Betel leaf extract and its major component hydroxychavicol promote osteogenesis and alleviate glucocorticoid-induced osteoporosis in rats

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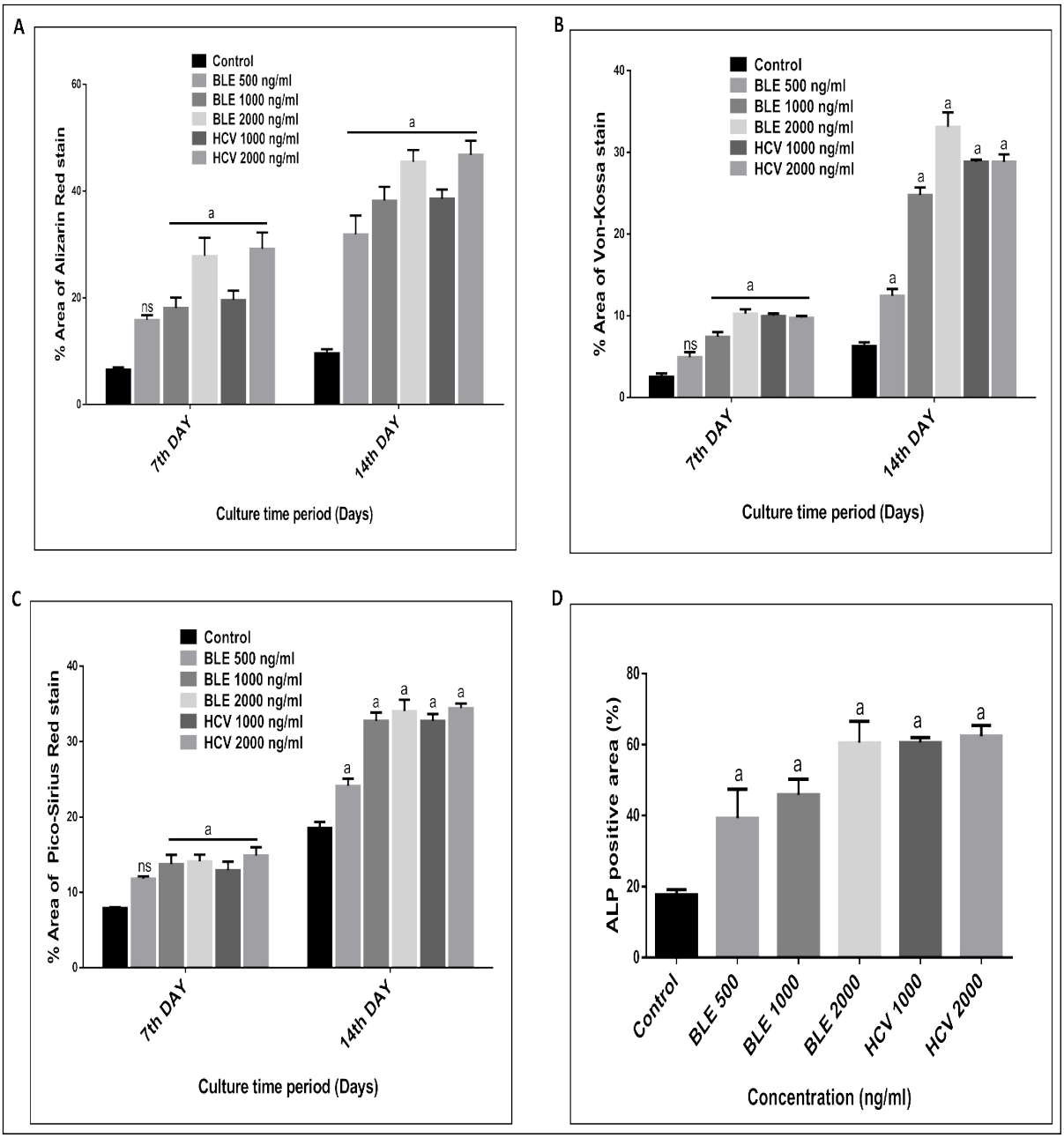
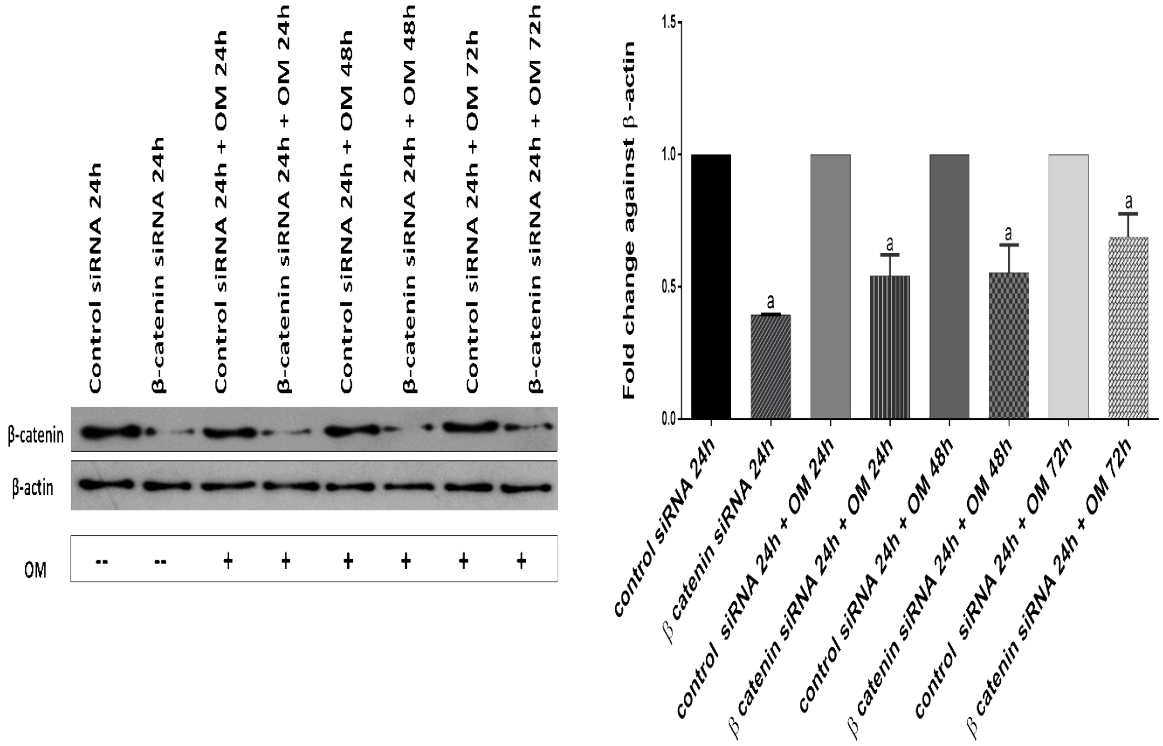
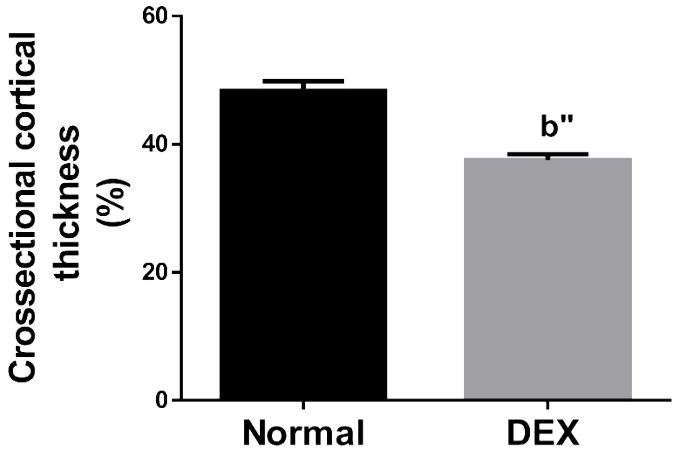


Supplementary Figure 1. Quantitative estimation of cells stained with various osteogenic markers. C3H10T1/2 cells were treated with BLE (500, 1000, 2000 ng/ml) and HCV (1000, 2000 ng/ml) for either 7 and 14 days (Fig. 4) or only 7 days (Fig. 5A) with OM in individual experiments. The corresponding stained area as shown in Fig. 4 and 5A were quantified with image J software. The bar graphs show the quantitative estimation of (A) Alizarin Red staining; (B) Von-Kossa staining; (C) Pico-Sirius Red staining as shown in Fig. 4 and (D) ALP staining

as shown in Fig. 5A. Results are mean ± SEM of three independent experiments., [a]$p<0.05$ versus respective vehicle treated control groups in each experiment. ns, non-significant; BLE, betel leaf extract; HCV, hydroxychavicol; OM, osteogenic media; ALP, alkaline phosphatase.



Supplementary Figure 2. Time dependent inhibition of β-catenin protein expression in response to its siRNA treatment. C3H10T1/2 cells were pre-treated with β-catenin siRNA and control siRNA for 24 h in PM. After 24 h the media was replaced with OM and incubated for different time intervals (24, 48 and 72 h post-siRNA treatment). Representative images showing immunoblots of β-catenin expression at different time intervals. The bar graph represents the mean relative arbitrary pixels intensities expressed as fold change with respect to β-actin (internal control) where the control siRNA or control siRNA + OM treated cells at respective time points were assigned a value of 1. Results are mean ± SEM of three independent experiments. $^{a}p<0.05$ versus control siRNA of respective groups. PM, proliferation media; OM, osteogenic media.



Supplementary Figure 3. Quantification of crossectional cortical thickness in various groups of animals. Data are mean ± SEM of n = 5 for each group; $^{b"}p<0.05$ versus normal untreated control. Dex, dexamethasone.