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Original Research Article

Anti-osteoporosis activity of Astragalus membranaceus Bunge extract in experimental rats

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Abstract

Purpose: *To investigate the anti-osteoporosis effect of Astragalus membranaceus (Fisch.) Bunge. extract (AMBE) in experimental rats.*
Method: *Female Sprague-Dawley rats were randomly divided into six groups: control group, ovariectomy (OVX) with vehicle group, OVX with 17β-estradiol (E2, 25 μg/kg/day) group, and OVX with AMBE doses (60, 120 and 240 mg/kg/day) groups. Daily oral administration of AMBE or E2 was started 4 weeks after OVX and lasted for 16 weeks. The bone mineral density (BMD) of L4 vertebrae and right femurs was evaluated. The length of each femur was measured with a micrometer, and the center of diaphysis was determined. Three representative L4 vertebrae were selected to evaluate trabecular microarchitecture. Serum alkaline phosphatase (ALP), urinary calcium (U-Ca), urinary phosphorus (U-P), urinary creatinine (Cr) and osteocalcin (OC) levels were measured.*
Results: *AMBE dose-dependently inhibited the bone mineral density (BMD) reduction of L4 vertebrae (0.27 ± 0.03 g/cm^2, $p < 0.05$) and femurs (0.23 ± 0.03 g/cm^2, $p < 0.05$) caused by OVX and prevented the deterioration of trabecular microarchitecture ($p < 0.05$), which were accompanied by a significant decrease in skeletal remodeling ($p < 0.05$) as evidenced by the lower levels of bone turnover markers. A higher dosage of AMBE treatment (240 mg/kg/day) increased U-Ca/Cr (0.27 ± 0.03 mmol/mmol), ALP (137.23 ± 16.72 U/L), U-P/Cr (4.18 ± 0.27 mmol/mmol) and OC (8.47 ± 0.26 mmol/L) levels (both $p < 0.05$).*
Conclusion: *The findings of this study indicate that AMBE prevents OVX-induced osteoporosis in rats.*

Keywords: *Astragalus membranaceus (Fisch.) Bunge, Osteoporosis, Ovariectomy, Bone mineral density*

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INTRODUCTION

Osteoporosis is a common systemic skeletal condition among older people. Currently, 2.2 million people have osteoporosis and for those aged 50 and over, up to one in four men and two in five women will experience a minimal trauma fracture [1,2]. Osteoporosis is a generalised skeletal disorder characterised by decreased bone mass and deteriorated bone architecture. Osteoporosis results in an increased susceptibility to bone fractures, and accelerated bone loss is correlated with an increased post-fracture mortality risk; thus, osteoporosis is a major health concern [3]. In the elderly, hip fractures are closely associated with mortality [4]. Hormone deficiency is known to impair cancellous metaphyseal bone and reduce BMD in humans and animals. Therefore, estrogen deficiency in post-menopausal women has been

regarded as a critical cause of this population's susceptibility to osteoporosis [5]. Osteoporosis is twice as common in women as in men [6-8].

Among recent drugs, adjuvant hormone antagonist therapies aromatase inhibitors in women who have undergone surgery for breast cancer, gonadotropin releasing hormone (GnRH) agonists in men with prostate cancer] need special attention. Treatment with these drugs results in a progressive decrease in BMD, although a role for independent factors for fracture risk cannot be excluded [9-13]. Other medicines that stimulate bone formation (e.g., growth hormone, sodium fluoride, and parathyroid hormone) or inhibit bone resorption (e.g., bisphosphonates and calcitonin) may prevent bone loss progression in established osteoporosis. However, these medications are not suitable for a large proportion of the world population, especially in developing countries, and these drugs have side effects, such as gastrointestinal reactions, cancers, osteonecrosis of the jaw, and reduced skeletal strength [14,15]. Consequently, there is a need to develop new drugs with improved therapeutic efficacy and fewer undesirable side effects.

Astragalus membranaceus (Fisch.) Bunge. has been widely used as an anti-osteoporosis herb in traditional medicine for many years in China [16-19]. This study was designed to investigate the anti-osteoporosis effect of AMBE in rats.

EXPERIMENTAL

Collection and preparation of *Astragalus membranaceus* extract

The herbal samples of *Astragalus membranaceus* (Fisch.) Bunge. were collected from Shiyan City, Hubei Province in China in May 2015. Taxonomic identification of the plant was performed by Professor ZhiHu of Changchun University of Chinese Medicine, in China. A voucher specimen (no. AMBE 201505016) was deposited in College of Pharmacy, Changchun University of Chinese Medicine, China for future reference.

One batch of herbal samples *Astragalus membranaceus* (Fisch.) was dried in an oven. AMBE was obtained by steeping the dried *Astragalus membranaceus* (Fisch.) in water at 60 ^{o}C three times for one hour each. Then the extracted fluid was dried in an oven and freeze-dried to obtain the last extract. One gram powder was equivalent to about 1.8 g crude samples. The yield was 55.67 %.

Animals and treatments

Female Wistar rats (weighing 200 ± 20 g) were provided by the Experimental Animal Center of Jilin Province (Certificate no. SYXK2003-0006). The animals had free access to feed and water, and were allowed to acclimatize for at least one week before use. The rat experiment was approved by the Animal Care and Use Committee of Changchun University of Chinese Medicine (approval ref no. 20100308) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [20].

Sixty rats were randomly divided into six groups of ten rats each: control group, ovariectomy (OVX) with vehicle group, OVX with 17ß-estradiol (E_2, 0.025 mg/kg/day) group, and OVX with AMBE doses (60, 120, or 240 mg/kg/day) groups.

BMD measurement

The BMD of L4 vertebrae and right femurs of rats was measured using dual-energy x-ray absorptiometry scanning. The measurement was expressed as gram of mineral contents per cm^2 of surface area.

Three-point bending test

After the animals were sacrificed by cervical dislocation, they were used for three-point bending test. The left femurs of rats were slowly thawed at room temperature. The length of each femur (distance from the intermalleolar to the intercondylar region) was measured with a micrometer, and the center of the diaphysis was determined.

Serum and urine specimen analysis

Serum alkaline phosphatase (ALP), urinary calcium (U-Ca), urinary phosphorus (U-P), and urinary creatinine (Cr) levels were measured on an automatic analyzer using a diagnostic reagent kit. Serum osteocalcin (OC) concentration was determined using a rat OC ELISA kit.

Statistical analysis

The data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA combined with Bonferroni's multiple comparison test using SPSS 18.0. Differences were considered statistically significant at $p < 0.05$.

RESULTS

BMD of L4 vertebrae and femur

BMD results for rat L4 vertebrae and femur are shown in Table 1. Compared with control group, OVX significantly decreased the BMD in the L4 vertebrae and femurs (both $p < 0.05$). However, AMBE treatment prevented the BMD decrease in OVX-induced L4 vertebrae and femurs (all $p < 0.05$) in a dose-dependent manner compared to the OVX group. E_2 also significantly increased the BMD of the L4 vertebrae and femurs (both $p < 0.05$).

Mechanical properties of femur

The femur mechanical testing result sees Table 2. Compared with the sham group, 16 weeks of estrogen deficiency significantly decreased the maximum load and maximum stress (both $p < 0.05$). Higher dosage of AMBE treatments (120 or 240 mg/kg/day) markedly prevented the OVX-induced tendency to decrease these parameters (both $p < 0.05$). E_2 also increased the above-mentioned biomechanical parameters, which were significantly higher than those of the OVX group (all $p < 0.05$).

Biochemical profile of rat serum and urine

The effects of AMBE on biochemical parameters in the serum and urine of OVX rats sees Table 3. Compared with the sham group, the levels of U-Ca/Cr, U-P/Cr, ALP, and OC were significantly increased in the OVX group (all $p < 0.05$). All three AMBE doses increased U-Ca/Cr and ALP levels (all $p < 0.05$) in a dose-dependent manner. Higher dosage of AMBE treatment (120 or 240 mg/kg/day) increased U-P/Cr and OC levels (both $p < 0.05$). Again, E_2 administration also reversed the above-mentioned increases, which were statistically significant.

Table 1: Effect of AMBE on BMD of L4 vertebrae and femur (n = 10)

Group	Dosage (mg/kg)	BMD of vertebrae (g/cm^2)	BMD of femurs (g/cm^2)
Control	-	0.29±0.02*	0.26±0.02*
OVX	-	0.18±0.03	0.12±0.03
E_2	0.025	0.24±0.04*	0.21±0.02*
L-AMBE	60	0.20±0.03*	0.15±0.03*
M-AMBE	120	0.25±0.03*	0.19±0.04*
H-AMBE	240	0.27±0.03*	0.23±0.03*

* $p < 0.05$ and ** $p < 0.01$ versus OVX group. L-AMBE: low dose of AMBE, M-AMBE: middle dose of AMBE, H-AMBE: high dose of AMBE

Table 2: Effect of AMBE on femur mechanical properties (n = 10)

Group	Dosage (mg/kg)	Maximum load (N)	Maximum stress (MPa)
Control	-	118.3±4.9*	216.7±5.4*
OVX	-	88.7±5.2	137.1±6.2
E_2	0.025	121.5±4.7*	194.3±5.6*
L-AMBE	60	92.3±5.4	162.5±6.8
M-AMBE	120	98.6±4.1*	182.2±5.6*
H-AMBE	240	116.1±4.5*	197.4±5.1*

* $P < 0.05$ and ** $p < 0.01$ versus OVX group. L-AMBE: low dose of AMBE, M-AMBE: middle dose of AMBE, H-AMBE: high dose of AMBE

Table 3: Effect of AMBE on biochemical of rat serum and urine (n = 10)

Group	Dosage (mg/kg)	U-Ca/Cr (mmol/mmol)	U-P/Cr (mmol/mmol)	ALP (U/L)	OC (mmol/L)
control	-	0.19±0.03*	3.27±0.31*	103.72±11.35*	6.71±0.31*
OVX	-	0.47±0.02	5.48±0.28	201.54±26.43	14.26±0.43
E_2	0.025	0.23±0.03*	4.33±0.25*	132.27±16.46*	8.54±0.27*
L-AMBE	60	0.41±0.03*	5.53±0.45	176.32±17.21*	12.35±0.32
M-AMBE	120	0.36±0.02*	4.62±0.39*	148.26±15.76*	11.64±0.28*
H-AMBE	240	0.27±0.03*	4.18±0.27*	137.23±16.72*	8.47±0.26*

* $p < 0.05$ and ** $p < 0.01$ versus OVX group. L-AMBE: low dose of AMBE, M-AMBE: middle dose of AMBE, H-AMBE: high dose of AMBE

DISCUSSION

Osteoporosis poses a significant public health issue. The use of drugs registered for the treatment of osteoporosis are recommended when the benefits overcome the risk. Despite the pharmacological and clinical advantages of HRT as a widely accepted therapeutic strategy for osteoporosis, serious side effects of long-term application have also been reported. Therefore, new therapeutic drugs for osteoporosis is urgently needed. In recent decades, Chinese medicine have been extensively investigated for their pharmacological effects related to bone protection. The present study is the first to demonstrate the beneficial effects of AMBE against OVX-induced osteoporosis in rats. In our study, high dose of AMBE significantly improved the bone mass, bone strength, bone microarchitecture, and bone turn-over in OVX-induced osteoporotic rats. These results revealed AMBE could be used as a natural alternative for treating osteoporosis.

Bone remodeling is the natural process that mediates changes in the traits that influence bone strength. Any interruption in bone remodeling, such as menopause, will disturb the balance between formation and resorption and cause bone mass loss [21,22]. We used OVX rats as an animal model for human osteoporosis *in vivo* experiments. It has been reported that statistically significant bone loss can be seen after 30 days of treatment [23]. Consistent with other studies, OVX caused significantly higher body weights in our present study, which may be attributed to fat deposition caused by the lack of estrogen [24]. Previous studies suggest that estrogen plays an important role in stimulating the differentiation of progenitor cells through the osteoblast lineage but not the adipocyte lineage [25].

The amount of bone present in the body, bone mineral content, and bone mineral density are parameters measured to determine whether a person is osteoporotic. Bone strength is dependent on both the quantity of minerals present (BMD) and the quality of the bone. Bone re-modelling is a major determinant of bone strength. Bone quality is a function of bone morphology and architecture as well as of bone material properties. Decreased BMD is one of the major factors jeopardizing bone strength, resulting in increased susceptibility to fractures [26]. Thus, BMD measurement can best predict fracture risk [27]. This study showed that OVX reduced BMD in the right femurs and L4 vertebrae, which are rich in trabecular bone, while treatment with AMBE dose-dependently prevented the decreases in BMD. Although BMD is among the strongest predictors of facture resistance, both empirical observations and theoretical analyses show that the biomechanical properties of bone and trabecular microarchitecture trabecular bone strength as well [28]. The present study revealed that higher doses of AMBE prevented the OVX-induced tendency toward decreased biomechanical parameters.

Furthermore, bone marker measurement plays a role in osteoporosis diagnosis and treatment [29]. Enhanced ALP, OC, U-Ca/Cr, and U-P/Cr levels indicated upregulation of bone turnover by OVX. The bone turnover markers above were dose-dependently reversed by AMBE, indicating a reduction in bone turnover rate after treatment of AMBE.

CONCLUSION

The findings of this study indicate that AMBE is effective in the treatment of osteoporosis in rats. Thus, AMBE has a potential to be developed as a natural alternative treatment agent to treat osteoporosis in humans.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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