Effects of dodder total flavone on polycystic ovary syndrome rat models induced by DHEA combined HCG

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Abstract
Aims: Explore the effects of dodder total flavone on polycystic ovary syndrome (PCOS) rat models induced by dehydroepiandrosterone (DHEA) combined human chorionic gonadotropin (HCG).

Methods: Except the blank group, the rest of the rats were injected with DHEA 6 mg/100 g on the back of the neck and 1.5 IU HCG each day, for 21 consecutive days. On the 16th day of modeling, vaginal smear was performed to select the model rats, which were randomly divided into model group, dacin-35 group, large, middle and small dose dodder total flavonoids groups, and given the medicine for three weeks. At the end of the last administration, take samples, so as to calculate the ovaries and uterus indexes, measure serum LH/FSH ratio, P, PRL and INS levels, fixed the uterus and pancreas in 10% formalin solution and stained with HE to observe the morphological changes of the organs. And measure the expression of TNF-α and IGF-1 proteins in ovaries by immunohistochemistry.

Results: Compared with the blank group, ovarian and uterine indexes, serum LH/FSH ratio, serum PRL and INS levels, ovary TNF-α and IGF-1 protein expression were significantly increased, and significant pathological changes were observed in the uterine and pancreatic tissues in model group (P < 0.01). While the serum P level decreased significantly (P < 0.01), Compared with the model group, the ovarian and uterine indexes, serum LH/FSH ratio, serum P, PRL and INS levels, ovary TNF-α protein expression were significantly decreased in large, middle and small dose dodder total flavonoids groups (P < 0.01); The expression of IGF-1 protein was decreased and uterus pathological changes were improved in different extents (P < 0.01 or P < 0.05), pancreas pathological changes were improved significantly (P < 0.01).

Conclusion: PCOS rat models was successfully replicated. Dodder total flavone can protect PCOS rats induced by DHEA combined HCG by different action pathways.

1. Introduction
Polycystic ovary syndrome (PCOS) is a reproductive endocrine metabolic disorder characterized by insulin resistance accompanied by persistent ovulation disorder and hyperandrogenemia (Elbohoty Ahmed et al., 2016). Western medicine believes that genetic factors, high luteinizing hormone, glycolipid metabolism disorder (Daghestani, 2018), hyperinsulinemia and insulin resistance (Giandalia), hyperandrogenemia (Li et al., 2018), low degree inflammatory reaction (Jamilian), obesity (Rezvan et al., 2018), abnormal ovarian function and hyperpuberty are the etiology and pathogenesis of PCOS (Leng and Wei, 2018; Zuo and Fu, 2018). PCOS belongs to the category of “late menstruation” and “amenorrhea” in traditional Chinese medicine (TCM). In TCM, ovum is called “reproductive sperm”, TCM believes that kidney storage sperm, leading to menstrual production. Deficiency of kidney leads to deficiency of qi, blood and liver qi stagnation. In turn, it leads to spleen apoplexy, which leads to different TCM syndromes of PCOS. Based on clinical data mining, kidney deficiency ranks first among the TCM syndromes of PCOS, and is often accompanied by blood deficiency, blood stasis, liver depression and other syndromes (Huang et al., 2018). TCM emphasizes the combination of dialectic and disease differentiation. For the root cause of PCOS disease is kidney deficiency, it is necessary to use Chinese medicine to supplement kidney for intervention. Dodder is a kind of traditional Chinese medicine commonly used in the treatment of PCOS. Studies have shown that its main chemical component flavonoids
can have a multidirectional effect on the endocrine function of hypothalamic-pituitary-gonads, such as improving the level of estrogen in the body, protecting spermatogenic cells of male rats, and improving glycolipid metabolism (Zhao et al., 2018; Ren et al., 2018; Huang et al., 2016). However, there is no relevant research report on the intervention of total flavonoids from dodder in PCOS. Exogenous increase of DHEA level can create a high androgen state in patients with polycystic ovary syndrome, resulting in decreased secretion of follicle-stimulating hormone in the body, follicular development blocked, follicular atresia and lack of ovulation (Liu et al., 2018). HCG can play the role of LH, and can simulate follicular development blocked, follicular atresia and lack of ovulation (PCOS) due to gonadal axis disorder, aggravating the PCOS secretion disorder. Therefore, making the PCOS rats induced by DHEA combined with HCG as the research object, to observe the effect of total flavonoids of dodder on PCOS rats.

2. Experimental materials

2.1. Drugs and reagents

Dodder total flavone, Nanjing zelang biotechnology co. LTD, the total flavonoid content of dodder was 51.25% by UV detection. Diane-35 (Ethynylestradiol and Cyproterone Acetate Tablets), Schering GmbH-Co. Produktions KG, batch no:333A; Dehydroepiandrosterone (DHEA), Aladdin, batch no: B1227012; Human chorionic gonadotropin (HCG), Hangzhou animal medicine factory, batch no: 20170623SR. Anti-IGF-1 polyclonal antibody, Wuhan bode bio-engineering co. LTD, batch no: 161008, Veterinary drug approval number: veterinary drug (2010) 110201281.

2.2. Experimental animals

SD rats, SPF level, male, 23 days old, 70 subjects. Jinan Pengyue experimental animal breeding co. LTD. License no: SCXK (Lu) 20150005; Animal certificate no: 37009200006387; Training certificate no: 1612; Animal facilities use certificate no: 00013517.

2.3. Experimental apparatus

BX61 electric microscope, OLYMPUS, Japan; AR1140/C electronic analytical balance, Ohaus (Shanghai) co. LTD; Type 680 enzyme marker, Bio-rad company, USA; KDC-160HR high speed refrigerating centrifuge, HKust innovation LTD; TGL-168, high-speed table centrifuge, Shanghai anting science instrument factory; HWS12 electric thermostat water bath, Shanghai yiheng scientific instruments co. LTD.

3. Experimental method

3.1. Modeling and drug delivery (Miao et al., 2018)

Ten rats were randomly selected as the blank group, the rest of the rats were injected with DHEA 6 mg/100 g on the back of the neck and 1.5 IU HCG each day, for 21 consecutive days. On the 16th day of modeling, vaginal smear was performed to select the model rats. Rats with successful modeling were selected and randomly divided into model group, dacin-35 group (0.3392 mg kg⁻¹), large, middle and small dose dodder total flavonoids groups (200 mg kg⁻¹, 100 mg kg⁻¹, 50 mg kg⁻¹), 10 in each group, and given the medicine for three weeks. The blank group and the model group were given the same amount of solvent.

3.2. Test indicators and methods

Two hours after the last administration, draw blood from abdominal aorta, and serum was separated to measure LH/FSH ratio, P, PRL and INS. Ovaries and uterus were weighed so as to calculate viscera index. Uterus and pancreas were fixed in 10% formalin solution for HE staining and observe morphological changes. The aprotinin expression of TNF-α and IGF-I in the ovary was measured by immunohistochemistry.

4. Statistical methods

SPSS21.0 statistical software was used to statistically process the experimental results. The measurement data are expressed as mean value ± standard deviation (x ± s). Single factor analysis of variance was used for inter-group comparison. The homogeneity of variance was tested by LSD method, and the heterogeneity of variance was tested by Games-Howell method. The hierarchical data is validated with Ridit.

5. Experimental results

5.1. Effect of dodder total flavone on ovary index and uterus index of PCOS rat models induced by DHEA combined HCG

As shown in Table 1: Compared with the blank group, the ovary index and uterus index of the model group were significantly increased (P < 0.01). Compared with the model group, dacin-35 group, large, middle and small dose dodder total flavonoids groups could significantly reduce the ovary index and uterus index of PCOS rats (P < 0.01).

5.2. Effect of dodder total flavone on serum LH/FSH ratio of PCOS rat models induced by DHEA combined HCG

As shown in Table 2: Compared with the blank group, the serum LH/FSH ratio in the model group increased significantly (P < 0.01). Compared with the model group, the serum LH/FSH ratio in the dacin-35 group, large, middle and small dose dodder total flavonoids groups decreased significantly (P < 0.01).

5.3. Effect of dodder total flavone on serum P, PRL and INS levels of PCOS rat models induced by DHEA combined HCG

As shown in Table 3: Compared with the blank group, PRL and INS levels in the serum of the model group increased significantly (P < 0.01), serum P level decreased significantly (P < 0.01). Compared with the model group, the serum PRL and INS levels decreased significantly (P < 0.01), P level increased significantly in (P < 0.01).

5.4. Effect of dodder total flavone on TNF-α and IGF-I protein expression in ovari of PCOS rat models induced by DHEA combined HCG

As shown in Table 4: Compared with the blank group, the mean optical density of TNF-α and IGF-I protein in ovary of the model group increased significantly (P < 0.01). Compared with the model group, the mean optical density of TNF-α and IGF-I in ovary was...
The uterus tissues of each group were measured according to the semi-quantitative standard, and the results were shown in Table 5.

As shown in Table 5: By Ridit test, the uterine endometrium in the blank group was normal. Compared with the blank group, the endometrial glands showed significant hyperplasia and inflammatory infiltration in the model group \((P < 0.01)\). Compared with the model group, the pathological changes of uterus in rats was significantly improved in the dacin-35 group, large, middle dose dodder total flavonoids groups (\(P < 0.01\)), small dose dodder total flavonoids group could obviously improve the pathological changes of uterus in rats (\(P < 0.05\)).

5.6. Effect of dodder total flavone on islet tissue of PCOS rat models induced by DHEA combined HCG

Pathological observation of islet tissue in rats (see Fig. 2): In the blank group, the islet shape and structure of the rat pancreas were normal, and the cells were arranged in a compact and orderly manner. In the model group, abnormal lesions appeared in the pancreas islet tissue. It suggests that patients with polycystic ovary syndrome may have islet tissue lesions, and islet lesions may also promote the occurrence of polycystic ovary syndrome through relevant mechanisms, aggravating the patient’s condition. Under the light microscope, it is obvious that the islet volume of PCOS model group is larger than that of the blank group. However, the islet volume of each group which were given drugs decreased significantly. The pancreas of each group of animals was selected to measure 10 pancreas islets, and the mean value of the widest and narrowest part of the pancreas was obtained by measuring microscope, then the diameter of the pancreas islet was obtained, and the measured results were shown in Table 6.

The uterine tissues of each group were measured according to the semi-quantitative standard, and the results were shown in Table 5.

As shown in Table 5: Compared with the blank group, the pancreatic islet diameter in the model group increased significantly (\(P < 0.01\)). Compared with the model group, dacin-35 group, large, middle and small dose dodder total flavonoids groups could significantly reduced the diameter of pancreatic islets (\(P < 0.01\)).
6. Discuss

The clinical manifestations of PCOS are highly heterogeneous, and it is a chronic endocrine disorder with multiple causes and systems. PCOS can cause non-ovulation infertility and ovulation disorder infertility, cause long-term complication such as amenorrhea, glucose and lipid metabolism disorder, endometrial hyperplasia or cancer, endanger the long-term health of the body (Gui et al., 2018; Siddamalla et al., 2018). TCM emphasizes the combination of dialectic and disease differentiation. There is no unanimous con-

Table 4
Effect of dodder total flavone on TNF-α and IGF-1 protein expression in ovaries of PCOS rat models (mean light density).

| Group               | n   | Dose (mg/kg) | TNF-α       | IGF-1       |
|---------------------|-----|--------------|-------------|-------------|
| Blank group         | 10  | —            | 0.260 ± 0.018 | 0.203 ± 0.008 |
| Model group         | 10  | —            | 0.307 ± 0.015 | 0.222 ± 0.009 |
| Dacin-35 group      | 10  | 0.339        | 0.256 ± 0.010 | 0.201 ± 0.006 |
| Dodder total flavone| 10  | 200          | 0.253 ± 0.015 | 0.201 ± 0.005 |
|                    | 10  | 100          | 0.268 ± 0.023 | 0.206 ± 0.010 |
|                    | 10  | 50           | 0.258 ± 0.010 | 0.210 ± 0.011 |

Note: Compared to the blank group, A represents P < 0.05, A^A represents P < 0.01; Compared to the model group, *represents P < 0.05, **represents P < 0.01.

Table 5
Effects on uterine tissue of PCOS rat models.

| Group               | n   | Dose (mg/kg) | – | * | ** | *** |
|---------------------|-----|--------------|---|---|----|-----|
| Blank group         | 10  | —            | 10| 0 | 0  | 0   |
| Model group         | 10  | —            | 0 | 0 | 1  | 2   |
| Dacin-35 group      | 10  | 0.339        | 8 | 1 | 1  | 0   |
| Dodder total flavone| 10  | 200          | 6 | 3 | 1  | 0   |
|                    | 10  | 100          | 5 | 3 | 2  | 0   |
|                    | 10  | 50           | 4 | 4 | 1  | 0   |

**A** = normal, endometrial gland epithelial cells in a single column, the surface of the villi arranged neatly; **A**+ glandular epithelial cells are basically normal, occasional inflammatory cells, slight chronic inflammation; **A**++ glandular epithelial cells were mildly proliferated, a small number of uterine cavity or deep gland were dilated, most inflammatory cells were observed, and moderate chronic inflammation was observed; **A**+++ glandular epithelial cell hyperplasia, most uterine cavity or deep gland dilate, a large number of inflammatory cells, moderate-severe chronic inflammation.

Fig. 1. Effect of dodder total flavone on uterine tissue of PCOS rat models induced by DHEA combined HCG (A: Blank group; B: Model group; C: Dacin-35 group; D: Large dose dodder total flavone group; E: Middle dose dodder total flavone group; F: Small dose dodder total flavone group).
clusion on the etiology and pathogenesis of PCOS. Most scholars believe that renal, liver and spleen functions jointly influence PCOS, and mostly treated from the perspective of kidney (Wang et al., 2017). Common TCM syndromes of PCOS include kidney deficiency and blood stasis, kidney deficiency and sputum stasis, kidney deficiency and phlegm dampness, and phlegm dampness block, kidney deficiency can be seen almost throughout the PCOS. PCOS belongs to the categories of “infertility”, “amenorrhea”, “avulsion” and “menstrual disorder” in TCM. Systematic cluster analysis showed that gynecological diseases such as female infertility, menstrual disorder, amenorrhea, ovulation free, polycystic ovary syndrome in Chinese and western medicine were the first category of diseases related to kidney deficiency (Liu et al., 2012), which further verified the essence of kidney deficiency in PCOS patients. Throughout the history of Chinese medicine for various gynecological diseases, there is no lack of application of Chinese medicine for tonifying kidney. In the treatment of PCOS, TCM mostly use the methods of tonifying kidney, which reflects the principle of “regulating menstruation, must tonify kidney”.

The classification of PCOS in Chinese medicine and the rule of drug use were summarized in the literature, the results showed that kidney deficiency was widely considered to be the main pathogenic factor of the disease. And kidney-tonifying Yang medicine frequently appeared in the treatment of the disease. However, the clinical application of tonifying kidney-yang medicine in PCOS is often combined with other drugs in the form of prescription. In order to further clarify the rationality of kidney-tonifying Chinese medicine intervention and treatment of PCOS, and confirm its efficacy, it is necessary to study single Chinese medicine. In view of this phenomenon, it is proposed to use kidney-tonifying Yang medicine to treat gynecological diseases. Based on different animal models to confirm the curative effect of kidney-tonifying Chinese medicine commonly used in clinical prescription, it has been found that Chinese medicine for kidney-yang has significant effects on perimenopausal syndrome (Fu et al., 2018), perimenopausal depression (Liu et al., 2012), uterine dysplasia and dysmenorrhea. Dodder is a kind of traditional Chinese medicine commonly used for kidney-tonifying, which has many pharmacological effects, such as regulating immunity, anti-aging, protecting liver, regulating reproductive system, protecting cardiovascular and cerebrovascular, lowering blood glucose. Flavonoids are its main active components (Miao et al., 2017). In this study, the polycystic ovary syndrome model of rats was established by DHEA combined HCG to explore the intervention effect of total flavonoids of dodder on PCOS rats.

PCOS patients have certain follicular excretion disorder, which to some extent leads to ovarian hyperplasia (Zhang et al., 2016; Wang et al., 2012) and increased uterine volume. The total flavonoids of dodder could reduce ovarian and uterine indices, so as to improve the PCOS rat model. High LH/FSH (follicular stimulating hormone) level is one of the typical characteristics of PCOS. High LH (luteinizing hormone) inhibits follicular stimulating hormone

| Table 6 Effect of dodder total flavone on islet tissue of PCOS rat models induced by DHEA combined HCG (x ± s). |
|---|
| Group | n | Dose (mg/kg) | Islet diameter (µm) |
| Blank group | 10 | — | 142.28 ± 12.78 |
| Model group | 10 | — | 241.52 ± 18.32 \(^{AA}\) |
| Dacin-35 group | 10 | 0.339 | 153.07 ± 8.80 \(^{**}\) |
| Dodder total flavone | 10 | 200 | 177.24 ± 8.15 \(^{**}\) |
| 10 | 100 | 178.83 ± 9.72 \(^{**}\) |
| 10 | 50 | 182.08 ± 15.22 \(^{**}\) |

Note: Compared to the blank group, \(^{*}\)represents \(P < 0.05\), \(^{AA}\)represents \(P < 0.01\); Compared to the model group, \(^{*}\)represents \(P < 0.05\), \(^{**}\)represents \(P < 0.01\).
(FSH) function, making granulocytes flavinized, small sinus follicle development stopped, and ovarian polycystic changes. The increase of serum PRL and LH/FSH ratio is a manifestation of endocrine disorder, and the reduction of serum PRL and LH/FSH ratio can regulate the endocrine disorder in PCOS patients, thereby delaying the progress of the disease and improving the disease (Yin et al., 2018). Because PCOS ovaries do not ovulate regularly, they cannot secrete progesterone, and the amount of progesterone can regulate the endocrine disorder in PCOS patients, thereby delaying the progress of the disease and improving the disease (Xu and Xiong, 2016; Wei and Burren, 2017), and their serum INS level are increased to different degrees. PCOS is a low-grade inflammatory reaction, which can damage the capillary endothelium and pancreatic islet cells, and damage the ovary, leading to polycystic changes and abnormal hormone secretion. Therefore, improving the inflammatory response is the key to the treatment of PCOS (Jiang et al., 2018). Inflammatory factors in PCOS patients can indirectly promote androgen synthesis by inducing insulin resistance (IR), among which TNF-α is one of inflammatory cytokines studied most (Lee et al., 2018). High level insulin-like growth factor-1 (IGF-1) can enhance the activity of cytokrome P450sc17 enzyme, stimulate the follicular membrane to synthesize excessive androgens, and aggravate hyperandrogenemia (Chen et al.; Zhong et al., 2018). Pregnancy rate is related to endometrial receptivity and endometrial thickness, and endometrial dysplasia leads to a reduction in pregnancy rate (Zeng et al., 2017; Azemi et al., 2018; Lungu et al., 2017; Kumar and Kumar, 2017; Gokharman et al., 2017; Gao et al., 2017; Xu et al., 2018). The results showed that total flavonoids of dodder could reduce serum LH/FSH ratio, PRL and INS levels and increase serum P levels, decrease the levels of TNF-α and IGF-1 proteins in ovarian tissue. Which indicating that it can achieve the intervention effect on PCOS model rats by improving the endocrine, inflammatory reaction, insulin resistance, uterus and islet pathological changes.

In conclusion, total flavonoids of dodder can decrease the ovary and uterus index, affect the secretion of serum hormones (LH/FSH, PRL, INS and P), regulate endometrial hyperplasia protein expression (TNF-α and IGF-1); improving endometrial hyperplasia, inflammatory infiltration and islet tissue lesions, so as to achieve the protective effect on PCOS rat models induced by DHEA combined HCG.

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