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A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2016; 11: S72-S81
ISSN: 1991-0088

Effects of *Curculigo orchioides* total glucosides in mouse peri-menopause model of related organization and organs morphology

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Article Info

Received: 11 January 2016
Accepted: 2 March 2016
Available Online: 5 March 2016

DOI: 10.3329/bjp.v11iS1.26948

Cite this article:
Shan C, Shuo T, Ming B, Shaoyan L, Jiaojiao J, Mingsan M. Effects of *Curculigo orchioides* total glucosides in mouse perimenopause model of related organization and organs morphology. Bangladesh J Pharmacol. 2016; 11: S72-S81.

Abstract

The purpose of this study was to examine the **e**ffects of *Curculigo orchioides* total glucosides in mouse perimenopause model. The castrated mice were treated with different doses of soybean isoflavone soft capsule, Gengnian'an, *C. rhizome* total glycoside. Hypothalamus, thymus, spleen and uterus were collected and then fixed in 10% formaldehyde, HE staining, to observe the pathological changes of morphology. The results indicate that compared with the model group, soybean isoflavone soft capsule, gengnianan capsule and big, medium, small dose of *C. rhizome* total glycoside group could significantly improved the pathological changes in mouse uterus, hypothalamic, thickening of the thymic cortex and significantly increased the number of lymphocytes in the cortex, increased the volume of splenic nodule ($p<0.01$), in which the effect of large dose of *C. orchioides* total glycosides group was the best. In conclusion, *C. orchioides* total glucosides in mice caused by peri-menopausal pathological changes of uterine, hypothalamus, spleen and other organs have good improvement effect.

Introduction

Perimenopausal syndrome is a clinical common disease. Its incidence is increasing year by year (Su et al., 2014). Western medicine thinks women ovarian secretion of estrogen levels are closely related with the occurrence of perimenopausal syndrome, follicle stimulating hormone increased and estradiol decreased leading to other system changes, resulting in a series of diseases (Qi, 2014). Research has shown that, perimenopausal women with cardiovascular disease incidence increased significantly (Weng, 2013) and there is significant correlation with the levels of estradiol (Zhu et al., 2015).

This paper was presented in the 3rd International Conference on Biomedicine and Pharmaceutics in Zhuhai, China, on December 11-13, 2015.

Perimenopausal syndrome have no separate records in Traditional Chinese Medicine (TCM). According to TCM it belongs to hysteria, depression, irregular menstruation and some other diseases (Man et al., 2011). The modern Chinese Medicine called it as menopausal syndromes (Ge et al., 2015; Li et al., 2014).

TCM holds that the human body is composed of a multi-level structure of the organic whole, local lesions mostly overall physiological function disorder in the local reflection. Studies have found that menopausal syndrome can be divided into yin deficiency of liver and kidney, heart kidney disharmony, Yang deficiency of spleen and kidney, deficiency of yin and Yang of some virtual types (Sun and Zhou, 2010), treating in theory of dialectic.

Materials and Methods

Animals

Mice of clean grade, Kunming, female, 20-25 g, were provided by the experimental animal center of Hebei Province (Certificate No. 1209021; Laboratory certificate No. SYXK [%] 2010-001).

Drugs and reagents

Curculigo rhizome total glycoside was provided by the Henan University of Traditional Chinese Medicine chemistry room (batch number ZI20120824, the content of 52.3%). Gengnian'an capsule was collected from Shanxi Star Pharmaceutical Co. Ltd. (production batch number 120303). Others were soybean isoflavone, vitamin E soft capsules (product of Weihai Ziguang Biological Technology Development Co. Ltd., batch number: 12040301), sodium carboxymethyl cellulose (Tianjin Hengxing Chemical Reagent Co. Ltd., batch number 20110728), chloral hydrate (Tianjin Municipality Kemi'ou Chemical Formulation Development Center, No. 20120606), penicillin sodium for injection (North China Pharmaceutical Co. Ltd., 4000000 units, batch number c1107702), formaldehyde solution (Yantai Double Chemical Co., batch number: 20120701), 0.9% sodium chloride injection (Zhengzhou Yonghe Pharmaceutical company, batch number: 12030501).

Apparatus

Electronic, Shanghai Mingqiao Medical Instrument Ltd Co., model: JY601; electronic analytical balance, Ohaus (Shanghai) model: AR1140/C; electric microscope, the Japanese company OLYMPUS, model: BX61.

Experimental methods

One hundred mice were randomly chosen as blank, sham operation treatment and perimenopausal period model. Intraperitoneal injection of 10% chloral hydrate (0.03 mL/10 g) was used to induce anesthesia. From the back of the mice to the end of the rib, shearing at the intersection of axillary midline and lateral spine from about 1 cm, after disinfection of skin incision and dorsal muscle incision about 0.5-1 cm, in the view of a white light visible fat, the ovary was embedded in it. Using tweezers pull gently gripping cellulite at the out of incision, separate fat, it could be seen a thin like irregular yellow red ovary.

The first ovarian fallopian tube (including fat) was ligated using thread. After ovariectomy, to take advantage of the uterine horn back into the abdominal cavity, suture the muscle and skin, with the method of bilateral ovariectomy.

Intramuscular injection of penicillin 200,000 U/kg (each 0.1 mL) was given after operation in order to prevent infection for 3 consecutive days. Operation start by only the mouse vaginal smear examination after 5 days, 1 times a day, continuous for 5 day to determine whether complete resection of ovary. The mice showed emotional reaction was abandon. Sixty mice were ovariectomized completely and then random divided into 6 groups for the experiment respectively model group, gengnianan capsule group, soybean isoflavone soft capsule group, low, medium and high doses of Curculigo rhizome total glycoside. A total of 10 mice were taken as the blank group.

Each animal were given the corresponding drugs in tenth days after operation. Gengnianan capsule group was administrated with gengnianan capsule suspension 675 mg/ kg, soybean isoflavone soft capsule group was fed with soybean isoflavone soft capsule suspension 250 mg/ kg, big, in, small dose of Curculigo rhizome total glycoside respectively were gavaged by groups of big, medium and small doses of Curculigo rhizome total glycoside, 400 mg/kg, 200 mg/kg, 100 mg/kg. The blank group and the model group were given the same volume of distilled water (intragastric administration, 1 time a day, continuous dosing for 21 days).

Two hours after the last administration (fasting 12 hours), the mice were sacrificed by cervical dislocation. The thymus, spleen, thymus, spleen, uterus were removed. The uterus, hypothalamus, brain tissue were fixed in 10% formaldehyde solution, paraffin embedding, sectioning and HE staining. Histological changes were observed under the light microscopy.

Statistical analysis

Data was analyzed by SPSS 17.0 statistical package for statistical analysis of medical data. The measurement data was said with the mean ± standard deviation. The groups were compared with using single factor analysis of variance, variance homogeneity used the method of LSD, heterogeneity of variance using Games-Howell and Ridit test.

Results

Uterine tissue morphology

According to the different degrees change of the experiment of mice endometrial, glands and muscle layer, using semiquantitative criteria, the pathological morphology was divided into four levels. The experimental mice uterus were measured (Table I).

The model group mice showed significant pathological lesions in uterus in comparison to blank group ($p<0.01$). The drug significantly improved the uterine pathological lesions ($p<0.01$), in which the effect of large dose of Curculigo rhizome total glycoside group was the best.

Table I

Effect on perimenopausal mice uterine tissue morphology

Group	n	-	+	++	+++
Blank	10	10	0	0	0
Model	10	0	0	1	9
Soybean isoflavone soft capsule	10	8	2	0	0
Gengnianan capsule	10	6	4	0	0
High dose of Curculigo rhizome total glycoside	10	9	1	0	0
Medium dose of Curculigo rhizome total glycoside	10	6	4	0	0
Low dose of Curculigo rhizome total glycoside	10	3	5	2	0

" - " the endometrial epithelium, glands, muscles, serosal layer were normal; " + " part of endometrial epithelial cells and glands atrophy, muscles, serosa were normal; " + + " the endometrial epithelium, glands atrophy, muscle atrophy were slightly, normal endometrial epithelial "+ + + "; cells, glands atrophy, muscle atrophy, normal serosa

Table II

Effect of Curculigo rhizome total glycoside on perimenopausal changes in hypothalamic pathology in mice

Group	n	-	+	++	+++
Blank	10	10	0	0	0
Model	10	0	0	2	8
Soybean isoflavone soft capsule	10	4	6	0	0
Gengnianan capsule	10	1	8	1	0
High dose of *Curculigo rhizome* total glycoside	10	8	2	0	0
Medium dose of *Curculigo rhizome* total glycoside	10	4	5	1	0
Low dose of *Curculigo rhizome* total glycoside	10	1	6	3	0

" - " cranial nerve nuclei of the hypothalamus in the cytoplasm, cell volume showed no abnormal changes, glial cells were normal; " + " a few cells of brain nuclei of hypothalamus decreased volume reduction, glial cells were basically normal; " + + " some cytoplasm cranial nerve nuclei of hypothalamus decreased volume reduction, a few glial cells shrinkage; " + + + " most of the cytoplasm of cranial nerve nuclei of the hypothalamus significantly reduced volume reduction, glial cells shrinkage or disappearance

Hypothalamic morphology

According to the different degrees of pathological change of hypothalamic neurons on mice, using semi quantitative standards, they were divided into four levels. The experimental mice hypothalamus were measured (Table II).

By Ridit test, from the table, compared with the blank group, model group mice hypothalamus had significant pathological lesions ($p<0.01$). Compared with the model group, each treatment group significantly improved the pathological lesions of the hypothalamus of mice ($p<0.01$). The best effect was the large dose of Curculigo rhizome total glycoside.

Thymus tissue morphology

With the micrometer scale, the experimental group each mouse thymic cortex thickness and the narrowest part are measured, the mean are obtained and then use the micrometer scale baseline measured the number of lymphocytes that are pressured at baseline in the thymic cortex thickness and the narrowest part, the mean are obtained, the results are shown in Table III.

From the chart shows, compared with the blank group, model group's thickness of thymic cortex and cortex of mice lymphocyte numbers decrease significantly ($p<0.01$), indicating the mice thymus volume atrophy in perimenopausal period model. Compared with the model group, each treatment group could significantly thickened cortex of thymus and a significant increase in the number of lymphocytes in the cortex ($p<0.01$), in which the effect of large dose of Curculigo rhizome total glycoside group is the best (Ashwini et al., 2015; Li et al., 2015)

Spleen tissue morphology

The micrometer scale baseline falls in the spleen nodule, splenic nodule size thickness in the central artery as the center on both sides are measured, the average number; at the same time, calculate the number of colonies on both sides of the lymphocytes at baseline, the average number (Table IV).

From the chart shows, compared with the blank group, model group mice splenic nodule volume and cell number is significantly decreased ($p<0.01$), description of perimenopausal model mice spleen atrophy.

Table III

Effect on perimenopausal mice thymus tissue morphology

Group	n	The thickness of thymic cortex (μm)	The number of lymphocytes in the cortex (a)
Blank	10	38.0 ± 2.0[a]	85.8 ± 8.1[a]
Model	10	21.5 ± 1.6	43.5 ± 4.1
Soybean isoflavone soft capsule	10	39.6 ± 2.5[a]	65.8 ± 3.4[a]
Gengnianan capsule	10	54.5 ± 2.6[a]	79.6 ± 8.0[a]
High dose of *Curculigo rhizome* total glycoside	10	61.0 ± 1.5[a]	107.3 ± 11.1[a]
Medium dose of *Curculigo rhizome* total glycoside	10	41.3 ± 2.1[a]	57.7 ± 3.2[a]
Low dose of *Curculigo rhizome* total glycoside	10	41.7 ± 1.9[a]	52.8 ± 4.0[a]

Data are mean ± SD; Compared with model group, [b]p<0.05, [a]p<0.01

Table IV

Effect on perimenopausal mice spleen symptom patterns

Group	n	Dose (mg/kg)	Splenic nodules (μm)	Number of lymphocytes (a)
Blank	10	–	23.2 ± 1.3[a]	49.0 ± 2.8[a]
Model	10	–	18.6 ± 1.4	28.6 ± 1.4
Soybean isoflavone soft capsule	10	250	35.8 ± 1.6[a]	64.4 ± 2.2[a]
Gengnianan capsule	10	675	26.3 ± 2.4	43.7 ± 3.0[a]
High dose of Curculigo rhizome total glycoside	10	400	26.7 ± 1.2[a]	48.4 ± 1.6[a]
Medium dose of Curculigo rhizome total glycoside	10	200	24.3 ± 1.3[a]	42.8 ± 1.6[a]
Low dose of Curculigo rhizome total glycoside	10	100	20.0 ± 1.0[a]	42.1 ±3.2[a]

Data are mean ± SD; Compared with model group, [b]p<0.05, [a]p<0.01

Compared with the model group, the treatment group can significantly increase the volume of splenic nodule ($p<0.01$); and the number of lymphocytes was significantly increased ($p<0.01$), and it is related to dose .

Discussion

Perimenopausal syndrome refers to some women's impairment and pathological changes of multiple system due to ovarian function decline or loss (Cao, 2009). A suitable animal model has the vital significance for the study of the etiology and pathogenesis of the disease and the objective evaluation of drug effect (Li et al., 2012). This study used ovariectomized animal model, the animal model of climacteric syndrome animal model is used, the success rate is high, stable and reliable, and the clinical symptoms of coincidence degree is higher, it can be used to study the factors related to estrogen (Sun and Jin, 2015). Modern clinical for perimenopausal syndrome have endocrine, immune, neurotransmitters and other theories, which focus on endocrine theory development (Yu et al., 2012), endocrine theory thinks that the hypothalamus-pituitary-ovarian axis maintains the female reproductive endocrine system relatively stable (Yin et al., 2013), if the reproductive endocrine disorders, it can cause a variety of plant nerve function disorder (Huo and Kong, 2014). If ovarian function failure, it will cause the disorder of hypothalamus and autonomic nervous function (Wang et al., 2010). Immune theory thinks that immunity decreased showed correlation with estradiol level decreased, while estradiol increased the content of β-EP increased hypothalamic secretion, enhance immunity. The thymus and spleen, as an important immune organ, the weight index and morphological changes also have close relationship with the perimenopausal period (Li and Wang, 1991; Wang et al., 2002). Modern research shows that the ovariectomized rat uterus was significantly thinner and the uterine wall thickness is increased significantly after treatment (Pan et al., 2015). In this study pathological section of uterus, thymus, spleen, hypothalamus are observed, analysis of effects of different drugs on pathological changes in these tissues, to explore the influence of the relevant organs of the perimenopausal syndrome, so as to reveal the characteristics of peri menopausal syndrome.

Curculigo orchioides is the Curculigo rhizomes of red spider lily family plant, pungent, hot, toxic. It has some effects like the dissipation of carbuncle swollen, kidney, strong bones and muscles, essence and blood, expelling wind and other effects. It is commonly used in the treatment of kidney yang deficiency, impotence, spermatorrhea, cold pain in the lumbar spine, urinary frequency and urgency, cold, sore throat, asthenia metrorrhagia and metrostaxis and pain and other clinical symptoms (Yang and Ye, 2011). Modern pharmacological studies suggest that *C. orchioides* can improve the response of the pituitary of female rats to LRH and LH, significantly increased uterus, ovary weight of normal rats (Wong et al., 2001). Research has shown that *C. orchioides* extracts possess estrogenic activity in ovariectomized immature rats (Vijayanarayana et al., 2007). Curculigoside is one of the quality indexes of the constituents of Rhizoma curculiginis (Huo et al., 2012), is one of the main components of Rhizoma curculiginis Yang, there are a lot of reports think that the kidney tonifying traditional Chinese medicine for the treatment of perimenopausal syndrome clinical effect is better, so the subject research on the effect of *C. orchioides* total glucosides to perimenopausal mice model.

The experimental results show that the Curculigo rhizome total glycoside can significantly improve the mouse pathological lesions in uterus, hypothalamus. The cortex thickness and number of thymus lymphocytes are increased significantly. It can improve the pathological changes of thymus. Spleen nodule volume and number of lymphocytes are increased significantly. It has the positive correlation with dose. The effect of large dose of Curculigo rhizome total glycoside group is the best. The experimental results suggest that perimenopausal syndrome has a close relationship with the immune system.

Our laboratory advocated the anti-treatment adjustment (Liu et al., 2011; Liu and Miao, 2012). This study used the drug of kidney yang to treat female diseases. kidney yang drugs can improve the regulatory mechanism of the body, so that the inherent function of the recovery of the body and play a positive role in the treatment. It is consistent with the clinical reports of perimenopausal syndrome pathogenesis (Ling et al., 2015; Nie and Chen, 2015). It also provides new methods and ideas for the treatment of perimenopausal syndrome.

Clinical study shows that the ER Xian decoction is an effective prescription in the treatment of perimenopausal syndrome (Su, 2012; Xia et al., 2015) and *C. orchioides* has played an important role.

Conclusion

C. orchioides total glucosides in mice caused by perimenopausal pathological changes of uterine, hypothalamus, spleen and other organs have good improvement effect.

Conflict of Interest

The authors declare that they have no competing interest.

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Supplementary images

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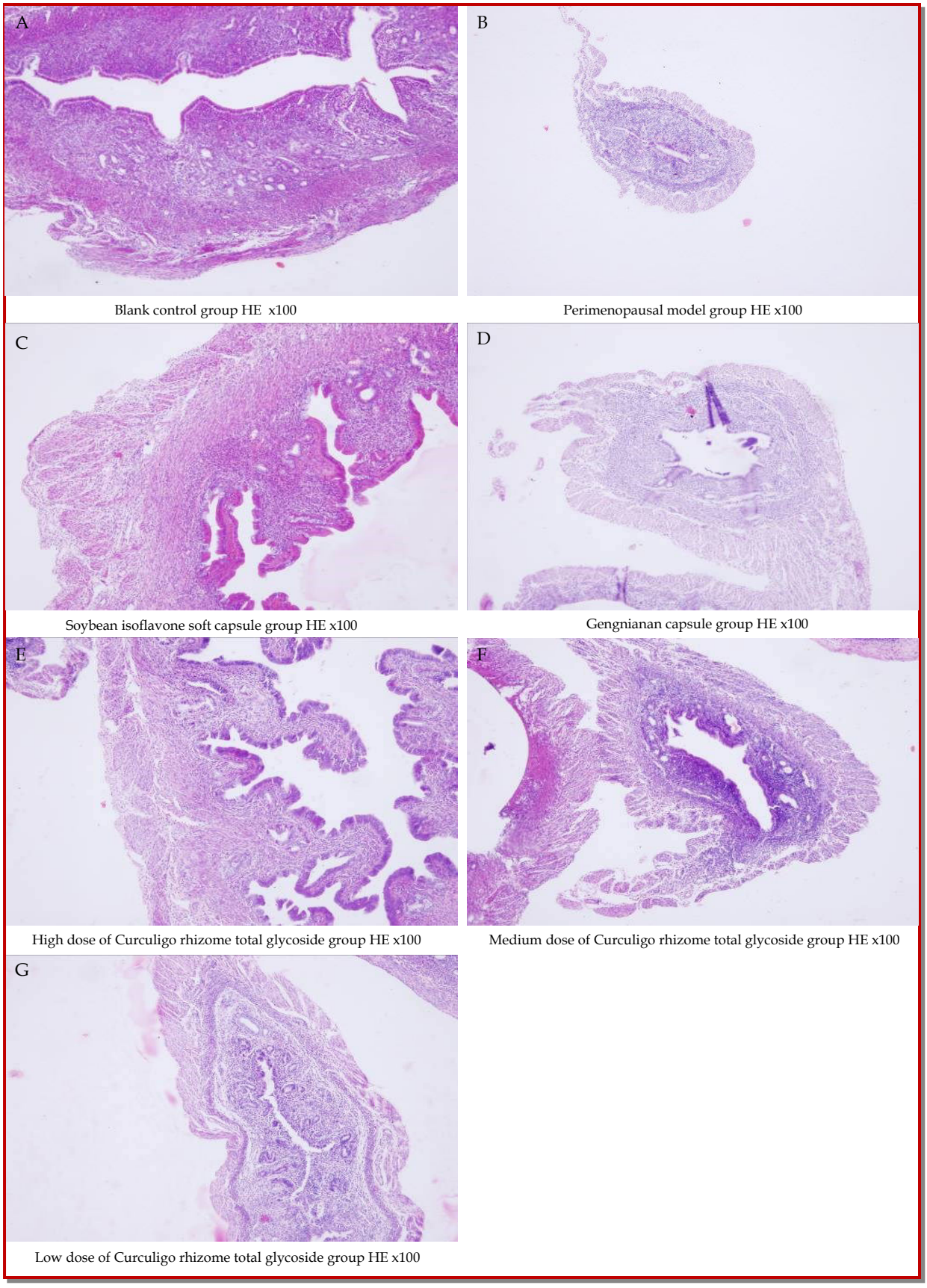

Figure 1: Effect of Curculigo rhizome total glycoside on mice model of perimenopausal uterine tissue morphology

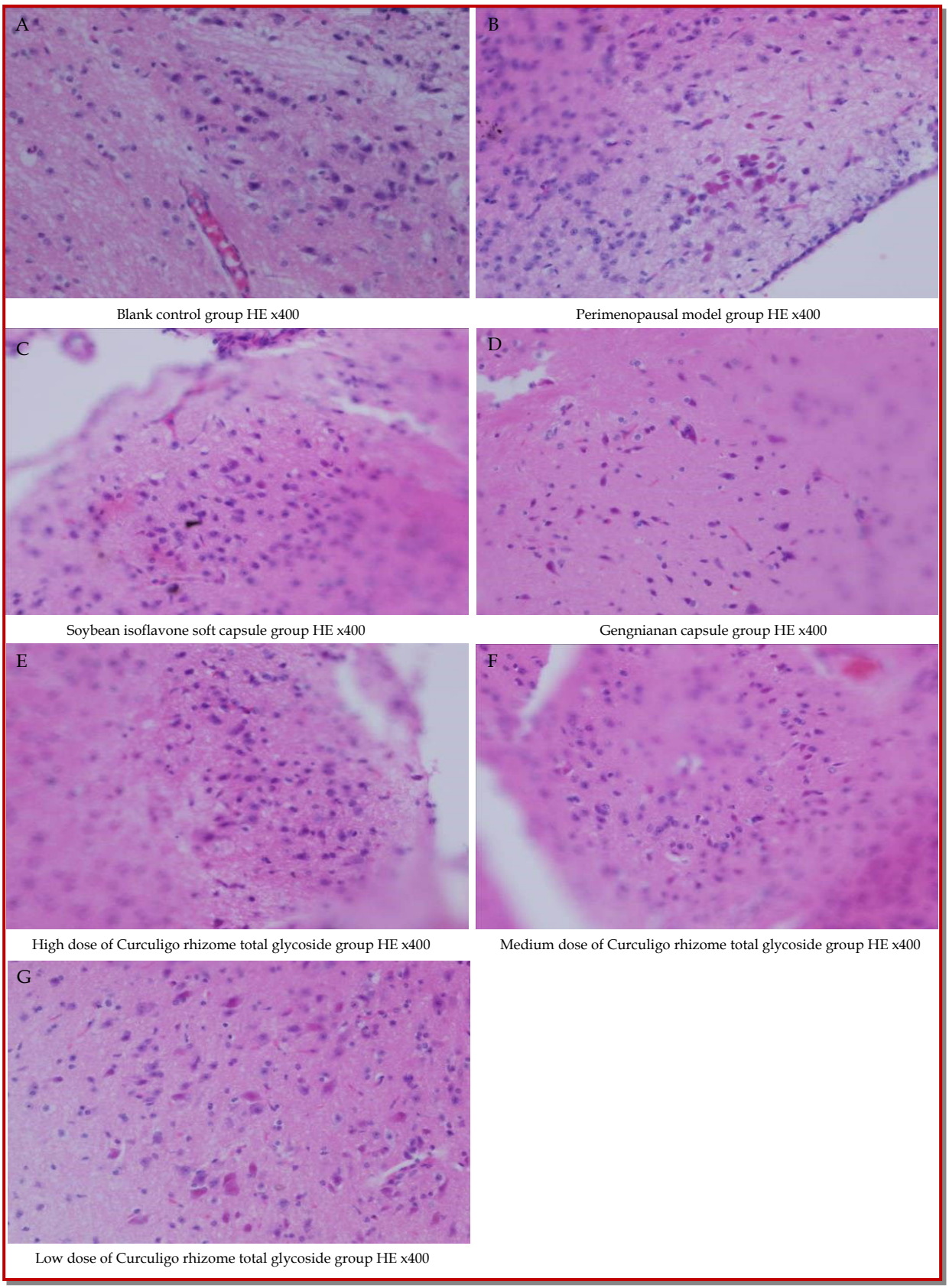

Figure 2: Effect of total glucosides of *Curculigo orchioides* in mouse hypothalamus morphological perimenopausal model

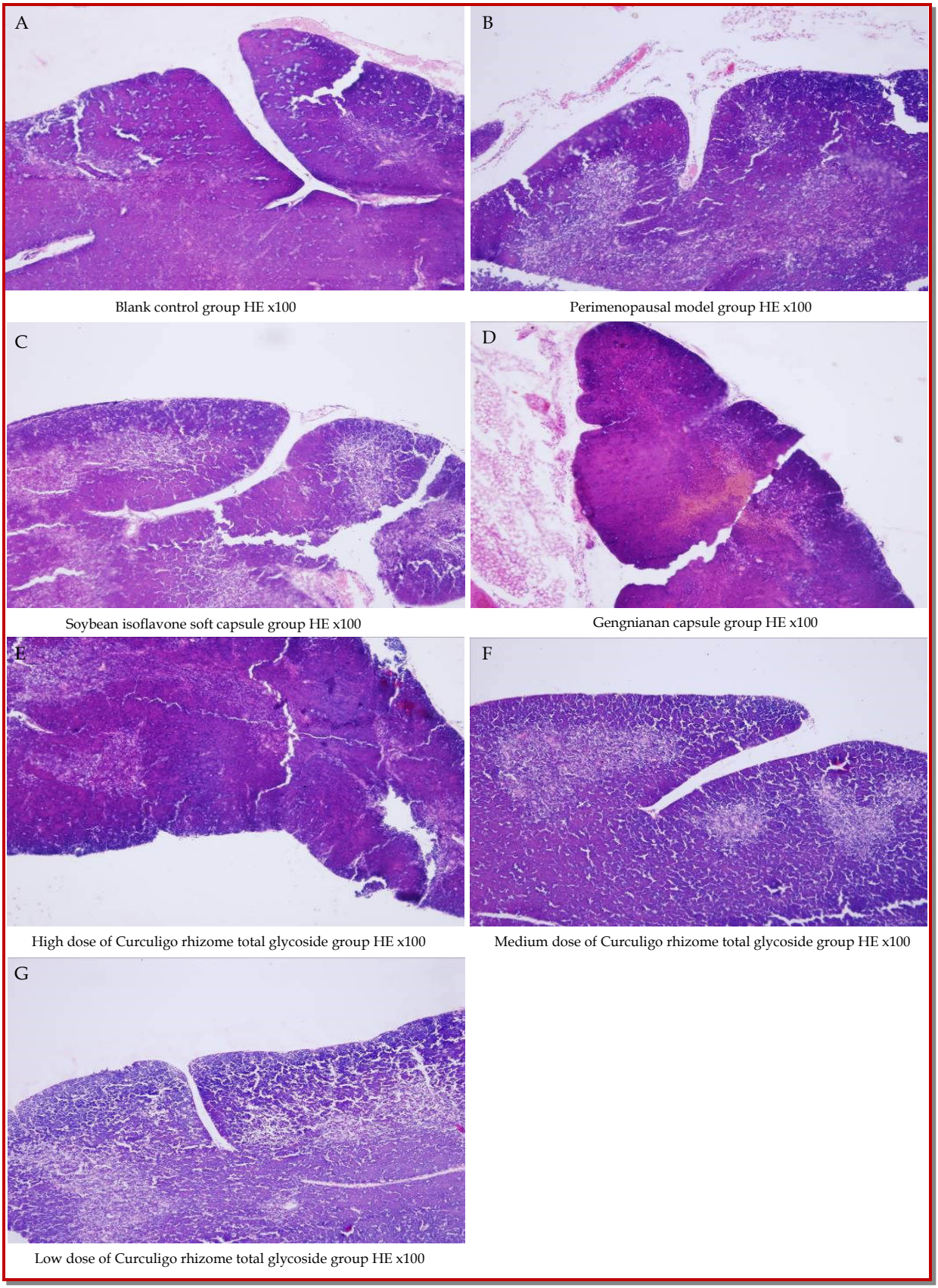

Figure 3: Effect of TGC on perimenopausal mice thymus tissue morphology

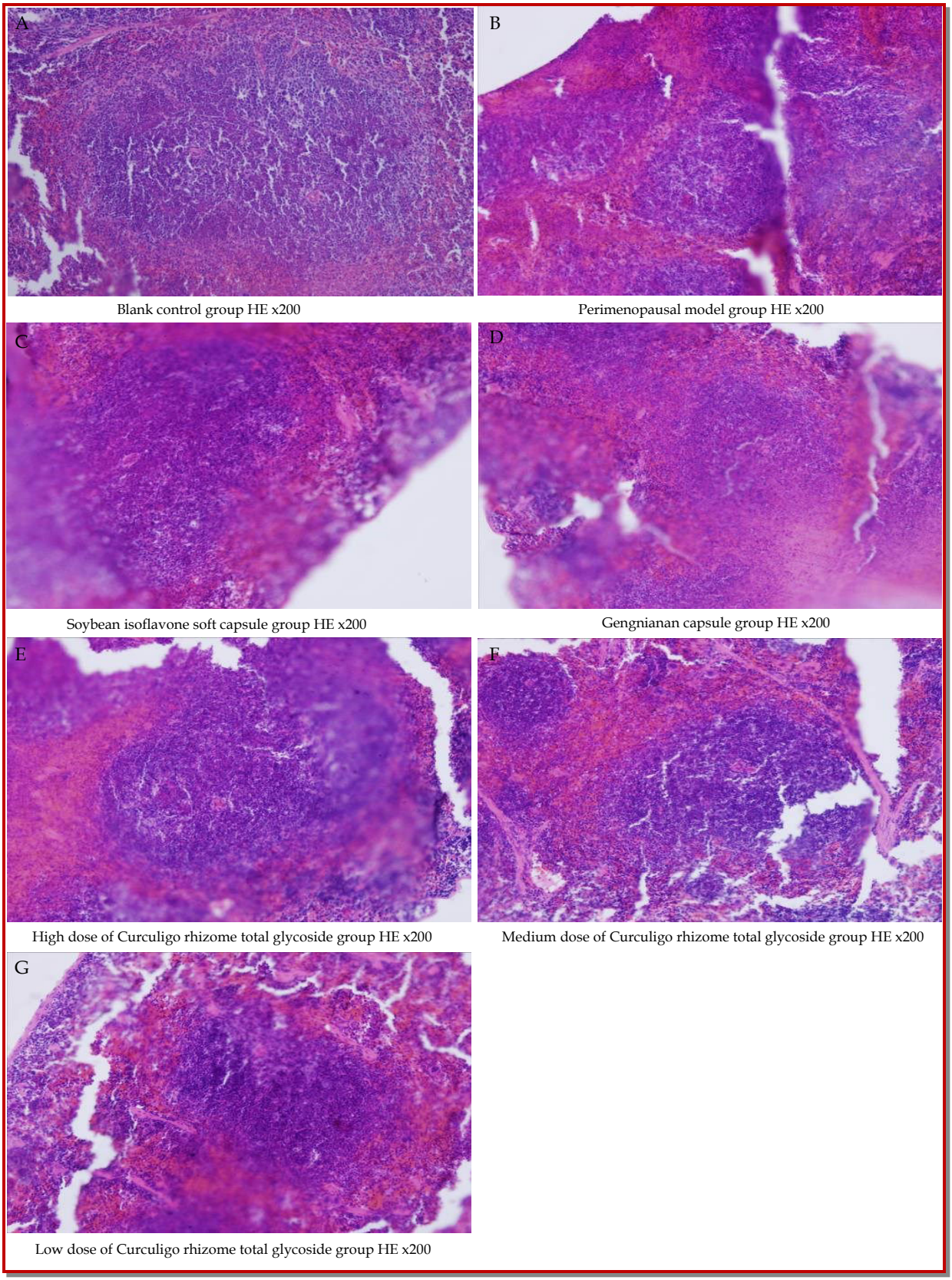

Figure 4: Effect of TGC on perimenopausal model mouse spleen tissue morphology

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