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Original Research Article

Evaluation of Epimedium brevicornum Maxim extract for anti-osteoporosis activity in rats

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Abstract

Purpose: *To evaluate the therapeutic effect of Epimedium brevicornum Maxim. extract (EBME) on ovariectomy-induced osteoporosis in rats.*
Methods: *The rats were divided into six experimental groups, viz, control (group 1) and five ovariectomy-induced (OVX) groups. The OVX groups include OVX-inducing agent only group (group 2), OVX with 17ß-estradiol (E_2, 25 µg/kg/day, group 3), OVX with 60 mg EBME/kg body weight/day (group 4), OVX with 120 mg EBME/kg body weight/day (group 5) and OVX with 240 mg EBME/kg body weight/day (group 6). The treatment started for the OVX groups with a single weekly dose of OVX inducing agent for 4 weeks, followed by oral daily dose of E_2 (group 3) or EBME (groups 4, 5 and 6) for another 16 weeks. Bone mineral density (BMD) of the 4th lumber vertebrae (4LV) and right femur of each rat was estimated. BMD determination was preceded by the measurement of the length of the femur and identification of diaphysis (center). Trabecular microarchitecture was assessed via three representative 4LV. The other parameters measured in this study were serum alkaline phosphatase (ALP), urinary calcium (U-Ca), urinary phosphorus (U-P), urinary creatinine (U-SCr) and osteocalcin (OC) levels.*
Result: *The results showed that the BMD decrease induced by OVX in 4LV and femur was significantly mitigated by high dose of EBME. EBME also protected the trabecular microarchitecture against OVX-associated deterioration, evidenced by decreased bone turnover marker levels in 4LV at high EBME dose. Trabecular number (Tb-N, 3.7 ± 0.2), trabecular thickness (Tb-Th, 0.082 ± 0.011), and trabecular spacing (Tb-Sp, 0.17 ± 0.01) of the highest dose EBME-treated OVX rats '4LV were significantly ($p < 0.05$) different from the corresponding values of EBME-free OVX rats.*
Conclusion: *The results reveal that administration of high doses of EBME lasting for 16 weeks not only protected against OVX-induced osteoporosis in rats but was also without the risk of endometrial hyperplasia. Thus, the extract may be a better alternative to other agents in current use for the treatment of postmenopausal osteoporosis in elderly women. However, its efficacy and safety require further investigations.*

Keywords: *Epimedium brevicornum Maxim., Postmenopausal osteoporosis, Ovariectomy, Bone mineral density*

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INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by decreased bone mass and deteriorated bone tissue. The disease causes bone fragility with increased susceptibility to fractures [1]. Based on the data from World Health Organization (WHO), millions of people are now suffering from osteoporosis throughout Europe, the USA, and China [2]. The morbidity rises dramatically with increasing life span. Accordingly, the risk of osteoporotic fractures and the associated costs correlates positively with advancing age. Hip fracture is among the major causes of death of the elderly [3].

Hormone deficiency is known to impair proper development of metaphyseal cancellous bone strength as well as reduction in bone mineral density (BMD) in humans and animals. Presently the hormone whose deficiency is widely believed to be linked to osteoporosis in post-menopausal women is estrogen [4]. Osteoporosis is twice as common in women as in men, and approximately one in three women over 50 years old experience an osteoporotic fracture [5].

The most common therapy for postmenopausal osteoporosis is hormone replacement therapy (HRT) [6]. Nonetheless, HRT is a health management approach fraught with risk, the major one is the induction of cancer of the reproductive tissues [7]. Besides HRT, there are other drugs currently used for the treatment of osteoporosis which are neither readily available to, nor affordable by a large proportion of the world population in developing countries. Again, the drugs have side effects, such as gastrointestinal reactions, cancer induction, osteonecrosis of the jaw, and reduced skeletal strength. Therefore, the search for alternative therapeutic agents capable of prompting osteogenesis and/or impeding bone resorption but devoid of untoward side effects has continued to hold the attention of research scientists [8,9].

It is well known that *Epimedium brevicornum* Maxim. displays kidney-toning and anti-osteoporosis effects, and is widely used herb in China for the treatment of nephrasthenia syndrome [10], osteoporosis, [11] and bone fracture [12].

In this study, an in-depth investigation of *Epimedium brevicornum* Maxim. extract (EBME) as a possible anti-osteoporosis agent in ovariectomy (OVX) - induced osteoporosis in rats was carried out.

EXPERIMENTAL

Preparation of *Epimedium brevicornum* Maxim. extract

The herb*, Epimedium brevicornum* Maxim., was collected in Nanning City, Guangxi Province, China, in October 2015. It was identified by Professor Lin He, and a voucher specimen (no. EBME 201509015) was deposited at the herbarium of College of Pharmacy, Wenzhou Medical University. One batch of the herbal sample of *Epimedium brevicornum* Maxim. was dried in an oven, and thereafter, immersed in water at 60 ^{o}C for 1 h to obtain the initial extract. This extraction process was repeated thrice, and the pooled *extracts were freeze*-dried. One gram of the extract powder was obtained from approximately 1.6 g herbal sample, giving a yield of 62.5 %.

Treatment of animals

Healthy, three-month-old female Sprague-Dawley rats (weight 200 ± 20 g) were supplied by the Experimental Animal Center of Zhejiang Province (Certificate no. SYXK2002-0005). They had free access to feed and water, and were allowed to acclimatize to the laboratory conditions for at least one week, before the study commenced. The animal studies were approved by the Animal Care and Use Committee of Wenzhou Medical University (approval ref no. 20100407). The animal studies were carried out according to Directive 2010/63/EU guidelines for the use of animals for scientific purposes [13].

The sixty rats used for this study were divided into six experimental groups of ten rats each. They were group 1, the control group, and the ovariectomy (OVX) groups (groups 2-6). Group 2 rats received only the OVX agent. Group 3 rats were the OVX category given 17ß-estradiol (E_2, 25 mg/kg/day). Group 4 rats were the OVX group, given 60 mg EBME/kg/day. Groups 5 and 6 were also OVX rats, but were given 120 and 240 mg EBME respectively /kg/day. The treatments started with single weekly dose of OVX agent for 4 weeks (all OVX rats), followed by oral daily dose of E_2 (group 3 rats) or EBME (groups 4, 5 and 6) for another 16 weeks.

Bone mineral density measurement

Dual-energy X-ray absorptiometry scanning (DEXA, GE Healthcare, USA) for small animal measurements was employed for the estimation of the BMD of the 4th lumber vertebrae and right femurs. The results of the measurements were

presented as mineral contents (g)/surface areas (cm^2).

Three-point bending test

The rats were sacrificed by cervical dislocation and thereafter the femur bones were carefully disarticulated and cleaned. Afterwards the length of each femur and the diaphysis were respectively, determined.

Biochemical assay

After each rat has been sacrificed by cervical dislocation, urine was obtained from it by stimulating the dorsal region, near the hind limbs. An automatic analyzer (Ciba-Corning 550, USA) was used for the measurement of the levels of serum alkaline phosphatase (ALP), urinary calcium (U-Ca), urinary phosphorus (U-P), and urinary creatinine (U-Cr) using diagnostic reagent kits. An osteocalcin (OC) ELISA kit (San Clemente, CA, USA) was used for the assay of serum osteocalcin level.

Statistical analysis

The results are shown as mean ± SD. One-way ANOVA combined with Bonferroni's multiple comparison test were used for data comparison while SPSS 16.0 software was used for statistical analysis. Criterion for statistical significant was set at $p < 0.05$.

RESULTS

BMD of 4th lumbar vertebra and femur

The values of the BMDs of the 4th lumber vertebrae and femurs are presented in Table 1. These results show that the BMDs of the 4th lumber vertebrae and femurs were significantly ($p < 0.05$) reduced as a result of ovariectomy, when compared to the control group. However, EBME treatment caused significant ($p < 0.05$) increase in BMDs of the 4th lumber vertebrae and femurs of OVX-induced rats compared with OVX-induced rats not treated with EBME. The increase was in a dose-dependent manner.

Mechanical characteristics of femur

The results of the mechanical characteristics of the femur bones are presented in Table 2. Compared with the control group, the tolerable maximum load and maximum stress limits decreased significantly ($p < 0.05$) in the estrogen deficient (ovariectomy) rats. However, the higher doses of EBME (80 or 160 mg/kg/day) caused significant ($p < 0.05$) increase in these parameters when compared to the EBME-free OVX rats. E_2 also increased these biomechanical parameters, compared to those of the OVX group significantly ($p < 0.05$). The effect of H-EBME on maximum load was similar to that of E_2.

Table 1: Effect of EBME on BMD of 4th lumber vertebrae and femurs

Group	Dosage (mg/kg)	BMD of vertebrae (g/cm^2)	BMD of femurs(g/cm^2)
Control	-	0.25 ± 0.03*	0.23 ± 0.03*
OVX	-	0.14 ± 0.03	0.12 ± 0.03
E_2	0.025	0.20 ± 0.03	0.18 ± 0.03
L-EBME+	60	0.19 ± 0.04*	0.15 ± 0.02*
M-EBME	120	0.21 ± 0.02*	0.18 ± 0.03*
H-EBME	240	0.24 ± 0.01*	0.21 ± 0.02*

*$P < 0.05$ and **$p < 0.01$ versus OVX group; +L / M / H-EBME: low / middle / high dose of EBME (n = 10 rats)

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

Group	Dose (mg/kg)	Maximum load (N)	Maximum stress (MPa)
Control	-	131.5 ± 5.3*	203.5 ± 6.5*
OVX	-	94.7 ± 4.5	164.3 ± 5.6
E_2	0.025	123.6 ± 5.2*	184.7 ± 4.8*
L-EBME+	60	98.2 ± 4.9	153.6 ± 4.4
M-EBME	120	108.2 ± 5.2*	169.3 ± 5.1*
H-EBME	240	114.3 ± 5.1*	173.1 ± 5.0*

*$P < 0.05$ and **$p < 0.01$ versus OVX group; + L/M/H-EBME: low/middle/high dose of EBME

Table 3: Effect of EBME on morphometric parameters in L4 vertebrae

Group	Dose (mg/kg)	Tb-N (1/mm)	Tb-Th (mm)	Tb-Sp (mm)
Control	-	5.2 ± 0.3*	0.094 ± 0.014*	0.12 ± 0.01*
OVX	-	2.7 ± 0.4	0.061 ± 0.012	0.38 ± 0.02
E_2	0.025	4.3 ± 0.4*	0.085 ± 0.014*	0.16 ± 0.01*
L-EBME+	60	2.9 ± 0.3	0.069 ± 0.015	0.29 ± 0.03
M-EBME	120	3.6 ± 0.3*	0.073 ± 0.012*	0.25 ± 0.02*
H-EBME	240	3.7 ± 0.2*	0.082 ± 0.011*	0.17 ± 0.01*

*$P < 0.05$ and **$p < 0.01$ versus OVX group. + L/M/H-EBME: low/middle/high dose of EBME (n = 10 rats)

Table 4: Effect of EBME on biochemical parameters in the serum and urine

Group	Dose (mg/kg)	U-Ca/Cr	U-P/Cr	ALP (U/L)	OC (mmol/L)
Control	-	0.24 ± 0.02*	3.3 ± 0.3*	107.5±12.5*	7.6 ± 0.3*
OVX	-	0.48 ± 0.03	5.8 ± 0.4	231.2 ±26.8	15.1 ± 0.5
E2	0.025	0.28 ± 0.02*	4.3 ± 0.2*	138.4±18.7*	9.6 ± 0.4*
L-EBME+	60	0.33±0.0.02*	5.5 ± 0.4	176.2±16.5*	11.3 ± 0.3
M-EBME	120	0.37 ± 0.01*	4.3 ± 0.3*	154.5±15.2*	10.7 ± 0.2*
H-EBME	240	0.26±0.0.02*	4.5 ± 0.2*	143.3±16.5*	8.8 ± 0.3*

*$P < 0.05$ and **$p < 0.01$ versus OVX group. + L / M / H-EBME: low / middle / high dose of EBME (n = 10 rats).

Biochemical parameters of serum and urine specimens

Data presented in Table 4 show the effects of EBME on the biochemical parameters of OVX rats. Compared to the control group, U-Ca/Cr, U-P/Cr, and serum ALP, and OC levels were significantly ($p < 0.05$) increased in the OVX group. However, EBME significantly decreased the U-Ca/Cr and ALP levels ($p < 0.05$) in a dose-dependent manner. Higher dosage of EBME (120 or 240 mg/kg/day) decreased the U-P/Cr and OC levels significantly ($p < 0.05$) relative to the non-OVX control. Again, E_2 administration also reversed these changes significantly. The effect of E_2 on these parameters is similar to that of the highest dose of EBME.

DISCUSSION

Bone re-modeling is an important physiological process that exerts changes and impacts on bone strength [1]. Any interruption in bone remodeling, caused by menopause for example, will disturb the balance between formation and resorption with attendant loss in bone mass [14]. Therefore, OVX rats were used as animal model for human osteoporosis *in vivo* experiments. It has been reported that statistically significant bone loss can be seen after 30 days of exposure to OVX inducing agents [15], so treatment was initiated 4 weeks after OVX. Consistent with other studies, one the findings in the present study is that OVX caused significantly higher body weights gain, which may be attributed to fat deposition caused by the lack of estrogen. It has been proposed previously that the differentiation of progenitor cells may greatly rely on estrogen via the osteoblast lineage but not the adipocyte lineage [16].

Decreased BMD is believed to exert harmful impact on bone strength, leading to increased susceptibility to fractures [17]. Thus, measurement of BMD is can provide information for evaluating bone fracture risk [18,19]. The results in the present study showed that OVX reduced BMD in the right femurs and 4th lumber vertebrae, which are rich in trabecular bone. Also found was that EBME hindered the reduction of BMD in a dose-dependent manner. Although BMD is among the strongest predictors of bone susceptibility to facture, both empirical observations and theoretical analyses indicate that the biomechanical properties of bone and trabecular microarchitecture also affect trabecular bone strength [20]. Three-point bending tests of the left femurs in our study indicated that the higher extract doses (80 or 160 mg/kg/day) prevented the OVX-induced tendency toward decreased biomechanical parameters.

Measurements of structural parameters using micro-CT also showed that treatment with EBME effectively restored the trabecular micro-

architectural properties compared to the OVX group. In addition, measurement of bone markers is an important approach to osteoporosis diagnosis and treatment [21]. Bone mass loss, as evidenced by enhanced levels of ALP, OC, U-Ca/Cr, and U-P/Cr, was an indication of the capacity of OVX to increase bone turnover. The alterations in values of bone turnover markers analyzed in this study were dose-dependently reversed by EBME. Evidently, the results presented here suggest that EBME is likely to be an effective agent for the treatment of osteoporosis in menopausal women.

CONCLUSION

The findings of this study show that EBME mitigates OVX-induced osteoporosis in rats. Therefore, the plant extract requires further investigation for possible management of osteoporosis in menopausal women.

DECLARATIONS

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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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