SYNERGY EFFECT OF α-PHELLANDRENE AND 5-FLUOROURACIL ON ANTI-PROLIFERATION IN COLORECTAL CANCER CELLS

α-水芹烯與 5-氟尿嘧啶抑制人類大腸癌細胞株增生之協同作用

MASTER THESIS

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FOOD TECHNOLOGY DOUBLE DEGREE MASTER PROGRAM

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Submitted to the Department of Food Technology, Soegijapranata Catholic University,
and Department of Food and Nutrition, Providence University
In partial fulfillment for obtaining the Master's double degree

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ABSTRACT

Alpha-phellandrene (α-PA), an important component of dill, has been shown to have many physiological effects, including chemoprevention. However, the synergy effect of α-PA with chemotherapy medicines is limited. Colorectal cancer (CRC) ranked second as the most common cause of death globally, and it has a high incidence rate in Taiwan and Indonesia. One of the most frequently used medicines for chemotherapy for colorectal cancer is 5-fluorouracil (5-FU). Unfortunately, high doses and long-term use of 5-FU can generate side effects, such as those toxicity and inflammation, for CRC patients. This study investigates the synergy effect of α-PA combined with 5-FU on anti-proliferation in human colorectal HT-29 cancer cells. This study analyzed cell viability, cell proliferation, cell cycle and its regulators, apoptosis and its regulators, and cell death via the NF-κB pathway towards HT-29 after 50, 100, or 250 μM α-PA combined 5 μM 5-FU treatment for 72 h. Our results show that combining 250 μM α-PA and 5-FU treatment can reduce cell viability and fluorodeoxyuridine (FdU) incorporation level more than the 5-FU alone group ($p<0.05$). Reducing FdU levels means the combination of α-PA and 5-FU has a synergy effect inhibiting cell proliferation. Cell proliferation is also inhibited by 250 μM α-PA combined 5-FU due to cell cycle arrest in the G1 phase ($p<0.05$). As compared to the 5-FU treatment alone group, the combination treatment of 100 and 250 μM α-PA and 5-FU can more efficiently activate p21, 50 and 100 μM α-PA combined 5-FU can significantly decrease CDK2 levels ($p<0.05$), and 250 μM α-PA combined 5-FU can significantly reduce CDK-4 levels ($p<0.05$) in HT-29 cells. Combination of 50, 100, and 250 μM α-PA and 5-FU significantly reduce Wnt and p-β-catenin expression level, induce β-catenin translocation, and significantly reduce Wnt/β-catenin ratio ($p<0.05$) in HT-29 cells. Besides, this combination treatment of 50, 100, and 250 μM α-PA and 5-FU can more efficiently induce both early (by Annexin V stained) and late (by PI stained) apoptosis by reducing the mitochondria membrane potential (MMP), increasing Bax, Cytochrome C, Caspase-8, Bid, Caspase-9, and Caspase-3, also reducing Bcl-2 than the 5-FU treatment alone ($p<0.05$). The combination treatment of 50, 100, and 250 μM α-PA and 5-FU also more efficiently activates the NF-κB pathway by inducing p-IκB, NF-κB (p65) translocation, and NF-κB DNA binding. NF-κB pathway activation resulted in the translocation of NF-κB in the mitochondria, reducing the

binding of HK-2 and VDAC-1 complexes and leading to apoptosis. These results show that α-PA is a safe phytochemical that is not toxic to human cells and has efficiency and high potential as a synergic chemotherapy component of 5-FU. These synergic effects reduce cell proliferation, induce apoptosis via intrinsic and extrinsic apoptotic pathways, and activate the NF-κB signaling pathway.

Keywords: α-PA, 5-FU, apoptosis, cell proliferation, HT-29,