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Antiovulatory and Estrogenic Activity of Stem of *Musa paradisiaca* in Female Albino Rats

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ABSTRACT

Our aim is to evaluate the effect of petroleum ether, chloroform, acetone, ethanol and aqueous extracts of stem of *Musa paradisiaca* Linn (*Musaceae*) on the estrous cycle and to identify the estrogenic activity of most active ethanol extract in female albino rats. Plant extracts were tested for antifertility activity in female albino rats at two dose level 250 and 500 mg/kg respectively. The effective ethanol extract were further studied on estrogenic activity in rats. Histological studies of the uterus were carried out to confirm their estrogenic activity. The result of study revealed that the treatment of rats with extract of *M. paradisiaca* Linn at a dose of 250 and 500 mg/kg body weight for five days cause a prolonged diestrous stage of the estrous cycle with consequent temporary inhibition of ovulation. The ethanol extract was most effective in interrupting the normal cycle of rats ($P < 0.05 < 0.01 < 0.001$). This later exhibited prolonged diestrous stage of the estrous cycle with consequent temporary inhibition of ovulation. The antiovulatory activity was reversible on discontinuation of treatment. The ethanol extract showed significant estrogenic and antiestrogenic activity. All findings suggest that the antifertility activity of ethanol extract of *M. paradisiaca* Linn.

INTRODUCTION

Increased population is one of the critical problems of the developing countries like in India. This population explosion will have negative impact on our economic policies which may simultaneously misbalance our socioeconomic infrastructure (Kamath and Rana, 2002). Thus, control of human fertility in the sense of its limitation is the most important and urgent of all biosocial and medical problems confronting mankind today (Darney, 1997). In recent years there has been a considerable interest in plant with possible antifertility effect (Vishnukanta and Rana, 2010). In the modern system of medicine, about 25% of prescriptions contain active principle derived from plants. Plant kingdom therefore, holds a great promise for the discovery of new and effective anti-fertility agents (Rahaman and Azahari, 2012). *Musa paradisiaca* (Family: Musaceae) is fast growing herbaceous perennials arising from underground rhizomes, native in tropical Asia and cultivated throughout India and surrounding islands (Swathi D et al, 2011).

M. paradisiaca is used in inflammation, in skin disease, as anaesthetic, as anthelmintic, in bronchitis, in kidney problems, in blood & vaginal disease. Banana is rich in vitamins capable of preventing and curing disease due to Vitamin A deficiency (Kirtikar and Basu, 1975). The plant contains tannic acid, gallic acid, butonates, pentanoates, proteins, polyphenol oxidase, zinc copper, magnesium, chromium, pectin etc. All part of plant has medicinal value (Nirmala et al, 2012). The methanolic & ethanolic extract of root showed potent antiestrogenic activity (Anonymous, 1985; Kumar et al., 2012).

In the ethanobotanical claim, *Musa paradisiaca* Linn. pseudostem and root are used to control child birth or for fertility regulation in India. In Mirzapur district of India, tribal women use pseudostem of banana for contraception. Pseudo stem is crushed and mixed with jaggery and made into pills. Each pill is taken early in the morning for nine days after menstruation (Maurya, 2004). Literature reviews indicated that antifertility activity of stem of *M. paradisiaca* Linn. has not been scientifically evaluated so for. In view of this, the present study was aimed at evaluating the antifertility activity stem of *M. paradisiaca* Linn in female albino rats.

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MATERIALS AND METHODS

Plant

Stem of *Musa paradisiaca* were collected from Jawer Jhalawar district, Rajasthan. Identification of the plants was done by Dr. S. N. Mishra (Sr. Scientist), K.N.K College of Horticulture. A voucher specimen (BRNCP/07/2010/ *Musa paradisiaca* stem) have been deposited in the herbarium of the Department of Pharmacognosy, BRNCP, and Mandsaur. The material was dried under shade, powdered mechanically and stored in air tight container.

Preparation of extracts

The powdered material of stem of *Musa paradisiaca* was extracted using pet ether (60 – 80°C) for 72 hr and successively extracted with chloroform, acetone, ethanol and water for 72 hr each in a soxhlet apparatus. The extracts were evaporated under reduced pressure to obtain solid masses and the percentage yield of the extract was calculated for each extract after drying The extracts were evaporated under reduced pressure to solid masses and the percentage yield of extracts was found to be 2.9%, 2.8%, 2.3%, 8.6 and 10.2 % w/w respectively**.**

Preliminary Phytochemical screening

In order to determine the presence of various phytoconstituents, a preliminary phytochemical study (colour reaction) with extracts was carried out by using the standard procedure given by (Khandelwal, 2005).

Experimental animals

Female albino rats (Wister strain weighing 150-200 g) were used for antiovulatory activity and immature female rats of 21-23 days old, were used for estrogenic activity. The animals were housed in standard environmental condition of temperature (21± 2 °C), humidity (55 ± 10%) and a 12-h light dark cycle. The rats were acclimatized to laboratory hygienic conditions for 10 days before stating the experiment. Animal study was performed in the Division of Pharmacology, B R Nahata College of Pharmacy, Mandsaur, with due permission from the Instutuinal Animal Ethics Committee (918/ac/05/CPCSEA/53).

Acute Toxicity studies

Plant extracts of *M. paradisiaca* stem were studied for acute oral toxicity according to the guidelines set by Organization for Economic Co-operation and development (OECD) guideline number 420. Female Wistar rats (150–180 g) were used for this study. Extract at different doses up to 2,000 mg/kg (p.o.) of the test samples were administered and treated animals were observed for behavioural changes. There was no toxic reaction found. Based on acute toxicity result, 250 mg/kg and 500 mg/kg for antifertility study were selected.

Antifertility activity

Antiovulatory activity

Experiments were carried out in female Wistar rats weighing (150-180 g). The vaginal smear of each rat was examined daily between 9-10 A.M for 15 days to select the animals showing regular cycles (4-5 days). The selected rats were divided into 11 groups of six animals each. The extracts were administered orally for five days to cover one regular estrous cycle. Group I received vehicle (1% Tween 80) and served as control. Group II- XI received Petroleum ether, chloroform, acetone, ethanol and water extracts of stem of *Musa paradisiaca* Linn (*Musaceae*) at 250 and 500 mg/kg body weight. Vaginal smear from each animal was observed every morning between 9-10 A.M for five days of treatment and subsequently for 15 days (Sheeja et al , 2009).

Estrogenic and antiestrogenic activity

The extract with antiovulatory activity was further evaluated for estrogenic activity and antiestrogenic activity (Ghosh et al, 2011). Immature female Wistar strain rats, 30-35 day old, weighing between 35 and 45 g, were divided in to 6 groups of six rats each. The first group served as control and received the vehicle only (1% Tween 80). The second group received ethinyl estradiol (standard) in distil water at a dose of 0.02 mg/kg body weight. The third and fourth group received the most active ethanolic extract of *Musa paradisiaca* Linn stem at two dose level 250 and 500 mg/kg body weight, respectively. The groups fifth and sixth received ethinyl estradiol in addition to a test dose of the ethanolic extract of stem at the same dose. All the above treatment were given for three days (p. o.) (Sheeja et al , 2009).

Histopathology

On the fourth day, the rats were sacrificed by decapitation, the uteri dissected out and surrounding tissues removed and washed with normal saline. The uteri were blotted on filter paper and weight quickly on a sensitive balance and fixed in Bouin's solution for 24 h. The paraffin-embedded tissues were cut at 5 mm thickness and stained with hematoxylin-eosin solution. The sections were examined microscopically for histological observation (Ghosh et al, 2011).

Statiscal analysis

Statistical analysis was carried out by one-way (ANOVA) followed by Dunnett-test. Results were expressed as mean ± SEM from six rats in each group. P values $p<0.001$ were considered significant.

RESULTS

Preliminary Phytochemical investigation

The phytochemical screening of different extracts revealed the presence of various constituents as shown in Table 1.

Table. 1: Preliminary phytochemical studies of various extracts of *M . paradisiaca* stem.

Plant extracts	Constituents
Mp.S -P	Fats, steroids
Mp.S -C	Steroids, glycosides
Mp.S -A	Tannins, triterpenoids, flavonoid
Mp.S -E	Carbohydrates, glycosides, Tannins, glycosides, flavonoid, saponin
Mp. S -W	Carbohydrates, glycosides, Tannins, flavonoid, saponin

Mp. S - *Musa paradisiaca* Stem, P- Petroleum ether (60-80ºC), C- Chloroform, A-Acetone, E- Ethanol, W- Aqueous.

The result from the cytological and reproductive screening (Table 2) in the present study revealed that the ethanolic extract of *Musa paradisiaca* stem could be responsible for the antifertility effect. Treatment of mice with extract of 250 and 500 mg/kg body weight for 15 days caused a prolonged estrous cycle with significant increase in the duration of diestrous phase (Table 2) and elongation of estrus stage in treatment with higher dose (500 mg/kg body weight).

It is observed that in the control group of animals treated with 1% Tween 80 which was used as a vehicle in the present experiment all the six animals manifested normal cyclical oestrus phase throughout the study period. The estrous cycle in rats with ethanolic extract at dose level of (250 mg/kg), normal cyclical oestrus phase was absent in all the six animals after 4-5 days on an average. With higher doses in 2nd group (500 mg/kg) estrous phase disappeared more quickly i. e within 3 days on an average. This estrous suppressing effect of ethanol extract lasted for some period of drug treatment and even after discontinuation of the drug. In the group treated with 250 mg/kg ethanol extract, estrous suppressing effect lasts for about 10 to 14 days and in the 2nd group treated with 500 mg/kg ethanol extract estrous suppress for about 15 to 22 days. From the above observation it is seen that ethanol extract of *Musa paradisiaca* leaves caused suppression of the estrous phase in female albino rats in a dose dependent, reversible manner. Since estrous phase in animal is a manifestation of ovulation, it may be presumed that suppression of estrous phase in albino rats is due to suppression of ovulation, suggesting an antiovulatory effect of the drug in the experimental group of animals.

Estrogenic activity and antiestrogenic activity

The effect of estrogenic effect of ethanol extract is shown in Table 3.Oral administration of ethanol extract at 250 and 500 mg/kg body weight caused a significant increases in uterine weight in immature rats **(**Vs control p< 0.001).The thickness of endometrium was significantly increased when compared to the control rats (Figure 1, 2 & 3). The uteri of these rats were inflated and full of fluid resembling the proestrous/estrous uterus. The epithelium of the endometrium consisted of spindle shaped cells with basal nuclei.

Table. 3: Estrogenic and antiestrogenic activity of stem of *Musa paradisiacal.*

Groups	Dose (mg/kg)	Uterine weight (mg/100g body weight)
Control	(Tween-80, 1%)	47.50 ± 1.25
Ethinylestradiol	0.02	145 ± 3.787***
Mp.S-E	250	68. 43 ± 1.24***
Mp.S-E	500	83.22 ± 2.02***
Ethinylestradiol+ Mp.S-E	0.02+250	165.31 ± 0.34***†
Ethinylestradiol+ Mp.S-E	0.02+500	142.22 ±3.65***†

Mp.S - *Musa paradisiaca* stem
P- Petroleum ether (60-80ºC), C- Chloroform, A- Acetone, E- Ethanol, W- Aqueous. Each value represents the mean ± S.E.M. (n=6); $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ Vs Control. $^{\dagger}P < 0.05$ Vs Ethinyl estradiol (Dunnett's' test).

The stroma consisted of loose and edematous fibroblast-type cells with edema. The control rats shows closed vagina whereas the treated rats showed an open vagina. Examination of the vaginal smears of the treated rats revealed predominantly cornified and nucleated epithelium cells. The administration of ethanol extract aggraved a significant increases in the uterine weight, signifying the estrogenic activity. However, when treated with ethinyl estradiol, it lowers the effect of estrogenic activity produced by ethinyl estradiol (Figure 4) comparatively the ethanol extract was found to be more active.

Histopathology

Histological examination of the uteri were carried out in the ethanol extract of *Musa paradisiaca* stem treated groups of animals with an idea to substantiate the experimental findings.

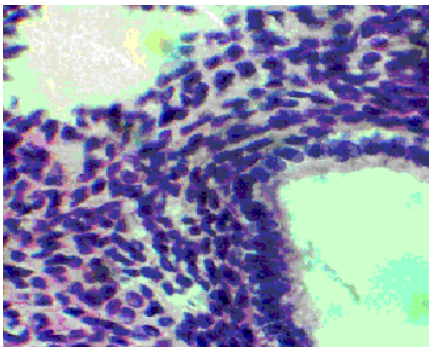

Fig. 1: Photomicrograph of a transverse section of the uterus of control rats.

Table. 2: Effect of treatment of various extracts of *M paradisiaca* on estrous cycle for 5 days in rats.

Treatment	Dose mg/kg	Duration of cycle (Days)	Duration of different phases of estrous cycle (days)			
			Proestrous (days)	Estrous (days)	Metestrous (days)	Diestrous (days)
Control	---	4.35 ± 0.02	0.84 ± 0.17	0.83 ± 0.17	0.83 ± 0.31	1.83 ± 0.40
Mp.S -P	250	4.24 ± 0.55	0.63 ± 0.30	0.61 ± 0.16	1.03 ± 0.35	1.35 ± 0.91
	500	4.36 ± 0.16	0.77± 0.50	0.84 ± 0.31	1.08 ± 0.22	1.70 ± 0.73
Mp.S -C	250	3.69 ± 03.6	0. 51 ± 0.49	0.67 ± 0.28	0.93 ± 0.32	1..51 ± 0.30*
	500	3.83±0.21	0.67 ± 0.32	0.59 ± 0.13	0.89 ± 0.33	1.36 ± 0.87*
Mp.S -A	250	5.25 ± 0.49*	0.66 ± 0.47	0.67 ± 0.15	0.88 ± 0.32	2.50 ± 0.99
	500	5.21 ± 0.89*	0.66 ± 0.21	0.65 ± 0.27	0.78 ± 0.38	2.40 ± 1.00
Mp.S -E	250	5.77 ± 0.35**	0.25± 0.11*	0.82 ± 0.30	0.67 ± 0.37	4.33 ± 0.54**
	500	5.57 ± 0.22***	0.38 ± 0.38*	0.51 ± 0.12	0..85 ± 0.25	3.89 ± 0.85***
Mp.S -W	250	4.26 ± 0.25	0.65 ± 0.32	1.02 ± 0.34	1.06 ± 0.34	1.34 ± 0.76
	500	4.24 ± 0.02	0.52 ± 0.21	0.73 ± 0.22	1.09 ± 0.24	2.26 ± 0. 78

Mp.S - *M. paradisiaca* Stem
Each value represents the mean ± S.E.M. (n=6); * $P < 0.05$. ** $P < 0.01$, ***P <0.001 vs control. (Dunnetts s't' test)

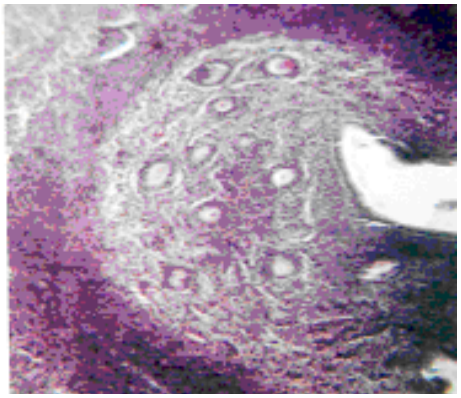

Fig. 2: Photomicrograph of a transverse section of the uterus of ethanol extract at 250 mg/kg p.o -treated rats with stroma consisting of loose fibrous tissues with edema.

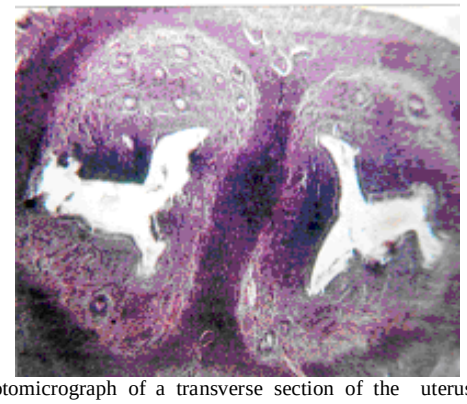

Fig. 3: Photomicrograph of a transverse section of the uterus of ethanol extract, 500 mg/kg p.o-treated rats, with increase in the thickness of the endometrium.

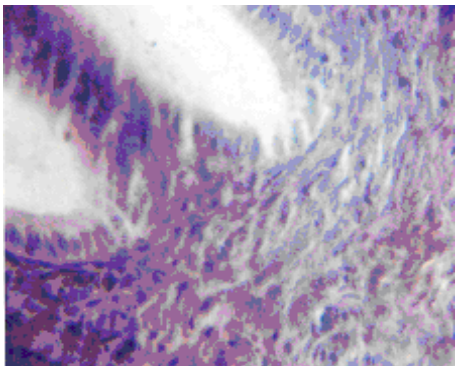

Fig. 4: Photomicrograph of a transverse section of the uterus of ethinyl estradiol (0.02 mg/kg p.o)-treated rats showing proliferation stage.

DISCUSSION

Research on fertility regulating plants has been given priority by Central Drug Research Institute (CDRI) Lucknow and Indian Council of Medical Research (ICMR) New Delhi, in recent years, but so far not a single plant product is marketed, which can be used as anti-fertility agent, in this direction the efforts have been made on the anti-fertility activity of stem of *Musa paradisiaca*. In the present study, stem of *Musa paradisiaca* was tested for its antiovulatory activity and for estrogenic activity. Among the five extracts tested at two different doses, the ethanol extract at 500 mg/kg body weight was more potent in their antiovulatory activity, which was further studied for estrogenic activity. The ethanol extract of *Musa paradisiaca* stem exhibited significant ($P < 0.05$, $P < 0.01$, $P < 0.001$) antifertility activity. The rat has a characteristic short estrus cycle of 4 to 5 days in phases which make them ideal for reproductive studies. The presence and absence of four cell types and the relative proportion of each cell type, determine the stages of the estrous cycle. An estrous cycle is a rhythmic reproductive cycle in sexually matured female mammals and is influenced by the release of gonadotropin releasing hormone from the hypothalamus, gonadotropins from the pituitary gland and six hormones from the gonads. While female indicating a quiescent uterus and resting vaginal epithelium. A proestrous smear will have many epithelial cells with granular cytoplasm, indicating a rapidly growing vaginal epithelium and also the preovulatory stage. Withdrawal of the treatment did not indicate any significant change either in the four phases of the estrous cycle, or in the duration of the cycle. An irregular pattern of estrous with a prolonged diestrus and consequently a reduced number of ova in the ovary was attributed to administration of *Musa paradisiaca* stem extract. The prolongation in the diestrous phase explains the remote possibility of the rats getting pregnant. The reversible nature of the antifertility activity of the extract is explained through the observation that there was no significant change in the diestrous and the estrous cycle after withdrawing the extract from those of the control. As a result, the extracts provoked inhibition of the ovulation with consequent reduction of the cyclicity (Table 2). The extract with antiovulatory activity was further studied for its estrogenic activity and antiestrogenic activity. Ethanolic extract of stem of *Musa paradisiaca* was found to posse significant estrogenic activity as indicated by increase in uterine weight, vaginal cornification and uterotropic responses. In immature female rats, when compared to control, but not significantly greater than standard in dose dependent manner (Table 3). The stem extract acted as estrogen when given alone but when given with ethinyl estradiol it exhibited slight antiestrogenic activity. This shows that the extract acted as competitive antagonist to the much more potent ethinyl estradiol. It is well known fact that estrogenic substances inhibit pregnancy by suppressing the level of both follicular stimulating hormone (FSH) and luteinizing hormone (LH), which in turn prevent the implantation. Estrogen and progesterone are the hormones responsible for histology and functional modifications of female genital tract (Anderson et al, 1972). Preliminary phytochemical studies indicated the presence of carbohydrates, glycosides, tannins, glycosides, flavonoid, saponin. According to the literatures, flavonoids and saponins are known to exhibit antifertility activity. The non-steroidal compounds with estrogenic activity including flavonoids (flavones, flavonones and isoflavonoids) alkaloids, phenolics, occur in variety of plants are well documented as anti-fertility agents (Khushalani et al, 2006).The ethanol extract displayed significant activity when compared with controls, indicating that flavonoids could be responsible for the activity. In the present study the histological evidence of the uterus treated with ethanolic, extracts clearly

supports an unfavorable uterine milieus, showing obliterated lumen with loose stroma, increased height of luminal epithelium and stimulated uterine gland in respective extracts, therefore from the present findings it can safely be said that all the extracts possesses estrogenic activity in dose dependent manner.

CONCLUSION

The results of the present study indicate that the ethanol extract of *Musa paradisiaca* stem have significant antifertility activity. The antifertility effect of ethanol extract of *Musa paradisiaca* Linn stem appears to be possibly due to its antioestrogenic effect, either by blocking the estrogen receptors or by diminished estrogen synthesis. The ethanol extract of leaves of this plant could be used to induce abortion and can further be developed into a contraceptive.

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