UNIVERSITI TEKNOLOGI MARA

EXOPOLYSACCHARIDES FROM *Bifidobacterium pseudocatenulatum* ATCC 27919 INDUCED AUTOPHAGY AND APOPTOSIS IN Caco-2 CELLS

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ABSTRACT

The application of probiotics and its components as an adjuvant or alternative treatments for cancer is an ongoing research. Exopolysaccharide (EPS) is one of the probiotic components which have been reported to play vital parts in the modulation of cell cycle and apoptosis in cancer cells. In this regards, EPS from *Bifidobacterium pseudocatenulatum* was explored for its potential to induce autophagy and apoptosis in human colon cancer (Caco-2) cells. Studies on EPS specifically from *Bifidobacterium pseudocatenulatum* ATCC 27919 has yet to be comprehensively examined, especially on the underlying mechanism of anti-cancer potential. Therefore, this study aimed to determine the effects of EPS from *Bifidobacterium pseudocatenulatum* ATCC 27919 exposure in Caco-2 cells on cell death mechanisms that include apoptosis and associate it with autophagy. The anticancer potentiality of the EPS were evaluated using cytotoxicity assay and microscopy observation in the Caco-2 cells after exposure to 1, 5, and 10 mg/ml EPS for 24 and 48 hours. Additionally, the effects of EPS on both apoptosis (cell cycle assay, annexin V and dead cell assay, DAPI staining and immunoassay (ELISA)) and autophagy (LC-3 assay and immunoassay) were determined respectively. At molecular level, the alteration in gene expression of apoptotic and autophagic mechanisms were quantified by qPCR. Furthermore, the association between the apoptosis and autophagy mechanisms was also quantified by qPCR. The obtained data confirmed the cytotoxicity effects of EPS in the Caco-2 cells by significantly ($p<0.001$) reducing the viability from 67 to 9%. From the viability assay and IC_{50} calculated, 5 mg/ml of EPS was chosen for further experiments. Thirty (30) μM of Rapamycin was used as the positive control and showed a more significant ($p<0.001$) reduction in cell viability which is 38%. Microscopy observation and morphological assessments exhibited typical morphological features of apoptosis in EPS-exposed Caco-2 cells, such as shrinkage of cells, condensed nuclei, and loss of shape. An early phase of apoptosis and cell cycle arrest at the G2/M phase were detected in EPS-exposed cells. qPCR assay showed a significant increase in mRNA of apoptosis markers such as cleaved Caspase-3, Bcl-2-associated X (BAX), and Poly (ADP-ribose) polymerase -1 (PARP-1). They were enhanced relative to Rapamycin-exposed cells compared to unexposed cells. Besides, autophagy was also detected, whereby autophagic LC3-II protein was observed after 24 hours of exposure to EPS on Caco-2 cells. In contrast, mRNA expression of Beclin-1 (upregulated) and Bcl-2 (downregulated) by qPCR assay also indicated the occurrence of autophagy. Detection of protein showed that Sequestosome 1 (SQSTM1) /p62 protein was also enhanced in EPS-exposed cells at 24 hours. The upstream activation of the autophagy event was evident by the increased phosphorylated AMP-activated protein kinase (AMPK) protein with the reduced phosphorylated mechanistic Target Of Rapamycin (mTOR) protein. The potential association node between the mechanisms was demonstrated by examining GRP78 mRNA expression. GRP78 has been recognized to be involved in Endoplasmic reticulum (ER) stress-induced autophagy. GRP78 was significantly upregulated ($p<0.001$) in EPS-exposed Caco-2 cells. In conclusion, autophagy was suggested as a cytoprotective response in Caco-2 cells against stress, as it preceded the apoptosis upon EPS exposure in Caco-2 cells. In this study, it is also suggested that both autophagy and apoptosis are associated with antagonistic effects in which suppressing autophagy has enhanced the apoptosis mechanism in the Caco-2 cells.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Non-communicable diseases (NCDs) are long-term illnesses caused by a mix of genetic, physiological, environmental, and behavioural characteristics. According to World Health Organization (WHO), cancer is one of the main NCDs. It has been reported that in Malaysia, colorectal cancer (CRC) is the second (16.9%) and third (10.7%) most common cancer among males and females respectively (Veettil *et al.*, 2017). Additionally, with over 1.8 million new cases worldwide in 2018 and current treatment regimens, research has also emerged for alternative interventions for CRC. This is due to certain limitations associated with the current treatments such as existing systemic toxicity, unsatisfying response rate, unforeseeable innate and acquired resistance and low tumour specific selectivity (Xie *et al.*, 2020). The alternative interventions for anti-cancer have been explored from many sources including from microorganisms namely probiotics. Besides, potential approach in anti-cancer research also involves metabolites produced by the probiotics such as short chain fatty acids (SCFA), enzymes, and exopolysaccharides (EPS) (Kvakova *et al.*, 2022). In the recent years, studies on EPS have been proven to be effective against cancer. It includes inhibitory effects on the proliferation of several colon cancer cell lines (Liu *et al.*, 2017; Zhou *et al.*, 2017).

EPS are polysaccharides composed of homopolymers or heteropolymers synthesized by microorganisms and secreted into the extracellular environment. They are particularly important against desiccation, phagocytosis, cell recognition, phage attack, antibiotics or toxic compounds and osmotic stress (Khalil *et al.*, 2022). Microbial-derived EPS, specifically EPS extracted from Lactic Acid Bacteria (LAB) have displayed diverse health benefits including anticancer, antioxidant, and immunomodulatory (Makino *et al.*, 2006; Laiño *et al.*, 2016; Deeb *et al.*, 2018; Adebayo-Tayo *et al.*, 2019). LAB are probiotics that inhabit the animals and human's gastrointestinal tract which depends on species, age, and location within the gut (Nayok,