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SHORT COMMUNICATION

Effect of *Asparagus racemosus* Rhizome (Shatavari) on Mammary Gland and Genital Organs of Pregnant Rat

S. K. Pandey[1,*], A Sahay[1], R. S. Pandey[2] and Y. B. Tripathi[2]

[1]Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India
[2]Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

***Asparagus racemosus* (AR) Willd (family Liliaceae) is commonly known as Shatavari. The alcoholic extract of its rhizome was administered orally to adult pregnant female albino rats at a dose of 30 mg/100 g body weight, daily for 15 days (days 1–15 of gestation). The macroscopic findings revealed a prominence of the mammary glands, a dilated vaginal opening and a transversely situated uterine horn in the treated group of animals. The weight of the uterine horns of the treated group was found to be significantly higher ($p < 0.001$) but the length was shorter ($p > 0.01$). Microscopic examination of the treated group showed proliferation in the lumen of the duct of mammary gland. It was obliterated due to hypertrophy of ductal and glandular cells. Hyperplasia of the glandular and muscular tissue and hypertrophy of the glandular cells were observed in the genital organs. The parenchyma of the genital organs showed abundant glycogen granules with dilated blood vessels and thickening of the epithelial lining. The oviduct in the treated group showed hypertrophied muscular wall, whereas the ovary revealed no effect of the drug. The results suggest an oestrogenic effect of Shatavari on the female mammary gland and genital organs. Copyright © 2005 John Wiley & Sons, Ltd.**

Keywords: Asparagus racemosus; mammary gland; Shatavari; oestrogenic, herbals; phytoestrogen, Ayurveda.

INTRODUCTION

Asparagus racemosus (AR) Willd, (family Liliaceae) is an important medicinal plant of Ayurveda and is known as Shatavari. Traditionally it is used as a health tonic, aphrodisiac and as Rasayana medicine (possessing properties of rejuvenation). The oil is used for massage in pain, skin disorders and muscular weakness (Pandey and Chunekar, 1998). AR increased the mucosal defensive factors such as mucus secretion, cellular mucus, life span of cells and also showed a significant antioxidant effect (Sairam *et al*., 2003). Saxena and Chourasia (2001) reported the isolation of a new isoflavone from its rhizome. Earlier it was observed that the extract of Shatavari rhizome increased the weight of postpartum rats (Sabin *et al*., 1968). However, it is also used in pregnancy, lactation and various gynaecological disorders (Dalvi *et al*., 1990; Sharma *et al*., 1996). Rao (1981) reported the usefulness of the root extract in mammary gland carcinoma. Interestingly, even after a long traditional use, little scientific data are available for this plant. Therefore a systematic study on the efficacy and action of this drug in the pregnant female rat has been carried out, and the present study is focused on the possible oestrogenic action on the mammary gland and sex organs.

* Correspondence to: Dr S. K. Pandey, Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India.
E-mail: pandeyskbhu@rediffmail.com
Contract/grant sponsor: Banaras Hindu University.

MATERIALS AND METHODS

Female adult albino rats (C.F. strain) obtained from the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varansi, were maintained at normal room temperature with 45%–55% humidity on a normal diet. The female rats were mated with adult male rats and the next morning, pregnancy was confirmed by the examination of a vaginal smear. The sperm positive day was marked as day 0 of the pregnancy. The pregnant female rats were randomly divided into treated and control groups.

Dried AR rhizomes were procured from the Ayurvedic Pharmacy of the Institute and its authenticity was verified on standard pharmacognostical parameters, according to the Indian Pharmacopoeia of Indian Medicinal Plants. Voucher specimens are preserved in the Department of Medicinal chemistry for future reference (MC-39). The rhizome was powdered and then exhaustively extracted with ethanol in a continuous soxhlet extractor for 35 h. The yield was 16.75%.

The experimental dose was worked out on the basis of the adult human dose (20 g/day). The rhizome extract was given orally with the help of a gavage tube to animals of the treated group from day 1 of pregnancy up to 15 days in a dose of 30 mg/100 g body weight per day. The control animals received 1 ml drug-vehicle (10% of Tween-80 in water). The mammary glands and the vaginal orifice of both groups were observed on day 15 of gestation for any change in shape, size and colour. The animals were killed on day 16 of pregnancy for the macro and microscopic examination of the

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mammary gland and the genital organs. Gross examination of these organs were carried out, photographed and then fixed in 10% neutral formalin for histological preparation. The microsections of 7 μm thicknesses were cut and stained with PAS and heamatoxylin/eosin for histological findings.

RESULTS

The mammary glands were found to be prominent and pigmented in the treated animals, compared with the control group (Fig. 1A and 1B). The vaginal opening in the treated group of rats showed dilatation, dryness and a violet colour, while it was moist, non-dilated (closed) and pink in the control group (Fig. 1A and 1B). The vertically situated uterine horns showed a gap between the embryonic sacs in the control group, whereas the treated group showed thickened, shortened and transversally situated uterine horn with enlarged embryonic sacs, which were in close contact with the adjacent sac (Fig. 1C and 1D). The differences in the average uterine weight of the two groups as well as the uterine weight index were calculated and found to be statistically significant ($p < 0.001$), whereas the length of both right and left uterine horns were significantly reduced ($p > 0.01$) in the treated group (Table 1). The ovary and oviduct of both groups showed no gross differences (Fig. 1C and 1D).

Microscopic examination

The effect of the AR rhizome extract revealed obliteration of the lumen of the proliferated duct and hypertrophy of the periductal glandular tissue containing secretory alveoli in the treated animals (Fig. 2B). The vagina of the treated animals showed thickening of the stratified squamous epithelium, well-defined hypertrophied muscular tissue and numerous dilated blood vessels in the lamina propria and circular muscle layer (Fig. 2C and 2D). All layers of the uterine wall in the treated group showed thickening, especially the muscular layer, which contained numerous dilated blood vessels. Hypertrophied decidual basalis showed finger-like projections in the uterine cavity of the treated group (Fig. 3B). The parenchyma of the treated uterus appeared dense with glycogen granules (Fig. 3D). The oviduct of the treated animal showed hypertrophied muscular walls and longitudinal mucous folds, projecting deeply into the lumen of the duct (Fig. 4B). The

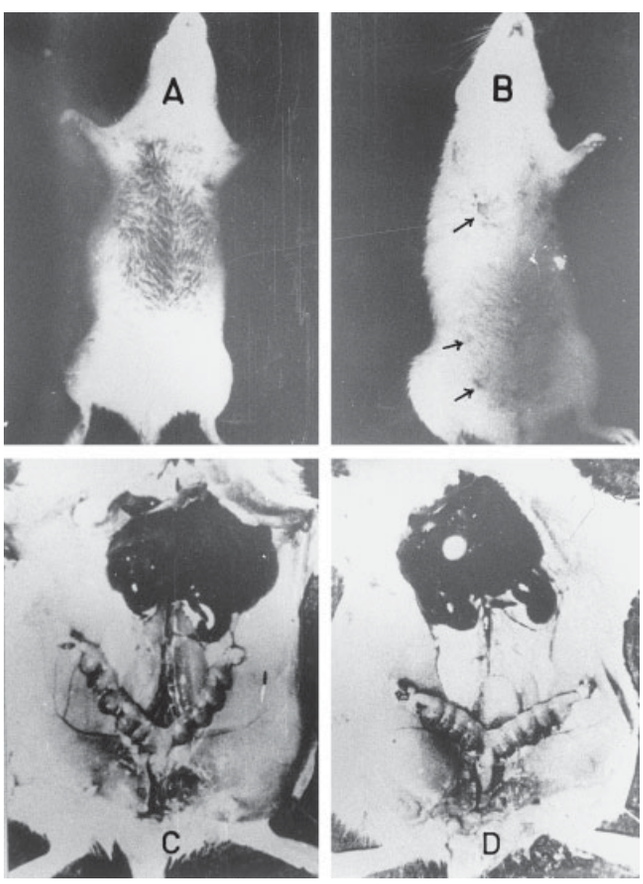

Figure 1. A. Photograph of control pregnant rat showing mammary glands and closed vaginal orifice. B. Photograph of treated pregnant rat showing prominent mammary glands (arrow) with dilated vaginal orifice. C. Photograph of control pregnant rat showing internal genital organs with embryonic sac. D. Photograph of treated pregnant rat showing thickened, shortened and transversely lying uterine horns. The large embryonic sac showing close contacts with one another and not shows any changes in oviduct and ovary.

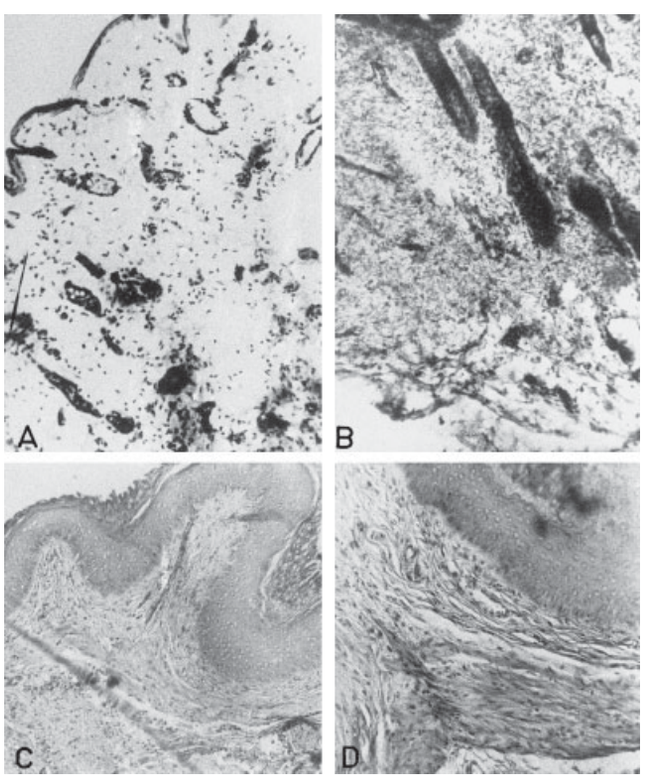

Figure 2. A. Section of the parenchyma of mammary gland of control pregnant rat showing ducts surrounding with periductal glandular tissue. H & E × 150. B. Section of the parenchyma of mammary gland of treated pregnant rat showing proliferation of glandular tissue and obliteration of the ducts with proliferated periductal glandular tissue. H & E × 150. C. Section of the vagina of control pregnant rat showing stratified squamous epithelium and subepithelial fibrous and muscular region. H & E × 150. D. Section of the vagina of treated pregnant rat showing thickening of the epithelium and hypertrophy of epithelial, stromal and muscular cells. The hypertrophied muscular layer is clearly defined with numerous dilated blood vessels. H & E × 150.

Table 1. Effect of AR rhizome on uterine weight index and length of uterine horn in pregnant rats

	Control (n = 16)		Treated (n = 16)		
	Range	Mean ± SD	Range	Mean ± SD	p value
Body weight (g)	175–190	183.40 ± 4.05	198–230	210.00 ± 8.19	<0.001
Uterine weight (g)	0.68–1.31	1.06 ± 0.16	1.41–1.87	1.76 ± 0.14	<0.001
Uterine weight index	0.37–0.71	0.58 ± 0.08	0.71–0.88	0.85 ± 0.04	<0.001
Length (mm) of right horn	20–35	29.19 ± 4.59	15.0–29.0	23.94 ± 4.28	>0.01
Length (mm) of left horn	16–30	24.44 ± 4.11	11.0–24.0	19.50 ± 3.67	>0.01

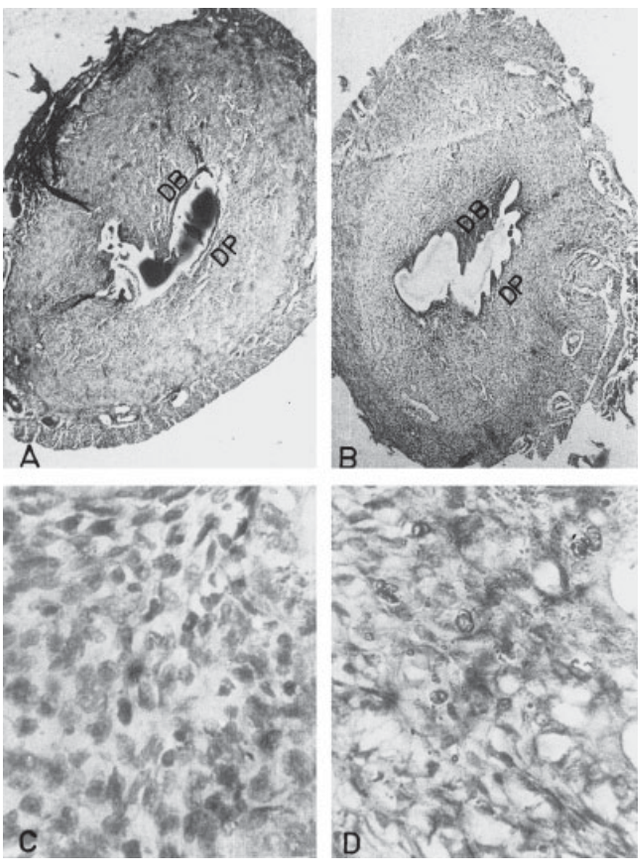

Figure 3. A. Section of the uterus of control pregnant rat showing embryo in the lumen of uterus with decidua basalis (DB) and parietal (DP). H & E × 60. B. Section of the uterus of treated pregnant rat showing empty lumen. Decidua basalis and fragmented decidua parietalis projected deeply into the lumen. Thick compact layers of the uterine wall showing hypertrophy of muscular and stromal cells with dilated blood vessels. H & E × 60. C. Section of the uterus of control pregnant rat showing dilated blood vessels and glycogen deposition in the parenchyma. PAS × 600. D. Section of the uterus of treated pregnant rat showing glycogen granules densely appeared in the parenchyma. PAS × 600.

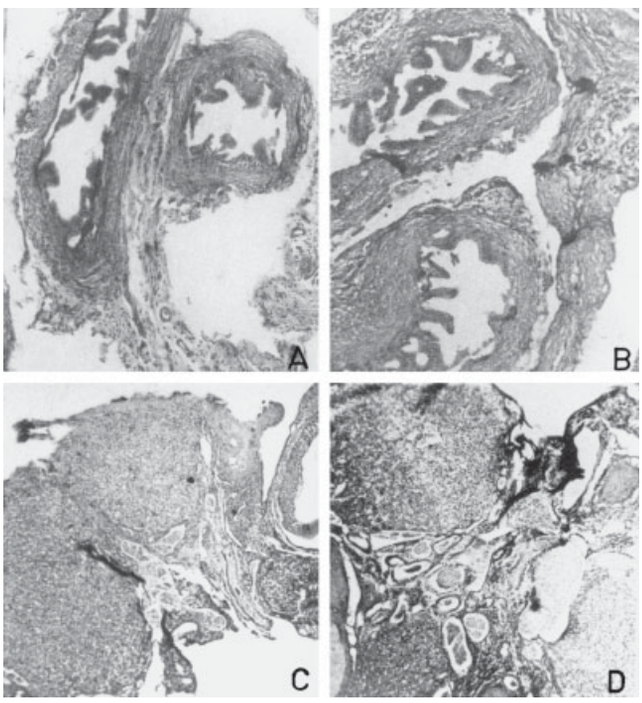

Figure 4. A. Section of the oviduct of control pregnant rat showing muscular wall and lumen. H & E × 60. B. Section of the oviduct of treated pregnant rat showing thickening of the muscular wall and the longitudinal mucous folds deeply projected into the lumen. H & E × 60. C. Section of the ovary of control pregnant rat showing large convoluted corpus luteum containing hypertrophied lutein cells along with numerous round colloidal bodies. H & E × 60. D. Section of the ovary of treated pregnant rat representing the same features as seen in control. H & E × 60.

ovary of both the control and treated rats showed large convoluted corpus luteum, which contained hypertrophied lutein cells. Numerous dark rounded colloid bodies appeared in the ovaries of both groups (Fig. 4).

DISCUSSION

The oestrogenic effect of various Indigenous drugs has been documented earlier (Tiwari *et al*., 1976). *Asparagus racemosus* (AR) has been used only clinically and shows oesterogenic effects in adult virgin female mammary glands and genital organs of rats (Sahay and Pandey, 2003). The alcoholic extract of AR rhizome increases the size of the mammary glands with a dilated vaginal orifice in virgin rats. The present study shows prominent and pigmented mammary glands with a dilated and pigmented vaginal orifice, after long-term therapy with AR rhizome extract. Microscopically well-established secretory lobules were observed, containing secretory alveoli, which obliterated the lumen of ducts. This finding may be due to the hypertrophy of ductal and periductal glandular cells. Numerous dilated blood vessels, a known oestrogenic effect in humans (Haslam, 1988), was also found.

Gaitonde and Jetmalani (1969) observed a clinical physiological effect of AR on female genital organs and an antioxytocic action on uterine muscle and mammary gland. The macro and microscopic effects of

this drug on the genitalia of pregnant mammals has not been reported before. The present study revealed thickening of all the layers of the genital organs, especially the muscular layer. This may be due to hypertrophy of the glandular, stromal and muscular cells and hyperplasia of the stromal tissue along with numerous dilated blood vessels, especially in the muscular wall. Earlier, similar effects of AR have been reported in the genital organs of adult virgin rats (Sahay and Pandey, 2003), whereas Arriaza *et al*. (1989) revealed similar findings of oestrogen on the genital organs of the female. It has been reported that the alcoholic extract of AR rhizome contains significant amounts of steroid sapogenins (Subrahmaniam and Nair, 1968), which may be responsible for the oestrogenic effect. Oestrogen has no effect on the pregnant human ovary, and the same findings were observed in the present study also. This is the first histological report of the effects of AR rhizome, which showed oestrogenic effects on the pregnant female mammary gland and the genital organs of the rat. Further study will elucidate whether the effect is directly due to one of the ingredients of AR rhizome, or indirectly by activating the signalling pathway of oestrogen synthesis.

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