Original Research

Experimental Evaluation of Oestrogenic activity of *Rajahpravartini vati* - A Herbo-mineral formulation

Bhagyalakshmi B R[1], Keshava D V[2], Shobha G Hiremath[3], Pradeep K Prajapati[1]

[1] Dept. of Rasashastra & Bhaishajya Kalpana, I.P.G.T. & R.A., Gujarat Ayurved University, Jamnagar.

[2]Dept of Kayachikitsa, ALN Rao Memorial Ayurveda College, Koppa.

[3]Dept. of Rasashastra & Bhaishajya Kalpana, Government Ayurveda Medical College, Bangalore-09.

Corresponding Authors Email: drbhagyalakshmibr@gmail.com

Abstract

Background: ***"Rajahpravartini vati" (RPV)*** is a classical Herbo-mineral formulation indicated for Amenorrhoea and Dysmenorrhoea.

Objectives: To evaluate *Rajahpravartini vati* for Oestrogenic activity using rat models of uterotrophic bio-assay.

Materials and Method: Immature female rats were randomly divided into five groups as control, standard and three different dose levels of RPV (1/2TED [Therapeutic equivalent dose], TED, 2TED). All these groups were administered with respective medicines for seven consecutive days. Body weight, vaginal openings were noted on everyday during the treatment. On the 8th day rats were sacrificed and uterus was collected. Wet and Dry uterine weights were taken. The obtained results were expressed as Mean ±SEM and unpaired "t" test was applied to compare the effect of test drug with control and standard groups.

Results: Results showed significant oestrogenic activity of RPV at all dose levels, however lower doses (1/2TED) showed highly significant oestrogenic activity in all parameters. Histo-pathologically the trail drug at low doses showed increased endometrial thickness when compared with control group suggesting oestrogenic activity.

Conclusion: Thus the trial drug RPV showed significant oestrogenic activity in all three dose levels better at low doses.

Key words - Oestrogenic activity, *Rajahpravartini vati*, Uterine weight, Uterotrophic bioassay.

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Introduction

Factors effecting female reproductive health is an important area of research. There are probably a number of reasons for this development. Young and fertile females are more participating in the general working life. This may have the effect on female health in general and reproductive health in particular. It is well documented that shift work and sleep deprivation have significant effects on female hormonal status which in turn may be one of the reasons for such health effects.[1] Amenorrhoea and dysmenorrhoea are very common among working women due to hormonal disturbances**.** *Rajahpravartini vati* is the *Khalveeya rasa yoga* prescribed for Amenorrhoea and Dysmenorrhoea containing equal amounts of *Shuddha Tankana* (purified borax), *Shuddha Hingu* (purified *Ferula asafoetida* Linn*), Shuddha Kaseesa* (purified Ferrous Sulphate), and *Kanya sara* (solid extract of *Aloe vera* Linn) which are triturated with *Kumari swarasa* (juice of *Aloe vera* Linn.)[2]

Tankana (borax) one of the ingredients is said to posses *Streepushpajanana* property (inducing menstruation/ ovulation).[3] *Kaseesa* (Ferrous sulphate) cures *Rajodbhava shoola*(Dysmenorrhoea).[4] *Hingu* (*Ferula asafoetida* Linn) is having *Artava janaka* (inducing

menstrauation) property.[5] Ferutinin one of the component of *Hingu* is a Phytoestrogen.[6] The studies showed that ferutinin exerts an oestrogenic activity in ovarectomized non oestrogen primed female rats.[7] *Kanya Saara* is having *Raja pravartaka* (inducing menstruation), *Stree pushpa janaka* (inducing menstruation/ovulation)[8]property. It is rich in Anthraquinone which is emmenogogue and uterine stimulant.[9] These do contain phytoestrogen.[10] Betacystosterol, cholesterol, fatty acids, composterol are the phytoestrogens present in Kumari. The aqueous extracts of Kumari are used to treat infertility. It is also having anti androgenic and oestrogenic activities and reduces the secretion of gonadotrophins. [11]

All the ingredients of RPV are having identical properties like *Katu rasa* (pungent taste), *Ushna veerya* (hot in potency), *Sara* (mobileness) *Teekshna* (sharp) *guna* and *Pitta vardhaka (*aggravates *pitta)*. It is also noted that all are having *Raja pravartana,* and *Streepushpa janana* property along with some drugs containing phytoestrogens. Till now no research work is carried on RPV experimentally. Synthetic oral estrogens have many side effects like nausea, bloating, weight gain, fluid retention, mood swings etc. They even increase the risk of developing breast cancer, endometrial cancer, thromboembolism.[12] But the phytoestrogens can overcome by these adverse effects. Thus an attempt was made to evaluate the oestrogenic activity of *Rajahpravartini vati* experimentally.

Materials and Methods

Collection of Raw materials and processing of raw materials

Raw materials of PRV were collected from local market, and *Kumari* (Aloe vera) was collected from botanical garden. All raw materials were identified and checked for genuinity.

The ingredients were processed by different methods before using them in the formulation. *Hingu* was purified by frying with *Ghrita*[13] *Kaseesa* was purified by triturating with *Nimbu Swarasa* (*Citrus acida*)[14]and *Tankana* was purified by *Bharjana* (frying) method.[15]

Purified *Hingu, Kaseesa, Tankana*, and *Kanya Sara* were properly mixed in proportion to form a homogenous mixture and subjected to *Bhavana* (trituration) with *Kumari Swarasa*. The pills of 375mg (3 *Ratti*) each were prepared.

Experimental Study

Animal experiment was carried out after obtaining permission from Institutional Animal Ethics Committee (IAEC) **(GCP/IAEC/16/2010-2011, 11th Feb.2011).** Female immature Wister Albino Rats weighing 60-80 gm were obtained from Drug Testing Laboratory, Bangalore. Test drug RPV was prepared in the Pharmacy Govt Ayurvedic Medical College Bangalore and Standard drug 17 alpha Ethinyl oestradiol was obtained from Sigma Aldrich Company, Bangalore.

Immature Female Wister Albino Rats weighing 60-80 gm were housed in the animal house of Pharmacy College with standard laboratory diet rat pellets. For their drinking purpose tap water *ad libitum* was used. Temperature in the experimental animal room was maintained at 22-25^0 C, with humidity between 50-70%. Daily lighting sequence of 12 hours light and 12 hours dark was maintained.

Posology

Immature female Wister albino rats were divided into five groups containing six animals in each group. Human dose of *Rajahpravartini Vati* is three ratti (375mg/day).[1] The dose selection for animals was done on the basis of body surface area ratio using the table of Paget and Barnes (1969). [16] The grouping and dose for respective groups are shown in Table 1.

Experimental model: Uterotrophic bioassay in Rodents was carried out as per OECD/OCDE guidelines 440.[17]

Procedure

The animals were kept fasting for 12 hours before administering the drug. Then animals were weighed and

respective drugs were administered orally to all groups. Trail drug was made suspension in distilled water and administered depending on weight of animal. Standard drug Ethynyl oestradiol was dissolved in olive oil, and administered at the dose of 0.03mg/kg wt single dose/day. The drugs were administered to respective groups for seven consecutive days. General clinical observations along with vaginal opening and body weight were carried out daily at the same time each day.

On the 8th day all the animals were sacrificed by chloroform anaesthesia .Uteri were dissected and each uterus was transferred to weighed petridish. Then the uterus with luminal fluids was weighed to the nearest 0.1mg (Wet uterus weight). Later each uterus was individually processed to remove the luminal fluid. Both uterine horns were cut longitudinally. The uterus was placed on filter paper and gently pressed with another paper to remove the luminal fluid. The uterus without the luminal contents were weighed to the nearest 0.1mg (Dry uterine weight). Weight of wet and dry uterus were noted and expressed in mg/100gm body weight of animal.

Later the uterus was fixed in 10% neutral buffered formalin for further Histopathological examination.

Statistical analysis

The data generated from the experimental study was represented as Mean ±SEM and subjected to unpaired t test. Results of trail groups were compared with standard and control determine significant difference between groups at $P < 0.05$.

Observations and results

Vaginal opening is also one of the parameter to assess the oestrogenic activity. 100% of animals showed vaginal opening in Standard Group, 66.6% in 1/2TED(Therapeutic Equivalent Dose) and 2TED group, TED 50%, 16%in control at the end of 7th day. This shows the test drug is having mild to moderate oestrogenic activity when compared control group (Table 2). Body weight of animal before and after treatment showed significant increase in all groups (Table 3). Mean uterine weight at low dose (1/2TED) showed high significant result when compared with the control (<0.001), whereas 2TED and TED showed significant results when compared with the control group (Table 4). Observations on endometrial thickness showed significant increase in endometrial thickness in 1/2TED group (Table 5).

Discussion

Rajahravartini vati is useful in *Rajarodha* and *Kashtartava.*[1] All the Ingredients of RPV are having identical properties like *Katu rasa*, *Ushna veerya*, *Sara Teekshna guna* and *Pitta vardhaka.* All these properties remove obstruction in the passage and do *Sroto Shodhana* (cleansing the channel). By this there is improvement in *Stanika Arthavagni* and *Upadhatu Arthava*. *Ushna guna* increases the secretion of glands thus improves proliferative phase of menstrual cycle.

Arthava Janana, Rajahpravartana, and *Rajorodha nashana* are the properties attributed to RPV. Hormonal influence plays an important role in maintaining both ovulatory and menstrual cycle. The primary changes in uterine tissue are governed by ovarian hormones oestrogen and progesterone. oestrogen is responsible for growth and proliferation of endometrial glands, vascularisation of superficial endometrium and thickening of endometrium.[18] A variety of phytoestrogens also binds to the oestrogen receptors and could induce oestrogenic actions.[19] The ingredients like *Hingu* and *Kumari* contain Phytoestrogens.[6,10] Studies on ferutinin a component of *Hingu* exerts an oestrogenic activity in ovariectomized non oestrogen primed female rats.[7] Aloe Vera sap also showed favourable effects on oestrogen synthesis due to its phytoestrogen components such as beta sitosterol, and increase the oestrogen level.[11] In another study to examine the effect of Aloe vera extract on pregnant rat ovaries, it was found that this plant causes minimal weight gain, increased vasculogenesis around the secondary follicles. Results showed it has similar effect of oestrogen and follicle stimulating hormone.[20]

Vaginal opening, wet and dry uterine weight are the reliable

measures of oestrogenicity.[21]Vaginal opening is also one of the parameter to assess the oestrogenic activity. 100% of animals showed vaginal opening in standard group, 66.6% in 1/2TD and 2TD group, TD 50%, 16%in control. This shows the test drug is having mild to moderate oestrogenic activity when compared control group.

Wet and dry uterine weights are reliable measures on oestrogenicity. The wet weight includes the uterus and the luminal fluid contents. The blotted weight is measured after the luminal contents of the uterus have been expressed and removed. Wet uterine weight is an early marker of oestrogen action where water imbibitions are seen due to enhanced micro vascular permeability. However some suggests that substances that directly stimulate mitotic activity are the index of an oestrogenic activity. [22] This response is followed by a weight gain due to tissue growth. The uterotrophic response of the test drug RPV in TED doesn't displayed many oestrogenic characteristics. However mean uterine weight at low dose (1/2TED) showed high significant result when compared with the control (<0.001), whereas 2TED and TED showed significant results when compared with the control group. The difference of relative wet and dry uterine weight indicates oestrogenic activity in lower doses of test drug when compared with high doses. Standard group showed high significant uterotrophic response when compared with all groups with p value <0.001. Thus RPV displayed oestrogenic activity at all doses but more significant at lower doses, because biological responses to Phytoestrogens occur at lower doses not at higher doses. [23] There are two isoform receptors for estrogens ERa and ERb. Binding to ERa at AP-1 can also enhance transcription. However, the same compound binding to ERb at AP-1 can inhibit transcription. Ligands can be oestrogenic or antioestrogenic depending on tissue type. Phytoestrogen interactions with these receptors are unknown. Phytoestrogen affinity to receptor or enzyme binding site determines the physiological concentration to initiate a response. [24] Ferutinin also displays oestrogenic or antioestrogenic activity through ER alpha in the hypothalamus depending on the absence or presence of oestrogen binding.[7] This may the reason that TED group did not show much oestrogenic activity.

Histopathological Studies

Epithelial proliferation is a very sensitive index for assessing oestrogenic activity [25] and in present study standard drug treated animals revealed it. However treatment with test drug at ½ TED showed significant enhancement of epithelial proliferation in comparison with control group. But TED group did not show significant proliferation of endometrium because sometimes enzymes involved in oestrogen synthesis and metabolism may be inhibited by phytoestrogens.

Thus it could be concluded that RPV may be having moderate Oestrogenic activity at all doses more significant in lower doses. Significant results in all parameters like body weight, vaginal opening, wet and dry uterine weight in comparison to control proven the oestrogenic activity of the trial drugs. The presence of Phytoestrogens in RPV may be responsible for oestrogenic activity of the trail drug.

Conclusion

Thus the trial drug RPV showed significant oestrogenic activity in all three dose levels better at low doses in all studied parameters like body weight, vaginal opening assay, uterotrophic bioassay. Histo-pathologically the trail drug at low doses showed increased endometrial thickness when compared with control group suggesting oestrogenic activity.

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Table 1. Showing grouping of animals with dose

Sl.No	Group	Treatment	Dose
1	A	Normal control – Plain water	1ml/kg wt/day
2	B	Standard, Ethynyl estradiol	0.03mg/kg wt/day
3	C	1/2TED of RPV	16.87mg/kg wt /day
4	D	TED of RPV	33.75mg/kg Wt/day
5	E	2TED of RPV	67.5mg/kg wt/day

Table 2: Showing the observation on vaginal opening on the 7th day

Group	No of animals showed vaginal opening on 7th day	Percentage of opening
A	1	16%
B	6	100%
C	4	66.6%
D	3	50%
E	4	66.6%

Table 3: Observations on Body weight of animals

Group	WBT (Mean ±SEM)	WAT (Mean ±SEM)	P value
A	72.5±2.141	80±2.887	<0.001,***
B	70±1.290	79.1±2.21	0.002**
C	80±2.581	96.6±2.110	0.011**
D	74.33±2.027	95±3.410	0.003**
E	78.33±2.449	95.83±2.006	<0.001***

*** Statistically highly significant, ** statistically significant,
WBT-Weight of animal before treatment in gm, WAT – Weight of animal after treatment in gm

Table 4: Observations and results on Wet and Dry uterine weights

Group	WUW (Mean ± SEM)	P value	DUW (Mean ± SEM)	P value
A	112.10 ± 7.402		96.310 ± 4.672	
B	252.9±21.467	<0.001***	236.65± 19.19	<0.001***
C	238.98±21.438	<0.001***	209.54±20.697	<0.001***
D	131.035 ± 24.825	0.482*	100.802± 22.032	0.846*
E	209.10 ± 26.032	0.005**	192.08 ± 24.546	0.003**

*** - statistically highly significant, ** statistically significant, * statistically insignificant

WUW -Wet uterine weight in mg/100gm weight of animal,
DUW - Dry uterine weight in mg/100gm weight of animal.

Table 5: Observations on Histopathological studies (Endometrial thickness)

Group	Mean Endometrial thickness ±SEM in µm
A	7.6 ±1.46
B	11.2 ±2.15
C	10.1 ±1.94
D	7.3 ±1.40
E	8.6 ±1.65

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