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## IMMUNOLOGY AND MICROBIOLOGY

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### Phytoestrogen Effects on Vaginal Microbiocenosis of Sprague-Dawley Rats

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Phytoestrogens present in the plants endemic for Chile were studied. The effects of phytoextract [specimen of preparation No. 181 (fraction b)] on target tissues were similar to those of estradiol. The preparation inhibited the stimulatory effect of estradiol on vaginal lactobacilli population.

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**Key Words:** *phytoestrogens; lactobacilli; vaginal flora; estradiol; eosinophils*

Normal microflora of the urogenital tract is an important component of female health during all periods of life. Correction of microbiocenosis is particularly important during the postmenopausal period, when changes in the hormonal profile are paralleled by reduction of the numbers of some of its components providing colonization resistance of the female genital tract. Direct relationship between this phenomenon and estrogen level necessitates search for substances for substitute hormonal therapy. This strategy is possible because lactobacilli are the main agents of the vaginal microbiocenosis and due to their high acid production and active adhesion to the vaginal epithelium they prevent the involvement of opportunistic and pathogenic microorganisms in the cenosis [2]. Estrogens stimulate the proliferation of vaginal epithelium. It accumulates glycogen, a nutritive substrate for lactobacilli. This fact determines the hormone dependence of multiplication of these bacteria. In addition, increase in the

numbers of estrogen-dependent receptors [1] is associated with activation of lactobacilli adhesion, this, in turn, leading to the formation of protective biological membrane on the surface of vaginal mucosa.

The need in substitution hormonal therapy during the postmenopausal period suggested search for phytoestrogens as an alternative to endogenous hormones.

The aim of this study was to evaluate the effects of preparation No. 181 (fraction b) (suggested by the Chile scientists), extracted from a local plant containing phytoestrogen, on vaginal microbiocenosis. The study was carried out within the framework of the an agreement between Russia and Chile on cooperation, at Laboratorio de Endocrinología Experimental y Patología Ambiental del Programa de Fisiopatología, Facultad de Medicina de la Universidad de Chile (head: Prof. Titular, Dr. A. N. Tchernitchin).

#### MATERIALS AND METHODS

Double blind experiments were carried out with 17 $\beta$ -estradiol (Sigma E8875) and pharmacopoeian phytoestrogen Klimadynon (Bionorica) — ethanol extract from *Cimicifuga racemosa* L. rhizome, which served as the reference drugs.

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The algorithm of the experiment was as follows. Young (21 days) Sprague-Dawley rats were injected with estrogens. The study was carried out on young rats in order to rule out the effects of endogenous estrogens. After 24 h the animals were sacrificed and 3 series of histological sections were prepared: tissues adjacent to the cervix uteri, middle part of the horn, and distal fragments. The sections were stained by hematoxylin and eosin. The degree of infiltration of the uterus by eosinophils, status of the myometrium, and endometrial edema were evaluated. Eosinophils [4], myocytes, and endometrial epithelial cells [3] were counted and the mean counts per visual field were estimated.

Group 1 (control) animals were injected with saline, group 2 with 17 $\beta$ -estradiol, group 3 with preparation No. 181 (fraction b), group 4 were injected with preparation No. 181 (fraction b) together with 17 $\beta$ -estradiol, group 5 received Klimadynon (reference drug), and group 6 were injected with Klimadynon together with 17 $\beta$ -estradiol. Each group consisted of 5 rats.

## RESULTS

Increase in the mean count of eosinophils in uterine sections was interpreted as an increase of tissue infiltration by eosinophils. Reduction of myocyte count was interpreted as a manifestation of myometrial hypertrophy, as the myocytes increased in size in this case and their count in the visual field decreased. A similar reduction of the mean count of cells in the basal layer of the uterine mucosa in the endometrium indicated an increase of the cell-to-cell space, which was an evidence of edema.

Estradiol increased eosinophil infiltration of the uterus almost 2-fold, caused proliferation of the myometrium and endometrial edema (Table 1). Injection of preparation No. 181 (fraction b) did not lead to eosinophil infiltration of the uterus, but caused proliferation of the myometrium and endometrial edema.

Klimadynon (phytoestrogen) caused a reduction of eosinophil infiltration of the uterus in comparison with the control, was inessential for myometrial proliferation, but caused endometrial edema.

A combination of estradiol and preparation No. 181 (fraction b) led to an effect similar to that of estradiol alone. The reference drug Klimadynon completely canceled the stimulatory effect of estradiol on eosinophil proliferation, but did not modify high proliferation of the myometrium and endometrial edema.

Hence, the biological effect of preparation No. 181 (fraction b) was most similar to estradiol effect; obviously, it modulated the estrogen-dependent receptors, and hence, at the next stages of the study the phytoestrogen effects on vaginal microbiocenosis were evaluated for this drug.

Stage 2 was carried out on 2 groups of animals, 28 per group. Group 1 comprised ovariectomized rats and group 2 consisted of intact animals. Each group was subdivided into 4 subgroups, 7 per subgroup. Two subgroups 1 (controls) were injected with 0.2 ml saline, two subgroups 2 received 500  $\mu$ g/kg 17 $\beta$ -estradiol, subgroups 3 received 0.2 ml (1.0 mg dry substance) of the studied phytoestrogen, and animals of subgroups 4 received 17 $\beta$ -estradiol together with preparation No. 181 (fraction b).

The absolute counts of lactobacilli in the rat vaginas reduced significantly after ovariectomy (Table 2). Injection of estradiol to these animals restored the microbiocenosis to the status before ovariectomy. Injection of estradiol to intact animals did not change their bacterial flora.

Phytoestrogen No. 181 (fraction b) caused no changes in the level of lactobacilli in ovariectomized or intact animals. On the other hand, injected together with estradiol to intact animals, it reduced the level of lactobacilli, but caused no changes in the vaginal microbiocenosis in ovariectomized rats.

These data suggest a competitive effect of preparation No. 181 (fraction b) under conditions of 17 $\beta$ -es-

**TABLE 1.** Changes in Uterine Tissues of Experimental Animals after Estradiol and Phytoestrogen Treatment

Cell counts (mean per visual field)	Group				Klimadynon	Klimadynon+ 17 $\beta$ -estradiol
	control	17 $\beta$ -estradiol	preparation No. 181 (fraction b)	preparation No. 181 (fraction b)+ 17 $\beta$ -estradiol		
Eosinophils	2.45 $\pm$ 0.50	4.76 $\pm$ 0.50	2.18 $\pm$ 0.30	5.68 $\pm$ 1.30	0.43 $\pm$ 0.09	0.34 $\pm$ 0.10
Myocytes	131.4 $\pm$ 1.7	70.4 $\pm$ 1.7	104.3 $\pm$ 1.7	112.5 $\pm$ 2.2	127.1 $\pm$ 2.2	117.4 $\pm$ 1.7
Basal layer cells of uterine mucosa	94.03 $\pm$ 2.10	49.10 $\pm$ 1.05	85.31 $\pm$ 1.86	84.32 $\pm$ 2.01	104.19 $\pm$ 0.8	101.26 $\pm$ 0.80

**TABLE 2.** Changes in the Counts of Lactobacilli in Vaginal Smears from Experimental Animals Treated by Estradiol and Preparation No. 181 (fraction b)

Subgroup	Preparation	Intact animals	Ovariectomied rats
1 (control)	Saline	26.43±1.46	0.95±0.46
2	17 $\beta$ -estradiol	24.28±1.15	20.78±1.17
3	Preparation No. 181 (fraction b)	26.60±1.23	1.125±0.490
4	Preparation No. 181 (fraction b)+17 $\beta$ -estradiol	12.80±1.05	1.41±0.46

tradiol interactions with estrogen receptor. Reduction of lactobacillus multiplication can be due to extrusion of the endogenous hormone by the phytoestrogen in intact animals. On the other hand, its binding to estradiol-fixing receptors does not allow estradiol stimulation of the bacterial flora.

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