LETTER TO THE EDITOR

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The proliferation and differentiation of pre-osteoblastic MC3T3-E1 cells from Vietnamese drug formulations

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DEAR EDITORS

A public heath problem, osteoporosis, is recognized as the prevalent disease, and mainly causes for impairment and loss mass of bone. It closely related the balance between bone formation by osteoblasts and resorption by osteoclasts during the remodeling cycle of bone.[1,2] Hence, pharmaceutical therapies are looking for the potential agents to stimulate osteoblastic bone formation, as well as inhibit osteoclastic processes.[3]

In Vietnamese traditional medicine, the combination of the three species, including *Dillenia Indica* (local name "So"), *Schefflera racemosa* (local name "May tang"), *Adiantum raddianum* (local name "Cut nich"), were widely used as a herbal drug of osteoarthritis by several ethnic minorities in the mountainous areas of Laocai-Vietnam. However, up to now, no studies improve the roles of these crucial medicinal herbal plants. In current paper, we set out to investigate the effects of Vietnamese drug formulations of three plants on the proliferation and differentiation of pre-osteoblastic MC3T3-E1 cells through golden criteria, including cell viability, alkaline phosphatase activity (ALP), collagen content, and mineralization (calcium deposition). The dried leaves of these herbal plants were used as materials, whereas ultilizing water-ethanol (1:1, v/v) as a solvent for extraction. According to tradditional uses, we herein proposed the preparation of three drug formulations CT1-CT3, followed by the material rates, comprising formulation CT1 (3 : 1 : 1, *D. Indica* : *S. racemosa* : *A. raddianum*), formulation CT2 (2 : 1 : 1, respectively), formulation CT3 (1 : 1 : 1, respectively). The osteoblastic protocols were performed as described by.[4,5]

The fluctuation of viablity of pre-osteoblastic MC3T3-E1 cells cultured with or without the presence of three formulations were displayed in Figure 1A. Data clearly revealed that, at the concentrations 50 and 100 µg/mL and 3 days incubation, only CT2 increased while the remainders failed to do so. Therefore, The formulation CT2 can be claimed responsible for next steps. A biochemical method, alkaline phosphatase activity, can be seen as a obsteoblast phenotype marker, which provided quantitative information for the initial differentiation.[4,6] At the concentrations 50 and 100 µg/mL, the drug formulation CT2 significantly promoted the increases of ALP activity up to 7.5 and 20% after 7 days, respectiely, as compared to that of the control (Table 1). Collagen acted as a predominant product in bone cell during the osteoblastic differentiation.[2] The effects of CT2 on the synthetic collagen were investigated, using Sirius red based colorimetric experiment. As shown in Table 1, accounting for 6 and 25% in the accumulation of collagen was induced by this drug formulation at the corresponding concentrations of 50 and 100 µg/mL and after 10 days treatment. Histochemical analysis also confirmed that, under two concentration conditions, pre-osteoblastic MC3T3-E1 cells took higher colors than that of untreat group (Figure 1B). During the osteoblastic differentiation, calcium accumulated in the matrix and cell layer of pre-osteoblastic MC3T3-E1 cells, the observation of mineralization was normally assessed by Alirazin red staining.[2,4,5] After 15 days treament, the formulation CT2 (50 and 100 µg/mL) notably increased the mineralization content of respective 14 and

Table 1 **Effects of drug formulation CT2 (50 and 100 µg/mL) on ALP activity, collagen content, and mineralization of MC3T3-E1; Data are expressed as mean ± SEM (n = 3), *$P < 0.05$, **$P < 0.01$ versus control (only MC3T3-E1)**

Concentration (mg/µL)	ALP activity (%)	Collagen content (%)	Mineralization (%)
100	119.70 ± 3.94**	124,55 ± 6.74**	128.65 ± 5.02**
50	107.49 ± 0.61*	105,83 ± 5.56	114.38 ± 2.74*
0	100.00 ± 0.94	100.00 ± 0.85	100.00 ± 2.89

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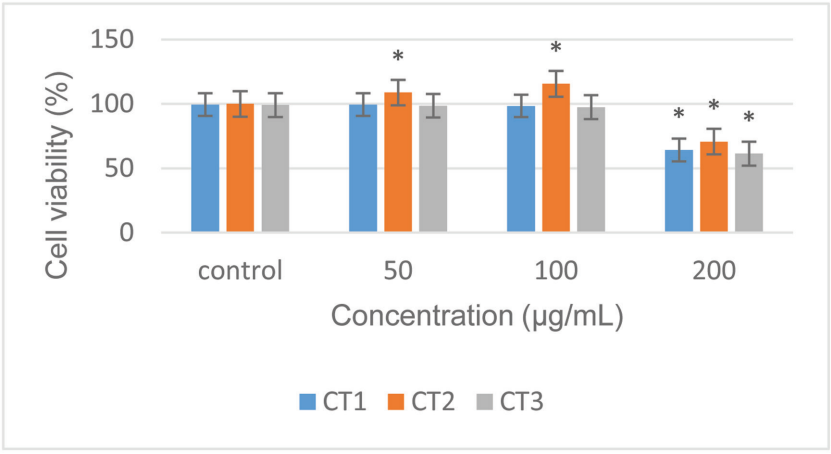

Figure 1A Effects of drug formulation CT1-CT3 (50 , 100 and 200 µg/mL) on viability of MC3T3-E1; Data are expressed as mean ± SEM (n = 3), $^{*}P < 0.05$ versus control (only MC3T3-E1)

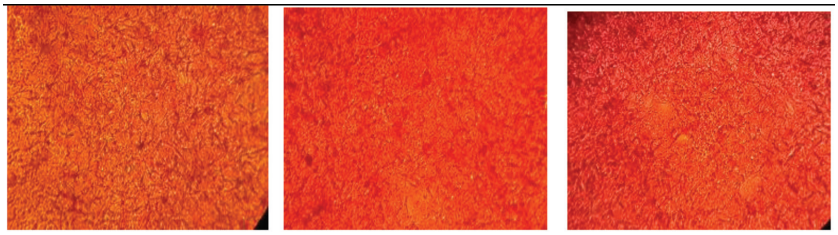

Figure 1B Sirius red staining: a) only MC3T3-E1, b) MC3T3-E1 + CT2 (50 µg/mL), c) MC3T3-E1 + CT2 (100 µg/mL)

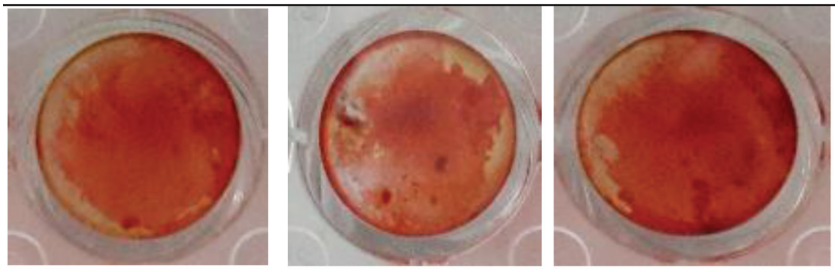

Figure 1C Alizarin red staining: a) only MC3T3-E1; b) MC3T3-E1 + CT2 (50 µg/mL); c) MC3T3-E1 + CT2 (100 µg/mL)

29%, especially the bright red color was observed at 100 µg/mL (Table 1 and Figure 1C).

Taken together, at concentration 100 µg/mL, Vietnamese combination drug of three plants (2 : 1 : 1, *D. Indica* : *S. racemosa* : *A. raddianum*) evidently generated the prospective values in the treatment of bone related diseases. Therefore, extensive researches in either further biological experiments or phytochemical investigations are expected.

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