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A preliminary study on the effects of Xiang Shao granules on reproductive endocrinology in drugged ovariectomised rats

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Abstract

Objective To establish a rat model of pharmacological ovariectomy by GnRH-a injection and to preliminarily investigate the reproductive endocrine effects of Xiangshao granules on pharmacologically ovariectomized rats. Methods: A rat model of pharmacological ovariectomy was established by injecting female rats with Gonadotropin-releasing hormone agonist(GnRH-a).The rats were randomly divided into four groups: GnRH-a injected saline group (GnRH-a + NS); GnRH-a injected oestradiol group (GnRH-a + E2); GnRH-a injected Xiangshao granule group (GnRH-a + Xiangshao), and the control group of saline-injected rats (NS + NS). The number of rats per group was 6.According to observations of the rats' vaginal smears, modelling was determined as successful. Then corresponding drug gavage intervention was administered for 28 days, and rat body weight and anal temperature were measured every other day to adjust the drug intervention amount according to body weight changes. Plasma sex hormone levels (E2, FSH, LH), uterine weight, uterine index and endometrial histomorphological changes, ovarian weight, and ovarian index and ovarian histomorphological changes were measured in each group after the gavage.

Results (1) Plasma sex hormone levels (E2, FSH, LH) of the GnRH-a + NS, GnRH-a + E2, and GnRH-a + Xiangshao granule groups were significantly lower than the NS + NS group ($P < 0.001$), while the E2 level of the GnRH-a + E2 group was higher than that of the GnRH-a + Xiangshao granule group ($P < 0.05$). The FSH level of the GnRH-a + E2 group was significantly lower than that of the GnRH-a + Xiangshao granule group ($P < 0.05$). The LH level of the GnRH-a + E2 group was significantly lower than those in the GnRH-a + NS and GnRH-a + Xiangshao granule groups ($P < 0.001$, $P = 0.001$). The LH and FSH levels of the GnRH-a + NS and GnRH-a + Xiangshao granule groups were not significantly different ($P > 0.05$). (2) Compared with the NS + NS group, the uterine weight and uterine index, and ovarian weight and ovarian index of GnRH-a injected rats in each model all significantly decreased ($P < 0.001$). Between the groups, the uterine weight and uterine index, and ovarian weight and ovarian index of GnRH-a + E2 and GnRH-a + Xiangshao granule groups were all significantly higher than those of the GnRH-a + NS group ($P < 0.001$, $P < 0.05$). The uterine weight and uterine index, and ovarian weight and ovarian index of the GnRH-a + E2 group increased compared with the GnRH-a + Xiangshao granule group ($P < 0.05$). (3) Compared with the NS + NS group, the number of primordial follicles of the GnRH-a + NS, GnRH-a + E2, and GnRH-a + Xiangshao granule groups increased significantly and the number of growing follicles and mature follicles significantly decreased. (4) Rats' uterine wall of the NS +

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NS and various GnRH-a groups was significantly thinner, with the endothelial layer atrophied, while the uterine wall of the GnRH-a + E2 and GnRH-a + Xiangshao granule groups was thicker obviously, with the number of vaginal folds and blood vessels also increasing. Specifically, the uterus and vagina improvements in the GnRH-a + E2 group were more obvious than in GnRH-a + NS and GnRH-a + Xiangshao granule groups.

Conclusion GnRH-a injection can reduce the levels of sex hormones E2, FSH, and LH in rats, causing perimenopausal symptoms such as hot flashes, while Xiangshao and E2 granules could significantly improve such symptoms and exert a slight oestrogenic effect, to a lesser extent than E2 does.

Trial registration Not applicable.

Keywords GnRH-a, Pharmacological oophorectomy, Perimenopausal syndrome, Xiangshao granules

Introduction

Endometriosis [1] usually refers to endometrial tissues (glands and mesenchyme) appearing in tissues outside the uterine cavity and infiltrating and growing there, causing shedding and bleeding with the menstrual cycle. It is a gynecological chronic inflammatory disease that affects 5%-10% of women of childbearing age globally [2, 3]. Its clinical symptoms mainly include dysmenorrhoea, non-menstrual pelvic pain, and infertility. According to statistics, about 50%-80% of women with dysmenorrhoea and 50% of women with infertility suffer from endometriosis [4, 5], which seriously affects the quality of life. Despite being a benign disease, endometriosis has the malignant biological behaviors of infiltration, metastasis, and recurrence, which seriously troubles clinicians. Early diagnosis and treatment of endometriosis are crucial to preventing the progression of malignancies and complications such as infertility and pain associated with the condition. Consequently, identifying new, rapid, and non-invasive biomarkers is essential for diagnosis. However, despite numerous recent studies on potential markers, definitive results have yet to be established [6–8]. Its treatment mainly includes drug therapy and surgery [9, 10]. Drug therapy aims to inhibit the growth of ectopic endometrium [11], but is often ineffective for patients with moderate-to-severe endometriosis. Surgery is the most common means of endometriosis treatment, but has a high rate of postoperative recurrence. It has been reported that the recurrence rate of endometriosis 5 years after surgery can reach 18.9%-40% [12–14], and the rate of patients readmitted to hospital for surgery 4 years after surgery is about 27% [15, 16]. Therefore, postoperative drug treatment is extremely important to prevent recurrence.

There are a variety of postoperative medications, of which gonadotropin-releasing hormone agonists (GnRH-a) are now recognised as the most effective in the treatment of endometriosis [17–19]. GnRH-a has a high affinity for and binds to pituitary GnRH receptors, leading to a decrease in pituitary secretion of hormones, thus reducing ovarian oestrogen production. This down-regulation of pituitary function can lead to severe hypo-estrogenism, and thus temporary amenorrhoea. This ovarian function inhibition method through drugs is referred to as "pharmacological oophorectomy" [20, 21]. This down-regulation of the pituitary gland and the inhibition of gonadotropin release to maintain the hypo-estrogenic state often cause perimenopausal symptoms in patients. Hot flashes and sweats have been reported in about 90% of these women, and bone loss occurs in 2.5% to 5% of the women after 4-6 months of treatment [22, 23]. In view of this, the safety and efficacy of GnRH-a treatment beyond 6 months is controversial, which undoubtedly limits the clinical efficacy of GnRH-a. The proposed reverse addition theory can remedy this deficiency by adding low-dose estrogen and progesterone [17, 24]. However, long-term hormone use may damage liver and kidney functions, increase phlebothrombosis risks, and increase the chances of endometrial cancer or breast cancer [25]. Influenced by traditional thinking, hormone replacement therapy is less accepted in China, with poor patient compliance. Therefore, other safe and effective reverse additive drugs are urgently needed.Xiangshao granules have been shown to be efficacious as a drug for the relief of perimenopausal symptoms. We therefore considered whether it would have the same effect on patients undergoing pharmacological oophorectomy?

Xiangshao granule is a compound preparation [26] composed of 10 traditional Chinese medicines, such as Fragrant herb and white peony, and a national Class III new drug (traditional Chinese medicine). It is effective in relieving perimenopausal symptoms in women. However, current studies focus on the treatment for healthy naturally menopausal and perimenopausal women, with fewer studies on the treatment of pharmacological menopause. Therefore, we hypothesized that the Xiaongshao granules could also rapidly relieve perimenopausal symptoms such as hot flashes and sweating caused by pharmacological menopause. This study proposed to establish a rat model of pharmacological

oophorectomy induced by GnRH-a treatment by means of GnRH-a injection, with physiological saline (NS) injection as a control. The intervention was carried out by using Xiangshao granules and estradiol drugs to further understand the effect of paeoniflora granules on female reproductive endocrine secretion.

Materials and methods

Materials

1.1 Experimental animals

Twenty-four female Sprague-Dawley (SD) clean-grade rats, with an average weekly age of 6-8 weeks, were selected and purchased from Shanghai Bicaikewing Biotechnology Co., Ltd. and approved by the Ethics Committee for Laboratory Animal Welfare. The experiments formally began after the rats had been acclimated to the rearing environment for 1 week. All experimental operators of the experimental animals in this study possessed the Laboratory Animal Induction Certificate. All experimental operations complied with the Regulations of the People's Republic of China on the Management of Laboratory Animals issued by the National Science and Technology Commission of China and the Regulations on the Management of Laboratory Animals issued by Jiangsu Provincial Government.

1.2 Experimental reagents

(1) Gonadotropin-releasing hormone agonist (GnRH-a): Generic name: Treprostinil acetate; trade name: Diphereline. Its main ingredient is treprostinil acetate. Excipients: Polypropylene cross ester ethyl cross ester copolymer, mannitol, carboxymethylcellulose, polysorbate 80. Specification: 3.75mg/capsule. Manufacturer: Beaufor Ipsen Pharmaceuticals Limited, France.
(2) Xiangshao granules: Main ingredients: White peony, fragrant herbs, neem seeds (fried), *chaihu*, *chuanxiong* rhizome, fructus aurantii, half-sia (ginger-made), cardamom, woodruff, and liquorice. Excipients: Sucrose, betacyclodextrin, dextrin. Specification: 4 g/bag. Manufacturer: Yangzijiang Pharmaceutical Group Sichuan Hairong Pharmaceutical Co., Ltd. Batch No.: State Standard Z20050425.
(3) Estradiol Valerate: Generic name: Estradiol Valerate Tablets; trade name: *Bu Jia Le*. Main ingredient: Estradiol Valerate (E2). Specification: 1 mg/capsule. Manufacturer: Bayer Healthcare Co., Ltd. Guangzhou Branch.
(4) Others

1.3 Enzyme-linked immunosorbent assay (ELISA) reagents

1.4 HE staining reagents

Methods

Animal modelling

(1) GnRH-a injectable drug ovarian denudation animal modelling: 18 SD adult female rats. GnRH-a (Triptorelin Acetate for Injection, Diphereline) was administered intramuscularly at 0.06 mg /kg for 7 consecutive days, and after that maintained for 21 days at 1/5 of the starting dose.
(2) Saline injection control group: Six adult female SD rats were injected with saline 0.06 mg/kg intramuscularly for 7 consecutive days, after which the injection was maintained at 1/5 of the starting dose for 21 days. The saline control group was designed to reduce the error associated with drug injection.

Modelling result determination

To determine whether GnRH-a drug-induced modeling succeeds or not, vaginal smears of rats were taken at a fixed time daily during the 7 days after GnRH-a injection to observe the vaginal secretion cells under the microscope and determine the motility cycle.

Experimental drug gavage intervention

Pharmacological interventions started after successful modelling (2 weeks of continuous GnRH-a injection) in each group. Estradiol valerate (E2) and Xiangshao granules were processed and dissolved in sterile saline to form a turbid solution for rats to be gavaged. Rats were weighed every other day to adjust the dosage according to the body mass changes.

Experimental programme and grouping

After the modelling of experimental rats was completed, 24 rats were randomly divided into 4 groups with 6 rats each, depending on different drug intervention protocols:

(1) GnRH-a injected saline group (GnRH-a + NS): Carry out GnRH-a injection for 2 weeks and then gavage with 0.06 mg/kg saline for 28 consecutive days.
(2) GnRH-a injected oestradiol group (GnRH-a + E2): Carry out GnRH-a injection for 2 weeks and then gavage with oestradiol valerate E2 for 28 consecutive days.
(3) GnRH-a injected Xiangshao granule group (GnRH-a + Xiangshao granule): Carry out GnRH-a injection for 2 weeks and then gavage with 60 mg/kg Xiangshao granules for 28 consecutive days.

(4) Physiological saline injection control group (NS+NS): Carry out intramuscular saline injection at 0.06 mg/kg for 2 weeks and then gavage with 0.06 mg/kg physiological saline for 28 consecutive days.

Body temperature and body weight measurements of rats in each group

After successful modelling, the anal temperature and body weight of rats in each group were measured every other day. The average body temperature of each group was calculated and plotted as a "temperature-time curve". Meanwhile, the body weights of rats were weighed and recorded every other day and a "body weight-time curve" was plotted according to the average body weights.

Experimental rat execution, sample collection and testing

Rats in each group were injected intraperitoneally with 1% pentobarbital sodium and then decapitated to take rat specimens at the end of the drug intervention in each group. Blood (serum and plasma, respectively) was taken from the abdominal veins of the rats, and the uterus and ovaries were taken out after saline perfusion.

(1) Uterine weight and uterine index: After rats in each group were executed, an intact uterus was dissected and removed to measure the uterus wet weight and calculate the uterine index according to the uterine index formula (Uterine index (UMI) = Wet weight of the uterus / Body weight (%)). Data were then statistically analyzed.
(2) Ovary weight and ovarian coefficient: After rats in each group were executed, the intact bilateral ovaries of rats were dissected and removed. The ovarian coefficient was calculated according to the ovarian coefficient formula (Ovarian coefficient = Wet weight of ovary / Body weight (%)). Data were then statistically analyzed.
(3) Use ELISA assay to detect changes in the expression levels of E2, FSH, and LH in plasma.
(4) Use HE staining to detect morphological changes in endometrium.
(5) Use HE staining to detect morphological changes in the ovary and observe follicular development.

SPSS 26.0 statistical software was used, and measures conforming to normal distribution were described by X±S, and the t-test was used for two groups of data. Multi-group data were tested using the ANOVA test: measures not conforming to normal distribution were described as the median (P25-P75). The Mann-Whitney U test was used for two groups of data and the Kruskal-Wallis test for multiple groups of data. $p < 0.05$ was considered a statistically significant difference.

Results

Analysis of vaginal smear results

The results showed that the vaginal cell smears of rats in the control group (NS + NS group) exhibited changes in the estrous cycle (see Fig. 1). The cells in the smears were mostly polygonal superficial cells with less cytoplasm and a few epithelial cells. On the contrary, no changes in vaginal cell smears were observed in the estrous cycle for rats in the GnRH-a model group, which stayed in the inter-estrous phase, and the cell smears were more homogeneous, mainly with leukocytes as well as rounded and non-nucleated polygonous epithelial cells (see Fig. 1).

The results showed that the vaginal cell smears of rats in the control group (NS+NS group) showed changes in the estrous cycle (see Fig. 1), and the cells in the smears were mostly polygonal superficial cells with less cytoplasm and a few epithelial cells; on the contrary, there were no changes in the estrous cycle in the vaginal cell smears of the rats in the GnRH-a model group, which only stayed in the inter-estrous phase, and the cell smears

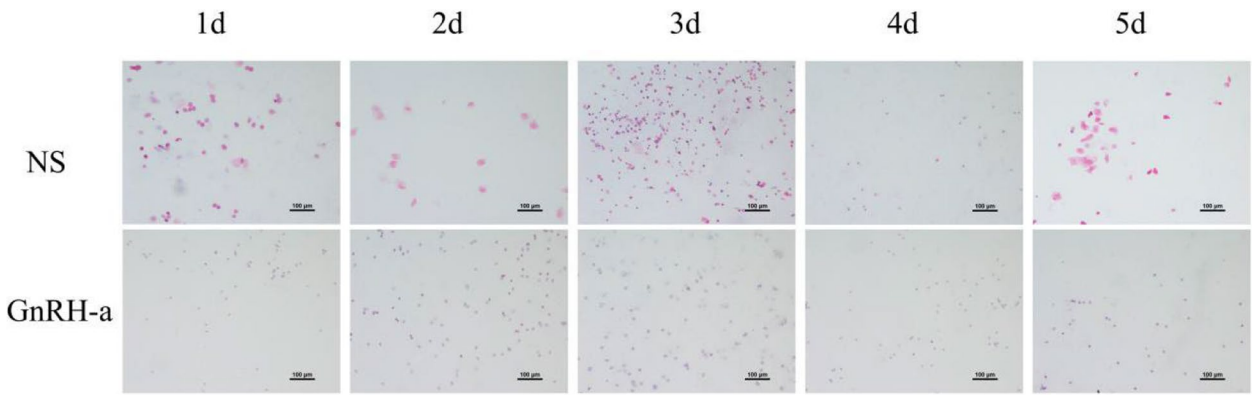

Fig. 1 Vaginal cell smears of rats in NS + NS group and GnRH-a model group

were more homogeneous, mainly with leukocytes and rounded epithelial cells (see Fig. 1). On the other hand, in the GnRH-a model group, the vaginal cell smears showed no changes in the estrous cycle and only remained in the inter-estrous phase.

Trends of body weight changes in rats

During the 2-week feeding period of rat modelling, the body weight of GnRH-a-injected rats (GnRH-a + NS, GnRH-a + E2, GnRH-a + Xiangshao granules) increased compared with that of the control group (NS + NS). Among the three groups with pharmacological interventions, the body weight of rats in the GnRH-a + NS group increased more significantly than in the GnRH-a + E2 and GnRH-a + Xiangshao granule groups. For details, see Fig. 2 (a "Weight-time trend" of rats was plotted based on the recorded results). During the 14 days of rat modelling, the weight of rats in the GnRH-a injection group increased significantly compared with that in the control group (NS + NS), whereas during the drug intervention period (18d-42d), rats in the GnRH-a + E2 and GnRH-a + Xiangshao granules group increased weights slower than those in the GnRH-a + NS group did. This phenomenon suggests that E2 and Xiangshao granules can effectively slow down GnRH-a-induced weight gain in rats.

Trends in rat body temperature

During the 2-week feeding period of rat modelling, the body temperature of GnRH-a-injected rats (GnRH-a + NS, GnRH-a + E2, GnRH-a + Xiangshao granules) increased obviously compared with that of the control group (NS + NS). Among the three groups with pharmacological interventions, the body temperature of rats in the GnRH-a + NS group increased more significantly than in the GnRH-a + E2 and GnRH-a + Xiangshao granule groups. For details, see Fig. 3 (a "Temperature-time trend" of rats was plotted based on the recorded results). During the 14 days of rat modelling, the body temperature of rats in the GnRH-a injection group increased significantly compared with that in the control group (NS + NS), whereas during the drug intervention period (20d-42d), rats in the GnRH-a + E2 and GnRH-a + Xiangshao granules group exhibited an obvious drop in body temperature, and this drop trend was similar between the GnRH-a + E2 and GnRH-a + Xiangshao granules groups. This phenomenon suggests that GnRH-a injection can cause the rats to have increased body surface temperature similar to perimenopausal hot flashes, and E2 and Xiangshao granules can rapidly improve this situation. Moreover, Xiangshao granules can improve

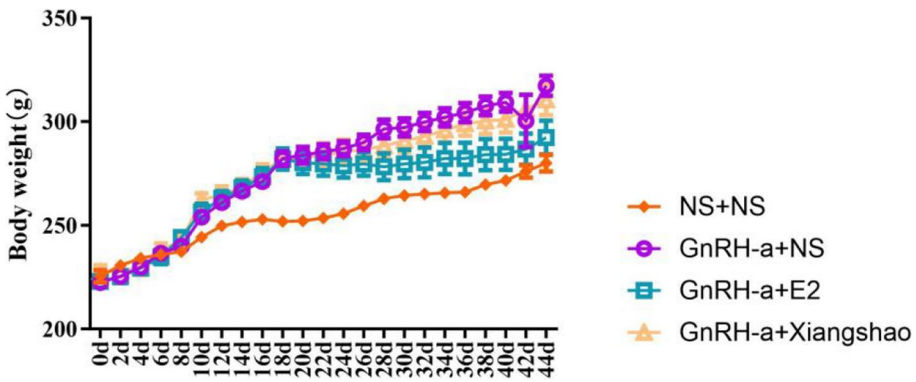

Fig. 2 "Body weight - time trend" for rats (*n*=24)

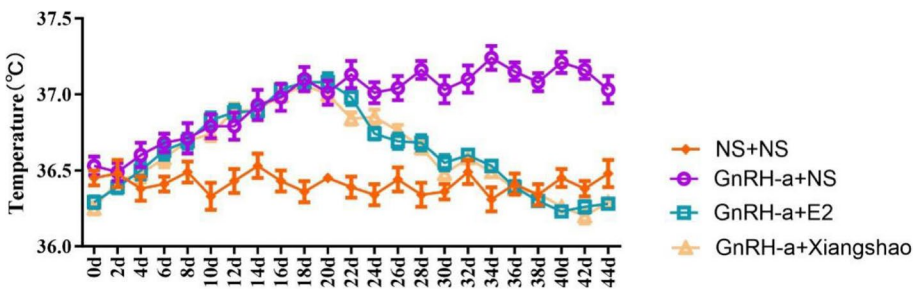

Fig. 3 Rat "Temperature-Time Trend Graph" (*n*=24)

the perimenopausal hot flashes symptoms induced by GnRH-a to the same extent as E2.

Serum sex hormone levels of rats after different drug interventions in each group

At the end of the drug gavage intervention, the serum E2 levels of GnRH-a-injected rats in all three groups (GnRH-a + NS, GnRH-a + E2, and GnRH-a + Xiangshao granules) were all significantly lower than those of the NS + NS group ($P < 0.001$). Between the groups, the E2 levels of the GnRH-a + E2 and GnRH-a + Xiangshao granules groups were significantly higher than those of the GnRH-a + NS group ($P < 0.001$, $P < 0.05$), and E2 levels of the GnRH-a + Xiangshao granules group dropped significantly compared with the GnRH-a + E2 group ($P < 0.05$). At the end of the drug intervention, the serum FSH levels in the GnRH-a + NS, GnRH-a + E2, and GnRH-a + Xiangshao granules groups were significantly lower than those of the NS + NS group ($P < 0.001$). The FSH levels in the GnRH-a + E2 group showed a slight downward trend compared with those in the GnRH-a + NS group, but the difference was not statistically significant ($P > 0.05$). The FSH levels in the GnRH-a + E2 group were significantly lower than those in the GnRH-a + Xiangshao granules group ($P < 0.05$). There was no statistically significant difference between FSH levels in the GnRH-a + NS and GnRH-a + Xiangshao granules groups ($P > 0.05$). At the end of the drug intervention, the serum LH levels in the GnRH-a + NS, GnRH-a + E2, and GnRH-a + Xiangshao granules groups were all significantly lower than those in the NS + NS group ($P < 0.01$, $P < 0.001$, $P < 0.001$), and the LH levels in the GnRH-a + E2 group were significantly lower than those in the GnRH-a + NS and GnRH-a + Xiangshao granules group ($P < 0.001$, $P < 0.05$). The serum LH levels in the GnRH-a + Xiangshao granules group dropped slightly compared with those in the GnRH-a + NS group, but the difference was not statistically significant ($P > 0.05$) (see Table 1, Fig. 4). The test results suggested that GnRH-a injection could achieve the expected effect of establishing a perimenopausal rat model, and this animal model successfully registered a significant decrease in E2, FSH, and LH levels in rats. Meanwhile, Xiangshao granules could increase the E2 level in vivo, meaning that Xiangshao granules might have a certain stimulating effect on the ovary or have a certain estrogen-like effect, which could produce a small amount of estrogen, but its effect was significantly lower than that of estrogen, indicating the estrogen-like effect is relatively weak, and that Xiangshao granules will not increase the FSH and LH levels Tables 2 and 3.

Table 1 Comparison of plasma sex hormone (E2, FSH, LH) levels after drug intervention in each group of rats

Group	N	E2 (pg/ml)	FSH (IU/L)	LH (IU/L)
NS+NS	6	34.20±8.60	2.72±0.32	2.03±0.25
GnRH-a+NS	6	1.45±0.38***###	1.45±0.38***	1.55±0.24***###
GnRH-a+E2	6	1.16±0.34**	1.16±0.34***	0.91±0.13***
GnRH-a+ Xiangshao	6	1.70±0.36***#	1.70±0.36***#	1.48±0.46***##

** $P < 0.01$

*** $P < 0.001$vs NS+NS

# $P < 0.05$

$P < 0.01$

$P < 0.001$ vs GnRH-a+E2

Morphological changes in uterine index and endometrial tissue of rats after drug intervention in all groups

After drug intervention, the uterine index of GnRH-a-injected rats in all three groups (GnRH-a + NS, GnRH-a + E2, and GnRH-a + Xiangshao granules) was significantly lower than that of the NS + NS group ($P < 0.001$). The uterine index of rats in the GnRH-a + E2 and GnRH-a + Xiangshao granules groups was significantly increased compared with that of the GnRH-a + NS ($P < 0.001$ and $P < 0.05$). The uterine index of rats in the GnRH-a + E2 group was significantly higher than that in the GnRH-a + Xiangshao granules group ($P < 0.05$) (see Fig. 5).

Morphological changes of endometrial tissues after drug intervention

At the end of the drug intervention, the endometrial tissues of rats in each group were subjected to a pathological section while processed with HE staining, and the results showed the following. The endometrium of rats in the NS + NS group consisted of a single layer of columnar epithelial cells, tubular glands were distributed in the lamina propria, glandular epithelium was a single layer of columnar shape, cell morphology had no abnormality, and no oedema, atrophy, or hyperplasia was observed. The uterine wall of rats in the GnRH-a injection group was significantly thinner, the endometrial layer was atrophied, and the epithelial cells were flattened and partially atrophied. The uterine wall of rats in the GnRH-a + E2 and GnRH-a + Xiangshao granules groups showed a thickness increase compared with that of rats in the GnRH-a + NS group. The endometrial epithelial cells were in the shape of columns or cubes, and the cells were not edematous but with atrophic thinning to varied degrees in the GnRH-a +

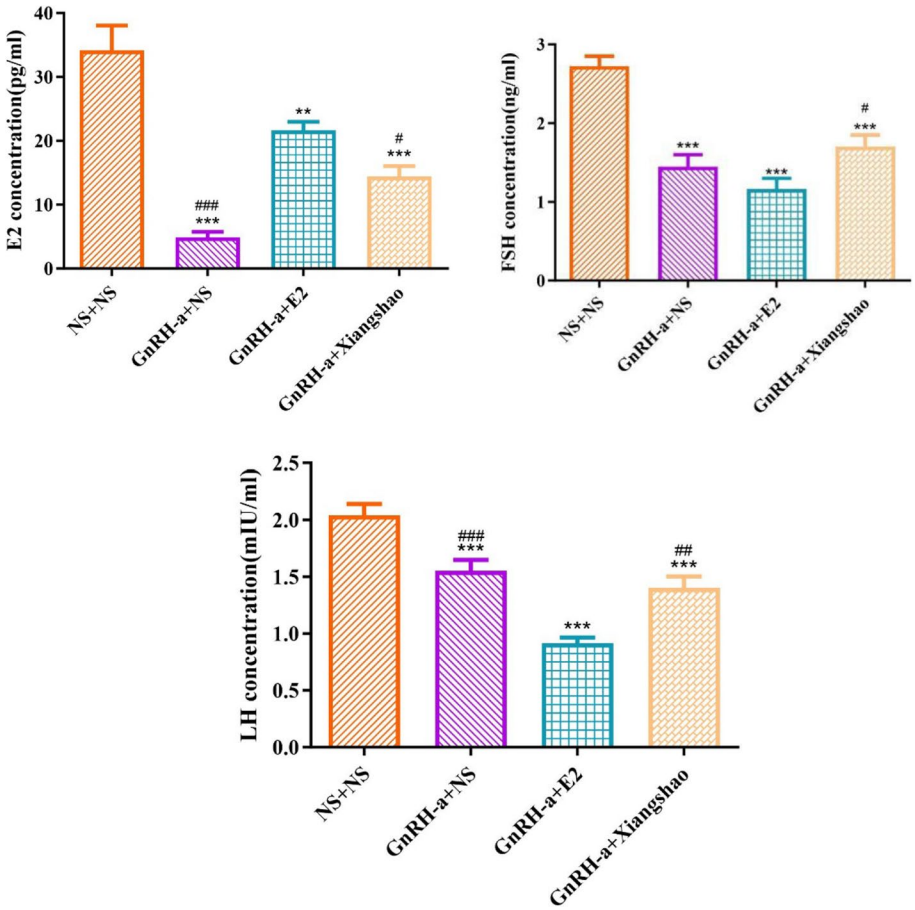

Fig. 4 Plot of plasma sex hormone levels after therapeutic intervention in each group of rats (**P* <0.05, ***P* <0.01 ****P* <0.001vs NS+NS, #*P* <0.05, ##*P* <0.01, ###*P* <0.001 vs GnRH-a+E2)

Table 2 Uterine wet weight and uterine index in rats after drug intervention

Group	N	Uterus wet weight	Uterus index
NS+NS	6	0.5355±0.1314	0.192±0.051278
GnRH-a+NS	6	0.17425±0.01846***###	0.055±0.005353***###
GnRH-a+E2	6	0.354625±0.048718	0.122±0.020585***
GnRH-a+Xiangshao	6	0.28025±0.029305***#	0.091±0.011101***#

*** *P* <0.001vs NS+NS

# *P* <0.05

P <0.001 vs GnRH-a+E2

Table 3 Ovarian wet weight and ovarian index in rats after drug intervention

Group	N	Ovarian wet weight	Ovarian index
NS+NS	6	0.204625±0.027682	0.0732±0.010204
GnRH-a+NS	6	0.093625±0.017353***###	0.0294±0.004776***###
GnRH-a+E2	6	0.13675±0.01836***	0.0468±0.005377***
GnRH-a+ Xiangshao	6	01185±0.015793***#	0.0385±0.006576***#

*** *P* <0.001vs NS+NS

# *P* <0.05

P <0.001 vs GnRH-a+E2

NS group compared with the GnRH-a + NS group (see Fig. 6).

Morphological changes in ovarian index and sub-ovarian tissues of rats after drug intervention in each group

After drug intervention, the ovarian index of GnRH-a-injected rats in all three groups (GnRH-a + NS, GnRH-a + E2, and GnRH-a + Xiangshao granules) was significantly lower than that of the NS + NS group ($P < 0.001$). The ovarian index of rats in the GnRH-a + E2 and GnRH-a + Xiangshao granules groups was significantly increased compared with that of the GnRH-a + NS ($P < 0.001$ and $P < 0.05$). The uterine index of rats in the GnRH-a + E2 group was significantly higher than

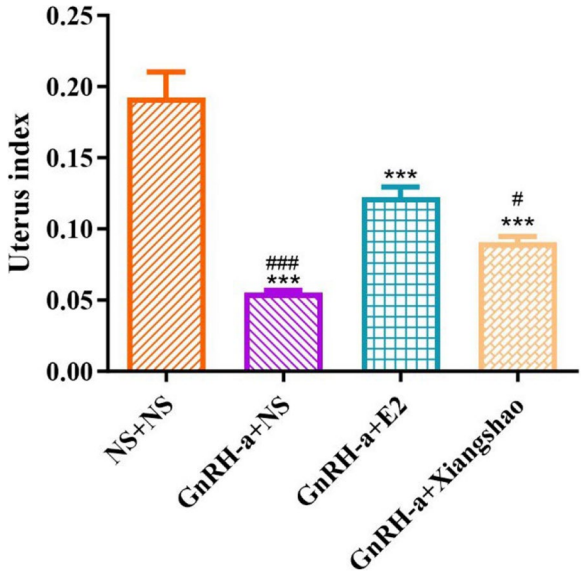

Fig. 5 Post-treatment uterine index graph for each group of rats

that in the GnRH-a + Xiangshao granules group ($P < 0.05$) (see Fig. 7).

Morphological changes of ovarian tissues after drug intervention

At the end of the drug intervention, the complete ovaries of rats in each group were taken out for pathological sections and HE staining at the same time. Morphological changes of ovarian groups were observed under the microscope. The observations showed the following. The follicle structure was normal in the NS + NS group, with visible follicles at all levels. The follicle structure was normal in the GnRH-a + NS, GnRH-a + E2, and GnRH-a + Xiangshao granules groups, and the numbers of growing and mature follicles was obviously reduced, with more primordial follicles (see Fig. 8).

Discussion

Drug GnRH-a, which inhibits oestrogen synthesis, can be used to control recurrence after endometriosis surgery. However, long-term use of GnRH-a can lead to secondary perimenopausal reactions, which can cause discomfort such as hot flashes and sweating, emotional irritability, and poor sleep, constituting the main reasons for patients to give up treatment. Despite a good solution to perimenopausal symptoms, the reverse-addition therapy requires long-term use of hormones, which may lead to liver function damage, venous embolism, and vascular diseases, and increase the risks of endometrial cancer, ovarian cancer, and breast cancer, among other diseases. Therefore, finding effective and safe counter-additive drugs is an urgent need.This study used GnRH-a injection to establish a pharmacological ovariectomy rat model, and vaginal smears were made to observe changes in rats' vaginal cells to determine whether the modelling succeeded. In this study, the rats' vaginal smears showed that the changes in rats' estrous cycle in the GnRH-a group disappeared, and that the vaginal cells showed a persistent inter-estrous phase, with the cells mainly being rounded outer primordial layer ones with a large rounded nucleus, indicating that the modelling was successful and further confirming that GnRH-a could induce the hypoestrogenic state in the body of the rats. In addition, the results of this study showed that the body temperature of rats was on the rise after the GnRH-a injection

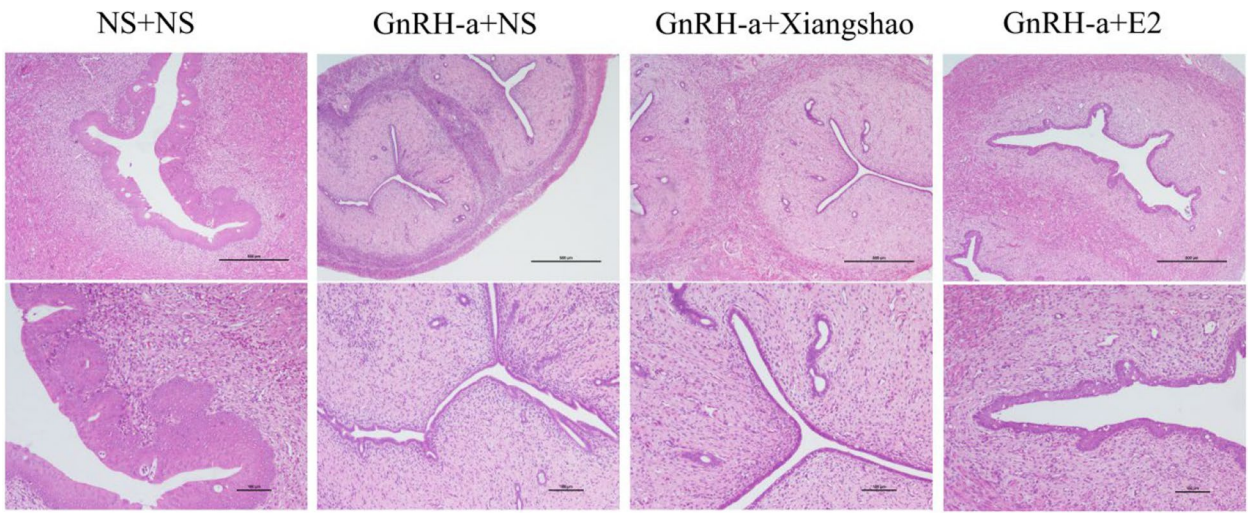

Fig. 6 Endometrial tissue sections of rats in each group after drug intervention (Scale bar 20×/5×)

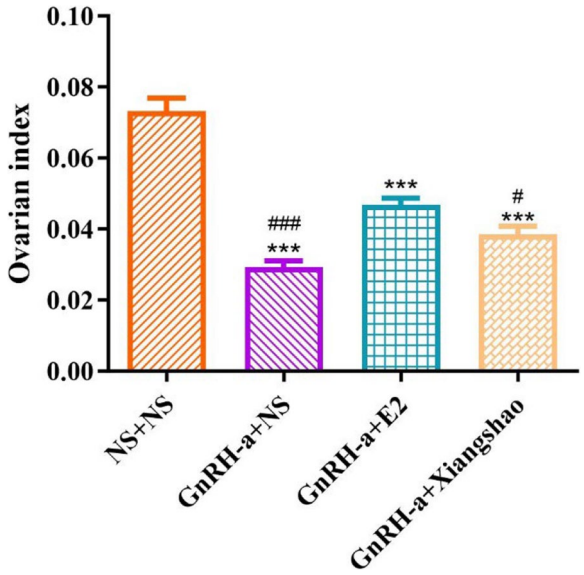

Fig. 7 Post-treatment ovarian index graph for each group of rats

and that with E2 and Xiangshao granules intervention was on the decline, and the two methods posted similar degrees of drop. This further verified that GnRH-a could cause perimenopausal hot flashes, sweating and other discomforts in the perimenopausal period, and Xiangshao granules and oestradiol could both improve this symptom, with the degree of hot flashes improvement similar. Therefore, Xiangshao granules can quickly relieve the symptoms of perimenopausal hot flushes. The results of this study showed that GnRH-a injection could increase the body weight of rats significantly and reduce the levels of serum sex hormones E2, FSH, and LH at the same time, lowering the uterine index and ovarian index of rats. It was found that the endometrium of the rats injected with GnRH-a was obviously atrophied, the numbers of growing and mature follicles dropped significantly, and primordial follicles increased. After E2 and Xiangshao granules intervention, the body weight of rats was significantly reduced, which indicated that Xiangshao granules had the same effect as estradiol in alleviating the perimenopausal weight gain induced by GnRH-a. Meanwhile, after the intervention of Xiangshao granules, E2 levels of the rats increased, but to a lesser extent than that of the E2 intervention group, which further indicated that Xiangshao granules might have the effect of stimulating or improving the function of the ovary, or have a slight estrogen-like effect, which was consistent with the study by Li et al. who studied the effects of Xiangshao granules on perimenopausal ovarian function [25]. However, HE staining of rat endometrium showed no significant endometrial hyperplasia in the Xiangshao granules intervention group, which indicated that the estrogen-like effect of Xiangshao granules may be weak and have a smaller effect on the endometrium. After the drug intervention, the serum FSH and LH levels of rats were not different from those of the control group, indicating that Xiangshao granules may not exert anti-perimenopausal effects through an estrogen-like effect.

Xiangshao granule is a Class III new drug (traditional Chinese medicine) marketed in China in recent years. It is extracted from 10 purely natural plant medicines, such as fragrant herbs, white peony, neem seeds, chaihu, chuanxiong rhizome, fructus aurantii, and cardamom [27], and has effects of complementing the Qi (the vital energy that circulates through the body at

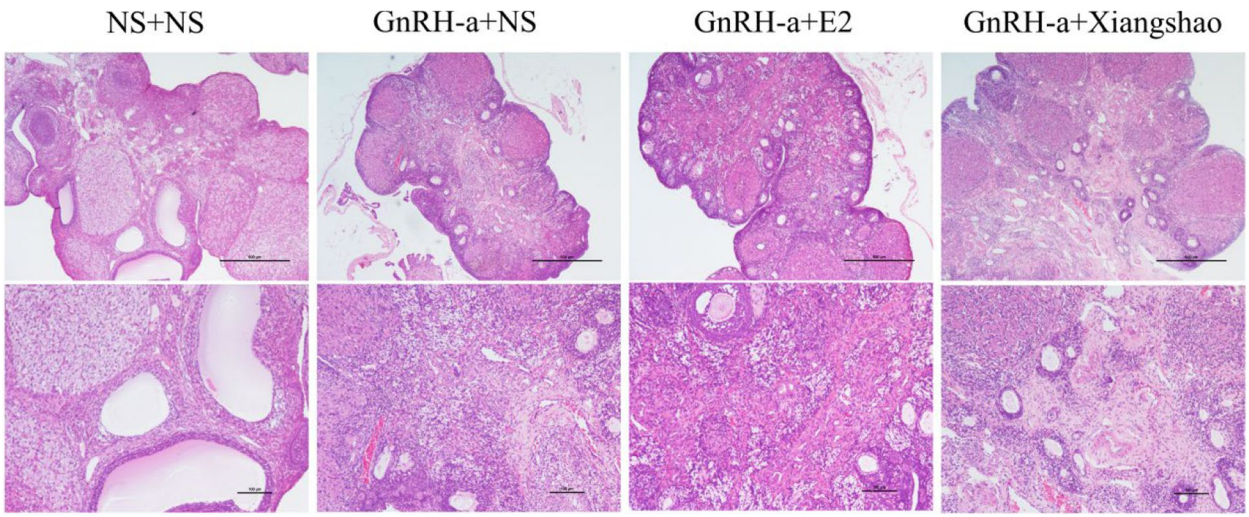

Fig. 8 Bilateral ovarian tissue sections of rats in each group (Scale bar 20× /5×)

all times according to Traditional Chinese Medicine), soothing liver, and relieving the pain. Clinical phases II and III studies have proved [28] that the Xiangshao granules have good efficacy in treating perimenopausal syndrome and premenstrual syndrome and can quickly relieve the symptoms of perimenopausal syndrome, such as hot flashes and sweating, depression, and emotional irritability. However, little research has been done on perimenopausal symptoms caused by pharmacological menopause. In view of this, we can deduce that Xiangshao granule also antagonizes the symptoms of postoperative GnRH-a treatment for patients with endometriosis. This study aims to investigate the clinical efficacy of Xiangshao granules in antagonizing perimenopausal symptoms caused by postoperative GnRH-a treatment in patients with endometriosis, using oestradiol as a reference, in the hope that it can become a novel alternative to reverse-addition therapy after GnRH-a treatment with higher safety and reliability. The pathway through which Xiangshao granules act on perimenopausal symptoms remains unclear and requires further research. It is also a focus of our future research efforts.

In summary, GnRH-a injection can cause low FSH, LH, and E2 levels in perimenopausal rats, which can lead to perimenopausal-like symptoms such as hot flashes and weight gain. These symptoms are more in line with the perimenopausal clinical discomfort caused by GnRH-a. It was found that Xiangshao granules can improve the symptoms such as hot flashes quickly and effectively. Although Xiangshao granules can cause estrogen elevation in rats, according to endometrial HE staining, it wouldn't cause endometrial hyperplasia. Therefore, although Xiangshao granules have an estrogen-like effect, the effect is weak, without increasing the risks of endometrial hyperplasia, breast cancer, and other related diseases. However, it can achieve the same effect as estrogen to alleviate perimenopausal symptoms. Meanwhile, as mentioned above, both Xiangshao granules and estradiol can alleviate perimenopausal symptoms such as hot flashes and have certain commonalities. The difference is that estradiol improves the symptoms through direct exogenous estrogen, while Xiangshao granules have a smaller estrogenic effect. However, the latter may act through different pathways. This shows that Xiangshao granules can effectively alleviate the perimenopausal symptoms due to GnRH-a, without increasing the risk of endometrial cancer, breast cancer, renal vein thrombosis, and other diseases, being a safe clinical alternative.However, the molecular mechanism of the action of paeoniflora granules is not clear, and further studies are still needed.

Conclusion

GnRH-a injection can reduce the levels of E2, FSH, and LH in rats, causing perimenopausal symptoms such as hot flushes, which can be significantly ameliorated by E2 and Xiangshao granules. Xiangshao granules do not exert their anti-perimenopausal symptomatic effects through the estrogen-like effect, but through other pathways.

Authors' contributions

Huimin Tang and Qiucheng Jia wrote the main manuscript text, Xiangmei Zhong, Wanying Chen, Yihan Wu Analysied data and mapping, Weiwei Wei and Hong Zheng literatured review, Jiming Chen Designed Experiments and Instruction. All authors reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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