Saudi Pharmaceutical Journal 25 (2017) 537–547

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Phenylethanoid Glycosides of Cistanche on menopausal syndrome model in mice

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ARTICLE INFO

Article history:
Available online 10 May 2017

Keywords:
PGC
Menopausal syndrome
Mice

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, and 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 E_2, T and decrease the levels of LH, FSH in the serum ($P < 0.01$); LPGC could improve the levels of E_2 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 naturelt. 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

Abbreviations: MPS, menopausal syndrome; PGC, Polyethylene Glycosides of Cistanche; HPGC, high doses of Phenylethanoid Glycosides of Cistanche; MPGC, medium doses of Phenylethanoid Glycosides of Cistanche; LPGC, low doses of Phenylethanoid Glycosides of Cistanche; T, testosterone; E2, Estradiol; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2017.04.020
1319-0164/

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 accompanies 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 (Ia, 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 Miao, 2011), by regulating the hypothalamic-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 Miao, 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-pituitaryadrenal function, and promote the release of neurotransmitters and hormones in the body; secondly, it has the function of anti-fatigue, improving the body function. The effect of the replenishing kidney of Cistanche is different with the general kidney drugs such as Epimedium brevicornum Maxim, Morinda officinalis How. Cistanche does not hurt the yin, but replenishing kidney. It does not appear with fireness, dry mouth and other symptoms for the long-term use.

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 deserticolaY. 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), E_2 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 the 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)