e - ISSN - 2249-7668
Print ISSN - 2249-7676

International Journal of
Pharmacology & Toxicology

www.ijpt.org

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

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**ABSTRACT**

Several plants are traditionally used as contraceptive agents by the rural people in India. Musa sapientum Linn. Pseudostem is one of the folk claimed medicinal plant used as an antifertility agent by tribal women in Mirzapur district, U.P. The present study was carried out to evaluate the antifertility activity of the plant by carrying out pharmacological studies with the EEMS extract. The oral administration of EEMS was screened for its antifertility effect at the doses of 200 and 400mg/kg. The extract shown the significant increase in the uterine weight of an immature ovariectomised rats. It also causes significant increase in the diameter of the uterus and thickness of the endometrium which resembles the estrogenic activity of the extract. The extract also exhibits the anti-implantation activity causing the decrease in the number of implants. The significant decrease in the FSH levels in extract treated groups compared with those of control animals indicates hormonal imbalance and disturbance in ovulation through suppression of FSH. Thus, the EEMS exhibited significant antifertility activity which could be by estrogenic and antiimplantation activity.

**Keywords:** Antifertility Activity, Musa, Anti-Implantation, FSH.

## 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.

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. Approximately 75% of the population in emerging nations receive herbal medical health care, compared with over half of the population in developed nations, particularly for lifestyle-related diseases. The herbal medicine market is also benefiting from a structural change in how it can generate financial gain for different countries and their business sectors, which is why more companies are providing medical coveragefor herbal medical care [1, 2].

A review of the literature indicated the potential benefits of the use of a number of plants/preparations for fertility regulation [3]. Some local contraceptive agents have also been described in traditional medicine. An attempt has been made to document Musa sapientum that is usually prescribed as antifertility agent or have been tested for their activity in vitro or in vivo.

## MATERIALS AND METHODS

### 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).

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**Plant material**

The Pseudostem of *Musa sapientum* was collected from the surroundings of chennai, India and authentified by a certified botanist. 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 vaccuam under reduced pressure and dried in dessicator. Thus the ethanolic extract of *Musa sapientum* pseudo stem (EEMS) was prepared [4].

**Preliminary Phytochemical Screening**

The Preliminary Phytochemical Screening has been conducted on the plant extract to determine the phytochemicals present in the extract [4].

**Experimental Procedure**

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 [5] and animals were grouped as follows given in table 1

**Estimation of anti-Estrogenic activity**

Colony-bred Wistar female rats, aged 21-23d, weighing between 35-45g were bilaterally ovariectomised under sterile conditions [7]. They were divided into four groups consisting of six rats each. All the animals were treated with the respective doses of the drugs for 7 days. On day 8, the rats were sacrificed by pentobarbitone anaesthesia, the uteri were dissected out, and the exact weight of the uteri were recorded. The two horns of the uterus were separated; one of the horns was used for investigation of biochemical changes. The diameter of the uterus, thickness of endometrium, was measured using a calibrated ocular micrometer [6].

**Anti-implantation activity**

Colony-bred virgin female Wistar rats (150-200 g) were used. The vaginal smears from each rat were monitored daily. Only the rats with normal estrous cycle were selected for the experiment. The female rats were caged with male rats of proven fertility in the ratio of 3:1 in the evening of proestrous and examined the following day for the evidence of copulation. Rats exhibiting the copulation plug or thick clumps of spermatozoa in their vaginal smears were separated and that day was designated as day 1 of pregnancy, and those rats were divided into three groups containing six rats in each group. All the animals were treated with the respective concentrations of the test extracts from day 1 to 7 of pregnancy and on day 10, laparotomy was performed under pentobarbitone anaesthesia using sterile conditions. The uteri were examined to determine the number of implants. [8]

**Estimation of Uterotonic activity**

The animal was sacrificed and the abdomen was opened. The uterine horns were cut at their junctions with the fallopian tubes, and placed in a dish containing De Jalon´s solution. For each experiment a uterine strip was set up in a thermostatically regulated organ bath that contains the Dejalon's solution, which was maintained at about $37^0$ C and gassed with air. The uterine strip was tied with the tissue holder and the contractions were recorded with the help of student Kymograph. A tension of 1gm was applied to the tissue and was allowed to equilibrate for at least 30 min before starting the test. Oxytocin was used as a stimulant to record contractions. A dose response curve was plotted with Oxytocin at a final bath concentration each of 40ng/ml, 80ng/ml, 160ng/ml and 320ng/ml. Each time, the added Oxytocin was left in contact with the tissue or 30 seconds and then washed with De Jalon's solution.

Isotonic contractions of the uterine muscle with different extract concentrations were recorded, and concentration- response curves were constructed. The extract additions were cumulative. In the same way, the concentration- response curve of the standard drug, oxytocin was constructed. The effect of the extract after thorough washing of the preparation was observed and recorded [9].

**Estimation of reproductive hormones**

Blood samples were collected from the caudal vein of the animal. Four hundred microliters of blood samples were collected from each animal for hormonal assay; 10 µL of serum was used for each hormonal assay. Serum 17β-estradiol and Follicle Stimulating Hormone concentrations were measured by ELISA microwell kits [10].

**Statistical analysis**

All values were expressed as mean ± SEM. and data were analysed by Student's *t* – test using the software Graphpad Instant.

**Table-I: Animal grouping and Dosing**

| GROUPS | TREATMENT | DOSE |
|---|---|---|
| I (control) | Vehicle ( Tween 80) | 5ml/kg |
| II (standard) | Standard drug (Ethinyl estradiol) | 1µg/rat/day s.c |
| III (Test 1) | EEMS I | 200mg/kg p.o |
| IV (Test 2) | EEMS II | 400mg/kg p.o |

**Table 2: Histological changes in the uterus and endometrium after treatment with ethanolic extract of the Musa sapientumLinn.Pseudostem (EEMS).**

| Treatment | Uterine weight mg/100gm body weight | Diameter of uterus (µm) | Thickness of endometrium (µm) |
|---|---|---|---|
| Control | 91.55 ± 1.283 | 370.316 ± 2.768 | 114.83 ± 2.822 |
| Standard (Ethinyl estradiol) | 160.66 ± 1.542*** | 685.32 ± 3.949*** | 317.16 ± 1.740*** |
| EEMS (200mg/kg) | 113.83 ±7.040* | 454.73 ± 2.656*** | 176.33 ± 1.85** |
| EEMS (400mg/kg) | 140.66± 10.791** | 545.083 ± 2.538*** | 239.83 ± 1.40*** |

**N=6 animals in each group; *** P< 0.001, **P<0.01 ,when compared with control; Values were expressed as mean ± SEM.**

**Table 3: Effect of ethanolic extract of Musa sapientumLinn.pseudostem on implantation in rats**

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

**N=6 animals in each group; *** P< 0.001, **P<0.01 ,when compared with control; Values were expressed as mean ± SEM.**

**Table 4: Effect of Ethanolic extract ofMusa sapientumLinn. Pseudo stem (EEMS) on the serum reproductive hormones.**

| Treatment | No. of Implants | No.of Litters |
|---|---|---|
| Control | 26.83 ± 0.4773 | 8.00 ± 0.365 |
| EEMS (200mg/kg) | 29.66 ±0.333** | 5.83 ± 0.307** |
| EEMS (400mg/kg) | 32.16 ± 0.477** | 4.0 ± 0.258** |

**Figure 1: Estrogenic activity of the ethanolic extract of Musa sapientum Linn. pseudostem (EEMS)**

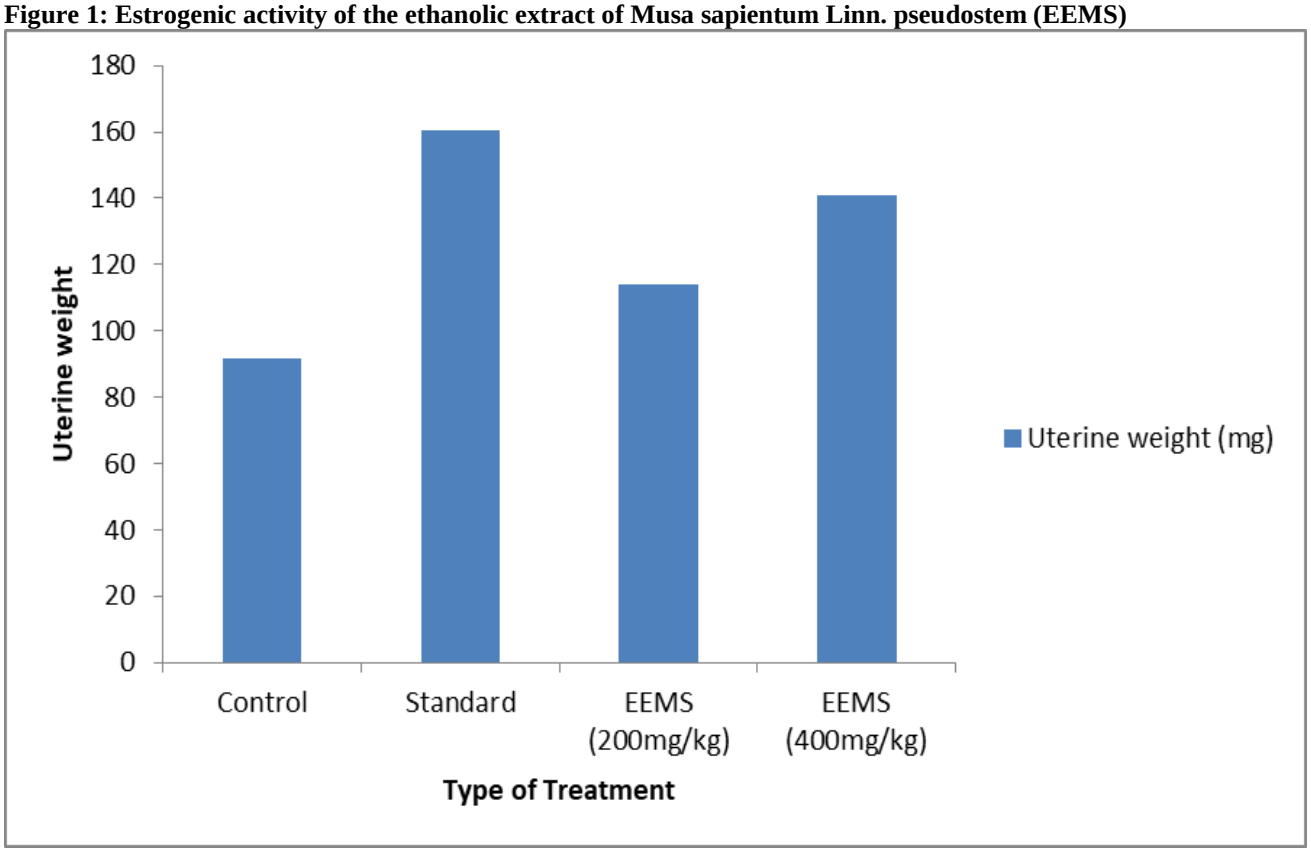


**Figure 2: Histological changes in the uterus and endometrium after treatment with ethanolic extract of the Musa sapientumLinn. Pseudostem (EEMS).**

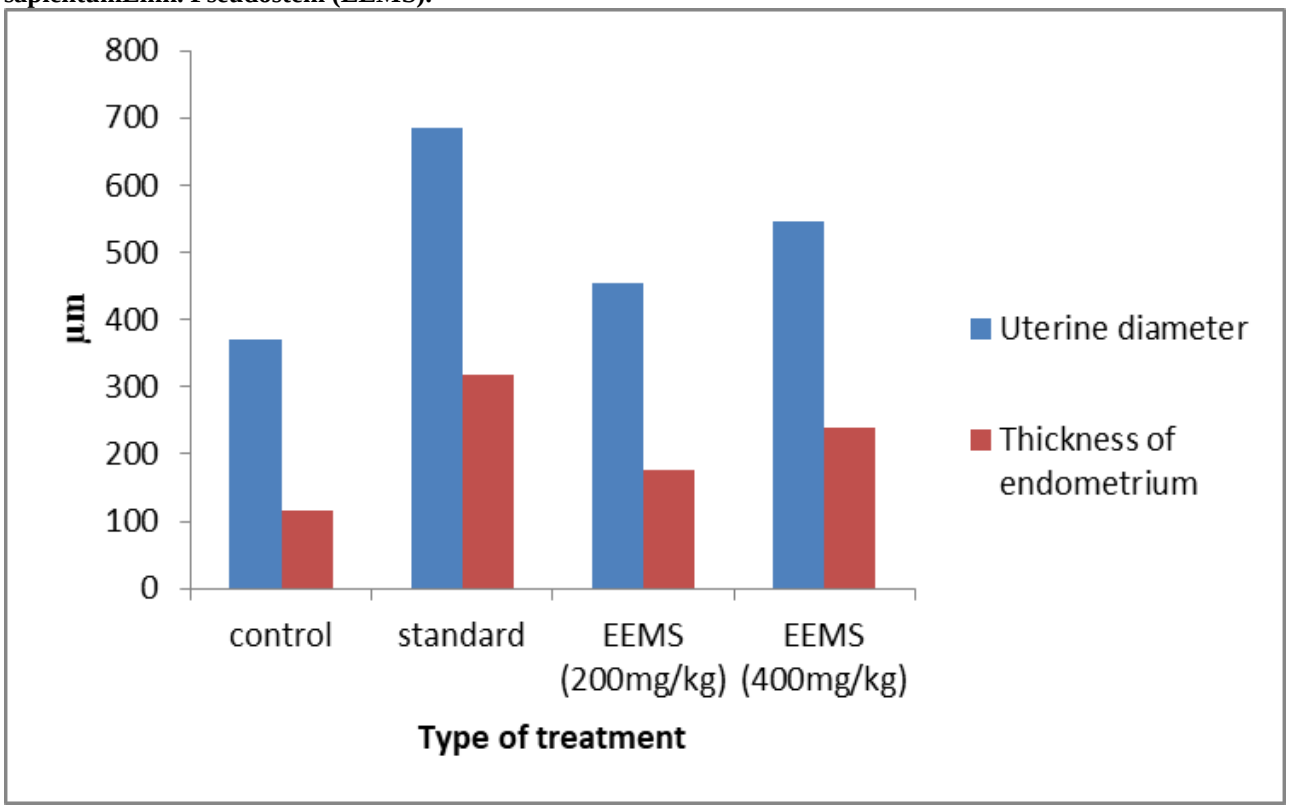



**Figure 3: Effect of ethanolic extract of Musa sapientum Linn. pseudostem on implantation in rats**

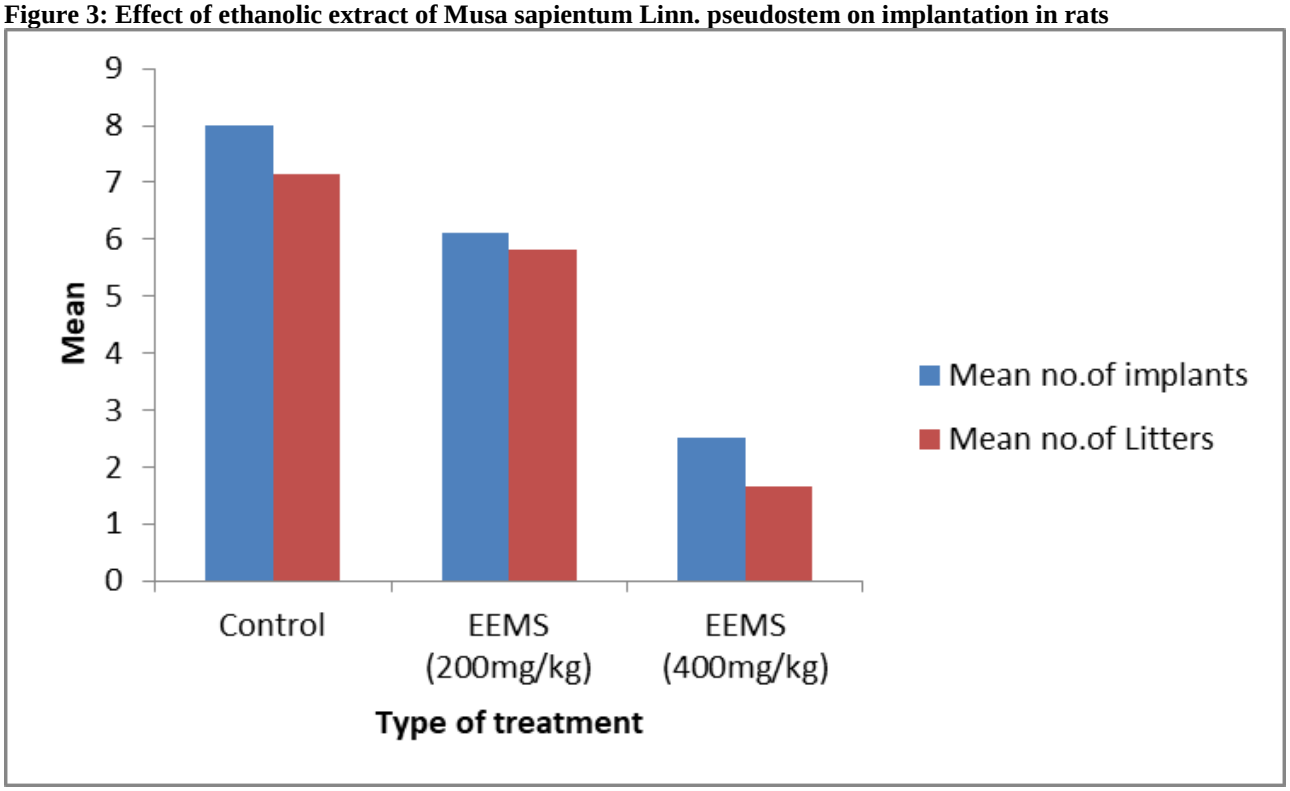
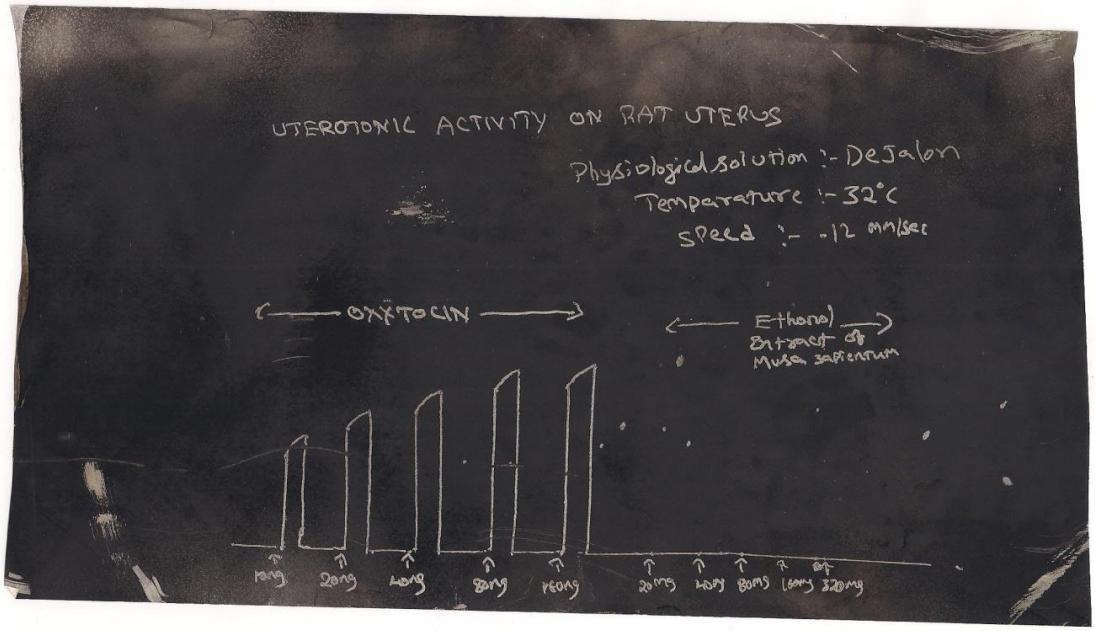


**Figure 4: In vitro uterotonic activity on rat uterus**




## RESULTS

### Preliminary Phytochemical Investigation

The revealed results of the preliminary phytochemical screening of the ethanolic extract of Musa sapientumLinn.pseudostem gave positive results for alkaloids, flavanoids, carbohydrates, glycosides, saponins.

### Estrogenic activity of EEMS

The effect of the ethanolic extract of Musa sapientumLinn.pseudostem (EEMS) on the immature ovariectomised rat uterus is shown in table 2. 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

### Anti – implantation activity

The ethanolic extract of *Musa sapientum* Linn.pseudostem was found to reduce the number of implants significantly when compared with the control group.

### Effect of ethanolic extract on serum reproductive hormones

The extract shown that there was significant increase in the serum estrogen levels and decreased levels of FSH.

### Uterotonic activity of Ethanol extract

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.

## 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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