Effect of Jianpi Bushen formula on aromatase-inhibitor-associated bone loss after menopause

Dai Yan, Chen Qingqing, Guan Ruodan, Xu Rui, Qiu Chang, Song Xue, Guo Qianqian, Wang Zhiyu, Chen Qianjun

Abstract

OBJECTIVE: To investigate the effect of Jianpi Bushen (JPBS) formula on aromatase inhibitor (AI)-associated bone loss after menopause.

METHODS: Six-month-old female rats were randomly divided into 6 groups: a sham group, an ovariectomized (OVX) group, an OVX treated with exemestane and 3 OVX groups each treated with a different dose of JPBS formula. Bone mineral density (BMD) at the lumbar vertebrae, histology, bone markers and serum levels of estrogen were assessed. Furthermore, a cohort study was conducted in 130 postmenopausal women with breast cancer that had undergone treatment with AIs. The subjects were given JPBS + caltrate D or caltrate D only, administered orally. BMD at the lumbar vertebrae and femoral neck and bone markers were evaluated in both control and herbal treatment groups at baseline and 12 months.

RESULTS: Experimental results indicated that a high dose of JPBS significantly increased the trabecular bone area percentage (Tb.Ar %) and broadened the trabecular thickness (Tb.Th). The JPBS formula enriched the carboxyterminal propeptide of type I procollagen and increased serum estrogen level significantly. The clinical investigation revealed that bone loss was decreased in the group treated with JPBS vs control (BMD T score at lumbar vertebrae, 3.9% increased vs 14.58% decreased, respectively, \( P = 0.004 \) and BMD T score on femoral neck, 1.8% decreased vs 22.45% decreased, respectively, \( P = 0.008 \)). Besides, JPBS formula elevated N-middle osteocalcin and decreased type I collagen cross-linked C-terminal telopeptide.

CONCLUSION: JPBS formula prevented aromatase-inhibitor-associated bone loss after menopause by inhibiting bone resorption and promoting bone formation.

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Keywords: Aromatase inhibitors; Menopause; Bone resorption; Osteogenesis; Jianpi Bushen formula

INTRODUCTION

Breast cancer has become the most common cancer in Chinese women. More than 1.6 million people are diagnosed and 1.2 million people die of the disease each...
year. Third-generation aromatase inhibitors (AIs), including exemestane (EXE), anastrozole and letrozole, have been replacing tamoxifen (TAM) as adjuvant therapy for postmenopausal women with hormone receptor-positive breast cancer due to their superior efficacy shown in several head-to-head trials. However, AIs accelerate bone loss at least 2-fold in women treated with them when compared to healthy women of the same age. As a result, AIs increase the incidence ratio of bone fractures. The accelerated bone loss and increased incidence of bone fractures is called aromatase-inhibitor-associated bone loss (AIBL). Patients taking AIs experience significantly more bone fractures than those taking TAM. The fracture ratio is about 7%-11% with AIs over 3-5 years. The widespread use of AIs to treat hormone receptor-positive breast cancer in women presents a challenge for maintaining good bone health. Bone fractures not only worsen quality of life, but also increase medical expenses and mortality. According to reported statistics, the average cost after an osteoporosis-related hip fracture is around 20 000-30 000 RMB for hospitalization in China and increases approximately 6.14% per year. Notably, medical care costs after hospitalization are even higher. In the UK, mean hospital costs during the first year after a hip fracture are about £14 163. Women that experienced a clinical vertebral or hip fracture have a 6- to 9-fold increased risk of mortality. The higher number of fractures and the older of the patients, the higher mortality risk. Therefore, novel and safe strategies to prevent and treat AIBL are urgently needed. Currently, AIBL management includes lifestyle adjustments, calcium and vitamin D supplements, bisphosphonates and Denosumab. It is suggested that all patients undergoing treatment with AIs seek advice regarding exercise and calcium/vitamin D supplements. Bisphosphonate therapy is recommended for patients with a low bone mineral density (BMD) T-score and a high risk of fractures. Although bone loss is caused by various factors such as calcium malabsorption, obstruction of active vitamin D synthesis and dysfunction of both, calcium/vitamin D supplements have limited effects in treating and preventing AIBL. For patients that do not reach the intervention criteria of bisphosphonates therapy, calcium/vitamin D supplements fail to reverse the decrease in BMD. In addition, strict dosing guidelines for oral bisphosphonates lead to poor compliance and persistence. Moreover, limited evidences is available that supports the use of bisphosphonates for treating AIBL. Intravenous administration of bisphosphonates is inconvenient, must be done for the long-term, and might result in mandibular necrosis, renal insufficiency, hypocalcemia and other side effects. Denosumab, a new type of bone resorption inhibitor, is a monoclonal antibody, which acts on the receptor activator of the NF-κB ligand. It has been shown to increase bone density and reduce the risk of fractures, but limited efficacy data are available for its use in treating AIBL and it is very expensive. Overall, effective strategies with fewer side effects are urgently needed to treat AIBL.

Traditional Chinese medicine (TCM) is widely used for breast cancer treatment in Asian countries. Statistics suggest that the proportion of patients using traditional Chinese medicine in combination with medical treatments are 37.9%, 42.2% and 39.5% for stage 0-II, stage III and stage IV breast cancer, respectively. The widespread application of TCM is attributed to the belief that it can modulate the inner microenvironment of humans by balancing immunity and alleviating toxic effects, thus improving quality of life. Preliminary clinical studies in China have indicated that traditional medicine tends to retard AI-associated bone loss. Furthermore, animal studies and clinical investigations have shown that a kidney-nourishing and spleen-invigorating prescription had positive effects in postmenopausal osteoporosis. Since AIBL and postmenopausal osteoporosis are both types of estrogen-dependent bone loss, kidney-nourishing and spleen-invigorating prescriptions can theoretically prevent AIBL. Based on previous studies, the Jianpi Bushen (JPBS) formula, consisting of kidney-nourishing and spleen-invigorating herbs, was developed and its effects on AIBL after menopause were tested in female rat models and in a clinical trial in the current study.

MATERIALS AND METHODS

Experimental medicine

The JPBS formula was established based on the TCM theory of tonifying the kidney and invigorating the spleen. The JPBS formula consists of 9 herbs: Huangqi (Radix Astragali Mongolici) 30 g, Shanyao (Rhizoma Dioscoreae Opposita) 15 g, Baizhu (Rhizoma Ararctylidis Macrocephalae) 10 g, Yinyanghuo (Rhizoma Atractylodis Brevicornus) 15 g, Shudihuang (Radix Rehmanniae Praeparata) 10 g, Duzhong (Cortex Eucommiae) 15 g, Buguzhi (Fructus Pononaleae) 15 g, Roucongrong (Herba Cistanches Deserticola) 15 g, and Gouqizi (Fructus Lycii) 10 g. All herbs were authenticated and prepared by the Department of Pharmacy, Guangdong Provincial Hospital of Chinese Medicine. An aqueous extract was prepared by boiling the herbs 2 times to make decoctions at 3 different doses (1.4, 2.8 or 5.6 g/mL) for oral administration. Briefly, the 9 herbs were mixed, put into an automatic TCM decocting machine and marinated in water for approximately 20 min. The ingredients were boiled twice for 1 h. Lastly, the medical liquid was filtered twice and evaporated using a rotary evaporator at 100 °C. The concentrated liquid was freeze dried into a powder and dissolved using water into 3 different doses. Exemestane (Nantong Pharmacy Company of Hainan Province, No. 20120501) was dissolved in distilled water a concentration of 3.3 mg/mL. The formula was administered via daily oral gavage for

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Bone turnover markers were measured using commercially available kits, as specified by the manufacturers. Bone formation markers, including serum bone gla protein (BGP), alkaline phosphatase (ALP), and carboxyterminal propeptide of type I procollagen (PINP) were measured by electrochemiluminescence immunoassay (Roche Diagnostics Ltd., Shanghai province, China). Bone resorption markers, including Tartrate-resistant Acid Phosphatase (TRAP) and type I collagen cross-linked C-terminal telopeptide (B-CTx) were measured by enzyme-linked immunosorbent assay (Yaji Biotechnology Ltd., Shanghai province, China). Serum levels of estrogen were measured by radioimmunoassay.

**Clinical study design and participants**
A prospective cohort study was conducted to evaluate the efficacy of JPBS Formula for treatment of aromatase-inhibitor-associated bone loss in postmenopausal breast cancer women. The study design and protocol were approved by the ethical committee of Guangdong Provincial Hospital of Chinese Medicine. The ovariectomized model (OVX) was used to imitate the postmenopausal status of women with breast cancer. The animals were randomly divided into 6 groups (n = 16 rats/group) as follows: group 1, the sham group (distilled water 3 mL/d); group 2, OVX group (distilled water 3 mL/d); groups 3-5, OVX + EXE (1.3 mg·100 g⁻¹·d⁻¹) + JPBS (1.4, 2.8, or 5.6 g·100 g⁻¹·d⁻¹), called JPBSL, JPBSM, and JPBSH respectively; group 6, OVX + EXE group (1.3 mg·100 g⁻¹·d⁻¹). All rats were anesthetized using chloral hydrate and ovariectomized, except the sham group. All rats were acclimatized for 4 weeks prior to the experiment. Medicines were administered by oral gavage.

**Dual-energy X-ray absorptiometry (DXA) analysis**
The lumbar spines of individual animals were scanned by DXA (Hologic QDR; 4500 A) using the regional high resolution scan mode. The bone mineral content and area was measured and BMD was calculated automatically at bone mineral content/area.

**Histology analysis**
All rats were euthanized by excessive anesthetic injection before histology analysis. The lower 1/3 ends of the left femurs were collected from rats in all groups. The bone marrow cavity was exposed by coronary incision in the left femurs were collected from rats in all groups. All rats were euthanized by excessive anesthetic injection at bone mineral content/area.

**Biochemical analysis**
Bone turnover markers were measured using commercially available kits, as specified by the manufacturers. Bone formation markers, including serum bone gla protein (BGP), alkaline phosphatase (ALP), and carboxyterminal propeptide of type I procollagen (PINP) were measured by electrochemiluminescence immunoassay (Roche Diagnostics Ltd., Shanghai province, China). Bone resorption markers, including Tartrate-resistant Acid Phosphatase (TRAP) and type I collagen cross-linked C-terminal telopeptide (B-CTx) were measured by enzyme-linked immunosorbent assay (Yaji Biotechnology Ltd., Shanghai province, China). Serum levels of estrogen were measured by radioimmunoassay.
were assessed every 6 months. The follow-up time was 12 months.

Statistical analysis

All data are presented as mean ± standard deviation (±). All data were processed using SPSS 19.0 (IBM Corp. Released 2010, IBM SPSS Statistics for Windows, Version 19.0, Armonk, NY, USA). In normally distributed and analysis of variance was conducted, followed by post hoc Fisher’s least significant difference or Kruskal-Wallis H test to test the differences between groups. A P value < 0.05 was considered as statistically significant.

RESULTS

JPBS formula improved bone microstructure in the rat model

Histology analysis showed a significant reduction in Tb.Ar% and Tb.Th at 16 weeks after ovariectomy in the OVX group compared to the sham control (P = 0.005 and 0.006, respectively). The high dose of JPBS formula (5.6 g/mL) prevented the reduction in Tb.Ar% and Tb.Th compared with the OVX group (P = 0.021 and 0.010, respectively). Results in the OVX-EXE group were not significantly different from those in the OVX group. There were no significant differences in Tb.N and Tb.Sp between the 6 groups (Table 1). Femoral bone microstructure analysis indicated that trabecular bone was thin and sparse and that the adipose tissue was rich with erythrocytes, sparsely distributed in medullar cavities, in the OVX and OVX + EXE groups. However, in the herbal treatment groups, trabecular bone was thick with smaller spatia. Additionally, the medullar cavities were filled with enriched erythrocytes, especially in the JPBSH group (Figure 2).

JPBS formula promoted bone formation and inhibited bone resorption

In OVX rats (Table 2), the level of serum PINP was decreased while the expression of serum β-CTX was significantly increased 16 weeks after oophorectomy. PINP was a significant 28.85% higher in OVX + EXE group compared with the OVX group (P < 0.05). Interestingly, the JPBS formula further increased PINP in OVX + EXE rats. The increases in PINP were 40.35% and 53.85% in the JPBSM and JPBSH groups respectively (P < 0.01), indicating that the
JPBS formula increased the level of PINP in a dose-dependent manner (Figure 3A). There were no significant differences in the levels of BGP, TRAP or ALP among the 6 groups.

**JPBS formula raised serum estrogen in OVX rats treated with exemestane**

Serum estrogen in the OVX group was significantly decreased 16 weeks after oophorectomy. There was no sta-

### Table 1 | Histomorphometric analysis in six groups of rats (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Tb.Ar (%)</th>
<th>Tb.N (n/mm)</th>
<th>Tb.Th (μm)</th>
<th>Tb.Sp (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>44.5±7.4’</td>
<td>18.3±3.2</td>
<td>24.7±2.4’</td>
<td>31.8±9.5</td>
</tr>
<tr>
<td>OVX</td>
<td>35.8±10.8’</td>
<td>18.0±4.2</td>
<td>20.2±4.4’</td>
<td>42.0±30.6</td>
</tr>
<tr>
<td>JPBSL</td>
<td>37.8±6.6’</td>
<td>18.1±3.0</td>
<td>21.1±2.9</td>
<td>35.9±9.9</td>
</tr>
<tr>
<td>JPBSM</td>
<td>36.0±5.0</td>
<td>17.5±2.6</td>
<td>20.9±2.5</td>
<td>37.8±8.3</td>
</tr>
<tr>
<td>JPBSH</td>
<td>42.6±8.3’</td>
<td>17.9±3.3</td>
<td>24.2±4.9’</td>
<td>33.9±11.9</td>
</tr>
<tr>
<td>OVX+EXE</td>
<td>40.3±6.2</td>
<td>17.9±3.3</td>
<td>23.2±5.1</td>
<td>34.4±7.8</td>
</tr>
</tbody>
</table>

Notes: sham group: sham-operated and received distilled water 3 mL/d for 12 weeks; OVX group: bilaterally ovariectomized and received distilled water 3 mL/d for 12 weeks; JPBSL, JPBSM, JPBSH group: bilaterally ovariectomized and received JPBS at dose of 1.4, 2.8, 5.6 g·100 g⁻¹·d⁻¹ for 12 weeks; OVX + EXE group: bilaterally ovariectomized and received exemestane of 1.3 mg·100 g⁻¹·d⁻¹ for 12 weeks. Tb.Ar: trabecular bone area; Tb.N: trabecular number; Tb.Th: trabecular thickness; Tb.Sp: trabecular separation; OVX: ovariectomized; JPBSL: low dose of JPBS; JPBSM: middle dose of JPBS; JPBSH: high dose of JPBS; JPBS: Jianpi Bushen formula; EXE: exemestane. Compared with the OVX group, *P* < 0.05.

Figure 2 Microstructure of trabecular bone in the rat model (hematoxylin-eosin staining, x 40)

A: picture is selected from sham group which were sham-operated and received distilled water. Trabecular bone is thick and rich. B: picture is selected from OVX group which were bilaterally ovariectomized and received distilled water. Trabecular bone become thin and sparse. C-E: pictures are selected from JPBSL, JPBSM, JPBSH group which were bilaterally ovariectomized and received JPBS at dose of 1.4, 2.8, 5.6 g·100 g⁻¹·d⁻¹. The trabecular bone is thick and rich, particularly in picture E. F: picture is selected from OVX + EXE group which were bilaterally ovariectomized and received exemestane at dose of 1.3 mg·100 g⁻¹·d⁻¹. OVX: ovariectomized; JPBSL: low dose of JPBS; JPBSM: middle dose of JPBS; JPBSH: high dose of JPBS; JPBS: Jianpi Bushen formula; EXE: exemestane.

### Table 2 | Bone turnover markers in the six groups of rats (pg/mL, x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>PINP</th>
<th>BGP</th>
<th>ALP</th>
<th>TRAP</th>
<th>β-CTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.688±0.401’</td>
<td>0.08±0.012</td>
<td>0.347±0.214</td>
<td>0.123±0.068</td>
<td>0.110±0.021’</td>
</tr>
<tr>
<td>OVX</td>
<td>0.518±0.301’</td>
<td>0.087±0.010</td>
<td>0.322±0.120</td>
<td>0.108±0.034</td>
<td>0.134±0.033</td>
</tr>
<tr>
<td>JPBSL</td>
<td>0.637±0.209’</td>
<td>0.092±0.015</td>
<td>0.362±0.108</td>
<td>0.113±0.043</td>
<td>0.146±0.131</td>
</tr>
<tr>
<td>JPBSM</td>
<td>0.727±0.162’</td>
<td>0.096±0.011</td>
<td>0.300±0.124</td>
<td>0.112±0.090</td>
<td>0.131±0.054</td>
</tr>
<tr>
<td>JPBSH</td>
<td>0.800±0.381’</td>
<td>0.090±0.012</td>
<td>0.324±0.143</td>
<td>0.126±0.047</td>
<td>0.149±0.067</td>
</tr>
<tr>
<td>OVX+EXE</td>
<td>0.670±0.192’</td>
<td>0.093±0.011</td>
<td>0.329±0.135</td>
<td>0.101±0.024</td>
<td>0.145±0.033</td>
</tr>
</tbody>
</table>

Notes: sham group: sham-operated and received distilled water 3 mL/d for 12 weeks; OVX group: bilaterally ovariectomized and received distilled water 3 mL/d for 12 weeks; JPBSL, JPBSM, JPBSH group: bilaterally ovariectomized and received JPBS at dose of 1.4, 2.8, 5.6 g·100 g⁻¹·d⁻¹ for 12 weeks; OVX + EXE group: bilaterally ovariectomized and received exemestane of 1.3 mg·100 g⁻¹·d⁻¹ for 12 weeks. PINP: carboxyterminal propeptide of type I collagen; BGP: bone gla protein; ALP: alkaline phosphatase; TRAP: Tartrate-resistant Acid Phosphatase; β-CTX: type I collagen cross-linked C-terminal telopeptide; JPBS: Jianpi Bushen formula. Compared with the OVX group, *P* < 0.05.
tistically significant difference in serum estrogen between OVX and OVX + EXE rats, however the levels of serum estrogen were significantly elevated in all 3 herbal treatment groups (Figure 3B), indicating that JPBS formula might improve AIBL via increasing the concentration of estrogen.

DISCUSSION

The successful establishment of an animal model can be judged by estrogen level, endometrial thickness, bone mass, bone tissue histomorphometric analyses, and bone biochemical markers. Serum estrogen, as well as TbAr% and Tb.Th decreased significantly 16 weeks after oophorectomy in the OVX rats. In addition, the levels of the bone formation marker, PINP was the same before and after treatment ($P = 0.737$). The BMD T-score in the herbal treatment group remained stable after 12 months, unlike the control group in which BMD T-score declined after 12 months. A detailed summary is shown in Figure 5. There was 1 case of breast cancer recurrence each in the treatment and control groups.

Figure 3 Effect of the herbal JPBS on PINP and serum estrogen on the rat model

A: results are PINP in the 6 groups of rats. B: results are Serum estrogen in the 6 groups of rats. sham group: sham-operated and received distilled water 3 mL/d for 12 weeks; OVX group: bilaterally ovariectomized and received distilled water 3 mL/d for 12 weeks; JPBSL, JPBSM, JPBSH group: bilaterally ovariectomized and received JPBS at dose of 1.4, 2.8, 5.6 g·100 g$^{-1}$·d$^{-1}$ for 12 weeks; OVX + EXE group: bilaterally ovariectomized and received exemestane of 1.3 mg·100 g$^{-1}$·d$^{-1}$ for 12 weeks. PINP: carboxyterminal propeptide of type I collagen; OVX: ovariectomized; JPBSL: low dose of JPBS; JPBSM: middle dose of JPBS; JPBSH: high dose of JPBS; JPBS: Jianpi Bushen formula; EXE: exemestane. $P < 0.05$, compared with OVX group; $P < 0.05$, compared with OVX + EXE group.

Clinical characteristics of subjects

A total of 65 patients in each group completed the study. Baseline characteristics of both groups are displayed in Table 3. There were no significant differences in age, age at menopause, body mass index, BMD T score, blood chemistry or treatments (chemotherapy, radiotherapy and endocrine drugs) between 2 groups.

JPBS formula retarded AIBL

Although there was no significant difference in BMD among the 6 groups in the animal study (Figure 4), clinical study revealed a promising result. There was a significant decrease in BMD T-scores in the control group (13.58% and 22.45% decreases at the lumbar vertebra and femoral neck, $P = 0.005, 0.002$, respectively) after 12 months, however there was no significant difference in BMD T-scores in the herbal treatment group (3.9% increase and 1.8% decrease at the lumbar vertebra and femoral neck, $P = 0.276, 0.789$, respectively). The difference in vertebral and femoral neck BMD T-scores between the control and herbal treatment groups was significant ($P = 0.004, 0.008$, respectively). Bone turnover markers were also tested in the herbal treatment group (Table 4). The level of serum $\beta$-CTx increased 9.25% and the expression of $\beta$-CTx decreased 21.7% compared to baseline levels ($P = 0.030, 0.002$, respectively). Contrary to results in the animal model, the level of serum PINP was the same before and after treatment ($P = 0.737$). The BMD T-score in the herbal treatment group remained stable after 12 months, unlike the control group in which BMD T-score declined after 12 months. A detailed summary is shown in Figure 5. There was 1 case of breast cancer recurrence each in the treatment and control groups.

Accumulated evidence has shown that single herbs, such as Yinyanghuo (Herba Epimedii), Duzhong (Cortex Eucommiae), Buguzhi (Fructus Psoraleae), Roucongong (Herba Cistanche), are capable of preventing osteoporosis by balancing bone metabolism. Laboratory experiments have also indicated that herbal formulas, such as spleen-invigorating formula, kidney-tonifying formula, and spleen-invigorating and kidney-tonifying formula can prevent osteoporosis in humans. This has been shown for the spleen-invigorating and kidney-tonifying formula, in particular. In addition, clinical studies have demonstrated that the spleen-invigorating and kidney-tonifying formula effectively prevents postmenopausal osteoporosis. Since AIBL and postmenopausal osteoporosis are both types of estrogen-dependent bone loss, the JPBS formula should also theoretically prevent AIBL. Results of the current study suggest that administration of 1.1 g/mL of the JPBS formula prevented reductions in Tb.Ar% and Tb.Th in OVX rats. Notably, the JPBS formula increased bone mass and improved the bone microstructure as evidenced by thick and close trabecular bone. However, there was no significant difference in BMD of rats that received herbal treatment, which is consistent with some previous domestic research in China. The lack
of an effect on BMD may could be due to the short intervention time, although 16 weeks of drug administration is the usual duration for studies done abroad.\textsuperscript{16,19} Contrary to the results of the animal portion of the study, the clinical results demonstrated that the BMD at lumbar vertebra and femoral neck remained stable in the JPBS-treated group, but decreased significantly in the control group. These data confirm the efficacy of the JPBS formula in preventing bone loss in postmenopausal women taking AIs in order to avoid the occurrence of fractures.

Bone metabolism markers are the chemical products produced by bone formation and resorption, and thus are a manifestation of bone formation and resorption. Results in this study indicated that exemestane increased the serum PINP level in OVX rats, implying that it had a slight effect in promoting bone formation, since PINP reflects the formation of type I collagen. One of the metabolites of exemestane, 17-H-EXE, is effective in preventing bone loss and might be responsible for this result.\textsuperscript{20} The JPBS formula further increased the level of PINP in OVX rats treated with EXE, especially in the high dose group. These results suggest that the JPBS formula efficiently prevented bone loss by enhancing bone formation. Moreover, the clinical results indicated that the JPBS formula promoted bone formation and inhibited bone resorption, thus disrupting the unbalanced bone metabolism. Under normal circumstances, bone metabolism markers fluctuate within a normal range. When the bone metabolic balance is broken and bone resorption accelerates, the bone formation system is activated. As a result, bone metabolism conversion is more active resulting in higher levels of bone formation and resorption markers. This phenomenon is called the high conversion status of bone metabolism.\textsuperscript{20} In the herbal treatment group of clinical study, the level of serum β-CTx was (0.59 ± 0.25) pg/mL (normal value: 0-0.6 pg/mL). In addition, the serum PINP level was quite a bit higher than normal value (51.69 ± 20.07) pg/mL vs 0.371 ng/mL. These data indicate that bone metabolism was in the high conversion status. The serum β-CTx level decreased by 21.67% in OVX rats treated with JPBS and caltrate D at the same dose with herbal treatment group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Herbal treatment group</th>
<th>Control group</th>
<th>P value</th>
</tr>
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<tr>
<td>Age (years)</td>
<td>56.4±4.7</td>
<td>57.1±4.9</td>
<td>0.45</td>
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<tr>
<td>Menopause age (years)</td>
<td>48.9±2.3</td>
<td>49.1±2.3</td>
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<td>Body mass index (kg/m^2)</td>
<td>23.2±2.6</td>
<td>23.8±2.1</td>
<td>0.13</td>
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<tr>
<td>BMD T score at lumbar vertebra</td>
<td>−1.5±0.6</td>
<td>−1.4±0.5</td>
<td>0.21</td>
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<tr>
<td>BMD T score at femoral neck</td>
<td>−1.1±0.4</td>
<td>−1.0±0.6</td>
<td>0.25</td>
</tr>
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<td>Liver function abnormalities (n)</td>
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<td>0</td>
<td>-</td>
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<tr>
<td>Kidney function abnormalities (n)</td>
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<td>-</td>
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<td>Chemotherapy regimens (n)</td>
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<td></td>
<td>Doublet chemotherapy</td>
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<td>Three-drug chemotherapy</td>
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<td>Letrozole</td>
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<tr>
<td></td>
<td>Anastrozole</td>
<td>16</td>
<td>21</td>
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Notes: herbal treatment group: postmenopausal women with breast cancer treated with AIs (exemestane 25 g/d or letrozole 2.5 mg/d or Anastrozole 1 mg/d) and caltrate D 0.75 g/d. They also took the JPBS twice daily; control group: postmenopausal women with breast cancer treated with AIs and caltrate D at the same dose with herbal treatment group.
Consistent with a study by Paul et al., it was also found that exemestane did not reduce serum estrogen in OVX rats, which might be attributed to the under-developed peripheral tissue aromatase system in rats. On the contrary, the JPBS formula increased the serum estrogen in the OVX + EXE, especially in the high dose group. Some studies have also found that particular herbs elevated serum estrogen levels and that such effects might be attributed to the wild existence of phytoestrogens in Chinese herbs, which are believed to have estrogen-like functions. In addition, active herbal ingredients directly affect bone cells and promote their proliferation and differentiation by regulating the expression of osteoprotegerin mRNA in osteoblast and core binding factor α1 mRNA in bone tissue. Some studies have also reported that the herbs protect against bone loss by regulating the secretion of cytokines such as IL-1 and IL-6, thus affecting calcium metabolism and the balance of trace elements. Therefore, enhancing estrogen-mediated bone metabolism...
may be an important therapeutic strategy for treating AIBL. Estrogen replacement therapy can have harmful consequences in the prognosis of breast cancer patients and herb integrative therapy has become a safe and promising alternative strategy in order to avoid AIBL. In this study, the serum estrogen levels in all 3 herbal treatment groups was lower than that in the control group, indicating that the estrogen modulating effects of JPBS was moderate and should be safe for use in patients with hormone-dependent breast cancer. In addition, JPBS appeared to have little influence on the disease free survival of patients and no liver or kidney function abnormalities were observed in patients in the clinical portion of this study.

In conclusion, the current study suggests that the JPBS formula prevents AIBL after menopause by inhibiting bone resorption and promoting bone formation. JPBS slowed the decrease of BMD in postmenopausal women taking AIs as adjuvant endocrine therapy. The efficacy of JPBS might be associated with its moderate estrogen-promoting activity. However, the efficacy and reduction in the incidence of fragility fracture incidence should be evaluated in patients undergoing longer-term JPBS treatment. In addition, further exploration into the active compounds in JPBS, which target estrogen production, is needed.

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