Effect of Gengnianchun Recipe (更年春方) on Bone Mineral Density, Bone Biomechanical Parameters and Serum Lipid Level in Ovariectomized Rats*

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ABSTRACT
Objective: To observe the effect of Gengnianchun Recipe (更年春方, GNC) on bone mineral density (BMD), bone biomechanical parameters and serum lipid level in the bilaterally ovariectomized (OVX) rats and to explore the prophylactic and therapeutic action of GNC on ovariectomy induced osteoporosis and hyperlipidemia. Methods: OVX SD rats, 10-12 months old, were divided into different groups and fed with GNC 2 g/d, GNC 1 g/d and Nilestriol 0.125 mg/week, respectively for 4 months to observe the change of BMD and bone biomechanical parameters of the lumbar vertebrae, and the serum levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), and to compare the effect of the two drugs on the morphology of the uterus. Results: There was marked reduction in BMD and biomechanical parameters in lumbar vertebrae (P<0.01) and increase of serum TC and LDL-C levels (P<0.01) in rats after OVX. GNC or Nilestriol significantly improved the decreased BMD and biomechanical parameters of the lumbar vertebrae (P<0.05 or P<0.01), and reduced the serum TC and LDL-C levels (P<0.01). In the Nilestriol group, the wet weight of uterus got increased obviously (P<0.01), the number of uterine glands increased, uterine columnar epithelium thickened, and the mitotic figures in the epithelial stroma and myointimal cells augmented. But no such effect in wet weight and morphology of uterus was found in the GNC group. Conclusion: GNC could increase the BMD and biomechanical parameters of the lumbar vertebrae, reduce the serum TC and LDL-C levels, yet produce no adverse reaction in stimulating proliferation and hypertrophy of uterus.

KEY WORDS Gengnianchun Recipe, postmenopausal osteoporosis, bone mineral density, bone biomechanics, total cholesterol, low-density lipoprotein cholesterol

Gengnianchun Recipe (更年春方, GNC), a TCM compound formulated on the basis of the principle of nourishing Shen (腎), softening Gan (肝), clearing Xin (心)-fire by Affiliated Obstetric and Gynecology Hospital, Fudan University, was proved to be effective in treating climacteric syndrome in women¹, and in improving structural deterioration of bone trabecula in ovariectomized (OVX) rats²,³. The present study was designed to observe the effect of GNC on bone mineral density (BMD), bone biomechanical parameters and serum lipid level in OVX rats.

METHODS

Drugs and Animals
GNC, composed of twelve Chinese herbs including Rehmannia glutinosa Libosch, Anemarrhena asphodeloides Bge, Chinemys reevesii, Lycium chinensis Mill, Epimedium brevicornum Maxim, and Phellodendron chinense Schneid, etc., was made into oral liquid with the concentration of 1 g crude drugs/ml by Department of Pharmacognosy, Fudan University. Nilestriol was produced by Shanghai Hualian Drug Co. Ltd., batch number: 030901.

Sixty 10-12 months old female Sprague Dawley rats with a mean body weight of (400 ± 50) g were obtained from Department of Experimental Animal, Fudan University, which were raised as clean animals.

Animal Grouping and Model Establishment
The rats were randomly divided into five groups

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with twelve rats in each group: the sham-operative group (S group), the control group (C group), the Nilestriol group (E group), the high dose GNC group (H group), and the low dose GNC group (L group). The model was established after anesthetization with 100 mg/ml of ketamine (200 mg/kg body weight) peritoneally as described in the reference(4). The operation on rats in the S group was cutting off some fatty tissues around the ovary without excising ovary, while that on rats in the other four groups were all ovariectomized bilaterally. The administration of testing drugs started one week after the operation with GNC in high dose (2 g/day) and low dose (1 g/day) to rats in group H and L respectively, which was equivalent to 20 and 10 times the dose for human adult; while rats in the E group were fed with Nilestriol 0.125 mg, once per week, equivalent to 20 times the dose for human adult, and to group S and C only saline 1 ml/day. The treatment lasted for 4 consecutive months. Blood samples were collected before starting treatment and after terminating the 4 months’ treatment, and then rats were sacrificed and their uterus and lumbar vertebrae were cut out immediately for examination.

Items and Methods of Observation

1. Histomorphology
   The wet weight of uterus was measured. A 5-mm segment of one uterine horn was fixed for 24 h in 10% formalin, dehydrated in a graded ethanol series, cleared with xylene, embedded in paraffin, sectioned at 8 μm and stained with hematoxylin and eosin (HE). Photographs of uterine sections were taken by a microscope equipped with a digital camera.

2. BMD
   BMD of lumbar vertebrae (L2) was measured on a physical densitometer (Mettler Toledo AG204, Switzerland). Prior to measuring, all soft tissues and the bone marrow were removed and the samples were baked for 1 h at 120°C.

3. Biomechanical parameters of bone
   Biomechanical parameters of the lumbar vertebrae (L3), including maximal load, maximal stress, and elastic module, were evaluated by compressive test which was performed on a material testing system (INSTRON 5543, USA). Prior to testing, all soft tissues including the bone marrow were removed.

4. Serum lipid level
   Fasting blood serum was collected for measurement of the levels of blood lipids, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), with enzymatic method on automatic analyzer (HITACHI 7060, Japan).

Statistical Analysis
   Data was expressed as mean ± standard deviation and was evaluated for its significance by one-way ANOVA with Stata 7.0 software.

RESULTS

Changes of Wet Weight and Morphology of Uterus in Each Group

See Table 1 and Figure 1. Uterine wet weight declined obviously after OVX (P<0.01). Compared with group C, significantly increased weight was shown in group E (P<0.01). However, there was no significant difference in wet weight of group H and group L when compared with that in group C (P>0.05).

Compared with those in group S, in group C the uterine columnar epithelium got atrophied, the number of uterine glands reduced, and the mitotic figures in the epithelium reduced. Compared with those in group C, in group E the columnar epithelium got thickened obviously, showing the pseudostratified form, with increased uterine glands and augmented mitotic figures in epithelial stroma and myointimal cells, but no significant difference was found in comparison of the morphological pictures in group C with those either in group H or in group L.

<table>
<thead>
<tr>
<th>Group</th>
<th>Wet Weight of Uterus (g)</th>
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<tbody>
<tr>
<td>S</td>
<td>0.962±0.141</td>
</tr>
<tr>
<td>C</td>
<td>0.202±0.552*</td>
</tr>
<tr>
<td>E</td>
<td>0.375±0.046</td>
</tr>
<tr>
<td>H</td>
<td>0.175±0.016</td>
</tr>
<tr>
<td>L</td>
<td>0.198±0.038</td>
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</tbody>
</table>

Notes: * P<0.01, compared with group S; △ P<0.01, compared with group C

Comparison of BMD of L2 and Biomechanical Parameters of L3 Vertebrae

See Table 2. BMD of L2 vertebrae and biomechanical parameters in L3 vertebrae including maximal load, maximal stress and elastic module decreased significantly in OVX rats (P<0.05 or P<0.01). Compared with group C, obvious increases