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Review

A traditional Chinese herbal preparation, Er-Zhi-Wan, prevent ovariectomy-induced osteoporosis in rats

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ABSTRACT

Ethnopharmacological relevance: Er-Zhi-Wan (EZW), a classic Chinese formulation, which contains Fructus Ligustri Lucidi (FLL) and Herba Ecliptae (HE). EZW is widely used to prevent and treat various kidney diseases for its actions of nourishing the kidney and strengthening tendon and bone. Although recent reports indicate that EZW restrains osteoclastic bone resorption, its effects on the protection against define OVX-induced bone loss in mature rats have not been systematically investigated.
Materials and methods: Sixty 3-month-old female Sprague–Dawley rats were randomly assigned into sham-operated group (Sham) and five OVX subgroups, OVX with vehicle (OVX); OVX with Estradiol Valerate (EV, 0.4 mg/kg body weight/day); OVX with EZW of graded doses (9.0, 4.5, or 2.25 g/kg/day). Daily oral administration of EV and EZW on 5th week for 26 weeks. Bone turnover markers (Serum alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BALP), osteocalcin (OCN), deoxypyridinoline (DPD)), other parameters, including serum calcium (S-Ca), serum phosphorus (S-P), urine calcium (U-Ca), phosphorus (U-P), and bone mineral density (BMD) of the femur, 4th lumbar vertebra and tibia, bone biomechanical properties and trabecular microarchitecture parameters were measured.
Results: Administration of EZW could significantly prevent ovariectomy-induced bone loss, biomechanical reduction, deterioration of trabecular microarchitecture and the body weight gain without affecting the weight of the uterus, and increased S-Ca, S-P levels, decreased level of bone turnover markers and U-Ca, U-P levels in ovariectomized rats.
Conclusion: The present study indicated that EZW had a definite antiosteoporotic effect without hyperplastic effect on uterus, and it might be a potential alternative medicine for treatment of postmenopausal osteoporosis.

Contents

Abbreviations: ALP, Serum alkaline phosphatase; ANOVA, one-way analysis of variance; BALP, bone-specific alkaline phosphatase; BMD, bone mineral density; BV/TV, bone volume over total volume; Conn.D, connectivity density; DPD, deoxypyridinoline; DXA, dual-energy X-ray absorptiometry; EZW, Er-Zhi-Wan; EV, Estradiol Valerate; FLL, Fructus Ligustri Lucidi; HE, Herba Ecliptae; MicroCT, microcomputed tomography; HRT, hormone replacement therapy; OCN, steocalcin; OVX, ovariectomized; S-Ca, serum calcium; SD, Sprague–Dawley; Sham, sham-operated; SMI, structure model index; SPF, specific-pathogen-free; S-P, serum phosphorus; Tb.N, trabecula number; Tb.Sp, trabecula separation; Tb.Th, trabecula thickness; U-Ca, urine calcium; U-Cr, urine creatinine; U-P, phosphorus; VOI, volumes of interest.

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1. Introduction

Osteoporosis is a disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures (Epstein, 2006). The incidence of osteoporosis increases with age and occurs most frequently in postmenopausal women due to the dramatic estrogen withdrawal associated with menopause (Shih et al., 2001). Hormone replacement therapy (HRT) has proven to be efficacious in preventing bone loss and reducing the incidence of skeletal fractures in postmenopausal women (Stevenson, 2005). However, long-term HRT has estrogen-like side effects (Prelevic et al., 2005). Traditional Chinese medicines have been used in prevention and treatment of postmenopausal osteoporosis. Since these medicines are prepared from medicinal plants they have fewer side effects and for long term use (Cao et al., 2008). The traditional use of these herbal medicines can be cost-effective alternatives. Currently, their international acceptance as a regiment for prevention and treatment of osteoporosis require extensive research using modern technology.

Er-Zhi-Wan (EZW) is a common traditional Chinese formulation, which contains two herbs viz. Fructus Ligustri Lucidi. and Herba Ecliptae, has been developed as a restorative formula for hundreds of years. An equal weight ratio (1:1) of Fructus Ligustri Lucidi to Herba Eclipta is widely used to prevent and treat various kidney diseases for its actions of nourishing the kidney and strengthening tendon and bone (Lu, 2007) Modern research shows that antiosteoporotic activity of EZW is carried out mainly by restraint of osteoclastic bone resorption, to develop a new anti-osteoporotic agent for clinic usage (Zhang et al., 2008). Current knowledge whether EZW has potential antiosteoporotic activity in rats should be evaluated. Therefore, the aim of this study was to systematically evaluate whether EZW have the functions of treatment for osteoporosis induced by OVX in rats.

2. Methods

2.1. Preparation of EZW

Dried Fructus Ligustri Lucidi and Herba Ecliptae were purchased from a local herbal drug store in Xi'an, China, identified as the fruit of *Ligustrum lucidum* Ait. (Oleaceae) and the aerial parts of *Eclipta prostrate* L. (Asteraceae), respectively, by Professor Sun (Biomedicine Key Laboratory of Shaanxi Province, Northwest University, Xi'an, China). The voucher specimen of Fructus Ligustri Lucidi (KMFLL-01-20) and Herba Ecliptae (KMHE-01-20) were deposited at the Department of Medicinal Chemistry of the Fourth Military Medical University (Xi'an, China).

EZW contains two herbs viz. Fructus Ligustri Lucidi and Herba Ecliptae by weight ratio (1:1), EZW was prepared according to the National Pharmacopoeia Commission of P.R. China (Zhonghua Renmin Gongheguo wei sheng bu yao dian wei yuan hui, 2005). The extraction for Fructus Ligustri Lucidi was performed as follows: 1000 g of Fructus Ligustri Lucidi was broken into coarse powder, the powder was immersed in 1–10 (W:V) 75% alcohol for 1 h, and then boiled in a distillation apparatus, for 1 h. This process was repeated twice. The preparation was filtrated and evaporated using a rotary evaporator at 80 °C to get 1000 ml extracting solution, at a concentration of 100%, by weight of the starting materials, stored at 4 °C until use; The extraction for Herba Eclipta was performed as follows: 1000 g of Herba Eclipta was immersed in distilled water for 0.5 h, and then boiled in a distillation apparatus, for 1 h. This process was repeated twice. The preparation was filtrated and evaporated using a rotary evaporator at 80 °C to get 1000 ml extracting solution at a concentration of 100%, by weight of the starting materials, stored at 4 °C until use; The two herbal concentrates were added (1:1; V:V), respectively, mixed together, for the final product, EZW.

2.2. Animals and treatments

Female Sprague–Dawley (SD) rats were purchased from the Experimental Animal Center of the Fourth Military Medical University. Sixty 3-month-old virgin Sprague–Dawley specific-pathogen-free (SPF) female rats (SIPPR-BK Experimental Animal Ltd., China) (average body weight 230 ± 20.0 g) were housed in animal house at 22 °C and with a 12-h light and 12-h dark cycle. During the experimental period, all the rats were pair-fed, and allowed free access to distilled water and fed with standard rat chow (SIPPR-BK Experimental Animal Ltd., China). The acclimatized rats underwent either bilateral laparotomy (Sham, $n = 10$) or bilateral OVX (OVX, $n = 50$). Four weeks after recovering from surgery, the OVX rats were randomly divided into five groups: OVX with vehicle (OVX, $n = 10$); OVX with Estradiol Valerate (EV, $n = 10$, 0.4 mg/kg body weight/day); OVX with EZW of graded doses (EZW-H, $n = 10$, 9.0 g/kg body weight/day); (EZW-M, $n = 10$, 4.5 g/kg body weight/day) and (EZW-L, $n = 10$, 2.25 g/kg body weight/day). The experimental dose for EV and EZW in the present study was equivalent to the corresponding clinical prescription dose for a 60 kg human subject. Vehicle, EV, EZW was administrated orally through a custom-made stomach tube, which started on 5th week after OVX for 26 weeks.

The body weight of the animals was recorded weekly during the experimental period. Urine sample was collected in metabolic cages without providing food 1 day before euthanizing the animals and acidified with 2 ml 1 mol/l HCL. After