UNIVERSITI TEKNOLOGI MARA

MECHANISM OF APOPTOSIS: COMPARATIVE ANALYSES OF SELECTED POLYPHENOLS AND SYNTHETIC STILBENE

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ABSTRACT

Polyphenols such as curcumin, resveratrol and gallic acid have anticancer potential to suppress proliferation of variety of tumour cells. Nowadays, research and development of new cancer chemotherapeutic drugs has escalated tremendously. Therefore, the need for new chemotherapy with less or no side effects are much sought after. For the same objective, this study was aimed to investigate comparatively the mechanism of apoptosis induced by these pure polyphenols and a novel synthetic stilbene, 3, 4, 10-trimethoxystilbene (S2) on different types of cancerous cell lines. The antiproliferative effects were determined via MTS assay followed by annexin V and PI staining in flow cytometry to determine the stages of cell death resulting from either apoptotic or necrotic processes and also their cell cycle arrest. The results were further supported by the apoptosis gene expression via RT-PCR and the levels of caspase (caspase-3/7, -8 and -9) to determine their pathway of apoptosis. Cytochrome c release was also measured to elucidate the apoptotic pathway of the compounds and Bid (BH3 Interacting Domain Death Agonist) levels was determined on HepG2 cells treated with gallic acid. Results of the MTS assay showed that the IC_{50} value of S2 against human hepatocelullar carcinoma (HepG2) cells was lower than curcumin, gallic acid and resveratrol with 0.14 ± 0.01 μM, 35.8 ± 2.5 μM, 33.4 ± 1.6 μM and 51.2 ± 1.1 μM, respectively. S2 even demonstrated high selectivity index (WRL68/HepG2) of 8.7, compared to others. Lower doses of S2 were able to induce higher percentage of early apoptosis compared to the other compounds. Curcumin, resveratrol and S2 have been shown to induce cell cycle arrest at S phase while HepG2 treated with gallic acid were arrested at G_1/G_0 phase. Activities of caspases-3 and -9 were also detected on HepG2 cells treated with S2, curcumin and resveratrol while caspases-3 and -8 were detected on HepG2 treated with gallic acid. All of the tested compounds were able to upregulate the expression of the apoptotic genes (*p53, Bax and caspase 3*) and downregulate the anti-apoptotic gene, *bcl-2*. S2 showed a 3-fold higher ratio of Bax/Bcl-2 compared to resveratrol indicating higher potential of S2 in inhibiting proliferation of hepatoma cells. Cytochrome c was detected in HepG2 cells treated with curcumin, resveratrol, gallic acid and S2 and significant ($p<0.05$) increase of Bid was found in the cells of HepG2 treated with gallic acid. As a conclusion, curcumin, resveratrol and S2 have the potential to induce apoptosis on HepG2 cells via mitochondrial pathway and S phase cell cycle arrest. On the other hand, gallic acid was able to trigger apoptosis through Fas signaling pathway (caspase-8 activation) involving Bid and cytochrome c which induced G_1/G_0 phase-arrested cells.

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CHAPTER ONE
INTRODUCTION

1.1 OVERVIEW

In Malaysia, based on data from International Agency for Research on Cancer, in 2012, the number of people that are newly diagnosed with cancer per year was 37400 cases with incidence of 143.6 in every 100,000 people were recorded. At the same time, people with age of 75 and below had 15% risk of getting cancer and led to almost 21,700 deaths per year.

Therefore, many approaches are needed to reduce the occurrence of cancer and following deaths, mainly via the primary and secondary prevention strategies (Kelloff, 2000). The primary prevention strategy is to remove causative agents and modify lifestyle that could lead to the risks of cancer, such as smoking cessation, healthy diets and screening tests for detection of precancerous (Pezzuto, 2008; Steward & Brown, 2013). Cancer chemoprevention which is the secondary prevention, involves the intervention of natural and/or synthetic agents to inhibit the development or spread of malignant tumour through various pathways (Pezzuto, 2008). One of which is the induction of apoptosis (Noonan *et al.*, 2007).

Apoptosis, or programmed cell death, is a normal phenomenon in the development of multicellular organisms. Death of cells is a response that caused by variety of stimulation and during apoptosis it occurs in controlled and regulated template. Apoptosis is a process in which cells play an active role in their own death and specific signals leading to the cells to undergo distinctive changes occur in the cell. This makes it different from necrosis in which uncontrolled cell death leads to lysis of cells and inflammatory responses, causing serious health problems (Dash *et al.*, 2006).

Caspases (family of proteins) were activated in the early stages of apoptosis. These proteins breakdowns are the key cellular components that are required for normal cellular function which included structural proteins in the cytoskeleton and nuclear proteins such as DNA repair enzymes. It also activates other degradative enzymes such as DNases, which begins to cleave the DNA in the nucleus. Apoptotic