SHORT COMMUNICATION
Stimulatory Constituents of Eclipta prostrata on Mouse Osteoblast Differentiation

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One flavonoid, diosmetin (1), and two isoflavonoids, 3′-hydroxybiochanin A (2) and 3′-O-methylorobol (3), were isolated from the methanol extract of Eclipta prostrata L. by a bioactivity-guided fractionation technique using primary cultures of mouse osteoblasts as an in vitro assay system. All three compounds significantly increased osteoblast differentiation as assessed by the alkaline phosphatase activity.

Keywords: Eclipta prostrata; Compositae; isoflavonoid; osteoblast differentiation; alkaline phosphatase.

INTRODUCTION

Osteoporosis is a metabolic bone disease characterized by a reduction in bone mass and deterioration in bone architecture, resulting in increased bone fragility and its susceptibility to fractures (Rodan and Martin, 2000). Osteoporosis is known to be caused by a relative increase in bone resorption over bone formation. In bone formation, osteoblasts play a crucial role through the proliferation and differentiation (Rodan and Martin, 2000). During differentiation, osteoblasts produce almost all of the constituents of the bone matrix and direct its subsequent mineralization (Manolagas, 2000). Therefore, stimulation of osteoblast differentiation has been suggested to be an important therapeutic approach for the prevention and/or treatment of osteoporosis.

To find natural products having a stimulatory activity on osteoblast differentiation, several hundreds of plant extracts were screened employing primary cultures of mouse calvarial osteoblasts as an in vitro assay system. In the course of screening, a methanol extract of the aerial parts of Eclipta prostrata was purchased from Kyung-dong Market, Seoul, Korea in June 2004, and identified by Dr. Jong Hee Park, a professor of the College of Pharmacy, Pusan National University. A voucher specimen (SNUPH-EP2004-06) has been deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

Extraction and isolation. The aerial parts of E. prostrata were purchased from Kyung-dong Market, Seoul, Korea in June 2004, and identified by Dr. Jong Hee Park, a professor of the College of Pharmacy, Pusan National University. A voucher specimen (SNUPH-EP2004-06) has been deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

Plant material. The aerial parts of E. prostrata were purchased from Kyung-dong Market, Seoul, Korea in June 2004, and identified by Dr. Jong Hee Park, a professor of the College of Pharmacy, Pusan National University. A voucher specimen (SNUPH-EP2004-06) has been deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

Extraction and isolation. The aerial parts of E. prostrata (9 kg) were extracted three times with 80% MeOH to obtain a methanol extract (831 g). The methanol extract was suspended in H2O and partitioned successively with n-hexane, CHCl3, EtOAc and n-BuOH. The CHCl3 fraction (77 g) was subjected to CC over silica gel eluted with a 70 eV ionizing potential. TLC and column chromatography (CC) were carried out on precoated silica gel F254 plates (art. 5715, Merck), RP-18 F254 plates (art. 15423, Merck), silica gel 60 (230–400 mesh, Merck) and Sephadex LH 20 (18–110 µm, Pharmacia Co. Ltd).

Material and methods. General experimental procedures. The 1H and 13CNMR measurements were carried out in a Bruker AMX 400 spectrometer operating at 300 and 100 MHz, respectively. Solvent signals were used as internal standards. 1H–1H COSY, HMQC and HMBC NMR experiments were performed on the same spectrometer. EI-mass spectra were obtained on a VG Trio 2 spectrometer with a 70 eV ionizing potential. TLC and column chromatography (CC) were carried out on precoated silica gel F254 plates (art. 5715, Merck), RP-18 F254 plates (art. 15423, Merck), silica gel 60 (230–400 mesh, Merck) and Sephadex LH 20 (18–110 µm, Pharmacia Co. Ltd).

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Assessment of osteoblast differentiation and proliferation. ICR mice (kept at 20–23 °C; 12 h light cycle; food, Agribrand Purinar Korea, and water ad libitum) were provided by the Laboratory Animal Center, Seoul National University. All experiments were conducted according to the guidelines of the Committee on the Care and Use of Laboratory Animals of the Seoul National University. Murine calvarial osteoblasts were obtained from the calvariae of neonatal mice 1–2 days after birth by the sequential collagenase digestion method (Lee et al., 2006). Osteoblasts were maintained in DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin for 1 week and then seeded at a concentration of $1 \times 10^6$ cells/cm². Osteoblast differentiation was induced by changing the medium containing 50 μg/mL ascorbic acid and the cells were treated with vehicle or compounds to be tested. After 3 days, the media were changed with fresh medium containing each compound and maintained for a further 4 days. For the assessment of the ALP activity, the cells were rinsed with phosphate-buffered saline and lysed in 0.01% sodium dodecyl sulfate in PBS followed by sonication. After clarification by centrifugation, the cell lysates were assayed for ALP activity using an alkaline phosphatase assay kit (Youngdong Pharmaceutical Co., Korea). Each value was normalized with the protein content of cell lysate, measured using bicinchoninic acid with bovine serum albumin as a standard. The proliferation of osteoblasts was assessed by the MTT assay. The evaluation of statistical significance was determined by the one-way ANOVA test with a value of $p < 0.05$ considered to be statistically significant.

RESULTS AND DISCUSSION

In a continuation of the search for natural products having stimulatory activity on osteoblast differentiation, the methanol extract of aerial parts of *E. prostrata* was found to increase the ALP activity significantly in primary cultures of mouse calvarial osteoblasts. Bioactivity-guided fractionation of the methanol extract of *E. prostrata* resulted in the isolation of one flavonoid (1) and two isoflavonoids (2 and 3). The structures of these compounds were identified as diosmetin (1), 3′-hydroxybiochanin A (2) and 3′-O-methylorobol (3) by direct comparison of their physicochemical and spectroscopic data with those previously reported (Roberts et al., 2004; Shen et al., 1993; Hosny and Rosazza, 1999). All three compounds are reported from this plant for the first time.

The effects of these flavonoids on osteoblast differentiation was evaluated in primary cultures of mouse calvarial osteoblasts. All three compounds significantly
increased the ALP activity at concentrations ranging from 1.0 to 25.0 μM (Fig. 2A). However, these compounds did not show significant effects on osteoblast proliferation (Fig. 2B).

Isoflavonoids are known to exert many biological and pharmacological activities. Related to bone, isoflavonoids such as daidzein and genistein have been reported to increase osteoblast differentiation, i.e. ALP activity in an in vitro system (Kanno et al., 2004). Isoflavonoids also have exerted beneficial effects against bone loss in various experimental models (Arjmandi et al., 1996) and in a clinical study (Morabito et al., 2002). Consistent with the previous reports, in the present study, two isoflavonoids and one flavonoid isolated from E. prostrate also exerted stimulatory activity on osteoblast differentiation. These results suggest a possibility of therapeutic potentials for osteoporosis, although the mechanism of these compounds has not been verified fully yet.

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REFERENCES


