CHARACTERIZATION OF THE ANTITUMOR ACTIVITY OF THE *Piper cernuum* SPECIES IN ORAL SQUAMOUS CELL CARCINOMA CELLS (OSCC)

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Introduction

Oral squamous cell carcinoma (OSCC) is one of the 10 most common types of cancer. OSCC has more than 300,000 new cases reported each year (Vigneswaran and Williams 2014). Although progress has been made in cancer research and therapy, the survival rate has not improved significantly in recent years (SCULLY AND BAGAN, 2009). Plants of the genus *Piper* are used in traditional medicine to treat cancer, and they present a diversity of phytochemicals with cytotoxic potential (ESSID et al. 2015).

Method

Crude methanol extract of *Piper cernuum* leaves (CEPCL) and hexane, dichloromethane and ethyl acetate chemical partitions were produced (HPPCL, APPCL, DPPCL). Chromatographic partitions were prepared from the dichloromethane chemical partition of *Piper cernuum*. Partitions and fractions were tested for clonogenic and cell viability (MTT assay), using carboplatin as positive control. OSCC cell lines (SCC4, SCC9, SCC25) and primary human fibroblasts were used for all assays. From the dose-response curves of the compounds and from the non-linear regression the concentration values required for 50% of cells inhibition (IC_{50}) (GraphPadPrism 5) were calculated. The selectivity index (SI) was calculated as: IC_{50} of normal cells/IC_{50} of tumor cells, an SI > 2 indicates that the compound is selective. Acute toxicity tests were performed in mice according to the CEUA / UFF # 982 protocol.

Results / Discussion

In the clonogenic test, it was observed that CEPCL significantly reduced cell density of the SCC9 line at all concentrations tested (1.5 and 25 µg/mL) and reduced cell viability (IC_{50} = 106.3 ± 0.06 µg/mL) when compared to the control (IC_{50} = 225.3 ± 0.09 µg/mL). Although all partitions showed cytotoxicity, the dichloromethane partition (DPPCL) was the most active (IC_{50} ≅ 47 µg/mL). This partition induced reduction of cell number, and membrane permeabilization (6.5 times more cells than control), and it was not hemolytic and not acutely toxic in mice. Seven chromatographic partitions of DPPCL were analyzed for its cytotoxicity, from which the fractions 9 (IC_{50} = 40.25 µg/mL) and 12 (IC_{50} = 79.64 µg/mL) were the most active. Fractions 9 and 14 were partitioned again, each one with 5 new partitions. All new fractions were tested and it was seen that 09.07 and 14.05 were the most selective (SI = 2.03 and SI = 2.53, respectively).

Conclusion

After analysis it can be concluded that the extracts, partitions and fractions of *Piper cernnum* were active against the cell lines of SCC9. In addition, *Piper cernnum* dichloromethane chromatographic partitions tested on SCC9 cells had a significant inhibitory effect on the viability of oral squamous cells. The next steps will be to determine which major substances are present in the most active and selective partitions and cell death pathway.

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