and 1455.5 mg/Kg body weight had an anticonceptive effect for a period of three gestational periods. However, at a dose of 485.5 mg/kg body weight the number of pups littered was significantly reduced as compared to the control with a degree of protection of 2 gestational periods (Table 3). There was no abnormality observed in the pups over 12 weeks. The pups from test group were significantly heavier and longer than that of the control (Table 4).

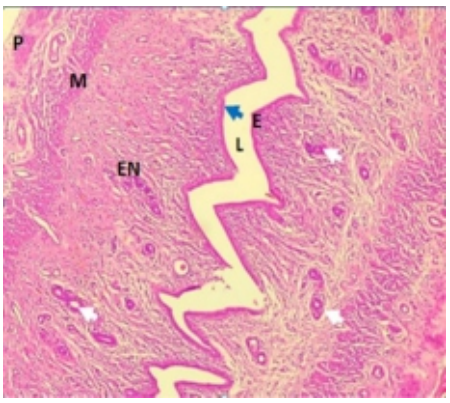

Figure 1: Section of uterus of ovariectomised immature rats of control group (1ml/rat/day of normal Saline)
Uterus showing normal lumen (L), endometrium (E) and myometrium (M). The endometrium which is lined by single layer of columnar epithelium with small amount of cytoplasm (blue arrowhead). There are glands in the endometrium (white arrowhead). (H & E 100×)

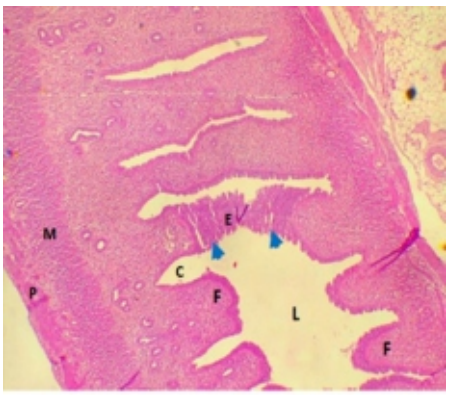

Figure 2: Section of uterus of ovariectomised immature rats of reference group (0.1mg/kg of 17β estradiol

alone)Reference uterus showing dilated uterine lumen (L) and increase endometrial fold (F) and dilated glandular lumen (#) with cuboidal epithelium and epithelial lining hyperplasia (E and blue arrowhead), Endometrium (E), Myometrium (M) and Perimetrium (P). (H & E 100×)

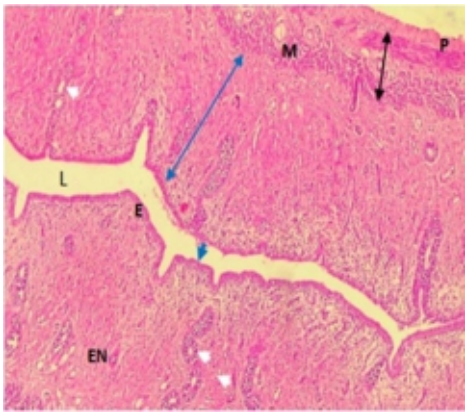

Figure 3: Section of uterus of ovariectomised immature rats of low dose group (485.5 mg/kg body weight *A. comosus* methanol leaf extract only)
Section of uterine tissue showing slit-like Lumen (L), a layer of mild atrophic cuboidal epithelium (E), non-prolilferative endometrium (EN, blue double headed arrow) with few tubular glands (white arrow head), myometrium (M, black two end arrow) and perimetrium (P, black arrowhead), (H & E 100×).

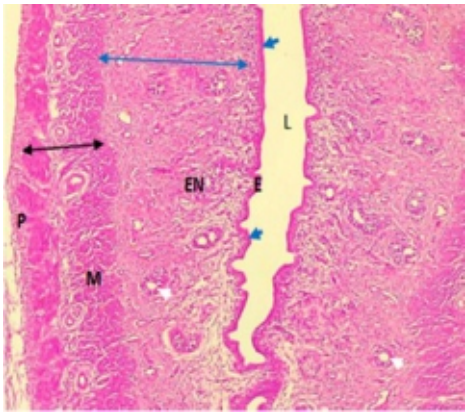

Figure 4: Section of uterus of ovariectomised immature rats of medium dose group (970.0 mg/kg body weight *A. comosus* methanol leaf extract only)
Section of uterine tissue showing a wider lumen (L), a mildly hypertrophic epithelium (E), endometrium with lesser folds and crpts (blue two end arrow, EN) myometrium (black two end arrow, M) and perimetrium (black arrowhead, P). Also seen are average number of uterine glands.

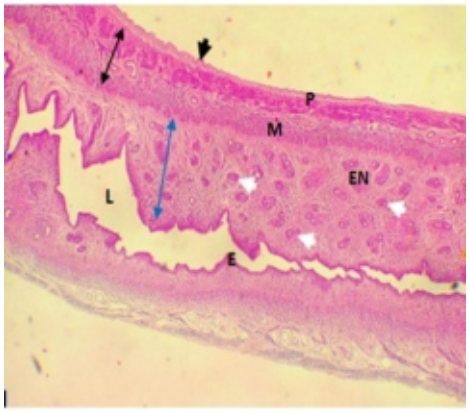

Figure 5: Section of uterus of ovariectomised immature rats of high dose group (1455.5 mg/kg body weight *A. comosus* methanol leaf extract only)
Section of uterine tissue showing a wider lumen (L), a mildly hypertrophic epithelium (E), endometrium (blue two end arrow, EN) myometrium (black two end arrow, M) and perimetrium (black arrowhead, P). Also, the number of uterine glands appears to be more (H & E 100×)

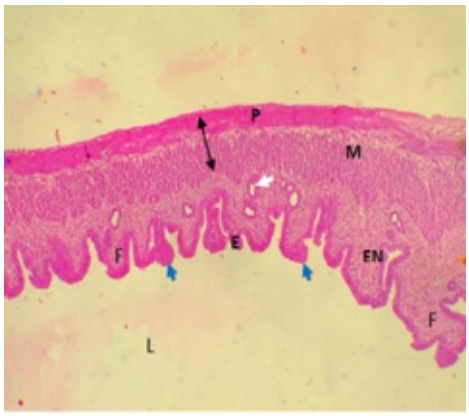

Figure 6: Section of uterus of ovariectomised immature rats of low dose group (485.5 mg/ kg body weight of *A. comosus* methanol leaf extract) + 0.1mg of 17β estradiol.
Uterus showing showing dilated lumen (L), epithelium (E) is hypertrophied, endometrium (EN) has developed into several folds (F) with evidence of crypts (red arrowhead) (blue two end arrow), myometrial layer (black two end arrows, M) has increased. Also seen are uterine dilation with and cystic formation (white arrow head). (H & E 100×)

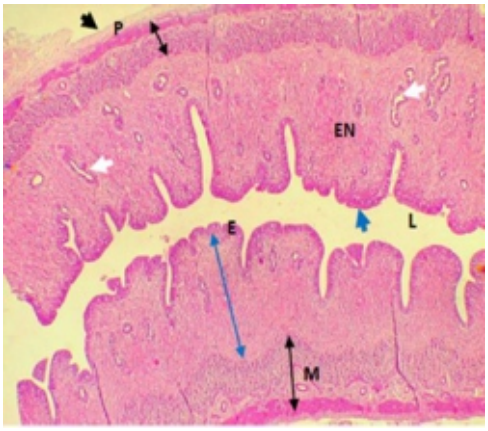

Figure 7: Section of uterus of ovariectomised immature rats of medium dose group (970.0 mg/Kg body weight of *A. comosus* methanol leaf extract) + 0.1mg of 17β estradiol.
Uterus showing showing dilated lumen (L), hypertrophied epithelium (E), endometrium (EN) has developed into some folds (F) with evidence of crypts (red arrowhead, C) (blue two end arrow), myometrial layer (black two end arrows, M) has increased. Also seen are uterine dilation with and cystic formation. (H & E 100×)

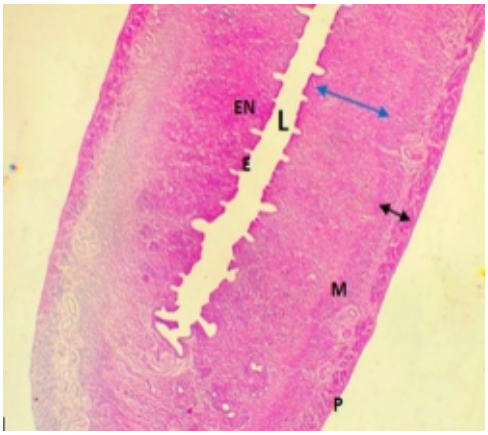

Figure 8: Section of uterus of ovariectomised immature rats of high dose group (1455.5 mg/Kg body weight of *A. comosus* methanol leaf extract) + 0.1mg of 17β estradiol.
Uterus showing a reduced lumen (L), epithelium (E) is sloughed off, endometrium (EN) has minimal folds (F) with a few crypts (red arrowhead) (blue two end arrow), myometrial (M) layer (black two end arrows) has reduced with many glands. There is no uterine dilation with and cystic formation.
(H & E 100×)

Histological analysis

The control (Figure 1) showed a fairly sized lumen of the uterus with simple columnar epithelium overlying a thick lamina propria. The lining epithelium extended into the lamina propria forming tubular glands. Dispersed in the lamina propria are cross-sections of glands. The functionalis layer of the uterus was closer to the lumen while the layer just above the myometrium is the basalis layer. The endometrium, which consist of the lining epithelium, lamina propria, coiled arteries and uterine glands were situated above the myometrium with compact bundles of smooth muscle with numerous blood vessels and interstitial connective tissue. This section of uterine section (Figure 1) was in the follicular stage.
The uterine section of the reference group of animals showed the uterus in a luteal phase with a wider lumen (Figure 2). The epithelium had proliferated, appearing somewhat as stratified columnar epithelium. The functionalis and basalis layers are thicker due to increased glandular secretion and oedema in the lamina propria. The uterine glands were also hypertrophied and tortous, their lumen dilated due to deposition of nutritive secretory material (Figure 2). The arteries were also prominent and congested. The muscular layer was thicker compared to control.
The histological features of the section of ovariectomised immature rats of the low dose, medium, and high dose groups of *Ananas cosmosus* methanol leaf extract only showed a dose dependent graded increase in the lumen size, height of columnar cell making up the epithelium (that is, dose dependent epithelial hyperplasia) increase in number and size of glands and width of lamina propria, respectively (Figures 3, 4 and 5). The increase in height of columnar cells resulted from increase in the cytoplasm of the columnar cells. The myometrium and perimetrium were almost the same in all the groups.
The uterine sections of the rats administered low, medium and high dose of the extract with 17β estradiol respectively showed gradual dose-dependent reduction in sizes of lumen, height and layers of epithelium as the 'stratified' epithelium seen in the reference group (Figure 2) had totally sloughed off and was reduced to a single layer in the high dose + 17β estradiol group (Figure 8). The sizes

of the endothelium in these groups were reduced with increase in dose as there was reduction in the size of lamina propria and numbers and sizes of glands. The number of crypts and tortuosity reduced in similar manner. The myometrium decreased in size too when compared to the reference group. This shows that the extract has a strong anti-oestrogenic activity.

Discussion

There was a mild oestrogenic effect of the methanolic extract of *Ananas comosus* as it caused significant increase in the uterine weight in immature rats compared to the control, but with no vaginal opening and cornification. The extract antagonized the effect of ethyl estradiol when administered together as the weight of uterus was less than that of the group given ethyl estradiol alone. Simultaneous administration of ethyl estradiol and methanolic extract caused an increase in the uterine weight when compared to the control but this was less than that produced by ethyl estradiol. Uterine diameter and thickness of the epithelium, also were more prominent. These observations were confirmed by the diameter of uterus, thickness of endometrium and height of endometrial epithelium when compared to the reference and control. The extract had a weak oestrogenic effect when given alone but a strong antioestrogenic effect when given with the reference. The strong anti-oestrogenic effect of the *A. comous* methanol leaf extract is also confirmed with the reduced uterine weight and volume between the treatment groups and reduced concentration of oestrogen compared to control though inversely proportional to the dose. With increase oestrogen concentration, FSH and LH were decreasing suggesting the antagonistic effect of the extract. The mild oestrogen increase also effected an increase in the uterine weight compared to control. Pattanayak and Mazumder (2009) had similar results to the plant under study with the plant extract of *Dendrophthoe falcate*.[20] The ethanolic extract of *Derns brevipes* variety *corica* initially had a weak estrogenic activity alone but a strong anti-oestrogenic activity when given with ethyl estradiol.[21] This was also seen in the aqueous extract of *Piper betle*.

The anti-oestrogenic activity may be attributed to the negative feed-back inhibition of the hormonal regulation. The steroidal glycosides and saponins are responsible for anti-oestrogenic activity as these are reported to reduce the activity of estrogen induced enzymes in several estrogen targeted tissues.[22] Anti-oestrogenic substances may disrupt the endogenous oestrogen and progesterone which may result in fertility failure.[23] For the anti-oestrogenic effect to bare, the methanolic extract of *Ananas cosmosus* acted as a competitive antagonist to estradiol.[21]

Ananas cosmosus methanol extract prevented conception totally in the medium and high dose group of animals while the animals in the low dose had reduced number of pups compared to control. This has confirmed the contraceptive activity of *Ananas cosmosus* leaf extract whose potency increases with concentration.

Antifertility of plants or antifertility potential of some plants are possible because of the presence of phytochemicals such as flavonoids, terpenoids, alkaloids and steroidal saponins as they interfere with implantation and induce abortion.[22,24] Plants that have contraceptive and abortifacient properties may act through the rapid expulsion of the fertilized ova from the fallopian tubes; inhibition of implantation due to disturbance in oestrogen and progesterone balance and feotal abortion, lack of supply of nutrients to the uterus and embryo.[25] The methanolic extract of *Ananas cosmosus* at 970.0 mg/kg body weight and 1455.5 mg/kg body weight prevented implantation of any fertilized ovum in the uterine horn. However, at the 485.5 mg/kg body weight dose, the number of implants on pregnancy day 10 were carried to term, implying that the extract has no abortifacient activity in the later part of pregnancy. The extract caused a 73.8% anti-implantation activity in the low dose and a 100% activity in the medium and high doses. The extract possibly made the uterus unreceptive and since the implantation is time bound, this resulted in failure. This is supported by reduced concentrations of oestrogen and progesterone hormones compared to control and reducing uterine weight and volume with increasing dose of extract. Moreso, the histology of the uterus showed somewhat reduced uterine and thinning of the epithelium with increased doses. The extract possibly maintained the integrity of the blastocyst as the number of

implants counted were carried to term. This may be responsible for the pups delivered by the low dose group of animals despite the uterine unreceptiveness.

Several reports have documented that the petroleum and benzene extracts of *Ananas cosmous* leaves exerted some abortifacient activity mainly between day 1 and 10 of pregnancy but had no effect from day 13-16 of pregnancy after implantation.[26] This inhibition of implantation between day 1-day 10 of pregnancy was seen in the ethanolic extract of *Achyarnthes asprea* when given orally from day 1 to day 7 of pregnancy at a dose of in 5 out of 6 rats.[27] Also, the ethanolic extract of *Calotropis procera* root administered orally at a dose 250mg/kg from day 1 to day 7 of pregnancy effected a 100% anti-implantation activity as no implantation sites were seen in the uterine horns on the day 10th after laparotomy.[28] This effect may be due to the anti-zygotic and blastocytotoxic effects of the extract.[29]

There was no gross abnormality observed in the pups littered at birth and for 12 weeks although the weight and length of pups delivered from female rats given the extract was significantly more than those from the control rats. According to Hu *et al*. (2011), the extract of pineapple leaves were safe in the rat during embryonic development even at high doses.[30] It did not cause any significant reduction in maternal body weight or caused maternal organ weight alteration, reduced female fertility, disturbed fetal growth and development or caused teratogenic effects as done by cyclophosphamide. The ethanolic extract of *Denis brevipies* variety *coriacaea* at a dose of 600 mg/kg, given from day 1 to day 7 of pregnancy had been reported to have no morphological effect with no appreciable change in weight in experimental animals.[21] The petroleum ether extract of Acalypha indica which significantly inhibited pregnancy at a dose of 300 mg/kg body weight and 600 mg/kg body weight had no toxic effects in the pups littered.[29]

Conclusion

The study showed that ***Ananas comosus*** **methanol leaf extract** prevented conception through its anti-implantation and anti-oestrogenic effects. The leaf extract showed no signs of teratogenicity on the foetus up to twenty (20) day old.

Acknowledgement

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