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RESEARCH

Anti-obesity potentiality of *Lactiplantibacillus plantarum* E2_MCCKT isolated from a fermented beverage, *haria*: a high fat diet-induced obese mice model study

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Abstract
Obesity is a growing epidemic worldwide. Several pharmacologic drugs are being used to treat obesity but these medicines exhibit side effects. To find out the alternatives of these drugs, we aimed to assess the probiotic properties and anti-obesity potentiality of a lactic acid bacterium E2_MCCKT, isolated from a traditional fermented rice beverage, *haria*. Based on the 16S rRNA sequencing, the bacterium was identified as *Lactiplantibacillus plantarum* E2_MCCKT. The bacterium exhibited in vitro probiotic activity in terms of high survivability in an acidic environment and 2% bile salt, moderate auto-aggregation, and hydrophobicity. Later, E2_MCCKT was applied to obese mice to prove its anti-obesity potentiality. Adult male mice (15.39 ± 0.19 g) were randomly divided into three groups (n = 5) according to the type of diet: normal diet (ND), high-fat diet (HFD), and HFD supplemented with E2_MCCKT (HFT). After four weeks of bacterial treatment on the obese mice, a significant reduction of body weight, triglyceride, and cholesterol levels, whereas, improvements in serum glucose levels were observed. The bacterial therapy led to mRNA up-regulation of lipolytic transcription factors such as peroxisome proliferator-activated receptor-α which may increase the expression of fatty acid oxidation-related genes such as acyl-CoA oxidase and carnitine palmitoyl-transferase-1. Concomitantly, both adipocytogenesis and fatty acid synthesis were arrested as reflected by the down-regulation of sterol-regulatory element-binding protein-1c, acetyl-CoA carboxylase, and fatty acid synthase genes. In protein expression study, E2_MCCKT significantly increased IL-10 expression while decreasing pro-inflammatory cytokine (IL-1Ra and TNF-α) expression. In conclusion, the probiotic *Lp. plantarum* E2_MCCKT might have significant anti-obesity effects on mice.

Keywords Adipocytogenesis · Anti-obesity · Lipid profile · Probiotic

Introduction

Obesity is a growing epidemic worldwide. According to the World Health Organization (WHO), globally, 1.9 billion (39%) or more are overweight people, and 650 million (13%) of those are obese (Haththotuwa et al. 2020). The incidence rate of this epidemic is higher in women (41.88%) than in men (38.67%) in India (Venkatrao et al. 2020). Heredity and obesogenic factors such as improper diet patterns (excessive high-calorie food intake), lower physical activity, and a secondary lifestyle lead to obesity development along with a slow metabolism process (Pradeepa et al. 2015). Obesity also increased the incidence rate of several life-threatening diseases such as heart disease, type-2 diabetes mellitus (T2DM), cancer, and Alzheimer's disease (Fabbrini et al. 2010; Luppino et al. 2010; Abenavoli et al. 2019). The hypertrophy of adipose tissue and its ectopic deposition surrounding the heart and coronary arteries impairs angiogenesis, leading to local tissue hypoxia and necrosis. This condition contributes to left ventricular hypertrophy and remodelling, ultimately contributing to heart failure

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(DeMarco et al. 2014). Moreover, adipose tissue-derived free fatty acids (FFAs) and superoxides caused hypertension via sympathetic nerve activation, increasing the left ventricular mass, stroke volume, and cardiac output (Ding et al. 2019). Dietary lipids and lipidomics can play a significant role in the development of T2DM associated with the dysbiosis of the gut microbiota. Free fatty acids (FFAs) and their derivatives stimulate protein kinase C, initiating Ser/Thr phosphorylation of insulin receptor substrates 1 (IRS-1). This process reduces the typical Tyr phosphorylation of IRS-1, consequently hindering the regulation of the glucose transporter GLUT4. Such interference induces insulin resistance, compromising glucose tolerance (Lauterbach et al. 2017). Additionally, high levels of leptin and low adiponectin levels disrupt β-cell function and suppress insulin secretion, eventually contributing to the development of type 2 diabetes mellitus (T2DM) (Könner et al. 2012; Jin et al. 2023). In addition, the association between obesity and various cancers, including breast, colon, rectum, oesophagus, stomach, gallbladder, uterus, pancreas, and ovary, is multifaceted. Excessive fat accumulation leads to dysfunction of adipose tissue, resulting in increased production of pro-inflammatory cytokines, sex hormones, and lipid metabolites, as well as impaired profiles of adipokines and insulin resistance (Liu et al. 2021). This altered adipose tissue is a source of inflammation, fibrosis, cancer-associated adipocytes, and adipocyte progenitors. These factors collectively contribute to tumour initiation, growth, and recurrence (Aune et al. 2016). Obesity has been linked to changes in both brain structure and function, cognitive impairments, and an increased risk of developing dementia and Alzheimer's disease (AD) (Arnoldussen et al. 2014). Excessive fat accumulation in adipose tissue could reduce the normal blood supply into the brain, leading to vascular injury (Dorrance et al. 2014). Therefore, it creates ischemia in the hippocampal regions (CA1, CA3, and CA4), caudate nucleus, cerebellum, and layers III, V, and VI of the neocortex in the brain (Payabvash et al. 2011). The heightened baseline metabolic activity of the hippocampal regions decreases oxygen and glucose supply, potentially contributing to increased memory loss. Moreover, leptin and other cytokines cause long-term peripheral inflammation linked to reducing white matter, which impairs neuronal connections in the brain (Arnoldussen et al. 2014; Kiliaan et al. 2014).

Currently, orlistat, sibutramine, phentermine/topiramate, etc. drugs have been used as appetite suppressants (anorexic) and absorption preventives (mainly dietary lipids) for obesity or overweight management. However, there is a need to minimize the use of the above-mentioned drugs because of their lower efficacy and unexpected side effects in obese or overweight patients. Orlistat is the first prescribed medicine for obesity management. Rather than curbing hunger, it disrupts gastrointestinal lipase activity and obstructs enzymatic function. Consequently, the enzyme cannot break down triglycerides (TG) (Leung et al. 2003; Derosa et al. 2012). Therefore, this medicine primarily affects the gastrointestinal system and encompasses diarrhea, faecal incontinence, oily spotting, flatulence, bloating, and dyspepsia. Notably, there has been a report of severe liver injury or liver failure associated with orlistat use over the past decade (Derosa et al. 2012). The European Medicines Agency's (EMEA) Committee opted to suspend the drug Sibutramine due to emerging evidence of an elevated risk of heart attacks and strokes in obese patients, highlighted by data from the Sibutramine Cardiovascular Outcomes Trial (James et al. 2010). Phentermine/topiramate drug has several side effects, such as cardiovascular diseases, fetal toxicity, and suicidal ideation (Kim et al. 2014). In this regard, an alternative treatment is needed, and it is supposed to directly modulate energy homeostasis. Probiotic treatment is an alternative approach to manage obesity and related disorders (Choi et al. 2019; Das et al. 2022).

Lactic acid bacteria (LAB) are ubiquitous and frequently used as probiotics, which can be defined as non-pathogenic living microbes which provide positive health benefits to the host if an appropriate amount is consumed (Morelli and Capurso 2012). LABs are abundant in fermented foods and present in the natural intestinal microbiota of humans and most animals. LAB is mostly colonized in human gastrointestinal tract and elevates the intestinal functions (Pessione et al. 2012; Korcz et al. 2018). LAB exerts different health-beneficial effects such as promotion of intestinal activity, improvement of digestion, inhibition of the growth of potential pathogens, prevention of diarrhea, production of conjugated fatty acids, and synthesization of vitamins, etc. (Wallace et al. 2011; Das et al. 2022). *L. plantarum* DSM 15313 and its fermented blueberries have the ability to reduce blood pressure of both non-N^G-nitro-L-arginine methyl ester (non-L-NAME) and N^G-nitro-L-arginine methyl ester (L-NAME) treated rats. Hence, this particular strain could potentially be utilized to mitigate the risk of cardiovascular diseases by regulating blood pressure (Ahrén et al. 2015). The administration of *L. plantarum* led to a significant reduction in levels of total cholesterol, c-glutamyl transpeptidase, LDL, glucose, homocysteine, and IL-6 in postmenopausal women (Barreto et al. 2014). Furthermore, *L. plantarum* NCU116 exhibited potential capabilities to regulate lipid metabolism, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), as well as influence the morphology of the liver and adipose tissues in high-fat diet feed rat (Barreto et al. 2014; Li et al. 2014). The pre-intake of *L. plantarum* J26 restored the gut microbial balance, improved the intestinal barrier functions that reduce hepatic LPS levels. It attenuated the activation of the TLR4-mediated MAPK signaling pathway and thus

reduced the generation and development of liver inflammation (Li et al. 2022). Moreover, the extracellular products of *L. plantarum* and *L. plantarum* 78010 exhibited anticancer effects by enhancing the trans-epithelial electrical resistance of H4 cells and reducing the secretion of pro-inflammatory cytokines such as IL-6 and IL-8 (Dimitrovski et al. 2014; Wang et al. 2014). Therefore, LAB exerts anti-obesity effects by regulating adipogenesis-related gene expressions (Park et al. 2011). Probiotic may alter the microbial structure in the gut, lipid metabolism pathway in the liver, and increase nutrient absorption and thus help in triglyceride reduction. Moreover, probiotic may influence the inflammatory factors and oxidative stress (Nihei et al. 2018). Probiotic bacteria could modulate the intestinal metabolites such as SCFAs which can be transported to liver through blood and regulate lipid metabolism (Soto et al. 2018). Probiotics could regulate the adipocyte division, adipose tissue volume, and cell size by activating the PPAR pathway; ultimately relieve obesity (Long et al. 2020). Few studies proved the anti-obesity potentiality of LAB and their mechanisms of action are less reported delivering insufficient efficacy.

In this study, we isolated lactic acid bacteria from a rice-based fermented beverage, *haria,* which is very popular in different parts of India. Still, this beverage has not received significant scientific attention. In its preparation, the low-graded rice is boiled up to charring, followed by cooling and the addition of a starter culture, *bakhar*. The mixture is then put into an earthen pot and fermented for 3–5 days (Ghosh et al. 2014). After that, the glutinous material is diluted with drinking water and consumed by the people. According to the traditional beliefs of tribal people, this beverage could prevent dysentery, diarrhea, acidity, amebiosis, vomiting and many gastrointestinal ailments. Recent studies showed bio-augmentation of LAB in *haria* (Ghosh et al. 2015a, 2021). Hence, we used this beverage to isolate the LAB. Though numerous LABs participated in the *haria* fermentation, the health-beneficial effects of individual bacteria have not been studied. Moreover, the health benefits of wild-type food-borne LAB are in huge demand as they are mostly safe and can colonize the human gastrointestinal tract. Considering that, this study aimed to evaluate the probiotic properties of *Lactiplantibacillus plantarum* E2_MCCKT isolated from *haria* and its anti-obesity potentiality on the high-fat diet-induced obese mice.

Materials and methods

Chemicals

All chemicals were obtained from Hi-Media Laboratories, India except Folin-Ciocalteu Reagent, TEMED, acrylamide, bis-acrylamide, bovine serum albumin, and ammonium persulfate which were procured from Sisco Research Laboratories, India.

Sample collection

Twenty-three *haria* (a rice-based fermented beverage) samples were collected aseptically from the different villages of Paschim Medinipore district, West Bengal, India. The main ingredient of this beverage is low-grade boiled rice which is mixed with a starter culture (*bakhar*) and allows fermenting for 3–5 days in an earthen pot (Ghosh et al. 2014). It is a controlled fermentation process where LAB play a major role. The collected fermented samples were put into an ice box, taken to the laboratory, and maintained at − 20 °C for further work.

Isolation and identification of LAB from *haria*

LAB was isolated as previously described by Ghosh et al. (2015b). One gram of each sample was diluted with 9 ml of PBS (0.2 M, pH-6.8) solution. The appropriate dilution (0.1 ml) was spread on Rogosa SL agar media (containing 0.132% acetic acid) and incubated anaerobically at 37 °C for 24 h. Thereafter, randomly selected colonies were purified on Rogosa SL agar plate and stored at 4 °C for further use. Twelve numbers of LAB colonies were obtained from 23 numbers of *haria* samples. Out of twelve isolates, E2_MCCKT was selected based on cumulative probiotic score (pH tolerance, bile salt tolerance, autoaggregation ability, and hydrophobicity activity). Then, the overnight grown culture of LAB isolate was identified by 16S rRNA sequencing. At first, genomic DNA was extracted by using Hi-Pura Genomic DNA purification Kit (Hi-media). The extracted DNA was then quantified by Nanodrop (Eppendorf Biospectrometer, Part No.-6135000904) at 260/280 nm. The existing DNA was further confirmed by a 1.2% agarose gel electrophoresis system. The LAB-specific primer set Lact-F (5′-AGCAGTAGGGAATCTTCCA-3′) and Lact-R (5′-ATTYCACCGCTACACATG-3′) were applied for amplification (Ritchie et al. 2008, 2010; Ghosh et al. 2015b). The polymerase chain reaction (PCR) was performed by the following steps—5 min initial denaturation at 94 °C, following 35 cycles, 30 s denaturation at 94 °C, 45 s annealing at 60 °C, 1 min extension at 72 °C, and 7 min final extension at 72 °C. A 340 bp single distinct amplified band was visualized by using agarose gel electrophoresis. The purified PCR amplicon was used for Sanger Sequencing and Bi-directional DNA sequencing with mentioned LAB-specific primers by ABI-3500Dx genetic analyzer using a BDT v3.1 Cycle sequencing kit. The forward and reverse sequence data of the partial 16S rDNA gene was used to make a single contig by using BioEdit (7.2). After BLASTN, the first ten sequences were taken and aligned by using the multiple sequence alignments

in CLUSTAL W in MEGA 11, followed by the construction of a phylogenetic tree by using the Neighbour-Joining Method in MEGA 11. According to the phylogenetic tree, the isolated bacterium was *Lactiplantibacillus plantarum* and the strain number was E2_MCCKT.

Survivability in an acidic environment

Probiotic bacteria must endure the journey through the stomach, where gastric acid secretion is a key defence mechanism against the ingested microbes (Pithva et al. 2014). A *Lactobacillus* strain must withstand the hostile gastrointestinal environment to attain potential probiotic status. The intragastric pH of the harsh gastrointestinal environment ranges from 1.6 to 4.5 in the daytime, increasing to 2.4–6.7 after a meal (Zhang et al. 2022). Therefore, the present study mimicked the preparation of an artificial stress condition (pH 2.0, 3.0, and 6.8) like the human gastrointestinal system. The survivability of LAB isolate in different pH's was performed as described by Charteris et al. (1998). The different pH solutions, such as pH 2.0 (adjusted with HCL), pH 3.0 (adjusted with HCL), and the pH 6.8 (no HCL added), were prepared by using 0.2 M PBS buffer. Thereafter, each solution was sterilized by autoclaving at 15 psi (temperature 121 °C) for 15 min. The LAB suspension (10^9 CFU/ml) were dissolved with 1 ml of each solution and incubated at 37 °C at individual pH levels for 0, 30, 60, and 120 min time intervals, respectively. Appropriate dilution of these treated samples was plated on Rogosa SL agar. The plates were incubated anaerobically (5% CO_2) at 37 °C for 48 h to enumerate the colony-forming units (CFU).

Survivability in bile salt condition

The survivability of the isolate in bile salt conditions was executed by the method described by Vinderola and Reinheimer (2003). The concentration of intestinal bile acid is about 0.1–2% (Amara et al. 2019). Artificial bile salt solution was prepared by mixing appropriate amount of bile salt mixture (Hi-media, India) with 0.2 M PBS (pH 6.8) to reach the concentration of 0.3 and 2% bile. The control bile salt (0%) was prepared without bile salt. LAB suspensions (10^9 CFU/ml) were dissolved in 1 ml of each prepared bile salt solution and incubated for 0, 30, 60, and 120 min time intervals, respectively, and appropriate dilution of these treated samples were plated on Rogosa SL agar. The CFU was enumerated after 48 h of anaerobic incubation (5% CO_2) at 37 °C.

Auto-aggregation assay

The auto-aggregation ability of the isolated LAB was tested following the method of Zommiti et al. (2017). The LAB suspensions (10^9 CFU/ml) were washed twice using 0.2 M PBS (pH-6.8) and resuspended in the same solution. Thereafter, the sample was left standing and incubated at 37 °C in a CO_2 (5%) incubator. The upper aqueous layer was checked spectrophotometrically at OD_{600nm} for one-hour, two-hour, three-hour, four-hour, and five-hour time intervals. The auto-aggregation percentage was calculated by the following equation:

$$\text{Autoaggregation } \% = \left[1 - \left(A_t/A_0\right) \times 100\right]$$

where A_t denotes the final absorbance, and A_0 denotes the initial absorbance.

From this assay, the isolated strain could be classified into three groups: < 20%, low auto-aggregation, ≥ 20–70%, moderate auto-aggregation, and > 70%, high auto-aggregation.

Hydrophobicity of strain

The adhesion ability of the isolated LAB was determined following the method of Rokana et al. (2018). The LAB suspensions (10^9 CFU/ml) were harvested by 10 min centrifugation at 8000 rpm from the overnight grown culture. After washing twice with 0.2 M PBS (pH-6.8), the LAB suspensions (10^9 CFU/ml) were redissolved in PBS and in an equal volume of n-hexadecane (1:1). The sample was, then, left to stand for 30 min in an anaerobic condition (5% CO_2) at 37 °C for phase separation. The spectrophotometric measurement of the upper aqueous layer was made at OD_{600nm} after one-hour, two-hour, three-hour, four-hour, and five-hour, respectively. A decrease in absorbance was used to determine the percentage of hydrophobicity, which was computed using the following equation:

$$\%\text{ of cell surface hydrophobicity} = \left(1 - A_1/A_0\right) \times 100$$

where A_1 denotes the final absorbance, and A_0 denotes the initial absorbance.

The strain could be classified into three groups from this assay: < 20%, low hydrophobicity, ≥ 20–70%, moderate hydrophobicity, and > 70%, high hydrophobicity.

Antibiotic sensitivity test

According to CLSI (Clinical and Laboratory Standard Institute) guidelines, different groups of antibiotics with different doses [ampicillin (AMP, 10 µg), chloramphenicol (CHL, 30 µg), polymyxin B (PB, 300 units), tetracycline (TE, 10 µg), and streptomycin (S, 25 µg)] were used to determine the sensitivity or resistant pattern. The antibiotic sensitivity of the isolated LAB was determined by using the Kirby-Bauer method (Sharma et al. 2017). Briefly, a freshly overnight grown bacterial culture (0.1 ml) was spread on previously prepared Rogosa SL agar medium and was allowed

to keep for 5 min. The antibiotic discs were put aseptically on the plates at a particular distance using sterile forceps and incubated anaerobically (5% CO_2) at 37 °C for 24 h. After 24 h of incubation, the zone of inhibition (ZOI) was measured using the zone reader (Hi-Antibiotic Zone Scale, Hi-media, India) on the undersurface of the petri dish, and the results were interpreted as per Clinical and Laboratory Standard Institute (CLSI) guidelines. The CLSI (2015) recommended that if the ZOI is less than 14 mm, it could be considered as resistant (R); those having a ZOI between 15 and 19 mm might be considered intermediate (I), and those with a > 20 mm of ZOI considered susceptible (S).

Cumulative probiotic scores

According to FAO/WHO recommendations, the commercial probiotic products and standard strains of probiotic bacteria were evaluated by using the cumulative probiotic score. The specific parameters were chosen based on the in vitro probiotic characterization (Sharma and Sharma 2017). Therefore, the cumulative probiotic score method was used to select a potent probiotic LAB. Several reports have also used the probiotic score method to select suitable probiotic bacteria (Tambekar and Bhutada 2010; Gautam and Sharma 2015). The scores were determined on the basis of growth under acidic condition (pH 2.0), growth under bile salt condition (2%), autoaggregation ability, and hydrophobicity activity. Scoring points (0, 1, and 2) were applied for the assessment of test parameters where "0" denotes the lowest value and "2" denotes the highest value.

Animal selection for experiment

The Institutional Animal Ethical Committee of Midnapore City College approved the animal experiments (MCC/IAEC-KG/10/23-004). The present study selected only male albino mice, as the male mice exhibit metabolic disease better than the female mice (Mauvais-Jarvis et al. 2017). Moreover, a recent study suggested that male mice acquired significantly more weight than the female mice in a high-fat diet-induced obesity model (Stapleton et al. 2024). The initial body weight of all the male albino mice was 13.59 ± 0.56 g, and they were habituated for 10 days in a controlled environment (32 ± 2 °C and 50% moisture) with a 12 h light/dark cycle. The mice were given normal food along with free access to water *ad libitum*. Based on the diet and therapy, mice were randomly picked up and differentiated into three different groups (n = 5) (Lee et al. 2018; Choi et al. 2020; Kim et al. 2022). The first group was the Normal Diet (ND), which received a food made of carbohydrates, protein, and fat (64.2:22.3:13.5) ratio. The second group was the High Fat Diet (HFD), which received lab-prepared food containing carbohydrates, protein, and fat (38.9:22.2:38.9) ratio, and the last/final group was the High Fat Treatment group (HFT), which was given high-fat diet for 8 weeks along with probiotic *Lp. plantarum* E2_MCCKT (10^9 CFU/ml) for last 4 weeks. The probiotic bacterium was treated to the HFT group after obesity development at 4 weeks. Some other studies have also revealed that 10^9 CFU of probiotic bacteria per day has an effective dose for obesity management (Kang et al. 2013; Lin et al. 2020). At the end of the experiment (after 8-week study periods), the mice were anesthetized with cervical dislocation and carefully dissected. Blood samples were collected by orbital plexus puncture, and liver samples were collected for gene expression study, and stored at − 20 °C until the analysis.

Determination of body weight analysis

The body weight gain was recorded at the end of every week for eight weeks, as per protocol, to investigate the effects of given probiotics on obese mice. A skin-fold calliper was used to measure the body length of the experimental mice (Central; Model no. 6420). Body mass index (BMI) was calculated using a conventional method and expressed as g/cm^2 (Ray et al. 2018).

Histopathology of liver

A single-blind study was used to assess the liver histopathology using hematoxylin and eosin (H&E) staining for liver fat analysis. Briefly, a slice of the liver was fixed in 10% (v/v) formalin after mice scarification. After that, it was thoroughly processed and embedded in paraffin. The thickness of the liver sections was 4–5 µm when stained with hematoxylin and eosin. The slide was inspected by an expert under a photo-microscope (40X, magnifications; Olympus, Tokyo 163-0914, Japan) to observe the fat deposition and the lipid droplets were quantified by using Image J software (National Institute of Health and the Laboratory for Optical and Computational Instrumentation) (Karimi et al. 2015).

Biochemical study of serum sample

Blood serum was collected by centrifugation process (2500 rpm for 5 min) and was used for the biochemical study. Total serum cholesterol, serum triglycerides, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), serum glutamic-oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT) levels were quantified by using a kit of Enzopak (India) as per instruction given there.

TNF-α estimation using ELISA

TNF-α is a pro-inflammatory cytokine, secreted from the perivisceral fat, which is responsible for developing inflammation during obesity or its related disorders. The serum TNF-α level was determined by using an enzyme-linked immunosorbent assay kit as per the manufacturer's instruction (Wuhan Fine Biotech Co. Ltd, China).

Gene expression study by using semiquantitative PCR

mRNA was extracted from liver tissues (50–100 mg) by using a Hi-Pura Total RNase Miniprep Purification kit (Hi-media, India). Total RNA (1 µg) was converted to cDNA by a Hi-cDNA synthesis kit (Hi-media, India). The standard PCR amplification was performed by using 10% cDNA and gene-specific primers (Supplementary Table 2). The cDNA served as a template in a 30 µl reaction mixture and was processed using an initial step at 94 °C for 5 min, followed by 30–32 amplification cycles (94 °C for 30 s; 55–60 °C for 45 s; 72 °C for 1 min) and a final elongation for 10 min at 72 °C. After completion of semi qPCR analysis, a five-microliter reaction mixture along with one microliter of 6X gel loading buffer was mixed and electrophoresed in agarose gel [1.2% (w/v) and 2 µl EtBr]. The specific DNA band was analyzed in Gel Imaging Densitometer (GS-700) using Molecular Analyst Software (version 1.5; Bio-Rad).

Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) for mRNA expression

Furthermore, the target gene expression was analysed by qPCR (G8830A; AriaMx Real-Time PCR; Agilent Technologies, India) that utilized a previously synthesized cDNA (1 µl) as a template, along with 2X SYBR-Green Mixture (10 µl; Agilent Technologies, India) containing gene-specific primers 1 µl (Supplementary Table 2) and the final volume was adjusted to 20 µl by using molecular biology grade water. The qPCR procedure involved 35 cycles with the following steps: 95 °C denaturation for 30 s, annealing at 55–60 °C for 1 min, and extension at 72 °C for 30 s. The expression of the target gene was quantified relative to that of the housekeeping genes GAPDH. To end, the reaction tubes was kept at 4 °C. The fold change of target gene expressions was calculated by using $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen's 2001).

Analysis of inflammatory and anti-inflammatory cytokines expression

Adipose tissue was collected from the abdomen after separating from the skin. Thereafter, it was homogenized and lysed with ice on RIPA buffer (50 mM Tris–HCl, 150 mM NaCl, 5 mM EDTA, and Titron-X100 (1%) and cocktail protease inhibitors). The protein quantification was done according to the Lowry method. Briefly, 1 ml NaOH (1 N) was taken in a test tube and heated for few seconds. Thereafter, 1 ml of sample was mixed in the same test tube and incubated for 3–4 min. Moreover, 5 ml of alkaline $CuSO_4$, and $C_4H_4KNaO_6{\cdot}4H_2O$ were added followed by incubation at 37 °C for 10 min. Finally, Folin-Ciocalteu reagent (0.5 ml) was mixed to produce blue-green colour after 30 min incubation and that was detected by OD_{750nm}. Then, 50 µg protein samples were placed in a loading buffer and were boiled for 5 min, followed by SDS-poly-acrylamide gel electrophoresis, and blotting via nitrocellulose membrane (BioRad, China). The membrane was then treated with primary antibody for an overnight period at 4 °C after blocking with 5% BSA for an hour at room temperature. The primary antibodies such as β-actin (Abgenex, India), interleukin (IL)-1Ra (Santa Cruz Biotechnology, USA), peroxisome proliferator-activated receptor (PPAR)-α (Santa Cruz Biotechnology, USA), and interleukin (IL)-10 (Santa Cruz Biotechnology, USA) were used in this study. After that, the membrane was treated for an hour with a secondary anti-mouse antibody conjugated with HRP (Abgenex, India). The exact protein bands were detected by using DAB.

Statistics

All triplicate experimental data were expressed as the mean ± standard error (SE). The significant differences in all experimental data were analyzed using Tukey's one-way ANOVA. The statistical significance was defined by $p < 0.05$. Data analysis was done using the Sigma Plot 12.5 program (USA).

Results

Isolation and identification of LAB

Out of twelve isolates, E2_MCCKT was selected based on cumulative probiotic score (Supplementary Table 1). The 16S rRNA sequencing was then used to identify the E2_MCCKT strain. A 340 bp fragment of the partial 16S rRNA sequence was analyzed for the interference of the evolutionary history using the Neighbour-Joining method. The 16S rRNA sequence of E2_MCCKT showed high similarity with *Lactiplantibacillus plantarum* T17 (NCBI Accession Number: MG739432) and it also clustered with different strains of *Lactobacillus plantarum* in phylogenetic analysis. Hence, the E2_MCCKT (NCBI Accession Number: OQ073780) might be a strain of *Lp. plantarum* (Fig. 1).

Probiotic properties of the isolated strain, in-vitro

Survivability in acid medium

Probiotic microorganisms need to survive in the gastrointestinal environment. The isolated strain was able to survive at pH 2 (98.4%), pH 3 (99.3%), and pH 6.8 (100%) after 120 min of the incubation period (Fig. 2A). The isolated strain showed a high survivability rate in pH 3 than pH 2. The survivability rates (at pH 2 and 3) were statistically non-significant compared to control pH 6.8.

Bile salt tolerance test

A probiotic strain should survive and grow in the intestinal tract. It must be able to withstand intestinal bile, a major antimicrobial component. In the small intestine, the bile salt concentration varied from around 0.3–2%, depending on the individual food ingestion. The isolated strain exhibited strong bile salt resistance and was able to withstand 0.3% and 2% bile salt for 120 min**.** The survivability rate of the selected strain at 0.3% bile was 98.8%, and similarly, the survivability rate was 97.69% at 2% bile salt after 120 min (Fig. 2B).

Autoaggregation and hydrophobicity

These are the two dominant criteria for accessing the adhesion ability of probiotic bacteria on intestinal epithelium walls (Rahman et al. 2008). The isolated *Lp. plantarum* E2_MCCKT exhibited 52.52% autoaggregation ability and 38.08% surface hydrophobicity. This result indicated that

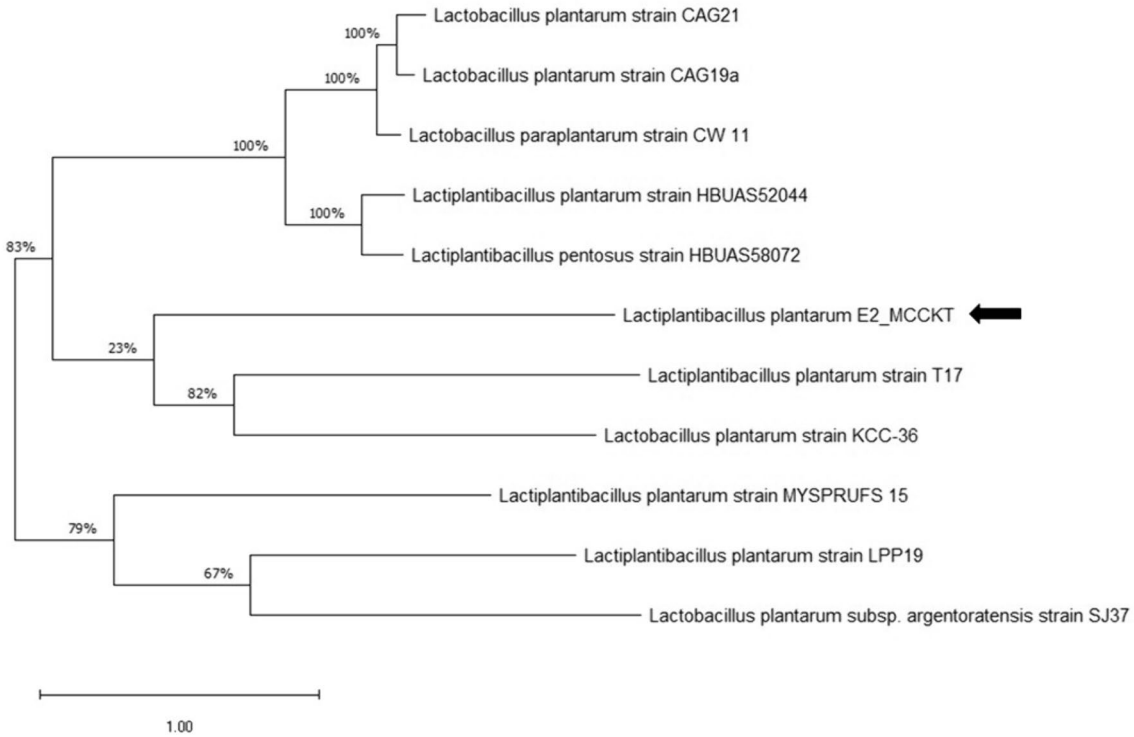

Fig. 1 Phylogenetic tree of the dominant lactic acid bacterial isolate E2_MCCKT strain. According to the tree, it belongs to *Lp. Plantarum*

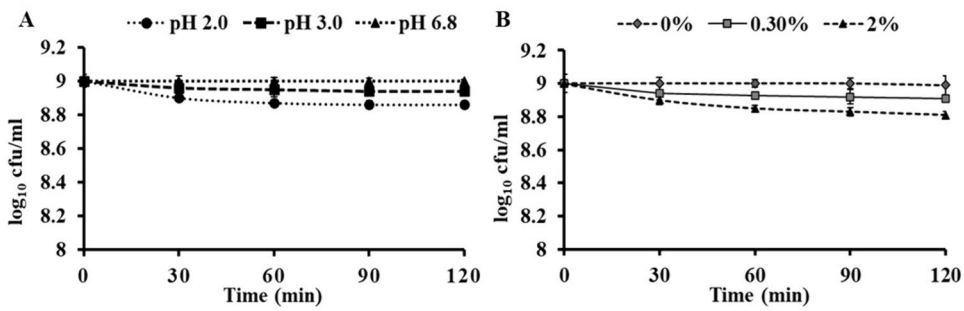

Fig. 2 Survivability of *Lp. plantarum* E2_MCCKT in acidic condition **A**, and bile salt **B** in different time duration. Values are expressed as Mean ± SE

the isolated bacterium might potentially adhere to human intestinal epithelial cells.

Antibiotic sensitivity test

Antibiotic sensitivity assay of the isolated *Lp. plantarum* E2_MCCKT was carried out by using different antibiotics. As per CLSI guidelines, the bacterium *Lp. plantarum* E2_MCCKT was intermediately sensitive to ampicillin (10 µg, ZOI 15.5 mm) (β-lactams), chloramphenicol (30 units, ZOI 14.2 mm) (macrolide), and resistant to polymyxin-B (300 units, ZOI 12.09 mm) (colistin), tetracycline (10 µg, ZOI 12.3 mm) (tetracyclines), and streptomycin (25 µg) (aminoglycosides).

Anti-obesity activity of *Lp. plantarum* E2_MCCKT

Body weight and serum lipid profile analysis of *Lp. plantarum* E2_MCCKT on HFD induced obese BALB/c mice

The body weights of three different mice groups were nearly equivalent initially [Normal diet (ND), 15.44 ± 0.44 g; high-fat diet (HFD), 15.61 ± 0.64 g; high-fat treatment (HFT), 15.14 ± 0.81 g]. The final average body weights (after eight weeks of study period) of ND, HFD, and HFT were 28.40 ± 0.92, 35.80 ± 0.77, and 30.60 ± 0.99 g, respectively (Fig. 3). The BMI was significantly increased (1.66-fold) in the high-fat diet group (0.48 ± 0.01 g/cm^2) comparing to the normal diet group (0.33 ± 0.02 g/cm^2) after eight weeks (Fig. 3). However, BMI was reduced (1.57-fold) in the probiotic treatment group HFT (0.41 ± 0.01 g/cm^2) than HFD at eight weeks (or after 4 weeks of *Lp. plantarum* E2_MCCKT treatment, Fig. 3). The tested lipid profile was higher (50–70%) in HFD group than ND group (excluding HDL cholesterol), whereas, the lipid profile was considerably reduced after E2_MCCKT treatment in HFT group than HFD group. Total serum cholesterol (up to 38.69%), serum triglyceride (up to 40.00%), and LDL cholesterol (38.22%) were found to be lowered in the HFT compared to HFD. The marked reduction in serum cholesterol on the HFT was mainly confined to the VLDL fraction (46.45% reduced in the HFT group) (Table 1). However, HDL cholesterol levels were significantly increased (60.58%) after *Lp. plantarum* E2_MCCKT supplement (Table 1).

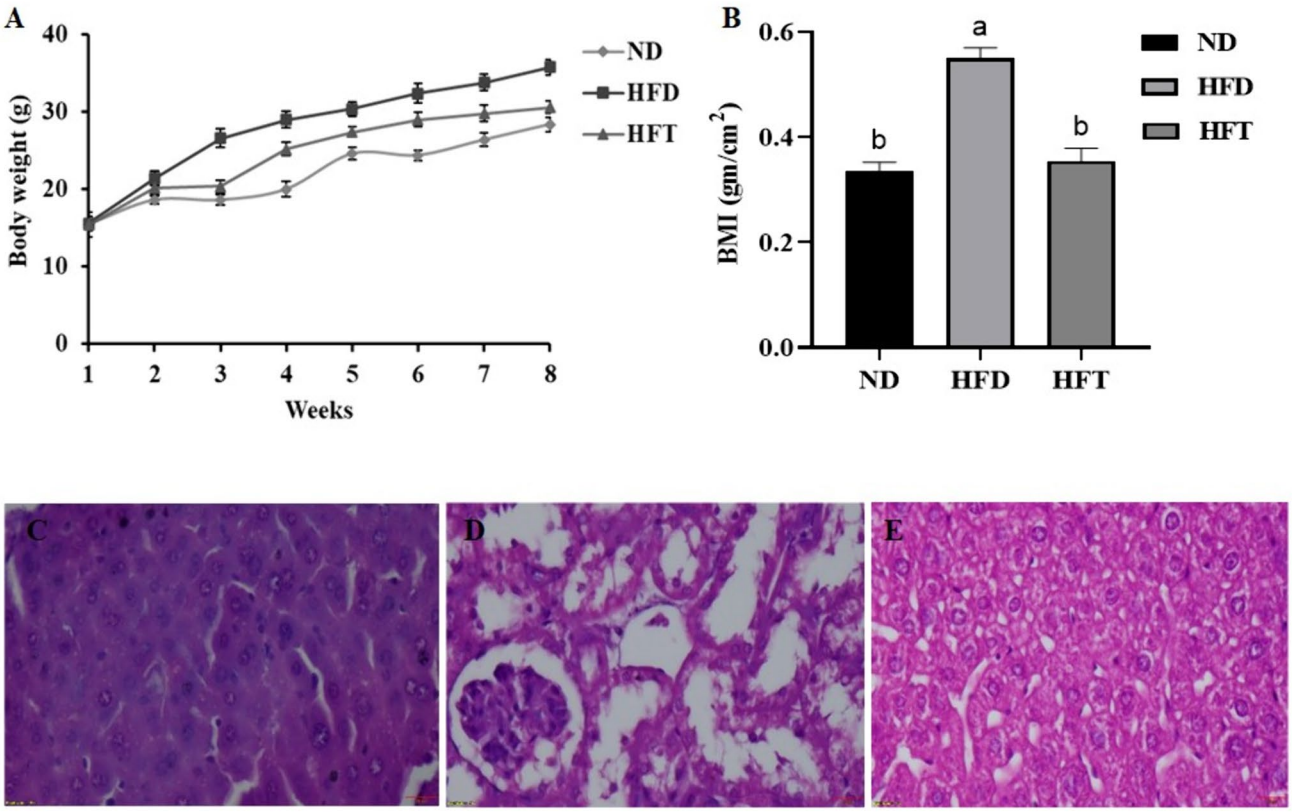

Fig. 3 Assessment of body weight **A**, and BMI **B** in different experimental groups (n = 5). Values are expressed as Mean ± SE. Data with different superscript letters are significantly different at $p < 0.05$ levels. Liver histoarchitecture of different groups mice fed a normal diet (ND, **C**), mice fed a high-fat diet (HFD, **D**), mice fed a high-fat diet with *Lp. plantarum* E2_MCCKT supplement (HFT, **E**). The observation was made under 40× magnification

Analysis of liver function and liver histology

The SGOT and SGPT levels of serum are reliable measures to evaluate the efficient spectacle of liver function and were markedly increased in the HFD group. The SGOT levels were 32.14, 53.49, and 33.70 U/l in ND, HFD, and HFT, respectively (Table 1). Similarly, 36.93, 59.52, and 26.57 U/l of SGPT levels were observed in ND, HFD, and HFT, respectively (Table 1). The histological study revealed the free fat accumulation in the hepatocyte cells of HFD mice (Fig. 3), whereas a significant reduction of fat deposition was observed in the HFT group (Fig. 3). Moreover, the percentage of liver droplets was observed higher in HFD group (48.80%) in compare to ND (4.01%) while significantly reduction was observed in HFT group (31.12%) after the *Lp. plantarum* E2_MCCKT supplement.

Gene expression study of lipid metabolism

The genes associated with adipogenesis and lipogenesis/lipolysis were inspected. First, semiquantitative PCR was done by using densitometer analysis of the lipogenetic/lipolytic marker genes (Supplementary Fig. 1). Further, mRNA expression of that marker genes was studied by using qRT-PCR as confirmatory analysis and found the similar observation as qPCR result (Fig. 4). The key activation factors of β-oxidation pathways such as PPAR-α, acyl-CoA oxidase (ACO), and carnitine palmitoyl-transferase 1 (CPT1) were significantly higher in HFT groups (1.68, 1.88, and 1.57-fold, respectively) in comparison to HFD group (Fig. 4). On the other hand, mRNA expression of adipocytes disintegrating and lipogenic transcriptional factors, such as sterol regulatory element-binding protein—1c (SREBP-1c) and their downstream products such as acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) were found lower in HFT group (2.23, 2.54, and 1.61-fold, respectively) than HFD group (Fig. 4). The expression of ANGPTL4 (angiopoietin-like protein 4) was significantly improved (2.7-fold) by *Lp. plantarum* E2_MCCKT treatment (HFT) group (Fig. 4). Moreover, the leptin expression was significantly increased (4.01-fold) in the HFD group (Fig. 4), and the expression of adiponectin was notably reduced (1.89-fold) in the HFD group (Fig. 4).

Table 1 Analysis of serum lipid profile and liver function test-related biomarkers in different experimental groups

Parameters	ND	HFD	HFT
Total cholesterol (mg/dl)	$85.37^{b} \pm 1.32$	$232.46^{a} \pm 1.91$	$89.49^{b} \pm 1.71$
Triglyceride (mg/dl)	$91.88^{c} \pm 2.52$	$278^{a} \pm 2.11$	$111.63^{b} \pm 1.70$
VLDL (mg/dl)	$21.52^{b} \pm 1.86$	$41.35^{a} \pm 1.30$	$19.21^{b} \pm 1.66$
LDL (mg/dl)	$58.90^{b} \pm 1.55$	$141.94^{a} \pm 1.75$	$54.21^{b} \pm 2.47$
HDL (mg/dl)	$62.72^{a} \pm 2.73$	$39.25^{b} \pm 0.68$	$64.65^{a} \pm 0.62$
SGOT (U/l)	$32.14^{c} \pm 0.69$	$53.49^{a} \pm 0.96$	$33.70^{b} \pm 0.94$
SGPT (U/l)	$36.93^{b} \pm 0.55$	$59.52^{a} \pm 1.09$	$26.57^{c} \pm 0.76$

Each value represents the mean (n = 5). The values without a common superscript letter are significantly different at ($p < 0.05$) levels from each other in the respective row

ND normal diet, *HFD* high fat diet, *HFT* high fat diet with *Lp. plantarum* E2_MCCKT, *VLDL* very low density of lipoprotein, *LDL* low density of lipoprotein, *HDL* high density of lipoprotein, *SGOT* serum glutamic-oxaloacetic transaminase, *SGPT* serum glutamic-pyruvic transaminase

Inflammatory and anti-inflammatory cytokines expression

The relative protein expression of PPAR-α, IL10, leptin, and IL-1Ra were assessed. The expressions of PPAR-α (Fig. 5) and IL10 (Fig. 5) were increased by 1.11-folds and 1.19-folds, respectively, in the HFT group compared to the HFD group, whereas the expression of leptin receptor (Fig. 5) and IL-1Ra (Fig. 5) was significantly reduced (1.09-fold and 1.19-fold) in HFT group than HFD group. TNF-α is produced by macrophage activation in the acute phase of inflammation. In an observation, the TNF-α level was significantly higher (4.18-fold) in the HFD group (173.813 pg/ml) than HFT (76.89 pg/ml) and ND (41.58 pg/ml) group ($p < 0.05$, Fig. 5).

Discussion

In this study, 12 LAB strains were isolated from *haria*, a traditional rice-based fermented beverage. *Lp. plantarum* E2_MCCKT was selected on the basis of their probiotic score (Supplementary Table 1). The isolated strain showed high survivability at pH 3 (99.3%) and pH 2 (98.4%), which were in good agreement with the findings of Guo et al. (2010) and Zhang et al. (2022). There are some workable mechanisms behind the acid tolerance response or survivability in the acid medium of the probiotics such as alkaline substance production by H^{+}-ATPase pathway activation, proton pumps, and cell membrane changes through macromolecule protection (Wu et al. 2013). Huang et al. (2016) reported that *L. plantarum* ZDY2013 could regulate pH homeostasis by repulsion of the protons from the intracellular environment. In addition, the arginine deiminase system helps glutamic acid decarboxylation with alkaline substance production, which is the main system used in pH homeostasis. Moreover, genes and proteins act as key components for cellular protection that help the probiotic in pH adaptation (Huang et al. 2016). In connection to this, the current result indicated that the bacterium might withstand gastric stresses and could survive for longer periods in high acid-containing food such as yoghurt, pickles, vinegar etc., without changing

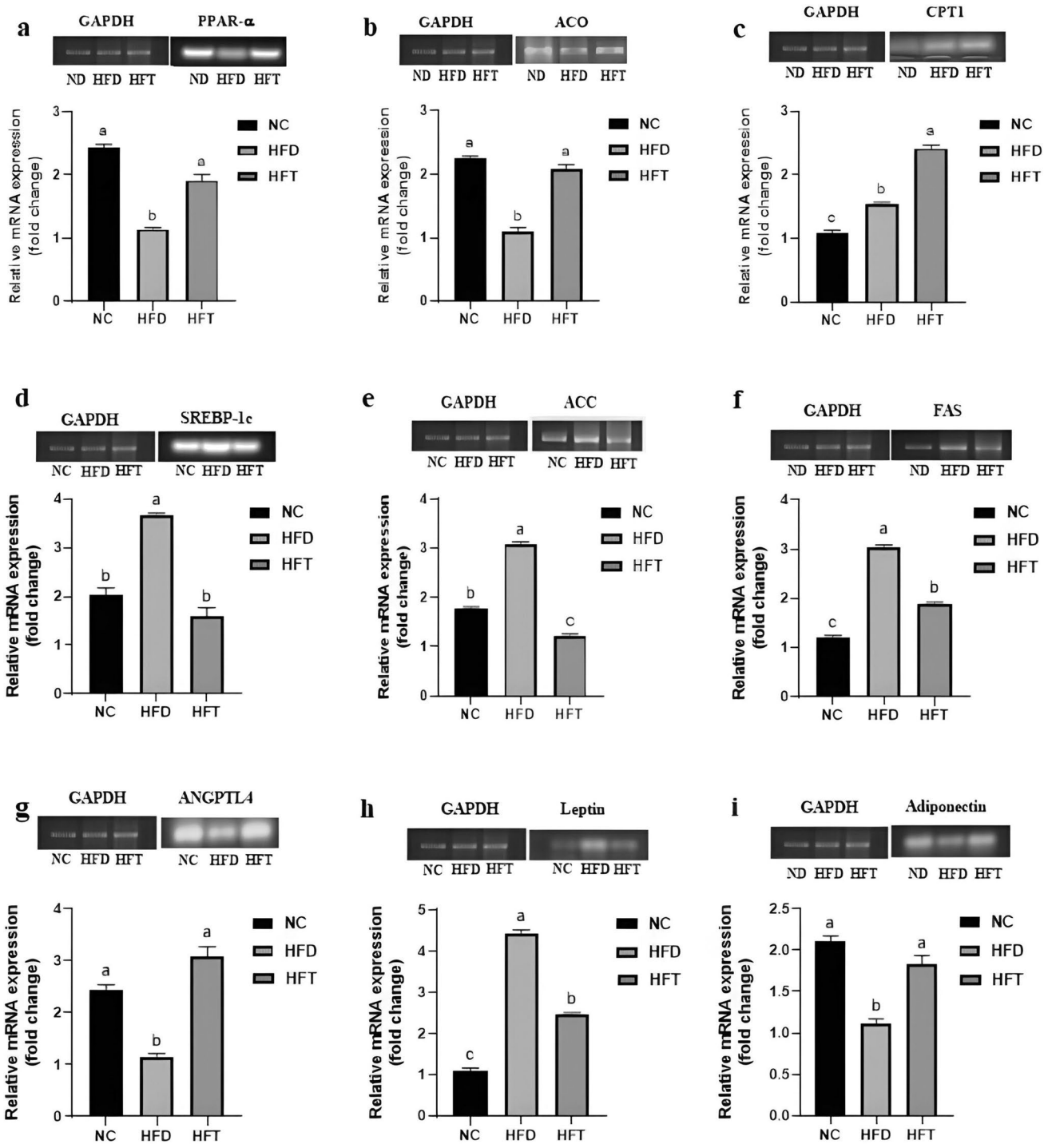

Fig. 4 Relative mRNA expression (fold change) of obesity and fatty acid metabolism (synthesis and oxidation)-related genes in mice liver samples was measured by qRT-PCR: PPARα **a**; ACO **b**; CPT1 **c**; SREBP-1c **d**; ACC **e**; FAS **f**; ANGPTL4 **g**; Leptin **h**; and Adiponectin **i**. Values are expressed as Mean ± SE (n = 5). Data with different superscript letters are significantly different at $p < 0.05$ levels. *ND* normal diet, *HFD* high-fat diet, *HFT* high-fat treatment

their number (Wang et al. 2010). The bile salt concentration of the intestine is 0.1–2% (Amara et al. 2019). The strain E2_MCCKT could tolerate 0.3% (98.8% survivability after 2 h incubation) and 2% bile salt (97.69% survivability after 2 h incubation) which is a strong antimicrobial substance present in the small and large intestine. In case of an ideal probiotic, the microorganisms should survive in bile conditions present in the small and large intestines and to

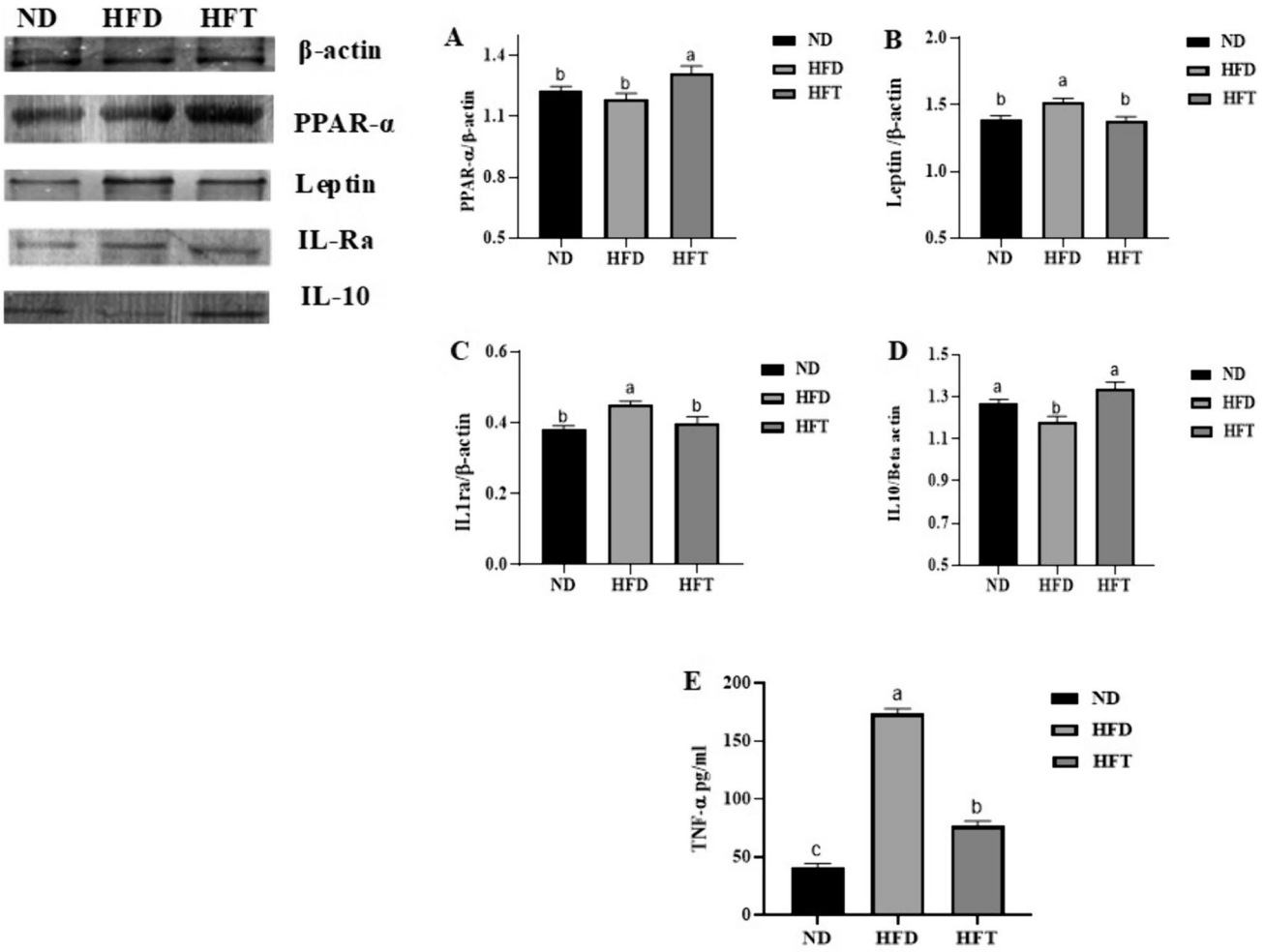

Fig. 5 Inflammatory and anti-inflammatory cytokine expression (PPAR-α **A**, Leptin **B**, IL-Ra **C**, IL-10 **D**) in adipose tissue compared to β-actin expression. Expression of TNF-α level in high-fat diet-induced obese mice (n = 5). The concentration of TNF-α was measured by ELISA. Values are expressed as Mean ± SE. Data with different superscript letters are significantly different at $p < 0.05$ levels

prove as bile resistant microorganisms. The conjugated bile acids are secreted into the duodenum. Ideally, conjugated and deconjugated bile has strong anti-microbial activity on Gram-positive and Gram-negative bacteria. A study of *Lp. plantarum* HOM3204 and *Lp. rhamnosus* GG showed strong survivability in bile salt (Zhang et al. 2022). Zhou et al. (2019) revealed that *Lp. plantarum* LP-115 triggered the bile salt hydrolase gene expression that improved the bile salt tolerance by changing the fatty acids composition of the plasma membrane. The isolated bacterium might have the ability to produce a bile salt hydrolase (BSH) enzyme, which could catalyze and convert conjugated bile salt to its conjugated form and release free amino acids (Begley et al. 2006). Probiotic strains' autoaggregation and cell surface hydrophobicity activity are frequently connected with their possible capacity to adhere to the intestinal epithelium and mucous membranes by homotypic interactions (Trunk et al. 2018). Autoaggregation and cell surface hydrophobicity help in bacterial fortification and colonization in the gut. On the other hand, bacterial adhesion in the human gut is a complex event depending on the cellular charges of both humans and bacteria, surface proteins, and extracellular polysaccharides (Huligere et al. 2023). The isolated strain showed 52.52% autoaggregation and 38.08% hydrophobicity, clearly indicating the isolate's ability in colonization and adhesion to the epithelial cells of the gastrointestinal tract, as suggested by Abushelaibi et al. (2017). Zhang et al. (2014) also found a similar pattern of autoaggregation and hydrophobicity of *L. plantarum* C51. *L. fermentum* KKL1 showed moderate hydrophobicity activity (Ghosh et al. 2015b). Therefore, autoaggregation and cell surface hydrophobicity might help alleviate the host infection (Jeong et al. 2021). According to the previous study, *Lactobacillus* spp. could adhere to the intestinal wall competitively against pathogens, which helps to minimize the inflammatory effects on the host (Dhanani and Bagchi 2013). The bacterium was found

to be intermediately sensitive to ampicillin (10 µg), chloramphenicol (30 units), and resistant to polymyxin-B (300 units), tetracycline (10 µg), and streptomycin (25 µg). This result was comparable to the report of Sharma et al. (2017), where *L. plantarum* (D7) was most resistant to intermediate pattern of the tested antibiotics. It can be assumed that the *Lp. plantarum* E2_MCCKT strain may carry the antibiotic resistance genes in its genome. Clearly, a detailed analysis is needed to find out the antibiotic resistance mechanism of the isolate. However, the bacterium may help to restore and maintain the normal gut microbiota during antibiotic therapy. Therefore, the isolated *Lp. plantarum* E2_MCCKT might exhibit in vitro probiotic activities.

Later *Lp. plantarum* E2_MCCKT was applied in mice to evaluate its anti-obesity potentialities. After the study period of eight weeks, the final average body weight and BMI were observed to be high in HFD, which may indicate that daily food consumption was significantly higher in HFD mice than in ND mice. The daily food intake capacity was regularly monitored for 8 weeks (data not shown), and this capacity was significantly reduced after *Lp. plantarum* E2_MCCKT treatment in the HFT group with all the mentioned parameters compared to HFD group. Therefore, the decrease in body weight indicates a reduction in energy expenditure, which may result from either a decrease in food intake or an increase in energy expenditure stimulation (Karimi et al. 2015). Moreover, the total serum cholesterol, triglyceride, LDL, and VLDL were found to be highest in the HFD group compared to other groups, whereas the treatment of *Lp. plantarum* E2_MCCKT could significantly lower the mentioned parameters, and those were comparable with the ND group (Table 1). Conversely, the HDL level in the HFT group was significantly increased after probiotic treatment compared to the HFD diet (Table 1). The increased levels of triglycerides, TC, LDL, and decreased HDL cholesterol are markedly associated with dyslipidemia which significantly raise the chances of coronary artery disease (CAD) (Dixon et al. 2012). A high-fat diet reduces insulin sensitivity in healthy, non-obese individuals, which may lead to the development of T2DM by altering the gut-brain axis and gut microbiota (Ağagündüz et al. 2023a, b, c). Probiotic *Lp. plantarum* E2_MCCKT and their metabolites might regulate the glucose homeostasis by maintaining the gut-brain axis. Several studies have also reported that high body weight and serum cholesterol level (excluding HDL) are associated with obesity and can be altered by probiotic treatment (Kang et al. 2013; Miyoshi et al. 2014; Ray et al. 2018; Das et al. 2022). Moreover, chronic obesity could increase the ectopic fat accumulation in the tissue, liver, and muscles, resulting in the increment of energy influx due to adipose tissue failure (Lomonaco et al. 2013). The probiotic *Lp. plantarum* E2_MCCKT might inhibit fat synthesis and accumulation. Moreover, probiotic bacteria could bind with the cholesterol to reduce the absorption in the intestine and may mobilize more cholesterol to re-synthesize bile salts (Liong et al. 2007). LAB produced SCFAs also inhibit fat absorption in the animal body (Daniele et al. 2014). Hence, the probiotic bacteria *Lp. plantarum* E2_MCCKT might help in lowering serum cholesterol levels, obesity and obesity-associated disorders.

Elevated SGOT and SGPT levels were observed in the HFD group (Table 1), which might indicate liver cell injury or damage due to fat accumulation and adipocyte proliferation (Jung and Choi 2014). Adipose tissue secretes fatty acids in obese condition entirely into the liver, resulting in fatty liver and dyslipidemia in the blood, which was ameliorated after *Lp. plantarum* ATG-K2 treatment (Lee et al. 2021). Serum SGOT and SGPT levels were found significantly lower (1.58 and 2.24-fold) in the HFT group after treatment of *Lp. plantarum* E2_MCCKT (Table 1). Hence, the bacterium could decrease obesity-induced liver damage.

To explore the mechanism of *Lp. plantarum* E2_MCCKT against obesity and related disorders, the genes related to adipogenesis and lipogenesis were checked. Peroxisome proliferator-activated receptors (PPARs) are an important regulator for energy expenditure which is activated by probiotics and accelerate fatty acid oxidation (Nakamura et al. 2016). Alternatively, PPARs (including α, β, γ) are also involved in the development of obesity by altering various physiological and pathological processes (Khajebishak et al. 2019). PPAR-α is a transcription factor of PPARs super family which is up-streamed during fatty acid oxidation, where CPT1 is up-streamed as a key target gene of PPAR-α. In the liver, CPT1 expression is regulated by PPAR-α and both PPAR-α/CPT1 pathway of lipid metabolisms which are directly related to obesity development (Gan et al. 2020). It enhances the fatty acid transportation into mitochondria and β-oxidation by specific CPT1 expression in muscles and liver. Alternatively, PPAR-α can alter the mitochondrial lipid metabolism (including β-oxidation and ω-oxidation) with the help of acetyl coenzyme A oxidase to inhibit obesity (Gan et al. 2020). The present study demonstrated that the gene expression of PPAR-α, ACO, and CPT1, the key regulating enzymes in β-oxidation were increased by 1.68, 1.88, and 1.57-fold, respectively, after *Lp. plantarum* E2_MCCKT treatment in the HFT group than the HFD group (Fig. 4). In connection to this, lower expression of SREBP-1c, ACC, and FAS genes by 2.23, 2.54, and 1.61-fold, respectively in the HFT group clearly indicated the suppression of adipogenesis and lipogenesis (Ray et al. 2018; Lee et al. 2021) (Fig. 4). According to Cai et al. (2020), *L. plantarum* FRT10 could alleviate the lipid metabolism by increasing hepatic PPAR-α and CPT1-α expression by downregulating SREBP-1 and tDGAT1 expression in obese mice. Moreover, *L. plantarum* KC28 significantly up-regulated PGC1-α and CPT1-α and down-regulated ACOX-1, PPAR-γ in a diet-induced obese

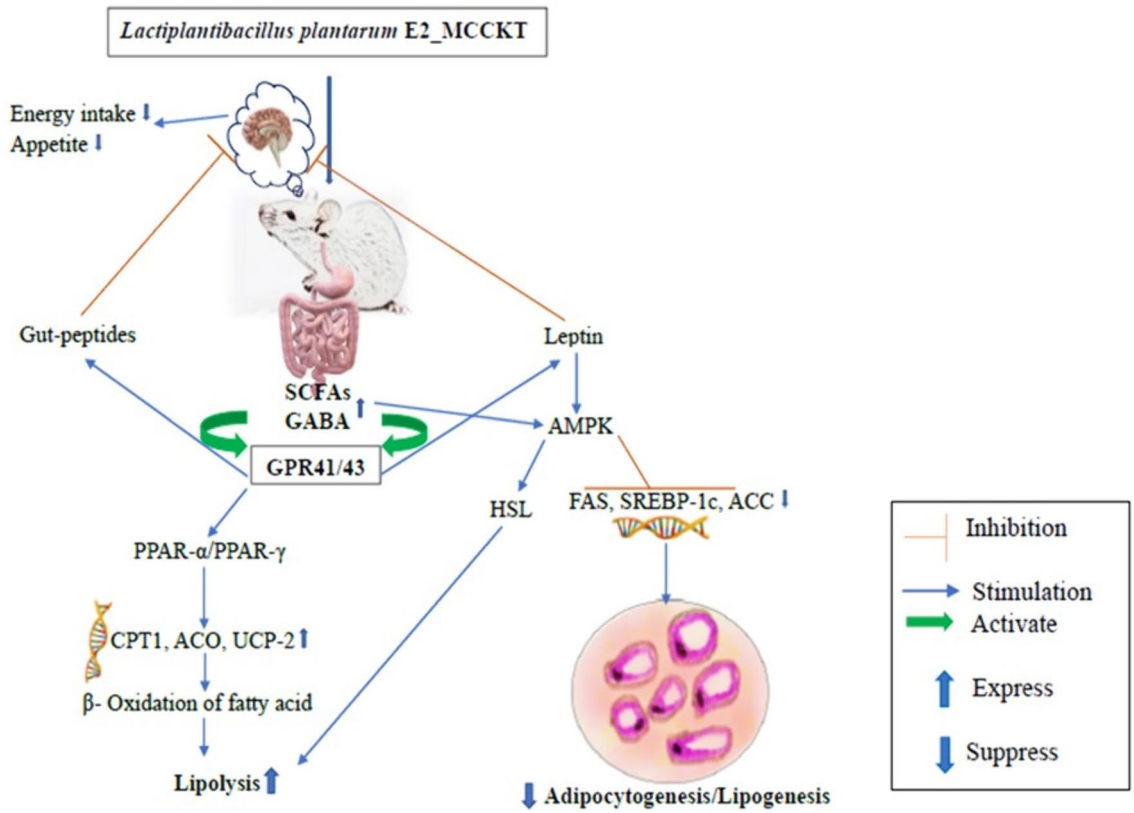

Fig. 6 The hypothetical mechanism and actions of *Lp. plantarum* E2_MCCKT in intestinal fermentation of SCFA/GABA production to regulate lipogenesis and lipolysis process

C57BL/6 mice model (Huang et al. 2021). *L. plantarum* CQPC01 could down-regulate C/EBP-α and PPAR-γ expression in obese mice (Gan et al. 2020). *L. plantarum* LMT1-48 cell extract inhibited adipocyte differentiation and lipid accumulation by down-regulating the adipogenic genes PPAR-γ, C/EBP-α, FAS and FABP4 in 3T3-L1 cells in an cellular assay (Choi et al. 2020). Furthermore, *L. plantarum* LMT1-48 strain was also downregulated the lipogenic genes PPAR-γ, HSL, SCD-1, and FAT/CD36 in the liver, resulting in the reduction of body weight and fat volume in HFD fed obese mice (Choi et al. 2020). Thus, PPARs may be an important target for evaluating the regulatory effect of *L. plantarum* on obese patients.

ANGPTL4 is a protein that regulates lipid metabolism via lipoprotein lipase (LPL) activity. The elevated expression of ANGPTL4 (2.7-fold) might lower the plasma cholesterol level and triglyceride in HFT mice (Fig. 4). These findings were comparable to the previous studies (Ray et al. 2018; Choi et al. 2019). The isolated bacterium *Lp. plantarum* E2_MCCKT might exhibit an anti-obesity effect by increasing the β-oxidation and suppressing adipocytogenesis and lipogenesis.

Adipocytes and enterocytes secrete the polypeptide hormone leptin in the small intestine (Brennan and Mantzoros 2006). It helps to regulate energy balance by inhibiting hunger due to the stimulation of the hypothalamus in the brain, thus helping to diminish fat storage in adipocytes and decrease body weight (Fradinho et al. 2014). The leptin gene expression (4.01-fold) and protein (1.09-fold) were significantly higher in the HFD group than the ND group, whereas after the treatment of *Lp. plantarum* E2_MCCKT could significantly downregulate the leptin expression (Figs. 4, 5). The downregulation of the leptin gene (1.81-fold) and protein (1.10-fold) might be associated with *Lp. plantarum* E2_MCCKT produced SCFAs, which could manage the host appetite by regulating the expression of gut hormones through the gut-brain axis (Lin et al. 2020). Hong et al. (2015) also found a similar type of leptin expression when they treated high fat-induced mice with probiotic *L. plantarum* DKL 121. Adiponectin is a type of adipokine produced by adipose tissue and regulates tissue inflammation (Whitehead et al. 2006). The increased levels of adiponectin in the HFT group in comparison to the HFD group denote the inhibition of lipid biosynthesis in obesity development (Fig. 4). Upregulation of adiponectin in the HFT group could protect against high-fat diet-induced lipotoxic effects and lipid accumulation (Combs et al. 2004).

During obesity, inflammatory and pro-inflammatory cytokine levels were significantly increased with high leptin expression (Bastard et al. 2006). Due to the obesity, the HFD group mice had higher expression of pro-inflammatory markers such as IL-1Ra (1.16-fold) and TNF-α (4.18-fold) comparing to ND, whereas downregulation of IL-10, an anti-inflammatory cytokine (1.07-fold) was observed in HFD group (Fig. 5). Application of *Lp. plantarum* E2_MCCKT significantly reduced the expression of IL-1Ra (1.19-fold) and TNF-α (2.26-fold) while increasing the expression of IL-10 (Fig. 5). The probiotic bacteria or their products might increase the IL-10 expression, and that would block the activated macrophages and reduce pro-inflammatory cytokines expressions such as IL-1Ra and TNF-α. Hence, the administration of *Lp. plantarum* E2_MCCKT could significantly alleviate obesity-associated inflammation.

Conclusion

The present study has demonstrated that the isolated probiotic *Lp. plantarum* E2_MCCKT had a significant effect on the suppression of adipogenesis and lipogenesis-related genes. It could enhance fatty acid β-oxidation by PPARs activation, increasing leptin expression, and suppress appetite (Fig. 6). The lipolysis pathway could be activated by suppressing the adipogenesis/lipogenesis process. Thus, it could prevent obesity in terms of reduction of body weight, BMI, and serum lipid profile (excluding HDL) in obese mice. Moreover, supplementation of probiotic *Lp. plantarum* E2_MCCKT could increase anti-inflammatory cytokines and decrease obesity-induced inflammation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11274-024-03983-3.

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Author contributions TKD, TP, AK, AD, PK, SG, and KG have collected samples and executed the experiments. KG, SP, and SC analyzed the data. KG and KM made a work plan and supervised the work. TKD, KM, and KG wrote the manuscript. All authors have reviewed, edited, and approved the final manuscript.

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Data availability The partial genome sequence of *Lactiplantibacillus plantarum* E2_MCCKT has been submitted to NCBI (Accession No. OQ073780).

Code availability Not applicable.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval The Institutional Animal Ethical Committee of Midnapore City College approved the animal experiments (MCC/IAEC-KG/10/23-004).

Consent to participate Not applicable.

Consent for publication All authors agree to publish.

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