Original Article

Evaluation of osteoporosis prevention by adlay using a tissue culture model

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Adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) is a grass crop, which has been used in traditional Chinese medicine and also as a nourishing food. Recently, some studies have indicated that adlay possesses some pharmacological effects including anti-allergic, anti-mutagenic, hypolipemic, and anti-diabetic effects. However, the effect of adlay on osteoporosis is still unknown. In this study, we investigated and evaluated the effect of adlay seed on the osteoporosis prevention. The methods of in vitro cultures of neonatal rat calvaria tissues or adult rat femoral metaphyseal tissues of bones isolated from normal or ovariectomized female rats were used for further investigation. Treatment with water extract of adlay seed could reverse the decreased alkaline phosphatase activities and calcium levels and increased tartrate-resistant acidic phosphatase activities induced by parathyroid hormone in cultured metaphyseal tissues. In ovariectomized rats, the alkaline phosphatase activities and calcium levels were significantly decreased and tartrate-resistant acidic phosphatase activities were increased in femoral metaphyseal tissues as compared with sham-control. Treatment with water extract of adlay seed could counteract these effects in ovariectomized rats. Taken together, these findings imply that adlay is capable of reversing the osteoporotic status in rats, and may be a helpful healthy food for osteoporosis prevention.

Key Words: adlay, extract, osteoporosis, tissue culture, ovariectomized rats

INTRODUCTION

Adlay (*Coix lachryma-jobi*) is a grass crop that has long been used in traditional Chinese medicine and also used as a nourishing food. The seed of adlay has been used in Asian countries for the treatment of warts, chapped skin, rheumatism, female endocrine system and neuralgia, and as an anti-inflammatory or antihelmintic agent. Recent studies has shown that adlay exerts various pharmacological activities on immune and gastrointestinal systems,[1-4] and possesses antiproliferative and chemopreventive effects[5,6] and hypolipidemic and hypoglycemic abilities in diabetic status.[7] Adlay oil has also been reported to reduce leptin in adipose tissue and LDL levels in the rats.[8] The water extract of adlay seed has recently been shown to exhibit anti-obesity effects through neuroendocrine modulation.[9] However, there is little known about the effect of adlay on the bone system or osteoporosis.

In the present study, the effect of water extract of seeds of *Coix lachryma-jobi* L. var. *ma-yuen* Stapf on the osteoporosis prevention was studied. The methods of *in vitro* cultures of neonatal rat calvaria tissues or adult rat femoral metaphyseal tissues of bones isolated from normal or ovariectomized female rats were used for further investigation. The changes of alkaline phosphatase and tartrate-resistant acidic phosphatase activities and calcium levels in these cultured tissues after the exposure of water extract of adlay seeds were examined.

MATERIALS AND METHODS

Plant material and sample preparation

Adlay was purchased from a farmer who planted Taichung Shuenyu No. 4 (TCS4) of *Coix achrymal-jobi* L. var. *ma-yuen* Stapf in Taichung, Taiwan. The air-dried adlay seeds were dehulled, blended into powder, and screened through a 20-mesh sieve (aperture = 0.94 mm). The powder of adlay (100 g) was extracted with 1 L of methanol; the plant material was filtered off, and the methanolic extracts were combined and concentrated to dryness under reduced pressure by a rotatory vaccum evaporator (Eyela, Tokyo, Japan). After methanolic extraction, the plant material was continuously extracted with warm water at 50^{o}C for 30 min. The plant material was filtered off to obtain warm water extract. This fraction was dried using a freeze-dryer

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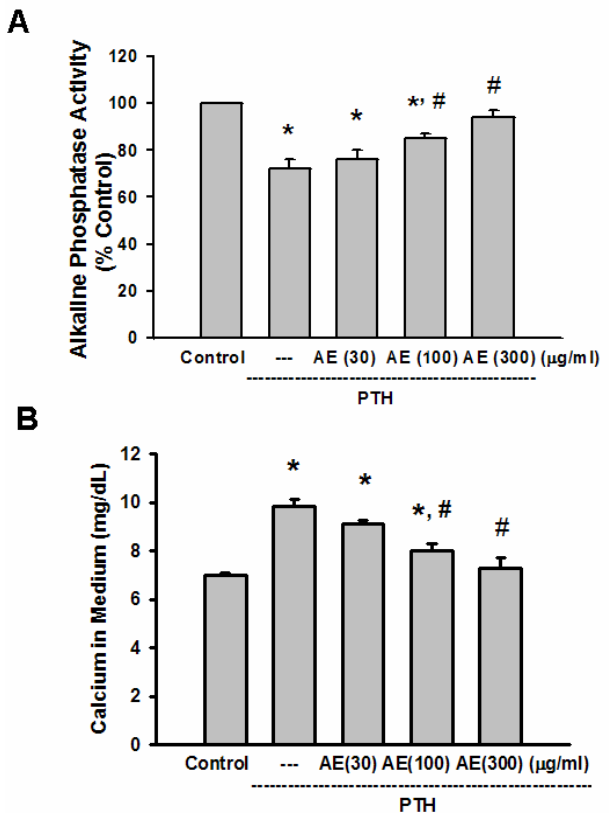

Figure 1. Effects of adlay extracts on alkaline phosphatase activities and calcium levels in cultured neonatal rat calvaria tissues. Adlay extracts treated neonatal rat calvaria tissues for 48 hours in the presence or absence of parathyroid hormone (PTH, 10^{-7} M), and the alkaline phosphatase activities in bone and calcium levels in medium were measured. Data are presented as mean±S.E.M. (n≥4). *: $p< 0.05$ as compared with control. #: $p< 0.05$ as compared with PTH alone. (A) alkaline phosphatase activity, (B) calcium levels.

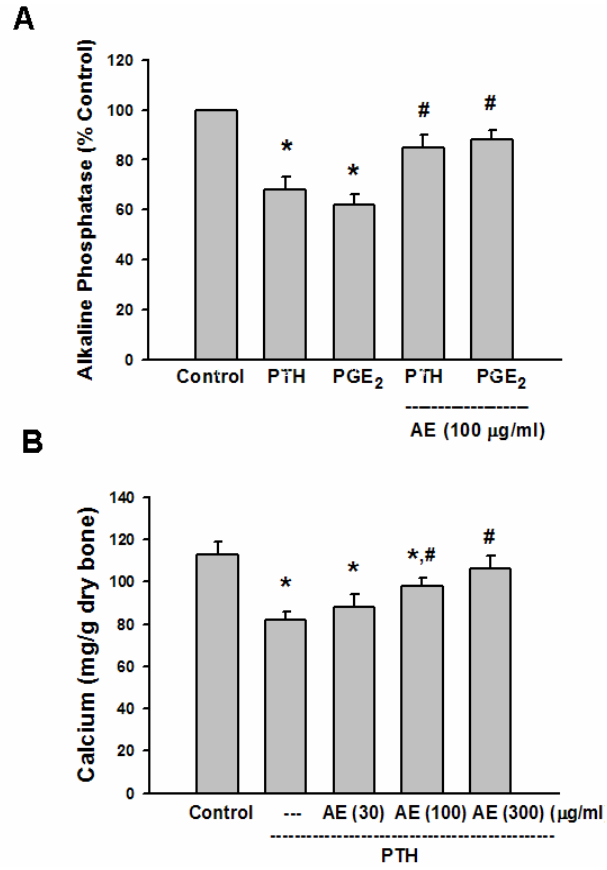

Figure 2. Effects of adlay extracts on alkaline phosphatase activities and calcium levels in cultured adult rat femoral metaphyseal tissues. Adlay extracts treated femoral metaphyseal tissues for 48 hours in the presence or absence of parathyroid hormone (PTH, 10^{-7} M) or prostaglandin E_2 (PGE_2, 10^{-5} M), and the alkaline phosphatase activities and calcium levels were measured. Data are presented as mean±S.E.M. (n≥4). *: $p< 0.05$ as compared with control. #: $p< 0.05$ as compared with PTH or PGE_2 alone. (A) alkaline phosphatase activity, (B) calcium levels.

(model SFD-25, Chang Juing Co., Kaohsiung, Taiwan). These fractions of dehulled adaly were stored at -20 °C until use.

Neonatal rat calvaria and adult rat femoral metaphyseal tissues cultures

The 3 days or 4-week old Wistar rats were obtained from Laboratory Animal Center of the College of Medicine, National Taiwan University. The procedures of the animal study, including the raising, feeding, and the whole surgical processes were approved by the Committee of Animal Study in National Taiwan University. The neonatal rat calvaria and adult rat femoral metaphyseal tissues were isolated and cultured in αMEM medium containing 10% FBS, 100 units/ml penicillin and 2.2 g/L $NaHCO_3$ for 48 hours in the presence or absence of water extract of adlay seeds. In some experiments, the ovariectomy operation was performed in adult female rats under anesthesia. At 4 weeks after ovariectomy, the rats were sacrificed and then the femoral metaphyseal tissues were isolated and cultured.

Measurement of bone alkaline phosphatase and tartrate-resistant acidic phosphatase activities

Alkaline phosphatase and tartrate-resistant acidic phosphatase activities in bone tissues were determined as previously described.[10] The bone tissues were homogenized and then centrifuged at 600 x g for 5 minutes. The supernatants were used for measurement of the enzyme activity. The protein content was measured using a commercial assay kit (BCA™ Protein Assay Kit; PIERCE).

Measurement of Bone Calcium

The calcium levels in medium or bone from tissue cultures were measured. Bone tissues were dried for 16 hours at 120°C, weighed, and then dissolved in nitric acid solution, followed by 100 times dilution with distilled water. The calcium levels were determined by Raichem® colorimetric assay (Hemagen Diagnostics, Inc., San Diego, CA).

Statistical analyses

The values given in this article are presented as mean SEM. All analyses were performed by analysis of variance followed by a Fisher's least significant difference test. A p value of less than 0.05 was viewed as statistically significant.

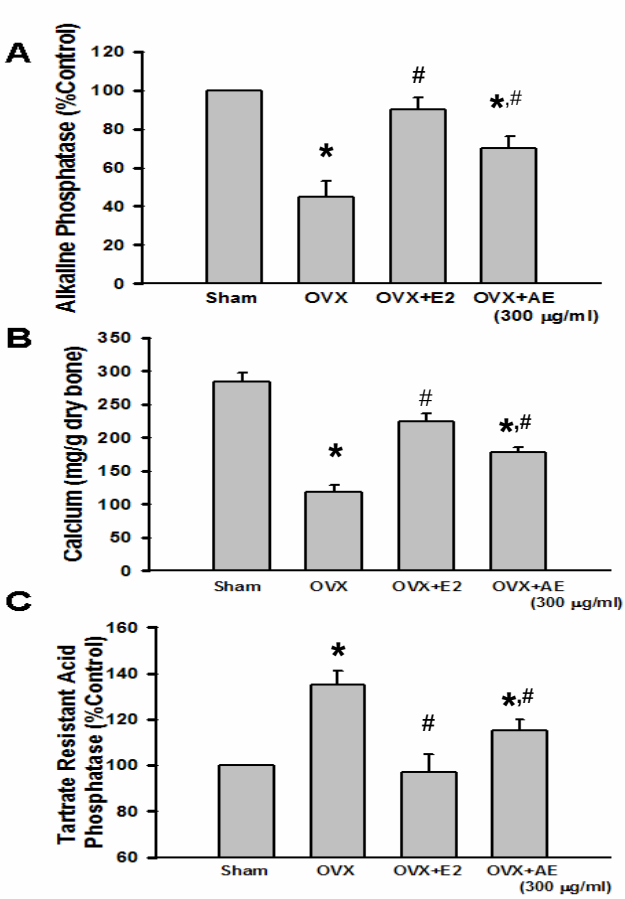

Figure 3. Effects of adlay extracts on alkaline phosphatase activities, calcium levels and tartrate-resistant acidic phosphatase activities in femoral metaphyseal tissues isolated from ovariectomized rats (OVX). After ovariectomy for 4 weeks, the rats were sacrificed by cervical dislocation. In some experiments, ovariectomized rats treated with 17β-estradiol (E2, 10 μg/kg/day, s.c.) for 2 weeks before sacrifice. The femoral metaphyseal sections of bones were collected for tissue culture. Adlay extracts treated femoral metaphyseal tissues for 48 hours, and the alkaline phosphatase activities, calcium levels and tartrate-resistant acidic phosphatase activities were measured. Data are presented as mean±S.E.M. (n=4). *: $p< 0.05$ as compared with control. #: $p< 0.05$ as compared with OVX group. (A) alkaline phosphatase activity, (B) calcium levels, (C) tartrate-resistant acidic phosphatase activity.

RESULTS

As shown in figure 1, treatment with water extract of adlay seed (30-300 μg/mL) in cultured neonatal rat calvaria for 48 hours could reverse the decreased alkaline phosphatase activity in bone (Fig. 1A) and increased calcium level in medium (Fig. 1B) induced by parathyroid hormone (10^{-7} M) in a dose-dependent manner. Similarly, treatment with water extract of adlay seed (100 μg/mL) in cultured femoral metaphyseal tissues for 48 hours significantly reversed the decreased alkaline phosphatase activity induced by parathyroid hormone (10^{-7} M) or prostaglandin E_2 (PGE_2, 10^{-5} M) (Fig. 2A). Water extract of adlay seed (30-300 μg/mL) could also antagonize the decrease of calcium level in bone induced by parathyroid hormone (10^{-7} M) in a dose-dependent manner (Fig. 2B).

In ovariectomized rats, the alkaline phosphatase activities and calcium levels were significantly decreased and tartrate-resistant acidic phosphatase activities were increased in isolated femoral metaphyseal tissues as compared with sham-control. Treatment with 17β-estradiol (10 μg/kg/day, s.c.) for 2 weeks could counteract these effects in ovariectomized rats (Fig. 3). Moreover, treatment with water extract of adlay seed (300 μg/mL) in cultured femoral metaphyseal tissues isolated from control and ovariectomized rats for 48 hours significantly reversed the alterations in the alkaline phosphatase activities, calcium levels and tartrate-resistant acidic phosphatase activities (Fig. 3).

DISCUSSION

Bone remodeling, an incorporated interaction between the bone resorption and bone formation, plays an important role in the bone homeostasis. The bone remodeling has been modulated by various factors, including parathyroid hormone, 1,25-dihydroxyvitamin D3, sex hormones, calcitonin, nitric oxide, prostaglandins, and a lot of growth factors and cytokines, e.g., RANK, RANKL, and OPG etc.[11-14] In the present study, we found that water extract of adlay seed could reverse the alterations in alkaline phosphatase activities and calcium levels induced by parathyroid hormone in cultured neonatal rat calvaria tissues and adult rat femoral metaphyseal tissues. Moreover, water extract of adlay seed could also counteract the decreased alkaline phosphatase activities and calcium levels and increased tartrate-resistant acidic phosphatase activities in femoral metaphyseal tissues of bones isolated from ovariectomized rats. These findings imply that adlay is capable of reversing the osteoporotic status in rats, and

may be a helpful healthy food for osteoporosis prevention. However, the cellular and molecular mechanisms need further investigating in the future.

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AUTHOR DISCLOSURES

Rong Sen Yang, Wenchang Chiang, Yi Hsiang Lu and Shing Hwa Liu, no conflicts of interest.

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