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The Cancer Control Challenge:
Better Understanding It to Better Face It

TITLE: CYTOTOXIC EVALUATION OF OILS EXTRACTED AT DIFFERENT STAGES OF MATURATION FROM THE PIPERACEAE FAMILY IN MODELS OF DIFFUSE GASTRIC CANCER.

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Introduction: Gastric cancer, the fifth most incident in Brazil, still represents a significant public health challenge. Conventional chemotherapies present adverse effects that limit their efficacy. In this context, the search for new alternatives becomes essential. Essential oils from the *Piperaceae* family have been gaining prominence due to their immunomodulatory, anti-inflammatory, and antiproliferative properties. **Objectives:** To evaluate *in vitro* antineoplastic activity of *Piperaceae* family essential oils, extracted at different maturation stages, on a diffuse-type gastric adenocarcinoma model. **Methods:** The ACP02 cell line was cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (37°C, 5% CO^2). Essential oils from *Piperaceae* (OEPA, OEPVD, and OEPV) were obtained by hydrodistillation using a Clevenger apparatus. The assays included: cell viability analysis by MTT assay with seeding density of 3×10^3 cells/well and treatment with the three oils at concentrations ranging from 100 to 1.15 µg/mL for 72 hours, followed by spectrophotometric reading at 570 nm. For cell death pattern evaluation, cells were treated with IC_{50} and $½IC_{50}$ of each oil, differentially stained with fluorophores, and analyzed by fluorescence microscopy to assess percentages of viable, apoptotic, and necrotic cells in the ACP02 lineage. Negative (untreated) and positive controls (1µM Doxorubicin) were included. Data were analyzed by two-way ANOVA followed by Bonferroni's test ($p<0.05$). **Results:** The MTT tests showed that the oils had a cytotoxic effect on the ACP02 strain at all stages of maturation. OEPVD and OEPA

exhibited the least cytotoxicity *in vitro*, requiring average inhibitory concentrations (IC_{50}) of 38.41µg/mL and 43.61µg/mL, respectively, to significantly reduce cell viability. OEPV, on the other hand, showed a more significant reduction in viability compared to the different oils, with an IC_{50} of 14.43µg/mL. It is worth noting that OEPV showed potent activity from the lowest concentration tested (7.2µg/mL), reaching an effect comparable to the positive control (chemotherapy) at 14.4µg/mL (IC_{50}), with a significant reduction in cell viability. The results of the cell death pattern showed that all essential oils from the Piperaceae family (OEPVD, OEPA and OEPV) showed dose-dependent apoptotic activity in ACP02 cells. OEPVD induced a significant increase in apoptosis at both ½IC_{50}(19.2 µg/mL) and IC_{50} (38.4 µg/mL). OEPA showed a similar effect, with greater apoptosis at 21.8 µg/mL (½IC_{50}), and 43.6 µg/mL (IC_{50}). In all cases, the response was predominantly apoptotic. It was observed that the levels of cell death by necrosis were not significant in the three types of oils at different concentrations. **Conclusion:** *Piperaceae* essential oils, especially OEPV, showed potential anti-tumor effects against gastric cancer, inducing apoptosis with low necrosis, indicating potential for complementary therapies. Further studies are needed to confirm these findings.

Keywords: Gastric cancer; Essential oils; Apoptosis; Cell death.