Ovarian Failure-Resistant Effects of Catalpol in Aged Female Rats

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Catalpol, an iridoid glycoside obtained from various natural sources, has many biological functions. However, its ovarian failure-resistant effect has scarcely been studied. The present study used senile 14-month-old Sprague-Dawley female rats to examine the in vivo ovarian failure-resistant activity of catalpol. Daily oral graded doses of catalpol (1, 3, or 5 mg/kg/d) for 4 weeks significantly increased the levels of serum 17\(\beta\)-estradiol (E\(_2\)) and progesterone (P4) but reduced follicle-stimulating hormone and luteinizing hormone levels. Electron microscopic analysis and flow cytometry showed that catalpol significantly retarded apoptosis of the ovarian granulocytes of the rats. These findings suggest that catalpol works on the sex organs by nourishing ovarian tissues and improving both the quality and quantity of follicles, thus leading to rebalanced E\(_2\) and P4 levels in aged rats so that catalpol has a direct in vivo antiaging effect on the rat ovarian system.

Key words catalpol; ovarian recession; menopausal symptom

Menopause, a naturally occurring phenomenon in women between the ages of 40 and 60 years, represents the cessation of menstrual cycles due to loss of estrogen and progesterone secretion. Research into the main cellular and molecular aspects of ovarian follicle ageing that lead to menopause include a wide range of important factors. Follicle ageing is characterized by the impairment of specific functions of oocytes and granulosa cells, as well as general cellular dysfunctions including reduced mitochondrial activity, energetic failure, and changes in gene and protein expression. It is speculated that the level of cellular decline is sufficient to make ovarian follicles and ovulated oocytes more susceptible to apoptosis. A significant number of women entering menopause exhibit symptoms, including hot flushes, sleep disorders, irritability, depression and anxiety, which can lead to significant disruptions in the course of daily life. In extreme cases, such symptoms lead women to seek medical attention. The possibility of side-effects, including a transitory increased risk of heart disease at the start of treatment and increased risk of endometrial or various forms of cancer, which cause many women to seek alternatives, has led most doctors to disagree on the utility of hormone replace therapy (HRT) in all menopausal patients. On the basis of the risks and findings from the Women’s Health Initiative, the U.S. Preventive Services Task Force published a recommendation against the routine use of HRT for the prevention of chronic conditions in postmenopausal women.

Recently, herbal medicines origin without hormonal replacement have been attracting a great deal of attention as alternative and supplemental medicines because of their proven therapeutic efficacy and low risk of side-effects. Many extracts from the herbal agents have been proven to be useful in the treatment of age-associated diseases. Rehmannias, an important traditional Chinese herbal medicine which is widely used to replenish vitality, strengthen the liver, kidney, heart, and for treatment of a variety of ailments like diabetes, anemia, and urinary tract problems. There have been growing evidences that the extract from the root of Rehmannia possesses significant neuroprotective activity. Catalpol (Fig. 1), an iridoid glycoside, was isolated from the fresh root of Rehmannia with column chromatography method in our laboratory. It exists broadly in many plants all over the world and has many biological functions such as anti-inflammation, protection of liver damage, and reduction of elevated blood sugar, but its ovarian failure resistant effect to our knowledge has been scarcely studied. In this study, we investigated the in vivo ovarian failure resistant activity of catalpol.

MATERIALS AND METHODS

Animals A total of 48 Sprague-Dawley (SD) female rats (SPF grade; Sino-British Sippr/BK Lab) were included in the study. Forty of these rats were 14 months old (250–300 g), while the other eight were 4 months old (180–220 g). The animals were maintained under regulated environmental conditions (temperature, 22±2°C; humidity, 45–55%; 12 h light/dark cycle) and provided with a standard diet and water ad libitum and processed in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986 and its associated guidelines. The 14-month-old SD female rats were observed for four continuous estrous cycles using a smear with vaginal exfoliated cells followed after staining with hematoxylin and eosin for four continuous estrous cycles using a smear with vaginal exfoliated cells followed after staining with hematoxylin and eosin which were obtained between 9:00 and 10:00 h. The ageing female model was established using a vaginal cytological technique involving an extended estrogenic cycle, continuous estrous, and repeated fake pregnancy in cells.

Preparation of Catalpol Catalpol was separated from the traditional Chinese herbal medicine Rehmannia glut-
**RESULTS**

**Effect on Uterine, Ovarian, and Pituitary Indices**
In the present study, the initial body weights of the five groups (except normal group) were similar. The body weights increased continuously in all groups. At the end of the study, the rats in E₂-treated group and the three catalpol-treated groups showed no increases in body weight, which were not different in control group compared to normal group \( (p<0.01, p<0.01, \text{and } p<0.05, \text{respectively}) \), indicating that apolexis resulted in atrophy of the brain, ovary and adrenal. But the the pituitary and uterine indices of control group were not seen to have significantly difference from normal group \( (p>0.05) \). 17β-E₂, high dose catalpol, medium dose catalpol, and low dose catalpol administration prevented ovary index loss compared to control group \( (p<0.05, p<0.01, p<0.01, \text{and } p<0.05, \text{respectively}) \) but still resulted in a lower ovary index than that of normal group \( (p>0.05) \; \text{Fig. 2B).} \)

**Effect of Catalpol on the Ovarian Ultrastructure of Aged Female Rats**
Healthy Normal Rats (Fig. 3) Electron microscopy with low resolution disclosed that the granulocytes in normal group of reproductive potential were 12–15 μm in diameter, with irregular oval shape and arrayed next to each other. The granulocytes possessed one or two reticular nucleoli with normal chromatin. The cytoplasm of the granulocytes was found to contain many dark and round secretory granules with a diameter of 0.3–0.8 μm; the Golgi complexes were electronically stained. Under electron microscopy with high resolution, a large quantity of utricular smooth endoplasmic reticulum (ER) was observed in the cytoplasm of the granulocytes, and the mitochondria dispersed inside showed clear tabulate cristae in a regular array; the Golgi complexes were normal in shape; no apoptotic cells were found.

Model Rats (Fig. 4) Compared with normal group, the granulocytes size of control group became small-diameter of 8 μm or so. The numbers of organelles and secretory granules decreased, and secretory granules became vacuoles; the
quantity of smooth ER and mitochondria in the cytoplasm was significantly reduced; the size of mitochondria was smaller and cristae were not visible; the Golgi complexes were found in disordered state. The sinusoidal gap widened among the granulocytes and myelination-like modifications appeared.

Catalpol-Treated Rats (Fig. 5) Compared with control group, the organelles in the cytoplasm of catalpol-treated group which treated with catalpol 3 mg/kg were more abundant, and the structure normal. Secretory granules were seen. The size of the granulocytes rejuvenated to that of normal group, and the nucleoli of the cells became apparent with X chromatin. The quantity of smooth ER increased and they looked like round theca. The mitochondria were abundant, and showed clear transverse cristae. The Golgi complexes looked normal and secretory granules were rare. The appearances are similar to other catalpol-treated groups.

Estrogen-Treated Rats (Fig. 6) Compared with control group, the size of the granulocytes increased, averaging...
Fig. 5. The Ovarian Ultrastructure of Catalpol-Treated Group, Treated with Catalpol 3 mg/kg

Fig. 6. The Ovarian Ultrastructure of E2-Treated Group

Fig. 7. Effect of Catalpol on Apoptosis of Ovary Granule Cells in Aged Female Rats by Flow Cytometry
(A) Normal group, treated with vehicle; (B) control group, treated with vehicle; (C) E2-treated group, treated with 17β-estradiol 0.18 mg/kg; (D) H-catalpol-treated group, treated with catalpol 5 mg/kg; (E) M-catalpol-treated group, treated with catalpol 3 mg/kg; (F) L-catalpol-treated group, treated with catalpol 1 mg/kg.
8–12 μm in diameter. Large amounts of mitochondria, smooth ER and Golgi bodies existed in the cytoplasm. The structure of the organelles looked normal. Secretory granules were seen. Vacuolar granules and sinusoidal gaps among granulocytes were abundant.

**Effect of Catalpol on Apoptosis of Ovary Granule Cell in Aged Female Rats** Cell apoptosis was determined by a double staining method using flow cytometry. In apoptotic cells, the phosphatidyl serine (PS), which is confined to the inner layer of the plasma membrane in healthy cells, is exposed to the outer plasma membrane. Annexin V, a Ca^{2+}-dependent protein with high affinity for PS, was used to detect apoptotic cells. Meantime, the viable cells were distinguished from the dead ones using PI. As shown in Fig. 7, UL represent the proportions of dead cells; UR represent the proportions of late apoptotic cells; LL represent the proportions of living cells; LR represent the proportions of early apoptotic cells. After administration, the proportions of total apoptotic cells (late and early apoptotic cells) for the ovary granule cell were 25.70%, 86.65%, 47.04%, 29.28%, 46.12%, and 63.28% in normal group (Fig. 7A), control group (Fig. 7B), E2-treated group (Fig. 7C), H-catalpol-treated group (Fig. 7D), M-catalpol-treated group (Fig. 7E), L-catalpol-treated group (Fig. 7F), respectively. The samples of each group repeat that three more times. As shown in Fig. 8, control group had the highest levels of apoptosis rate (p<0.01), which were coincident with the apoptosis of ovarian granulosa cell. It was also evident that three doses of catalpol (p<0.01) and 17β-E₂ (p<0.01) administration restored the apoptosis of ovarian granulosa cell, compared with those of control group. Moreover, this adjustment of catalpol is superior to that of 17β-E₂.

**Effect of Catalpol on Hormone Levels of Aged Female Rats** In the present study, the initial hormone levels of the five groups (except normal group) were similar. The effects of the different diet supplements on plasma mean levels of E₂, P₄, FSH, and LH are shown in Fig. 9. It can be seen control group had the lowest levels of E₂ and P₄, and the highest levels of FSH and LH (p<0.01), which were coincident with the gradual failure of ovulation and hyposcretion or deficiency of sex hormones in aged rats. Catalpol significantly increased the levels of E₂ (p<0.01) and P₄ (p<0.01) and reduced the levels of FSH (p<0.05) and LH (p<0.05) in the aged rats compared with control group. Moreover, this adjustment of catalpol on the levels of P₄ and LH are superior to those of 17β-E₂.

**DISCUSSION**

Catalpol, separated from *Rehmannia glutinosa* has shown protective effects in many experimental models of neurodegeneration. As is well known, the pituitary-adrenal gland-ovary system in the rat is a good tool for the investigation of emergent responses in neuroendocrinology. The present study...
focused on the pharmacological effects of catalpol on the ovary system of aged rats. From this experimentation results, it is found that use of catalpol resulted in significant increases of serum E2 and P4 levels in the aged rats, and restores of serum FSH and LH levels. Specifically, the adjustment of catalpol over P4 and LH is superior to that of 17β-E2. The increased ovarian indices in the catalpol-treated group perhaps correspond to neurobiological factors and the greater nutrition that ovarian tissues obtained, confirmed that catalpol’s mechanism is not estrogenic.

Moreover, the electronic microscopy analysis revealed that new follicles were formed after the administration of catalpol. Follicles are associated with the generation of internal hormonal agents. If catalpol had only estrogenic activities, the levels of P4, FSH, and LH should not have undergone significant changes. Rejuvenation of the granulocytic ultrastructure is further evidence to support that catalpol nourishes and harmonizes ovarian tissues to regain hormonal balance. From the electron microscopic studies, it was seen that catalpol inhibits the apoptosis of cells by modulating granule synthesis and producing endogenous estrogens. From the flow cytometry studies, it was seen that catalpol reduces apoptosis of cells by modulating the apoptotic mechanism of granulocytes. All the results revealed that catalpol reinvigorates the morphogenesis and function of organelles, such as ER and mitochondria, and granulocytes, and reduce the apoptotic rate of granules.

In summary, catalpol works on the sex organs by nourishing ovarian tissues and improving both the quality and quantity of follicles, thus leading to rebalanced E2 and P4 levels in the aged rats. But it is unclear that catalpol has a regulating activity of ovarian tissues via expression of apoptosis-related molecules or other signal transduction pathways, which in turn are coupled to catalpol’s differential pharmacological activity in a quantitative and temporal fashion, are involved in the production of endogenous E2 rather than the action of FSH and LH. This puzzle is being addressed in another ongoing study we are conducting by means of molecular biological techniques.

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REFERENCES


