UNIVERSITI TEKNOLOGI MARA

# DILLAPIOLE EFFECTS ON APOPTOSIS IN HUMAN NASAL EPITHELIAL CARCINOMA, RPMI 2650 CELLS INVOLVES BCL-2 AND CASPASE -8 SIGNALLING PATHWAY

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MSc

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# AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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# ABSTRACT

The incidence of developing cancers of nasal cavity or paranasal sinuses is higher among the Southeast Asian compared to European countries. In Malaysia, they occurred as early as in the third and fourth decade of life with no sex predominance. However, the current treatments such as surgery, chemo-, radio- and adjuvant therapies are toxic with various undesirable side-effects . Dillapiole, 4, 5-dimethoxy-6-prop-2-enyl-1,3-benzodioxole is a phenylpropanoid extract of *Peperomia pellucida* was highly cytotoxic to cancer cells such as estrogen receptor-positive and -negative breast cancers cells but not to normal cells. However, its effect on cancers in the head and neck region remains ambiguous. In the present study the cytotoxic effect of dillapiole on human nasal epithelial carcinoma, RPMI 2650 cells and the underlying mechanism was investigated. Normal human gingival fibroblast, HGnF cells was used as a comparison. The cell cytotoxicity effect of dillapiole was determined using WST-1 assay; and validated by MTT and trypan blue exclusion assays. Flow cytometric analysis of cells stained with annexin-V-FITC/PI was used to determine the cell death mechanism induced by dillapiole. The expression of CASP8 and BCL2 proteins were assessed by enzyme-linked immunosorbent assay (ELISA). Cisplatin and untreated cells were used as positive and negative controls, respectively. Dillapiole was more cytotoxic and selective to RPMI 2650 cells compared to HGnF cells. Its action was dose dependent. Respective $IC_{50}$ and $IC_{75}$ of 46 µM and 125 µM were obtained for RPMI 2650 cells but none for HGnF cells up to 150 µM. On the contrary, respective $IC_{50}$ of 8 µM and 70 µM were obtained in cisplatin treatment on both RPMI 2650 and HGnF cells. The results from MTT and trypan blue exclusion assays were in accordance with WST-1. At the respective $IC_{50}$ and $IC_{75}$ concentrations, dillapiole induced apoptosis in RPMI 2650 cells by $27.83 \pm 2.35$ % ($p<0.05$, $n=3$) and $58.86 \pm 8.45$ % ($p<0.05$, $n=3$), while cisplatin induced $92.8 \pm 2.5$% ($p<0.05$, $n=3$) apoptosis at its $IC_{50}$ concentration. At the same dose of treatments as above, dillapiole and cisplatin up-regulated the expression of pro-apoptotic CASP8 protein and down-regulated anti-apoptotic BCL2 protein in RPMI 2650 cells. These findings indicated that similar to cisplatin, dillapiole activated apoptosis in RPMI 2650 cells via both the extrinsic and intrinsic pathways through the involvement of CASP8 and BCL2. Findings from this study may provide crucial roles for enhancement into *in vivo* studies and good baseline reference for future downstream experimental of gene and protein study.

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