Effect of cynomorium flavonoids on morphology of perimenopausal depression mice model

Tan Wang, Mingsan Miao*, Yan Li, Min Li, Ying Zhang, Shuo Tian

Henan University of Chinese Medicine, Zhengzhou 450008, China

Available online 24 April 2016

KEYWORDS
Cynomorium flavonoids; Thymus; Spleen; Uterus; Tissue morphology

Abstract  Objective: In this report, the effects of cynomorium flavonoids on mouse model of perimenopausal depression were investigated. Method: 60 ovariectomized female mice were randomly divided into 6 groups evenly: high, medium and low doses of cynomorium flavonoids groups (400 mg kg⁻¹, 200 mg kg⁻¹, 100 mg kg⁻¹), Gengnian’an capsule group (675 mg kg⁻¹), soy isoflavones soft capsule group (250 mg kg⁻¹), and model group. Give the corresponding drug five days after surgery once a day, consecutive thirty days. The model group and control group were given the water of same volume. The model related groups were applied with different stress for consecutive eighteen days. Kill the mice and remove the thymus, spleen, uterus and one hand of brain when it is 2 h after the last administration in mice of each group. Observe the histological changes of each group under light microscope. Results: By observing the pathological section, compared with model group, the pathological changes of the uterus and hypothalamus of mice were significantly improved. The thymic cortex markedly thickened, volume of splenic nodule also significantly increased, and the number of lymphocytes significantly increased (p < 0.01). Simulation results show that the high dose of cynomorium flavonoids group has the best effective. Conclusion: Cynomorium flavonoids on mouse uterus, hypothalamus, thymus and spleen lesions have a significant role in the improvement. Cynomorium flavonoids have a good therapeutic effect on mice with perimenopausal depression.

1. Introduction

Perimenopausal depression is a mood disorder that occurs during the perimenopause period. They become prone to mood depression, unresponsiveness, slow thinking, irritability, and pessimism (Ma, 2013). Nowadays, the incidence of perimenopausal depression is on the rise. Over the years, to investigate the effect of cynomorium flavonoids on a mouse model of perimenopausal depression is more and more important. In this report, the effects of cynomorium flavonoids on mouse model of perimenopausal depression were investigated (Tan, 2014).

* Corresponding author at: No. 1, Jinshui Road, Henan University of Chinese Medicine, Zhengzhou 450008, China. E-mail address: miaomingsan@126.com (MS. Miao).

Peer review under responsibility of King Saud University.

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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 this report, the effects of cynomorium flavonoids on mouse model of perimenopausal depression were investigated.

2. Materials

2.1. Animal

KunMing mouse, female, 27–30 g. Provided by Wuhan Experimental Animal Center, Certificate of Quality No.: 00009520.

2.2. Drugs and reagents

Cynomorium flavonoids, extracted by Henan University of TCM medicine Laboratory, No. 20110303; Gengnian’an capsule, Changehun YingPing Pharmaceutical Co., Ltd., No. 2011020104; Soybean isoflavones Soft capsule, produced by GARLANDFOND (US) HEALT HLAREER IND. GROUP,INC, No. 00683209; Chlortal hydrate, produced by Tianjin Kemioi chemical preparation development center, No. 20111018; and Cefazolin sodium for injection, produced by Zuhuai Federal Pharmaceutical Co., Ltd., Zhongshan branch, No. 90801302.

2.3. Instrument

Electronic balance, Produced by Shanghai MinQiao Medical Instrument Co., Ltd. No. JY601; Electronic analytical balance, Produced by Ohaus (Shanghai) Ltd. No. AR1140/C.

Electric thermostatic water bath, Produced by Shanghai a constant Scientific Instrument Co., Ltd. No. HWS12; Intelligent discharge instrument, Produced by Shanghai Institute of Nuclear Research Institute of the Fourth Ring instrument, No. Sn-895B.

3. Method

Take 80 mice of Kunming, female mice, 27–30 g, 10 female mice were randomly divided into control group, and others made perimenopausal depression: Removing both of the ovaries, and ligating fallopian tubes (including fat) to make the mice of perimenopausal depression. They are being intramuscular injection cefazolin sodium once a day, for 3 d (20 u/mL, 0.1 mL each one) to prevent infection. Vaginal smears examination one by one 5 d after surgery to determine ovarian removal. Abandon the mice whose smears show emotional responses (Zhang et al., 2013). 60 ovariectomized female mice were randomly divided into 6 groups evenly: large, medium and small dose of cynomorium flavonoids groups (400 mg kg⁻¹, 200 mg kg⁻¹, 100 mg kg⁻¹, 20 mg kg⁻¹, 10 mg kg⁻¹, 5 mg kg⁻¹), Gengnian’an capsule group (675 mg kg⁻¹, 33.75 mg mL⁻¹), Soy isoflavones Soft capsule group (250 mg kg⁻¹, 12.5 mg mL⁻¹), and 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 d. Each cage is with 1 mouse, and there are 9 kinds of stressors in a random application on the mice everyday continuously for 18d and each stimulation cannot be consecutive: (1) 5 min (160 Hz)horizontal oscillation; (2) swimming in ice water (4°C, 5 min); (3) heat stress (45°C, 5 min); (4) shake (1 time/s, 5 min); (5) clip tail (1 min); (6) fasting (24 h); (7) all night lighting (24 h); (8) prohibition of drinking water (24 h); and (9) Behavior limitations (6 h). Mice were dissected at 29 days of administration. At the same time, Remove and weigh thymus, spleen, uterus, and calculate the organ index (organ index = organ wet weight mg/mice weight). Take the brain and isolated hypothalamus. Observe the changes of the structure and morphology of the groups under the light microscope. Data analysis used SPSS 13.0 for statistical treatment l

4. Result

4.1. Effect of the index of viscera in the model mice

From Table 1, Compared with the control group, thymus and spleen index of model group significantly reduce (p < 0.05), and Uterine index decreases significantly (p < 0.01). Prove that the thymus, spleen and uterus tissue of the mouse model of the peri menopausal syndrome were observed. Compared with the model group, soybean isoflavone soft capsule group and gengnianan capsule group can significantly improve the thymus, spleen and uterus index (p < 0.05); middle dose Cynomorium flavonoids group can significantly increase the thymus, spleen and uterus index (p < 0.01); middle dose Cynomorium flavonoids can significantly improve the animal model of thymus gland, uterus index (p < 0.05), and significantly increase the spleen index (p < 0.01); small dose of Cynomorium flavonoids can obviously increase the thymus index and spleen index (p < 0.05), and significantly increase the uterus index (p < 0.01).

4.2. Effects of the morphology of the uterus in the model mice

After Ridit test, it was found that the model group was significantly more than that in the control group (p < 0.01), and the model group was successful in the model of peri-menopausal mice. Ratio, in which the each of the drugs can significantly improve the mouse uterus pathological changes (p < 0.01), including large and middle doses of Cynomorium flavonoids group has the best effect. The pathological sections are seen in Appendix A (see Table 2).

4.3. Effects of the morphology of the hypothalamus in the model mice of the peri menopausal syndrome

Through Ridit test, Compared with the control group, the model mice hypothalamus changes prominently (p < 0.01). Compared with the model group, the drug groups could significantly improve the mice pathological lesions (p < 0.01), whose large and middle doses of cynamorium flavonoids
group have the best curative effect. The pathological sections are shown in Appendix B (See Table 3).

### 4.4. Effects of the morphology of the thymus in the model mice

From Table 4, compared with the control group, there was no significant difference. With the model group, drug group could markedly thick thymic cortex ($p < 0.01$); soy isoflavone soft capsule group can significantly increase the number of lymphocytes in the cortex ($p < 0.05$), high and low doses of Cynomorium flavonoids can significantly improve the number of lymphocytes in the cortex ($p < 0.01$). The pathological sections are shown in Appendix C.

### 4.5. Effect of the shape of the spleen in the model mice

From Table 5, Compared with the control group, the volume of spleen in the model group was significantly decreased ($p < 0.01$), and the number of lymphocytes decreased significantly ($p < 0.01$), which indicates that the spleen atrophy after
the mice were perimenopausal decreases. Compared with the model group, the dosage of Cynomorium flavonoids, Gengnian’an capsule group, and soy isoflavone soft capsule group can significantly increase the volume of splenic nodule (p < 0.01), and significantly increase the number of lymphocytes (p < 0.01), and large dose of Cynomorium flavonoids group has the best effect. The pathological sections are shown in Appendix D.
5. Discussion

The effects of receptor metabolism, endocrine, autonomic nerve and other factors, depend mainly on the physical and psychological aspects of the body and the psychological aspects of the abnormal, the mood swings, and the high degree of depression in patients (Lin et al., 2014). Because estrogen secretion is leading to abnormal perimenopausal the main influence factors and stressful life practice are significant factors of promoting depression (Li et al., 2013), so this experiment takes the removal of both ovaries, then the mice were continued stress which was made in perimenopausal depression model (Miao et al., 2016). Due to castrated mice after a drop in estrogen levels, atrophy of uterus and immunity decreased through the observation and study of Cynomorium songaricum flavone on mouse uterus, thymus gland, hypothalamus and spleen influence to explore the therapeutic effect of C. songaricum flavone of Menopause Depression.

Ancient medical that peri menopausal depression. This is the kidney, disease lies in the heart, and the uterus, involving eight and liver and spleen qi, and central link is heart kidney physiological reproductive axis misalignments (Zhao et al., 2013). Women enter the peri menopausal period, due to ovarian function decline, the uterus index decreased, estrogen levels decreased, due to estrogen has a role in assimilation, can promote protein synthesis to make uterine weight, while the mouse uterine vascular expansion, water intake increased, the uterine weight and body weight ratio increased, so the
Chinese traditional medicine by observing the uterus index to investigate whether the drug has estrogen activity. And the level of estrogen in blood decreased, the pituitary feedback mechanism disappeared, so that the normal hypothalamic pituitary ovarian axis (HPOA) of the imbalance between (Mo and Ning, 2014), resulting in the body structure and physiological function of the body, which is the most prominent of the immune system function is significantly decreased (Zhang et al., 2013), thymus is immune system of the central organs, and spleen is also an important way to improve immune function, so the spleen and stomach weakness can cause menstrual and reproductive changes, but also one of the important factors leading to the peri menopausal syndrome (Han and Lu, 2012b). Cynomorium water extract of Part III can enhance CTX immunity inhibition of mouse nonspecific and specific immunity function (Yu et al., 2012), and has certain protective effect on immune imbalance body; kidney yang drugs can balance hormone levels on ovarian axis and gonadal axis can play a regulatory role (Su et al., 2014). In this experiment, through the observation of peri Menopause Depression Model, Mice’ tissue morphology changes C. songaricum flavone on peri menopausal depression treatment effect.

The experimental results show that compared with the model group, dose of C. songaricum flavone group mice uterus coefficient was significantly higher than that of Cynomorium flavonoids that have estrogenic activity; the thymic cortex markedly thickened, volume of splenic nodule also significantly increased, and the number of lymphocytes increased significantly, said Ming in C. songaricum flavone can increase the immunity of the body. In addition to hypothalamic pathology tissue lesions had significant improvement effect, and the effect of total flavonoids of high dose group is the best. This study suggests that Cynomorium flavonoids can effectively improve the mouse uterus, thymus, spleen and hypothalamic lesions, perimenopausal mice have significant therapeutic effect and TCM clinical treatment of depression and antidepressant drug research provides pharmacological basis, new methods and new ideas.

Acknowledgements

The research work was supported by the National Natural Science Foundation of China (Grant No. 81274154) and the Collaborative Innovation Center for the creation of new Traditional Chinese medicine and genuine regional drug deep processing in Henan University of Chinese Medicine (2012) 188-2.

Appendix A
See Fig. A1.

Appendix B
See Fig. B1.

Appendix C
See Fig. C1.

Appendix D
See Fig. D1.

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