Original Article

Effect of Dandelion Extracts on the Proliferation of Ovarian Granulosa Cells and Expression of Hormone Receptors

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Abstract

Background: In the current society, infertility related to age has become a social problem. The *in vitro* fertilization (IVF) success rate in women with poor ovarian response (POR) is very low. Dandelion extract T-1 (DE-T1) is an effective component of the extract from the leaves and stems of *Taraxacum officinale*, which is one of the medicines used in some patients with POR, but its molecular mechanism remains unclear.

Methods: Following IVF, ovarian granulosa cells (GCs) of sixty patients were extracted and divided into normal ovarian response (NOR) and POR groups. GCs were cultured in a dose-dependent and time-dependent manner with DE-T1, proliferation of GCs was determined by Cell Counting Kit-8 assay, and mRNA levels of insulin-like growth factor 1 receptor (IGF-1R), luteotropic hormone receptor (LHR), follicle-stimulating hormone receptor (FSHR), LHR, and CYP19A1 (aromatase) were determined by quantitative polymerase chain reaction. Progesterone and estradiol (E2) concentrations were determined by enzyme-linked immunosorbent assay.

Results: The cell viability gradually increased with the progressive increase in the DE-T1 concentration. Compared with the control group (without DE-T1), the mRNA expressions of FSHR, LHR, IGF-1R, and CYP19A1 were upregulated after the addition of DE-T1, especially in the 2.5% DE-T1 group ($P < 0.01$). The expression of IGF-1R was upregulated approximately 25 times (24.97 ± 4.02 times) in the POR group with 2.5% DE-T1. E2 and progesterone levels increased with the increasing DE-T1 concentration. There were highly significant differences in the E2 and progesterone secretion between the NOR and POR groups ($P < 0.01$).

Conclusion: DE-T1 may promote steroid hormone synthesis by promoting GC proliferation and upregulating GC receptor expression, thereby improving ovarian endocrine function.

Key words: Dandelion Extracts; Follicle-Stimulating Hormone Receptor; Human Granulosa Cells; Insulin-Like Growth Factor 1 Receptor; Proliferation; Steroidogenesis

Introduction

In the current society, infertility related to age has become a social problem. Approximately 10% of women experience an accelerated loss of follicles before the age of 32 years.[1] Although various strategies have been used to improve ovarian response, the optimal approach for poor responders remains controversial, and many Chinese people have begun to seek adjuvant therapies in traditional Chinese medicine after *in vitro* fertilization (IVF) failure. Dandelion extract T-1 (DE-T1) is one of such medicines.

DE-T1 is an effective component of the extract from the leaves and stems of *Taraxacum officinale,* which is widely used as an anti-inflammatory and antiviral therapy in obstetrics and gynecology.[2] Animal studies have shown that 500 μg/ml of DE-T1 can significantly increase the release of LH and follicle-stimulating hormone (FSH) from the pituitary glands of mice (secretion volume increased

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.235864

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Received: 11-02-2018 **Edited by:** Li-Shao Guo
How to cite this article: Wang T, Xue B, Shao H, Wang SY, Bai L, Yin CH, Zhao HY, Qi YC, Cui LL, He X, Ma YM. Effect of Dandelion Extracts on the Proliferation of Ovarian Granulosa Cells and Expression of Hormone Receptors. Chin Med J 2018;131:1694-701.

by 70–90%). DE-T1 can significantly upregulate the expression of the estrogen receptor in the uterus and FSH receptor (FSHR) in the ovaries of mice and increase the secretion of estradiol (E2).

We hypothesized that the therapeutic effects of herbal product DE-T1 could possibly be associated with influence on reproductive hormone levels or the expression of their receptors or the viability of granulosa cells (GCs). This study aimed to verify these three considerations through *in vitro* experiments.

Methods

Participants

Mural GCs were aspirated after the oocyte retrievals of infertile patients undergoing IVF. The cells were collected and processed only after receiving signed informed consent from each patient for the use of cells that would have otherwise been discarded. This study was approved by our local Institutional Review Board. The patients' cells were divided into two groups based on the patients' ovarian responses: normal ovarian response (NOR) and poor ovarian response (POR) group. Poor responders had basal serum FSH levels >10 U/L and antral follicle count <5. We excluded patients with infertility diagnoses related to conditions such as polycystic ovary syndrome or endometriosis, which might affect the quality of GCs. All patients were treated with a gonadotropin-releasing hormone agonist protocol; human chorionic gonadotropin (hCG; 6500 U, Serono, USA) was administered when the leading follicle reached 18–20 mm in diameter together with at least three follicles of 16 mm detected by ultrasonography. Oocyte retrieval was performed 36 h later under transvaginal ultrasound guidance, and only mature oocytes were collected.

Cell purification and culture

After oocyte isolation, mural GCs were collected from residual follicular aspirates and washed three times with phosphate-buffered saline (PBS). To decrease the variability between women, GCs from 3 to 5 patients within the same group were pooled together when possible, depending on the number of retrievals that day. The cells were centrifuged at 2000 rpm for 10 min and separated from the blood cells using 40%/80% Percoll gradient (Sigma-Aldrich, St. Louis, MO, USA). The pellet was suspended with PBS and centrifuged again for 10 min at 1000 rpm. Then, the PBS was removed, and the cells were re-suspended with 1 ml of Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (DMEM/F12) (Invitrogen, Carlsbad, CA, USA). The purified cells were counted using a hemocytometer, and cell viability was determined using trypan blue (BD Biosciences, San Jose, CA, USA) before seeding.

Experimental protocol

The cells were plated in 24-well plates (100,000 live cells/well) and cultured in DMEM/F12 supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. The cells were maintained in the culture at 37.5°C in a humidified atmosphere of 5% carbon dioxide and 20% oxygen. After 24 h, the medium was replaced with fresh medium containing different concentrations of taraxacum extract DE-T1 (Wellness Advance Institute, Osaka, Japan), which were chosen based on gradient (v/v). The experiments were performed in duplicate or triplicate, depending on cell availability.

Proliferation assay

The mural GCs were cultured in 96-well plates at a density of 1.5×10^4 cells per well. After being cultured for 24 h, triplicate wells were treated with different concentrations of the taraxacum extract. Then, the cells were cultured for 48–72 h. Next, the culture medium was replaced with fresh medium containing 10 μl of the Cell Counting Kit-8 (CCK-8) assay, and the plates were incubated at 37.5°C for 4 h. The absorbency of each well was measured at 240 nm.

RNA isolation and real-time polymerase chain reaction

Total RNA was extracted from the GCs using a Quick-RNA Micro Prep Kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. The mRNA concentrations were assessed using a NanoDrop 2000C spectrophotometer (Thermo Scientific, Waltham, MA, USA). Aliquots of 1 ml of RNA were used for the reverse transcription using a high-capacity complementary (c)-DNA RT kit (Thermo Scientific) according to the manufacturer's instructions. The fluorescent SYBRGreen polymerase chain reaction (PCR) mix (Thermo Scientific) was used to quantify the PCR products. Specific primers for the genes of interest (insulin-like growth factor 1 receptor [IGF-1R], FSHR, luteotropic hormone receptor [LHR], and CYP19A1) were added, and a final volume of 10 ml was used for the PCR step. β-Actin was used as a reference (normalization) gene for each sample.

The primers were generated according to NCBI using BLAST search of the GenBank Database and were all intron-exon spanning to avoid genomic DNA contamination. These were FSHR: forward 5'-CTTCTGACCTCCCGAGGAAT-3' and reverse 5'-GGTCCCCAAATCCTGAAAAT-3'; LHCGR: forward 5'-CCCTGAGCCCTGCAACT-3' and reverse 5'-TTGACAGGGAGGTAGGCAAG-3'; and CYP19A1: forward 5'-GAGAATTCATGCGAGTCTGGA-3' and reverse 5'-GCCGAATCGAGAGCTGTAAT -3'.

Amplification and detection were performed using the StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA, USA) with the following profile: 1 cycle at 95°C for 10 min, and 40 cycles each at 95°C for 10 s and 60°C for 50 s. Aliquots of 1 ml of cDNA were used per reaction in a 10 ml total reaction volume. All samples were run in triplicate. The same quantitative real-time PCR protocol was used for all the genes analyzed. Relative quantification of the mRNA levels was performed using the comparative Ct method with B-actin as the housekeeper gene. To obtain the relative gene expression data (control group), the formula ΔΔCt method was used. Results are expressed as fold-change with respect to the experimental control without DE-T1.

Measurement of hormone levels

The E2 and progesterone concentrations in the culture medium were measured in duplicate according to the manufacturer's instructions using enzyme-linked immunosorbent assay kits (Cayman Chemical Co., Ann Arbor, MI, USA). The assay sensitivities were 15 and 10 pg/ml for E2 and progesterone, respectively. The intra-assay coefficient of variation (CV) and inter-assay CVs were 7.3% and 7.7%, respectively, for progesterone, and the intra-assay CV was 14.3 for E2.

Statistical analysis

Each experiment was run at least in triplicate, and the data are expressed as mean ± standard deviation (SD). All statistical analyses were performed using the software SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Statistical comparisons of the mean values between groups were performed using the Student's *t*-test, and multiple comparisons were performed using one-way analysis of variance (ANOVA). The differences were considered statistically significant when $P < 0.05$.

Results

Participants

Sixty women younger than 38 years were prospectively enrolled (thirty diagnosed as having POR and thirty diagnosed as having NOR met the study inclusion criteria). The patient characteristics of the two groups (NOR and POR) are summarized in Table 1. The mean values for age and body mass index did not differ significantly between the two groups. However, significant differences were observed in the antral follicle count and FSH level on the third cycle day and in the serum E2 on the day of the hCG injection between the two groups. Furthermore, the number of eggs retrieved significantly differed between the two groups ($P < 0.001$).

Morphologic observation of granulosa cells of the normal ovarian response group *in vitro*

Under an inverted phase contrast microscope, the GCs appeared spherical when just vaccinated, and they began adhering to the wall 8 h later. The adherence rate reached 70% at 24 h and reached 90% after culture for 2 days. The cellular morphology was irregular, with cells being star like or spindle shaped. The cells were connected to each other by intercellular-extended pseudopodia. The nuclei were large and round, and the cytoplasm was full and had high transparency. The cells showed clustered growth. For GCs planked with uniform cell density, after 24 h of preculture, DE-T1 was added according to gradient concentrations. At 48 h, the proliferation and extension degree of GCs in the group treated with DE-T1 were significantly higher than those were in the control group [Figure 1].

Effect of dandelion extract T-1 on *in vitro* proliferation of human granulosa cells

Effect of different concentrations of dandelion extract T-1 on the viability of granulosa cells cultured *in vitro*

After GCs were cultured with different concentrations of DE-T1 for 48 h, the CCK-8 assay was used to test cell viability. The results showed that, for GCs in the NOR group within a certain concentration range, the cell viability gradually increased with the progressive increase of DE-T1 concentration, suggesting that DE-T1 could promote the proliferation of GCs, and this effect was positively correlated in a dose-dependent manner. After the 48 h incubation of cells with DE-T1, the proliferative activity was higher than that in control group, with a statistically significant difference observed at different concentrations ($P < 0.05$). The proliferative activities in the 1.25% ($P = 0.003$), 2.5% ($P = 0.007$), and 5% groups ($P = 0.001$) were significantly higher than that in the control group [$P < 0.01$; Figure 2].

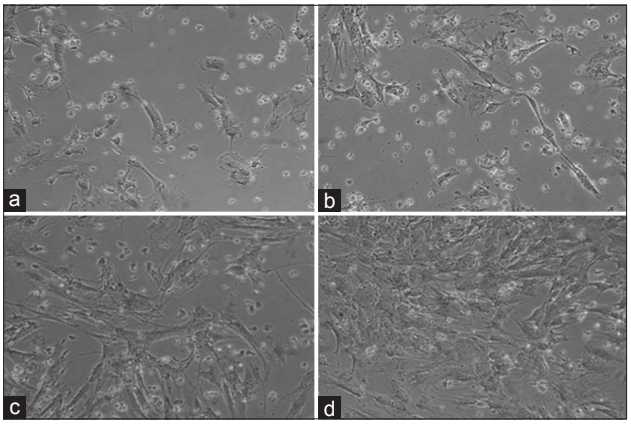

Figure 1: Microscopic images of ovarian granulosa cells cultured *in vitro* from the normal ovarian response group for 48 h (×200). (a) Control group (without DE-T1); (b) 1% DE-T1; (c) 5% DE-T1; (d) 10% DE-T1. DE-T1: Dandelion extract T-1.

Table 1: Comparison of characteristics between the NOR group and POR group

Variable	NOR ($n = 30$)	POR ($n = 30$)	t	P
Age (years)	31.73 ± 3.93	33.36 ± 3.68	1.659	0.102
BMI (kg/m^2)	22.83 ± 2.03	23.49 ± 2.37	1.144	0.257
AFC	11.10 ± 2.69	4.43 ± 1.33	−12.579	<0.01
bFSH (mU/ml)	6.35 ± 1.82	11.92 ± 5.17	5.552	<0.01
Eggs retrieved	9.93 ± 2.32	3.43 ± 0.93	−12.898	<0.01
Peak estradiol (pg/ml)	2490.19 ± 550.53	1179.92 ± 294.55	−11.494	<0.01

Data are expressed as mean ± SD. BMI: Body mass index; AFC: Antral follicle count; bFSH: Basal follicle-stimulating hormone; NOR: Normal ovarian response (bFSH <10 mU/ml or AFC = 5–15); POR: Poor ovarian response (bFSH >10 mU/ml or AFC <5); SD: Standard deviation.

Granulosa cell changes in patients with low responses after the addition of dandelion extract T-1 *in vitro*, followed by culture for different days

After 1 and 2 days of culture of the GCs from patients with low responses with DE-T1 *in vitro*, the GCs showed a trend of slow proliferation over time. Then, they gradually aged, and cell viability gradually decreased. The aging and death as well the decline in the proliferation ability of GCs were significantly delayed in the DE-T1 addition group than in the control group. Significant differences were found after 1 day ($P = 0.014$), 2 days ($P = 0.005$), and 3 days ($P = 0.002$) of culture [$P < 0.01$; Figure 3].

Effect of dandelion extract T-1 on the mRNA expressions of follicle-stimulating hormone receptor, luteotropic hormone receptor, insulin-like growth factor 1 receptor, and *CYP19A1* in granulosa cells

Normal ovarian response group

The mRNA expressions of FSHR, LHR, IGF-1R, and CYP19A1 of GCs after the addition of DE-T1 showed an increasing trend in the NOR group compared with the control group. The mRNA expression level of FSHR was higher in the 1.25% DE-T1 concentration group than in the control group ($P = 0.017$). Significant differences were found in the mRNA expression levels of FSHR ($P = 0.009$), IGF-1R ($P = 0.003$), and CYP19A1 ($P = 0.001$) between the 2.5% DE-T1 group and the control group ($P < 0.01$). The mRNA expression levels of IGF-1R ($P = 0.004$) and CYP19A1 ($P = 0.003$) of GCs were significantly higher in the 5% DE-T1 group than in the control group [$P < 0.01$; Figure 4a and 4b].

Poor ovarian response group

The mRNA expression levels of FSHR, LHR, IGF-1R, and CYP19A1 of GCs after the addition of DE-T1 showed an increasing trend in the POR group than in the control group. Extremely significant differences were found in the mRNA expression levels of FSHR ($P = 0.001$), IGF-1R ($P = 0.001$), and CYP19A1 ($P = 0.001$) between the 2.5% dose group and the control group ($P < 0.01$), and no difference was found between the 2.5% dose group and the NOR group. The mRNA expression level of CYP19A1 ($P = 0.011$) was significantly higher in the 1.25% dose group than in the control group ($P < 0.05$). Significant differences were found in the mRNA expression levels of LHR ($P = 0.014$) and IGF-1R ($P = 0.003$) between the 5% dose group and the control group [$P < 0.01$; Figure 4c and 4d].

Difference between normal ovarian response and poor ovarian response with 2.5% dandelion extract T-1

Under different DE-T1 concentrations, there was a difference in the gene expression of GCs between the NOR group and the POR group. With the 2.5% DE-T1 concentration, LHR was upregulated by 4.78 ± 0.50-fold in the POR group, which was significantly higher than that in the NOR group (3.58 ± 0.12, $P < 0.05$). In the POR group, IGF-1R significantly upregulated (24.97 ± 4.02) than in the NOR group (3.29 ± 0.51; $P < 0.01$) and CYP19A1 were upregulated and highly significantly different in the POR group (1.49 ± 0.12) than in the NOR group (1.06 ± 0.09, $P < 0.01$). FSHR was upregulated and significantly higher in the NOR group than in the POR group [$P < 0.05$; Figure 5].

Effect of dandelion extract T-1 on progesterone secretion of granulosa cells

In the culture medium of GCs in the NOR group, the progesterone level increased with an increasing concentration of DE-T1. When the DE-T1 concentration reached 5%, there was a statistically significant difference in the secretion of progesterone ($P = 0.021$). A statistically significant difference was found in the secretion of progesterone in the POR group when the DE-T1 concentrations were 2.5% ($P = 0.027$) and 5% [$P = 0.002$; Figure 6a and 6b].

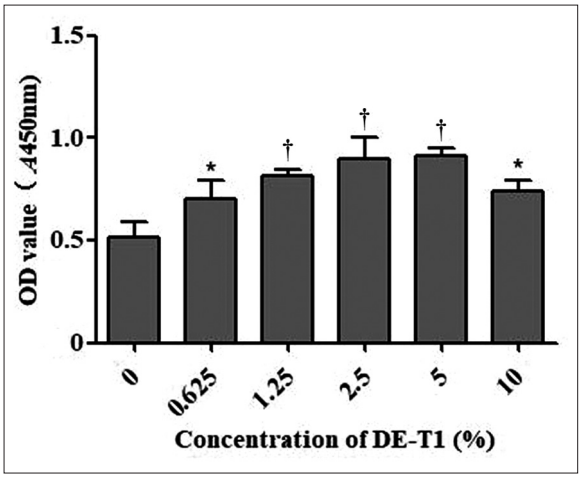

Figure 2: Changes in ovarian granulosa cells from the normal ovarian response group cultured with taraxacum at increasing doses (0.625–10%) for 48 h, with cell viability tested using the CCK-8 assay. Data are shown as mean ± SD compared to a control without DE-T1. *$P < 0.05$, †$P < 0.01$. CCK-8: Cell Counting Kit-8; DE-T1: Dandelion extract T-1. SD: Standard deviation.

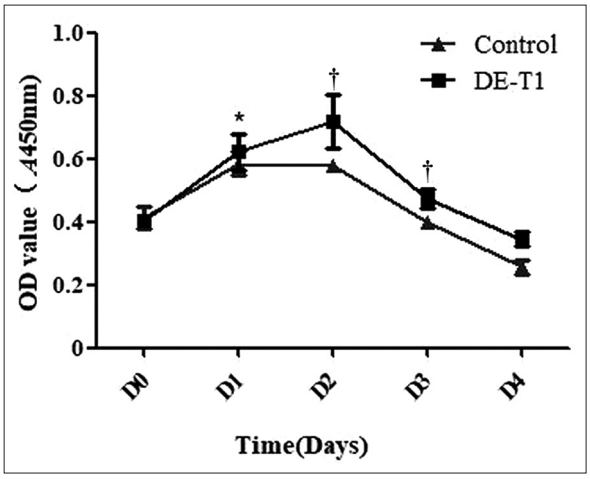

Figure 3: Changes in ovarian granulosa cells of the poor ovarian response group cultured with 5% taraxacum over increasing amounts of time (1–4 days), with cell viability tested using the CCK-8 assay daily. Data are shown as mean ± SD compared to a control without DE-T1. *$P < 0.05$, †$P < 0.01$. CCK-8: Cell Counting Kit-8; DE-T1: Dandelion extract T-1; SD: Standard deviation.

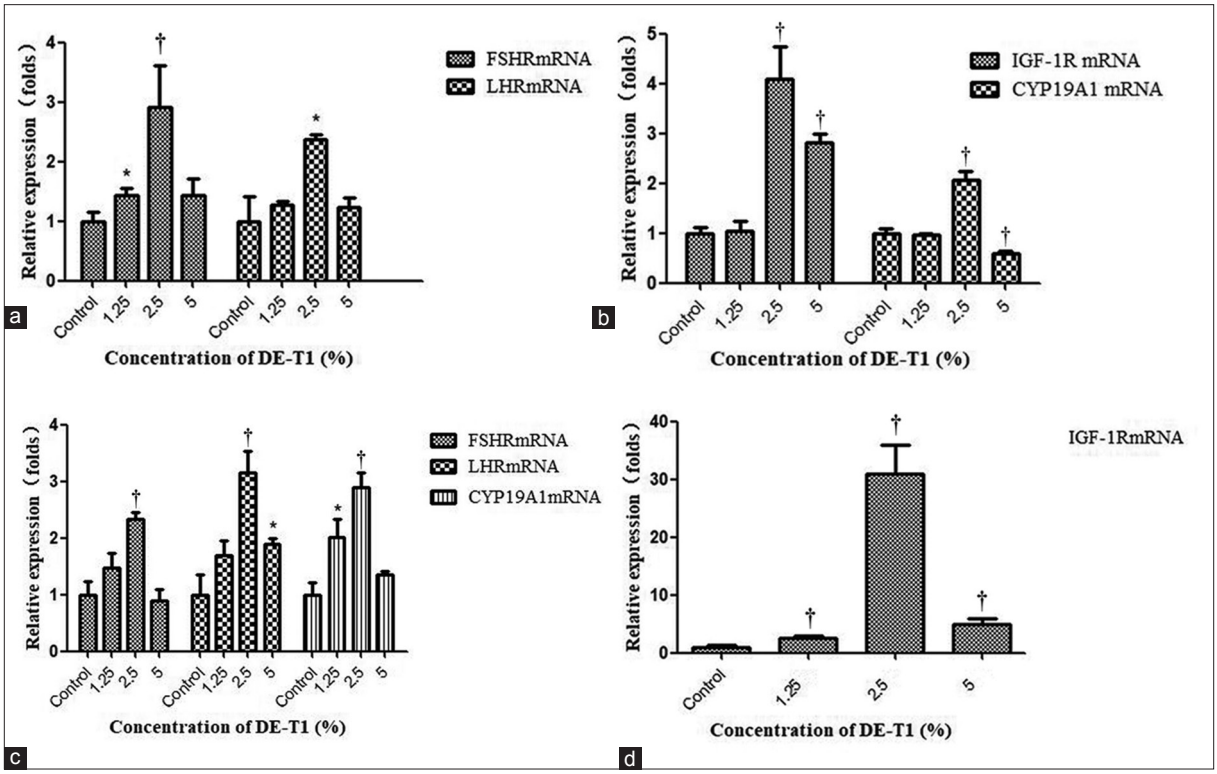

Figure 4: Effect of taraxacum administration on mRNA levels of (a) FSHR and LHR; and (b) IGF-1R, CYP19A1 (aromatase) in ovarian GCs of the NOR group. Effect of taraxacum administration on mRNA levels of (c) FSHR, LHR, and CYP19A1 (Aromatase); and (d) IGF-1R in GCs of the POR group, mRNA levels in GCs were measured using RT-PCR of extracted RNA and normalized against β-actin. The measured taraxacum levels are presented as percentage. Data are shown as mean ± SD compared to a control without DE-T1. *$P < 0.05$, †$P < 0.01$. FSHR: Follicle-stimulating hormone receptor; LHR: Luteotropic hormone receptor; GCs: Granulosa cells; NOR: Normal ovarian response; POR: Poor ovarian response; RT-PCR: Real-time polymerase chain reaction; SD: Standard deviation.

Effect of dandelion extract T-1 on estradiol secretion of granulosa cells

In the culture medium of GCs in the NOR group, E2 increased with increasing concentrations of DE-T1. A statistically significant difference was found in the secretion volume of E2 when the DE-T1 doses were 2.5% ($P = 0.016$) and 5% ($P = 0.003$). In the POR group, when the DE-T1 doses were 2.5% ($P = 0.004$) and 5% ($P = 0.002$), a significant difference was found in the secretion of E2 in GCs [$P < 0.01$; Figure 6c and 6d].

Discussion

GCs are the main functional cells of the ovary, and the proliferation and differentiation of these cells can be regarded as markers of follicular development. The study conducted by Nakahara *et al*.[3] suggested that compared to women with normal ovarian function, patients with declining ovarian reserve have slower proliferation and accelerated apoptosis of GCs. In women with normal ovarian function, there is a positive correlation between the FSHR signaling pathway and the FSH regulator gene PPAP/CYP19A1, whereas in patients with POR, such correlation is disturbed or even blocked.[4] GC apoptosis is a marker of antral follicular atresia. It is considered that GC apoptosis plays an important role in the cycle of ovulation promotion and exerts an adverse effect on the clinical pregnancy rate of the cycle. Therefore, promoting the proliferation of GCs or reducing their apoptosis may improve ovulation induction outcomes. FSH (through the CAMP-ERK-1/2 pathway) promotes the proliferation of GCs[5] and stabilizes the GC skeleton through the cAMP-PKA pathway, thereby increasing the number of GCs.[6] This study showed that, within certain concentration ranges, DE-T1 can enhance the proliferative activity of GCs cultured *in vitro* and delay the decreases in the proliferative activity of these cells. It was speculated that DE-T1 may have FSH-like effects and promote the proliferation of GCs.

GCs primarily expresses FSHR in the human body, and the number of FSHR directly affects the responsiveness of patients to ovulation-inducing drugs. The study conducted by Doherty *et al*.[7] confirmed that compared to patients with NOR, patients with POR have decreased expression of FSHR on the surface of GCs. Hu *et al*.[8] also confirmed that the distribution density of FSHR on the surface of GCs in patients with low ovarian reserve decreased. In clinical practice, the ideal oocyte number for retrieval was obtained by increasing the dosage of gonadotropin, but the effect was not satisfactory. However, a study showed that the FSH level in the follicular fluid of patients with POR is higher than that in patients with NOR.[9] It was believed that large doses of ovulation-stimulating drugs could indeed increase the secretion of local stimulating hormones in the follicle, but there are a lower number of FSHR s on the

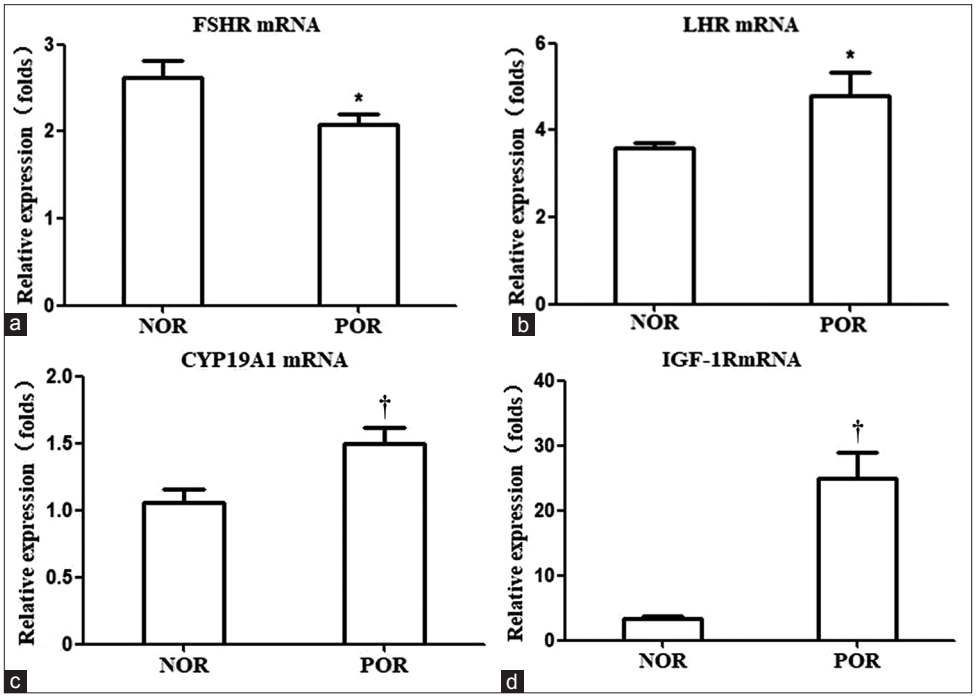

Figure 5: Effect of administering 2.5% DE-T1 on the mRNA levels of (a) FSHR, (b) LHR, (c) CYP19A1 (aromatase), and (d) IGF-1R. mRNA levels between ovarian granulosa cells of the NOR and POR groups were measured using qRT-PCR of extracted RNA and were normalized against β-actin. DE-T1 levels are presented as percentage. Data are shown as mean ± SD compared to a control without DE-T1. *$P < 0.05$, †$P < 0.01$. DE-T1: Dandelion extract T-1; FSHR: Follicle-stimulating hormone receptor; LHR: Luteotropic hormone receptor; NOR: Normal ovarian response; POR: Poor ovarian response; qRT-PCR: Quantitative real-time polymerase chain reaction; SD: Standard deviation.

surface of granular cells of patients with POR, leading to poor outcomes. Hence, it was considered that increasing the expression of FSHR on GC surfaces is an important way to improve ovarian response to ovulation-stimulating drugs, reduce drug dosages, and improve clinical outcomes.[10] This study showed that, in both the NOR and POR groups, the expression of FSHR significantly increased with and without the introduction of DE-T1 into the GC culture. Therefore, the level of upregulation of the expression of FSHR in the NOR group was slightly higher than that in the POR group (2.61 ± 0.20 vs. 2.08 ± 0.10), indicating that DE-T1 could enhance the expression of FSHR on GCs, and that it is effective for infertile patients with normal ovarian responsiveness and those with poor ovarian responsiveness. This is speculated to be an important mechanism by which DE-T1 assists in ovulation induction and improving clinical ovarian responsiveness.

CYP19A1 is the key enzyme-associated gene of P450 aromatase, and activated GCs synthesize E2 under the function of P450 aromatase. Samardzija *et al*.[11] found that, by inhibiting the expression of LHR and CYP19A1 on the GC surfaces of mice, the secretion of E2 in GCs can be inhibited, thereby inhibiting ovulation in mice.

Ginther *et al.*[12] found that in FSH/FSHR-knockout and IGF-1-knockout mice, follicular development was blocked in the preovulatory stage. Dierich *et al.*[13] studied IGF-1 and the relationship between its receptor and embryo quality. These results also showed that the IGF-1 expression level is high in embryos with high growth potential. The IGF-1 concentration in follicular fluid is positively correlated with the quality of the oocytes,[13] and the biological function of IGF-1 is realized by its binding to specific IGF-1 receptors on GC surfaces. One study showed that, by increasing the number of IGF-1 receptors on GC surfaces, FSH can synergistically increase the synthesis of DNA and proteins, thus promoting the maturation and differentiation of ova and GCs.[14] This can also significantly increase cell cleavage and improve embryo development. Studies have also suggested that IGF-1 and its receptors play an important role in the proliferation, differentiation, and ovulation function of GCs and are closely related to embryo quality.

Through whole-genome sequencing, Stocco *et al.*[15] found that nearly 50% of the regulating effect of FSH on GCs is attributable to IGF-1R. After IGF-1 receptor expression was inhibited, the expression of some of the genes, including CYP19A1 mediated by FSH, was reduced. Under the mediation of FSH, CYP19A1 expression was upregulated nearly 20 times, but half of that can be inhibited by AEW (IGF-1R inhibitor). This study showed that the expressions of CYP19A1, IGF-1R, and LHR in GCs after the addition of DE-T1 significantly increased compared to those in the control group. Therefore, it was considered that DE-T1 may upregulate the mRNA expression of important receptors such as FSHR, LHR, and IGF-1R on GC surfaces. This would likely further enhance the synergistic effect of such hormones in follicular development and strengthen regulation of the transcription of the downstream target gene P450 aromatase. As a result, more estrogen will be produced

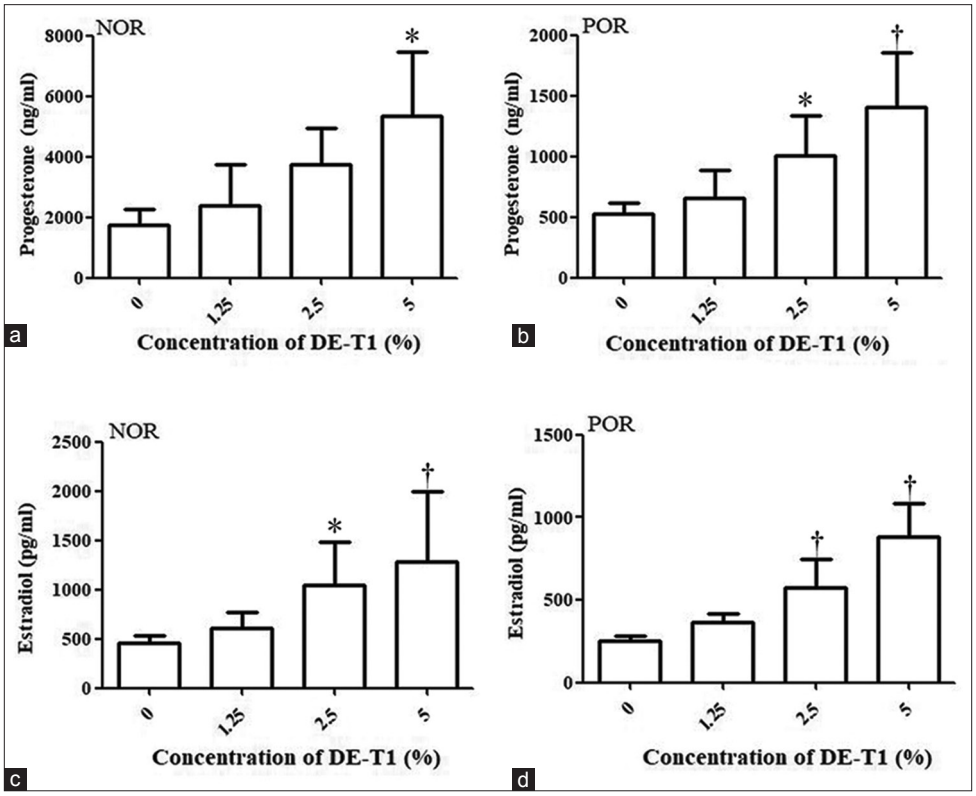

Figure 6: (a) Progesterone level at different concentrations of DE-T1 in the NOR group and (b) the POR group by the ELISA method. (c) Estradiol level at different concentrations of DE-T1 in the NOR group and (d) the POR group by the ELISA method. Data are shown as mean ± SD compared to a control without DE-T1. *$P < 0.05$; †$P < 0.01$. DE-T1: Dandelion extract T-1; NOR: Normal ovarian response; POR: Poor ovarian response; ELISA: enzyme-linked immunosorbent assay; SD: Standard deviation.

in the ovary, which contributes to follicular growth and development and ovulation through the positive feedback effect. We found that the expressions of LHR, CYP19A1, and IGF-1 receptors in GCs were more sensitive to DE-T1 in the POR group than in the NOR group, and the expression of IGF-1R in GCs of the DE-T1 group increased nearly 25 times. Therefore, it was speculated that IGFs may be the key to the sensitivity of patients with low responses to DE-T1. The PI3K/AKT signaling pathway is a classic downstream activation pathway of IGF-1R, and DE-T1 may activate the differentiation of GCs through this pathway. Further in-depth studies should be conducted to elucidate these mechanisms.

The interactions between FSHRs and the activation of intracellular signal transduction pathways lead to the proliferation and differentiation of GCs. Under the stimulation of FSH and the synergistic hormones, GCs produce E2, which has a pleiotropic effect. It can promote the proliferation of GCs, play a crucial role, and function in a synergistic manner with FSH to promote the expression of FSHR and LHR, thereby enhancing the activity of aromatase. The levels of E2 and progesterone hormones in the GC culture medium were measured in the experiment, and it was found that the secretion volumes of E2 and progesterone in GCs were significantly higher in the DE-T1 group than in the control group. At the same level of drug concentration, hormone secretion was greater in the NOR group than in the POR group. Estrogen and progesterone are important female reproductive hormones; thus, it was speculated that DE-T1 may sustain the further development and maturation of oocytes in follicles by promoting GCs to synthesize more steroid hormones, which may be beneficial for early embryonic development after ovulation.

This study had some limitations. The GCs used were from patients who underwent IVF ovulation treatment and were luteinized after IVF oocyte pick-up, so the cells' proliferations were relatively weak. Cells most relevant to follicular development should be the cumulus granular cells in antral follicles, but it is difficult to obtain these in the clinical setting. Based on previous animal experiments, the effect of DE-T1 was detected in a luteinized granular cell model.

Currently, as a Chinese medicine preparation, DE-T1 has been rarely reported to assist in ovulation induction or enhancing the expression of reproductive hormone receptors. Compared to the previous DE-T1 experiments in animal models, the focus of this experiment was the primary culture of human ovarian GCs *in vitro*, thus verifying the medicinal value of investigating the T1 mechanism of action of DE-T1.

In summary, it was speculated that DE-T1 could promote the proliferation of GCs, thereby increasing the mRNA expression of FSHR and IGF-1R on GC surfaces and enhancing the sensitivity of GCs to ovulation-stimulating hormones and the synergetic hormones. It was also

considered that DE-T1 could regulate the transcription of the downstream target gene P450 aromatase, thus promoting the ovary to produce more estrogen, contributing to follicular growth and development. DE-T1 may be used to assist clinical ovulation therapy and reduce the dosage of ovulation-inducing drugs, especially for improving the ovarian function of women with adverse reactions to ovulation-inducing drugs. In addition, DE-T1 could be used to achieve an ideal retrieved oocyte number and improve the clinical outcomes of IVF. Additional basic experiments and evidence of clinical applications are required to confirm the clinical application and value of DE-T1 in assisted reproduction technologies.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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蒲公英提取物对促进人卵巢颗粒细胞增殖及激素受体表达的研究

摘要

背景：研究蒲公英提取物（DE-T1）对颗粒细胞增殖、甾体激素合成及相关受体表达的影响。
方法: 收集行体外受精-胚胎移植（in vitro fertilization - embryo transfer，IVF-ET）卵巢低反应组（poor ovary response，POR）和卵巢正常反应组（normal ovary response，NOR）患者各30例的卵泡液，并提取颗粒细胞，原代培养24 h后分别在含DE-T1低（1.25%）、中（2.5%）、高（5%）剂量的培养液内培养孵育48~60h，采用CCK-8法检测颗粒细胞活性。荧光定量PCR方法检测颗粒细胞胰岛素样生长因子1受体（insulin-like growth factor-1 receptor，IGF1-R）、促卵泡激素受体（follicle-stimulating hormone receptor, FSHR）、黄体生成素受体（luteinizing hormone，LHR）和CYP19A1mRNA的表达。采用酶联免疫吸附法（ELISA）测定颗粒细胞培养液中孕酮（progesterone，P）、雌二醇（Estradiol，E2）浓度。
结果：1、在一定浓度范围内（0.625%~5%），颗粒细胞活性随蒲公英提取物剂量增加而增强，其中1.25%、2.5%、5%DE-T1组，细胞增殖活性均显著优于未添加组（$P<0.01$）。2、DE-T1加药后与空白对照组相比，FSHR、IGF-1R、CYP-19A1表达均有剂量依赖性的增高趋势。尤其在2.5%DE-T1加药组低反应患者LHR、IGF-1R、CYP-19A1mRNA表达上调倍数，均显著高于正常反应组，其中低反应组IGF-1R表达上调近25倍（24.97±4.02）。3、DE-T1加药后，正常反应及低反应两组颗粒细胞培养基中，雌激素和孕激素分泌均随DE-T1加药浓度的增加而升高。其中NOR组当DE-T1浓度达到2.5%时其雌二醇分泌水平较不加药组相比，有显著差异（$P<0.05$），5%浓度时雌激素分泌出现极显著差异（$P<0.01$），同时（5%浓度DE-T1下）孕激素的分泌量水平也有显著差异（$P<0.05$）；在2.5%及5%浓度下，POR组在2.5%及5%浓度下，细胞雌二醇、孕激素水平二醇较空白组的分泌量均有显著增高（分别为$P<0.01$、$P<0.05$）。
结论：蒲公英提取物DE-T1可以促进颗粒细胞的增殖，促进FSHR、LHR、IGF-1R、CYP-19A1 mRNA的表达，促进卵巢颗粒细胞类固醇激素的合成。