CYTOTOXICITY AND SELECTIVITY OF THE *Equisetum hyemale* (*E. hyemale*) EXTRACT IN ORAL SQUAMOUS CELL CARCINOMA

Queiroz, L. N[1,2*]; Silva, D. P. D[1], Pereira, M. T. M[1,3].; Pascoal, A. C. R. F.[1,3]; Sawaya, A. C. H. F[4]; Moreira, D. L.[5]; Robbs, B. K.[1,2]

[1]*Laboratório Multiusuário de Pesquisa Biomédica, Instituto de Saúde de Nova Friburgo, Universidade Federal Fluminense, Nova Friburgo, Brasil*

[2] *Programa de Pós-Graduação em Ciências Aplicadas a |Produtos para Saúde, Faculdade de Farmácia, Universidade Federal Fluminense, Niterói, Brasil*

[3] *Programa de Pós-Graduação em Ciências e Biotecnologia, Instituto de biologia, Universidade Federal Fluminense, Niteroi, Brasil*

[4]*Faculdade de Ciências Farmacêuticas, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil*

[5]*Departamento de Produtos Naturais, Instituto de Tecnologia Farmacêutica, Fundação Oswaldo Cruz, Rio de Janeiro, Brasil*

**lucasnicolaunf@gmail.com*

Introduction

Cancer was the second leading cause of death in 2018, and oral squamous cell carcinoma (OSCC) stands out for itshigh mortality and low survival rate, and also for the poor treatment evolution in the last 30 years (World Health Organization, 2018; Scully and Bagan 2009). Therefore, the development of new treatments becomes necessary. Plants from the *Equisetum* genus are popularly used in the treatment of various diseases, they have anti-inflammatory, antioxidant and antimicrobial effects (Churqui; Lind *et al.*, 2018, Li; Wang *et al.*, 2012).Theaim of this study was to perform phytochemical analysis of the *E.hyemale* stem (EHS), evaluation of the antiproliferative effect and determination of the cell death pathway induced bythe extracts in the OSCC.

Method

Crude ethanol extract was prepared from the *Equisetum hyemale* stem and clonogenic and cell viability assays were performed with MTT using SCC9. Subsequently, the crude extract of stem was partitioned into the liquid/liquid hexane (EHH), dichloromethane (EHD) and ethyl acetate (EHA) fractions. Then, the MTT assay was repeated using SCC4, SCC 25 cells and primary culture human fibroblasts. The IC_{50} was calculated by non-linear regression curve using the GraphPadPrism 5 software. Cell morphology was analyzed through microscopy and cell death analyzed in the propidium iodide (P.I) labeling assay. Hemolysis tests were performed with goat blood. The concentration of phenols and flavonoids were quantified based on gallic acid and quercetin standards, respectively. Acute toxicity tests were performed on C57 Black/6J according to the CEUA/UFF #982 protocol. The fractions were analyzed using Ultra-High Liquid Chromatography (AcquityUHPLC) coupled with mass spectrometer (TQD Acquity).

Results / Discussion

The IC_{50} for the crude extract (200.6±0.09 µg/ml) was calculated through the clonogenic and MTT assays. In the cell viability assay with the partitions, the $IC_{50}$for EHA (64.5±0.072 µg/ml) was calculated, but the EHH and EHD fractions did not reach 50% inhibition in the tested concentrations, indicating a concentration of the cytotoxic compound in the EHA. Carboplatin chemotherapy (280,4 ±0,05 µg/ml) was used as a positive control. To investigate the type of death, the morphology of cells treated with EHA was analyzed by microscopy where smaller number of cells, increased cytoplasm, and large amounts of

granules and vacuoles were observed. The EHA partition was shown to be less hemolytic than the control, with reduced absolute number of cells in culture and induced permeabilization of the tumor cells.The EHA was also tested on tumor lines SCC4 and SSC25 showing a similar IC_{50}, 54.53 ± 0.047µg/mg (SCC24) and 64 ± 0.014µg/ml (SCC25).The EHA partition in normal Fibroblast cells had an IC_{50} of 133.5 ± 0.02 µg/mL and Carboplatin 523 ± 0.034 µg/mL. The SCC9 EHA demonstrated selectivity index (SI) of 2,070 in comparison with Carboplatin (SI 1,865). When a substance has an SI index > 2 it can be considered selective; however, if SI < 2, this substance is cytotoxic to both tumor cells and normal cells (Mahavorasirikul; Viyanant *et al.*, 2010). The EHA fraction presented phenol (0.41±0.17 µg/mg) and flavonoids (0.85±0.10 µg/mg). These partitions were selective when tested on normal primary fibroblasts and had showed no macroscopic changes in the animals. Azelaic acid was the only substance specifically identified in the EHA until now but the LS / MS results are still under analysis.

Conclusion

After analyzing the results, it can be concluded that the *Equisetum hyemale* stem extract has a cytotoxic effect against oral squamous cells, with the EHA fraction being the most selective. LC-MS/MS analysis is being performed to identify possible active compounds.

Acknowledgments

The authors would like to acknowledge the Brazilian agencies that fund our research: CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPERJ (Emergency Support for Stricto Sensu Graduate Programs and Courses in the State of Rio de Janeiro Project E -26/200.930/2017) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code 001.

Bibliographic References

- WHO. Cancer. https://www.who.int/news-room/fact-sheets/detail/cancer, 2018. Acesso em: 01/07/2020.
- Scully C, Bagan J. 2009. Oral squamous cell carcinoma overview. Oral oncology. 45(4-5):301-308.
- Mahavorasirikul, W.; Viyanant, V.; Chaijaroenkul, W.; Itharat, A. *et al.2010.* Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells in vitro. BMC Complement Altern Med, 10, p. 55.
- Churqui, M. P.; Lind, L.; Thörn, K.; Svensson, A. *et al.* 2018. Extracts of *Equisetum giganteum L* and Copaifera reticulate Ducke show strong antiviral activity against the sexually transmitted pathogen herpes simplex virus type 2. J Ethnopharmacol, 210, p. 192-197
- Li, H.; Wang, P.; Liu, Q.; Cheng, X. *et al. 2012.* Cell cycle arrest and cell apoptosis induced by *Equisetum hyemale* extract in murine leukemia L1210 cells. J Ethnopharmacol, 144, n. 2, p. 322-327.