

OESTROGENIC ACTIVITY OF JAYANTI AND VANSA AN EXPERIMENTAL STUDY

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ABSTRACT

Phytoestrogens, sometimes called "dietary estrogens", are a diverse group of non-steroidal plant-derived polyphenolic compounds. They exhibit structural similarity and mimic the effect of naturally occurring estrogen compounds in the body. Their endocrine activity, as well as their potential influence on other biologic pathways, has led to considerable interest from an epidemiological standpoint. They are advocated as safe substitute to estrogen in women, especially in menopausal syndrome where HRT as treatment modality with its increased risk of breast cancer, heart disease, and stroke outweighs its benefits. Today the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. The present study was designed to study the estrogenic effect of *Sesbenia sesban*, (*Jayanti*) and *Bambusa arundinaceae*, (*Vansa*) in ovariectomised female albino rats by analyzing body and uterine weight changes, vaginal cell cornification, and histopathology characteristic of estrogen-induced responses. Results are suggestive of considerable estrogenic activity and they can be considered as good source of phytoestrogens.

Keywords: Phytoestrogen, menopause, *S. sesban*, *Jayanti*, *B. arundinaceae*, *Vansa*

INTRODUCTION

Natural menopause as defined by the World Health Organization (WHO) is the "permanent cessation of menstruation resulting from the loss of ovarian follicular activity"⁽¹⁾. According to U.S. Census data from 2000, there are about 37.5 million women reaching or currently at menopause (ages 40 to 59)⁽³⁾ and an estimated 85 percent report one or more symptoms as a result of the hormonal changes such as hot flushes, perspiration, dyspareunia, pruritus vulvae, frequency of urine, dryness of skin etc. and psychological symptoms like irritability, anxiety, fluctuation of mood, disturbed sleep, headache without any

known pathology⁽²⁾, prompting nearly 10 percent of them to seek medical help⁽³⁾. HRT so far has been advocated as choice of treatment but Publication of the Women's Health Initiative (WHI) trial results in 2002 significantly reduced physician and patient confidence in and acceptance of hormone replacement therapy (HRT) as an appropriate treatment option for menopause⁽⁴⁾. It has generated an interest to look out for safe alternatives other than HRT. Phytoestrogens are plant derived substances that are structurally and functionally similar to estrogen. They are converted into weak estrogenic substances in the gastrointestinal tract and exhibit

estrogenic activity in body by acting on estrogen receptors. They have both weak estrogen and anti-estrogenic activity therefore; they are termed as natural SERMs (selective estrogen receptor modulators). Their SERMs like action makes them very useful in various indications⁽⁵⁾. They are heavily promoted as the “natural alternative” to estrogen replacement therapy for postmenopausal women or women who have undergone a hysterectomy. In Ayurveda, menopause does not find its mention by name, though all the classics have described the cessation of menstruation occurring at the age of 50 years⁽⁶⁾, as natural ageing process which requires no therapeutic. In the present study two plant products reputed to affect female fertility in folkloric medicine have been selected to investigate their estrogenic activity.

Bambusa arundinaceae, member of family Graminae (Poaceae), common name Bans or bamboo, Sanskrit name Vansa is a perennial plant. Its leaves, roots, grains and gum are reported to be medicinally important. In folklore medicine, tribal women in and around Salem in Tamil Nadu chew leaves of *B. arundinaceae* Retz. in the morning and evening for 1-3 days to induce abortion of an early pregnancy⁽⁷⁾. An ethanolic extract of tender shoots of *B. arundinaceae* (at 300mg/kg/day) to adult male rats impaired the structural and functional integrity of epididymis⁽⁸⁾. The seeds of *B. arundinaceae* are believed to enhance fertility by the Kani tribes of Kanyakumari, Western Ghats, so the seeds are in great demand in pharmaceutical industry to manufacture drugs to improve fertility⁽⁹⁾. Flowers are used in antifertility and seeds are used in dysmenorrhea and scanty periods⁽¹⁰⁾. The leaves of *Bambusa arundinacea* have been

reported to be anti-inflammatory and antiulcer⁽¹¹⁾, are emmenagogue, antileprotic, febrifuge, and bechic, used in hemoptysis⁽¹²⁾. It is reported to have anti-diabetic activity also⁽¹³⁾. Roots of this plant are used for preparation of an ointment which is said to be a folk remedy for cirrhosis and hard tumors, especially tumors of the abdomen, liver, spleen and stomach⁽¹⁴⁾. The root (burnt root) is applied to ringworm, bleeding gums, painful joints⁽¹²⁾. Seeds are acrid laxative, used in strangury and urinary discharges⁽¹⁵⁾. Bark is beneficial for treatment of skin troubles⁽¹⁶⁾ and is used for skin eruptions⁽¹²⁾.

Sesbania sesban, commonly known as Jayanti, Sanskrit name Jaya and English name Common sesban, belongs to family Leguminaceae. Different parts of this plant are reported to have various medicinal properties such as anti-inflammation and antioxidant effect, abortion and antifertility agent, antimicrobial activity. One study suggests inserting *S. sesban* seed paste (15 g) placed in cotton swab into vagina causes abortion in females (human beings). In addition, taking fresh root decoction twice a day for 3 to 4 days following menstrual phase serves as an antifertility agent⁽¹⁶⁾. *S. sesban* seed powder hinder the ovarian normal function, change the uterine structure and prevent implantation, thus, control the fertility of female⁽¹⁸⁾, seeds are shown to have antifertility activity⁽¹⁹⁾. Kandha tribe of India uses the root extracts as contraceptive⁽²⁰⁾, seeds have healing and antifertility properties⁽²¹⁾. The poultice of *S. sesban* leaves are used to cure boils abscesses and absorption of inflammatory rheumatic swellings⁽²²⁾.

OBJECTIVE

To evaluate the effect of methanol extract of *Sesbania sesban* (Jayanti) and *Bambusa arundinaceae* (vans) in ovariectomised female albino rats.

MATERIALS AND METHODS

Plant material

Sesbania sesban seeds were powdered and successively extracted in a soxhlet apparatus with methanol for 24 hours. The methanol extract was concentrated under reduced pressure to syrup and was further freed from solvent in desiccators till a constant weight was obtained. The resultant thick syrupy mass was then used to compute for doses based on a constant volume per body weight basis. An aqueous solution prepared was administered orally for experimental purpose.

Bambusa arundinaceae leaves and tender shoots were air dried, powdered and successively extracted in a soxhlet apparatus with methanol for 24 hours. The methanol extract was obtained into aqueous solution by the same method as *S. sesban*. Aqueous solution was used for experimentation purposes. Drugs were obtained from Dravya Guna department, BHU, and drug extraction was carried out at Department of Medicinal Chemistry, BHU, Varanasi.

Animal

For present study female albino rats, common name Sprague dawley rats, species *Rattus rattus*, were selected. As it breeds easily under standard laboratory conditions and much is known about their reproductive biology. Thirty healthy animals with a weight range of 145 to 200 gms, having normal estrus cycle of 5- 6 days duration were selected and kept under standard laboratory conditions. Rats were supplied with standard pellet diet and water *ad*

libitum. The animals were acclimatized to laboratory hygienic conditions for 10 days before starting the experiment. Experiment was carried out at Zoology Department, BHU.

Ethics statement

The approved standard procedures and the institutional animal ethical committee (Central Animal House of Banaras Hindu University (Registration No-542/AB/CPCSEA) guidelines were followed throughout the experiments. The study was approved by the Institutional Animal Ethical Committee of the Central Animal House of Banaras Hindu University.

Study design

Initial weights of the animals were taken and were bilaterally ovariectomized following standard rodent ovariectomy procedure. A period of 10 day was allotted for wound healing and acclimatization. Objective was to remove the endogenous source of estrogen secretion from the body. Study of vaginal smear cytology was done every day and when smear showed the presence of leucocytes confirming the almost complete suppression of circulating estrogen they were classified into 3 groups of 4 animals each.

Group 1- received 200mg/kg body wt. of *S. sesban* daily for 10 days. (At least 2 reproductive cycle in animals)

Group 2- received 200mg/kg body wt. of *B. arundinaceae* daily for 10 days.

Group 3- received 1 ml of aqueous solution for 10 days (Control).

During the period of drug administration, vaginal smear cytology was studied daily in the morning hours, at the same time between 9-10am each day.

Weights of the rats were taken after 8 day of drug administration and again on 12th day,

i.e. at the end of the treatment. Uterus along with vagina was dissected out and fixed in Bouin's fluid. After successive dehydration in alcohol, weight was taken.

For histological study uterus and vagina were fixed in Bouin's fluid, embedded in paraffin wax, serially sectioned at 6mm and stained with Elarlich's haematoxylin and eosin, slides were studied under microscope.

OBSERVATIONS

Vaginal smear- cellular differentiation

Cornification is histological and cytological change that occurs in the vaginal epithelium especially of a rodent in response to stimulation by an estrogen and that have been used as a bioassay for estrogenicity of chemicals. Vaginal smear of the rats were taken daily between 9am -10am at the same time every day during the course of experiment. Immediately after taking the smear slides were prepared and studied under microscope. Observation of the cell types as leucocytes, epithelial cells and cornified cells were made and their percentage presence in the slide was recorded. A raw score on a scale of 1 through 5 was assigned for cell populations ranging from entirely leucocytes (indicating a diestrus stage) to entirely cornified (indicating an estrous stage). Mean of the cell types from three animals present in each group is shown in respective graphs.

Group 1: Animals in this group were treated with S.sesban, an increase in the average number of cornified and epithelial cells was observed. After treatment, cornified cells reached the level comparable to normal approximately on day 4 and on days 9 onwards percentage of cornified cells in vaginal smear was more than that observed in normal. Epithelial cells reached

the level comparable to normal on day 4 and it was more than normal after 8 days of treatment. The increase was maintained throughout study indicating good estrogenic activity of S.sesban.

Group 2: Animals in this group were treated with B. arundinaceae, an increase in the average number of cornified and epithelial cells was observed. Cornified cells reached the level comparable to normal on day 3 and level remained same throughout the treatment period. Epithelial cells reached the level comparable to normal on day 4 and the level remained comparable to normal throughout the treatment period indicating the estrogenic activity.

Control: Animals in this group were given aqueous solution. Decline in the average number of cornified and epithelial cells after vehicle treatment as compared to before ovariectomy was observed. This indicates loss of estrogenic activity after ovariectomy and treatment did not improve this.

Body weight of animals

The body weight of the animals was measured initially before starting the treatment and after 8 days of treatment and again at the end of the treatment. There was an increase in the weight of the animals during the study period suggesting absence of pharmacological or any toxic effect. Observations made are shown in Table 1

Uterine weight of animals

At the end of treatment, animals were sacrificed and their uterus along with vagina was dissected out and fixed in Bouin's fluid. After successive dehydration in alcohol weight was taken. As far as weight is concerned no significant changes in the weight of uterus was observed. **Table 2**

Uterine histology

Uterus of normal rat showed well developed endometrium and myometrium. Endometrium is lined with single layer of epithelium lining containing cuboidal cells; uterine gland is well proliferated containing secretions. Ovariectomy lead to suppression of endometrium. Uterine glands are also reduced in number. Treatment with *S.sesbane* and *B.arundinaceae* for 12 days in ovariectomised rat led to stimulation of uterus. Uterus showed an increase in size and uterine histology indicates sign of stimulation. Endometrium also showed increase in size. Uterine glands are well proliferated containing secretory products in their lumen. Epithelial lining consist of cuboidal cells as shown in histological pictures.

DISCUSSION

Administration of methanolic extract of *S.sesbane* seeds and *B. arundinaceae* leaves and shoots in bilaterally ovariectomised animals induced vaginal cornification and stimulation of uterus indicating estrogenic activity. Vaginal cytology is good indicator to confirm the effect of estrogen. After animals were ovariectomised vaginal smear showed leucocytes confirming almost complete suppression of circulating estrogen. Such animals were when treated with *S. sesbane* showed appearance of cornified cells on fourth day and average number of cornified cells remained high (higher than normal) throughout the treatment period. Ovariectomised animals were when treated in similar way with *B.arundinaceae* showed appearance of

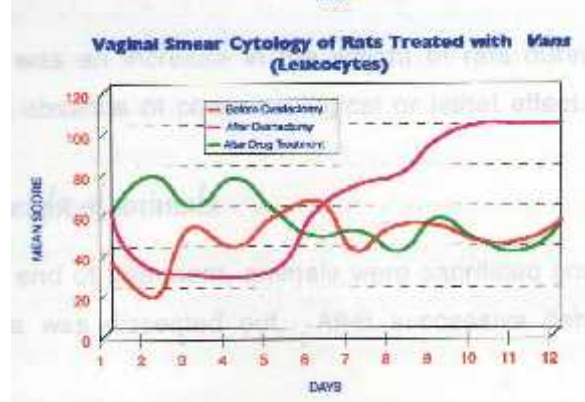
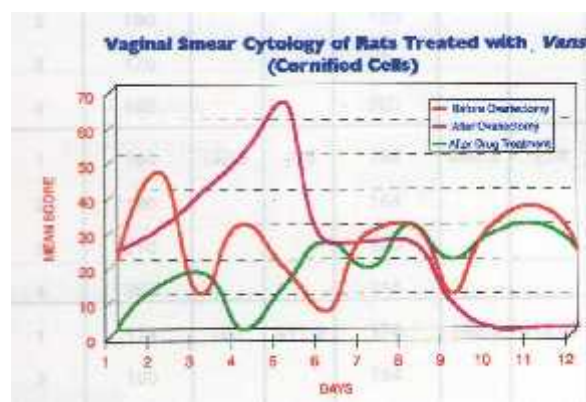
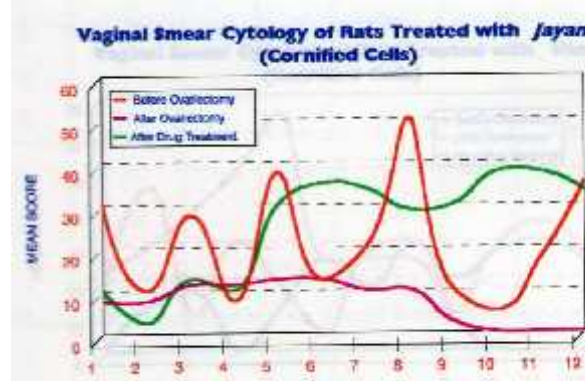
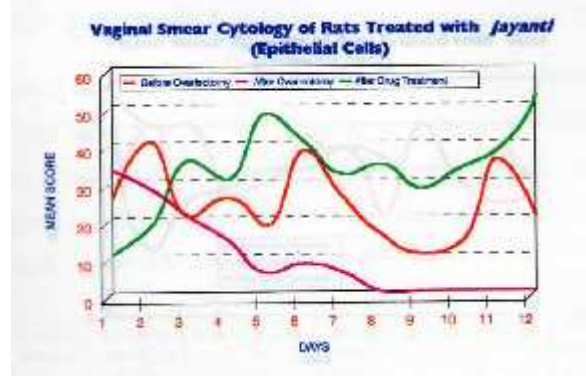
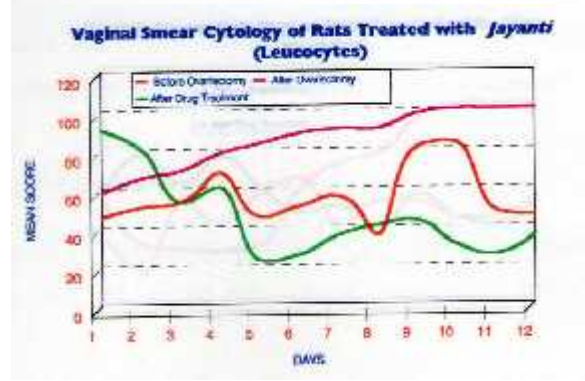
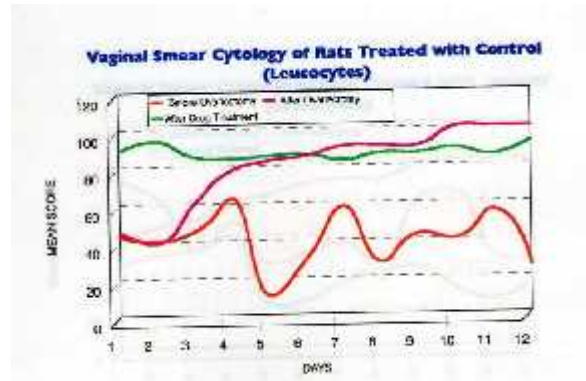
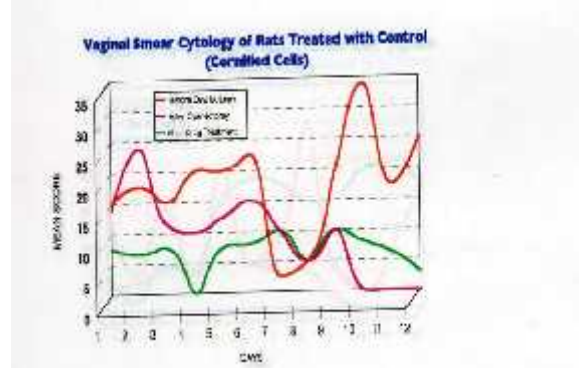
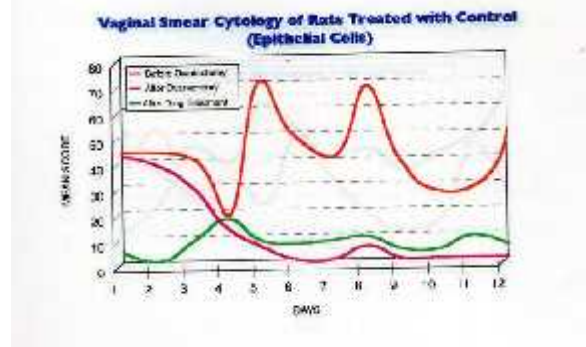
cornified cells and remained comparable to normal animal throughout the course of treatment. Cornification of the vagina in the estrous cycle has been found to be caused by estrogen⁽²³⁾. Vaginal cornification has been induced when estrogen is applied to female mice^(24, 25), rats⁽²⁶⁾ and immature rats⁽²⁷⁾.

T.S. of uterus on histological study showed features of uterine stimulation. Uterine endometrium showed cuboidal cells in epithelial lining of endometrium and an increase in size of uterine glands, their proliferation and presence of secretory products in their lumen.

It does not significantly increase either animal or uterine weight. As it did not suppress the body weight of the animals, the effect of the drug may not be toxic. *S. sesbane* and *B. arundinaceae* could be advocated for therapeutic uses as a good source of phytoestrogen.

CONCLUSION

Use of natural products is preferred compared to synthetic as they are considered more in harmony with the human body. The two plants undertaken in present study are easily available and can be given orally with good results and with minimum side effects. *S.sesban* and *B.arundinaceae* could be advocated as a good and safe source of phytoestrogen. This is a preliminary study and further chemical and pharmacological study, receptor response study and mating studies are suggested.



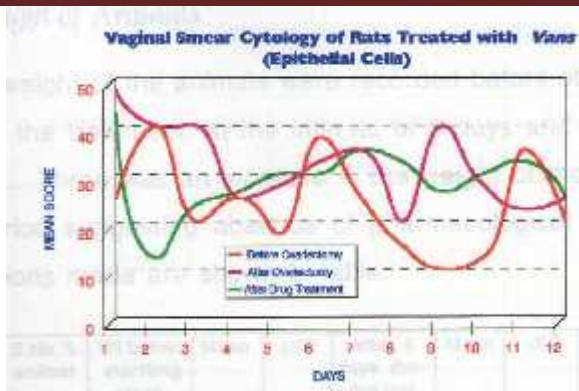


Table 1: Body weight of animals

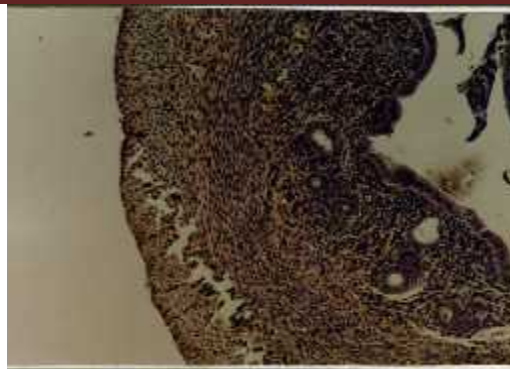
| Drug | S. No. of animal | Wt. before starting treatment | Mean | SD | After 8 days of treatment | Mean | SD | After 12 days of treatment | Mean | SD |
|-----------------------------|------------------|-------------------------------|------|------|---------------------------|------|------|----------------------------|------|------|
| Control | 1 | 190 | 183 | 9.5 | 194 | 181 | 11.1 | 190 | 195 | 12.7 |
| | 2 | 180 | | | 190 | | | 128 | | |
| | 3 | 170 | | | 174 | | | 180 | | |
| | 4 | 190 | | | 200 | | | 210 | | |
| <i>Sesbania sesban</i> | 1 | 180 | 193 | 15 | 186 | 203 | 20 | 186 | 207 | 28.4 |
| | 2 | 180 | | | 184 | | | 180 | | |
| | 3 | 210 | | | 230 | | | 240 | | |
| | 4 | 200 | | | 210 | | | 220 | | |
| <i>Bambusa arundinaceae</i> | 1 | 174 | 180 | 11.2 | 178 | 187 | 12.5 | 180 | 191 | 12.2 |
| | 2 | 190 | | | 194 | | | 200 | | |
| | 3 | 190 | | | 200 | | | 200 | | |
| | 4 | 168 | | | 174 | | | 178 | | |

Table 2: Uterine weight of animals

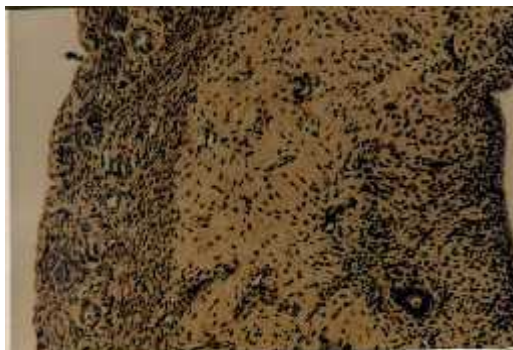
| S. NO | Name of the drug | Wt. of the Uterus in mg | Mean | SD |
|-------|-----------------------------|-------------------------|-------|-------|
| 1 | Control | 0.001216 | 0.152 | 0.036 |
| 2 | | 0.001895 | | |
| 3 | | 0.001782 | | |
| 4 | | 0.001218 | | |
| 1 | <i>Sesbania sesbane</i> | 0.001261 | 0.125 | 1.56 |
| 2 | | 0.001275 | | |
| 3 | | 0.001241 | | |
| 4 | | 0.001245 | | |
| 1 | <i>Bambusa arundinaceae</i> | 0.00178 | 0.137 | 0.02 |
| 2 | | 0.00129 | | |
| 3 | | 0.00122 | | |
| 4 | | 0.0012 | | |



T S of Rat Uterus



T S of Rat Uterus (Treated with *Jayanti*)



T S of Rat Uterus (overiectomised)



T S of Rat Uterus (Treated with *Vansa*)

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