

UNIVERSITI TEKNOLOGI MARA

**MECHANISMS UNDERLYING
ANTICANCER EFFECT OF
LACTOBACILLI (LAB 12 AND LABPC)
AGAINST HUMAN COLORECTAL
CANCER**

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of the requirements for the degree of
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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 result of my own work, unless otherwise indicated or acknowledge as referenced work. This thesis has not been submitted to any academic institution or non-academic institution for any degree or qualification.

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ABSTRACT

Chemotherapy plays a crucial role in treatment of colorectal cancer (CRC). Its effectiveness, however, is often hampered by toxicity-limiting therapeutic effect and drug resistance. Probiotics are thought to be able to prevent CRC through changing of colonic microbiota. This study examined the anticancer of Malaysian LAB and unravel the underlying mechanisms against CRC. Twelve LAB isolated from locally fermented food were screened for anticancer against HCT 116, HT-29 and DLD-1 using MTT assay. CRC cells treated with either LAB strain eliciting the most potent anticancer or *Lactobacillus casei* strain Shirota (reference strain; LABPC) were subjected to detection of apoptosis by AO/PI staining. The mode of cell death was confirmed by annexin V-FITC/PI. The *in vitro* findings were validated using a HCT 116 tumour xenograft model (n=6/group). Oral administration (10^9 cfu/ml) of LAB 12 and LABPC, either in freeze dried form (FD) or fermented in soymilk (SM), was performed daily before and after tumour inoculation. Tumours were then excised and homogenized for nitric oxide (NO) production, pro-inflammatory cytokine levels (IL-1 α , IL-8 and VEGF), antioxidant activity (SOD, CAT and GSH) and caspase-3 activation. The metabolic profiling of serum was also performed using LC/MS Q-TOF and differential expression of the metabolites was established by clustering analysis. Anticancer screening revealed that whilst both HT-29 and DLD-1 remained viable in the presence of all tested LAB, HCT 116 were particularly sensitive to treatment with *Lactobacillus plantarum* (LAB12) (IC₅₀=57.70% \pm 39.69) and LABPC (IC₅₀=44.87% \pm 18.63) fermented in RPMI. Confocal microscopy of AO/PI stained LAB12- and LABPC-treated HCT 116 confirmed the presence of apoptotic bodies, chromatin condensation and fragmentation as well as membrane blebbing. Subsequent detection of apoptosis found increased percentage of HCT 116 in early and late apoptotic (indicative of apoptosis) stages following 24, 48 and 72 h treatment with LAB12 (4.58-46.46%) and LABPC (6.72-51.45%), respectively when compared to untreated HCT 116. *In vivo* study showed reduction of tumour volume (41%-65%) and weight (22%-46%) in LAB12- and LABPC-treated mice. Tumour homogenates of LAB12- and LABPC-treated mice were presented with down-regulation of NO (57%-77%) and pro-inflammatory cytokine [IL-1 α (34%-71%), IL-8 (15%-67%) and VEGF (18%-64%)] as well as up-regulation of caspase-3 (16%-68%) and antioxidant enzymes [SOD (20%-37%), CAT (37%-46%) and GSH (43%-71%)] levels. Metabolic profiling of serum from LAB12- and LABPC-treated mice identified significant ($p < 0.05$) up-regulation of nine metabolites associated with antitumour activity and they include eicosapentaenoic acid (5.92-12.14), palmitic acid (8.93-9.12), undecanoic acid (1.46-5.86), docosapentaenoic acid (3.89-12.64), oleamide (12.52-13.59), dihydroxyvitamin D3 (5.71-6.14), dihydrosphingosine (13.96-14.09), phytosphingosine (14.99-15.61) and C16 sphinganine (15.99-16.91). The present study reports for the first time that the anticancer effect of LAB12 and LABPC was mediated through the induction of apoptosis. The apoptotic effect was associated with up-regulation of antioxidant activities and anticancer metabolites levels as well as down-regulation of nitric oxide and pro-inflammatory cytokines. These findings suggest the potential use of Malaysian LAB for prevention of CRC.

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CHAPTER ONE

INTRODUCTION

Colon cancer is one of the most common cancers worldwide, particularly amongst developed countries in the west (Wu et al., 2012). In Asian population, which includes Malaysians, there is a rapid increase in the incidence of colon cancer (Natrah, Ezat, Syed, Rizal & Saperi, 2012). The risk of developing this malignancy is greatly influenced by age, genetic, environment and change in dietary and lifestyle factors (Yamall, Crouch & Lewis, 2013). It is estimated that dietary factors such as high intake of red and processed meat as well as low levels of fiber contributed to approximately one half of all colon cancer cases (Wu et al., 2012; Yamall, Crouch & Lewis, 2013).

The current treatment regimens for colon cancer are extensive, and they include surgery and chemotherapy, both of which are highly dependent on tumour stage. When colon cancer is detected during the early stage, surgery is the preferred option. However, the success of this strategy is limited by development of scar tissue in the abdomen that can cause organ or tissue to stick together and block the bowel [American Cancer Society (ACS), 2014]. Metastatic cancers are usually treated by chemotherapy. In colon cancer, conventional chemotherapy is not as effective when compared to other cancers because drugs do not reach the target site in effective concentration (Mishra, Ramasamy & Majeed, 2012). The non-selectivity of anticancer agents causes high level of side effects and thus affects the patients' quality of life. Therefore, it is of great importance to discover and develop novel agents with low side effects, improved safety and maximum efficacy as well as the ability to overcome drug resistance (Yang et al., 2012).

There is evidence indicating that ingestion of probiotics is able to diminish the risk of cancer and inhibit tumour growth (Choi et al., 2006; Kim, Oh, Yun & Kim, 2010). Probiotics are living microorganisms, which can confer benefit on the host when consumed in sufficient amount (FAO/WHO, 2002). The most common probiotics include the lactic acid bacteria (LAB), with most attention focused on the members of genera *Lactobacillus* and *Bifidobacterium* (Florence et al., 2012). These organisms have been widely reported to exert many beneficial effects in that they stimulate the growth of