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Cistanche deserticola extract increases bone formation in osteoblasts.

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Objectives: *Cistanche deserticola Ma (CD)*, a native herb in China, is widely used in traditional medicine for various therapeutic treatments due to its sedative, analgesic and immunostimulatory activity. However, the effect of CD on bone cell function has not been determined, yet. We investigated the effect of CD on bone formation by cultured osteoblasts.

Methods:

***Cistanche deserticola* extract**

CD extract was purchased from Chuang Song-Zong Pharmaceutical Company (Kaohsiung, Taiwan). Extraction and isolation of CD were followed as previously described (on the basis of spectral data they were identified the chemical composition including: beta-sitosterol, daucosterol, succinic acid, triacontanol, acteoside, betaine and polysaccharose).

Measurement of mineralized nodule formation

Osteoblasts were cultured in medium containing vitamin C (50 mg/ml) and β-glycerophosphate (10 mM) for 2 weeks. After incubation with CD extract for 12 days, cells were washed and fixed in ethanol for 30 min. Calcium deposition was determined using alizarin red-S (pH 4.2) and eluted with 10% cetylpyridinium chloride. The cells were quantified by measuring absorbance at 550 nm and compared with a standard curve.

Ovariectomy-induced osteoporosis

Female ICR mice, four weeks old, 22–28 g, were used for this study. Mice were ovariectomized bilaterally under trichloroacetaldehyde (100 mg/kg) anaesthesia and control mice were sham-operated (Sham) for comparison. Bone mineral density and bone mineral content were measured after oral administration of various concentrations of CD extract every two days for four weeks. Total body bone mineral density and bone mineral content were determined by dual-energy X-ray absorptiometer (DEXA; XR-26; Norland, Fort Atkinson, USA).

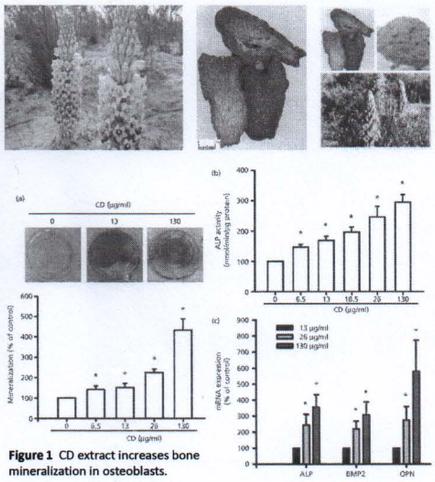

Figure 1 CD extract increases bone mineralization in osteoblasts.

Results: Here, we report that CD extract did not affect the proliferation, migration or wound healing activity of cultured osteoblasts, but did increase ALP, BMP-2, and OPN expression and bone mineralization. In addition, we show that the MAPK and NF-kB signaling pathways may be involved in the CD-mediated increase in gene expression and bone mineralization. In contrast, the CD extract did not suppress osteoclastogenesis *in vitro*. *Notably, treatment of mice* with CD extract prevented bone loss induced by ovariectomy *in vivo*.

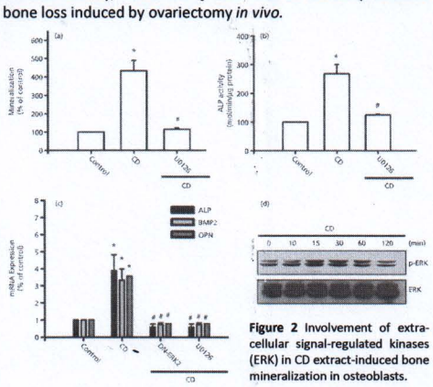

Figure 2 Involvement of extracellular signal-regulated kinases (ERK) in CD extract-induced bone mineralization in osteoblasts.

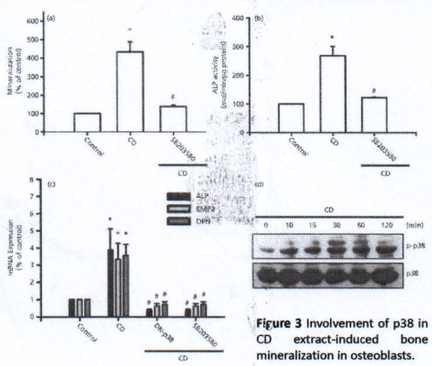

Figure 3 Involvement of p38 in CD extract-induced bone mineralization in osteoblasts.

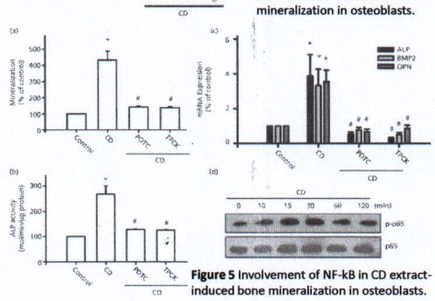

Figure 5 Involvement of NF-kB in CD extract-induced bone mineralization in osteoblasts.

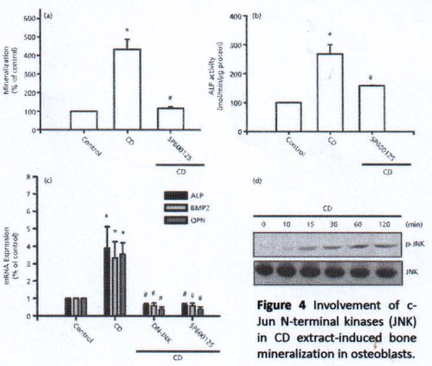

Figure 4 Involvement of c-Jun N-terminal kinases (JNK) in CD extract-induced bone mineralization in osteoblasts.

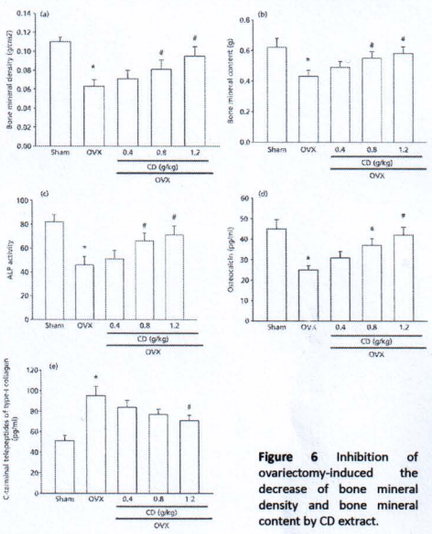

Figure 6 Inhibition of ovariectomy-induced the decrease of bone mineral density and bone mineral content by CD extract.

Conclusions: This study demonstrated that CD extract induced osteoblasts differentiation and maturation but not proliferation or migration. CD extract also increased ALP, BMP-2 and OPN expression and bone mineralization. We showed that the ERK, p38, JNK and NF-κB pathways are involved in CD extract-mediated bone formation and ALP, BMP-2 and OPN expression. Furthermore, CD extract prevented *in vivo* bone loss induced by ovariectomy. Therefore, CD may be beneficial in stimulating bone formation in the treatment of osteoporotic diseases.