Effects of methanolic and aqueous *Carica Papaya* leaf extracts in transcriptional and miRNA-mediated regulation of fetal hemoglobin

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Sickle cell disease (SCD) is a genetic blood disorder caused by mutations in β-globin gene that affects the shape and transport of red blood cells in blood vessels, leading to various clinical complications. The pharmacological reactivation of Fetal Hemoglobin (HbF) through compounds such as Hydroxyurea (HU), is one of the available treatments, however their safety concerns and expensive cost in low- and middle-Income countries limit their use. In this context, it is essential to study novel HbF-inducing compounds that have scarcer adverse effects and can be widely available, such as Carica papaya extracts, a medicinal plant with anti-oxidant and anti-inflammatory properties.

Therefore, the main aim of this work is to evaluate the effects of *Carica Papaya* leaf extracts (CPLE) in HbF reactivation. More specifically, we started by evaluating the effect of a methanolic CPLE extract in K562 cells (human immortalized myeloid leukemia cell line) at the proliferation rate and viability of the K562 cell line, compared to HU exposure. Subsequently, we analysed the expression levels of of *HBG1* and *HBG2* genes and that of its transcriptional and miRNA-mediated regulators.

In order to achieve these goals, the K562 cell line was first exposed for 72 hours to CPMLE at 500 μg/mL and for 24 hours to EMFCP (0.5; 50 and 100 μg/mL) and to HU (25 μg/mL). After exposure to natural compounds, the effects of gene expression were quantified from total RNA using RT-qPCR. The results have indicated that cell proliferation and viability were affected by CPMLE only at the concentration of 500 μg/ml, with no effects being observed at the lower concentrations analysed. Upon analysis of the expression levels of globins (*HBA*, *HBB*, *HBG1* and *HBG2*), HbF regulatory genes (*MYB*, *KLF1*, *BCL11A* and *BGLT3*) and miRNAs involved in the regulation of HbF we could observe more significant differences for the lower concentrations of extracts used, namely at the concentration of 0.5 μg/ml. As such we have decided to titrate down the concentration of methanolic leaf extracts used and furthermore, test the exposure to aqueous leaf extracts which possess different biological compounds that might lead to a differential regulation of the genes under analysis. Our preliminary results have revealed that at the concentrations of 0,05 ug/ml; 0,5 ug/ml and 5 ug/ml the cell viability and proliferation rates were not affected neither for methanolic neither aqueous extracts. We are currently analysing the expression levels of target and regulatory genes to determine the effect of both types of leaf extracts on regulators of HbF expression.

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