Purple pitanga (*Eugenia uniflora* L.) extract induces autophagy on activated hepatic stellate cells

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Introduction: Fibrosis is the essential pathophysiologic consequence of chronic liver injury and hepatic stellate cells (HSC) have consistently been shown to play a key role in hepatic fibrogenesis. The GRX cell line is a HSC model. Autophagy is a genetically programmed, evolutionarily conserved process coordinated by a family of genes, called Atg, that lead to the degradation of organelles and proteins. *Eugenia uniflora* L. is a tree that produced fruits called pitanga or Brazilian cherry, with have different flesh colors (purple, red and orange). This study aim evaluates the presence of autophagy in GRX cells treated with purple-fleshed pitanga extract (PFPE). Materials and methods: The cells were treated with 5, 50 and 100 µg of chlorogenic acid equivalents/mL of PFPE for 72 hours. Cytoplasmatic granular intensity was analyzed by flow cytometry. Quantification of acidic vacuolar organelles (AVOs), that is a typical feature of autophagy, was determined using acridine orange (AO) and quantified by flow cytometry. For mitophagy evaluating, cells were incubated with MitoTracker® Green FM (MTG) and Lysotracker Red DND-99 (LYSR), and analyzed by laser-scanning confocal microscopy. Ultrastructural analysis of cells was performed by Transmission Electron Microscopy (TEM). Results and discussion: We observed the increase of cytoplasmatic granularity in the cells treated with 50 and 100 µg/mL of PFPE. Autophagosomes were observed through AO staining, and cells treated with 50 and 100 µg/mL of PFPE with have a significant increased number of AVOs. The autophagy was confirmed by cell images using TEM that showed a great number of autophagosomes and autolysosomes in GRX cells treated with PFPE. Mitophagy was observed by colocalization of lysosomes and mitochondrias using confocal microscopy and this increase with PFPE concentration. Conclusion: These results suggested that PFPE induces autophagy in activated HSC that can be test with new therapy in fibrosis resolution. More studies are necessary to corroborate these findings.

Keywords: Liver Fibrosis; *Eugenia uniflora*; autophagy; Death Cell

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