

ANTITUMOR EFFECT OF *Equisetum hyemale* EXTRACT IN ORAL SQUAMOUS CELL CARCINOMA LINES, IN VITRO AND IN VIVO

Queiroz, L. N.^{1,2*}; Silva, D. P. D.¹, Pereira, M. T. M.^{1,3}; Da Fonseca, Anna C.¹, Pascoal, A. C. R. F.^{1,3}; Sawaya, A. C. H. F.⁴; Davyson L Moreira⁵; Robbs, B.K.^{1,2}

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

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

³Programa de Pós-Graduação em Ciências e Biotecnologia, Instituto de biologia, Universidade Federal Fluminense, Niterói, Brasil

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

⁵Departamento 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 2020, and oral squamous cell carcinoma (OSCC) stands out for the high mortality and low survival rate, and poor treatment evolution in the last 30 years (World Health Organization, 2020; Scully and Bagan 2009). Therefore, the development of new treatments becomes necessary. Plants of 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). The aim 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 by the extracts in the OSCC.

Material and Methods

Ethanollic crude extract was prepared from the stem of *Equisetum hyemale* and clonogenic assays and cell viability assays were performed with MTT using SCC9. Subsequently, the crude stem extract was partitioned into liquid/liquid fractions in hexane (EHH), dichloromethane (EHD) and ethyl acetate (EHA) and the MTT assay repeated using SCC4, SCC 25 cells and primary culture human fibroblasts. The IC₅₀ was calculated by non-linear regression curve in the GraphPadPrism 7 software. As a positive control, Carboplatin chemotherapy was used. The cell death pathway was analyzed with time-lapse, fluorescence assay by active 3/7 caspase, propidium iodide (P.I) and Hoechst. Hemolysis tests were performed with goat blood. Ultra-High Liquid chromatography (Acquity UHPLC) coupled with mass spectrometer (TQD Acquity) was performed for the fractions and the components identified through the Global Natural Products Social Molecular Networking. Acute toxicity tests were performed on C57 Black/6J according to the CEUA/UFF #982 protocol. SCC9 cells were inoculated into the flank of Balb nude (CEUA n°6497220421) for antitumor analysis.

Results and Discussion

Through the clonogenic and MTT assays with the crude extract IC₅₀ of 101.2 µg/ml was calculated. In the cell viability assay with the partitions we obtained the following mean IC₅₀ in tumor lines: Carboplatin= 53.23µg/ml, EHSa= 28.7µg/ml, EHSb= 253.3µg/ml and EHSd did not reach 50% inhibition, indicating a concentration of the cytotoxic compound in EHSa. Investigating the type of cell death induced by the EHSa, we observe in the morphological analysis, membrane blebbing and cell retraction,

increased active caspase 3/7 in the cells, it was positive for I.P and pyknotic nuclei, DNA fragmentation and phosphatidyl serine exposition were observed, strong indication of apoptotic cell death. The EHA partition was shown to be less hemolytic than the control. The EHA partition in normal Fibroblast cells had an IC₅₀ of 447 µg/mL, EHS_h 347.2 µg/mL and Carboplatin 337.2 µg/mL. Calculating the selectivity index comparing with the IC₅₀ of the normal cell we obtained: EHS=14.56, EHS_a=15.59, EHS_h=2.3 and Carboplatin=6.72. When a substance has an SI index > 2 it can be considered selective, however, if the value is SI < 2, that substance is cytotoxic to both tumor cells and normal cells (Mahavorasirikul; Viyanant *et al.*, 2010). The EHS_a partition did not show hemolytic activity and it caused moderate vacuolar degeneration, moderate cell binucleation and moderate portal hyperemia in the liver, but no necrotic spots were observed in the acute toxicity assay. EHS_a significantly reduced tumor growth (P>0.001) in the nude assay. In the phytochemical composition we identify 6 new substances never described in this species: 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.

Conclusion

After analysis of the results, it can be concluded that the *E. hyemale* stem extract has a cytotoxic effect against OSCC, with the EHS_a fraction being the most cytotoxic, selective and death occurs by apoptosis. The EHS_a fraction has low *in vivo* toxicity and has antumoral activity *in vivo* and 6 new substances for this species were identified.

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