STUDY ON THE ESTROGENIC EFFECT OF CLITORIA TERNATEA ROOT IN OVARY REMOVED MICE

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Abstract: The sexual cycle in mammals are controlled by some hormones secreted from the gonads and pituitary gland. In ovariectomised mice there is no estrus cycle as there is no ovary to secrete the sex hormones. Exogenous supply of estrogen can induce estrus in laboratory mice. The present investigation was aimed to study the estrogenic effect of root extract of the plant *Clitoria ternatea* in ovariectomised mice. Methanol extract was prepared in soxlet apparatus and final extract was prepared by evaporating the excess methanol in a rotary evaporator. Then adult female mice showing normal estrus cycle were ovariectomised to cease the cycle. The ovariectomised mice were divided into four groups as control, positive control, test-1 and test-2 having 5 animals in each group. All the groups were kept under similar environmental conditions and supplied with same food. Control group received normal saline, positive control group received 0.1µg synthetic estradiol-17β per kg body weight, test-1 and test-2 groups received 200 and 400 mg root extract per kg body weight respectively. All the groups were given their respective dose for 7 consecutive days via oral route except positive control which was given subcutaneously. During the treatment period vaginal smear was studied to observe the induction of estrus. At the end of the experiment vaginal smears of all the experimental groups were studies. Then all the animals were sacrificed under anesthesia and blood were collected by heart puncture. Serum Estrogen level was estimated by commercial kit. Uterine wet weight was recorded immediately after the mice were sacrificed. In the present investigation it was found that the cells of vaginal smears showed cornification in comparison to that of the control group. The wet weight of the uteri was increased significantly in all the tested groups. Serum level of estrogen was increased significantly in positive control and both the test groups in comparison to the control group. From the present study it can be assumed that the plant extract may have some estrogenic property.

Keywords: estradiol; ovariectomy; methanol extract; *Clitoria ternatea*

1. Introduction:

Clitoria ternatea Linn commonly known as Aparajita belonging to the family Fabaceae is a vigorous, strongly persistent, herbaceous perennial legume having twining fine stems, 0.5-3 m long. The plant is distributed almost throughout India, wild or cultivated, Western - north India, Egypt, Syria, Mesopotamia, Iraq, Persia, Arabia, Afghanistan etc. *Clitoria ternatea* L is a very well-known Ayurvedic medicine used for different ailments, which has been investigated scientifically in considerable detail. From ancient times “Shankhpushpi” is known as reputed drug of Ayurveda and reported as a brain tonic, nerve tonic and laxative. It is considered as a “Medhya-Rasayana” in Ayurvedic texts that comprises with herbs like *Convolvulus pluricaulis* (Convolvulaceae), *Evolvulus alsinoides* (Convolvulaceae), *Clitoria ternatea* (Fabaceae) and *Conscora decusata* (Gentianaceae). It is an Ayurvedic drug used for its action on the central nervous system, especially for boosting memory and improving intellect [1-2]. In Cuba decoction of roots alone or roots and flowers are considered emmenagogue. This mixture is made by placing a handful of cleaned and macerated roots in a bottle of water. A glass taken in the evening is said to promote menstruation and induce uterine contractions. A stronger dose of the same liquid is used as a vaginal douche [3]. The root juice is given in cold milk to remove the phlegm in chronic bronchitis. The roots and seeds prescribed internally in cough, disease of liver, spleen and rheumatic affections [4]. The roots are used to treat impotency & infertility and said to have aphrodisiac property in both male and female individuals [5-6]. Mammal ovary secrets two major hormones, estrogen and progesterone which are responsible for the reproductive functions. In absence of ovary or in clinical disorders of ovary, these hormones are either not present in them or present in negligible amount. There are many scientific evidences that exogenous estrogen can mimic the endogenous one. Exogenous source may be synthetic or plant origin. The present study was designed to estimate the serum level of estrogen and progesterone in *Clitoria ternatea* treated ovariectomised mice.

2. Methods:

2.1. Preparation of plant extract:

Prepared powder of root of *C. ternatea* L. was used to make the extract. Methanol extract of *C. ternatea* root (MECR) was prepared with 500 g of plant material in 1000 ml of methanol in a Soxhlet apparatus and run for 18 hours at 45^0-50^0 C. The extract was made solvent free in a Rotary Evaporator (Buchi, Switzerland).

2.2. Animals:

Healthy adult Swiss female albino mice (C3H); approximately 3 months of age and weighing 20-25g were used in the present study. They were maintained under uniform conditions of natural photoperiod (12hrs light/dark cycle), humidity (60-94%) and temperature (24°-32°C). All ethical norms were strictly followed during the experiments. Mice were subjected to bilateral ovariectomy under Ketamine-Xylazine (1:2) anesthesia. Animals were then kept under constant observation for at least 14 days.

2.3. Experimental protocol:

After ovariectomy, all the animals were divided into 4 groups as control, positive control, test-1 and test-2 each group having 5 animals. Control group received 1ml normal saline, positive control group received 1µg estradiol/kg bw. Test-1 and test-2 groups received 200mg and 400mg of root extract respectively /kg bw**.** All the treatments were given for 7 consecutive days and on day 8^{th} all the animals were sacrificed. During treatment vaginal smears were studied from day 3 till the last day. After sacrifice blood was collected and serum was taken for estimation of the hormones.

2.4. Study of vaginal smear:

Vaginal smears were prepared as per the method described by Emmens, 1941 and Kalita, 1998 [7-8].

2.5. Collection of blood samples:

Blood samples were collected using normal routine procedure (Heart puncture).Collected blood were then allowed to stand for 30 minutes to coagulate and then centrifuged at 3000 rpm for 15 min. Serum were collected and preserved for the estimation of different biochemical studies.

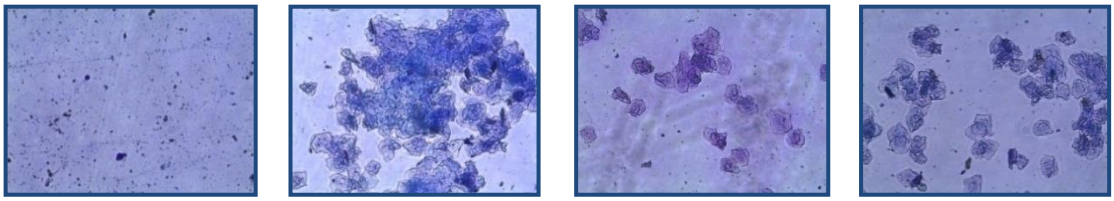
2.6. Estimation of serum estradiol level:

For the estimation of serum estradiol, blood was collected from all the experimental animals. Serum Estradiol was estimated using hormone estimation kit (bioMeriex SA) with ELFA (Enzyme Linked Fluorescent Assay) technique.

3. Results and Discussion:

3.1. Effect on vaginal cytology:

With the absence of ovary, estrus cycle was completely ceased in the experimental mice. Presence of cornified epithelial cells (mentioned here as estrus cells) in the vaginal smears of the animals indicated the effect of estrogenic compound. Control animals showed no cells of estrus from the beginning till the end of the experiment but, there was persistent estrus cells observed in positive control, test-1 and test-2 groups. In the positive control group cornifeid epithelial cells were observed from the 3^{rd} day of treatment and the number of the cells were gradually increased during the treatment period. On the other hand, estrus cells were observed from the 5^{th} day of treatment in the test-1 and test-2 groups. The number of estrus cells vary in the experimental groups. These results are consistent with the findings of other laboratories to screen the estrogenic compound. In a study, Drill (1966) reported that vaginal epithelial cell cornification in ovariectomized rodents could be induced only by estrogenic compounds. It is believed to be a definitive *in vivo* test for identification of estrogenic substances or complex mixtures [9]. *Citrus limonum* seeds extract when orally fed to immature ovariectomized rats for 7 days resulted in the increase in uterine weight as well as vaginal cell cornification [10].



Control E-2 Test-1 Test-2

Figure 1: Photographs showing distributions of Giemsa stained cornified cells in the ovariectomized mice from the vaginal smears on 8th day of experiment.

3.2. Effect on Uterine weight:

After 7 day continuous oral administration of the MECR dose, all the treated animals showed increased uterine weight when compared to the respective control. The increase in the uterine weight was observed in the morphology of the uterus as well as in wet weight. The wet weight of the uterus was measured after cleaning the additional fat bodies and it was converted to weight per 100g body weight of the animals. Care was taken not to lose any fluid inside the uterine lumen and intact uterus was taken for the weight profile. It was observed that, Control group showed 51.27±1.84 mg per 100g body weight of animal while, E_2 treated group showed 357.17 ± 17.57 mg per 100g bw. On the other hand, uterine weight of the 200 mg and 400 mg MECR treated groups were found to be 68.2±4.25 mg and 116.76±6.95 mg per 100g bw respectively. The increase in the uterine weight was measured with student t-test and found statistically significant (**p<0.01 and ***p<0.001) in all treated groups when compared to the control group. Highest significance level was observed for E_2 and 400mg MECR treated groups at p<0.001

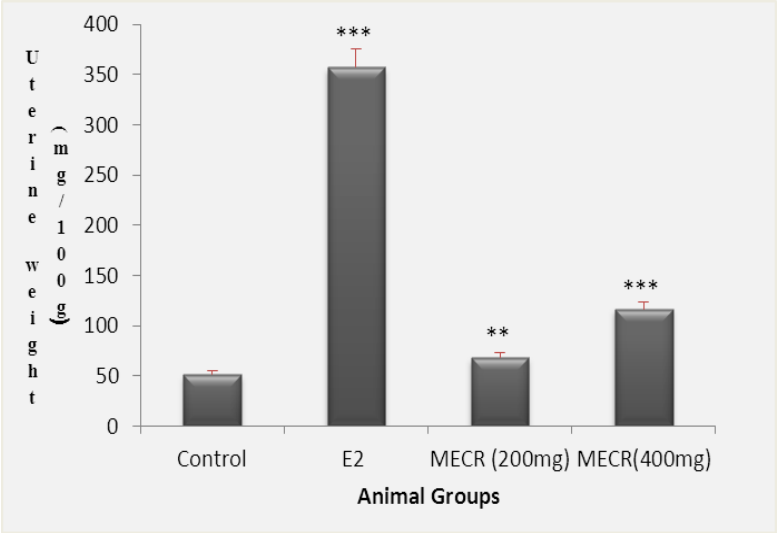
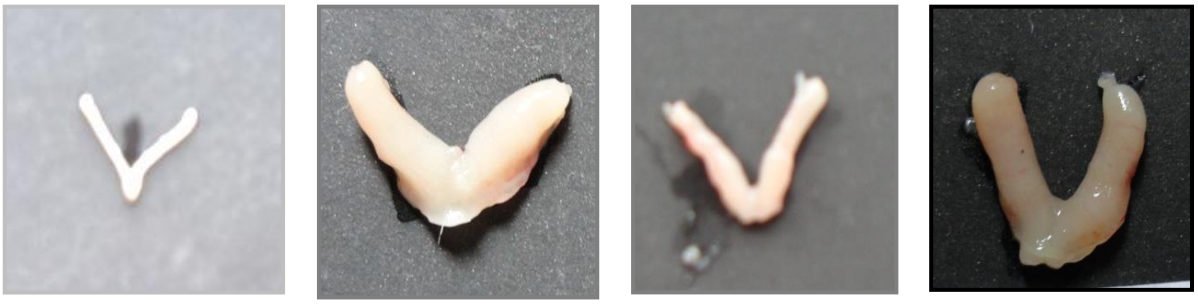


Figure 2: Effects of MECR on uterine wet weight (mg/100g BW) of adult ovariectomized mice. Data are expressed as Mean ± SEM (n=5/ group). Uterine weight was found to increase with the increasing dose of MECR and E_2 treated group showed highest uterine weight. Here **P<0.01 and ***P<0.001, by comparing all groups with the control group using Student's t-test.



A: Control **B: E_2** **C: 200 mg MECR** **D: 400 mg MECR**

Figure 3: Photographs showing the effect of MECR on morphology of uterus of adult ovariectomized mice. After sacrifice uterus from each animals was cut out very carefully and fat bodies around the uterus was removed. The intact uterus was then placed on a black board made up of non-absorbent material and took the photographs with a digital camera. The pictures clearly show the enlargement of the uterine volume in the treated mice in comparison with the control. E_2 treated group showed largest uterine volume and the control group showed constricted uterus. The MECR groups showed comparatively larger uterine volume than the control one.

Rodent uterotropic assay measured an increase in wet-weight of the uterus, is one of the established methods to determine estrogenicity of a chemical (whether it is plant derived or industrially synthesized) [11]. Most notable effects of phytoestrogens are a marked increase in weight of the uterus relative to body weight [12]. Santell *et al.*, 1997 reported that 375µg genistein per gram of diet induced a significant uterine weight gain in 60-day old ovariectomized rats [13]. In adult ovariectomized rats, administration of ethinyl estradiol markedly stimulated the uterus as measured by uterine weight gain [14]. Sheehan (1995) documented that increase in the weight of the uterus (a classic estrogen target site) is one of the ways to measure the biological activity of phytoestrogen [15].

3.3. Effect on serum estrogen level:

Serum estrogen level was measured in all the experimental groups. As endogenous estrogen was restricted with the removal of both the ovaries, the control group showed lowest amount of serum estradiol (27.59 ± 2.7 pg/ml). It was observed that, there was significant increase in serum estradiol level in all the treatment groups. Highest estrogen level was found in positive control group (151.8±7.97 pg/ml),test-1 group showed 50.184±4.72 pg/ml and test-2 group showed 103.94±5.11 pg/ml. Data were analyzed statistically with student t-test and found significant when compared to the control group. After ovariectomy there is depletion of endogenous estrogen level and administration of exogenous estrogens can elevate the level of serum estrogen. *Nigella sativa* L. seed extract when administered to ovariectomized rats for 3 weeks for studying the estrogenic effect, serum estrogen level was found to increase in the extract treated groups when compared to the control [16]. Aqueous extract of *Labisia pumila* was orally fed to ovariectomized rats for 60 days and found that the serum estrogen level increased compared to control. It was concluded that the plant is useful in the treatment of postmenopausal symptoms and estrogen deficiency-related disease [17].

4. Conclusion:

The present investigation indicated the presence of estrogen like substances in the plant extract and it can be concluded that the plant may be used in estrogen deficiency syndromes as in the above study.

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