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PHYTOTHERAPY OF EXPERIMENTAL ENDOMETRITIS: DOSE-RANGING OF ORTHILIA SECUNDA (L.) HOUSE EXTRACT

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This article presents a comparative analysis of the effectiveness of different doses of Orthilia secunda extract in treatment of experimental rat`s endometritis. Three doses were selected for the therapy: semi–therapeutic (0.04 mg/kg per day), therapeutic (0.09 and 0.21 mg/kg per day). The best histological results were achieved in the group treated with Orthilia secunda extract in the dose of 0.04 mg/kg. In the absence of therapy, the endometrium wasn`t restored.

***Key words**: treatment of experimental rat`s endometritis, Orthilia secunda (L.) House.*

Chronic endometritis is the only cause of infertility in 20% of cases [2, 10], miscarriage - in 50% [4]. The only unsolved problem of this nosology is the restoration of the morpho-functional potential of the endometrium, which is necessary for implantation of the fetal egg and gestation [3]. Phytotherapy, due to the multifactority of its effects, good tolerability, accessibility and low frequency of side effects, has long been the focus of attention of many researchers [7, 8]. For the treatment of chronic inflammatory diseases of the genitals in folk medicine for more than four centuries, Orthilia secunda (L.) House has been used [1, 6]. Earlier, there was performed a morphological study of the effectiveness of treatment of experimental endometritis, which is close to chronic, which showed that Orthilia secunda extract eliminates inflammatory processes and leads to a secretory transformation of the endometrium [5]. However, the choice of the dose of this phytopreparation remains unresolved.

Research objective: to establish the optimal therapeutic dose of Orthilia secunda extract for restoring the morphofunctional potential of the endometrium on the biological model of experimental endometritis.

Materials and methods

For the experiment, we took an extract of Orthilia secunda in the form of tablets 0.45 g, containing 2.77 mg of an extract of Orthilia secunda. Producer: OOO NPK Vegetable Resources, St. Petersburg; Registration Certificate (RLS 2017) - RU .77.99.11.003.E.003588.02.15). According to the annotation, Orthilia secunda extract is recommended to be applied at a dose of 0.09 mg/kg (2 tablets) or 0.14 mg/kg (3 tablets) per day. Taking into consideration the principle of phytotherapy for treatment with small doses [1], we decided to test a half dose of the extract - 2.77 mg (0.04 mg/kg) per day.

Taking into account that the course of phytopreparations therapy is 3-7 menstrual cycles of a woman, the duration of therapy was determined according to the estrous cycle of rats and constituted 21 days.

For the experiment, 80 female white outbred rats at the age of two months were used, with an average mass of 202.5±2.3 g. The animals were kept in standard vivarium conditions, with free access to water and food. The absence of males ensured that they had a resting phase of the estrous cycle (diestrus). After 10 days of quarantine, in all females there was induced endometritis by a single intrauterine injection of an aqueous formalin solution of 2% - 0.1 ml. Next, females were conditioned for three days to form an endometrial response. After that, all rats were divided into four groups. Three groups received an extract of an Orthilia secunda (diluted in two milliliters of distilled water) once a day, through a probe (intragastric).

The first group, 20 rats, received Orthilia secunda extract at a dose of 0.04 mg/kg per day; the second group - 0.09 mg/kg per day; the third - 0.14 mg/kg per day; the fourth group did not receive treatment of experimental endometritis.

All rats were executed the day after the end of the 21-day course of therapy, on the 34th day of the experiment, using craniocervical decapitation under ether anesthesia. All the clogged rats were taken up with material (uterus), fixed in neutral 10% formalin and carried out according to the classical method of making histological preparations with the coloring of hematoxylin and eosin.

Results and discussion

Rats of the first group receiving a half dose of Orthilia secunda extract (0.04 mg/kg/day) had stably normal activity throughout the experiment. At the time of the section, at the end of the experiment, weight gain was significantly increased (an increase in the average body weight from the initial 200.0±0.5 and 218.0±1.9, $p \leq 0.01$). By histological examination, the structure of the endometrium of the rats of the main group corresponded to the norm: the superficial epithelium (prismatic) and the gland epithelium (cubic) were completely restored. Inflammatory infiltration was not present. In the rats of the first group, secretory chang-

es in the endometrium were revealed: subnuclear vacuolization, edema of the stroma, the presence of a large number of capillaries in it (Figure 1).

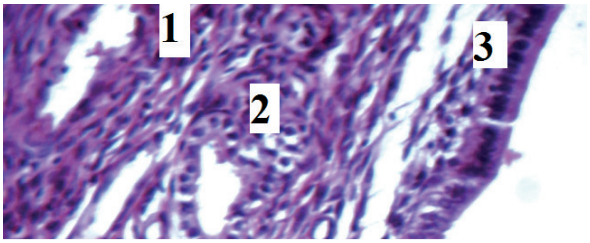


Figure 1. Endometrium of the white outbred rat of the 1st comparison group (treatment with a half dose of the extract of Orthilia secunda (0.04 mg kg per day)) on the 34th day of the experiment.
Staining with hematoxylin and eosin. Zoom x 200
1- Subnucular vacuolation, sparsity of stroma
2- Cubic epithelium of glands
3- Prismatic cover epithelium

In rats of the 2nd group, which received 0.09 mg/kg of extract per day during the experiment, normal activity was noted. They significantly increased in weight at the end of the experiment (202.0 0.9 and 219.0±1.8 g, p≤0.01). On the 34th day of the experiment, the endometrium of rats had a normal structure (rest phase, diestrus): fuzzy separation into the functional and basal layers and occupied 2/3 of the entire thickness of the uterus, lined with a single-layer prismatic epithelium with elongated hyperchromic nuclei. In the fibrous stroma mesenchymal spindle-shaped and lymphocyte-like cells were present. Straight uterine glands in the stroma are rarely located. Epithelium of the glands is single-rowed cylindrical with rounded nuclei (Figure 2).

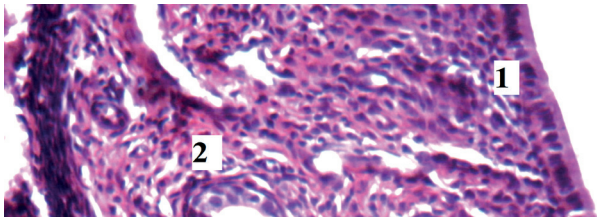


Figure 2. Endometrium of a white outbred rat of the 2nd comparison group (treatment with an extract of Orthilia secunda (0.09 mg/kg per day)) on the 34th day of the experiment. Staining with hematoxylin and eosin. Zoom x 200
1- Prismatic superficial epithelium
2- Cubic epithelium of glands

Rats of the third group, who received 0.14 mg/kg of Orthilia secunda extract per day, also had normal activity and significantly increased their weight at the end of the experiment (204.0±1.23 and 220.0±2.4 g, p≤0.01). In the histological study of the endometrium, the rats corresponded to the diestrus phaseб and in 35% of cases had a normal structure. In 65% of cases, destructive changes in the endometrium (flattening of the integument epithelium) and glands were detected. On the perimeter of the uterine cavity, rare glands with dystrophic epithelium and very small glands with hardly detectable epithelium were found. A pronounced congestion of stroma vessels was noted (Figure 3).

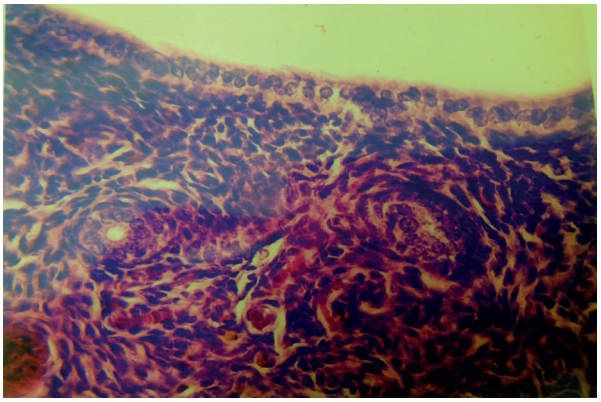


Figure 3. Endometrium of a white outbred rat of the 2nd comparison group (treatment with an extract of Orthilia secunda (0.14 mg/kg per day)) on the 34th day of the experiment. Staining with hematoxylin and eosin. Zoom x 200

Rats of the 4th group who did not receive treatment for experimental endometritis had a stably reduced activity, and at the time of the section, a significant decrease in the average body weight (200.0±2.26 and 190.0±3.25, p≤0.01) was recorded. The endometrium of rats did not take normal structure at the conclusion of the experiment. The regenerating cover epithelium was partially restored, flattened. The epithelium of the glands did not recover. Inflammatory infiltrate was located diffusely throughout the endometrium, single lymphoid cells were detected in myometrium. An increased cellularity of the stroma was noted: macrophages, eosinophils were detected in large numbers, lymphocyte-like endometrial stroma cells appeared. Sinusoidal capillaries sharply widened, capillary endothelium swollen (Figure 4).

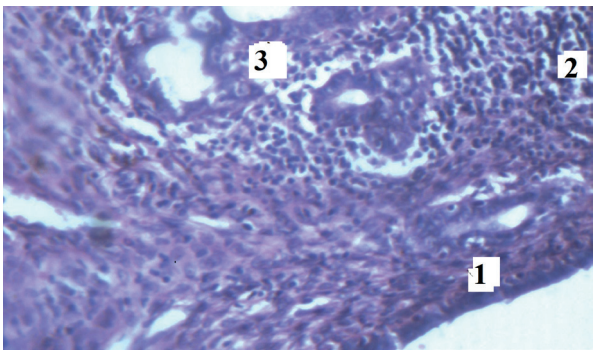


Figure 4. Endometrium of the uterine corn of a white outbred rat of the 4th comparison group (experimental endometritis without treatment) on the 34th day of the experiment. Staining with hematoxylin and eosin. Zoom x 200
1. Partially restored regenerating superficial epithelium
2. Inflammatory stromal infiltrate
3. Epithelium of glands

In the experiment, it was shown that the dose of Orthilia secunda extract 0.04 mg/kg per day is most effective for restoring the morphofunctional structure of the endometrium after the inflammatory stress (experimental endometritis), its full secretory transformation (forehead phase), is favorable for nidation of the fetal egg. A higher daily dose of Orthilia secunda extract (0.09 mg/kg, 0.14 mg/kg) does not lead to a secretory transformation of the endometrium, can cause minor hemorrhages in the tissues of the ovary and their hyperstimulation.

Conclusions

1. The most effective is the half dose of Orthilia secunda extract (0.04 mg/kg/day), as it leads not only to the normalization of the morphology of the endometrium, but also to the secretion of its reorganization.

2. Higher doses (0.09 and 0.14 mg/kg/day) are less effective because they do not lead to secretory changes in the endometrium, which is necessary for the implantation of the fetal egg.

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