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

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Introduction

Cancer was the second leading cause of death in 2018, and oral squamous cell carcinoma (OSCC) stands out for its high 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). 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.

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 IC50 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 IC50 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 IC50 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$_{50}$, $54.53 \pm 0.047 \mu g/mg$ (SCC24) and $64 \pm 0.014 \mu g/ml$ (SCC25). The EHA partition in normal Fibroblast cells had an IC$_{50}$ of $133.5 \pm 0.02 \mu g/mL$ and Carboplatin $523 \pm 0.034 \mu g/mL$. 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 ($0.41\pm0.17 \mu g/mg$) and flavonoids ($0.85\pm0.10 \mu g/mg$). 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.

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**Bibliographic References**