Cytotoxicity and apoptosis effects of curcumin analogue (2E,6E)-2,6-Bis(2,3-Dimethoxybenzylidine) Cyclohexanone (DMCH) on human colon cancer cells HT29 and SW620 in vitro

Colorectal cancer (CRC) is the third most common type of cancer worldwide and a leading cause of cancer death. According to the Malaysian National Cancer Registry Report 2012-2016, colorectal cancer was the second most common cancer in Malaysia after breast cancer. Recent treatments for colon cancer cases have caused side effects and recurrence in patients. One of the alternative ways to fight cancer is by using natural products. Curcumin is a compound of the rhizomes of Curcuma longa that possesses a broad range of pharmacological activities. Curcumin has been studied for decades but due to its low bioavailability, its usage as a therapeutic agent has been compromised. This has led to the development of a chemically synthesized curcuminoid analogue, (2E,6E)-2,6-bis(2,3-dimethoxybenzylidine) cyclohexanone (DMCH), to overcome the drawbacks. This study aims to examine the potential of DMCH for cytotoxicity, apoptosis induction, and activation of apoptosis-related proteins on the colon cancer cell lines HT29 and SW620. The cytotoxic activity of DMCH was evaluated using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) cell viability assay on both of the cell lines, HT29 and SW620. To determine the mode of cell death, an acridine orange/propidium iodide (AO/PI) assay was conducted, followed by Annexin V/FITC, cell cycle analysis, and JC-1 assay using a flow cytometer. A proteome profiler angiogenesis assay was conducted to determine the protein expression. The inhibitory concentration (IC50) of DMCH in SW620 and HT29 was 7.50 ± 1.19 and 9.80 ± 0.55 µg/mL, respectively. The treated cells displayed morphological features characteristic of apoptosis. The flow cytometry analysis confirmed that DMCH induced apoptosis as shown by an increase in the sub-G0/G1 population and an increase in the early apoptosis and late apoptosis populations compared with untreated cells. A higher number of apoptotic cells were observed on treated SW620 cells as compared to HT29 cells. Human apoptosis proteome profiler analysis revealed upregulation of Bax and Bad proteins and downregulation of Livin proteins in both the HT29 and SW620 cell lines. Collectively, DMCH induced cell death via apoptosis, and the effect was more pronounced on SW620 metastatic colon cancer cells, suggesting its potential effects as an antimetastatic agent targeting colon cancer cells.

Keyword: Colon cancer; Curcumin analogue; DMCH; Cytotoxic; Apoptosis; Cell cycle