RESEARCH ARTICLE

# Effect of Herba Epimedium Extract on Bone Mineral Density and Microstructure in Ovariectomised Rat

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## ABSTRACT

**Aim** To observe the effects of Herba Epimedium (HE) extract on bone mineral density (BMD) and microstructure in ovariectomised (OVX) rat.

**Methods** A total of 84 female Sprague–Dawley rats of 3-month-old were randomly divided into OVX group ($n = 70$) and sham group ($n = 14$). The osteoporotic model was established by ovariectomy. Twelve weeks after ovariectomy, when the osteoporosis (OP) was successfully affirmed by the BMD, the rats in OVX group was randomly divided into OVX group ($n = 14$), low-dose HE group ($n = 14$), middle-dose HE group ($n = 14$), high-dose HE group ($n = 14$) and positive group ($n = 14$). The BMD was measured by dual-energy X-ray. The microstructure of the bone was observed by micro-computed tomography.

**Results** Twelve weeks after ovariectomy, the BMD of femur of rats in OVX group was significantly lower than that of in sham group ($P < 0.05$). After 12 weeks of treatment, the BMD of femurs of rats in HE and positive groups were significantly higher than that in OVX group ($P < 0.05$), also the bone microstructure of rats in HE group has been recovered as the positive group.

**Conclusion** HE has strong therapeutical effect on OP, can improve the BMD and microstructure of bones.

## INTRODUCTION

Osteoporosis (OP) is a disease characterised by low-bone mass and deterioration of bone structure that causes bone fragility and increases the risk of fracture. OP has become a major health problem, which is expected to worsen with aging populations worldwide[1]. During the past three decades, a range of medications has been used for the prevention and treatment of OP, but some of them are entirely satisfactory. For example, bisphosphonates can lead to atypical femoral fractures and osteonecrosis of the jaw with long-term use, although it can effectively reduce the loss of bone mass; hormone replacement therapy is recommended only in women who also have menopausal symptoms, and some drugs like Raloxifene can increase the risk of blood clots and strokes. Denosumab, a fully human monoclonal antibody, acts as a new drug for the treatment of OP, has also many side effects include low calcium blood levels, infections, skin and jawbone problems, and pain in the muscles, back, arms and legs[2]. Therefore, it is always necessary to look for some novel therapies of treatment. Many herbs have been used in the treatment of OP and other bone diseases in China and other East Asian countries for many hundred years. Of all the natural alternatives currently undergoing investigation, Chinese medicines have received the most attention. *Herba Epimedii* (HE) is an important medicinal plant; it has been applied in various traditional Chinese formulations for thousands of years[3]. HE has been often used for the treatment of sexual dysfunction and OP[4].

To explore the effect of HE on OP, this study aims to evaluate the effects of HE in ameliorating bone loss and microstructure in ovariectomised (OVX) rats.

## MATERIALS AND METHODS

### *Animals and diets*

A total of 84 specific pathogen-free level female Sprague–Dawley rats were purchased (Animal Center of Guangdong Province, China) and acclimated



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to laboratory conditions for 2 weeks before the study. The animals were raised in an air-conditioned room at constant temperature (23 ± 2°C) and humidity (45–50%), with 12/12 h light–dark illumination cycles. The animals were fed in the Laboratory Animal Management Centre of Jinan University Medical College (Guangzhou, China). At the beginning of the study, the animals were 3 months of age with an average weight of 250 ± 20 g.

### Chemicals

HE (EPE-120215, purity: 98%) was purchased from Changsha Nutramax Company Limited (Changsha, China).

### Ovariectomy and administration of HE

The rats were randomly divided into two groups, the sham-operated group ($n$ = 14) and the OVX group ($n$ = 70). Bilateral ovariectomies were performed under pentobarbital sodium (0.15 mL/100 g, i.p.) anaesthesia. The sham-operated rats were subjected to surgery exposing the ovaries without moving. Three months later, the bone mineral density (BMD) of the femurs of all the rats were determined using GE Lunar Prodigy (GE Healthcare Bio-Sciences, Pittsburgh, USA) to evaluate whether the model had been successfully established.

Once the model is successful, the OVX rats were randomly divided into five groups of 14 animals: OVX group (double distilled water (0.2 mL/100 g, daily, administered orally)), positive group (70 mg/kg, weekly, administered orally) and OVX–HE groups (0.11, 0.33 and 0.99 g/kg, daily, administered orally) and treated for 12 weeks. The 0.11, 0.33 and 0.99 g/kg doses of HE were designated low (L-HE), medium (M-HE) and high (H-HE) groups, respectively.

After 12 weeks of treatment, the animals were then sacrificed using pentobarbital sodium, and the femurs were dissected and stored in a freezer at −80°C.

### Measurement of BMD

BMD (g/cm$^3$) is log-normally distributed for all groups at the femoral neck but normally distributed at the trochanter in men and postmenopausal women, so the assessment of BMD can be used to evaluate OP in the clinic. After 12 weeks of treatment, BMD (g/cm$^3$) was assessed by GE Lunar Prodigy (GE Healthcare Bio-Sciences, Pittsburgh, USA) using the small animal scan mode with the rats under pentobarbital sodium (0.15 mL/100 g, i.p.) anaesthesia. BMD (mg/cm$^3$) of the femur was measured in all the rats.

### Morphometric measurements

In recent years, micro-computed tomography (micro-CT) has become true for the evaluation of bone architecture. Compared with other imaging techniques, micro-CT is more effective for detecting early bone changes that allow fracture prediction and assessment of potential antiosteoporotic agents. The femurs were placed in the micro-CT scanning device (μCT 80; Scanco Medical AG, Switzerland) and the scanned images were obtained from different sections of the same specimen. Each plane was 20 μm. Morphometric parameters, including trabecular thickness (Tb.Th, μm), trabecular number (Tb.N, 1/mm) and trabecular separation (Tb.Sp, μm) were measured using the software and the bone tissue was reconstructed through 3D images.

### Statistical analysis

The statistical analyses were performed using SPSS software (version 19.0). The data are presented as mean value ± standard deviation (SD). The effects of HE in OVX rats were analysed by one-way analysis of variance (ANOVA), followed by Dunnett's test (post hoc analysis). $P$ values <0.05 were considered to be significant.

## RESULTS

### BMD

The BMD of OVX rats was significantly lower than that of sham-operated rats. After 12 weeks, the BMD of OVX rats treated with HE was significantly higher than that of the rats of untreated OVX rats (Table 1).

As shown in Table 1a, BMD of the femurs of OVX rats were decreased significantly compared with the sham-operated group. As shown in Table 1b, compared with the OVX group, administration of HE to OVX rats for 12 weeks significantly restored BMD. $^{*}P < 0.05$ vs. sham-operated, $^{**}P < 0.01$; $^{▲}P < 0.05$ vs. OVX; $^{▲▲}P < 0.01$.

### Morphometric analysis

Morphological observations were quantitated by the histomorphometric analysis of longitudinal cross-sections collected 2 cm away from the proximal femur

**Table 1** Effects of OVX and HE on BMD (g/cm$^2$), ($\bar{x} \pm S$, $n$ = 10).

| Groups | Left femur | Right femur |
|---|---|---|
| a: Effect of OVX on BMD | | |
| Sham | 0.310 ± 0.013 | 0.340 ± 0.020 |
| OVX | 0.247 ± 0.005* | 0.262 ± 0.006* |
| b: Effect of HE on BMD in OVX rats | | |
| Sham | 0.280 ± 0.04 | 0.256 ± 0.04 |
| OVX | 0.197 ± 0.01* | 0.200 ± 0.03* |
| Positive | 0.252 ± 0.03▲▲ | 0.248 ± 0.02▲▲ |
| L-HE | 0.262 ± 0.02▲▲ | 0.264 ± 0.02▲▲ |
| M-HE | 0.257 ± 0.02▲▲ | 0.275 ± 0.01▲▲ |
| H-HE | 0.281 ± 0.01▲▲ | 0.284 ± 0.04▲▲ |

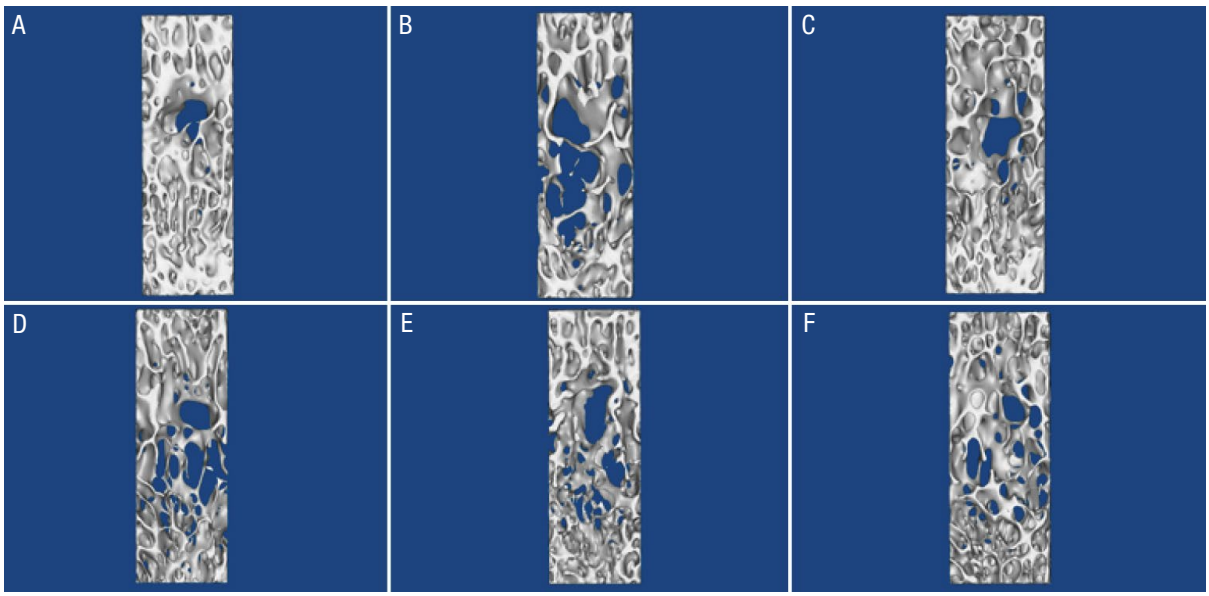


**Fig. 1** Micro-computed tomography analysis for the (A) sham, (B) OVX, (C) positive, (D) L-HE, (E) M-HE and (F) H-HE groups. OVX: ovariectomy, HE: Herba Epimedium, sham: sham-operated, positive: treated with alendronate sodium, L-HE: low-dose HE (0.11 g/kg/day), M-HE: medium-dose HE (0.33 g/kg/day), HI-HE: high-dose HE (0.99 g/kg/day).

**Table 2** Effects of HE on bone microstructure ($\bar{x} \pm S$, $n = 10$).

| Groups | Tb.N (1 mm$^{-1}$) | Tb.Sp (μm) | Tb.Th (μm) |
|---|---|---|---|
| Sham | 248.244 ± 25.94 | 1.750 ± 0.56 | 96.959 ± 9.97 |
| OVX | 101.911 ± 16.89* | 5.038 ± 0.27* | 56.822 ± 6.95* |
| Positive | 158.438 ± 16.70▲▲ | 4.095 ± 0.45▲ | 70.921 ± 4.52▲▲ |
| L-HE | 163.375 ± 40.59▲▲ | 4.161 ± 1.01▲ | 62.834 ± 6.62 |
| M-HE | 175.586 ± 26.42▲▲ | 4.378 ± 1.26 | 51.419 ± 6.39 |
| H-HE | 236.395 ± 51.23▲▲ | 3.453 ± 0.58▲▲ | 67.181 ± 7.31▲▲ |

Tb.N: trabecular number, Tb.Th: trabecular thickness, Tb.Sp: trabecular separation, OVX: ovariectomy, HE: Herba Epimedium, sham: sham-operated, positive: treated with alendronate sodium, L-HE: low-dose HE (0.11 g/kg/day), M-HE: medium-dose HE (0.33 g/kg/day), HI-HE: high-dose HE (0.99 g/kg/day). *$P < 0.05$, **$P < 0.01$ vs. sham-operated, ▲$P < 0.05$ vs. OVX, ▲▲$P < 0.01$.

using micro-CT. Marked bone loss was observed in the OVX rats compared with the sham-operated group (Fig. 1A). In comparison with the sham group, the bone trabecula is loose, fractured and exhibits loss in the OVX group (Fig. 1B). Following oral administration of Fosamax (Fig. 1C) and HE (Fig. 1D–F), the number of bone trabecula was clearly increased, and the bone trabecula was more densely concatenated than that in the OVX group. The effect of H-HE is the best.

### *Effects of HE on bone microstructure*

As shown in Table 2, compared with the sham group, Tb.N and Tb.Th decreased significantly in the OVX group ($P < 0.05$); whereas, Tb.Sp increased significantly ($P < 0.05$). Compared with the OVX group, the H-HE group had significantly increased Tb.N and Tb.Th ($P < 0.01$), while Tb.Sp decreased significantly ($P < 0.01$); whereas, in the M-HE group, Tb.N increased significantly ($P < 0.05$), in the L-HE group, Tb.N increased significantly ($P < 0.01$) and Tb.Sp decreased significantly ($P < 0.01$). The effect of H-HE is the best.

## DISCUSSION

The main index for evaluating the effect of postmenopausal OP (POP) is the increase in BMD[5]. Estrogen deficiency can lead to a decline in BMD in both OVX rats and postmenopausal women[6–7]. The research shows that the treatment with HE effectively prevents the loss of bone mass caused by lack of estrogen[8].

Our study shows that the bone changes in OVX rats, especially decline in BMD, are similar to those in POP women. HE can significantly improve BMD in OVX rats.

Bone microstructure is one of the important factors affecting the quality of bone, which along with bone

mass represent bone strength[9]. In OVX rats, the trabecular bone was obviously thinner and shorter. The parts of the trabecular bone were destroyed or even disappeared. The number of trabecular decreased noticeably and there was a visible osteoclast (OC) reaction. While, treated with HE reduced trabecular bone lesions, especially in the secondary spongy areas and reduced the number of bone resorption pouches and OC. H-HE can also recover the Tb.N, Tb.Th and Tb.Sp just as positive group.

## CONCLUSION

This study has demonstrated that H-HE can effectively increase BMD of the femurs, increased Tb.N and Tb.Th, but reduced Tb.Sp, all of which contribute to the treatment of OP.

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