

Effect of total flavone of epimedium on the mice model of perimenopausal syndrome

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Abstract. To investigate the effect of total flavone of Epimedium (TFE) on the model of perimenopausal syndrome (PMS) in mice, and to lay the foundation for the development of the drug which is effective for the treatment of PMS. The castration method making PMS animal model, observe the high, medium and small dose of TFE on PMS model mice Viscera index, serum sex hormones and organ histomorphology. Compared with the blank group, the uterus index, serum E2 levels of model group decreased significantly ($P<0.01$), LH and FSH levels were significantly increased ($P<0.01$), endometrial thickness of mice in model group, muscle layer thickness, thickness of thymic cortex and cortical lymphocytes number, volume of splenic nodule were significantly reduced ($P<0.01$) which explained the model was successful. Compared with the model group, the drug group of TFE could different degree increase thymus index, uterus index, serum E2 level; reduce levels of LH and FSH; endometrium, the thickness of thymic cortex increased, increased volume of splenic nodule, and increase the number of lymphocytes. Conclusion: TFE has a significant effect on the treatment of PMS.

1 Introduction

Perimenopausal syndrome (PMS) is refers to the women in premenopausal and postmenopausal period, prone to sex hormone fluctuations or due to a decrease of include hot flushes, sweating, hot flashes, flushing, menstrual disorders, autonomic nervous system disorders, genitourinary tract atrophy, osteoporosis and so on a series of somatic and psychological symptoms [1]. Modern medicine mainly uses symptomatic and hormone therapy, but there are some adverse reactions, more taboo. In recent years, due to the accelerated pace of life, work pressure, the disease has the age of onset, the incidence of the upward trend, especially among the occupational female class. TCM treatment of PMS, the effect is significant, by adjusting the nerve - endocrine - immune system, regulating blood lipid, inhibit apoptosis, down regulating the expression of vascular endothelial growth factor (VEGF) mechanism to treat PMS and positive results, compared with the hormone replacement therapy (HRT) more with safety [2].

Herba Epimedium also known as epimedium, three branches of the Nine Leaves of grass, and the sheep hinge, etc., the small suckers division (Berberidaceae) Epimedium genus [3]. Xin, Gan, temperature, liver, kidney. Has the functions of Invigorating Kidney Yang, strong bones, rheumatism. TFE is the total flavonoids extracted from the stem and leaves of the herba, and the main active ingredient of the herba [4]. Pharmacological studies showed that the immune system and nervous system and other diseases have a certain role [5]. Modern

studies also showed that TFE can enhance the hypothalamic pituitary function and have estrogen like activity [6-8]. The castration method to observe the role of the female perimenopausal, in order for PMS prevention in the screening of a new drug, and hot fill Kidney Yang drugs to provide a new idea for the treatment of women's climacteric disease.

2 Materials

2.1 Animals

Clean grade mice, Kunming (KM) species, female, 22 ~ 25g, provided by the experimental animal center of Henan province. Certificate number: SCXK (Yu) 2010-0005.

2.2 Drugs

Epimedium (TFE content is 60%) was provided by Xi'an city garden plant biological agents, batch number: TY200080116; Gengnian'an capsule was purchased from Changchun Yingping Pharmaceutical Co., Ltd., batch number:120303; Benorcom soy isoflavone tablets was purchased from Nanning fresh life Biological Technology Co., Ltd., Nanwei Shi Zheng word (2006) the 450101-000389.

2.3 Reagents and instruments

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Pentobarbital sodium was provided by China Pharmaceutical Group Shanghai chemical reagent company, batch number: F20030816; formaldehyde was provided by Chinese Laiyang Shuangshuang Chemical Industry Co. Ltd., batch number: 20040519; Sodium Chloride Injection was provided by Zhengzhou Yonghe pharmaceutical Limited by Share Ltd, batch number: 20060302; penicillin sodium for injection was provided by North China Pharmaceutical Limited by Share Ltd, batch number: X0603113; estradiol radioimmunoassay kit was provided by Beijing widescape Science & Technology Co. Ltd., batch number: Zhunzi S20063125; luteinizing hormone radioimmunoassay kit was provided by Beijing widescape Science & Technology Co., Ltd, batch number: Zhunzi S20063073; follicle stimulating hormone radioimmunoassay kit was provided by Beijing widescape Science & Technology Co. Ltd., batch number: Zhunzi S20063072.

FA (N) /JA (N) series of electronic scales was purchased from Shanghai min bridge Precision Instrument Co., Ltd.; B SN-695 type smart radioimmunoassay was purchased from Shanghai nuclear Institute of the Japanese instrument a plant.

3 Methods

3.1 Modeling methods [9, 10]

Took 80 childbearing age nonpregnant female KM mice, of clean grade, 22~25g, were respectively divided into 7 groups, that's the blank group, model group, gengnian'an capsule group, soybean isoflavone group(SI), total flavonoids of Epimedium high, medium and low dose group(TFE-HD, TFE-MD, TFE-LD). Except the blank group, other groups mice were intraperitoneally injected with sodium pentobarbital 50mg/kg after anesthesia abdominal position fixed, and then from the back of the mice at the end of the rib, in the midaxillary line and the distance between the lateral spine about 1 cm at the intersection of shearing, disinfection after open skin and dorsal muscle about 0.5~1cm, incision in the view of visible a milky white shiny cellulite, ovarian or packets buried in it. With small forceps gently clamped cellulite pulled out of incision, separated from the fat and the group, you can see a ball of thread like irregular was red and yellow of the ovary. Clipping the ovarian fallopian tube (including fat) was established by thread ligation, and the ovaries were removed. After the operation to take advantage of the uterine horn back into the abdominal cavity in, the muscle was sutured and the skin, with the method of extraction on the other side of the ovary [11]. After surgery, intramuscular penicillin was injected with penicillin for 3 consecutive days. After surgery, the vaginal smear was performed at 5d, and the vaginal smears were performed each day for 1 times, for 5 consecutive days to determine whether the ovary was completely removed. The animals were randomly divided into 7 groups and each group has 10 animals.

3.2 Administration methods

CMC-Na dissolved in distilled water boiled into a 0.5% solution do as solvent, each of the following drugs mixed suspension which. Total flavonoids of Epimedium high, medium and low dose: 20mg/ml, 10mg/ml, 5mg/ml, according to 400 mg/kg and 200mg/kg, 100mg/kg gavage (equivalent to 30 times the amount of clinical drug, 15times, 7.5 times); Gengnian'an capsule: 33.75mg/ml, according to 675mg mg/kg, Ig (equivalent to 15times of the clinical dosage); soybean isoflavones: 6.25mg/ml, according to 125mg/kg gavage; model group and blank group given CMC-Na solution of the same volume, volume of administration for 0.2ml/10g, once a day for 3 weeks.

3.3 Observation and detection methods

Mice in each group at the end of the irrigation stomach 2H (fasting 15 hours), orbital venous sampling, separation of serum, the content of serum E₂, LH and FSH were measured by radioimmunoassay; mice were dissected, removal of the thymus, spleen, uterus, weighing the wet weight and calculate the index of immune organs (Viscera index = dirty wet weight / rat body weight); thymus, spleen, uterus, brain tissue fixed in 10% formalin solution and embedded in paraffin, section, he staining, light microscope observation histological changes in each group.

3.4 Statistical analysis

The SPSS 13.0 statistical software for Windows was used for data analysis. The measurement results were expressed as "mean \pm standard deviation ($\bar{x} \pm s$)". Comparison between groups was performed by the LSD method.

4 Results

4.1 Effects of TFE on organ index in PMS model mice

Compared with the blank group, the uterine index of the model group was significantly decreased ($P < 0.01$), and the menopausal model caused the uterine atrophy. Compared with the model group, TFE-HD could obviously increase the thymus index ($P < 0.05$); TFE-LD and TFE-MD could significantly improve the uterus index ($P < 0.05$); TFE-HD and SI group could significantly improve the uterus index ($P < 0.01$). The results are shown in Table 1.

4.2. Effects of TFE on PMS model mice serum sex hormone content

Compared with the blank group, the serum E₂ levels of model group decreased significantly ($P < 0.01$), LH and FSH levels were significantly increased ($P < 0.01$),

Table 1. Effect of TFE on mice spleen thymus, menopause, uterus index ($\bar{x} \pm s$).

Groups	n	Dose (mg/kg)	Thymus index (mg/g)	Spleen index (mg/g)	Uterus index (mg/g)
Blank group	10	—	2.493±0.661	3.761±0.699	1.289±0.331
Model group	10	—	2.074±0.355	3.227±0.391	0.269±0.119 ^{△ △}
Gengnian'an capsule group	10	675	2.398±0.516	3.550±0.807	0.475±0.319
SI group	10	125	2.412±0.489	3.325±0.581	0.433±0.089**
TFE-HD group	10	400	2.419±0.40*	3.231±0.898	0.594±0.201**
TFE-MD group	10	200	2.348±0.398	3.381±0.676	0.505±0.296*
TFE-LD group	10	100	2.311±0.764	3.342±0.808	0.379±0.178*

Note: compared with the blank group [△] $P < 0.05$, ^{△ △} $P < 0.01$; compared with the model group * $P < 0.05$, ** $P < 0.01$

Table 2. Effect of TFE on the content of E2, FSH, LH in the serum of PMS model mice ($\bar{x} \pm s$).

Groups	Dose (mg/kg)	E2 (pg/ml)	LH (IU/L)	FSH (IU/L)
Blank group	—	23.90±2.66	2.411±0.345	4.647±0.385
Model group	—	15.78±2.09 ^{△ △}	3.404±0.470 ^{△ △}	5.833±0.520 ^{△ △}
Gengnian'an capsule group	675	18.05±1.77**	2.921±0.217*	5.148±0.449*
SI group	125	21.36±1.77**	2.958±0.340	4.975±0.682**
TFE-HD group	400	21.72±1.41**	2.690±0.391*	5.052±0.242**
TFE-MD group	200	20.36±1.50**	2.830±0.299**	5.125±0.265**
TFE-LD group	100	19.80±0.93**	2.969±0.190*	5.145±0.187**

Note: compared with the blank control group [△] $P < 0.05$, ^{△ △} $P < 0.01$; compared with the model group * $P < 0.05$, ** $P < 0.01$

indicating that removal of the ovary of menopause induced by serum sex hormone level of disorder. Compared with the model group, the drug groups could significantly increase menopausal animal models of serum E₂ levels ($P < 0.01$); in Gengnian'an capsule and TFE-LD, TFE-HD could significantly reduce the elevated LH levels ($P < 0.05$), TFE-MD could significantly reduce the level of LH ($P < 0.01$); Gengnian'an capsule could significantly reduce the elevated FSH levels ($P < 0.05$), each dose group of TFE and IS could be significantly reduced FSH levels ($P < 0.01$), the results are shown in Table 2.

4.3 Effects of TFE on mice model of organ and tissue morphology of PMS

4.3.1 Effects of TFE on mice model of PMS uterine tissue morphology

Using micrometer measurements of endometrium and the thickness of the muscular layer of the wide and the narrow place, for the mean, by count method calculated endometrial glands in number, for the mean, and the results are shown in Table 3.

4.3.2. Effects of TFE on PMS model mouse thymus tissue morphology

The micrometer measurements of thymic cortex of the

wide and the narrow place, for the mean, with micrometer baseline calculating falls on the baseline number of lymphocytes. The results are shown in Table 4.

Table 3. Effect of TFE on mice model of PMS uterine pathological changes ($\bar{x} \pm s$).

Groups	n	Endometrium (μm)	Muscle layer (μm)	Gland (n)
Blank group	10	42.36±8.27	7.13±2.43	21.38±3.48
Model group	10	11.24±2.16 ^{△ △}	4.07±1.25 ^{△ △}	7.24±2.51 ^{△ △}
Gengnian'an capsule group	10	28.32±3.42* *	5.84±1.53 *	11.26±2.34**
SI group	10	16.60±1.50* *	5.50±1.29 *	11.49±2.56**
TFE-HD group	10	64.25±7.20* *	8.16±2.10 **	28.31±3.46**
TFE-MD group	10	44.21±6.24* *	5.23±1.52	25.32±3.16**
TFE-LD group	10	19.61±2.37* *	4.34±1.16	8.12±2.43

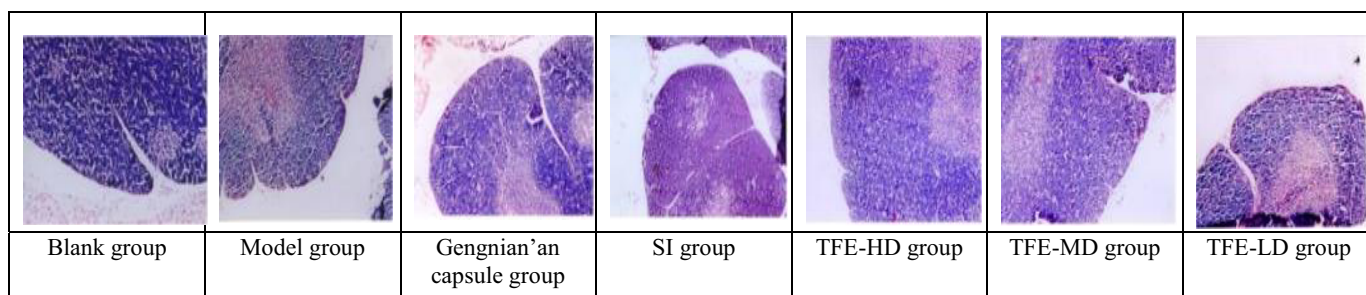
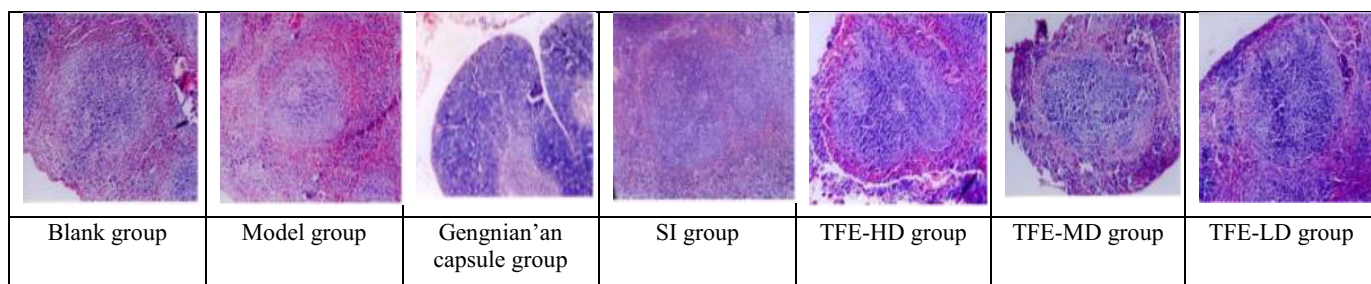
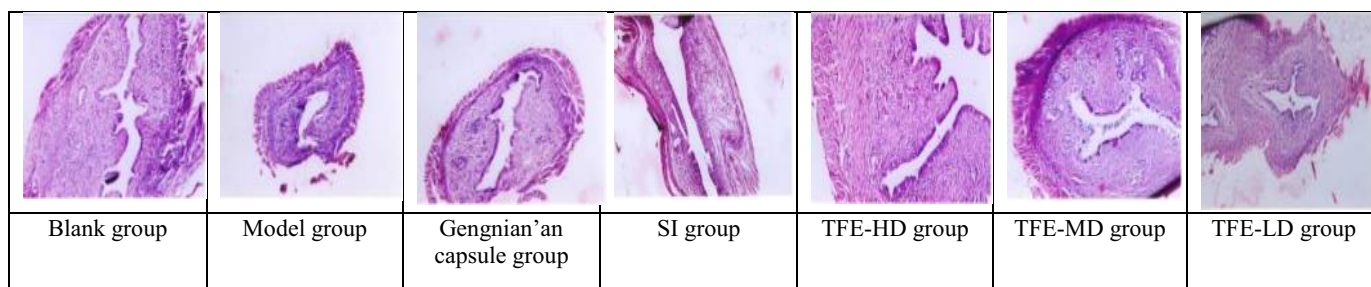
Note: compared with the blank control group [△] $P < 0.05$, ^{△ △} $P < 0.01$; compared with the model group * $P < 0.05$, ** $P < 0.01$

Table 4. Effect of TFE on PMS model mice thymus pathology change ($\bar{x} \pm s$).

Groups	n	Thymic cortical thickness (μm)	Cortical lymphocytes (n)
Blank group	10	39.47 \pm 10.26	62.38 \pm 17.36
Model group	10	27.29 \pm 12.30 [△]	38.17 \pm 11.22 ^{△△}
Gengnian'an capsule group	10	38.62 \pm 8.25*	54.06 \pm 10.26*
SI group	10	41.67 \pm 10.26*	68.74 \pm 11.20*
TFE-HD group	10	52.37 \pm 12.26**	64.26 \pm 16.06*
TFE-MD group	10	48.26 \pm 10.25**	58.35 \pm 14.25*
TFE-LD group	10	23.50 \pm 7.26	32.15 \pm 5.42

Note: compared with the blank control group [△] $P < 0.05$, ^{△△} $P < 0.01$; compared with the model group * $P < 0.05$, ** $P < 0.01$

4.3.3. Effects of TFE on PMS model mouse spleen

**Figure 1.** The pathological changes of thymus in the model of mice with peri menopausal period syndrome (HE \times 100).**Figure 2.** The pathological changes of the spleen in the mouse model of menopausal syndrome (HE \times 100).**Figure 3.** The pathological changes of the uterus in the mouse model of menopausal syndrome (HE \times 100).

tissue morphology

The micrometer baseline falls in the splenic nodules and in the central artery as the center were measured on both sides of the splenic nodule and the thickness of different sizes, for the mean; and calculating falls on the baseline on both sides of the lymphocytes in the number of a number, for the mean, and the results are shown in Table 5, Figure 1, Figure 2, Figure 3.

5 Conclusions

PMS also known as climacteric syndrome. Epidemiologic studies show that there are about 130 million climacteric women in China, and 60% of them are the syndrome of mild or mild menopause. Modern medicine usually uses hormone replacement therapy (HRT), the effect is rapid, the symptoms effect is obvious. However, the application of HRT has side effects, will increase the risk of heart disease, breast cancer and other diseases. At the same time, HRT belongs to the exogenous hormone supplement, rather than motivate the inner potential of the woman. At present, with the exploration and research of TCM, TCM has its unique characteristics and differentiation, so that the effect of this disease is greatly improved [12, 13].

Table 5. Effect of TFE on mice model of spleen pathological changes of PMS ($\bar{x} \pm s$).

Groups	n	Splenic corpuscle (μm)	Lymphocyte count (n)
Blank group	10	26.58±6.42	37.24±8.16
Model group	10	14.20±2.36 ^{△△}	16.38±3.45 ^{△△}
Gengnian'an capsule group	10	24.64±2.50**	42.12±4.25**
SI group	10	19.33±5.11**	20.46±3.28*
TFE-HD group	10	36.25±6.28**	58.45±6.48**
TFE-MD group	10	29.34±4.12**	51.30±5.20**
TFE-LD group	10	28.52±5.20**	49.22±6.15**

Note: compared with the blank control group [△] $P < 0.05$, ^{△△} $P < 0.01$; compared with the model group * $P < 0.05$, ** $P < 0.01$.

Modern medicine thinks PMS pathogenesis is the decline of ovarian function, estrogen levels reduce excessive caused by reducing the factors of hypothalamic pituitary ovarian axis or adrenal dysfunction induced by neurotransmitters, hormones, cytokines imbalance, increase the content of free radicals, immune mediators is around the main reason for the onset of menopause. The main endocrine, immune, free radicals and apoptosis and other theories, including the main endocrine theory research [14].

The levels of sex hormone in perimenopausal women were changed obviously, and the main expression of LH and FSH were increased, and E_2 decreased, so this experiment chose the uterine index and E_2 as the test index of this experiment. Modern pharmacological research show that the effective ingredients of epimedium and its extract has a wide range of physiological activity, as the TCM invigorating the kidney and strengthening yang traditional, due to its unique chemical composition and significant biological activity has been the hotspot of research at home and abroad.

At present, kinds of animal models of the domestic peripheral menopause syndrome include natural senescence, ovarian removal, X irradiation and destruction of ovarian three. Because of the short time of the animal model and the success rate of the model, the research used castration method for the PMS model [15].

TFE is effective and cheap, and Herba Epimedium is widely planted in China, to develop new drugs for TFE, and it is beneficial to the full application of Chinese

medicinal resources in China. This experiment through observed the effect of TFE on mice PMS animal model, explored the pharmacological effects and mechanism of action, with a view to develop a significant efficacy drugs for the treatment of women with PMS, also lay experimental basis for the development of an excellent therapeutic and effective drugs. TCM treatment of the effect of the PMS is sure, the prospect is broad, and it will be further studied, it will have very important theoretical and practical significance.

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