



ANTIFERTILITY ACTIVITY OF ETHANOLIC EXTRACT OF *MUSA SAPIENTUM* LINN. PSEUDOSTEM

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ABSTRACT

The oral administration of the ethanolic extract exhibited estrogenic activity as shown by the significant increase in uterine weight, diameter of the uterus and thickness of the endometrium in immature ovariectomised rats. The ethanolic extract shown the activity dose - dependently. In this study, we observed decrease in the FSH level and increase in the estrogen levels in the extract treated group when compared with those of the control animals. It may indicate the disturbances of the estrous cycle and ovulation through suppression of FSH might be due to the estrogenic activity of the extract.

KEYWORDS: Antifertility, *Musa sapientum* Linn.

1. INTRODUCTION

Antifertility agents are drugs that control fertility¹ and are also called oral contraceptives. These drugs affect and are involved in the menstrual cycle and ovulation in females. Estrogen and progesterone in combined form are given as birth control pills. The antifertility substance is deemed to be active in females when it prevents fertilization, prevents ovulation, implantation, and destroys the zygote or causes abortion. In males, it prevents spermatogenesis, inhibits testosterone, or affects the gonadotrophin of the organs or the mortality of sperm.

Herbal medicines and their derivatives have been incorporated into traditional medicine virtually since the beginning of recorded history. But it is only in recent times that the broader use of medicinal plants is beginning to garner acceptance in the more expansive international domain. There are certain bottlenecks in the process, including but not limited to the lack of quality control and toxicological studies, the imperative to increase product shelf life, and compliance with international regulatory standards that need to be overcome before their full market potential can be realized. Various medicinal plants have antifertility activities. The Unani system of medicine is an indigenous treatment, very prevalent in South Asia and popular among large populations in India, Pakistan, Bangladesh, and Sri Lanka. More specifically, Unani medicine is part of the South Asian culture where it is practiced among a large segment of the population.

1.1 The Next Generation Of Drug Based Birth Control

According to an article on Medicine net, some researchers trying to develop next generation of birth control for men and women.

- A. Career Pill
- B. Dry Orgasm Pill
- C. Reversible Inhibition of Sperm under Guidance (RISUG)
- D. Spermicidal Creams
- E. Vaccines against pregnancy

1.2 Need For Contraceptive / Antifertility Drugs

Antifertility drugs are not only required to limit the size of a family but also affect an individual as well as society's development in a multi-dimensional manner. A few indications for the use of antifertility drugs are as follows.

- A. Slowing population growth
- B. Birth Spacing
- C. Reducing infant mortality
- D. Preventing unintended pregnancy
- E. Preventing pregnancy-related health risks in women
- F. Later years of marriage

1.3 Estrous Cycle

The estrous cycle lasts for four to five days and can be divided into four stages as follows;

- A. Estrous
- B. Metoestrous
- C. Diestrous

D. Proestrous

1.4 Female Reproductive System

The menstrual cycle (of 28 days) is generally divided into three phases, with major events occurring in each phase. The 3 phases are,

- A. Menstrual (destructive phase)
- B. Proliferative (follicular) phase
- C. Secretory (luteal) phase

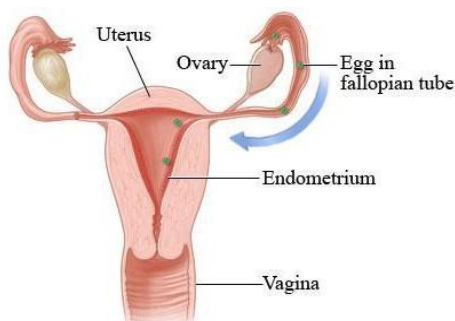


Fig. No. 1: Reproductive system.

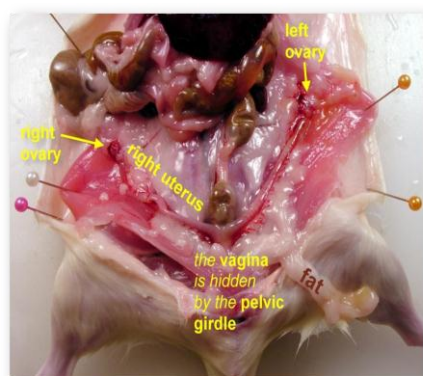


Fig No. 2: Female Rat Reproductive System.

2. PLANT PROFILE

- Botanical Name : *Musa sapientum* Linn
- Synonym : *Musa paradisiaca* L.,
Musa sapidisaca Jacob
- Family : Musaceae.

2.1 Botanical Description

- Class : Liliopsida
- Subclass : Commelinidae
- Order : Musales
- Family : Musaceae
- Genus : Musa

2.2 Vernacular Names

- Tamil : Vazhai, Vazha
- Hindi : Kela
- Sanskrit : Rambha
- English : Banana
- Telugu : Kadali, Artipandu

2.3 Geographical Distribution

Numerous varieties of each are cultivated in the tropical parts of Asia, Africa, and America; the wild parent is found at Chittagong, and other parts of tropical Asia.

2.4 Medicinal Uses

- Anti-ulcerogenic activity
- Hypolipidemic activity
- Diuretic activity
- Antacid activity
- Antihyperglycaemic Activity
- Analgesic activity
- Wound healing activity
- Antimicrobial activity
- Antihypertensive activity



Fig No. 3: *Musa Sapientum* Linn. Pseudostem.

3. MATERILAS AND METHODS**3.1 Preparation of Extract**

The pseudostem was shade-dried and coarsely powdered. The powder was first defatted with Petroleum ether, then filtered and dried. Measured amounts of the dried defatted powder was macerated in 95% ethanol and then subjected to the Soxhlet's apparatus for extraction. The marc was filtered and concentrated in vacuum under reduced pressure and dried in desiccator. Thus the ethanolic extract of *Musa sapientum* pseudo stem (EEMS) was prepared.

3.2 Animals

Female albino rats of wistar strain were used for all experiments. The animals were acclimatized to laboratory condition for not less than 10 days after their arrival. The animals were housed in groups under standard light/dark cycle with food and water provided *ad libitum*. All experimental procedures and protocols used in the study were received by Institutional Animal Ethical Committee (IAEC).

3.3 Preliminary Phytochemical Screening

The Ethanolic extract of *Musa sapientum* (EEMS) was subjected to preliminary chemical screening for their presence or absence of active phytochemical constituents.

3.4 Acute Toxicity Study

Acute toxicity study has been already reported and suggested that it does not exhibit any toxicity up to 2

gm/kg. From the toxicological study the dose was selected as 200mg/kg and 400 mg/kg.

3.5 Experiment Design

Table No. 1: Experimental Design.

GROUPS	NO.OF ANIMALS	TREATMENT	DOSE
I (control)	6	Vehicle (Tween 80)	5ml/kg
II (standard)	6	Standard drug (Ethinyl estradiol)	1µg/rat/day
III (Test 1)	6	EEMS I	200mg/kg p.o
IV (Test 2)	6	EEMS II	400mg/kg p.o

3.6 Pharmacological Study

1. Estrogenic Activity

✚ Procedure for Ovariectomy

2. Anti-Implantation Activity

3. Smearing Techniques

✚ Swab smear technique

✚ Pipette smear technique

4. Uterotonic Activity

5. Study Of Reproductive Hormones

✚ Estimation of estrogen levels

✚ Estimation of Follicle Stimulating Hormone (FSH) levels

flavanoids, carbohydrates, glycosides, saponins.

Table No. 2: Preliminary Phytochemical Test For EEMS.

S. No.	Phytochemical Tests	Results
1	Test for Alkaloids	+Ve
2	Test for Carbohydrates	+Ve
3	Test for Proteins	+Ve
4	Test for Steroids	-Ve
5	Test for Sterols	+Ve
6	Test for Phenols	-Ve
7	Test for Flavonoids	+Ve
8	Test for Gums and mucilage	-Ve
9	Test for Glycosides	+Ve
10	Test for Saponins	+Ve
11	Test for Terpenes	+Ve

+Ve: indicates the presence of compounds.

-Ve: indicates the absence of compounds.

4. RESULTS AND DISCUSSION

4.1 Preliminary Phytochemical Investigation

The revealed results of the preliminary phytochemical screening of the ethanolic extract of *Musa sapientum* Linn. pseudostem. Results were shown below. The ethanolic extract gave positive results for alkaloids,

4.2 Estrogenic Activity

Table No. 3: Estrogenic Activity Of The Ethanolic Extract Of Musa Sapientum.

Treatment (dose)	Uterine weight mg/100gm body weight
Control	91.55 ± 1.283
Standard (Ethinylestradiol)	160.66 ± 1.542***
EEMS (200mg/kg)	113.83 ± 7.040*
EEMS (400mg/kg)	140.66 ± 10.791**

Linn. Pseudostem (EEMS)

N=6 animals in each group.

***P<0.001, **P<0.01, *P<0.05, when compared with control.

Values were expressed as mean ± SEM.

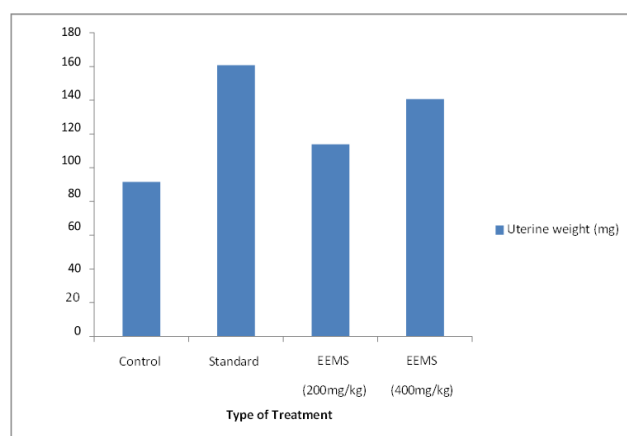


Fig. No. 4: Estrogenic Activity of The Ethanolic Extract of Musa Sapientum.

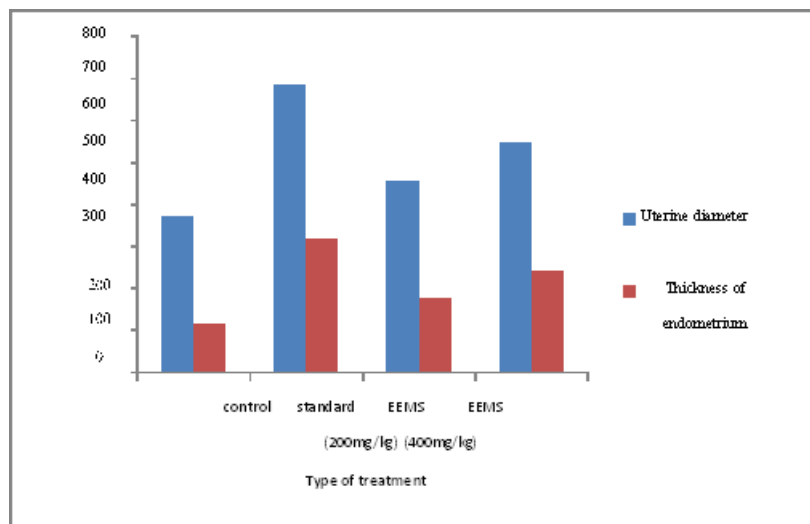
Linn. Pseudostem (EEMS)**Table No. 4: Histological Changes In The Uterus And Endometrium After Treatment With Ethanolic Extract of The *Musa Sapientum* Linn. Pseudostem (EEMS).**

Treatment (dose)	Diameter of uterus (μm)	Thickness of endometrium (μm)
Control	370.316 $\pm$ 2.768	114.83 $\pm$ 2.822
Standard (Ethinyl estradiol)	685.32 $\pm$ 3.949***	317.16 $\pm$ 1.740***
EEMS (200mg/kg)	454.73 $\pm$ 2.656***	176.33 $\pm$ 1.85**
EEMS (400mg/kg)	545.083 $\pm$ 2.538***	239.83 $\pm$ 1.40***

N=6 animals in each group.

*** P < 0.001, **P < 0.01, when compared with control.

Values were expressed as mean $\pm$ SEM.

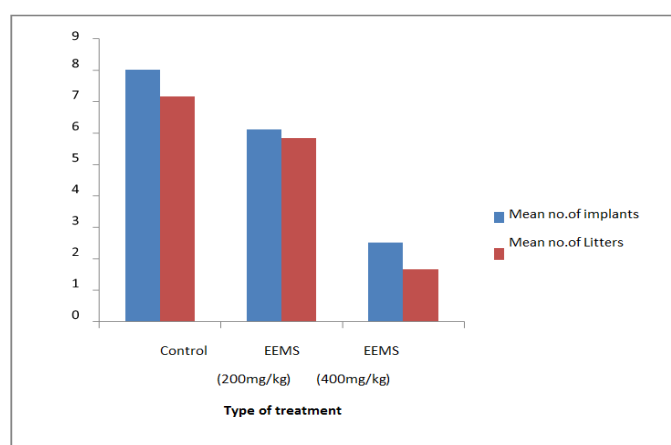
**Fig No. 5 Histological Changes In The Uterus And Endometrium After Treatment With Ethanolic Extract Of The *Musa Sapientum* Linn. Pseudostem (EEMS).****4.3 Study On Anti – Implantation Activity****Table No. 5: Effect of Ethanolic Extract Of *Musa Sapientum* Linn. Pseudostem On Implantation In Rats (EEMS).**

Treatment	Dose (mg/kg)	No. of Implants	No. of Litters
Control	-	8.0 $\pm$ 0.3651	7.16 $\pm$ 0.3070
EEMS	200	6.1 $\pm$ 0.3071**	5.83 $\pm$ 0.4773***
EEMS	400	2.5 $\pm$ 0.3650***	2.0 $\pm$ 0.2582***

N = 6 animals in each group.

*** P < 0.001, **P < 0.01, when compared with control.

Values were expressed as mean $\pm$ SEM.

**Fig. No. 6: Effect of Ethanolic Extract of *Musa Sapientum* Linn. Pseudostem on Implantation In Rats (EEMS).**

4.4 Effect of Ethanolic Extract on Serum Reproductive Hormones

Table No. 6: Effect of Ethanolic Extract Of *Musa Sapientum* Linn. Pseudo Stem (EEMS) On The Serum Reproductive Hormones.

Treatment	Estrogen (pg/ml)	FSH (ng/ml)
Control EEMS	26.83 ± 0.4773	8.00 ± 0.365
(200mg/kg) EEMS	29.66 ± 0.333**	5.83 ± 0.307**
(400mg/kg)	32.16 ± 0.477**	4.0 ± 0.258**

N=6 animals in each group.

**P < 0.01, when compared with control group.

Values were expressed as mean ± SEM.

4.5 Uterotonic Activity

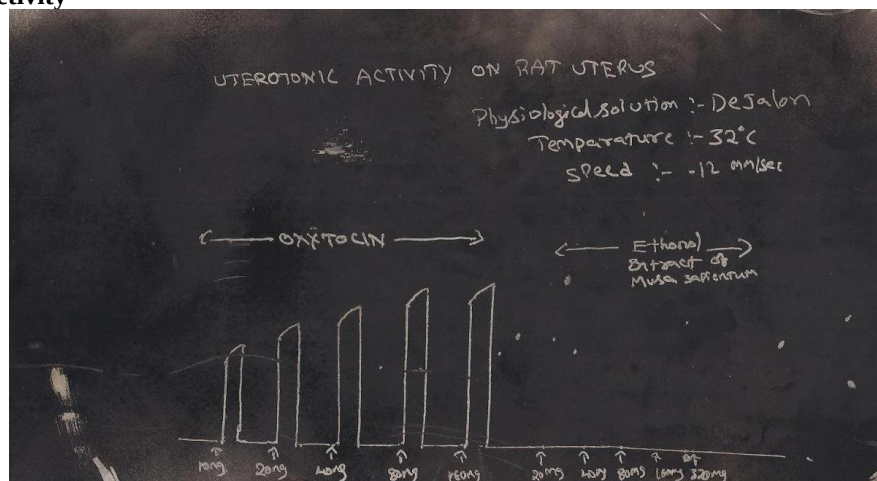


Fig. No. 7: In Vitro Uterotonic Activity on Rat Uterus.

4.6 Swab Smear Technique

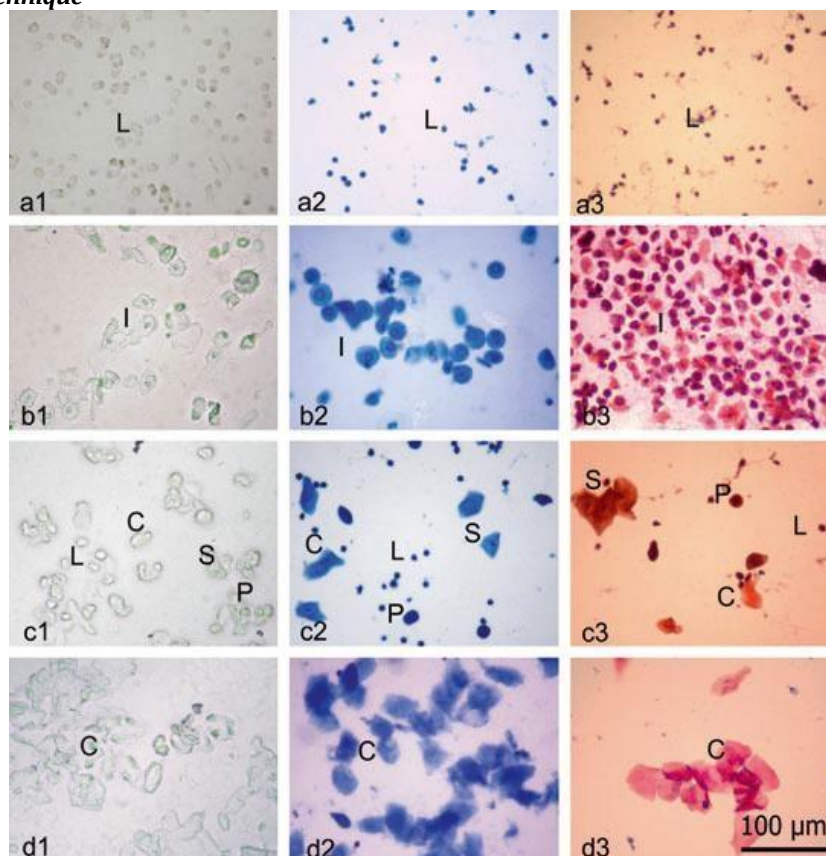


Fig. No. 8: Photomicrographs of Vaginal Smears From Female Rats With Different Staining Methods.

- ✚ Dioestrus phase is depicted in a1, a2 and a3,
- ✚ Pro-oestrus in b1, b2 and b3,
- ✚ Metoestrus in c1, c2 and c3,
- ✚ Oestrus in d1, d2 and d3
- ✚ a1,b1,c1,d1 - unstained smears
- ✚ a2,b2,c2,d2 - methylene blue stained smears
- ✚ a3,b3,c3,d3 - Papanicolaou stained smears
- ✚ Leucocytes (L), intermediate cell (I), cornified cell (C), parabasal cell (P) and superficial cell (S) are indicated.

DISCUSSION

In the present study, the ethanolic extract of the *Musa sapientum* Linn. pseudostem was tested for antifertility property at doses of 200 and 400mg/kg body weight. The effect of the ethanolic extract of *Musa sapientum* Linn. pseudostem (EEMS) on the immature ovariectomised rat uterus is shown in table 3 and table 4. Oral administration of the ethanolic extract at 400mg/kg body weight caused a significant increase in uterine weight in immature rats. The uterotrophic changes such as diameter of the uterus, thickness of the endometrium were significant when compared with control rats.

The ethanolic extract of *Musa sapientum* Linn. pseudostem was found to reduce the number of implants significantly when compared with the control group. The ethanolic extract of *Musa sapientum* Linn. pseudo stem did not cause any contractions of the uterus. Therefore the extract did not have the uterotonic activity. The extract shown that there was significant increase in the serum estrogen levels and decreased levels of FSH.

The negative feedback mechanism takes place due to increase in estrogen levels. The exact equilibrium of oestrogen and progesterone is essential for the implantation. Oestrogen stimulates the proliferation of the endometrium in preparation for the reception of a fertilized ovum. Large number of antifertility plant extracts is known to exhibit estrogenic activity in rats. Estrogenic substances may cause the expulsion of ova from tube, disrupt the functional equilibrium between the endogenous estrogen and progesterone which may results in failure of fertility.

The administration of ethinyl estradiol and ethanolic extract caused a significant increase in the uterine weight, when compared with control group. It also caused a highly significant increase in uterine diameter, thickness of the endometrium. The uterotrophic potency of the extract was less than that of ethinyl estradiol.

Musa sapientum Linn. Pseudostem has the folk claim for its contraceptive action. In Mirzapur district, the tribal women use the pseudostem of *Musa sapientum* Linn. as the contraceptive who used to take this early in the morning for nine days after menstruation. At this

period there is increased levels of estrogen (proliferative phase). Due to the estrogenic activity the extract might have produced the synergistic action and increases the level of estrogen leading to the suppression of the FSH (negative feed back mechanism) which leads to the disturbance in the Ovulation.

Flavonoids isolated from *Strigalutea* and *Strigaorobanchioides* possessed strong estrogenic and antifertility properties. Alkaloids and terpenoids present in *Ailanthus excelsa* exhibited the antifertility activity. Hence the flavonoids, alkaloids and terpenoids present in the pseudostem might be responsible for its antifertility activity.

5. CONCLUSION

The present study indicates that the ethanolic extract of *Musa sapientum* Linn. Pseudostem possess significant antifertility activity. The study demonstrated the possible therapeutic application of the pseudostem in fertility control. However, further study on the pharmacokinetic profile, safety and active principles of the plant have to be carried out.

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