

# Total glucosides of *Curculigo* rhizome to perimenopausal period mice model

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**Abstract:** To investigate the effects of total glucosides of *Curculigo* rhizome (TGC) to perimenopausal period (PMS) mice model. After removed the bilateral ovaries induced the PMS mice model, high, medium and low doses of TGC group were partly given TGC solution 400,200,100mg·kg<sup>-1</sup>, administered once a day, continuously 21 days. Compared with the model group (MG) mices, each dose of TGC group could significantly improve the activities of mice, increase thymus, uterus, spleen index(TI, UI, SI), the levels of testosterone(T), estradiol (E<sub>2</sub>), reduce the level of luteinizing hormone (LH), the high dose of TGC group(HD-C) group has the best effects. It prompted that TGC has the effect in treatment of PMS mice model, the HD-C group of TGC has the best effects.

**Keywords:** *Curculigo* rhizome total glycoside, perimenopausal period, independent activity frequency, the number of electric shocks to the darkroom, E<sub>2</sub>, T, LH, FSH.

## INTRODUCTION

With the augmentation of the social pressure, the occurrence of perimenopausal syndrome was increased, it has become a hot topic of modern medicine. According to investigation shows that it often occurs between 45 to 55 years old (Li *et al.*, 2015). Perimenopausal syndrome could lead to functional lesions of multiple systems (Wei *et al.*, 2013). Perimenopausal syndrome belongs to the category of Chinese medicine, "hysteria", "melancholia", "lily disease" *et al* (Ma *et al.*, 2015) Kidney deficiency is the basic cause of the perimenopausal syndrome (Zhang *et al.*, 2011) therefore the development of Nourishing Kidney Yang treatment of perimenopausal syndrome has important significance.

*Curculigo* is a perennial herb plant of Amaryllidaceae, and it has a long history in medicine. It has the effect of detumescence, replenishing kidney yang, strengthen muscles and bones, dispelling cold and dampness, replenishing essence and blood and so on. It usually used to treat the kidney yang deficiency, seminal emission and impotence, pain of sinews and bones and other clinical symptoms (Yang *et al.*, 2011). It has the study showed that it has the estrogenic activity of alcoholic extract of rhizomes of *Curculigo orchioides* (Vijayanarayana *et al.*, 2007). TGC is one of the quality indicators and the important components of replenishing kidney yang of *Curculigo* (Huo *et al.*, 2012). It has some reports about the traditional Chinese medicine of kidney tonifying to treat the perimenopausal syndrome and it has a better effects in clinical. This paper studies the mechanism of *Curculigo* to perimenopausal syndrome.

## MATERIALS AND METHODS

### *Experimental animal*

KM female mice (20~25g) were purchased from Hebei province of experimental animal center. Animal permit number: 1209021, the lab certificate No.SYXK (Henan) 2010-001.

### *Experimental materials and reagents*

TGC (Batch No. ZI20120824) was purchased from Nanjing Ze Lang Medicine Science and Technology Co. Ltd., lot number. GC (Batch No.120303) was purchased from Shanxi Tianxing Pharmaceutical Co., SI (Batch No. 12040301, Weihai Purple Biotechnology Development Co., Ltd.). Sodium carboxymethyl cellulose (Batch No.20110418,Tianjin Hengxing Chemical Reagent Co., Ltd.), Chloral hydrate(Batch No.20120627, Tianjin Institute of Fine Chemical Industry), Benzylpenicillin Sodium for Injection (Batch No.c1107702, North China Pharmaceutical Co., Ltd., the specification: 4 million units), formaldehyde Solution (Batch No.20120701, Yantai Shuangshuang Chemical Co., Ltd.), E2 ELISA assay kit (Batch No.20120901A, R&D Systems China ),T ELISA assay kit (Batch No.20120901A, R&D Systems China), LH ELISA assay kit (Batch No.20120901A, R&D Systems China), FSH ELISA assay kit (Batch No. 20120901A, R&D Systems China).

### *Experimental apparatus*

High-speed bench centrifuge, Shanghai Anting scientific instrument factory, Model: TGL-168; Mice autonomic activities test instrument, Chengdu taimeng science and technology limited company, Model: ZZ-6; Mouse dark instrument, Chengdu taimeng Technology Co., Ltd., Model: BA-200; Adjustable liquid shifter, Shanghai Leibo Analysis Instrument Co. Ltd.; enzyme mark instrument, USA BIO-RAD, Model: 680.

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### **Experimental methods**

According to the literature method made the PMS model (Li *et al.*, 2012): taken the female mice were 100, weight 22-25g, randomly selected 10 as a blank, did Sham surgery, the rest did the perimenopausal period mice model. After successful anaesthesia that model mice by used 10% chloral hydrate (30ml/kg) to intraperitoneal injection, abdominal fixed, then from the mice back under the bottom ribs, in the middle route of the axillary and about 1cm away from the spine lateral junction shearing on the left side, skin incision after disinfection and back muscle is about 0.5-1cm, incision view visible in a milky glow cellulite, ovary was embedding. Pull gently with tweezers and clamp cellulite incision, the separation of celluliteq, we can see a fine linear irregular was yellow red ovaries. When first the ovarian shearing time (including the fat) using thread ligation of fallopian tubes, to removed the ovaries, after the end of the operation, put the uterine horn back into the abdominal cavity, sutured the muscle layer and the skin layer, the same methods was used to remove the right ovary. Careful breeding, postoperative intramuscular penicillin 200000 u/kg (0.1 mL) in only in case of infection, continuous 3 day, one time a day. Five days after the operation, the model mice were one by one vaginally smears, one time a day, continuous 5 day, determined whether the ovaries removed. Abandoned to smear rendering emotional reaction in mice, choose the complete castrated mice were 60, and the mice were randomly divided into 6 groups for experimental use, it was MG group, the soft capsule of soybean isoflavone (SI group), Gengnianan capsules (GC group) and high, medium and low dose of TGC (HD-C group, MD-C group, LD-C group) group. A total of 10 mice were selected as the blank control (BC) group in the blank mice.

Animals in each group were administered corresponding drugs on the 10th day after surgery, the GC group were administered GC suspension as 675mg/Kg, SI group was administered SI suspension as 250 mg/ kg, HD-C group, MD-C group, LD-C group respectively were administered HD-C, MD-C, LD-C suspension as 400 mg/kg, 200 mg/kg, 100mg/kg. The BC group and the MG group were administered with the same volume of distilled water, 1 times a day and last 21 days.

After administration for 18 days, each groups measured the number of independent activities in 5 min, At the administration for 19~20d, determined the latency of the first time into dark room, and the number of electric shock in 5min. After the last administration 2h, take the blood, separation of serum, measured the level of E2, T, LH, follicle-stimulating hormone (FSH); And killed the mice, take the thymus, spleen, uterus tissue, weighing the wet weight and calculate the viscera index (viscera index = viscera wet weight mg/g) mice weight.

### **STATISTICAL ANALYSIS**

All the data was analyzed by SPSS 17.0 for statistical analysis of date, the data for the metrological data were measured using the mean add and subtract the standard deviation. The comparison between groups was analyzed by one-way ANOVA, F test meet the requirements, use the LSD analysis method, and if was not meet the requirements, use the Games-Howell analysis method. The date for the grade data was used the Ridit test.

### **RESULTS**

#### ***The effect of perimenopausal autonomic activities of mice***

From table 1, the date of the stand times and the activities times proved that the MC group mice was remarkably reduced compared with the BC group ( $P < 0.01$ ), the chart reflect the perimenopausal transition less curious about the new environment of model mice. The mice in HD-C group, MD-C group, LD-C group can remarkably improved the activity times compared with the MG group ( $P < 0.01$ ).

#### ***Dark incubation period and the number of electric shocks by the method of perimenopausal mice model***

From table 2, the time of incubation period and the number of electric shocks proved that the MG group mice were remarkably increased compared with the BC group ( $P < 0.01$ ), the chart reflect the perimenopausal mice model of memory is declining.

#### ***The effect of perimenopausal mice Viscera index***

From table 3, the uterus index proved that the MG group mice were remarkably decreased compared with the BC group ( $P < 0.01$ ), it indicating that the removal of ovaries made the uterine atrophy of the MPS mice. The mice in HD-C group, MD-C group, LD-C group can remarkably increased the TI, UI compared with the MG group ( $P < 0.01$ ). The mice in the MD-C group can remarkably increased the SI compared with the MG group ( $P < 0.01$ ). The mice in HD-C group, LD-C group can remarkably improve the SI compared with the MG group ( $P < 0.05$ ).

#### ***The effect of perimenopausal mice serum sex hormone content***

From table 4 and 5, the levels of E2 and T in serum proved that the MG group mice were remarkably decreased compared with the BC group ( $P < 0.01$ ), the levels of LH and FSH were remarkably higher compared with the BC group ( $P < 0.01$ ), it suggested the serum sex hormone levels was disorde in the MPS mice that remove the ovaries. All the different groups were remarkably increased the levels of T compared with the MG group ( $P < 0.01$ ); MD-C group can remarkably increased the serum E<sub>2</sub> levels compared with the MG group ( $P < 0.01$ ); HD-C group can significantly reduce the higher levels of LH compared with the MG group ( $P < 0.01$ ). Each dose group

**Table 1:** Effect of TGC on perimenopausal autonomic activities of mice( $\bar{x}\pm s$ )

Group	n	Activities (times)	Stand (times)
BC group	10	120.30±14.71 <sup>##</sup>	75.10±7.30 <sup>##</sup>
MG group	10	80.60±8.10	48.80±6.89
SI group	10	109.90±8.39 <sup>##</sup>	58.30±5.06 <sup>##</sup>
GC group	10	104.20±8.28 <sup>##</sup>	56.90±4.72 <sup>##</sup>
HD-C group	10	111.20±8.36 <sup>##</sup>	56.30±5.19 <sup>##</sup>
MD-C group	10	102.60±4.48 <sup>##</sup>	58.40±3.53 <sup>##</sup>
LD-C group	10	98.80±7.76 <sup>##</sup>	59.40±6.26 <sup>##</sup>

**Table 2:** Effect of TGC on perimenopausal mice latent period and number of electric shock chamber ( $\bar{x}\pm s$ )

Group	n	The incubation period (s)	Electric shock (times)
Blank group	10	95.80±7.315 <sup>##</sup>	2.80±1.03 <sup>##</sup>
Model group	10	32.70±4.715	6.80±1.69
SI group	10	95.20±8.066 <sup>##</sup>	4.30±1.57 <sup>##</sup>
GC group	10	106.60±8.514 <sup>##</sup>	4.10±0.99 <sup>##</sup>
HD-C group	10	103.00±8.692 <sup>##</sup>	3.10±0.99 <sup>##</sup>
MD-C group	10	89.50±9.560 <sup>##</sup>	3.30±1.16 <sup>##</sup>
LD-C group	10	87.50±8.847 <sup>##</sup>	4.10±1.29 <sup>##</sup>

**Table 3:** Effects of TGC on perimenopausal thymus, spleen, uterus index in the mice ( $\bar{x}\pm s$ )

Group	n	TI	SI	UI
BC group	10	3.564±0.437 <sup>##</sup>	4.262±0.985 <sup>##</sup>	3.022±0.684 <sup>##</sup>
MG group	10	2.925±0.442	3.152±0.324	0.625±0.116
SI group	10	3.862±0.431 <sup>##</sup>	4.205±0.900 <sup>##</sup>	1.602±0.251 <sup>##</sup>
GC group	10	3.961±0.478 <sup>##</sup>	4.293±0.969 <sup>##</sup>	1.583±0.186 <sup>##</sup>
HD-C group	10	3.615±0.457 <sup>##</sup>	4.034±0.833 <sup>#</sup>	1.599±0.197 <sup>##</sup>
MD-C group	10	3.766±0.383 <sup>##</sup>	4.376±0.874 <sup>##</sup>	1.505±0.263 <sup>##</sup>
LD-C group	10	3.465±0.428 <sup>##</sup>	4.028±0.526 <sup>#</sup>	1.418±0.732 <sup>##</sup>

**Table 4:** Effect of TGC to the level of E2,T in the PMS mice( $\bar{x}\pm s$ )

Group	n	E2(pmol/L)	T(ng/mL)
Blank group	10	30.829±3.348 <sup>##</sup>	16.363±1.451 <sup>##</sup>
Model group	10	17.232±1.622	9.902±1.362
SI group	10	24.120±3.276 <sup>##</sup>	14.626±1.686 <sup>##</sup>
GC group	10	22.449±3.237 <sup>##</sup>	14.563±2.602 <sup>##</sup>
HD-C group	10	23.610±4.215 <sup>##</sup>	14.144±2.428 <sup>##</sup>
MD-C group	10	23.890±4.049 <sup>##</sup>	14.136±1.942 <sup>##</sup>
LD-C group	10	21.620±5.632 <sup>#</sup>	13.636±2.346 <sup>##</sup>

**Table 5:** Effect of TGC to the level of LH, FSH in the PMS mice( $\bar{x}\pm s$ )

Group	n	LH(pg/mL)	FSH(mIU/mL)
BC group	10	913.571±92.278 <sup>##</sup>	28.984±5.315 <sup>##</sup>
MG group	10	1066.428±129.645	40.625±3.680
SI group	10	918.571±151.455 <sup>##</sup>	30.521±3.615 <sup>##</sup>
GC group	10	922.857±70.871 <sup>##</sup>	31.901±5.456 <sup>##</sup>
HD-C group	10	922.143±96.741 <sup>##</sup>	32.240±5.514 <sup>##</sup>
MD-C group	10	945.714±66.564 <sup>#</sup>	33.151±6.921 <sup>##</sup>
LD-C group	10	952.857±115.852 <sup>#</sup>	33.490±7.251 <sup>##</sup>

Note: compared with MG group <sup>#</sup> $P<0.05$  <sup>##</sup> $P<0.01$

can remarkably reduced the levels of FSH compared with the MG group ( $P<0.01$ ).

## DISCUSSION

Modern medicine research thought that the perimenopausal syndrome pathogenesis mainly include endocrine, immune, free radicals, cell apoptosis and other theory, mainly around the endocrine theory research. Because of perimenopausal women ovarian function gradually decreases, the secretion of estrogen also gradually reduce, progesterone secretion also gradually stop, thus stimulate the pituitary gland releases the FSH and LH, the levels of  $E_2$  were obviously declined. Studies have shown that perimenopausal syndrome caused by estradiol levels drop of sleep, depression and other symptoms have significant correlation (Li *et al.*, 2015; Wang *et al.*, 2015), some behavioral indicators such as number of activities, from the dark times automatically behavior index can reflect the perimenopausal syndrome memory, sleep, etc. Females in the process of aging, the endocrine, immune function also gradually reduce the weight of the relevant organ, the weight changes of the immune and endocrine organ can reflect out potential animal immunity, endocrine function. Soybean flavonoids belong to isoflavone estrogen, more years for the proprietary Chinese medicine, this study chose two positive medicine, although by comparing the two positive for confining the symptoms of menopause syndrome has a role, but Ann's effect is more obvious, more years indicated that the herbal medicine in treating perimenopausal syndrome has more advantages. In recent years, the two fairy soup treating perimenopausal syndrome is also common occurrence (Xia *et al.*, 2015; Su *et al.*, 2015; Du *et al.*, 2014), and is one of the medicinal herbs, *Curculigo* play an important role.

Now the medical community is actively looking for a reliable way to treat perimenopausal syndrome. Our laboratory advocated anti governance balance (Liu *et al.*, 2012), from the perspective of replenishing kidney yang treat the perimenopausal syndrome. This study reveals the mechanism and characteristics of total glucosides of *Curculigo* treatment of perimenopausal syndrome, and lay the foundation for the development and utilization of *Curculigo* treatment of perimenopausal syndrome, provide a reliable basis for the clinical application of *Curculigo* to treat the perimenopausal syndrome.

## CONCLUSION

*Curculigo* rhizome total glycoside had a good effect in treatment of perimenopausal mice model, and the effect of high dose of *Curculigo* rhizome total glycoside was the best.

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