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Cyperus Rotundus L. extract suppresses RANKL-induced osteoclastogenesis through NFATc1/c-fos downregulation and prevent bone loss in OVX-induced osteoporosis rat

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ABSTRACT

Ethnopharmacological relevance: *Cyperus Rotundus* L. (CyR) has been widely used for the treatment of gynecologic disorder. Recent studies have reported that CyR can prevent the formation of cystic follicles and ovarian malfunction. However, the effects of CyR on osteoclastogenesis and postmenopausal osteoporosis remain unknown.

Aim of the study: This study was aimed to investigate the preventive effects of CyR on RANKL-induced osteoclast formation and ovariectomy (OVX)-induced bone loss.

Materials and methods: In this *in vitro* study, we investigate the anti-osteoporotic effect of CyR on receptor activator of nuclear factor kappa-B ligand (RANKL)-induced osteoclastogenesis, the formation of tartrate-resistant acid phosphatase (TRAP) multinucleated cells, pit formation, transcription factors such as NFATc1 and c-Fos, and mRNA expression of osteoclast-associated genes were investigated. Forty 12-weeks female Sprague-Dawley rats for *in vivo* effect of CyR were used and OVX rat model was determined. The rats were randomly assigned into sham group and four OVX groups, *i.e.* OVX with D.W; OVX with estradiol (E2, 100 μg/kg/day), OVX with CyR-L (16 mg/kg/day), OVX with CyR-H (160 mg/kg/day). The treatment lasted for 8weeks.

Results: CyR inhibited osteoclast differentiation and pit formation in the RANKL-induced osteoclastogenesis of RAW 264.7 cells. Reverse transcription polymerase chain reaction analysis also showed that CyR reduced the mRNA expression of osteoclast-associated genes such as carbonic anhydrase II, TRAP, RANK, cathepsin K, matrix metalloproteinase 9, nuclear factor of activated T cells cytoplasmic 1 (NFATc1), and c-Fos. In addition, CyR decreased protein levels of NFATc1 and c-Fos. CyR inhibited trabecular bone loss in the femur caused by OVX.

Conclusion: The results of this study indicate that CyR inhibits the RANKL-induced osteoclast differentiation in RAW 264.7 cells and trabecular bone loss in OVX rats.

1. Introduction

Menopausal osteoporosis, a skeletal disorder characterized by decreased bone mass, increased bone fracture risk, and potential alteration of bone structure (Doherty et al., 2001; Yasothan and Kar, 2008), is caused by a failure of bone homeostasis. Bone homeostasis is regulated by the balance between new bone formation by osteoblast and bone resorption by osteoclasts (Alexander et al., 2001). Excessive bone resorption by osteoclast leads to several lytic bone diseases, such as postmenopausal osteoporosis, inflammatory arthritis, and metastasis of tumors to bone (Novack and Teitelbaum, 2008). Therefore, osteoclasts are useful targets for development of novel drugs against lytic bone disease.

Osteoclasts are multinucleated cells differentiated by the fusion of mononuclear progenitors of monocyte/macrophage; excessive osteoclast activity is the main cause of most adult skeletal diseases (Rodan and Martin, 2000). The differentiation of osteoclasts requires macrophage colony stimulating factor and receptor activator of nuclear factor-kB ligand (RANKL) (Theill et al., 2002). RANKL recruits tumor necrosis factor receptor-associated factor 6 after binding with RANK, a

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Table 1
Primer sequence and condition for RT-PCR.

Target genes	Primers (Forward, reverse)	Annealing Tm (°C)	PCR cycle	Product size (bp)
GAPDH	5′-ACT TTG TCA AGC TCA TTT CC–3′ 5′-TGC AGC GAA CTT TAT TGA TG–3′	57 °C	30	250
TRAP	5′-ACT TCC CCA GCC CTT ACT ACC G–3′ 5′-TCA GCA CAT AGC CCA CAC CG–3′	58 °C	32	381
NFATC1	5′-TGC TCC TCC TCC TGC TGC TC–3′ 5′-CGT CTT CCA CCT CCA CGT CG–3′	58 °C	32	480
c-Fos	5′-ATG GGC TCT CCT ATC AAC AC–3′ 5′-GGC TGC CAA AAT AAA CTC CA–3′	58 °C	33	480
CAII	5-CTC TCA GGA CAA TGC AGT GCT GA–3′ 5′-ATC CAG GTC ACA CAT TCC AGC A–3′	58 °C	33	411
Cathepsin K	5′-AGG CGG CTA TAT GAC CAC TG–3′ 5′-CCG AGC CAA GAG AGC ATA TC–3′	58 °C	28	403
MMP–9	5′- CGA CTT TTG TGG TCT TCC CC–3′ 5′-TGA AGG TTT GGA ATC GAC CC–3′	58 °C	40	263
RANK	5′- AAA CCT TGG ACC AAC TGC AC–3′ 5′-ACC ATC TTC TCC TCC CGA GT–3′	53 °C	32	344

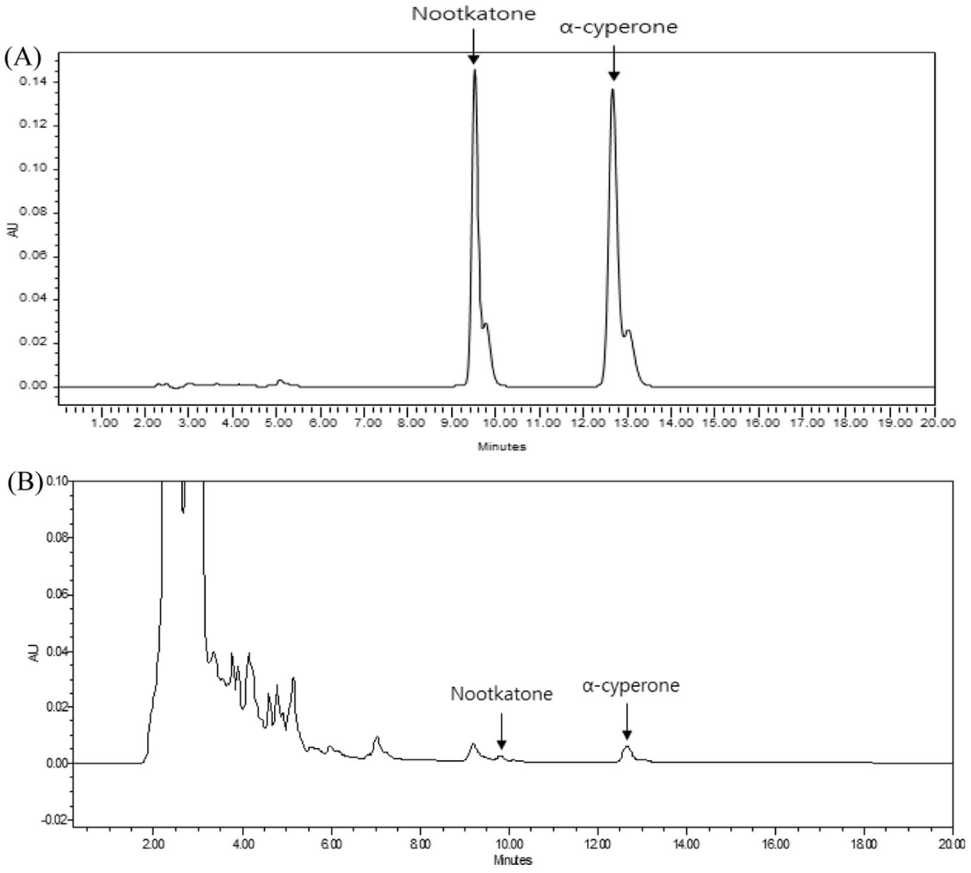

Fig. 1. HPLC Chromatograms of α-cyperone and nootkatone (A) and CyR (B).

receptor of RANKL, thus activating nuclear factor kappa beta and the mitogen-activated protein kinase pathway (Takayanagi, 2007; Theill et al., 2002). RANKL also activates the transcription factors, including c-Fos and nuclear factor of activated T cell cytoplasmic 1 (NFATc1) (Takayanagi, 2007). As a transcription factor of osteoclast differentiation, c-Fos is an essential factor in the expression of NFATc1. NFATc1, a master regulator of osteoclast differentiation, regulates the tartrate-resistance acid phosphatase (TRAP), matrix metalloprotease 9 (MMP-9), cathepsin K (CTK), and NFATc1 (Asagiri et al., 2005). Thus, the regulation of c-Fos and NFATc1 expression is expected to be useful in the treatment of osteoporosis.

Anti-osteoporotic agents such as calcitonin, bisphosphonate, vitamin D, and hormones (estrogen and parathyroid hormone) have been widely used for osteoporosis therapy. Among these agents, hormone