

Research on the Effects of the Extract of *Polygala fallax* Hemsl on Sex Hormones and β -EP in Perimenopausal Rat Models

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Abstract: Objective: To study the effects of the ethnic medicine *Polygala fallax* Hemsl with Guangxi characteristics on the sex hormones and β -EP in research objective perimenopausal rat models. **Methods:** 40 female SPF rats were randomly divided into 4 groups, including the normal, model, high-dose and low-dose groups. Rats of three groups except for the normal one were treated with perimenopausal modelling through the method of subcutaneous injection of compound 4-VCD for 15 consecutive days. Rats of the normal and model group were normally fed without any treatment. Rats of the high-dose and low-dose groups were administered by high- and low-dose intragastric administration of the extract of *Polygala fallax* Hemsl. According to the menstrual cycle of the vaginal smear of the rat, each menstrual cycle is a course of treatment and 6 consecutive courses of treatment would be given. The indexes of serum sex hormones (E2, FSH, LH) and β -EP of rats in each group were observed after treatment. **Results:** After the treatment of 6 cycles, for the levels of β -EP and E2, the model group was lowest ($P<0.05$), the normal group was highest ($P<0.05$); and the high-dose group was higher than the low-dose group; For the levels of FSH and LH, the normal group

was lowest ($P<0.05$), the model group was highest ($P<0.05$), and the high-dose group was lower than the low-dose group. **Conclusion:** Guangxi characteristic national medicine *Polygala fallax* Hemsl can effectively improve the levels of serum sex hormones and β -EP in perimenopausal rat models and relieve the related symptoms with a certain dose-effect relationship

Keywords: *Polygala fallax* Hemsl; Perimenopausal syndrome; Sex hormones; β -EP

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Perimenopausal syndrome, formerly known as menopausal syndrome, refers to a series of metabolic, endocrine and neurological changes that occur in women for the influence on the balance of the hypothalamic-pituitary-ovarian axis caused by the decrease in the secretion of sex hormones due to the decline of ovarian function before and after menopause^[1]. With a close relationship with its occurrence and development, sex hormones (E2, FSH,

LH)^[2] and β -EP^[3] were important indicators for the observation of perimenopausal syndrome. With the progress of the times, the acceleration of life rhythm and the increase of economic burden, the incidence of perimenopausal syndrome gradually increases with various and complicated symptoms, which causes serious physical and psychological problems for increasingly more perimenopausal women.

As shrub or small tree of Polygala, Polygalaceae, Polygala fallax Hemsl is widely distributed in Guangxi, Jiangxi, Hunan, Guangdong and Yunnan^[4]. Besides its root usually used as a medicine, it tastes sweet, slightly bitter, and mild-natured; and can be used to invigorate spleen to remove dampness, benefit qi and blood, and promote blood circulation to regulate menstruation. Also, it is always used to replace Astragalus and Codonopsis^[5]. It is commonly used in the treatment of physical weakness after illness, acute and chronic hepatitis, ageing, etc. Also, it is a common and speciality medicine for Yao, Miao, Zhuang and other minorities^[6]. Besides, some studies have shown that Polygala fallax Hemsl is also good medicine for gynaecology with the main chemical components of flavones, polysaccharides, and saponins. The total polysaccharides in different parts have estrogen activity^[7].

Whether Polygala fallax Hemsl can treat perimenopausal syndrome through sex hormones (E2, FSH, LH) and β -EP is the key issue discussed in this study.

1 Materials & Methods

1.1 Experimental materials

1.1.1 Experimental animals

40 female rats (Model: SD rats at the SPF grade; Source: Changsha Tianqin Biotechnology Co., Ltd.; Age: 6 weeks; Weight: 180-220g) were fed at the animal house of 22-25 °C. These rats were first fed conventionally, and the experiment started after one week of adaptation.

1.1.2 Reagents and equipment

The extract of Polygala fallax Hemsl was obtained by the Pharmacy Room of the Guangxi University of Chinese Medicine according to relevant requirements. Glass slide, normal saline, 1 ml syringe, cotton swabs, etc. were provided by the laboratory of Zhuang Medical College, Guangxi University of Chinese Medicine. 4-vinylcyclohexene dioxide (4-VCD); (Rat β -ET ELISA

KIT) kit (Shanghai Enzyme-linked Biotechnology Co., Ltd.); Research Inverted Microscope (DM18, Leica, Germany); Multiscan Spectrum (M200, Switzerland TECAN); High-Speed Tabletop Refrigerated Centrifuge (3k15, German sigma), etc.

1.2 Experimental Methods

1.2.1 Animal selection and grouping

The rats above were grouped and randomly divided into 4 groups with 10 rats in each group.

1.2.2 Model preparation and evaluation

Rats in the model, high-dose and low-dose groups were treated with subcutaneous injection of 80 mg/kg compound 4-VCD, and rats of 200 g were treated with administration of 0.2 ml (Concentration: 80 mg/ml), that is, 0.001 ml/g, for 15 consecutive days. Perimenopausal Rat Models were prepared. After 5 days of operation, rats in each group were treated with vaginal cytology smears for 5 consecutive days. According to the morphology of the vaginal cells under the light microscope, the existence of the oestrous cycle of the rats was judged. Besides the normal group, in the other three groups, the smears of the vaginal cells were consistent with the symptoms during the oestrous period, that is, a large number of white blood cells or a small number of epithelial and keratinocytes, then the modelling was successful. During the modelling period, no rats died and the data were all included in statistical analysis.

1.2.3 Therapeutic methods

Patients in the normal and model groups received no treatment. Patients in the high-dose group were administrated with high-dose extract of Polygala fallax Hemsl. Patients in the low-dose group were administrated with low-dose extract of Polygala fallax Hemsl.

1.3 Observation indicators and methods

1.3.1 Specimen collection

After 24 hours of the last administration, detached vaginal cells of rats in each group were taken for smears and all rats were weighed. Before the animals were killed, 5 ml of blood from the abdominal aorta of rats was injected into a common test tube. After standing and solidifying in a common test tube at 4 °C, the blood was centrifuged at low temperature to separate the serum and stored at -20 °C for use. At the same time, its

vaginal cells were smeared for the further HE staining. The staining experiment was carried out in Guangxi National Hospital, and the staining was observed under a microscope.

1.3.2 Serum β -EP detection

ELISA was used to detect according to the instructions in the manual.

1.3.3 Serum sex hormone index

It was sent to detect with radioimmunoassay in Nanjing Xin Fan Biology Technology Co., Ltd.

1.4 Statistical methods

The measurement data are expressed as mean \pm standard deviation (mean \pm SD). T test was used for comparison between groups in the case of normal distribution. Non-parametric test was used for non-normal distribution. $P < 0.05$ is considered as statistically significant difference. SPSS19.0 statistical software was used for data statistical analysis.

2 Results

2.1 HE staining of vaginal cell smears of rats in each group shown in Figure 1.

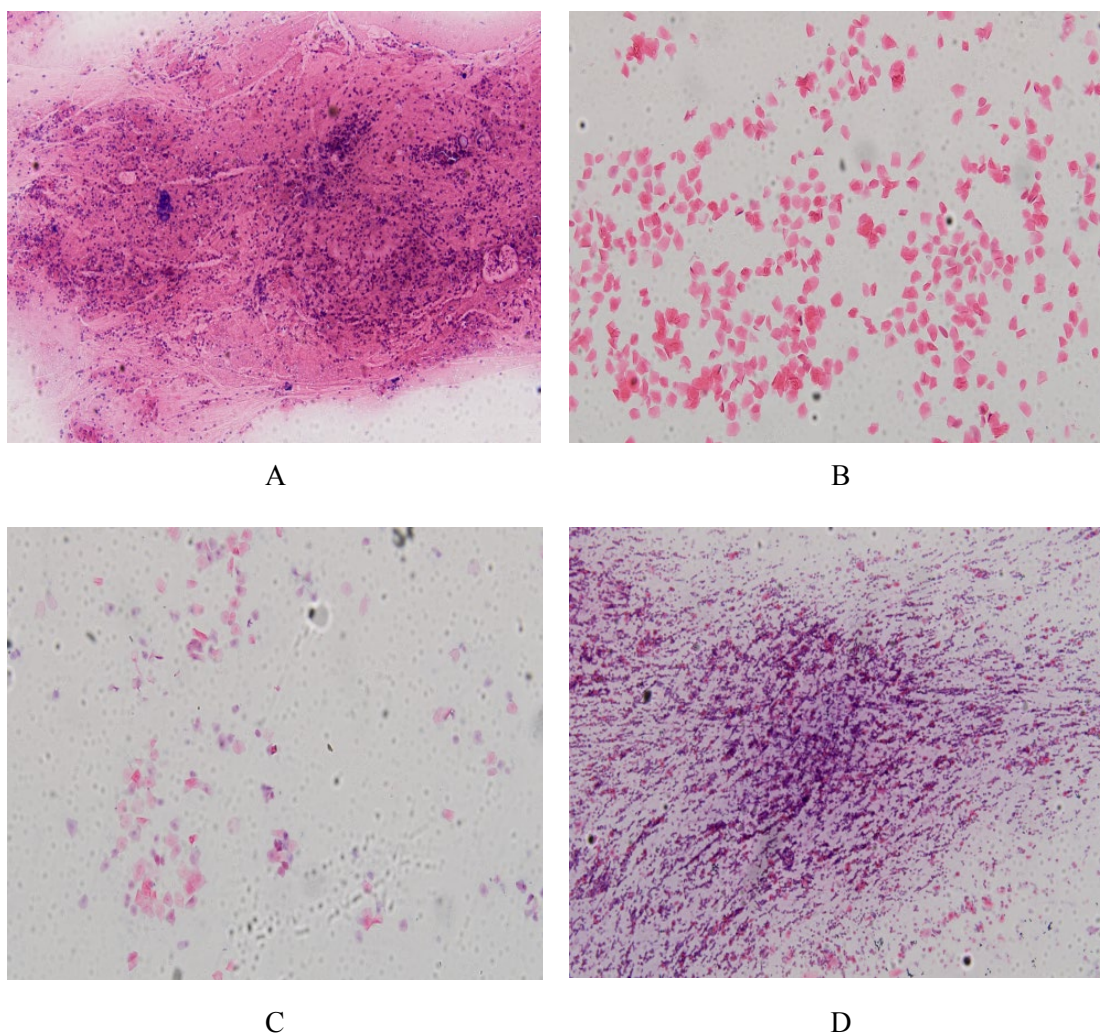


Figure 1. HE staining of vaginal cell smears of rats in each group (100x)

Figure 1 refers to the vaginal smear of a rat's oestrous cycle: A: Most were nuclear epithelial cells in the early period, accompanied with a small number of keratinocyte epithelial cells; B: All were keratinocytes with no nucleus, accompanied with a small number of epithelial cells; C: Most were keratinocytes and

leukocytes in the later period; D: Most were white blood cells in the inter-phase, with few mucosa and epithelial cells

2.2 Effects of *Polygala fallax* Hemsl on β -EP in perimenopausal rat models shown in Table 1.

Table 1. Comparison of serum β -EP content in each group of rats ng L-1, $x\pm s$

Group	Number of animals	β -EP
The normal group	10	184.097 0 \pm 42.3057**
The model group	10	110.5873 \pm 30.1545 ^{##}
The high-dose group	10	160.942 1 \pm 30.0527*
The low-dose group	10	135.884 3 \pm 22.6542 [#]

Note: Compared with the model control group, * P <0.05, and ** P <0.01; Compared with the normal group, [#] P <0.05, and ^{##} P <0.01.

2.3 Effects of Polygala fallax Hemsl on E2, FSH and LH in perimenopausal rat models shown in Table 2.

Table 2. Changes in hormone levels of rats in each group (n=10, $x\pm s$)

Group	Number of animals	E2 (pg/mL)	FSH (mIU/mL)	LH (mIU/mL)
The normal group	10	50.29 \pm 6.35	3.84 \pm 0.61	3.73 \pm 1.05
The model group	10	22.32 \pm 7.54*	7.35 \pm 0.73*	8.83 \pm 1.27*
The high-dose group	10	39.13 \pm 6.64*	4.02 \pm 0.37*	4.95 \pm 0.48*
The low-dose group	10	32.96 \pm 5.72 [#]	5.68 \pm 0.78 [#]	5.82 \pm 1.42 [#]

Note: Compared with the normal group, * P <0.05; Compared with the model group, [#] P <0.05.

3 Discussion

During the perimenopausal period, a key period for women's transition from fertility to old age, women show a series of clinical syndromes mainly caused by autonomic dysfunction for the decreased secretion of sex hormones, such as fever, depression, and osteoporosis. These are extremely harmful to their physical and mental health. Therefore, to explore new treatments has important practical significance^[8].

As a speciality and common medicine of Zhuang and Yao nationalities, Polygala fallax Hemsl has a wide range of pharmacological effects, including enhancing immune function, anti-ageing, and improving the body's anti-stress ability^[9]. Also, it has a strong estrogen activity.

Estradiol (E2) is an important regulator of estrogen, secreted by mature ovarian follicles. When women enter the menopause, E2 levels would decrease as ovarian function declines, result in an imbalance of the hypothalamic-pituitary-ovarian axis and further lead to the increase of the LH and FSH levels, which finally inhibited the follicular development and egg growth, while ovarian function experienced a decline^[5]; the lower level of E2 in the serum than the normal level, and the higher level of LH and FSH in the serum than the normal level^[10]. β -endorphin (β -EP) is a basic neurotransmitter that can inhibit GnRH. Studies have shown that the peripheral plasma β -EP levels of perimenopausal women were significantly reduced^[11], and caused hot flashes led by the reaction

of the body's temperature regulation centre. The decrease in estrogen levels causes the changes in the secretion of neurotransmitters in the hypothalamus and limbic system, and the decrease in the β -EP activity and dopamine tension decreases, resulting in increased norepinephrine activity and impaired 5-hydroxytryptamine secretion. These changes may cause hot flashes and sweat in the early phase, the urinary system and reproductive system atrophy in the mid-term phase, as well as neurological symptoms and memory loss in the late phase^[6]. Therefore, with a close relationship with perimenopausal occurrence and development, sex hormones (E2, FSH and LH) and β -EP are important indicators for the observation of perimenopausal syndrome.

According to this study, Polygala fallax Hemsl can effectively increase the levels of β -EP and E2 and reduce the levels of FSH and LH in perimenopausal model rats, and showed a better effect on the high-dose group than that of the low-dose group. That is, Polygala fallax Hemsl can improve perimenopausal syndrome and related symptoms, with a certain dose-effect relationship, and deserves clinical application and promotion.

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