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EVALUATION OF THE ANTITUMOR ACTIVITY *in vitro* AND *in vivo* OF THE *Piper cernuum* PLANT IN ORAL SQUAMOUS CELL CARCINOMA CELLS (OSCC)

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Introduction

Squamous cell carcinoma of the mouth or oral squamous cell carcinoma (OSCC) is a very common type of cancer worldwide [1] and has a 5-year survival rate of approximately 50% [2]. It is one of the 10 most common types of cancer [3]. The choice of treatment for OSCC is primarily based on the stage of the primary tumor, and the prognosis is also based on the stage of the carcinoma. The main forms of treatment consist of surgery, radiotherapy and chemotherapy [2]. To find new treatments, research is carried out in various plant genera. In fact, several drugs used today in the clinic are derived from plants [4]. Plants of the Piper genus are used in traditional medicine for the treatment of cancer and present a variety of phytochemicals with cytotoxic potential [5].

Material and Methods

Methanol crude extract of *Piper cernuum* leaves (CEPCL) and its liquid-liquid partitions of hexane, dichloromethane and ethyl acetate were produced. The chromatographic fractionation was prepared from the chemical partition of dichloromethane through high performance liquid chromatography giving rise to 7 chromatographic fractions (3, 5, 6, 9, 10, 12, 14), which were tested for clonogenic and cell viability (MTT) using carboplatin as a positive control. After the 7 fractions were tested, a new fractionation was performed from fractions 9 and 14, originating 10 new fractions (09-01, 09-03, 09-05, 09-07, 09- 09, 14-03, 14-05, 14-07, 14-09, 14-10). OSCC9 cells and primary human fibroblasts were used for all assays. Toxicity tests were performed in mice according to protocol CEUA/UFF#982. The administration of the carcinogen 4-Nitroquinoline1-oxide (4NQO) was performed in C57Bl/6 mice for 8 weeks. Thereafter, animals were treated for 8 weeks with intraperitoneal injection of the dichloromethane partition (DPPCL), 30mg/kg, twice a week. Then, macroscopic and histopathological analysis of the tongue of these animals was performed. Survival was analyzed by a Kaplan-Meier curve. In addition, the chemical profile of the most active and selective partitions was analyzed through LC-MS/MS and with the aid of the Global Natural Products Social Molecular Networking (GNPS) platform.

Results and Discussion

In the clonogenic test, it was observed that CEPCL significantly reduced the cell density of the OSCC9 tumor line and reduced cell viability when compared to control. Although all partitions showed cytotoxicity, the DPPCL was the most active. This partition induced reduction of cell number, induced membrane permeabilization (6.5 times more cells than control), was not hemolytic and not acutely toxic in mice. In the *in vivo* experiment, there was a statistical difference in the survival curve with $p>0.03$. Histological analysis of tumor progression revealed macroscopic and microscopic differences between 4NQO and 4NQO+DPPCL groups in the number and stage of tumors, indicating a possible therapeutic activity of this partition. Chromatographic fractions of DPPCL were analyzed and the fraction 9 (IC50 = 40.25, SI = 2.67) was the most active and the 14 (IC50 = 77.63, SI = 31.78) was the most selective on OSCC9 cells, while carboplatin presented IC50 = 322.30 and SI = 0.99. Fractions 9 and 14 were fractioned again. All new fractions were tested and it

was seen that 09-07 and 14-05 were active (IC50 = 74.71 and IC50 = 162.6, respectively), and the most selective (S=2.03 and SI=2.53, respectively) therefore, were selected for the continuation of the study. Through dereplication of MS data in GNPS, 3 substances were noted in fraction 09-07 and 9 in fraction 14- 05, one was detected in both fractions, 8 of these substances have cytotoxic activity against different tumor cell lines reported in the literature.

Conclusion

After analyzing the results, it was concluded that the extracts, partitions and fractions of *Piper cernuum* were active against the cell lines of OSCC9 and extended the life of mice with OSCC. Furthermore, chromatographic fractions of dichloromethane from *P. cernuum* tested on OSCC9 cells had a significant inhibitory effect on oral squamous cell viability.

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