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Effect of *Cynomorium* flavonoids on mouse model of perimenopausal depression

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Abstract

The effect of *Cynomorium* flavonoids on mice model of perimenopausal depression was studied. Ovariectomized female mice (n=70) were randomly divided into 7 groups evenly and treated with different concentrations (5-400 mg/mL) of *Cynomorium* flavonoids, gengnian'an capsule and soy isoflavones soft capsule. The model related groups were applied with different stress for consecutive 18 days. Behavior tests were performed on day 27, 28 and 29 of administration. The concentrations of estradiol, luteinizing hormone and follicle-stimulating hormone in serum as well as the 5-hydroxytryptamine (5-HT) and dopamine in brain homogenates were determined after the last administration. It was found that *Cynomorium* flavonoids have significant effects on the mice with perimenopausal depression.

Introduction

Perimenopausal depression is a mood disorder that occurs during the perimenopause period (Jia and Jin, 2014). The hormone levels in perimenopausal women fluctuate dramatically, and their abilities to adapt to environmental stress drop. They become prone to mood depression, unresponsiveness, slow thinking, irritability, and pessimism. Now-a-days, the incidence of perimenopausal depression is on the rise. A study on 1,280 perimenopausal woman aged 45-59 years was conducted by Beijing Union Medical College Hospital and it was found that 23.9% of the women had depression (Lin et al., 2011). This has led researchers to explore how to reduce the psychological and physiological symptoms in perimenopausal women and potentially develop drugs for the treatment of perimenopausal depression.

Cynomorium flavonoids are extracted from *Cynomorium*, which is a succulent perennial herb of a parasitic plant in the *Cynomorium* genus, *Cynomorium* family. They have multiple functions, such as scavenging free radicals, anti-oxidation, anti-aging and anti-stress, regulating immune and endocrine systems, and improving sexual function (Tian and Miao, 2014). In modern research, they have been used in preventive therapy for women's sexual apathy, infertility and sports fatigue (Sun and Zhou, 2010).

In this report, the effects and features of *Cynomorium* flavonoids on mouse model of perimenopausal depression were investigated.

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Materials and Methods

Animal

Kunming female mice (weight range 27-30 g) were provided by the Laboratory Animal Facility in Wuhan, No. 00009520 (mouse).

Drugs and reagents

Cynomorium flavonoids were produced by the Pharmaceutical Analysis Laboratory of Henan University of Chinese Medicine (content greater than 70%). The

followings were used: Gengnian'an capsule (Changchun Ying Ping Pharmaceutical Co., Ltd. No. 2011020104); soybean isoflavones soft capsule (Health Hlareer Ind. Group. Inc., No. 00683209); chloral hydrate (Tianjin Kemiou Chemical Preparation Development Center, No. 20111018); Cefazolin sodium for injection (Zhuhai Federal Pharmaceutical Co., Ltd. Zhongshan Branch, No. 90801302); Follicle stimulating hormone (FSH) radiation immunity analysis reagent case, luteinizing hormone (LH) radiation immunity analysis reagent case (Beijing Ke Meidongya Biological Technology Co., Ltd, No. 1211012); Serum estradiol radiation immunity analysis reagent case, No. 20120625; Dopamine (DA) quantitative detection reagent case (ELISA), 5 hydroxytryptamine (5-HT) quantitative detection reagent case (ELISA). It was produced by R and D, No. 1212010.

Instrument

The following instruments were used: Electronic analytical balance (AR1140/C; Ohaus, Shanghai Company); High speed bench centrifuge (TGL-168, Shanghai Anting Scientific Instrument Factory); Spontaneous activity of mice tester (ZZ-6, Chengdu Tem Science and Technology Co., Ltd0; Avoid dark instrument in mice (BA-200, Chengdu Tem Science and Technology Co., Ltd); Adjustable moving pipette (Thermo Scientific); 680 microplate reader (BIO-RAD).

Method

For the experiment, 80 female mice (27-30 g) were taken, and 10 mice were randomly selected as control group, while other mice were made model. Both ovaries were removed, and fallopian tubes (including fat) were ligated to make the mice of perimenopausal depression. They are intramuscular injection cefazolin sodium once a day, for 3 days (20 U/mL, 0.1 mL each one) to preventing infection. Vaginal smears were checked one by one for 5 days after surgery to determine if ovarian removal. The mice presenting the emotional reaction were rejected (Zeng et al., 2014). 60 ovariectomized female mice were randomly divided into 6 groups evenly: Large, medium and small dose of *Cynomorium* flavonoids groups (400, 200, 100, 20, 10 and 5 mg/mL), Gengnian'an capsule group (675 mg/kg, 33.75 mg/mL), soy isoflavones soft capsule group (250 mg/kg, 12.5 mg/mL), 5 days after surgery. The volume is 0.2 mL/10 g. Model group and control group were given the same volume of water, once a day continuously 30 days. Each cage had 1 mouse. There are 9 kinds of stressors in a random application on the mice every day continuously 18 days and each stimulation could not be consecutive: a) 5 min (160 Hz) horizontal oscillation; b) swimming in ice water (4°C, 5 min); c) heat stress (45°C, 5 min); d) shake (1 time/sec, 5 min); e) clip tail (1 min); f) fasting (24 hours); g) all night lighting (24 hours); h) prohibition of drinking water (24 hours); and i) behavior limitations (6 hours). The frequency of spontaneous activity of the mice in 5 min was documented on day 26. The avoiding latency and the number of being shocked in the dark room were determined on day 27. The sum of the fixed time of forced swimming test within 2-6 min was determined on day 28. The sum of the fixed time of tail suspension test in 2-6 min was determined on day 29. The last 2 hours after lavage (fasting, 15 hours) picked the eyeball blood, and separated the serum. The content of serum E2, LH, FSH was determined by radioimmunoassay. The mouse brain was separated and cut in two halves and prepared for brain homogenate, and determined the content of 5-HT, DA in the brain homogenate by ELISA.

Statistical treatment

Data analysis were done by SPSS 13.0 for statistical treatment 1 (Wei et al., 2012). Data were presented as mean ± SD, using one-way ANOVA as compared between groups.

Results

Comparing with control group, the activity times, stand number and the avoiding latency of model group mice had reduced significantly ($p<0.01$) (Table I). The number of being shocked has been increased ($p<0.01$). It shows that it's successful to build the mice model with perimenopausal depression. The result reflects that the model mice had less curious about the new environment, poor memories and despair to the adverse environment. Compared to model group, the large, middle, and small dose of *Cynomorium* flavonoids group could obviously increase the number of activities ($p<0.05$). The soy isoflavones soft capsule group could enhance the number of their activities ($p<0.01$). The large dose of *Cynomorium* flavonoids group could obviously extend the avoiding latency of dark avoidance method experiment ($p<0.05$). The large, middle dose of *Cynomorium* flavonoids group and the Gengnian'an capsule group could significant reduce the number of being shocked in the dark avoidance method experiment ($p<0.01$). The small dose of *Cynomorium* flavonoids group and soy isoflavones soft capsule group could obviously reduce the number of being shocked ($p<0.05$).

Table II shows the comparison to control group, the forced swimming fixed time and tail suspension fixed time of model mice were significantly prolonged ($p<0.01$). It shows that it's successful to build the mice model with perimenopausal depression. Comparing with the model group, large dose of *Cynomorium* flavonoids group could significantly reduce the forced swimming time of model mice ($p<0.01$), the middle, small dose of *Cynomorium* flavonoids group and

Table I

Effect of *Cynomorium* flavonoids on frequency of spontaneous activity, avoiding latency and the number of being shocked in the dark room of the mice of perimenopausal depression

Group	Dosage (mg/kg)	Activity (n)	Stand (n)	Avoiding latency (s)	The number of being shocked (n)
None	-	133.7 ± 27.6[b]	40.8 ± 6.3	111.7 ± 98.4[b]	3.5 ± 2.3[b]
Model (perimenopausal depression)	-	95.5 ± 15.2	35.4 ± 8.0	22.3 ± 21.9	6.0 ± 1.7
Soy isoflavones soft capsule	250	128.2 ± 19.2[b]	41.5 ± 11.7	49.0 ± 48.1	3.7 ± 2.1[a]
Gengnian'an capsule	675	111.9 ± 11.6	43.7 ± 5.3	78.1 ± 64.4	2.5 ± 1.8[b]
Cynomorium flavonoids (large dose)	400	117.9 ± 12.9[a]	42.1 ± 8.4	105.9 ± 97.7[a]	3.0 ± 1.7[b]
Cynomorium flavonoids (middle dose)	200	115.5 ± 23.6[a]	36.1 ± 13.1	71.7 ± 75.8	3.4 ± 2.5[b]
Cynomorium flavonoids (low dose)	100	117.3 ± 26.6[a]	42.3 ± 10.8	64.8 ± 78.4	3.9 ± 2.4[a]

Data are mean ± SD; Comparing with untreated diabetes group [a]p<0.05，[b]p<0.01

Table II

Effect of cynomorium flavonoids on forced swimming time and tail suspension fixed time of the mice model perimenopausal depression

Group	N	Dosage (mg/kg)	Forced swimming time (s)	Tail suspension fixed time (s)
None	10	-	60.0 ± 33.0[b]	73.2 ± 28.7[b]
Model (perimenopausal depression)	10	-	123.6 ± 27.7	135.8 ± 33.5
Soy isoflavones soft capsule	10	250	103.0 ± 30.0	108.7 ± 28.3[a]
Gengnian'an capsule	10	675	89.8 ± 27.8[a]	101.7 ± 25.0[b]
Cynomorium flavonoids (large dose)	10	400	87.4 ± 25.9[b]	108.0 ± 23.0[a]
Cynomorium flavonoids (middle dose)	10	200	90.3 ± 27.9[a]	111.8 ± 20.6
Cynomorium flavonoids (low dose)	10	100	89.4 ± 35.0[a]	107.8 ± 30.5[a]

Data are mean ± SD; Compare with model group: [a]p<0.05，[b]p<0.01

Gengnian'an capsule group could obviously reduce the forced swimming time of model mice (p<0.05). The large, small dose of *Cynomorium* flavonoids and soy isoflavones soft capsule group could reduce the tail suspension fixed time obviously (p<0.05), and Gengnian'an capsule group could significantly reduce the tail suspension fixed time (p<0.01).

Discern from Table III, comparing with control group, the level of 5-HT and DA in the brain homogenate of model mouse were significantly lower (p<0.01). It shows that it's successful to build the mice model with perimenopausal depression. Comparing with model group, large, middle dose of *Cynomorium* flavonoids group could elevate the level of 5-HT in the brain homogenate obviously (p<0.05). The Gengnian'an capsule group and soy isoflavones soft capsule group could rise the level of 5-HT in the brain homogenate significantly (p<0.01). The large, middle dose of *Cynomorium* flavonoids group, Gengnian'an capsule group and soy isoflavones soft capsule group could rise the level of DA in the brain homogenate significantly (p<0.01).

Discern from Table IV, compared to control group, the level of E_2 in the serum of model group decreased significantly (p<0.01). The level of LH and FSH were increasing significantly (p<0.01). It shows that it's successful to build mice model with perimenopausal depression, and the model mice were sex hormone disorder. Compared to the model group, the large dose of *Cynomorium* flavonoids group and soy isoflavones soft capsule group could significantly increase the level of E_2 (p<0.01), and the Gengnian'an capsule group could obviously increasing E_2 (p<0.05). The large, middle dose of *Cynomorium* flavonoids group, soy isoflavones soft capsule group and Gengnian'an capsule group could significantly reduce the level of serum LH and FSH (p<0.01). Small dose of *Cynomorium* flavonoids group could obviously reduced the level of serum LH and FSH (p<0.05).

Table III

Effect of cynomorium flavonoids on the 5-HT and DA level

Group	n	Dose (mg/kg)	5-HT (μg/g)	DA (μg/g)
None	10	-	1. 5 ± 0.2[b]	0. 7 ± 0.1[b]
Model (Perimenopausal depression)	10	-	1.1 ± 0.1	0. 4 ± 0.1
Soy isoflavones soft capsule	10	250	1.4 ± 0.1[b]	0.5 ± 0.1[b]
Gengnian'an capsule	10	675	1.3 ± 0.1[b]	0.5 ± 0.0[b]
Cynomorium flavonoids (large dose)	10	400	1.2 ± 0.1[a]	0.5 ± 0.1[b]
Cynomorium flavonoids (middle dose)	10	200	1.2 ± 0.1[a]	0.5 ± 0.1[b]
Cynomorium flavonoids (low dose)	10	100	1.2 ± 0.1	0.4 ± 0.1

Data are mean ± SD; Compared with model group: [a]p<0.05，[b]p<0.01

Table IV

Effect of cynomorium flavonoids on the content of E2, FSH, LH in the serum

Group	n	Dose (mg/kg)	E_2 (pg/mL)	LH (IU/L)	FSH (IU/L)
None	10	-	19.403 ± 2.490[b]	3.976 ± 0.680[b]	2.973 ± 0.370[b]
Model (perimenopausal depression)	10	-	10.882 ± 1.618	6.966 ± 1.109	4.45 ± 0.795
Soy isoflavones soft capsule	10	250	15.557 ± 2.942[b]	4.47 ± 0.704[b]	3.481 ± 0.759[b]
Gengnian'an capsule	10	675	12.872 ± 1.654a	5.31 ± 1.026[b]	3.556 ± 0.729[b]
Cynomorium flavonoids (large dose)	10	400	14.730 ± 2.289[b]	4.698 ± 0.673[b]	3.181 ± 0.568[b]
Cynomorium flavonoids (middle dose)	10	200	12.315 ± 0.903	5.706 ± 1.041[b]	3.44 ± 0.524[b]
Cynomorium flavonoids (low dose)	10	100	12.247 ± 1.043	6.107 ± 0.917a	3.694 ± 0.665a

Data are mean ± SD; Compared with model group: [a]p<0.05，[b]p<0.01

Discussion

The experiments results showed that *Cynomorium* flavonoids can significantly elevate the level of DA in brain homogenates and significantly lower the level of LH (Tan, 2014), indicating the improvement of hypothalamic function in perimenopausal depressed mice. Additionally, *Cynomorium* flavonoids can significantly lower FSH and enhance E_2 in serum, indicating the improvement of the ovarian function of the mice. Further, *Cynomorium* flavonoids can significantly increase the activity of the mice, indicating improvement of depression. the experiments demonstrated that *Cynomorium* flavonoids had a good therapeutic effect on mice with perimenopausal depression, and provided an experimental basis for the treatment of perimenopausal depression with *Cynomorium*.

Among the numerous hypotheses about perimenopausal depression, western medicine has focused its research on *in vivo* monoamine neurotransmitters and endocrine. Studies have generally agreed that reduced nerve function of 5-HT is one of the pathological bases of depression. Thus, based on the behavior tests, the expression levels of FSH, E_2, 5-HT and DA in mice, our study provided a theory basis for *Cynomorium* flavonoid treatment of mice with perimenopausal depression.

Recently, studies have shown that a lowered serum E_2 level *in vivo* was an independent risk factor for the initiation of perimenopausal depression in women (Wang and Peng, 2013). E_2 is a natural estrogen secreted by mature ovarian follicles. FSH is the most commonly used indicator to clinically measure ovarian reserve. When ovarian function begins to decline, FSH unstably increases. Since an increased FSH level can accelerate the growth of residual follicles, the secretion of estrogen is increased, and feedback inhibits the secretion of FSH (Yu et al., 2012). The decrease of serum E_2 level *in vivo* will negatively feedback elevate FSH, and further lead to significantly decreased expression of 5-HT and DA. Since abnormal secretion of estrogen is the most important factor for perimenopause and stress is a significant trigger for depression, the mouse model of perimenopausal depression based on continuous stress on ovariectomized female mice was set up (Freeman et al., 2013). Subsequently, the activity of the mice was studied, and changes to monoamine neurotransmitter levels in brain and hormone levels in the serum.

Conclusion

Cynomorium flavonoids had a good therapeutic effect on mice with perimenopausal depression.

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