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Research Article

ESTROGENIC ACTIVITY OF *MUCUNA PRURIENS* IN SWISS ALBINO MICE

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ABSTRACT
Various parts of *Mucuna pruriens* have been claimed to be useful in conditions like dysmenorrhoea, amenorrhoea, menorrhagia, infertility in females and lack of libido, stamina, sex drive, spermatorrhea, impotency in males. In present study, estrogenic activity of *M. pruriens* in mice was studied. The ovariectomy of selected mice was performed by flank method under mild ether anesthesia. Drugs under study were administered for fourteen days. One day after the administration of last dose, at necropsy, uteri were isolated, squeezed and weighed, under moist condition (freshly) for Assay of water uptake and under dehydrated condition for uterine weight assay**.** In present study, significant uterine weight gain was observed in moist and dry condition which might be due to the presence of estrogenic activity in the plant *M. pruriens* which was administered in the ethanolic extract form to the mice. Thus, it can be concluded that, the *M. pruriens* posses a good estrogenic activity.
KEYWORDS: *Mucuna pruriens*, ethanolic extract, ovariectomy, estrogenic activity, mice assay.

INTRODUCTION

The plant kingdom plays a major role in the life of man and animals. It serves as a source of food and medicines, which maintains health and vitality of individuals. Plants still maintains their original place as an important source in the treatment of various diseases. In recent years, all over the world, people have started realizing the side effects of the synthetic drugs. Special emphasis is therefore given towards the research and development of herbal drugs, which are economic and produce no adverse reaction.

In dairy animals it is very much necessary to maintain the health of reproductive system for optimum production. Several plants have been described in the indigenous medicine to be useful in various reproductive disorders.

M. pruriens is a widespread fodder plant in the tropics. It is an annual, climbing shrub with long vines that can reach over 15 m in length. When the plant is young, it is almost completely covered with fuzzy hairs, but when older, it is almost free of hairs. *M. pruriens* seeds contain high concentrations of levodopa a direct precursor of the neurotransmitter dopamine and it has long been used in traditional Ayurvedic Indian medicine for diseases including Parkinson's disease[1]. Various parts of *M. pruriens* have been claimed to be useful in conditions like dysmenorrhoea, amenorrhoea, menorrhagia, infertility in females and lack of libido, stamina, sex drive, spermatorrhea, impotency in males. In general, it is observed that, the plant is used as a traditional medicine in animals and also as an ingredient of various herbal products used for male reproductive disorders in human.

The present study was therefore planned to find out the estrogenic activity of *M. pruriens* in mice.

MATERIALS AND METHODS

The protocol of the study was approved by the Institutional Animal Ethics Committee of Krantisinh Nana Patil College of Veterinary Science, Shirwal - 412 801, Dist - Satara, Maharashtra state, India.

Plant Sample Collection and Identification

The whole plant of *M. pruriens* were collected from the surrounding locality of the institute, washed and dried under shade in laboratory. The plant material was identified for its authenticity and voucher specimens were kept in Pharmacology division for future reference.

Plant Extraction

Shade dried whole plants of *M. pruriens* were ground to powder form in grinder. The ethanol extract of powder was obtained by extracting in Soxhlet Extraction apparatus by continuous hot extraction method at 60 ^{0}C

for 35 hours and dried in rotary vacuum evaporator to get dark green extract.

Animals

Inbred Swiss albino mice (nulliparous and non-pregnant) of 08 – 10 weeks of age, weighing 25 ± 2 g maintained on standard managemental practices were procured from M/S. Raj Biotech, (Reg. no. 449/01/b/ CPCSEA), Bhor Dist. Pune (Maharashtra). They were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature 25 ± 2 ºC with dark/light cycle 12/12 h) throughout the experiment. The animals were provided with commercial pellet feed (Amrut Feed Industries, Pune) and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to experiment.

Amongst the procured Swiss albino mice, twenty adult female mice with regular estrous cycle were selected. The regular estruous cycle of the mice was confirmed based on the vaginal cytology[2]. The positive estrous stage was confirmed with almost all large, irregular cornified cells in the vaginal smear. Metestrous smear showed many cornified cells but also some leucocytes and epithelial cells, indicating the post ovulatory stage and desquamation of the vaginal mucosa. A diestrous phase was confirmed with few epithelial cells, mucus and few leucocytes and a proestrous phase was confirmed by many epithelial cells with granular cytoplasm.

The ovariectomy of selected mice was performed by flank method[3] under mild diethyl ether anesthesia. Fifth post-operative day onward vaginal smear were obtained for five consecutive days in morning (8.0 to 9.0 am) and evening (5.0 to 6.0 pm). The vaginal smear staining was carried out by using 5 % methylene blue stain for 30 seconds and depending upon vaginal smear cytology, eighteen mice showing continuous diestrous phase[2] were selected and randomly divided in three groups (Control, Test and Standard) containing six animals each.

Treatment

The actual study was started two weeks after ovariectomy.

Drugs under study were administered orally for fourteen days in morning (8.0 to 9.0 am), as per following schedule shown in Table 1. In present study, Prajana Capsule***** was used as a standard drug. 100 per cent heat synchronization was observed in cows after the administration of the Prajana capsule[4]. It was further reported that unlike with the use of Prostaglandins, the length of oestrus period was not reduced in the Prajana capsule treated cows, which made it possible to easily complete artificial insemination in the whole herd[4]. Therefore, it was decided to take it as a standard drug. The oral administration of the test material and the standard drug was carried out by using oral gavages needle. The dose of the test material 250 mg/kg body weight was fixed based on the pilot work in which doses of 100, 200, 300 and 400 mg/kg body weight were used in female mice. From the results of pilot study we observed that the optimum dose would be in between 200 to 300 mg/kg and thus we fixed the dose as 250 mg/kg as a final dose for the study.

One day (24 hrs) after the administration of last dose, all mice were fasted for 4 hours and then sacrificed by cervical dislocation method under deep diethyl ether anesthesia. At necropsy, uteri were isolated, squeezed and weighed, under moist condition (freshly) for Assay of water uptake[5] and under dehydrated condition (after dehydrating at 100 ^{0}C for 24 hrs) for uterine weight assay[6].

The results obtained were analyzed statistically using student "t" test[7].

RESULTS

In present study, as shown in Table 2, average weights of uteri in moist condition were 152.34 ± 0.62 mg in test group, 173.18 ± 0.36 mg in standard group of mice, which were significantly higher as compared to the weight (57.79 ± 0.14 mg) of uteri in control group of mice. The uteri weights in standard group of mice were significantly higher than that of test group of mice.

In dehydrated condition, the average weights of uteri in standard and test groups were 19.08 ± 0.09 and 18.8 ± 0.11 mg, respectively, with non significant difference. These uterine weights in both the groups were significantly higher as compared to the weights of uteri in control group (11.59 ± 0.06 mg) of mice.

DISCUSSION

In present study, an increase in the wet weight and dry weight of uteri in standard and the test group of mice proves to be the estrogenic activity of ethanolic extract of *M. pruriens*. It is also supported by the results obtained by others researchers too[8]. Administration of estrogen or progesterone to adult or ovariectomised rats is known to elevate significantly wet weight of uterus, cervix and vagina albeit the estrogen is found to exert more pronounced action[8].

An increase in wet weight of reproductive organs was also reported after administration of methanolic extract of *Calotropis procera* roots orally @ 250 mg.kg^{-1} body weight for three weeks in ovariectomised female rats and concluded to possess estrogenic activity[9].

There was an increase in uterine weight in ovariectomised female rats after oral administration of

black cohosh extract daily for three weeks[10]. Same results were also observed in mice after feeding of clover and alpha-alpha hay extract[11].

Increase in the weights of uteri in ovariectomised female can be explained as an estrogen stimulates the protein synthesis in uterine tissue[12]. Removal of ovaries results in loss of uterine protein, thus suggesting the estrogen dependency of uterine protein, and administration of exogenous estrogen to ovariectomised female rats restored some of the lost proteins that appeared in ovary intact rats[12].

In present study, the uterine weight gain was observed in moist and dry condition as well. This might be due to the estrogenic activity of the M. *pruriens* which was administered in the form of ethanolic extract to the mice.

CONCLUSION

From the results obtained in the present study and discussion thereafter, it can be concluded that, the *M. pruriens* posses a good estrogenic activity. However, further studies in large scale are required taking into consideration the different extracts of *M. pruriens* in detail.

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Table 1: Showing drug administration schedule.

Sr. No	Groups	Treatment
1.	Control	0.5 ml distilled water / mouse
2.	Test	6.25 mg ethanolic extract of *M. pruriens* in 0.5 ml distilled water per mouse (250 $mg.kg^{-1}$ body weight).
3.	Standard	2.5 mg Prajana Capsule in 0.5 ml in distilled water per mouse (100 $mg.kg^{-1}$ body weight).

Table 2: Weights of uteri (Mean ± SE) in moist and dehydrated condition

Sr. No.	Groups	Weights (mg) of uteri in moist (fresh) condition	Weights (mg) of uteri in dehydrated condition
1.	Control	57.79 ± 0.14^{a}	11.59 ± 0.06^{a}
2	Test	152.34 ± 0.62^{b}	18.8 ± 0.11^{b}
3	Standard	173.18 ± 0.36^{c}	19.08 ± 0.09^{b}

N = 6; Values with different superscript in a column differ significantly at ($p<0.01$).

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