



CASCAROSIDES, A NEW APPLICATION OF AUTOPHAGY INHIBITORS IN HUMAN LEUKEMIA CELL LINES

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Abstract: Cascarosides are the main constituents of *Rhamnus purshiana* (cascara-sagrada), a medicinal plant extensively used as a laxative. Prolonged use of cascara leads to melanosis coli, a disease caused by epithelial cells damage¹. Possible mechanisms proposed for melanosis involve the induction of apoptosis via mitochondrial outer membrane permeabilization, although the process of autophagy also appears to play an important role during the establishment of melanosis. **Objective:** This study aimed to evaluate the effect of crude extract of *R. purshiana* and its isolated cascariosides isomers on the process of autophagy and viability of two human leukemia cell lines. **Methodology:** The cascariosides were isolated from cascara sagrada by classical chromatography column (CCC) and high performance liquid chromatography (HPLC) and were identified by NMR (¹H, ¹³C, Dept and NOESY). For the autophagy assay, K562 cells (chronic myelogenous leukemia) and HL-60 (acute promyelocytic leucemia) were treated with different concentrations of cascariosides for 24h and then stained with acridine orange (AO) in order to be evaluate autophagy by flow cytometry and fluorescence microscopy. For the viability assay, cells were seeded in a 96-well plate and 24h after treatment, resazurin dye was added at a final concentration of 70µM, the amount of resazurin reduced by cells was determined by measuring its fluorescence (ex:579/em:584) in a SpectraMax i3 MiniMax Imaging Cytometer. **Results:** The cascariosides tested were more effective in inhibiting autophagy than cycloheximide, a protein synthesis inhibitor known to inhibit autophagy. Tunicamycin and rapamycin were used as positive control. The treatment with different concentrations of cascariosides in autophagy-inducing conditions (depletion of serum) showed a significant reduction of autophagy compared with the untreated control. The cascariosides isomers tested, also exhibited low cytotoxicity after 24h in all concentrations tested (1, 10, 50, 100, 500 and 100 µM), whereas the *R. purshiana* crude extract tested showed a moderate cytotoxicity. **Conclusion:** These results indicate the potential of cascariosides as autophagic inhibitors and provide new therapeutic opportunities for the treatment of leukemia. Nevertheless, further studies are needed to uncover their mechanism of action and explain their effects, thus enabling a future therapeutic application.

References

[1] Roerig, J. L. et al. 2010. Laxative Abuse Epidemiology, Diagnosis and Management. *Drugs*, 70 (12): 1487-1503.