Phenylethanoid Glycosides of Cistanche on menopausal syndrome model in mice

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Abstract

Cistanche is the traditional and precious Chinese herbal, with two thousand years of use history in China. It has the effect on tonifying kidney, strong supplement to the liver and kidney, replenishing essence and blood, known as the "desert ginseng". Here, we explored the mechanism of Phenylethanoid Glycosides of Cistanche (PGC) to the model mice of menopausal syndrome, as well as the therapeutic effect and characteristics of PGC to the menopausal syndrome. In this study, KM mice were reproduced by the complete resection of the ovaries on both sides of the back to establish the model mice of menopausal syndrome (MPS), and received distilled water or drugs, respectively. Model mice received distilled water. Mice received 200 mg/(kg day) high doses of Phenylethanoid Glycosides of Cistanche (HPGC), and 100 mg/(kg day) medium doses of Phenylethanoid Glycosides of Cistanche (MPGC), and 50 mg/(kg day) low doses of Phenylethanoid Glycosides of Cistanche (LPGC). After 21 days, it could determine the number of independent activities and the number of standing, the latent period of first entering the dark room, and the electric number. It also calculated the viscera index of uterus, thymus, spleen, measured the levels of estradiol (E2), testosterone (T), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in the serum. Furthermore, it observed the pathological changes of uterus, thymus, spleen and pituitary of mice. The results showed that behavioral indicators: Compared with the model group (MG), HPGC, MPGC, LPGC could increase the independent activities \((P < 0.01)\); HPGC, MPGC could increase the number of standing, the latent period of first entering the dark room, and reduce the electric number \((P < 0.01)\); LPGC could increase the number of standing \((P < 0.05)\); Viscera index: Compared with MG, HPGC, MPGC could increase the viscera index of uterus, thymus, spleen \((P < 0.01)\); LPGC could increase the viscera index of uterus \((P < 0.05)\); Serum index: Compared with MG, all groups could decrease the levels of LH in the serum \((P < 0.01)\); HPGC, MPGC could improve the levels of E2, T, and decrease the levels of FSH in the serum \((P < 0.05)\). Meanwhile, it had the trend to improve the levels of T in the serum. Pathological changes: Compared with MG, HPGC could significant improve the pathological changes of uterus, thymus, spleen and pituitary of mice; other groups also has a certain effect. The results indicated that PGC could improve the sex hormone disorder of MPS, and restore the function of uterus, thymus and spleen, with better therapeutic effect on MPS.

1. Introduction

Cistanche, the dried fleshy stems with scaly leaves of Cistanche deserticola Y. C. Ma and C. tubulosa (Schrenk) Wight (Chen et al., 2013), its a famous drug and widely used for thousands of years. It is originated from the “Shen Nong’s Herbal Classic” (Liu et al., 2013), salty in flavor and warm in naturet. It has the effect of tonifying kidney, strong supplement to the liver and kidney, replenishing essence and blood, moistening intestines to relieve constipation, and other efficacy. It is mainly used to treat the deficiency of kidney-yang (leading
to impotence), prospermia, cold sperm, and sterility due to cold in the uterus, constipation, enuresis and frequency of micturition, and other disease. Modern research shows that it can enhance human immunity, memory and learning ability with anti-aging, anti-inflammatory, anti-fatigue and other effects (Li et al., 2010; Rashid et al., 2017).

MPS is a syndrome of the autonomic nervous system dysfunction disorder caused by estrogen secretion disorder, and accompany by neuropsychiatric symptoms. It has different degrees of hot flashes, irritability, dizziness, tinnitus, palpitations, insomnia, etc., with visible osteoporosis, memory loss, cognitive impairment, cardiovascular and cerebrovascular diseases in the late period. The main reason is the gradual decline and disappearance of ovarian function (la, 2016). Currently the main use of hormone replacement therapy (HRT) for the treatment of MPS, direct supplement estrogen, long-term HRT can cause vaginal bleeding, breast tenderness, endometrial cancer, breast cancer and other adverse effects. In addition, the effect is not yet with satisfaction—the dose of estrogen on the immune system still has inhibited effect (Ma et al., 2016; Nawaz et al., 2017). Especially the recent discovery shows that it can increase the risk of cardiovascular and cerebrovascular disease and other serious side effects significantly limiting the clinical application. In the urgent market demand, more and more attention from the traditional Chinese (TCM) medicine to search for the treatment of MPS drugs (Halim and Phang, 2017; Mustafa et al., 2017). TCM treats the MPS with a long history (Zhang and Mao, 2011), by regulating the hypothalamus-pituitary-ovarian axis (HPOA), the ovarian function recovery, and the ovarian aging delay. It has found that the majority of drugs of kidney yang have a better therapeutic effect on the MPS (Wei and Mao, 2013).

Cistanche is the highest frequency of drugs replenishing kidney yang for the past dynasties (Tu et al., 2011). The main effective components of Cistanche are phenylethanoid glycosides, with androgen effect. It is the embodiment of modern medicine kidney yang (Zhao and Pan, 2013), with the highest active ingredients in the Cistanche (Yan et al., 2012). It is mainly through two ways to function (Wumaierjiang and Yao, 2016). Firstly, it has the function to strengthen the hypothalamus-pituitary-ovarian axis (HP0A), the ovarian function recovery, and the ovarian aging delay. It has been found that the majority of drugs of kidney yang have a better therapeutic effect on the MPS (Wei and Mao, 2013).

2. Material and methods

2.1. Material and reagents

Cistanche (Batch No.20130501) was purchased from Anhui, Dechang Pharmaceutical Pieces Co., Ltd. The samples were identified by Professor Chen Suiqing (Henan University of Chinese Medicine, identification of Chinese drug discipline) as the dried fleshy stems with scaly leaves of Cistanche deserticola Y. C. Ma, as well as the dried fleshy stems with scaly leaves of C. tubulosa (Schrenk) Wight, respectively. GC (Batch No.120303) was supplied by Shanxi star pharmaceutical Co., Ltd.; Echinacoside reference substance (Batch No.111670-200503) was supplied by the National Institute for the Control of Pharmaceutical and Biological Products; AB-8 macroporous absorption resin (Batch No.20130618) was supplied by the Tianjin Guangfu of Institute of Superfine Chemical Industry; Sodium carboxymethyl cellulose (Batch No.20120418, Tianjin Hengxing Chemical Reagent Co., Ltd.), Benzylpenicillin Sodium for Injection (Batch No.c1206807, North China Pharmaceutical Co., Ltd., the specification: 4 million units), 0.9% Sodium Chloride Injection (Batch No.1301265303, Chen Xin Pharmaceutical Co., Ltd; specifications: 250 ml), Chloral hydrate (Batch No.20120827, Tianjin Institute of Fine Chemical Industry), E2 ELISA assay kit (Batch No.20131001A, R&D Systems China), T ELISA assay kit (Batch No.20131001A, R&D Systems China), LH ELISA assay kit (Batch No. 20131001A, R&D Systems China), FSH ELISA assay kit (Batch No. 20131001A, R&D Systems China).

2.2. Sample preparation

2.2.1. Preparation methods

The procedure for sample preparation was as follows: By the literature methods (Gu et al., 2011), we were under the guidance of Feng Suxiang (Pharmaceutical analysis course discipline, Henan University of Chinese Medicine). The Cistanche crushed to the meal, by the methods of reflux extraction to extract 2 times with the amount of ethyl alcohol (the content of the ethyl alcohol was 70%). The time of reflux extraction was 1.5 h for once, and then combined the alcohol extraction liquid 2 times. The extraction liquid was decompressed and enriched without the alcohol flavor, and the distilled water was used to disperse (the concentration is 0.5 g/ml, as the sample solution. The sample solution was install into the AB-8 resin with flow (the ratio of the sample solution to resin was 1:10), after standing for 5 h until the sample solution was fully adsorbed. And then, first with 10 times of the column volume of distilled water to washed the AB-8 resin with the sample solution, it was abandoned to the water; Secondly, with 10 times of the column volume of the ethyl alcohol (the content of the ethyl alcohol is 10%), the impurities were removed; Thirdly, with 7 times of the column volume of the ethyl alcohol (the content of the ethyl alcohol was 60%) to elution, we collected the eluate and dried eluate, that is the powder of Phenylethanoid Glycosides of Cistanche.

2.2.2. Selection of determine wavelength

Select 0.5 mL control product solution, and add 5% sodium nitrite solution 1 ml to the control product. Then shake and quietly place for 6 min. After that, add 10% aluminum nitrate solution to the above mixture. Then, shake and quietly place for 6 min. Add 10% sodium hydroxide 10 mL to the above mixture, the volume of the mixture was fixed to 25 mL with the water. Shake and quietly place for 18 min, as control product solution. Choose 0.5 mL Phenylethanoid Glycosides of Cistanche solution, like the above method configuration, as the test solution. The blank sample was the blank solution except for the control product solution and the sample solution, like the above method configuration. In the UV spectrophotometer with the wavelength range of 200–800 nm, the full wavelength was used to scan the above 3 solutions. The control product solution and the sample solution had the maximum absorption peak at 507 nm, so the wavelength of 507 nm determined as the absorption wavelength.

After the Phenylethanoid Glycosides of Cistanche solution (1) and the Echinacoside reference substance (2) (superposition contrast), the color appeared (See the next Figure)
2.2.3. Method of the content detection

Precise weigh 1 mL waiting for the determination of solution, add 1 mL 5% sodium nitrite solution to the control product, shake and quietly place for 6 min. Then add 10% aluminum nitrate solution to the above mixture. Shake and quietly place for 6 min. Add 10 mL 10% sodium hydroxide to the above mixture, and the volume of the mixture was fixed to 25 mL with the water. Shake and quietly place for 18 min; the blank solution was prepared by the same method with the blank sample without sample solution. With the UV spectrophotometry to determine at the spectrophotometry of 507 nm.

2.2.4. Methodology examination

2.2.4.1. Preparation of the control solution and the sample solution. Preparation of the control solution: Precise weigh 3.06 mg echinacoside with drying to constant weight as the reference substance, and put it into the measuring flask of 25 mL. Then use 70% ethanol solution diluted to the scale, and shake the mixture, as the control solution. The content of the control solution was 0.1224 mg/ml.

Preparation of the sample solution: Precise weigh 5 mg Phenylethanoid Glycosides of Cistanche and put it into the measuring flask of 10 mL. Then use 70% ethanol solution diluted to the scale, and shake the mixture, as the sample solution.

2.2.4.2. Preparation of standard curve. Accurately draw the control solution of 0, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 mL, according to the "Selection of determining wavelength", and measured the absorbance. The regression equation was obtained by using the concentration to the absorbance: \( Y = 20.296X - 0.0015 \) \((r = 0.9994)\), and the linear range was 4.90–29.4 \(\mu g/ml\).

2.2.4.3. Precision test. Parallel determined the absorbance of the sample solution 6 times according to the item of “Method of the content detection”. After calculating the relative standard deviation of the absorbance, the absorbance was 0.299, 0.299, 0.298, 0.297, 0.297, and 0.297, and the average absorbance was 0.298, with RSD = 0.330% \((n = 6)\).

2.2.4.4. Stability test. Determine the absorbance of the sample solution after the solution was fully colored for 0, 15, 30, 50, and 60 min according to the item of “Method of the content detection”. After calculating the relative standard deviation of the absorbance, the absorbance was 0.300, 0.296, 0.295, and 0.295, and the average absorbance was 0.299, with RSD = 1.362% \((n = 5)\).

2.2.4.5. Repetitive testing. Prepare 6 sample solutions according to the item of “Preparation of the sample solution”, and calculate the absorbance and the content according to the item of “Method of the content detection”. The contents were 65.2%, 65.6%, 66.7%, 67.2%, and 66.5%, respectively, and the average content was 66.2%, with RSD = 1.142% \((n = 6)\).

2.2.4.6. Sample recovery rate test. Precision weigh 9 pieces of sample solution that the content was known, and each piece was 0.5 mL. Add 200, 250 and 300 \(\mu l\) standard solution into sample solution, respectively. Calculate the recovery rate and the relative standard deviation of Phenylethanoid Glycosides of Cistanche, and the recovery rates were 101.45%, 99.26%, 98.94%, 101.83%, 99.05%, 99.47%, 98.72%, 99.17%, and 100.64%. The average recovery rate was 99.84%, with RSD = 1.164% \((n = 9)\).

2.2.5. Determination the content of sample

Prepare 3 sample solutions according to the item of “Preparation of the sample solution”. Then, calculate the absorbance according to the item of “Method of the content detection” at the spectrophotometry of 507 nm, and calculate the content. Table 1 shows the results.

2.3. Experimental animals

KM female mice (20–25 g) were purchased from the Wuhan Institute of Biological Products. Animal Permit Number: 4200040000611, the lab certificate No. SYXK (Henan) 2010-001.

2.4. Experimental instrument

High speed tabletop centrifuge, Shanghai Anting scientific instrument factory, Model: TGL-168; Electronic analytical balance, Ohaus (Shanghai), model: AR1140/C; Mice autonomic activities test instrument, Chengdu Taimeng Science and Technology Limited Company, Model: ZZ-6; Mouse dark instrument, Chengdu Taimeng Technology Co., Ltd., Model: BA-200; Enzyme mark instrument, USA BIO-RAD, Model: 680; Electric microscope, Japanese OLYMPUS Company, Model: BX61.

2.5. Experimental animal model

100 KM mice (23–25 g) were weighted and intraperitoneal injection of 10% Hydrate of chlorine aldehyde (0.03 mL/10 g), abdominal fixation after anesthesia. 12 mice were randomly selected as the blank control group (BC), with the sham operation; others were conducted with the MPS model. Under the back of the mouse last rib, shear the mice hair at the intersection of the midaxillary line and distance from the lateral spine about 1 cm. After disinfecting, open the skin and the back muscle about 0.5–1 cm, we found a milky white fat mass in the visual field of incision, and the ovary was embedded in the fat. Gently pull the fat out of the incision with the tweezers, and separate the fat, it could be found that the thin thread irregular yellow red ovary. Firstly, the ovaries

**Table 1**

| Sample label | Absorbance | Content (%) | Average content (%) |
|--------------|------------|-------------|---------------------|
| 1            | 0.295      | 66.40       | 66.47               |
| 2            | 0.286      | 66.82       |                     |
| 3            | 0.294      | 66.18       |                     |
were under the fallopian tube (included fat) with a thin wire ligated, and then remove the ovaries. After the operation, the uterus was put back into the abdominal cavity. Stitch the muscles and skin, and the bilateral ovaries were removed with the same method. Careful feed after surgery, intramuscular injection of penicillin 200 thousand U/kg, in case infected, once a day for 3 days. After surgery for 5 days, the mice vaginal smears were tested, once a day for 5 days, in order to determine whether the ovaries were completely removed.

2.6. Experimental grouping

The mice had the estrous period by the vaginal smears were abandoned, and 72 mice with castration completely were randomly divided into five groups (n = 12 per group), namely model group (MG), GC, HPGC, MPGC, and LPGC.

2.7. Dispensing method

0.5% CMC: Sodium carboxymethyl cellulose 4 g, with distilled water as 800 ml. The drug dose of HPGC, MPGC, and LPGC were 200, 100, and 50 mg/kg, respectively (dosing volume: 0.1 ml/10 g). Weight the PGC of 2000 mg, 1000 mg, and 500 mg, dissolved with a small amount of 0.5% CMC. Then set the volume to 100 ml, and mix the suspension. The drug dose of GC was 675 mg/kg, with 9 pill of GC dissolved with a small amount of 0.5% CMC. Then set the volume to 40 ml, and mixed the suspension.

2.8. Drug administration

Animals in each group were administered corresponding to drugs on the 10th day after surgery. The GC group were administered GC suspension as 675 mg/kg, and the HPGC, MPGC, and LPGC groups were administered HPGC, MPGC, LPGC suspension as 200 mg/kg, 100 mg/kg, and 50 mg/kg. BC and the MG groups were administered with the same volume of 0.5% CMC solution, once a day for 21 days.

2.9. Determination the independent activities and the number of standing

Mice in each group administered for 18 days were put into the independent activity instrument, adapt to the environment for 1 min. Then determine the number of independent activities and standing within 5 min.

2.10. Determination the latent period of first entering the dark room and the electric number

Mice in each group were administered for 19 days, making the mice tail face the small door into the dark room into the determination box. After 24 h (administered for 20 days), determine the latent period of first entering the dark room and the electric number within 5 min.

2.11. Calculation of the viscera index

Mice after the last administration of 2 h (fasted for 12 h). After taking blood, the mice were sacrificed and dissected. Remove the thymus, spleen and uterus, and weigh the wet weight of the organ. Then calculate the viscera index (Viscera index = Organ wet weight mg/Body weight g).

2.12. Measurement of the content of E2, T, LH, FSH

Mice, after the last administration of 2 h (fasted for 12 h), were weighed and then taken blood. Separate the serum, and determine the content of E2, T, LH, and FSH in the serum as the specification.

2.13. Observation the morphological changes of uterus

According to the changes of the experiment mice of the endometrial, glands and the muscle layer, the semi-quantitative criteria was used to measure the uterus. The pathological organization morphology could be divided into four levels: the level of “−” meant that the endometrial epithelial cells, glands and muscle layer were normal; the level of “+” the small part of endometrial epithelial cells and glands were atrophy, and the muscle layer was normal; the level of “++” meant that part of endometrial epithelial cells and glands were atrophy, and the muscle layer had a little atrophy; the level of “+++” meant that the endometrial epithelial cells and glands were atrophy significantly, and the muscle layer was atrophy.

2.14. Measurement the thickness of thymic cortex

The mean thickness of the thymic cortex was measured by the micrometer to the mice.

2.15. Measurement the thickness of splenic nodule

The thickness of the splenic nodule of both sides was measured by the micrometer with the central artery as the center. Then calculate the average as the thickness of splenic nodule.

2.16. Calculation the number of the basophilic cell and the anterior pituitary cell

Calculate the number of the basophilic cell and the anterior pituitary cell in an area of 10,000 μm² square rectangular frame under high power field of vision.

2.17. Statistical analysis

All the data was analyzed by SPSS 17.0 statistical package for statistical analysis of medical data. The measurement data was used the mean add and subtract the standard deviation. (x ± s). The groups were compared by using single factor analysis (ANOVA) of variance, and the variance homogeneity was used the LSD analysis methods, the heterogeneity of variance was used the Games-Howell, and the date for the grade data was used the Ridit test.

3. Results

3.1. The effect of the independent activities and the number of standing in mice

Figs. 1 and 2 show the data of the independent activities and the number of standing. It demonstrates that MG mice is remarkably reduced compared with the BC (P < 0.01), reflecting the curiosity to the fresh environment is reduced. The mice in GC, HPGC, MPGC, and LPGC can remarkably improve the independent activities compared with the MG (P < 0.01); thoes in GC, HPGC and MPGC can improve the independent activities compared with the MG (P < 0.01); those in LPGC can improve the number of standing a little compared with the MG (P < 0.05).

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3.2. Effect of the latent period of first entering the dark room and the electric number

As shown in Figs. 3 and 4, the data of the latent period of first entering the dark room and the electric number demonstrated that MG mice are remarkably reduced compared with the BC ($P<0.01$), reflecting the decline of memory in the MPS mice. The mice in GC, HPGC, and MPGC can remarkably increase the latent period of first entering the dark room and reduce the electric number compared with the MG ($P<0.01$), as well as improve the memory.

3.3. Effect of the viscera index

As shown in Fig. 5, the data of the viscera index of uterus, thymus and spleen demonstrate that MG mice is remarkably reduced compared with the BC ($P<0.01$), reflecting the uterus and the immune organ of MPS mice are atrophied. The mice in GC, HPGC, and MPGC can remarkably improve the viscera index of uterus, thymus, and spleen compared with the MG ($P<0.01$). The mice in LPGC is a bit improved the viscera index of uterus compared with the MG ($P<0.05$).

3.4. Effect of the content of E2, T, LH and FSH

As shown in Fig. 6, the data of the level of E2, and T demonstrate that MG mice is remarkably reduced, and the data of the level of LH, and FSH demonstrate that MG mice is remarkably increased compared with the BC ($P<0.01$), it reflected the sex hormone levels in serum is disordered in the MPS mice. All groups mice are significantly decreased with the level of LH in serum compared with the BC ($P<0.01$). The mice in GC, HPGC, and MPGC can remarkably improve E2, T and decrease the level of LH, FSH in serum compared with the MG ($P<0.01$). The mice in LPGC can a bit improve E2 and decrease the level of FSH in serum compared with the MG ($P<0.05$).

3.5. Observation the pathological morphological changes of uterus

As shown in Figs. 7 and 8, with the micrometer scale, the morphological changes of uterus demonstrate that MG mice have the significant pathological changes in uterus compared with the BC ($P<0.01$). The mice in GC, HPGC can remarkably improve the pathological morphological changes compared with the MG ($P<0.01$). The mice in MPGC can improve the pathological morphological changes compared with the MG ($P<0.05$).

The changes of the pathological morphological are reported as the semi-quantitative criteria to measure the uterus, and the pathological organization morphology can be divided into four levels for each group and calculate the number of mice.
3.6. Measurement the thickness of thymic cortex

As shown in Figs. 9 and 10, with the micrometer scale, the data of the thickness of thymic cortex demonstrate that MG mice are significantly reduced compared with the BC ($P < 0.01$), indicating that the volume of thymus are decreased after establishing the MPS model mice. The mice in HPGC can remarkably increase the thickness of thymic cortex compared with the MG ($P < 0.01$).
mice in MPGC can increased the thickness of thymic cortex compared with the MG ($P < 0.05$).

3.7. Measurement the thickness of splenic nodule

As shown in Figs. 11 and 12, with the micrometer scale, the date of the thickness of splenic nodule demonstrate that MG mice are significant reduced compared with the BC ($P < 0.01$), indicating that the volume of spleen are decreased after establishing the MPS model mice. The mice in HPGC can remarkably increase the thickness of splenic nodule compared with the MG ($P < 0.01$). The mice in MPGC can increase the thickness of splenic nodule compared with the MG ($P < 0.05$).
3.8. Calculating the number of the basophilic cell and the anterior pituitary cell

As shown in Figs. 13 and 14, the date of the number of the basophilic cell and the anterior pituitary cell demonstrate that MG mice is significant reduced compared with the BC (P < 0.01), indicating that the number of the cell source of secretory gonadotropin is decreased after establishing the MPS model mice. All received drugs groups can remarkably increase the number of the basophilic cell compared with the MG (P < 0.01). The mice in GC, HPGC, and MPGC can remarkably increase the number of the anterior pituitary cell compared with the MG (P < 0.01).

4. Discussion

Records of the menopausal syndrome in ancient books are mainly scattered in the description of the “lily disease”, “zangzao” syndrome, “melancholia”, “abnormal menstruation” and others (Chen et al., 2015; Ma and Chen, 2015). For the woman over forty years, the Yin Qi was cut a half; Tiangui has dried up; the kidney Qi was gradually decline; the Yin Jing was deficiency; the balance of Yin and Yang were lose. Its main performance was as the menstrual disorders or menopause appear such as tidal fever and sweatiness, emotional irritability, restless, palpitation and insomnia, back pain, edema of the face and limbs, dizziness, tinnitus, and skin feeling like ant crawled disease. The strong decline of the kidney Qi is the intrinsic reason of the menstruation come or cut off. Kidney deficiency is the root reason of the menopausal syndrome (Tan et al., 2013), and the kidney is the origin of human life, called “congenital foundation”. The rise and fall of kidney essence dominate the growth and reproductive function of mature and decline (Wang and Huang, 2011). Studies have shown that the decline of ovarian function is the essence of kidney deficiency syndrome, and the imbalance of Yin and Yang of the kidney is closely related to the disorder of endocrine secretion and autonomic
nervous system dysfunction. The change of E2, FSH, and LH is an expression of kidney deficiency and kidney Yin and Yang imbalance (Shi et al., 2007). Tonifying kidney yang drugs are used to regulate the hormone level in the hypothalamus pituitary ovary axis, or play a direct role in regulating ovarian function. Modern Chinese medicine believes that the etiology and pathogenesis of the menopausal syndrome is the kidney deficiency (Razali and Said, 2017).

The study used the complete resection of the ovaries on both sides of the back reproduced the MPS mice model. 90% estrogen of female was secreted by the ovary, and the ovary was removed. It artificially caused the level of estrogen that was decreased by a sudden, and then to simulate the MPS. This model was the classic model, with high success rate, stable and reliable and so on. Through independent activities and the number of standing, the latent period of first entering the dark room and the electric number, we observed the improvement of the learning and memory function and the curiosity of the fresh environment by the medicine. The ovarian function was declined with the sex hormone secretion decreased. Furthermore, the gonadotropin was increased with MPS patients. The levels of E2, T in serum were lower than those person without the MPS symptoms, while the levels of LH, FSH were significantly higher than those person without the MPS symptoms. Therefore, through the measurement of E2, T, FSH and LH levels in serum, it could be used the visual indicators for identification the MPS, reflecting the effect of drugs to the MPS disease. The study showed that the ovarian function decline was the connotation of kidney deficiency, and the change of E2, T, FSH and LH was a kind of expression of kidney deficiency (Li et al., 2014; Cheng and Tian, 2012). Cistanche was through the effect of replenishing kidney to enhance immunity (Zhang et al., 2009; Shareef et al., 2017).

We used the kidney-replenishing medicine to treat MPS, which was an innovative. The results also showed that PGC could improved the disorder hormone level of MPS mice, and increase the levels of E2, T, LH and FSH in serum. Meanwhile, it could improve the pathological changes of uterus, thymus, spleen and pituitary of mice, conforming to the literature that the kidney deficiency is the fundamental in MPS.
5. Conclusion

Our study results indicated that the PGC had a good therapeutic effect for MPS—it could increase the independent activities and the number of standing, the latent period of first entering the dark room, the viscera of thymus, spleen and uterus, E2, T as well as reduce the electric number, LH, and FSH. The phenylethanoid glycosides were selected in Cistanche for high efficiency and convenience of the MPS research to improve the treatment of perimenopausal syndrome. Its quality standards is easy to control, conducive to the innovative research and industrialization development of drugs. It provides a new way of thinking for the treatment of MPS.

Conflict of interest

The authors have no conflict of interest to report.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (81274154); Henan Science and Technology Innovation Team (2012IRTSTHN011); Science and technology innovation team of Zhengzhou city (131PCXTD612); the key Medical Laboratory for the transformation of Chinese Medicine of Zhengzhou City (121PYFZK1820).
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