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Original Research Article

Anti-osteoporosis effect of Cistanche deserticola Ma extract in ovariectomized rats

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Abstract

Purpose: *To investigate the therapeutic effects of Cistanche deserticola Ma. extract (CDME) on ovariectomy-induced osteoporosis in rats.*
Methods: *Female Sprague-Dawley rats were randomly assigned to a control group and five ovariectomy (OVX) subgroups, that is, OVX with vehicle (OVX), OVX with 17ß-estradiol (E2, 25 µg/kg/day), and OVX with CDME doses (40, 80, or 160 mg/kg/day). Daily oral administration of E2 or CDME started 4 weeks after OVX and lasted for 16 weeks. Bone mineral density (BMD) of L4 vertebrae and right femur of rats was estimated, The length of each femur was measured, and biochemical analysis of serum and urine specimens were performed.*
Results: *CDME dose-dependently inhibited the reduction in BMD of L4 vertebrae (0.23 ± 0.02 g/cm^3, $p < 0.05$) and femurs (0.20 ± 0.03 g/cm^3, $p < 0.05$) 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.*
Conclusion: *This study indicates that CDME prevents OVX-induced osteoporosis in rats, and could be used for treating osteoporosis in elderly women.*

Keywords: *Cistanche deserticola, Osteoporosis, Ovariectomy, Bone mineral density, Femur*

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INTRODUCTION

Osteoporosis is the most common bone disease and is characterized by low bone mass, microarchitectural deterioration of bone tissue, and subsequent bone fragility with susceptibility to fracture [1]. Bone fracture risk typically increases in the hip, vertebral, and distal forearm bones. These fractures are not only painful but also disabling, leading to the need for nursing home care and increased mortality when compared to age matched populations [2]. Because of the high morbidity and mortality associated with osteoporotic fractures, treatment of osteoporosis prioritizes fracture prevention [3]. Hormone deficiency is known to impair cancellous metaphyseal bone and reduce bone mineral density in humans and animals; therefore, the estrogen deficiency in postmenopausal women has been regarded as a critical cause of this population's susceptibility to osteoporosis [4]. Osteoporosis is twice as

common in women as in men [5], and approximately one in three old women experience osteoporotic fracture in her lifetime [6,7].

Clinically, hormone replacement therapy (HRT) has been a popular therapeutic strategy designed for post-menopausal osteoporosis [8,9]. However, the long-term application of HRT has potential malignant effects on reproductive tissues [10]. Vitamin D and calcium are components of bone renewal, but supplementation has limited and inconsistent effectiveness and is often used in combination with other treatments [11].

Cistanche deserticola Ma. has been widely used as a kidney tonifying and anti-osteoporosis herb for treating osteoporosis for thousands of years in China [12-14]. Therefore, this study was performed to evaluate the effect of CDME on osteoporosis in rats.

EXPERIMENTAL

Preparation of *Cistanche deserticola* Ma. extract

The herbal samples of *Cistanche deserticola* Ma. were collected from Bozhou City, Anhui Province in China in September 2015. Taxonomic identification of the plant was performed by Professor Ping He of Zhejiang Chinese Medical University, China. A voucher specimen (no. CDME 201510012) was deposited in Zhejiang Chinese Medical University, China for future reference.

One batch of herbal samples of *Cistanche deserticola* Ma. was dried in an oven at 80 ℃. Aqueous extract of CDME was obtained by steeping the dried *Cistanche deserticola* Ma. in water at 70 ^{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 Sprague-Dawley rats (wt. 200 ± 20 g) were provided by the Experimental Animal Center of Zhejiang Province (Certificate no. SYXK 2003-0004). 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 Zhejiang Chinese Medical University (approval ref no. 20110506) 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 individuals: a control group and five ovariectomy (OVX) subgroups, that is, OVX with vehicle (OVX), OVX with 17ß-estradiol (E_2, 25 g/kg/day), and OVX with CDME (40, 80, or 160 mg/kg/day).

Bone mineral density (BMD) measurement

The BMD of the L4 vertebrae and right femurs was estimated using dual-energy x-ray absorptiometry scanning with small animal measurement. The measurements were expressed as grams of mineral contents per cm^2 of surface area. Scans were performed by the same blinded technician.

Three-point bending test

Before mechanical testing, the left femurs 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.

Biochemical analysis of serum and urine specimens

The levels of serum alkaline phosphatase (ALP), urinary calcium (U-Ca), urinary phosphorus (U-P), and urinary creatinine (Cr) 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 the mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA combined with Bonferroni's multiple comparison test using SPSS 16.0. Differences were considered statistically significant at $p < 0.05$.

RESULTS

BMD of L4 vertebrae and femur

Table 1 demonstrate that OVX significantly decreased the BMD in the L4 vertebrae and femurs compared to control group (both $p < 0.05$). Compared with the OVX group, CDME treatment obviously prevented the BMD decrease in OVX-induced L4 vertebrae and femurs ($p < 0.05$) in a dose-dependent manner.

Table 1: Effect of CDME on BMD of L4 vertebrae and femurs (n = 10)

Group	Dose (mg/kg)	BMD of vertebrae (g/cm^2)	BMD of femurs (g/cm^2)
Control	-	0.25±0.02*	0.22±0.04*
OVX	-	0.14±0.03	0.13±0.03
E_2	0.025	0.21±0.04*	0.17±0.04*
L-DME	40	0.16±0.03*	0.15±0.03*
M-DME	80	0.20±0.03*	0.18±0.04*
H-DME	160	0.23±0.02*	0.20±0.03*

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

Mechanical characteristics of femur

Table 2 revealed the results of the femur mechanical testing. Compared with the control group, 16 weeks of estrogen deficiency significantly decreased the maximum load and maximum stress (both p < 0.05). Higher dosage of CDME treatment (80 or 160 mg/kg/day) markedly decreased these parameters (both p < 0.05). E_2 also increased the biomechanical properties, which were significantly higher than those of OVX group (all p < 0.05).

Biochemical profile of serum and urine specimens

The effects of CDME on biochemical parameters in the serum and urine of OVX rats sees Table 4. Compared with control 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 CDME doses significantly prevented the increases in U-Ca/Cr and ALP levels (all p < 0.05) in a dose-dependent manner. Higher dosage of CDME treatment (80 or 160 mg/kg/day) significantly prevented the increases in U-P/Cr and OC levels (both p < 0.05).

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

Group	Dose (mg/kg)	Maximum load (N)	Maximum stress (MPa)
Control	-	131.4±6.2*	207.6±5.9*
OVX	-	89.7±5.1	145.4±5.3
E_2	0.025	121.6±4.8*	173.2±4.6*
L-CDME	40	92.8±4.5	149.6±5.8
M-CDME	80	103.7±5.8*	166.5±6.1*
H-CDME	160	117.6±4.9*	187.3±4.8*

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

Table 3: Effect of CDME on morphometric parameters in L4 vertebrae (n = 10)

Group	Dosage (mg/kg)	Tb-N (1/mm)	Tb-Th (mm)	Tb-Sp (mm)
Control	-	5.1±0.4*	0.093±0.013*	0.11±0.02*
OVX	-	2.4±0.5	0.059±0.015	0.42±0.04
E_2	0.025	4.3±0.3*	0.084±0.014*	0.22±0.03*
L-CDME	40	2.6±0.3	0.071±0.013	0.35±0.04
M-CDME	80	3.7±0.4*	0.075±0.014*	0.27±0.03*
H-CDME	160	4.5±0.3*	0.086±0.012*	0.17±0.02*

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

Table 4: Effect of CDME on biochemical parameters in the serum and urine (n = 10)

Group	Dose (mg/kg)	U-Ca/Cr (mmol/L)	U-P/Cr (mmol/L)	ALP (U/L)	OC(mmol/L)
Control	-	0.19±0.03*	2.8±0.4*	107.6±9.8*	7.3±0.3*
OVX	-	0.53±0.04	5.4±0.2	232.4±30.5	18.2±0.6
E_2	0.025	0.24±0.03*	3.3±0.3*	123.5±21.3*	10.5±0.4*
L-CDME	40	0.46±0.04*	4.9±0.4	187.1±19.3*	13.7±0.5
M-CDME	80	0.37±0.02*	4.1±0.3*	148.3±17.5*	11.4±0.4*
H-CDME	160	0.25±0.03*	3.2±0.4*	126.7±14.4*	8.8±0.3*

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

DISCUSSION

The aim of this study was to determine the potential effects of *Cistanche deserticola* Ma. extract (CDME) in osteoporosis therapy. Our results suggest that CDME could prevent bone loss. This study revealed that CDME significantly improved bone mass, bone strength, bone microarchitecture, and bone turn-over in OVX-induced osteoporotic rats, which was similar to that of E_2. These results suggest the potential role of CDME as a natural alternative for postmenopausal osteoporosis management.

Bone remodeling is the 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 [16]. Therefore, we used OVX rats as an animal model for human osteoporosis. It has been reported that statistically significant bone loss can be seen after 30 days of treatment [17], so treatment was initiated 4 weeks after OVX. 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. 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 [18].

Decreased BMD is one of the major factors that jeopardizing bone strength, resulting in increased susceptibility to fractures [19]. Thus, BMD measurement can best predict fracture risk. Results in the present study showed that OVX reduced BMD in the right femurs and L4 vertebrae, which are rich in trabecular bone, while treatment with CDME 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 influenced trabecular bone strength as well [20].

The measurement of bone markers plays a role in osteoporosis diagnosis and treatment [21]. Bone mass loss, as evidenced by enhanced levels of ALP, OC, U-Ca/Cr, and U-P/Cr, indicated upregulation of bone turnover by OVX. The bone turnover markers above were dose-dependently reversed by CDME, indicating a reduction in bone turnover rate after treatment of CDME. This study suggests that CDME is effective for treating osteoporosis in menopausal women.

CONCLUSION

The findings of this study indicate that CDME prevents OVX-induced osteoporosis in rats, and could be effective for treating osteoporosis in elderly women.

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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