Research Paper Biomedical

# Effect of *Mucuna Pruriens* Seed Extract on Uterus of Ovariectomized Mice

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**ABSTRACT**

*Mucuna pruriens is an annual, climbing shrub which has an important place among aphrodisiac herbs in India since the ancient times. The plant has been using traditionally for many medicinal purposes such as infertility, Parkinson's disease, loss of libido, antioxidant, anti venom, anti microbial etc. The present study was carried out to investigate the preliminary phytochemical analysis and evaluation of the effect on the uterus of ovariectomized mice. Methanol extract of the seeds was used for the study and applied to the mice for seven consecutive days. On the eighth day all the animals were sacrificed and the uteri were dissected out to see the effect of the extract on it. Result showed that the weight of the extract treated uteri was increased compared to that of the control mice along with the increase in the total uterine protein. Histological architecture of the uteri of the treated mice also showed changes compared to control.*

**Keywords :** *Mucuna pruriens*, **uterus, phytochemical analysis, methanol, extract**

**INTRODUCTION:**

*Mucuna pruriens* (Leguminoseae) is an annual herbaceous twining, climbing legume with flowers of white to dark purple in colour and hang in long clusters. The pods of Mucuna have hair-like needles covering the outside which cause intense irritation to the skin. Traditionally the plant has been using for many diseases all over the world. In India, the plant has been using against abortions, cancer, catarrh, cholera, cough, debility, diabetes, diarrhea, diuretic, dysentery, edema, fertility, gout, impotency, kidney stones, menstrual disorders, nervousness, scorpion sting, snakebite, sterility, tuberculosis, worms, and as an aphrodisiac and uterine stimulant. Pharmacological evaluation of various properties of the seeds of the plant has been reported by number of workers. It has been reported that the seeds have fertility enhancing property in male (Shukla *et al*., 2009; Abraham, 2011). It has been reported that the seeds have anti-venom (Tan *et al*., 2009; Guerranti *et al.,* 2002), antioxidant (Tripathi & Upadhyay, 2001; Rajeshwar *et al*., 2005; Dhanasekaran *et al*., 2008), anti-microbial (Rajeshwar *et al*., 2005), neuroprotective (Kasture *et al.,* 2009) and hypoglycemic (Bhaskar *et al*., 2008) properties. Various parts of *Mucuna pruriens* have been claimed to be useful in conditions like dysmenoorhoea, amenorrhoea, menorrhea, lack of libido and infertility in female (Shahaji, & Parnu, 2011). The present study was planned to find out the effect of methanol extract of seeds of *M. puriens* in ovariectomized mice.

**Materials and Methods:**

Whole plant and matured seeds of M. pruriens were collected from Barpeta district, Assam, India. Scientific identification of the plant was done in the Department of Botany, Gauhati University, Assam with herbarium. Methanol extract of the seeds was prepared in a soxlet apparatus. Preliminary phytochemical analysis was done with standard procedures (Trease & Evans, 1989 and Sofowara 1993). In the present study, mice (C3H) used were obtained from Department of Zoology, Gauhati University, Assam, India. Only females showing normal estrus cycle were selected. Ovariectomy was done by routine procedure using Ketamine-Xylazine anesthesia before 15 days of treatment. All ethical norms prescribed by the departmental ethical committee were strictly followed during the experiment. Acute toxicity was studied by the method followed by Handa & Sharma, 1990 before conducting the experiment and the dose for animals was fixed according to Alam et al., 2009. In the experiment, test compounds were routinely administered by intra-peritoneal injection in a volume of 100 µl at 24hrs interval for 7 consecutive days. Mice were divided into three groups each having 5 animals. Control group received 0.1% Tween-80, positive control group received 1 µg Estradiol 17-β per kg bw (body weight) and test group received 300 mg extract per kg bw daily. On the 8th day, all the animals were sacrificed and the uteri were removed very carefully keeping the uterine fluid intact. Wet weights of the uteri were recorded which were then converted to mg wt/100g bw and processed the uterine tissues for estimation of protein and histological study. Estimation of protein was done by method of Lowry et al., 1951 and histology was done by routine method using Eosin-Hematoxylene stain.

**Results:**

**Preliminary Phytochemical Investigation:** The preliminary phytochemical analysis of the seeds of the *M. pruriens* revealed the presence of protein, glycosides, alkaloids, steroids, flavonoids, phenols, tannins and carbohydrates.

**Acute Oral Toxicity Study:** The oral acute toxicity study revealed no mortality up to the dose 3000 mg/kg. Hence the dose 300 mg/kg body weight was fixed for the present study.

**Effect on uterine weight:** Table-1 showing the effect of the seed extract on relative weight of uterus. It has been recorded that the weight of extract treated uterus was increased in comparison with the control. This increase in uterine weight was statistically found significant.

**Effect on uterine protein:** Table-1 showing the effect of

methanol seed extract on uterine protein content. It has been seen that the amount of protein was increased in test and positive control groups when compared to the control. This increase was found statistically significant which was done by student's t-test.

**Changes in Histological Architecture:** Histological architecture of the extract treated uterus showed changes in the size of uterine lumen; change in the luminal epithelium and in the uterine glandular development. Fig-1 shows that the uterine lumen size was increased in both positive control and test group in comparison with the control group. The thickness of the luminal epithelium was increased in test group compared to that of control group. The folding of luminal epithelium was observed more in both positive control and *M. pruriens* treated groups compared to that of the controlled one. Development of uterine glands was seen in the positive control and test groups. No glandular development was seen in the control group.

**Table 1: Showing the effect of seed extract on uterus of ovariectomized mice. Data presented here as mean±SE. Statistical analysis was done by student's t-test and here *p< 0.5; **p<0.001 and ***p<0.0001.**

| Sl No. | Animal Groups | Uterine weight (mg/100g bw) | Uterine protein (mg/g tissue) |
|---|---|---|---|
| 1. | Control | 40.46±1.42 | 7.92±0.42 |
| 2. | Positive control | 215.77±9.45*** | 57.02±1.79*** |
| 3. | Test | 80.8±7.14** | 10.24±0.71* |

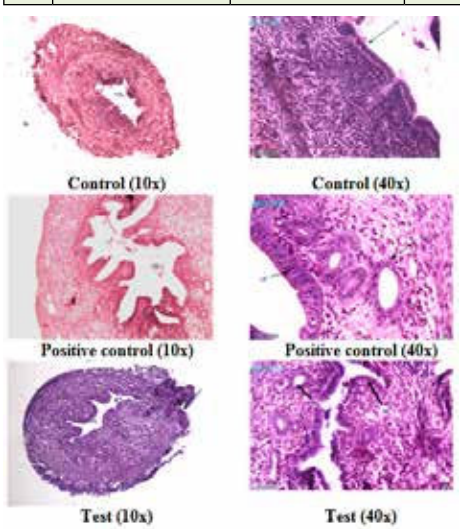


**Fig-1: Effect of seed extract of M. pruriens on histology of uterus in ovariectomized mice after 7 days treatment. Here UG=uterine gland; UL= uterine lumen; LE=luminal epithelium.**

**Discussion:**

The investigation of acute toxicity is an initial step for conducting any biological experiment. In the present study there was no changes in the mice after exposure to the extract upto a dose of 300mg/kg bw. Therefore it was assumed that the plant is safe for conducting experiments on animals. The present study revealed the effect of the methanol extract of *M. pruriens* seeds on the uterus of adult ovariectomized mice. The estrogenic effects of phytoestrogens have been reported by many workers both in-vivo and in-vitro. The Ishikawa estrogen bioassay was used earlier extensively by different workers to determine the estrogenic activities of many plant chemicals (Markiewicz *et al*., 1993, Kalita, 1998). Estrogens are steroid hormone with important functions in the regulation of specific sexual processes in the female. The present investigation showed the presence of steroids and flavonoids which showed the presence of estrogenic compound. The effect of the particular phytoestrogen would depend on the threshold dose and the duration of treatment. The red clover (*Trifolium prentense*) induced partial cornification of vaginal cells at the two highest doses of 500mg/kg and 750mg/kg on treatment for 21 days (Burdette *et al*., 2002). A classical mouse uterotrophic assay is simply a measure of the increase in uterine wet/dry weight after exposure to a chemical (Marky *et al*., 2001). However, some scientists have suggested that mitotic activity is the best index of an estrogenic effect (Marky, 2001). Thus, substances that directly stimulate mitotic activity in the female genital tract have estrogenic activity. In the present study, there was an increase in the uterine weight among *M. pruriens* treated subjects. Synthesis of protein was reported to be stimulated by estradiol (Takeda *et al*., 1988). Methanol extract of *Careya arborea* Roxb., roots when administered to adult ovariectomized mice for 14 days increased the uterine protein level (Kalita *et al*., 2011). Histological changes like increase in uterine lumen size, development of uterine glands, infolding of the lumen etc. indicated the estrogenic nature of the plant. Hence it can be concluded that methanol extract of seeds of *M. pruriens* may have some estrogenic effects.

**5. Acknowledgement:** Authors are indebted to the dept. of Zoology, Gauhati University and UGC for providing necessary facilities during this study.

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