International Journal Of

Recent Scientific Research

ISSN: 0976-3031

Volume: 6(12) December -2015

DETERMINATION OF ESTROGENICITY OF ASOCA PLANT (Saraca asoca Linn.) IN ADULT FEMALE OVARIECTOMISED MICE

Anindita Deka, Jogen Chandra Kalita, Yungkham Rajeevkumar Singh and Manalisha Deka

THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 12, pp. 7661-7664, December, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

DETERMINATION OF ESTROGENICITY OF ASOCA PLANT (*Saraca asoca Linn.*) IN ADULT FEMALE OVARIECTOMISED MICE

Anindita Deka*[1], Jogen Chandra Kalita[2], Yungkham Rajeevkumar Singh[3] and Manalisha Deka[4]

Animal Physiology and Biochemistry Lab, Department of Zoology, Gauhati University, Assam

ARTICLE INFO

Article History:

Received 16th September, 2015
Received in revised form 24th October, 2015
Accepted 23rd November, 2015
Published online 28st December, 2015

Key words:

Phytoestrogen, *Saraca asoka*, ovariectomy, estrus

ABSTRACT

The following study was carried to determine the phytoestrogenic property of Asoka plant, *Saraca asoca*, one of the foremost plants utilized from antiquity till date against different gynaecological ailments like uterine fibroids, relief of menstrual pain, treatment of menorrhagic etc. Oral administration of methanolic extract of bark of *Saraca asoca* was done in the ovariectomised mice at the dose of 500mg/kg body weight which showed marked induction of estrus, significant increase in uterine wet weight($p<0.0025$) and also marked change in uterine size. Hence, it may be inferred that the extract may contain phytoestrogenic compounds that led to the following changes in the uterus of ovariectomised mice. Thus, *Saraca asoca* can be used as a potent remedy for curing estrogen deficient diseases.

INTRODUCTION

Estrogen is a female sex hormones. It imparts its diverse effect on the growth, differentiation and function of many target organs, such as the mammary gland, uterus, vagina, ovary, testis, epididymis and prostate [Hewitt and Korach,2003; Rosselli and Dubey, 2006; Cooke *et al*,1991; Sauders PTK,2005; Ebling *et al*, 2000]. Estrogens also impart an important role in bone maintenance, the central nervous system, the cardiovascular system [Schomberg *et al*, 2005; Couse and Korach, 1999; Wang *et al*, 2003] and hypothalamic-pituitary axis [Hewitt and Korach, 2003]. The three major naturally occurring estrogens in human body are estrone (E1), estradiol (E2) and estriol (E3). Although it is found in both the sexes but its concentration is significantly higher in female. It is the main hormone that regulates menstrual cycle and prepares the uterus for pregnancy.

Environmental estrogens or xenoestrogens are chemicals that mimic some structural part of the physiological estrogen class of molecules, but are not endogenous to animal. This compound may act as inappropriate estrogens, and or could interfere with the action of endogenous estrogens. Environmental estrogens are compounds that are by-products of manufacturing (certain plastic or detergents), or agriculture chemicals (such as some pesticides) that can disrupt or inappropriately mimic many estrogenic processes in mammals [Mc Lachlan, 1993, Singleton and Khan, 2003]. Xenoestrogens can also be synthesized by plants such as isoflavons from soy, coumesterol from red clover, zearalenone from grain moulds or fungi and these compounds are reported to cause disruption of reproductive cycles when ingested [Burton and wells, 1998]. However, some phytoestrogens have been suggested as safe replacement of endogenous estrogens based on their consumption prevalence correlating with fewer estrogen-provoked diseases. In the hypoestrogenic post- menopausal woman with negligible endogenous estrogen levels, phytoestrogens occupy estrogen receptors and exert a weak estrogenic effect and help overcome the post menopausal symptoms. In a normally estrogenized reproductive female the exogenous phytoestrogens will compete at the receptor level with endogenous estrogens, thus inhibiting the effect of the endogenous hormone that help overcoming some menstrual problems [Mackey and Eden, 1998].

The complete ovariectomized (surgical removal of ovary) animal can serve as an animal model system in which there is no endogenous source of principle female hormone estrogen [Torrezan *et al*, 2008]. Thus, one can screen or assess the potency of certain industrial chemical or plant derive chemical (phytoestrogen) whether they are having estrogenic or anti estrogenic property or not. Using the above animal model, an

Corresponding author:* **Anindita Deka
Animal Physiology and Biochemistry Lab, Department of Zoology, Gauhati University, Assam

attempt is taken to check the phytoestrogenity of plant extract of *Saraca asoca*.

METHODS AND METERIALS

Plant materials and extract preparation

The plant materials of *Saraca asoca* Linn, used for the investigation of its estrogenic activity, were collected from the Gauhati University campus in the month of January. The collected plants were identified in the Department of Botany, Gauhati University. The collected barks were cleaned from dust and other materials, and then they were dried under the shade and grinded. About 200g the shade dried bark powder of *Saraca asoca* Linn was refluxed with methanol (70%) and distilled water in a Soxhlet extractor for 18 hrs in batches of 25g each cycle. The extracts obtained by the above techniques were concentrated by using rotarary evaporator and evaporation under controlled temperature. The yield obtained after evaporation was 10.2g. The dried bark extract of *Saraca asoca* was then stored in a desiccator for further use.

Table 1 Uterine wet weight of various groups of mice after 7 consecutive doses

Sl.no	Mice	Weight
1	Control – I	0.0044 g
2	Control – II	0.0052 g
3	Control—III	0.0047g
4	Estradiol – I	0.0321 g
5	Estradiol – II	0.0350 g
6	Estradiol –III	0.0328g
7	Treatment – I	0.0074 g
8	Treatment – II	0.0079 g
9	Treatment – III	0.0073g

Table 2 Post- hoc test for the uterine wet weights obtained in three groups of mice, Control, estradiol treated and A. asoka treated

Multiple Comparisons

Value Tukey HSD

(I) groups	(J) groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.0285333*	.0007538	.000	-.030846	-.026221
	3	-.0027667*	.0007538	.024	-.005079	-.000454
2	1	.0285333*	.0007538	.000	.026221	.030846
	3	.0257667*	.0007538	.000	.023454	.028079
3	1	.0027667*	.0007538	.024	.000454	.005079
	2	-.0257667*	.0007538	.000	-.028079	-.023454

*. The mean difference is significant at the 0.05 level.

Preparation of ovariectomised mice

C3H strain albino female mice were procured from the Animal House Facility; Department of Zoology, Gauhati University and due permission was taken from Animal Ethical Committee of Gauhati University. Animals were acclimatized to normal environmental condition in the laboratory for one week. Standard pallet diet with vitamins and mineral supplements (supplied by Agrivet Farm Care Division, Glaxo Smithkline, Chennai, India) and water was given ad libitum. The animals were of average body weight of 20-23 g. All the chemicals, glasswares and instruments were obtained from the Department of Zoology, Gauhati University. The procedure of ovariectomy was followed according to Kalita *et al*. The mice were ovariectomised via a dorsal incision under intramascular administration of proportionate ratio of xylazine and katamine, in the ratio of 2:1 [Kalita *et al*, 1998, Sikuler *et al*, 1985]. Two weeks later they were randomly grouped on the basis of the need of the experiment.

Grouping of animals

All the mice were divided into three groups namely,

Group A (Control group): In this group the mice were fed with 200ul 1% tween80, because 1%tween 80 was taken as the vehicle in the dosage treatment. (n=3).

Group B (Estradiol group): In this group, the mice were treated with estradiol, 0.1mg/kg body weight dissolved in 200ul of 1%tween 80. Since the average weight of the mice was 23 g so each mice received 0.0023mg estradiol dissolved in 200ul of 1%tween 80.(n=3)

Group C (Treatment Group): The mice were fed with Asoca extract, 500mg/kg body weight. The feeding volume for 23 g mice was prepared by dissolving 11.5mg in 200ul of 1% tween80.(n=3)

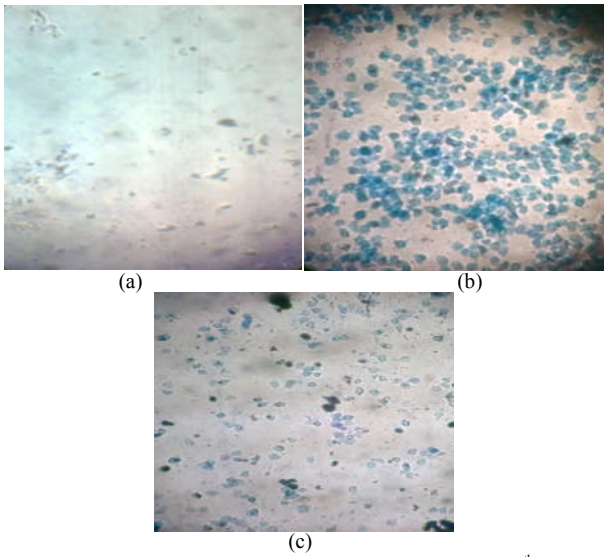
Phytoestrogenicity study

Two parameters were taken into consideration for checking the phytoestrogenicity. First is whether the extract can induce estrous cycle in ovariectomised mice. Determination of the stage of the estrus cycle was assessed by examining vaginal cytology [Byers *et al*, 2012]. The vaginal smears were taken using a small closed hairpin loop and inserted carefully into the vagina after dipping it in methanol. The loop was gently turned and rolled against the vaginal wall and was then removed. The mucous like secretion containing the cells was then mixed with a single drop of distilled water on a glass slide. When the drop dried, one drop of methanol was put with the help of a dropper. When it was dried, 2 to 3 drops of freshly prepared Giemsa stain was put to stain the cells present. It was then washed under slow running water after around 15 to 20 minutes. The smear was then observed under the microscope. The same process was repeated every day for all the animals.

Secondly was to study changes in uterine wet weight and uterine size after treatment with the extract. To compare the uterine size change and uterine wet weight half of the mice were sacrificed after 7 dose treatment. The mice, to be sacrificed, are made unconscious with the help of diethyl ether. Then it is sacrificed by cervical dislocation. The abdomen is cut open and the uterus is dissected out. Photographs of the uterus of the various group sacrificed on 8th day are snapped by Nikon camera (16 megapixels). (Image 2) The uterine wet weights (table I) are measured using a Sartorius immediately after dissection. Before measuring the uterus it is placed in a filter paper to soak excess fluid.

Statistical Analysis

Groups were analyzed with ANOVA to compare between them and post hoc test (Tukey test) to evaluate the significance.



(a) (b)

(c)

Figure 1 Vaginal smear of different groups of mice after 7th dose
(a) Vaginal smear of control group(after 7th day treatment)
(b) Vaginal smear of estradiol group (after 7th day treatment)
(c) Vaginal smear of asoka extract treatment group (after 7th day treatment)

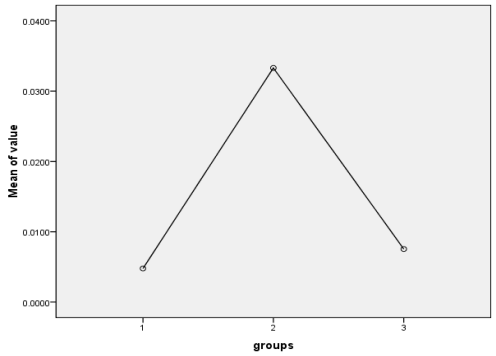


Figure 2 Mean plot of Asoka plant extract treated treatment and control uterine wet weights which has a significant difference ($p<0.025$)

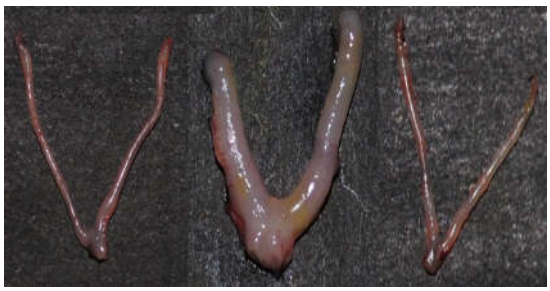


Figure 3 Showing the size variation of the uterus of various groups of mice after the 7th dose; a) The uterus of control group; b)The uterus of estradiol treated; c) The uterus of the treatment group

RESULTS

Induction of estrous cycle

The observations of the vaginal smears taken on the 8th day after 7 consecutive doses are as follows:

Control group

No estrus cells were seen. Only few leucocytes were found lying scattered

Estradiol group

Estrus cells were found. Contain hundreds of large cornified cells (squares) with degenerate nuclei. Masses of adherent cornified cells indicate the stage to be estrus

Treatment group

A good number of estrus cells were observed. Well nucleated epithelial cells along with leucocytes were found scattered. It indicated the proestrus phase preparing the uterus to undergo estrus.

Uterine wet weight

There is a marked increase in the weight of the uterus after treating with the extract. However the increase was more than the controlled but less than the estradiol treated. The results are shown in table I. Significant difference found among the different treatments applied on uterine wet weights of mice ($F_{2, 8}=871.67$; $p< 0.0001$). Futhermore, Post-hoc test (Tukey) [Table 2] showed that each treatment was significantly different from each other. Estradiol treated has the highest mean difference when campared to other two components (0.0001). Asoka plant extract treated treatment and control uterine wet weights also has significant difference ($p<0.025$) as shown in Diagram 1.

Uterine size

There was marked visible enlargement of the uterus size in the treatment group as compared to both the control and estradiol treated.

DISCUSSIONS

In the present study oral administration of bark extract of *Saraca asoca* was done in the ovariectomised mice at the dose of 500mg/kg body weight which showed marked induction of estrus (Image 1), significant increase in uterine wet weight (Table II and Diagram 1) and also marked change in uterine size (Image 2). So it may be inferred that the extract may contain phytoestrogenic compounds that led to the following changes in the uterus of ovariectomised mice. The present study is only a initial step towards establishing asoka plant as a potent estrogenic plant. Therefore, the active phtoestrogenic component *Saraca asoca* bark could be of interest for further development and research. More sensitive *in vitro* bioassays such as yeast bioassays, Ishikawa cell line, HPLC must be employed for the confirmation of the estrogenic compound present in *Saraca asoca*. Moreover analysis should be done at the gene level and the molecular mechanism of action of the phytoetrogens of asoka plant must be sort out for its best and effective contribution in modern therapeutic application.

References

1. Burton J L, Wells M; The effect of phytoestrogens on the female genital tract, *J Clin Pathol* (2002);55:401

2. Byers SL, Wiles MV, Dunn SL, Taft RA Mouse Estrous Cycle Identification Tool and Images. *PLoS* ONE 7(4) (2012) : e35538
3. Cooke PS, Young P, Hess RA, Cunha GR, Estrogen receptor expression in developing epididymis, efferent ductules, and other male reproductive organs. Endocrinology 128(6) (1991), 2874
4. Couse J F, Korach K S, Estrogen receptor null mice: what have we learned and where will they lead us? Endocr. Rev. 20 (1999), 358.
5. Ebling FJP, Brooks AN, Cronin AS, Ford H, Kerr JB, Estrogenic induction of spermatogenesis in the hypogonadal (hpg) mouse. Endocrinology 141 (2000), 2861.
6. Hewitt SC, Korach KS, Oestrogen receptor knockout mice: roles for estrogen receptors alpha and beta in reproductive tissue, Reproduction 125(2003), 143
7. Kalita JC, Milligan SR, Subramanium AV, Relative potency of xenobiotics estrogen in an acute in vivo mammalian assay, Environmental health Prospective,106(I) (1998) 23
8. Mackey R and Eden J, Phytoestrogens and the menopause. The *Journal of international menopause society*, Dec;1(4) ,(1998):302
9. McLachlan, Functional Toxicology: A New Approach to Detect Biologically Active Xenobiotics *J. A. Environ*. Health Perspect., 101(1993), 386.
10. Rosselli M, Dubey RK, Estrogen metabolism and reproduction – is there a relationship? *Journal für Fertilität und Reproduktion,* 16 (2006), 19
11. Saunders PTK, Does estrogen receptor β play a significant role in human reproduction? Trends in Endocrinology and Metabolism 16 (2005), 226.
12. Schomberg DW, Couse J F, Mukherjee A, Lubahn, D B, Sar M, Mayo K E, Korach KS, Targeted disruption of the estrogen receptor-a gene in female mice: characterization of ovarian responses and phenotype in the adult. Endocrinology 140(1999), 2733.
13. Singleton DW and Khan S A, Xenoestrogen exposure and mechanisms of endocrine Disruptions, Frontiers in Bioscience 8, (2003) 110
14. Sikuler E, Kravetz D, Groszmann RJ. Evolution of portal hypertension and mechanisms involved in its maintenance in a rat model. *Am J Physiol*.;248 (1985):G618
15. Torrezan R, Gomes RM, Ferrarese ML, de Melo FB, Ramos AM, Mathias PC, Scomparin DX, Treatment with isoflavones replaces estradiol effect on the tissue fat accumulation from ovariectomized rats, Arq Bras Endocrinol Metabol.;52 (2008) ,1489
16. Wang L, Andersson S, Warner M, Gustafsson J A, Estrogen receptor (ER) {beta} knockout mice reveal a role for ER{beta} in migration of cortical neurons in the developing brain. Proc. Natl. Acad. Sci. U. S. A. 100(2003), 703.

How to cite this article:
Anindita Deka *et al*., Determination of Estrogenicity of Asoca Plant (*Saraca asoca Linn*.) In Adult Female Ovariectomised Mice. *International Journal of Recent Scientific Research Vol. 6, Issue, 12, pp. 7661-7664, December, 2015*

****

ISSN 0976-3031