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# Effects of Erjing Pill on Serum Hormone Levels and Bone Histomorphology in Rats with Osteoporosis

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**Abstract: Objective:** To investigate the effects of Erjing Pill on serum sex hormone levels, bone mineral density, and learning and memory function in rats with osteoporosis, and to provide experimental evidence for its potential role in the prevention and treatment of osteoporosis and related cognitive impairment. **Methods:** Female Sprague–Dawley rats were randomly assigned to a sham-operated group, model group, alendronate sodium group, and Erjing Pill group. Except for the sham-operated group, all rats underwent bilateral ovariectomy to establish a postmenopausal osteoporosis model. One week after surgery, rats in the treatment groups received alendronate sodium or Erjing Pill intervention for 12 weeks. After treatment, femoral bone mineral density and bone mineral content were measured by micro-computed tomography (micro-CT). Histopathological changes in femoral bone tissue were observed using hematoxylin and eosin (H&E) staining. Serum levels of estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and gonadotropin-releasing hormone (GnRH) were determined by enzyme-linked immunosorbent assay (ELISA). Learning and memory function were evaluated using the Morris water maze test. **Results:** Compared with the sham-operated group, rats in the model group showed decreased serum E2 levels, abnormal changes in LH, FSH, and GnRH levels, significantly reduced bone mineral density, and marked histopathological damage in femoral bone tissue, characterized by sparse and thinned trabeculae, disorganized arrangement, and partial trabecular fracture ($P < 0.05$). Compared with the model group, both the alendronate sodium group and the Erjing Pill group showed varying degrees of improvement in serum sex hormone levels, increased bone mineral density, and alleviated bone histopathological damage. Notably, Erjing Pill exhibited significant effects in regulating hormone levels and improving bone microstructure ($P < 0.05$). **Conclusion:** Erjing Pill can improve sex hormone imbalance, increase bone mineral density, and attenuate histopathological bone damage in osteoporotic rats. Its protective effects may be related to endocrine regulation and delayed bone loss. Erjing Pill may represent a promising traditional Chinese medicine intervention for the prevention and treatment of osteoporosis.

**Keywords:** Erjing Pill; Postmenopausal Osteoporosis; Sex Hormones; Bone Mineral Density.

## 1. Introduction

Osteoporosis (OP) is a systemic skeletal disease characterized by reduced bone mass, destruction of bone microarchitecture, increased bone fragility, and a higher risk of fracture [1]. It can occur at any age but is more common with aging, particularly in postmenopausal women and older men. Among these, postmenopausal osteoporosis (PMOP) accounts for a substantial proportion of cases [1]. Epidemiological data indicate that osteoporosis affects a large population in China, with a prevalence of 32.1% in women aged over 50 years and 51.6% in women aged over 65 years [2]. With the rapid aging of the population, the burden of osteoporosis is expected to further increase, accompanied by a continuous rise in osteoporotic fractures and related healthcare costs [3]. PMOP is highly associated with fragility fractures, which can seriously impair quality of life and even lead to disability or death, making it a major public health concern among middle-aged and elderly women.

Estrogen deficiency is currently recognized as a central mechanism underlying the development and progression of PMOP. Dysfunction of the hypothalamic–pituitary–ovarian axis (HPOA) leads to abnormalities in estradiol (E2), follicle-stimulating hormone (FSH), and luteinizing hormone (LH), thereby disrupting bone metabolism and accelerating bone loss [4,5]. In recent years, increasing evidence has shown that FSH and LH are not merely indicators of ovarian function decline, but may also directly participate in the regulation of bone metabolism. In particular, FSH can bind to follicle-stimulating hormone receptors (FSHRs) expressed on osteoclasts and their precursors, activate downstream signaling pathways, promote osteoclast differentiation and bone resorption, and ultimately aggravate bone loss [6–9]. Therefore, modulation of HPOA-related hormone levels, especially interference with FSH-mediated bone resorption, may represent a potential strategy for the prevention and treatment of PMOP.

In traditional Chinese medicine (TCM), PMOP is classified under the category of "Guwei" (bone flaccidity), with kidney essence deficiency and marrow depletion regarded as the fundamental pathogenesis; accordingly, tonifying the kidney and replenishing essence is considered the principal therapeutic approach [10]. The role of the HPOA in regulating reproductive function and bone metabolism is broadly consistent with the TCM theory that "the kidney governs reproduction" and "the kidney governs bone and generates marrow." Previous studies have suggested that the material basis of the TCM concept "the kidney stores essence and governs bone" is closely related to the HPOA [11]. *Polygonatum sibiricum* has traditionally been used to tonify

the kidney, replenish essence, and strengthen bones and tendons. Modern studies have shown that *Polygonatum* and its polysaccharides can improve bone metabolism by promoting osteogenesis and inhibiting osteoclast activity, thereby increasing bone mineral density and trabecular bone mass [12–14]. *Lycium barbarum* also has the functions of nourishing the liver and kidney and replenishing essence and marrow, and its active components have shown anti-osteoporotic potential [15]. Erjing Pill, first recorded in *Shengji Zonglu*, is composed of *Polygonatum sibiricum* and *Lycium barbarum* and is traditionally used to tonify the kidney and replenish essence, which is consistent with the core TCM pathogenesis of PMOP [10,16].

Therefore, the present study established a rat model of PMOP to investigate the effects of Erjing Pill on serum E2, FSH, LH, and gonadotropin-releasing hormone (GnRH) levels, as well as bone mineral density and bone histomorphology, with the aim of providing experimental evidence for its potential application in the prevention and treatment of PMOP.

# 2. Materials

## 2.1. Animals

A total of 60 specific pathogen-free (SPF) female Sprague–Dawley (SD) rats, aged 6 months and weighing 350 ± 30 g, were purchased from the Hubei Provincial Experimental Animal Research Center (Animal License No. SCXK (E) 2023-0018). The animals were housed in the Animal Experimental Center of Huanggang Academy of Traditional Chinese Medicine under controlled conditions, including a room temperature of 20 ± 2°C, adequate ventilation, and a standard light/dark cycle, with free access to food and water. All animal procedures were approved by the Experimental Animal Ethics Committee of Huanggang Hospital of Traditional Chinese Medicine (Approval No. 202409005).

## 2.2. Herbal Materials

Erjing Pill consisted of 750 g of *Polygonatum* and 750 g of *Lycium barbarum*, both obtained from the pharmacy of Huanggang Hospital of Traditional Chinese Medicine. The herbal materials were authenticated and met the standards of the *Chinese Pharmacopoeia* (2015 edition).

## 2.3. Drugs and Reagents

Alendronate sodium tablets (Batch No. H20093921; 10 mg/tablet) were purchased from Guangdong Bidi Pharmaceutical Co., Ltd. Rat ELISA kits for estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and gonadotropin-releasing hormone (GnRH) were purchased from Wuhan Kezhu Biotechnology Co., Ltd. (Batch Nos. E-EL-R0050c and E-EL-R0089c).

## 2.4. Instruments

Micro-CT scanner (Scanco Medical, Switzerland); microplate reader (Thermo Fisher Scientific, USA); HI650 centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Ltd., China).

# 3. Methods

## 3.1. Preparation of Drugs

**Preparation of Erjing Pill lyophilized powder:**

A total of 750 g of *Polygonatum* and 750 g of *Lycium barbarum* were decocted according to the standard procedure specified in the *Management Standards for Traditional Chinese Medicine Decoction Rooms in Medical Institutions* issued by the National Administration of Traditional Chinese Medicine. Briefly, the herbs were soaked in eight volumes of ultrapure water for 30 min, brought to a boil for 10 min over high heat using an automatic decocting machine, and then simmered over low heat for 40 min. The decoction was filtered through a sterile gauze bag to remove residues, and the filtrate was then freeze-dried under reduced pressure at low temperature to obtain lyophilized powder. The powder was stored in a sealed desiccator and dissolved in double-distilled water (ddH2O) before use.

**Preparation of alendronate sodium suspension:**

Alendronate sodium tablets were ground into powder and suspended in ddH2O at a final concentration of 0.63 mg/mL before use.

## 3.2. Grouping, Model Establishment, and Drug Administration

After 7 days of acclimatization, the SD rats were randomly assigned to four groups: sham-operated group, model group, Erjing Pill group, and alendronate sodium group (0.184 mg/kg), with 15 rats in each group. Except for the sham-operated group, all rats underwent bilateral ovariectomy to establish a postmenopausal osteoporosis model. Rats were anesthetized by intraperitoneal injection of 1% pentobarbital sodium (0.03 g/kg) and fixed on the operating table. Longitudinal incisions were made bilaterally at 1–2 cm lateral to the midline on the dorsal side. After entering the abdominal cavity, the ovaries were identified in the fat pads, ligated, and bilaterally removed, followed by layered suturing. In the sham-operated group, the ovaries were preserved, and only an equivalent amount of periovarian adipose tissue was removed.

After surgery, all rats received intramuscular penicillin (1.6 mL/kg) once daily for 3 consecutive days. Food was withheld for 24 h after surgery, while water was provided ad libitum. One week after surgery, rats in the treatment groups were administered the corresponding drugs by gavage once daily for 12 consecutive weeks. Rats in the sham-operated and model groups received an equal volume of 0.9% saline by gavage.

**Table 1.** Preliminary ELISA results for serum FSH, LH, and E2 levels

| Group | FSH (IU/L) | LH (mIU/L) | $E_2$ (pmol/L) |
|---|---|---|---|
| Sham-operated group | 8.26±0.28 | 39.05±5.41 | 53.18±9.39 |
| Model group | 9.59±0.10△ | 49.51±3.63△ | 39.60±3.74△ |
| Alendronate sodium group | 9.19±0.46△ | 45.29±4.66△ | 42.15±10.48△ |
| Erjing Pill group | 8.37±0.86※ | 42.36±1.76※ | 46.75±6.55※ |

**Note:** $^{\triangle}P < 0.05$ vs. sham-operated group; $^{※}P < 0.05$ vs. model group.

## 3.3. Sample Collection and Processing

At the end of the treatment period, rats were anesthetized with intraperitoneal 1% pentobarbital sodium, and blood samples were collected from the abdominal aorta using vacuum blood collection tubes. Samples were centrifuged at 3000 rpm for 20 min, and the supernatant serum was collected

and stored at −80 ∘C-80\ ^\circ\mathrm{C}−80 ∘C until analysis.

The humerus and femur were isolated, and surrounding muscle and connective tissue were carefully removed. Part of the bone tissue was fixed in 4% paraformaldehyde for staining and bone density analysis, and the remaining part was wrapped in gauze and stored at −80 ∘C-80\ ^\circ\mathrm{C}−80 ∘C for subsequent analyses. For histological staining, bone tissues fixed in 4% paraformaldehyde were decalcified in 10% EDTA solution (15-fold volume) for 4 weeks, with the decalcifying solution changed every 3 days. Decalcification was considered complete when the bone could be easily penetrated with a needle. The tissues were then washed with distilled water and fixed in ethanol solution.

### 3.4. Effects of Erjing Pill on Bone Microstructure, Bone Mineral Density, and Bone Mineral Content in Ovariectomized Rats

Frozen femoral samples were thawed and fixed on the Micro-CT sample holder. Scanning was performed at a 360° rotation angle with a resolution of 14.8 μm to obtain continuous cross-sectional images of the proximal humerus. After scanning, trabecular bone in the region of interest, defined as 1.0 mm distal to the growth plate with a thickness of 2.0 mm, was reconstructed three-dimensionally for image acquisition. Quantitative analysis was performed using the built-in Micro-CT analysis software to determine bone mineral density and bone mineral content.

### 3.5. Effects of Erjing Pill on Femoral Histopathological Changes in Ovariectomized Rats

The left femur from each rat was fixed in 10% neutral formalin and then decalcified in 20% EDTA solution. After decalcification, the tissues were dehydrated, cleared, paraffin-embedded, and sectioned into continuous slices of approximately 4 μm thickness. Hematoxylin and eosin (H&E) staining was then performed, and histopathological changes in femoral tissue were observed under a light microscope.

### 3.6. Effects of Erjing Pill on Serum E2, LH, FSH, and GnRH Levels in Ovariectomized Rats

Serum samples were collected from each group, and the levels of estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and gonadotropin-releasing hormone (GnRH) were measured according to the instructions provided with the ELISA kits.

### 3.7. Statistical Analysis

Statistical analyses were performed using SPSS version 26.0. Data are presented as mean ± standard deviation (SD). After confirmation of normal distribution and homogeneity of variance, comparisons among multiple groups were performed using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test for pairwise comparisons. A value of $P < 0.05$ was considered statistically significant.

## 4. Results

### 4.1. Effects of Erjing Pill on Bone Morphology, Bone Mineral Density, and Bone Mineral Content in Ovariectomized Rats

As shown in **Figure 1**, compared with the sham-operated group, rats in the model group exhibited thinning of the cortical bone, sparse trabeculae, a reduced number of trabeculae, and marked disruption of bone microarchitecture. Compared with the model group, both treatment groups showed an increased number of trabeculae, a more intact structure, and overall improvement in bone morphology.

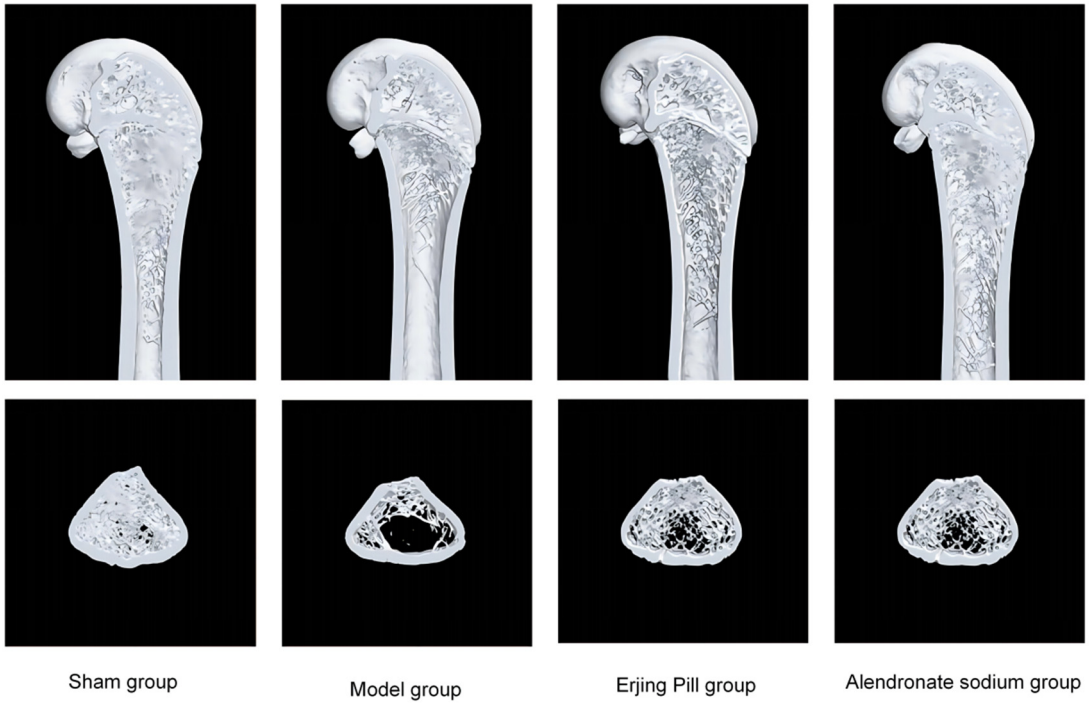



**Figure 1.** Effects of Erjing Pill on femoral bone microarchitecture in ovariectomized rats

### 4.2. Effects of Erjing Pill on Femoral Histopathological Changes in Ovariectomized Rats

As shown in **Figure 2**, the sham-operated group displayed uniform trabecular structure, regular arrangement, intact morphology, and a relatively small marrow cavity. Compared with the sham-operated group, the model group showed a reduced number of trabeculae, sparse arrangement, widened trabecular spaces, obvious enlargement of the marrow cavity, and increased fatty vacuoles, indicating marked histopathological damage in bone tissue. Compared with the model group, both treatment groups exhibited an increased number of trabeculae, more regular arrangement, reduced adipose tissue in the marrow cavity, and overall alleviation of histopathological damage.

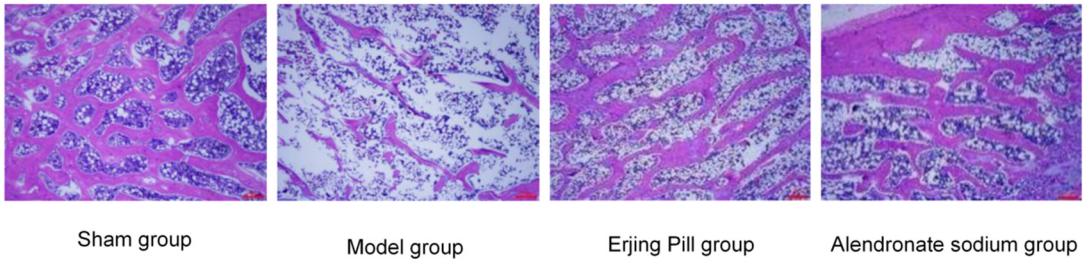



**Figure 2.** Effects of Erjing Pill on femoral histopathological changes in ovariectomized rats

### 4.3. Effects of Erjing Pill on Serum E2, LH, FSH, and GnRH Levels in Ovariectomized Rats

Compared with the sham-operated group, the model group showed decreased serum E2 levels and increased LH, FSH, and GnRH levels, with statistically significant differences ($\mathbf{P} < 0.05$ or $\mathbf{P} < 0.01$). Compared with the model group, the Erjing Pill group showed significantly increased serum E2 levels and significantly decreased LH, FSH, and GnRH levels ($\mathbf{P} < 0.05$ or $\mathbf{P} < 0.01$). The alendronate sodium group also improved these hormone levels to varying degrees. These findings suggest that Erjing Pill can ameliorate sex hormone imbalance in ovariectomized rats and improve dysfunction of the hypothalamic–pituitary–ovarian axis.

## 5. Discussion

The present study showed that ovariectomized rats in the model group exhibited decreased serum E2 levels, elevated FSH, LH, and GnRH levels, reduced bone mineral density, and marked histopathological damage to bone tissue, accompanied by impaired performance in the Morris water maze. After treatment with Erjing Pill, these abnormalities were improved to varying degrees, suggesting that Erjing Pill exerts a protective effect in rats with postmenopausal osteoporosis.

The core pathological basis of PMOP is ovarian failure after menopause and the resulting estrogen deficiency [4]. A reduction in E2 weakens its inhibitory effect on bone resorption, leading to an imbalance between bone formation and bone resorption, and ultimately causing bone loss and disruption of bone microarchitecture [1,5]. In the present study, the model group showed decreased E2 levels, reduced bone mineral density, and sparse, disorganized trabeculae, indicating successful establishment of the PMOP model and reflecting the typical pathological features of PMOP. After Erjing Pill treatment, E2 levels increased, bone mineral density improved, and bone histomorphology was ameliorated, suggesting that Erjing Pill may partially counteract estrogen deficiency and attenuate bone loss.

Recent studies suggest that FSH plays an important role in PMOP independent of estrogen deficiency. Elevated serum FSH levels are closely associated with reduced bone mineral density [6]. FSH binds to FSH receptors on osteoclasts and their precursors, activates downstream signaling pathways, promotes osteoclast differentiation and maturation, and enhances bone resorption, thereby aggravating bone loss [7–9]. In the present study, serum FSH levels were elevated in the model group but decreased after Erjing Pill treatment, suggesting that Erjing Pill may exert anti-osteoporotic effects by regulating HPOA-related hormone levels, particularly by suppressing abnormal FSH elevation. Notably, recent evidence has shown that FSH blockade may not only improve bone mass but also benefit cognitive function [17]. However, the present study did not assess FSHR expression or downstream signaling molecules, and the underlying mechanisms therefore require further investigation.

Bone mineral density and bone histomorphology are important indicators for evaluating the severity of osteoporosis [1]. In this study, the model group showed significantly reduced bone mineral density, and histological examination revealed decreased trabecular number, thinning, and fracture, indicating marked destruction of bone microarchitecture. In contrast, Erjing Pill increased bone mineral density and improved trabecular structure, suggesting that it can alleviate bone tissue damage and delay bone loss. These effects may be related to the active components of *Polygonatum* and *Lycium barbarum*, particularly polysaccharides, which have been reported to regulate endocrine function, inhibit bone resorption, and improve bone metabolism [12–15].

In addition, this study found impaired learning and memory performance in PMOP model rats, as reflected by prolonged escape latency in the place navigation test and reduced platform crossings and target quadrant retention time in the spatial probe test. These findings suggest that PMOP may be associated not only with abnormal bone metabolism but also with a certain degree of cognitive impairment. Previous studies have shown that estrogen is involved not only in bone metabolism but also in hippocampal synaptic plasticity and learning and memory processes. Moreover, elevated FSH levels may also contribute to cognitive dysfunction [17]. Thus, postmenopausal estrogen deficiency and endocrine imbalance may represent a common basis for both bone loss and cognitive decline. Improvement in Morris water maze

performance after Erjing Pill treatment suggests that Erjing Pill may protect learning and memory function while improving bone metabolism.

From the perspective of traditional Chinese medicine, PMOP belongs to the category of "Guwei" (bone flaccidity), and its fundamental pathogenesis lies in kidney essence deficiency [10,11]. According to TCM theory, the kidney governs bone and generates marrow; sufficient kidney essence nourishes the skeleton, whereas kidney essence deficiency leads to bone malnutrition and bone flaccidity. Erjing Pill, composed of *Polygonatum* and *Lycium barbarum*, is traditionally used to tonify the kidney and replenish essence, which is consistent with the core pathogenesis of PMOP. In the present study, Erjing Pill improved sex hormone levels, bone mineral density, bone histomorphology, and learning and memory performance in PMOP model rats, providing experimental support for the therapeutic principle of tonifying the kidney and replenishing essence in the treatment of PMOP.

Several limitations of this study should be acknowledged. First, only serum hormone levels were measured, and the FSH/FSHR-related pathway was not further validated at the molecular level. Second, this was an animal study, and the findings still require confirmation in clinical studies. Third, the mechanisms by which Erjing Pill improves learning and memory function were not explored in depth. Future studies should incorporate bone metabolism-related proteins and neurofunctional markers to clarify the underlying mechanisms.

In conclusion, Erjing Pill can improve sex hormone imbalance, increase bone mineral density, alleviate bone tissue injury, and partially ameliorate learning and memory impairment in PMOP model rats. Its protective effects may be associated with regulation of HPOA function, improvement of endocrine imbalance, and attenuation of bone loss.

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