Anti-resorptive effect of pilose antler blood (*Cervus nippon Temminck*) in ovariectomized rats

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Anti-bone resorption activity of pilose antler blood (*Cervus nippon Temminck*) were evaluated in ovariectomized Wistar rats. The rats were randomly divided into sham operated group (SHAM), ovariectomized group (OVX) and pilose antler blood treated group. The ovariectomized rats were treated with pilose antler blood orally in 4000µl/kg daily doses for 10 weeks. Compared with SHAM group, serum 17β-estradiol level decreased significantly and osteocalcin level increased significantly in OVX group, indicating successful model of osteoporosis. The experiments showed that the bone mineral density of the lumbar spine and left femur in OVX group decreased remarkably compared to SHAM group but normalized by treatment with pilose antler blood. Additionally, serum levels of insulin-like growth factor-1 and testosterone were lower obviously in OVX group than those in SHAM group but preserved by pilose antler blood treatment. However, no obvious changes in serum levels of calcium, phosphorus, total alkaline phosphatase and osteoprotegerin were observed among three groups. These results suggested that administration of pilose antler blood was effective in alleviating osteoporosis in ovariectomized rats.

**Keywords:** Bone mineral density, 17 β-estradiol, Insulin-like growth factor-1, Osteoporosis, Pilose antler blood, Testosterone

Osteoporosis is a kind of metabolic disease of bone characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. Both bone loss and fractures are fairly common in postmenopausal women\textsuperscript{1}. During the first decade after menopause, a sharp decrease in ovarian estrogen production causes an increase in bone resorption relative to bone formation and results in a rapid bone loss. Although antosteoporotic agents for postmenopausal osteoporosis may prevent further bone loss in established osteoporosis\textsuperscript{2}, they cannot restore bone mass that have been lost already and have some side effects, such as increasing the risk of breast cancer\textsuperscript{3} and cardiovascular disease\textsuperscript{4} with long-term hormone replacement therapy.

Velvet antlers or unossified horn cutting from deer have been used as oriental medicine for many centuries. Traditional medical reports and clinical observations from the Eastern world convincingly show that velvet antler is biologically active material and has been widely used to promote growth, boost immune function, prevent blood loss and chronic joint pain. Deer antler was beneficial in reducing the side effects of cancer treatments\textsuperscript{5,6}. In addition, Zhou \textit{et al.}\textsuperscript{7} found that velvet antler polypeptides promoted proliferation of chondrocytes and osteoblast precursors and fracture healing. Kim \textit{et al.}\textsuperscript{8} showed that deer antler aqua-acupuncture (DAA), prepared from the pilose antler of *Cervus korean* Temminck var *manchuricus Swinhoe* had some anti-bone resorption activity in adjuvant-induced arthritic rats. However, pilose antler blood is the blood which remains in the pilose antler while being sawed and also has lots of functions such as anti-fatigue activity, anti-aging activity, anti-radiation activity, promoting
metabolism and enhancing immunity. But little is known about its effect on bone growth. In the present study, the osteoporosis model was developed by ovariectomy in rats just like postmenopausal osteoporosis in woman and serum levels of 17 β-estradiol (E$_2$), insulin-like growth factor-1 (IGF-1), osteocalcin (OC), osteoprotegerin (OPG), testosterone (T), Ca, P, total alkaline phosphatase (ALP) and the bone mineral density (BMD) of the lumbar spine and femur in pilose antler blood-treated ovariectomized rats were observed.

**Materials and Methods**

*Preparation of pilose antler blood*—Pilose antler blood was the blood which remained in the pilose antler while being sawed and drawn from the pilose antler of male deer (*Cervus nippon Temminck*). The mixed blood sample from pilose antler was stored at -20°C before use.

*Animals*—Fifteen 10-week old female Wistar rats (180±10 g) were purchased from Vital River Experimental Animal Technology Inc (Beijing, China). Animals were randomly divided into 3 groups of 5 each and had free access to feed and water. They were acclimatized for one week before use. One group was sham operated (SHAM, group 1), and two other groups were subjected to bilateral ovariectomy (OVX, group 2 and 3). After 4 weeks of SHAM or OVX, one of OVX groups received pilose antler blood (4000 µl/kg daily, group 3) orally by using stomach tube for 10 weeks. All animal procedures in this study were carried out according to the guideline of the Institutional Animal Care and Use Committee of the Academy of Military Medical Sciences.

*Body weight*—Body weights of animals are presented in Fig. 1. Average body weight in OVX group was remarkably higher than that in SHAM group (P<0.05). Particularly, body weight of OVX group at the end of the experiment increased 53.4% while those in SHAM group increased 27.7% in average compared with initial body weights. However, body weight of pilose antler blood-treated group also increased 45.5% in average compared with initial body weights.

*Serum Ca, P and total ALP*—The serum Ca, P and total ALP levels of three groups are shown in Table 1. No significant changes were observed among three groups.

*Bone mineral density*—After sacrifice, the lumbar spine and left femur of rats of three groups were dissected out and carefully cleaned of soft tissue. The lumbar spine was stored in 0.9% NaCl at 4°C. The left femur was stored in 10% formalin. The bone mineral density was determined by dual-energy X-ray absorptiometry (DXA) (Excellplus, Norland Co. USA) with small animal software after each bone was picked out and made dry.

*Statistical analysis*—Data analysis was performed by SPSS program (Version 10.0). All results were presented as means±SD. Comparisons between groups were made by One-Way ANOVA. Statistical significances were defined as P<0.05.

**Results**

*Body weight*—Body weights of animals are presented in Fig. 1. Average body weight in OVX group was remarkably higher than that in SHAM group (P<0.05). Particularly, body weight of OVX group at the end of the experiment increased 53.4% while those in SHAM group increased 27.7% in average compared with initial body weights. However, body weight of pilose antler blood-treated group also increased 45.5% in average compared with initial body weights.

**Serum Ca, P and total ALP**—The serum Ca, P and total ALP levels of three groups are shown in Table 1. No significant changes were observed among three groups.

![Fig. 1—Effect of pilose antler blood on body weight in rats: SHAM (♦), OVX (■) and OVX treated with pilose antler blood (▲).*P<0.05 vs. SHAM group](image-url)
Serum E\textsubscript{2}, IGF-1, OC, OPG and T—The serum E\textsubscript{2} level decreased significantly ($P<0.05$) in OVX group compared to SHAM group, indicating successful model of osteoporosis caused by ovariectomy. However, there was no remarkable increase in serum E\textsubscript{2} level after pilose antler blood treatment (Fig. 2 a).

The serum levels of T and IGF-1 were lower significantly in OVX group ($P<0.05$) than those in SHAM group but preserved by pilose antler blood treatment ($P<0.05$). Serum OC level increased in OVX group ($P<0.05$) compared to SHAM group. But the serum level of OPG had no significant change among three groups (Fig. 2 b-e).

Bone mineral density—The bone mineral density of the lumbar spine and left femur of the rats are shown in Fig. 3. The lumbar spine and femur density in OVX group were lower significantly than those in SHAM group ($P<0.05$) but normalized by pilose antler blood treatment ($P<0.05$).

![Fig. 2](image-url)

![Fig. 3](image-url)

Table 1—Concentrations of serum Ca, P and ALP (mmol/L)

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>P</th>
<th>ALP</th>
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<tbody>
<tr>
<td>SHAM</td>
<td>2.51±0.09</td>
<td>1.95±0.19</td>
<td>181.6±44.84</td>
</tr>
<tr>
<td>OVX</td>
<td>2.57±0.04</td>
<td>2.04±0.16</td>
<td>189.2±39.84</td>
</tr>
<tr>
<td>OVX+pilose antler blood</td>
<td>2.46±0.08</td>
<td>2.03±0.38</td>
<td>190.6±34.37</td>
</tr>
</tbody>
</table>

SHAM, sham-operated; OVX, ovariectomy; OVX+pilose antler blood, ovariectomized and treated with pilose antler blood.

![Fig. 2](image-url)

![Fig. 3](image-url)

Fig. 2—Effect of pilose antler blood on serum (a) 17 \( \beta \)-estradiol, (b) testosterone, (c) IGF-1, (d) osteocalcin and (e) osteoprotegerin concentration in rats [Group 1=SHAM. Group 2=OVX, Group 3=OVX treated with pilose antler blood; $P<0.05$ *vs. Gr. 1, **vs. Gr. 2]

Fig. 3—Effect of pilose antler blood on bone mineral density (BMD) of (a) lumbar spine and (b) femur [Group 1=SHAM. Group 2=OVX, Group 3=OVX treated with pilose antler blood; $P<0.05$ *vs. Gr. 1, **vs. Gr. 2]
Discussion

To date, there has been no report that dietary supplementation with pilose antler blood can prevent osteoporosis. In the present study, the pilose antler blood treatment could increase the BMD of the lumbar spine and left femur in ovariectomized rats, which was associated with the high serum levels of IGF-1 as well as T, and played an important role in osteoprotection.

Estrogen is very critical for bone growth and estrogen receptors (ERs) are expressed by both osteoblasts and osteoclasts. The estrogen has both direct effects on osteoblasts, resulting in increased bone formation, and indirect effects, via an osteoblast-mediated interaction with pre-osteoclasts and osteoclasts, resulting in a decreased bone resorption. Trabecular BMD values decreased following ovariectomy but restored by estrogen treatment. In the present experiment, osteoporosis was developed by ovariectomy in female Wistar rats just like human postmenopausal osteoporosis due to E2 deficiency (P<0.05, OVX group). Further, the BMD of lumbar spine and femur in OVX group were significantly lower than those in SHAM group (P<0.05). However, the BMD of lumbar spine and femur increased remarkably after pilose antler blood treatment although E2 level was only slightly elevated (P<0.05). In addition to estrogen, androgen has important biological roles in young women, influencing bone and muscle mass. Pathophysiological states affecting ovarian and adrenal function or both may result in androgen deficiency in premenopausal women and low T in serum is associated with reduced bone mass and BMD. Although estradiol plays a greater role in maintenance of skeletal health than testosterone, androgens also have beneficial effects on bone via interaction with androgen receptors (AR) as well as estrogen receptors after aromatization to estradiol. In female, androgens may be converted into estrogen via the P450 aromatase enzyme complex which also expressed in bone tissue and therefore act as prohormones for estrogens. Therefore, administration of T alone or combined estrogen and T replacement resulted in an additional increase in bone density compared with estrogen alone in postmenopausal osteoporosis. Although the potential side effects of long-term androgen replacement and androgen and estrogen combination therapy will be a concern in postmenopausal women, androgen is an important hormone involved in bone growth through both the AR and ER-pathways. The present experiment showed that serum T level increased significantly after pilose antler blood treatment (P<0.05) in ovariectomized rats, and the BMD of lumbar spine and femur increased in pilose antler blood treatment group (P<0.05).

In the skeleton, IGF-1 is considered a major anabolic factor for the growth and maintenance of bone. IGF-1 can act as a systemic hormone or as an autocrine/paracrine growth factor modulating growth and differentiation of osteoblasts and play an important role in determining BMD. In vitro, studies demonstrated that both osteoblast and osteoclast had receptors of IGF-1. IGF-1 increased bone matrix production, induced osteoblast proliferation and differentiation and regulated bone resorption by effects on osteoclast differentiation and activation. In vivo, IGF-1 deficiency, such as in IGF-1 null (-/-) mice or a point mutation in exon 5 of the human IGF-1 gene, resulted in significant growth retardation and extremely low bone mineral density. Furthermore, genetically manipulated mice, with targeted overexpression of IGF-1 in mature osteoblasts, exhibited increased bone formation and enhanced trabecular and cortical bone volume. The present experiments showed that the serum IGF-1 level decreased in ovariectomized rats could be reversed significantly (P<0.05) and the BMD of lumbar spine and femur in ovariectomized rats also increased remarkably (P<0.05) by intaking pilose antler blood.

In summary, E2 deficiency, together with low serum T and IGF-1 levels resulted in the decrease of BMD in lumbar spine and left femur in OVX rats. However, pilose antler blood treatment could increase the BMD through enhancing the serum levels of T and IGF-1. Therefore, pilose antler blood supplementation had a beneficial effect on preventing osteoporosis because of its anti-bone resorptive activity.

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