UNIVERSITI TEKNOLOGI MARA

SYSTEMATIC REVIEW AND META-ANALYSIS OF HEPATOPROTECTIVE PROBIOTICS *IN VIVO* AND ELUCIDATION OF *LACTIPLANTIBACILLUS PLANTARUM* LAB12-INDUCED HEPATOPROTECTION AGAINST HIGH-FAT DIET-INDUCED METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE MOUSE MODEL VIA THE GUT-LIVER AXIS

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ABSTRACT

Metabolic dysfunction-associated fatty liver disease (MAFLD), previously known as non-alcoholic fatty liver disease (NAFLD), is a highly prevalent disease. To date, lifestyle modifications remain as the cornerstone for MAFLD management. During advanced stage of the disease, patients are prescribed with either vitamin E (i.e., α-tocopherol) or pioglitazone. Whilst the problems of lifestyle changes are associated with poor implementation and compliance, pharmacological therapies result in limited improvement of liver pathology and are often compromised by side effects. This raises the need for effective alternatives that are relatively easier to comply with and emerging functional food like probiotics appear to fit the bill given their increasingly recognised roles in delaying the progression of MAFLD. As such, this study had first systematically reviewed publications on hepatoprotection of probiotics in vivo based on the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. The literature search, which was performed through the Cochrane Library, EMBASE, PubMed/ Medline and Web of Science by using pre-defined keywords, had identified 4,841 studies, from which 53 were shortlisted based on the inclusion and exclusion criteria. All shortlisted studies for qualitative appraisal were presented with low/ unclear risk of bias. Only 24 studies were eligible for subsequent quantitative analysis using Review Manager Version 5.3. The primary outcome supported hepatoprotection of probiotics in the intervention group, which was demonstrated mainly through the significantly reduced severity of liver pathology based on the reduced total NAFLD Activity Score (NAS) (MD=-1.90; 95% CI=-2.25, -1.54, $p<0.00001$; $I^2=37\%$, $p=0.08$) when compared to the control group. This study then went on to elucidate mechanisms underlying hepatoprotection by *Lactiplatibacillus plantarum* LAB12 (LAB12) against high fat diet (HFD)-induced MAFLD in male C57BL/6J mice. The hepatoprotective effects of daily LAB12 supplementation over 20 weeks were evident through the significantly reduced ($p<0.05$) liver pathology [total NAS (2±1.2 vs 3 ± 1.8) and serum ALT (1.90 ± 0.40 ng/mL vs 2.91 ± 0.56 ng/mL) (-34.5%)] and hepatic total cholesterol (1.89 ± 1.12 mg/g vs 4.96 ± 2.85 mg/g) (-58.5%). The beneficial effects of LAB12 against HFD-induced MAFLD could be attributed, at least in part, to significantly reduced ($p<0.05$) hepatic SREBP-1 protein expression (-50%), hepatic TNF-α (0.00012 ± 0.00004 mg/g protein vs 0.0002 ± 0.0001 mg/g protein) (-45%) and IL-6 (0.0004 ± 0.0001 mg/g protein vs 0.0006 ± 0.0002 mg/g protein) (-35%) levels, intestinal permeability [lactulose/ mannitol ratio (0.24 ± 0.13 vs 0.84 ± 0.44) (-72%) and lipopolysaccharide level in hepatic portal blood (3.19 ± 1.57 ng/mL vs 7.17 ± 3.13 ng/mL) (-55%)] but significantly increased ($p<0.05$) serum butyrate (0.66 ± 0.17 mM vs 0.49 ± 0.08 mM) (+26%) when compared to the HFD control mice (HO). Interestingly, the LAB12-supplemented normal chow-fed mice (CL) were presented with significant increased ($p<0.05$) bacterial richness in the caecocolonic content and colonic butyrate (1.64 ± 0.71 mM, +40%) when compared to the chow control mice (CO) (0.98 ± 0.51 mM). Altogether, these findings strongly implied the usefulness of hepatoprotective LAB12 against HFD-induced MAFLD.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

The metabolic associated-dysfunction fatty liver disease (MAFLD), or formerly known as the non-alcoholic fatty liver disease (NAFLD) is a non-communicable disease that affects more than 30% of the world population (Xie et al., 2023; Younossi et al., 2023), with 19% and 38% increased mortality risks from all causes and cardiovascular diseases (CVDs), respectively (Xie et al., 2023). The conditions of the disease range from the accumulation of fat in the liver (i.e., steatosis) (≥5%), inflammation and the subsequent progression to non-alcoholic steatohepatitis (NASH) and the advanced, irreversible disease stage of fibrosis and cirrhosis, which are accompanied by metabolic syndromes of multisystemic metabolic dysfunctions, including dyslipidaemia, obesity or overweight, insulin resistance or hypertension (Adams et al., 2017; Byrne & Targher, 2015; Eslam et al., 2020b; Huang et al., 2021; Martin-Mateos & Albillos, 2021; Pipitone et al., 2023). Owing to its silent nature, MAFLD is often an incidental finding from elevated serum alanine aminotransferase (ALT), a sign of liver injury, in the clinics (Dyson et al., 2014).

Generally, the pathogenesis of MAFLD is associated with "multiple parallel hits" that are mainly governed by the gut-liver axis. More specifically, there is an interplay between the gut, the microbiota it harbours and the liver that drives MAFLD pathogenesis. It is increasingly recognised that overnutrition causes dysbiosis, the condition stemmed as the pathogenic factor of MAFLD and is associated with the metabolic profile of the disease (Leung et al., 2016). The diet-induced dysbiosis is also associated with reduced short-chain fatty acid (SCFA) production (Zhang et al., 2018) and increased intestinal permeability that promotes translocation of microbial metabolites to the liver (Mouries et al., 2019). On the other hand, overnutrition, which is associated with high-fat diet (HFD) or high-fructose diet, would result in free fatty acid (FFA) overload, leading to obesity and excessive supply of nutrients to the liver (Chakravarthy & Neuschwander-Tetri, 2020). The excessive nutrients would burden the metabolic sensing liver as well as its mitochondria and endoplasmic reticulum (ER).