The effect of curculigoside on mouse model of perimenopausal depression

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Abstract

Objective: To investigate the effect of curculigoside on mice with simulated perimenopausal depression.

Method: Gavage with high, medium, small dose of curculigoside once daily for 30 d consecutive days.
Record the related behavior index. The wet weights and viscera indexes of the mouse uterus, thymus, and spleen were measured. Half of the brain was homogenized and tested for 5-HT and DA concentrations. The levels of serum E2, T, FSH, and LH were measured as well. Finally, histological changes in the uterus, thymus, spleen, and hypothalamus were observed under a light microscope.

Result: curculigoside can enhance the activity and latency time of the mice, increase mouse memory, and decrease electric shocks and immobility times in the TST and FST experiments. Mice treated with curculigoside showed significantly enhancement in viscera indexes of the thymus, spleen, and uterus; significantly elevated levels of serum E2 and T; significantly increased brain 5-HT and DA concentrations; significantly decreased levels of serum FSH and LH; and improvements in the histopathological lesions of the uterus, hypothalamus, thymus, and spleen. The high dose of curculigoside produced the best results.

Conclusion: All doses of curculigoside are associated with reversing hormone (E2, T, FSH, and LH) disorders in perimenopausal syndrome and adjusting imbalanced 5-HT and DA levels, representing a therapeutic effect in perimenopausal depression.

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1. Introduction

Perimenopausal depression is a mood disorder that occurs shortly before or after menopause in women aged 56 to 66. The main symptoms of perimenopausal depression include emotional depression, anxiety and stress (Xu et al., 2015), accompanied by endocrine dysfunction, especially hypogonadism and senescence (Li and Tian, 2012).

As a commonly-used medicine to tonify yang, curculiginis can enhance kidney yang, strengthen bones and muscles, and alleviate coldness and wetness in the body. Curculigoside content is one of the quality indicators for curculiginis herbs, which have the pharmacological effects of promoting gonadal function, enhancing immunity, anti-cancer and anti-aging (Huo et al., 2012). Clinically curculiginis is used in the treatment of impotence, menopause syndrome, benign prostatic hyperplasia, and breast hyperplasia. Here, we report our findings about the effect of curculiginis on perimenopausal depression.

2. Experimental materials

2.1. Experimental animals

Kunming female mice with masses of 18–22 g, were obtained from Henan Experimental Animal Center, with a batch certificate conformity number of 0008495. The Laboratory Certificate of Conformity is SYXK (Henan) 2010-001.

2.2. Experimental reagents

Curculigoside was obtained from Nanjing Zelang Medical Technology Co., Ltd., with a lot number of ZI20120824 and curculigoside content of 52.3%. Gengnian’an Capsules were obtained from Shanxi Star Pharmaceutical Co., Ltd., with a batch number of 120303 and an approval number of Zhun Z14021848. Soy isoflavones Vitamin E Soft Capsules were obtained from Weihai...
Unisplendour Biotechnolog Co., Ltd., with an approval number of Jian G20080032 and a batch number of 12040301.

Chloral hydrate was obtained from Tianjin Kernel Chemical Reagents Development Center with a lot number of 20120606. Sodium carboxymethyl cellulose was obtained from Tianjin Hengxing Chemical Reagent Co., Ltd., with lot number of 20110728. Penicillin G sodium (four million IU) for injection was obtained from North China Pharmaceutical Co., Ltd., with a lot number of c1107702. Formaldehyde solution (AR) was obtained from North China Pharmaceutical Co., Ltd., with a lot number of 20110728. Penicillin G sodium (four million IU) for injection was obtained from Shanghai Yiheng Scientific Instrument Co., Ltd. An electric heated thermostatic water bath model HWS12 BA-200 was obtained from Chengdu Thai- meng Technology Co., Ltd. A passive avoidance apparatus model BX61 was obtained from Olympus (Japan).

### 2.3. Instrument

An electronic scale model JY601 was obtained from Shanghai Minqiao Medical Appliance Co., Ltd. An electronic analytical scale model AR1140/C was obtained from Ohaus Instruments (Shanghai) Co., Ltd. A high-speed desktop centrifuge model TGL-168 was obtained from Shanghai Anting Scientific Instrument Factory. An acrophotometer model ZZ-6 was obtained from Chengdu Thaimeng Technology Co., Ltd. A passive avoidance apparatus model BA-200 was obtained from Chengdu Thaimeng Technology Co., Ltd. An electric heated thermostatic water bath model HWS12 was obtained from Shanghai Viheng Scientific Instrument Co., Ltd. Adjustable pipettes were obtained from Shanghai Leibo Analytical Instruments Co., Ltd. A microplate reader model 680 was obtained from BIO-RAD (US). A motorized microscope (model BX61) was obtained from Olympus (Japan).

### 3. Method

#### 3.1. Modeling and administration

Modeling method. We randomly chose twelve mice from among 100 female Kunming mice with masses of 22–25 g to use as a control group that received a sham surgery treatment. The remaining mice were used for a perimenopause model.

Each mouse was anesthetized by an intraperitoneal injection of 10% chloral hydrate after weighing (0.03 mL/10 g) and received an abdominal bit fix. Then the mouse was sheared from the back of the last rib in the axillary line at about 1 cm lateral distance from the spine. After disinfection, the skin and back muscles were incised about 0.5–1 cm. A milky-white, shiny, visible section of cellulite was exposed, in which the ovary is embedded. First, the ovarian tube including cellulite was clipped with a thin ligature, and then the ovary was removed. The uterine horns were replaced in the abdominal cavity. Finally, the muscles and skin were sutured. The same treatment was applied to both ovaries. Each mouse received three daily intramuscular injections of 200 ku/kg (0.1 mL/mouse) penicillin to prevent infection. Starting on the fifth day after surgery, a vaginal smear was applied to each mouse daily for five consecutive days to verify the complete removal of the ovaries. Mice with estrus reactions shown by the smear result were discarded.

After five days of administration, the control group continued receiving normal food and water with five mice per cage, while the six test groups began randomly receiving different stresses every day, with one mouse per cage. Seven stress factors were applied randomly for eighteen consecutive days, with one stress a day and no identical stress for two consecutive days. The stress factors comprised: 1. wet litter (g litter, mL water), 2. ice swimming (4 °C, five minutes), 3. heat stress (45 °C, five minutes), 4. night lights (24 h), 5. tail clamping (one minute), 6. water deprivation (24 h), and 7. fasting (24 h).

Method of administration. All groups began to receive their corresponding medication on day ten after surgery. The concentration of the Gengnian’an suspension was 675 mg/kg. The concentration of the soy isoflavones suspension was 250 mg/kg. The concentrations of curculigoside with high, medium, and small dose were 400 mg/kg, 200 mg/kg, and 100 mg/kg, respectively. The control group and model group received distilled water instead. The dosing volume was 0.1 mL/10 g, and each mouse received gavage once daily for thirty consecutive days.

#### 2.2. Test items and method

Each mouse's spontaneous locomotor activities were measured for five minutes on the twenty-sixth day of administration. On the twenty-seventh day of administration, the latency time to enter the dark room as well as the number of electric shocks received by the mouse over five minutes due to entering the dark room were recorded to assess passive avoidance. The total immobility time from the second to sixth minute of the FST was recorded on the twenty-eighth day of administration. The total immobility time from the second to sixth minute of the TST was recorded on the twenty-ninth day of administration. Two hours after the last administration, which involved 12 h of fasting with water provided, each mouse was weighed and blood was collected via an eyeball removal procedure. The serum was separated and tested for estradiol (E2), testosterone (T), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) concentrations using ELISA kits. Finally, each mouse was sacrificed by cervical dislocation and anatomized.

The thymus, spleen, and uterus were extracted and wet weighed, and the viscera index was calculated (viscera index = viscera wet weight (mg)/mouse weight (g)). The brain was also extracted, and half of it was homogenized and measured for concentrations of monoamine neurotransmitter 5-hydroxytryptamine (5-HT) and dopamine (DA) using corresponding kits. The thymus, spleen, and remaining brain tissue were fixed in a 10% formaldehyde solution, embedded in paraffin, sectioned, and hematoxylin and eosin stain (HE) stained. Furthermore, the histological changes were observed under a light microscope.

#### 2.3. Statistical methods

Data was analyzed using the SPSS17.0 statistical package. Quantitative data was represented by mean ± standard deviation (±s). ANOVA was used to compare among groups; least significant difference test (LSD) was used for data with homogenous variance, and Games-Howell was used for non-homogenous variance. Ordinal data was tested using the Ridit analysis.

### 3. Result

#### 3.1. Effect on spontaneous locomotor activity of perimenopausal depression modeling mice

The test results for spontaneous locomotor activity for the different groups of mice are shown in Table 1.
Table 1 shows significantly lower activity and standing time for the model group mice than the control group (P < .01), indicating that the mice representing the perimenopausal depression model demonstrated less curiosity toward new environments. Compared to the model group, all administrative groups demonstrated significantly enhanced activity (P < .01). The mice in the high- and middle-dose curculigoside groups, as well as in the soy isoflavones group, show significantly enhanced standing times (P < .01), and the mice in Gengnian’an group show moderately significant enhancement in standing times (P < .05).

### Table 1

| Group                  | Dose (mg kg⁻¹) | Activity (n) | Standing times (n) |
|------------------------|----------------|--------------|--------------------|
| Control group          | –              | 123.50 ± 18.53 | 78.50 ± 7.29      |
| Model group            | –              | 80.90 ± 10.02 | 47.80 ± 7.67      |
| Soy isoflavones group  | 250            | 112.80 ± 8.85 | 58.50 ± 6.57      |
| Gengnian’an group      | 675            | 97.70 ± 6.09 | 52.90 ± 4.58      |
| High-dose curculigoside group | 400      | 110.80 ± 8.73 | 56.60 ± 4.40      |
| Middle-dose curculigoside group | 200   | 104.50 ± 7.88 | 60.50 ± 2.95      |
| Small-dose curculigoside group | 100      | 102.20 ± 7.15 | 55.20 ± 4.08      |

Note: Compared to the model group.
* P < .05.
** P < .01.

### 3.2. Effect on latency and number of electric shocks for mice modeling perimenopausal depression in passive avoidance

The passive avoidance test results are shown in Table 2. Table 2 shows that significantly lower latency for the model group mice, as well as a significantly higher number of electric shocks received, compared with the control group (P < .01), indicating reduced memory in perimenopausal depression modeling mice. Compared to the model group, all administrative groups demonstrate significantly prolonged latencies (P < .01) and significantly fewer electric shocks received (P < .01).

### Table 2

| Group                  | Dose (mg kg⁻¹) | Prolonged (s) | Number of electric shocks |
|------------------------|----------------|---------------|--------------------------|
| Control group          | –              | 95.78 ± 6.78  | 2.80 ± 1.03              |
| Model group            | –              | 32.95 ± 4.61  | 7.10 ± 1.91              |
| Soy isoflavones group  | 250            | 92.97 ± 4.84  | 5.00 ± 1.70              |
| Gengnian’an group      | 675            | 101.28 ± 9.59 | 4.40 ± 1.08              |
| High-dose curculigoside group | 400      | 99.61 ± 7.03 | 2.40 ± 1.27              |
| Middle-dose curculigoside group | 200   | 84.13 ± 7.27 | 3.60 ± 1.35              |
| Small-dose curculigoside group | 100      | 88.18 ± 7.72 | 4.10 ± 1.37              |

Note: Compared to the model group.
* P < .05.
** P < .01.

### 3.3. Effect on FST and TST for mice modeling perimenopausal depression

The FST and TST results are shown in Table 3. These results show that the model group displayed significantly higher immobility times than the control group in both the FST and TST (P < .01), indicating an increased desperation to adverse environments in model mice. Compared to the model group, all administrative groups exhibit significantly lower FST and TST times (P < .01).

### Table 3

| Group                  | Dose (mg kg⁻¹) | FST (s)       | TST (s)       |
|------------------------|----------------|---------------|---------------|
| Control group          | –              | 57.647 ± 16.830 | 74.072 ± 4.523 |
| Model group            | –              | 121.616 ± 15.724 | 124.717 ± 8.764 |
| Soy isoflavones group  | 250            | 101.689 ± 5.797 | 104.056 ± 6.564 |
| Gengnian’an group      | 675            | 89.331 ± 2.741 | 101.515 ± 4.592 |
| High-dose curculigoside group | 400      | 84.905 ± 5.928 | 100.096 ± 6.329 |
| Middle-dose curculigoside group | 200   | 94.483 ± 4.653 | 109.712 ± 8.596 |
| Small-dose curculigoside group | 100      | 94.091 ± 5.177 | 102.100 ± 4.313 |

Note: Compared to the model group.
* P < .05.
** P < .01.

### 3.4. Effect on monoamine neurotransmitter levels in mice modeling perimenopausal depression

The brain 5-HT and DA levels for each group are shown in Table 4. Both 5-HT and DA levels for the model group are significantly lower than those for the control group (P < .01), indicating success in our strategy for modeling chronic stress and depression. Compared to the model group, the mice in the high- and middle-dose curculigoside groups and the Gengnian’an group show significantly enhanced 5-HT levels (P < .01); the mice in the small-dose curculigoside group and the soy isoflavones group show moderately...
significant enhancement in 5-HT levels \((P < .05)\); and the mice in all of the curculigoside groups, the Gengnian’an group, and the soy isoflavones group show significantly enhanced DA levels \((P < .01)\).

### 3.5. Effect on the viscera index of mice modeling perimenopausal depression

The viscera indexes of the thymus, spleen, and uterus are shown in Table 5. These three viscera indexes are significantly lower in the model group than in the control group \((P < .01)\), indicating atrophy of the thymus, spleen, and uterus in model mice. Compared to the model group, mice in the high- and middle-dose curculigoside groups, the Gengnian’an group, and the soy isoflavones group all showed a significantly enhanced thymus viscera index \((P < .01)\); mice in the small-dose curculigoside group showed moderately significant enhancement in the thymus viscera index \((P < .05)\); mice in the medium- and small-dose curculigoside groups showed moderately significant enhancements in the spleen viscera index \((P < .05)\); and all administrative groups showed a significantly enhanced uterus viscera index \((P < .01)\).

| Group                  | Dose (mg kg\(^{-1}\)) | Thymus (mg g\(^{-1}\)) | Spleen (mg g\(^{-1}\)) | Uterus (mg g\(^{-1}\)) |
|------------------------|------------------------|-------------------------|-------------------------|-------------------------|
| Control group          | –                      | 3.626 ± 0.468\(^{*}\)   | 4.156 ± 0.543\(^{**}\)  | 2.930 ± 0.679\(^{**}\) |
| Model group            | –                      | 2.882 ± 0.302            | 3.038 ± 0.262            | 0.680 ± 0.145            |
| Soy isoflavones group  | 250                    | 3.703 ± 0.484\(^{*}\)   | 3.836 ± 0.607            | 1.771 ± 0.322            |
| Gengnian’an group      | 675                    | 3.993 ± 0.789\(^{*}\)   | 4.069 ± 0.610            | 1.591 ± 0.304            |
| High-dose curculigoside group | 400         | 3.774 ± 0.736\(^{*}\)   | 3.973 ± 0.764\(^{*}\)   | 1.584 ± 0.185\(^{*}\)   |
| Middle-dose curculigoside group | 200 | 3.710 ± 0.571\(^{*}\)   | 3.694 ± 0.448\(^{*}\)   | 1.559 ± 0.227\(^{*}\)   |
| Small-dose curculigoside group | 100       | 3.562 ± 0.542\(^{*}\)   | 3.597 ± 0.552\(^{*}\)   | 1.512 ± 0.236\(^{*}\)   |

Table 6

Effect on serum E\(_2\), T concentrations \((x \pm s, n = 10)\).

| Group                  | Dose (mg kg\(^{-1}\)) | E\(_2\) (pmol/L) | T (ng/mL) |
|------------------------|------------------------|-----------------|-----------|
| Control group          | –                      | 30.102 ± 2.150\(^{**}\) | 16.270 ± 1.853\(^{**}\) |
| Model group            | –                      | 20.738 ± 3.318   | 10.733 ± 1.290 |
| Soy isoflavones group  | 250                    | 27.623 ± 3.128\(^{*}\) | 14.750 ± 2.043\(^{*}\) |
| Gengnian’an group      | 675                    | 36.537 ± 4.210\(^{*}\) | 15.179 ± 2.334\(^{*}\) |
| High-dose curculigoside group | 400         | 27.254 ± 2.811\(^{*}\) | 14.722 ± 1.915\(^{*}\) |
| Middle-dose curculigoside group | 200 | 26.475 ± 1.914\(^{*}\) | 14.114 ± 2.191\(^{*}\) |
| Small-dose curculigoside group | 100       | 24.426 ± 2.164\(^{*}\) | 13.730 ± 2.126\(^{*}\) |

Table 7

Effect on serum FSH, LH concentrations \((x \pm s, n = 10)\).

| Group                  | Dose (mg kg\(^{-1}\)) | LH (pg/mL) | FSH (miu/mL) |
|------------------------|------------------------|------------|--------------|
| Control group          | –                      | 1102.000 ± 113.019\(^{*}\) | 25.864 ± 3.452\(^{*}\) |
| Model group            | –                      | 1494.000 ± 160.014\(^{*}\) | 35.916 ± 5.066\(^{*}\) |
| Soy isoflavones group  | 250                    | 1203.000 ± 139.686\(^{*}\) | 28.586 ± 3.665\(^{*}\) |
| Gengnian’an group      | 675                    | 1155.000 ± 115.590\(^{*}\) | 28.848 ± 4.553\(^{*}\) |
| High-dose curculigoside group | 400         | 1240.000 ± 123.918\(^{*}\) | 27.932 ± 5.251\(^{*}\) |
| Middle-dose curculigoside group | 200 | 1210.000 ± 106.249\(^{*}\) | 28.796 ± 4.392\(^{*}\) |
| Small-dose curculigoside group | 100       | 1226.000 ± 91.068\(^{*}\) | 29.895 ± 2.926\(^{*}\) |

Note: Compared to the model group.

\(^{*}\) \(P < .05\).

\(^{**}\) \(P < .01\).

Note: Compared to the model group.

\(^{*}\) \(P < .05\).

\(^{**}\) \(P < .01\).
3.6. Effect on serum sex hormone for mice modeling perimenopausal depression

The serum E2, T, FSH, and LH concentrations are shown in Tables 6 and 7. Serum E2 and T levels are significantly lower for the model group than the control group ($P < .01$), while serum FSH and LH levels are significantly higher for the model group than the control group ($P < .01$), indicating both successful modeling and disordered sex hormones in model mice. Compared to the model group, all administrative groups show significantly enhanced serum E2 and T levels ($P < .01$) and significantly reduced LH and FSH levels ($P < .01$).

Table 8
Effect on uterine morphology in mice modeling (unit:n).

| Group                        | Dose (mg kg$^{-1}$) | –  | +  | ++ | +++ |
|------------------------------|---------------------|----|----|----|-----|
| Control group                | –                   | 10 | 0  | 0  | 0   |
| Model group                  | –                   | 0  | 0  | 0  | 10  |
| Soy isoflavones group        | 250                 | 8  | 1  | 1  | 0   |
| Gengnian'an group            | 675                 | 3  | 6  | 1  | 0   |
| High-dose curculigoside group| 400                 | 7  | 3  | 0  | 0   |
| Middle-dose curculigoside group| 200                 | 8  | 2  | 0  | 0   |
| Small-dose curculigoside group| 100                 | 2  | 5  | 3  | 0   |

‘’–”: Endometrial epithelial cells, glands, muscle, and serosa are normal. ‘’+”: A small part of endometrial epithelial cells and gland are atrophic, but muscular and serous are normal. ‘’++”: Endometrial epithelial cells and gland are partially atrophic, myometrium is slightly atrophic, and serous is normal. ‘’+++”: Endometrial epithelial cells, gland, and myometrium are significantly atrophic, and serous is normal.

![Uterine morphology in mice modeling perimenopausal depression HE × 100.](image)
3.7. Effect on morphology in mice modeling perimenopausal depression

3.7.1. Effect on uterine morphology in mice modeling perimenopausal depression

The histopathological results were divided into four grades based on changes in endometrium, gland, and myometrium in the experimental mice, using semi-quantitative criteria. The determined mouse uterus morphology according to these grades is shown in Table 8 and Fig. 1.

According to the Ridit test, the data in Table 8 show that the model mice have significant histopathological uterine lesions compared with the control group ($P < .01$). All administrative groups demonstrate improved histopathological lesions compared to the control group.

Table 9
Effect on hypothalamus morphology in mice modeling (unit:n).

| Group            | Dose (mg kg$^{-1}$) | -- | +  | ++ | +++ |
|------------------|---------------------|----|----|----|-----|
| Control group    | –                   | 10 | 0  | 0  | 0   |
| Model group      | –                   | 0  | 0  | 2  | 8   |
| Soy isoflavones group | 250              | 6  | 2  | 2  | 0   |
| Gengnian’an group | 675                 | 6  | 4  | 0  | 0   |
| High-dose curculigoside group | 400         | 8  | 2  | 3  | 0   |
| Middle-dose curculigoside group | 200         | 7  | 3  | 0  | 0   |
| Small-dose curculigoside group     | 100                | 3  | 6  | 1  | 0   |

``-``: No abnormal change in cytoplasm and cell volume of hypothalamic nuclei, and glial cells are normal. ``+``: Cytoplasm and cell volume of few hypothalamic nuclei are reduced, and glial cells are normal. ``++``: Cytoplasm and cell volume of some hypothalamic nuclei are reduced, and few glial cells shrink. ``+++``: Cytoplasm and cell volume of most hypothalamic nuclei are reduced, and glial cells shrunk or are disappeared.
model group \((P < .01)\), and among them the medium-dose curculigoside group shows the best result.

### 3.7.2. Effect on hypothalamus morphology in mice modeling perimenopausal depression

The histopathology results were divided into four grades based on pathological changes in the experimental mice's hypothalamic neurons, using semi-quantitative criteria. The determined mouse hypothalamus morphology is shown in Table 9 and Fig. 2 according to this grading system.

According to the Ridit test, the data in Table 9 show that the model mice have significant histopathological hypothalamic lesions compared to the control group \((P < .01)\). All administrative groups demonstrate improved histopathological lesions compared to the model group \((P < .01)\), and among them the large-dose curculigoside group shows the best result.

### 3.7.3. Effect on thymus morphology in mice modeling perimenopausal depression

For each mouse, the thymus cortical thickness at the narrowest point was measured with a micrometer, and the mean value for each group was calculated. Furthermore, the number of lymphocytes at the thickness measurement point was determined, and the mean value for each group was again calculated. The results are shown in Table 10 and Fig. 3.

#### Table 10

**Effect on thymus morphology in mice modeling \((x \pm s,n = 10)\).**

| Group                | Dose (mg kg\(^{-1}\)) | Thymus cortical thickness (μm) | The number of lymphocytes at the thickness (n) |
|----------------------|------------------------|--------------------------------|-----------------------------------------------|
| Control group        | –                      | 36.33 ± 2.28**                | 98.77 ± 8.38**                                |
| Model group          | –                      | 25.83 ± 1.50                  | 56.33 ± 3.89                                 |
| Soy isoflavones group| 250                    | 62.87 ± 1.81**                | 172.39 ± 21.74**                             |
| Gengnian’an group    | 675                    | 49.87 ± 1.66**                | 119.61 ± 8.44**                              |
| High-dose curculigoside group | 400        | 30.64 ± 1.95**                | 82.66 ± 3.48**                               |
| Middle-dose curculigoside group | 200        | 33.58 ± 1.44**                | 64.33 ± 2.84**                               |
| Small-dose curculigoside group | 100        | 31.44 ± 1.55**                | 57.62 ± 2.43**                               |

Note: Compared to the model group.  
* \(P < .05\).  
** \(P < .01\).

![Fig. 3. Thymus morphology in mice modeling perimenopausal depression HE × 100.](image-url)
The data in Table 10 show significant reduced thymus cortical thickness in the model mice compared to the control group \( (P < .01) \), indicating thymic atrophy in model mice. Compared to the model group, all administrative groups demonstrate thickened thymus cortex \( (P < .01) \); the mice in the soy isoflavones group, the Gengnian’an group, and the high- and medium-dose curculigoside groups all show significantly increased cortical lymphocytes \( (P < .01) \), and the mice in the small-dose curculigoside group show a tendency for increased cortical lymphocytes.

### Table 11

| Group                  | Dose (mg kg\(^{-1}\)) | Splenic follicle (\(\mu m\)) | Numbers of lymphocytes (n) |
|------------------------|------------------------|------------------------------|----------------------------|
| Control group          | –                      | 27.91 ± 2.41\(^{**}\)       | 38.77 ± 1.78\(^{**}\)     |
| Model group            | –                      | 19.45 ± 1.86                | 26.79 ± 2.20               |
| Soy isoflavones group  | 250                    | 33.82 ± 1.26\(^{**}\)       | 67.14 ± 3.74\(^{**}\)     |
| Gengnian’an group      | 675                    | 31.77 ± 1.27\(^{**}\)       | 60.14 ± 5.35\(^{**}\)     |
| High-dose curculigoside group | 400               | 34.40 ± 1.27\(^{**}\)       | 57.72 ± 2.70\(^{**}\)     |
| Middle-dose curculigoside group | 200              | 28.73 ± 1.90\(^{**}\)       | 52.07 ± 5.03\(^{**}\)     |
| Small-dose curculigoside group | 100              | 29.88 ± 1.61\(^{**}\)       | 59.29 ± 3.38\(^{**}\)     |

Note: Compared to the model group.

\(^{*}\) \( P < .05 \).

\(^{**}\) \( P < .01 \).

### 3.7.4. Effect on splenic morphology in mice modeling perimenopausal depression

The thickness of the splenic follicle was measured from both sides, centered at the central artery and with the baseline of...
micrometer aligned with the splenic follicle. The mean value for each group was calculated. Meanwhile, the numbers of lymphocytes on both sides of the baseline were measured, and the mean value for each group was calculated. The results are shown in Table 11 and Fig. 4.

The data in Table 11 show significantly smaller splenic follicles ($P < 0.01$) and fewer lymphocytes ($P < 0.01$) in the model mice than in the control group, indicating splenic atrophy in the model mice. Compared to the model group, all administrative groups demonstrate enlarged splenic follicles ($P < 0.01$) and increased lymphocytes ($P < 0.01$).

4. Discussion

Perimenopausal depression refers to depression that first occurs during perimenopause, with emotional depression, anxiety, and tension constituting the main symptoms. Depression is predicted to present the greatest social and economic burden among all diseases other than heart attack worldwide by 2020 (He et al., 2014). Therefore, depression attracts increasing attention in society, especially in the medical community.

There is no detailed record of depression in Chinese traditional medicine; in fact, there is no such word as “depression” in the vocabulary of traditional medicine. However, the descriptions of melancholia, globus, lily disease, epilepsy, and Bentun fit the symptoms of depression very well (Yu and Tian, 2011). The major treatments for these diseases are based on herbal medicines for energy, the heart, and the spleen (Cheng et al., 2014). A large number of experiments in the early laboratory shows that tonifying kidney yang traditional Chinese medicine has a good therapeutic effect for perimenopausal depression (Miao, 2016a, 2016b). Curculigo is one of the tonics used to treat these diseases, affecting the kidney, liver, and spleen, suggesting its possible effectiveness for perimenopausal depression.

The monoamine hypothesis proposes that depression is mainly due to deficiency or insufficiency of monoamine neurotransmitters like norepinephrine (NE), 5-HT, and DA (Tong et al., 2015). Declining ovarian function reduces the secretion of estrogen and weakens the negative feedback toward the hypothalamus and pituitary, leading to dysfunction in the hypothalamic–pituitary–gonadal axis (HPO). Furthermore, it causes the imbalance of monoamine neurotransmitters such as 5-HT and DA in the central nervous system. Low levels of endogenous estrogen and central estrogen receptors are the keys to inducing depression (Zhang, 2010). Therefore, we investigated the levels of sex hormones such as E2, T, FSH, and LH, as well as the monoamine neurotransmitters 5-HT and DA, and we observed the effect of curculigoside on perimenopausal depression at the biological level.

TST and FST are interpreted as models of “behavioral despair,” and they were commonly used for initial antidepressant evaluation and rapid screening (Sun et al., 2014). Due to the interference of activity-enhancing drugs, the mice locomotor activity experiments aim to improve the selectivity and reliability of drug screening by TST and FST. The total immobility times of mice in our experiments reflect protest behavior and the degree of depression-induced despair. Chronic unpredictable mild stress (CUMS) can lead to changes in long-term behavior, neurochemistry, neuroimmunity, and neuroendocrine factors. This model simulates the important features of human depression; thus it can be considered an accurate model of depression. The CUMS model is the commonly-used experimental model for drug screening as well as investigating clinical efficacy mechanisms and pathophysiology (Wang et al., 2015). Therefore, this study uses experiments that direct reflect the therapeutic effect of curculigoside.

Our results indicate that curculigoside may enhance the activity and latency time of mice modeling perimenopausal depression. It may also increase mouse memory, leading to decreases in electric shocks and immobility times in the TST and FST experiments. Curculigoside can significantly enhance the visera indexes of the thymus, spleen, and uterus; significantly elevate the levels of serum E2 and T; significantly increase brain 5-HT and DA concentrations; significantly decrease levels of serum FSH and LH; and improve histopathological lesions in the uterus, hypothalamus, thymus, and spleen. Our results suggest that high dose of curculigoside are most effective. This study used the medicine curculiginis, which is commonly used in males. We hypothesized its therapeutic effect on perimenopausal depression based on the idea of yin and yang as the root for one another. We proposed a novel “anti-treatment” therapeutic strategy, providing experimental support and new ideas for the treatment of perimenopausal depression. Finally, we expanded the scope and direction of clinical uses for curculigoside.

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