In vivo activity of *Equisetum hyemale* extract against oral squamous cell carcinoma and phytochemical analysis.

Queiroz, L.N[1]; Fonseca, A.C.C[2] ; Wermelinger, G.F.[3]; Dutra, D.P.[3]; Pascoal, A.C.R.[3]; Sawaya, A.C.H [4]; Almeida, C.E.[3], Amaral, B.S.[5]; Moreira, D.L.[6]; Robbs, B.K[3]

[1] *Programa de Pós-Graduação em Ciências Aplicadas a Produtos para Saúde, Universidade Federal Fluminense, Niterói, RJ, Brazil. Electronic address: lucasnicolaunf@gmail.com.*

[2]*Programa de Pós-graduação em Odontologia, Universidade Federal Fluminense, Nova Friburgo, RJ, Brazil. Electronic address: fonseca.anna@gmail.com.*

[3] *Departamento de Ciências Básicas, Universidade Federal Fluminense, Nova Friburgo, RJ, Brazil. Electronic address: guilhermefwermelinger@gmail.com.*

[4] *Faculdade de Ciências farmacêuticas, Universidade Estadual de Campinas,Campinas, São Paulo, Brazil. Electronic address: alexandra.sawaya@fcf.unicamp.br*

[5] *Instituto Federal de Educação, Ciência e Tecnologia de São Paulo, São Paulo, Brazil.*

[6] *Departamento de Pesquisa, Instituto de Pesquisas Jardim Botânico do Rio de janeiro, Rio de Janeiro, Brazil. Electronic address: davysonmoreira@hotmail.com.*

Introduction

Oral squamous cell carcinoma (OSCC) is a public health problem in Brazil, with low efficacy of treatments, where new treatment options are essential[1]. Plants of the genus Equisetum are popularly used in the treatment of several diseases, including cancer[2]. The aim of this study was to perform the phytochemical analysis of the stem of *E. hyemale*, evaluate the antiproliferative effect, determine the cell death pathway induced by the extracts in OSCC, acute toxicity and in vivo antitumor activity.

Material and Methods

Crude ethanolic extract (EHS) and N-hexane (EHSh), dichloromethane (EHSd) and ethyl acetate (EHSa) were prepared from the stem of *E. hyemale*. Cell viability assays were performed with MTT. The IC_{50} was calculated by a nonlinear regression curve[3]. The cell death pathway was analyzed with time-lapse microscopy, active caspase 3/7 fluorescence assay and annexin-V/P.I. flow cytometry. Hemolysis tests were performed with human blood (CAAE: 43134721.4.0000.5626). Partitions were analyzed by LC-MS/MS and the components identified through Global Natural Products Social Molecular Networking[4]. Acute toxicity tests were performed in C57 Black/6J (CEUA/UFF #982). SCC9 cells were inoculated into the flank of nude Balb (CEUA #6497220421) for antitumor analysis. The cells used were SCC9, SCC4, SCC25 and primary cultured fibroblast. As a positive control, carboplatin chemotherapy was used.

Results and Discussion

Through the MTT assay, the extracts showed cytotoxic activity, the most active being the EHSa partition (IC_{50}=28.7µg/ml) and having a high selectivity index (15.59) compared to non-neoplastic cells. We identified morphological features of apoptosis membrane blebling and cell retraction, increased active caspase 3/7 in the cells, chromatin condensation. It has no hemolytic activity and low cytotoxicity in mice. EHSa significantly reduced tumor growth in the nude assay. The phytochemical identify where several glycosylated flavonoids among them 6 new never described in this species were annotated:

5-Hydroxy-3',4',7,8-tetramethoxyflavone; 5,4'-Dihydroxy-7,8,3'-trimethoxyflavone; 5,7-Dihydroxy-3',4'-dimethoxyflavone; 3',4',5,7-Tetramethoxyflavone; 5-Hydroxy-3',4',7-trimethoxyflavone; 5,4'-Dihydroxy-3'-7'-dimethoxyflavone. In conclusion, The EHSa fraction has low in vivo toxicity and has shown important activity *in vivo*. 6 new substances never described in *E. hyemale* were identified.

Conclusion

After analyzing the results, it can be concluded that the stem extract of *E. hyemale* has a cytotoxic effect against OSCC, with the EHSa fraction being the most cytotoxic and selective, and death occurs by apoptosis. The EHSa fraction has low toxicity in vivo and has shown significant in vivo activity, demonstrating the partitioning potential. Six new substances never described in this species were identified.

Acknowledgments

The authors would like to express appreciation for the support of the sponsors FAPERJ (E-26/210.915/2021), CAPES (Finance Code 001), PROPPI-UFF AND CNPq.

Bibliographic References

[1] Siegel, Rebecca L., et al. "Cancer statistics, 2023." *CA: a cancer journal for clinicians*, 2023, 17-48.
[2] Li, Hongxia, et al. "Cell cycle arrest and cell apoptosis induced by Equisetum hyemale extract in murine leukemia L1210 cells." *Journal of ethnopharmacology*, 2012, 322-327.
[3] W. Caldwell, Gary, et al. "The IC50 Concept Revisited". **:** Current Topics in Medicinal Chemistry,12, no 11, 2012, pp. 1282-1290(9).
[4] Mingxun W., Jeremy J, et al. "Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking." Nature biotechnology 4, no. 8 (2016): 828.