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Original Article

The Variation of Several Parameters in the Premature Ovarian Failure Model by D-Galactose and the Effect of Some Drugs on the Model: Fundamental Study

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ABSTRACT

Background and Aim: Premature Ovarian Failure (POF), or premature menopause affects 1-3% of women. Patients experience menopausal symptoms, genital dystrophy, high FSH and LH levels and low estradiol. Hormone replacement therapy and other treatments can help, but their side effects are debated. To better understand POF and develop effective treatments, we used D-galactose-induced models to test various drugs. **Methods:** Female Wistar rats (160-180 g, 16-18 weeks) were used to study D-galactose-induced Premature Ovarian Failure (POF). Rats were injected with 0.5 mL of 5% D-galactose subcutaneously for 30 days. Post-treatment, endometrium and myometrium thickness, ovary weight and volume and follicle number were measured. The POF models were then treated with *Styphnolobium japonicum* fruit extract, *Cimicifuga heracleifolia* extracts, folliculin and rigevidon and the karyopyknotic index, uterine gland number and size, ovary weight and follicle count were assessed. **Results:** In the D-galactose-induced POF models, endometrium and myometrium thickness, ovary weight and volume and mature follicle number significantly decreased. **Conclusion:** The effectiveness of drugs in reversing these effects was ranked as follows: folliculin, *Cimicifuga heracleifolia* extract, rigevidon and *Styphnolobium japonicum* extract.

Keywords: *Cimicifuga heracleifolia* extract, D-galactose, Folliculin, Premature ovarian failure, Rigevidon, *Styphnolobium japonicum* fruit extract.

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INTRODUCTION

Premature ovarian failure is an amenorrhea before the age of 40 and the primitive follicles of the ovary are very small or absent, the primitive follicles of the ovary are as numerous as normal women of age, but there is ovarian insufficiency that lacks the reactivity of the ovary to gonadotropin.[1] Women with POF may experience irregular or absent menstrual periods, hot flashes, night sweats, irritability and decreased libido, among other symptoms. The condition can arise due to genetic factors, autoimmune disorders, certain medical treatments like chemotherapy, or idiopathic causes where the reason remains unknown. Diagnosis typically involves a combination of clinical evaluation, blood tests to measure hormone levels and sometimes genetic testing. Detailed elucidation of the pathology and changes in the development of ovarian failure is a significant issue in protecting the health of middle-aged women and reducing the prevalence of disease.[2]

Styphnolobium japonicum has widespread uses in Chinese and European medicine. The chemical profile of SJ shows a broad variety of secondary metabolites with a wide array of pharmacological effects. It has various properties such as such as antibacterial, antihyperglycemic, anti-obesity, antihemorrhagic, antioxidant and anti-inflammatory.[3] A well-known traditional Chinese medicine, dried rhizome of *Cimicifuga heracleifolia* Kom. (*C. heracleifolia*) is widely used across Asian countries for its anti-inflammatory, antipyretic and analgesic properties.[4] Folliculin an tumor suppressor protein has found some use in involutional states. Similarly, rigevidon has found some utility in premature ovarian failure.[5,6]

We have developed a model of premature ovarian failure by D-galactose loading and studied to reveal the changes that occur in the uterus and ovary and the effect of some drugs on the model.

MATERIALS AND METHODS

Study Population

Female Wistar rats with 160-180 g were used as experimental animals.

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Grouping

Study group 1: Female Wistar rats received *Styphnolobium japonicum* fruit extract (150 mg/100 g, 60 days, catheter transfusion).

Study group 2: Female Wistar rats received *Cimicifuga heracleifolia* extract (150 mg/100 g, 60 days catheter transfusion).

Study group 3: Female Wistar rats received folliculin (2IU/100 g, 20 days, intramuscular injection).

Study group 4: Female Wistar rats received rigevidon (0.56 /100 g, 30 days catheter transfusion).

Materials

D-galactose was used to make premature ovarian failure model and *Styphnolobium japonicum* fruit extract, *Cimicifuga heracleifolia* extract, folliculin, rigevidon were used as study drugs on this model.

Methods

Method to elucidate variation of several parameters in the premature ovarian failure model by D-galactose

In 16-18 weeks old female rats with 160-180 g in body weight, 5% D-galactose 0.5 mL was subcutaneously injected in epigastric region for 30 days. After that, changes in thickness of endometrium and myometrium, weight and volume of ovaries and follicle count were observed. The thickness of endometrium was measured as the distance from the epithelial surface to the lamina propria and the thickness of myometrium was measured as the distance from the mucosal lamina propria to the perimetrium in the optical microscope field of view. The ovarian weight was measured by an electronic scale and it was converted into grams per 100g of body weight and the ovarian volume per 100 g of body weight was measured by the formula 4.19×[(L+S)/2] using the Long diameter (L) and Short diameter (S) of the ovary with calipers.

The ovarian follicle count was estimated by calculating the number of the primitive follicles, the development follicles, the mature follicles of three fields in 100 folds of view under the optical microscope of the ovarian tissue sample and calculated the average number per field.

Method to elucidate the effect of some drugs on the premature ovarian failure model by D-galactose

Styphnolobium japonicum fruit extract (150 mg/100 g, 60 days, catheter transfusion) was applied in study group 1, *Cimicifuga heracleifolia* extract (150 mg/100 g, 60 days catheter transfusion) was applied in study group 2, folliculin (2IU/100 g, 20 days, intramuscular injection) was applied in study group 3, rigevidon (0.56 μg/100 g, 30 days catheter transfusion) was applied in study group 4.

The karyopyknotic index of vaginal epithelial cells was measured by calculating the proportion of superficial and intermediate mature cells with unstructured chromatin and concentrated small nuclei by observing 100 vaginal epithelial cells at any three fields of 100-fold of view under the optical microscope of vaginal smear samples.

The uterine glands count was calculated in 400 folds of view under the optical microscope of the uterine tissue sample, the uterine glands size was averaged by selecting two of the largest and smallest of the uterine glands present in the sample and measuring its short diameter.

The ovarian weight was measured by an electronic scale and it was converted into grams per 100 g of body weight,

The ovarian follicle count was estimated by calculating the number of the primitive follicles, the development follicles and the mature follicles of three fields in 100 folds of view under the optical microscope of the ovarian tissue sample and calculated the average number per field.

Statistical Analysis of Data

All data were expressed as mean±Standard error. p value less than 0.05 was considered as statistically significant.

RESULTS

Changes of thickness of endometrium and myometrium

As shown in Table 1, the thickness of endometrium and myometrium in the model group was 19.5±2.1 μm, 135.3±12.7 μm respectively and was significantly lower compared with to the normal group ($p<0.05$).

Change of weight and volume of ovaries

As shown in Table 2, the weight and volume of ovaries in the model group was 0.028±0.003 g/100 g, 501.5±49.5 g/100 g respectively, was significantly smaller compared with to the normal group ($p<0.05$).

Changes of the follicle count

As a shown in Table 3, the mature follicle count in the model group was 0.5±0.07 head per a field, was significantly smaller compared with to the normal group ($p<0.05$).

Table 1: Changes of thickness of endometrium and myometrium ($\bar{X}$±SE, μm)

Groups	Endometrium thickness	Myometrium thickness
Normal (n=7)	35.1±3.8	198.3±21.7
Model (n=7)	19.5±2.1*	135.3±12.7*

*p<0.05 (compared with normal group).

Effect of drugs on the karyopyknotic index of the POF model

As shown in the Table 4, the karyopyknotic index in the study group 1, 2, 3 and 4 was 31.2±4.1, 34.4±4.5, 38.4±5.7, 36.1±5.2 respectively, was significantly higher compared with to the model group ($p<0.05$), but there was not significant difference between study groups.

Table 2: Change of weight and volume of ovaries (mean±SE).

Groups	Ovarian weight (g/100 g)	Ovarian volume (mm^3/100 g)
Normal (n=7)	0.039±0.004	755.3±93.5
Model (n=7)	0.028±0.003*	501.5±49.5*

*$p<0.05$ (compared with normal group).

Effect of drugs on the count and size of the uterine glands of the POF model

As shown in the Table 5, the uterine glands count in the study group 1, 2, 3 and 4 was 9.8±1.1head/field, 10.7±1.9 head/field, 13.4±2.4 head/field and 11.6±2.3 head/field respectively, was significantly larger compared with to the model group ($p<0.05$), but there was no significant difference between study groups.

Also, the uterine glands size in the study group 2, 3 and 4 was 24.4±1.5 μm, 25.8±1.8 μm, 24.6±1.4 μm respectively, was significantly higher compared with to the model group, but there was no significant different between the study groups.

The effect of drugs on the ovarian weight of the POF model

As shown in the Table 6, the ovarian weight in the study group 1, 2, 3 and 4 was tended to increase compared with to the model group, but there was not significant difference between model and study groups.

Table 3: Changes of the follicle count (mean±SE, head/field).

Groups	Primitive	Development	Mature
Normal (n=7)	48.7±7.1	7.9±1.1	1.1±0.22
Model (n=7)	33.6±3.8	6.4±0.7	0.5±0.07*

*$p<0.05$ (compared with normal group).

Table 4: The effect of some drugs on the karyopyknotic index of the premature ovarian failure model by D-galactose (mean±SE).

Groups	Applied drugs	Dose	Karyopyknotic Index
Normal (n=7)	-	-	19.7±2.8
Study group 1 (n=7)	*Styphnolobium japonicum* fruit extract	150mg/100g	31.2±4.1*
Study group 2 (n=7)	*Cimicifuga heracleifolia* Extract	150mg/100g	34.4±4.5*
Study group 3 (n=7)	Folliculin	2IU/kg	38.4±5.7*
Study group 4 (n=7)	Rigevidon	0.56μg/100g	36.1±5.2*

*$p<0.05$ (compared with normal group).

Table 5: The effect of some drugs on the count and size of the uterine glands of the POF model (mean±SE).

Groups	Applied drugs	Dose	Count (head/field)	Size (μm)
Normal (n=7)	-	-	5.7±0.9	19.3±1.7
Study group 1 (n=7)	*Styphnolobium japonicum* fruit extract	150mg/100g	9.8±1.1*	21.2±1.1
Study group 2 (n=7)	*Cimicifuga heracleifolia* Extract	150mg/100g	10.7±1.9*	24.4±1.5*
Study group 3 (n=7)	Folliculin	2IU/kg	13.4±2.4*	25.8±1.8*
Study group 4 (n=7)	Rigevidon	0.56μg/100g	11.6±2.3*	24.6±1.4*

*$p<0.05$ (compared with normal group).

Table 6: **The effect of some drugs on the ovarian weight of the POF model (mean±SE).**

Groups	Applied drugs	Dose	Weight (g/100 g)
Normal (n=7)	-	-	0.028±0.003
Study group 1 (n=7)	Styphnolobium japonicum fruit extract	150 mg/100 g	0.029±0.003
Study group 2 (n=7)	Cimicifuga heracleifolia Extract	150 mg/100 g	0.030±0.003
Study group 3 (n=7)	Folliculin	2IU/kg	0.034±0.004
Study group 4 (n=7)	Rigevidon	0.56μg/100g	0.031±0.003

NS.

Table 7: **The effect of some drugs on the ovarian follicle count of the POF model (mean±SE, head/field).**

Groups	Applied drugs	Dose	Primitive	Development	Mature
Normal (n=7)	-	-	33.6±3.8	6.4±0.7	0.5±0.07
Study group 1 (n=7)	Styphnolobium japonicum fruit extract	150 mg/100 g	39.8±3.1	5.2±0.5	0.7±0.07
Study group 2 (n=7)	Cimicifuga heracleifolia extract	150 mg/100 g	40.7±3.9	5.4±0.5	0.8±0.08*
Study group 3 (n=7)	Folliculin	2 IU/kg	43.4±4.4	5.8±0.8	0.9±0.10*
Study group 4 (n=7)	Rigevidon	0.56μg/100g	41.6±4.3	5.6±0.6	0.6±0.06

*p<0.05 (compared with normal group).

The effect of drugs on the ovarian follicle count of the POF model

As shown in Table 7, the count of primitive follicle and developmental follicle in the study group 1, 2, 3 and 4 were not significant compared to the model group, but mature follicle count in the study group 2 and 3 was 0.8±0.08 head/field and 0.9±0.1 head/field respectively, was significantly increased compared to the model group.

DISCUSSION

According to data, galactosemia causes premature menopause among women.[7] During galactosemia, accumulation of galactose and its wastes causes disorders of liver cells, eyes, kidneys, nervous and reproductive system. Some researchers have said that among the patients with galactosemia, due to deficiency of galactose-1-phosphate uridine converting enzyme, fibroconnective tissues were full inside ovarian cortex in ovary biopsy and develops primary menopause or POF.

According to our research, in premature ovarian failure models by D-galactose, the thickness of endometrium and myometrium, the weight and volume of ovaries, the number of mature follicles were clearly reduced compared with normal group. This shows that making model by D-galactose load is possible and it is more similar to human bodies compared to models by ovariectomy.

Unless there is no absolute contraindication for estrogen supplementation, the patients with POF should receive estrogen supplementation to prevent osteoporosis, cardiovascular disease, genitourinary dystrophy and to improve QOL. But there is a little useful data about advantages and disadvantages of estrogen supplementation.[8] A systematic review of 12 studies with 806 patients with premature ovarian failure shows that there is little data which shows hormone replacement therapy is useful to bone and cardiovascular system.[9] External gonadotropin supplementation has been studied as a method to improve the ovulation rate among patients with premature ovarian failure but its effect is poor,[10] and this gonadotropin supplementation can worsen autoimmune ovarian failure.[11] Long term use of hormone supplementation is not appropriate.

According to our research, *Styphnolobium japonicum* extracts, *Cimicifuga heracleifolia* extracts, folliculin and rigevidon shows positive effect on Karyopyknotic index of vaginal epithelial cells, the number of uterine glands, the size of uterine glands, the weight of ovaries and the number of follicles in premature ovarian failure models by D-galactose. Their order is folliculine, *Cimicifuga heracleifolia* extracts, rigevidon, *Styphnolobium japonicum* extracts.

Therefore, folliculine and rigevidon are effective to improve hypoestregenemia but *Cimicifuga heracleifolia* extracts and

Styphnolobium japonicum extracts are more promising due to several side effects of long-term use of folliculine and rigevidon.

CONCLUSION

The thickness of endometrium and myometrium, weight and volume of ovaries and mature follicle count in the premature ovarian failure model by D-galactose become significiantly smaller and the drugs in the following order that had a significant effect on the premature ovarian failure model by D-galactose was folliculin, *Cimicifuga heracleifolia* extract, rigevidone and *Styphnolobium japonicum* fruit extract.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATION

POF: Premature Ovarian Failure.

REFERENCES

1. Beck-Peccoz P, Persani L. Premature ovarian failure. Orphanet J Rare Dis. 2006;1:9. doi: 10.1186/1750-1172-1-9, PMID 16722528.
2. Jankowska K. Premature ovarian failure. Prz Menopauzalny. 2017;16(2):51-6. doi: 10.5114/pm.2017.68592, PMID 28721130.
3. Thabit S, Handoussa H, ElSayed NS, Breitinger HG, Breitinger U, Wink M. A fruit extract of *Styphnolobium japonicum* (L.) counteracts oxidative stress and mediates neuroprotection in Caenorhabditis elegans. BMC Complement Med Ther. 2023;23(1):330. doi: 10.1186/s12906-023-04149-8, PMID 37726773.
4. Hu L, Song X, Nagai T, Yamamoto M, Dai Y, He L, *et al.* Chemical profile of *Cimicifuga heracleifolia* Kom. and immunomodulatory effect of its representative bioavailable component, cimigenoside on poly(I:C)-induced airway inflammation. J Ethnopharmacol. 2021;267:113615. doi: 10.1016/j.jep.2020.113615, PMID 33242624.
5. Sevringhaus EL. The use of folliculin in involutional states. Am J Obstet Gynecol. 1933;25(3):361-8. doi: 10.1016/S0002-9378(33)90240-9.
6. Buckler HM, Healy DL, Burger HG. Does gonadotropin suppression result in follicular development in premature ovarian failure? Gynecol Endocrinol. 1993;7(2):123-8. doi: 10.3109/09513599309152491, PMID 8213226.
7. Guerrero NV, Singh RH, Manatunga A, Berry GT, Steiner RD, Elsas LJ 2nd. Risk factors for premature ovarian failure in females with galactosemia. J Pediatr. 2000;137(6):833-41. doi: 10.1067/mpd.2000.109148, PMID 11113841.
8. Committee on Gynecologic Practice. Committee Opinion No. 698: Hormone therapy in primary ovarian insufficiency. Obstet Gynecol. 2017;129(5):e134-41. doi: 10.1097/AOG.0000000000002044, PMID 28426619.
9. Burgos N, Cintron D, Latortue-Albino P, Serrano V, Rodriguez Gutierrez R, Faubion S, *et al.* Estrogen-based hormone therapy in women with primary ovarian insufficiency: a systematic review. Endocrine. 2017;58(3):413-25. doi: 10.1007/s12020-017-1435-x , PMID 29039146.
10. Awwad J, Farra C, Hannoun A, Abou-Abdallah M, Isaacson K, Ghazeeri G. Idiopathic premature ovarian failure: what is the most suitable ovarian stimulation protocol? Clin Exp Obstet Gynecol. 2013;40(3):327-30. PMID 24283158.
11. Tidey GF, Nelson LM, Phillips TM, Stillman RJ. Gonadotropins enhance HLA-DR antigen expression in human granulosa cells. Am J Obstet Gynecol. 1992;167(6):1768-73. doi: 10.1016/0002-9378(92)91773-4, PMID 1471696.

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