In vivo Wound Healing Effect of CrataBL: the New Pro-Angiogenic Protein Extracted from Bark of *Crataeva tapia*

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INTRODUCTION. Blood vessel formation constitutes an important step in threatening of severe disorders, like infarction and ulcerations. **OBJECTIVE:** To Investigate the *in vivo* wound healing effect of CrataBL and angiogenesis *in vitro*.
MATERIAL AND METHODS: Human umbilical vein endothelial cells (HUVEC) was treated with CrataBL and cell viability, migration and invasion were evaluated respectively by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and transwell colorimetric assays. Protein immunodetection, cytokines, nitric oxide liberation, glycosaminoglycans expression and adhesion were assayed as described procedures. For *in vivo* wound healing studies, circular lesions of 5 mm diameter were made in the dorsum-cervical region of C57BL/6 mice. After 8 days of treatment, lesion area was measured. **DISCUSSION AND RESULTS:** CrataBL stimulated metabolism of HUVEC in a dose-dependent manner and angiogenic phenotype *in vitro*, increasing 150% the tube formation with 10 µmol/L. CrataBL also demonstrated a chemoattractant effect on HUVEC, stimulating migration and invasion. Adhesion to collagen I was selectively stimulated, compared to collagen IV, fibronectin and laminin. The HUVEC conditioned medium with CrataBL showed a decrease in gelatinases activities by zymography, confirmed with immunodetection of matrix-metalloproteinase-2. The cell signaling demonstrated a remarkable increase in the expression of heparan sulfate, activation of α4, β1 and β3 integrins, and phosphorylation of FGFR2, specifically VEGFR2 Y996 isoform, with consequent activation of SRC-FAK and MAPK pathways. In culture medium conditioned with CrataBL, there were increases in FGF2 and IL-9 cytokines, but no significant effect on NO release. On *in vivo* wound healing assay the mice treated with CrataBL showed a faster healing process. **CONCLUSION:** CrataBL promotes neovascularization *in vitro* and enhances wound healing *in vivo*.

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