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Effects of probiotic fermented milk on management of obesity studied in high-fat-diet induced obese rat model

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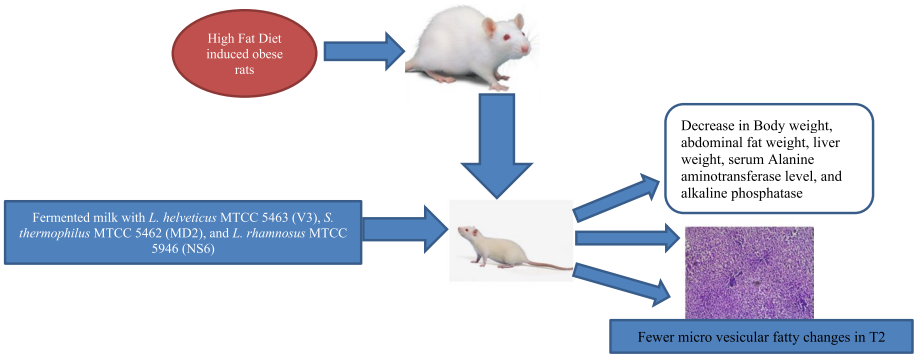
Abstract

The current study aimed to explore the hypothesis that probiotic bacteria are significantly involved in the control of obesity using Wistar rats as the test group by feeding high fat diets (HFD) induced obesity. A total of four groups of rats were considered viz., normal pellet diet fed (NC), HFD fed (DC), HFD fed rats treated with probiotic fermented milk with soy protein isolate (SPI) and whey protein concentrate (WPC) (T1), HFD fed rats treated with probiotic fermented milk without WPC and SPI (T2). Body weight, abdominal fat weight, liver weight, serum Alanine aminotransferase level, and alkaline phosphatase level significantly ($p < 0.05$) decreased after giving daily probiotic milk product supplementation with @ 2 ml per day for continuous 4 weeks. Whereas, C-reactive protein and Aspartate aminotransferase levels were not altered to a significant extent. The histology of the liver from the disease model group showed large lipid vacuoles deposited in the parenchyma cells. Product T2 confirmed fewer micro vesicular fatty changes and the appearance of T2 was better than T1. Overall, the in vivo study results indicated that the probiotic fermented milk exerted a better anti-obesity effect.

Keywords: Obesity, Probiotic, Fermented milk, High fat diet, WPC, SPI, Animal model

Graphical Abstract



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Introduction

The Latin term, obesitas, which means stout, plump, or fat, from where the word "obesity" originates. Obesity, according to medical term, is a condition in which the

body has stored excess fat to the point where it may have an adverse impact on health, diminishing life expectancy and/or increasing health issues. It is a medical disorder when extra body fat has built up to a level that could be harmful to the health (WHO 2015). Obesity and overweight are typically caused by either excessive calorie intake, insufficient exercise, or both. Additionally, a variety of genetic, environmental, and behavioural factors are significant contributors to obesity. It is the root cause of a number of fatal diseases, including diabetes, cardiovascular disease, non-alcoholic fatty liver disease (NAFLD), and a few types of cancer (Vucenik & Stains 2012). It is linked to a higher incidence of numerous illnesses in addition to mechanical impacts on the body (such as aggravating osteoarthritis and back pain from extra weight imposed on the skeleton) (Luppino et al. 2010). In many countries, it is a major risk factor for illness and mortality. Ballini et al. (2020) recently asserted that there were more than 2 billion overweight or obese people in the world (Narmaki et al. 2022).

Obesity has been linked to modifications in the structure and function of the gut microbiota (Mazloom et al. 2019). Inappropriate diet consumption has a negative impact on the host's health and is particularly associated to the emergence of obesity, changing both the function and composition of the gut microbiota. Probiotics have been discovered to alter the composition of the gut microbiota, promote gut integrity, and restore the microbial changes associated with obesity. (Mazloom et al. 2019). According to research so far, an obese person has a different composition of gut microbiota than a lean person, which has an impact on nutrient absorption and energy regulation. Therefore, modifying the gut microbiota by a diet rich in probiotics can be employed as an obesity treatment (Abenavoli et al. 2019).

According to Hill et al. (2014), "Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". The intestinal microbiota is also a part of the host's nutritional environment, and it has the ability to enhance the host's metabolic efficiency and, in some cases, act as a source of nutrients for the host. As a result, the intestinal microbiota benefits the host and develops a beneficial dependence with the host. (Alisi et al. 2014). Numerous studies have demonstrated that the development of obesity is significantly influenced by gastrointestinal dysbiosis, which is carried out by a high-fat and high-calorie diet (Caricilli & Saad, 2014; Ley et al. 2006). The number of the distal gut microbiota increases as a result of changes in intestinal microbial ecology, which encourages host adiposity (Bäckhed & Crawford, 2010; Turnbaugh et al. 2008). Colonization with intestinal microbes from obese mice resulted in increased body weight growth and fat buildup in germ-free mice compared to lean mice when microorganisms were added (Turnbaugh et al. 2006; Turnbaugh et al. 2008).

Modern convenience foods often contain high-sugar, high-fat ingredients so when consumed in excess can result in overeating, which aggravates obesity. Additionally, type 2 diabetes, cardiovascular, and cerebrovascular disorders, as well as other metabolic diseases, have mostly been associated with obesity (Chen et al. 2014). Hence, Metabolic diseases can be prevented by controlling lipid metabolism and managing obesity (Finicelli et al. 2019). An imbalance between energy expenditure and dietary energy intake leads to obesity, a chronic metabolic disease (Asano et al. 2019).

Various anti-obesity products have contained a variety of naturally derived milk components, including protein, calcium, essential fatty acids, as well as other natural nutritional elements. Whey and casein, two dairy proteins, may raise the threshold for satiety. Levels of circulating hormones that control appetite, such as glucagon-like peptide-1. It is better to consume soy protein before and during exercise. A unique amino acid composition is present in soy protein. In the current study, the antiobesity potential of probiotic fermented milk was examined by conducting an in vivo animal study with an indigenous cultures of Lactobacillus having potent probiotic potentials.

Materials and methods

Fermented dairy products preparation using selected lactic cultures and their maintenance

The LAB culture used in the production of fermented dairy products, i.e. *L. helveticus* MTCC 5463 (V3), *S. thermophilus* MTCC 5462 (MD2), and *L. rhamnosus* MTCC 5946 (NS6), (@ 2%, V3: MD2: NS6–1:1:1) were received from the Culture Collection of the Dairy Microbiology Department, SMC College of Dairy Science, Kamdhenu University, Anand, India. The LAB cultures were cultured in sterile reconstituted skim milk (10% Total Solids) and maintained at 5 ± 2 °C. The transfer was done at every week during the study.

Raw materials

Tonned Milk, Low heat skim milk powder of Sagar brand, India was purchased from the local market (Anand, India). Whey protein (WPC-70) was bought from Charotar Casein Company in Nadiad, Gujarat, India. Soy protein isolate (SPI) was used from SUPRO®120 IP.

Chemicals and glassware

During the entire study, glasswares of Borosil brand (Borosil Glass Works Ltd., Mumbai, India) and analytical grade and molecular biology grade chemicals were used.

Glassware and other materials were sterilized by usual procedures viz. 160-180 °C for 2 h in hot air oven, whenever required.

Protocol approval

The experimental protocol titled as "Evaluation of Functional Fermented Milk for Antiobesity Property on High Fat Diet Induced Obesity in Wistar Rats" was approved by the Ramanbhai Patel College of Pharmacy's Institutional Animal Ethics Committee (IAEC), Changa, Gujarat, India on 11/06/2019 in accordance with suggestions provided by the Ministry of Social Justice and Empowerment of India Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Protocol No. RPCP/IAEC/2019–20/R6).

Experimental design for conducting the study

In the study, probiotic fermented milks included (i) Probiotic fermented milk without WPC and SPI (T1) and (ii) Probiotic fermented milk enriched with WPC and SPI @ 2% (T2). The procedure followed in the preparation of both the products, had shown in Fig. 1. Every week, freshly prepared probiotic fermented milks were prepared to feed the rats and administered to the rats with 2 ml (Lactobacillus counts: 10^8–10^9 cfu/ml).

Method used for the preparation of probiotic fermented Milk

Induction of obesity in experimental animals

Animals used in the study In this study, adult male Wistar rats were obtained from the Zydus Research Centre in Changodar, Gujarat, India which with 200–250 g in weight. Polypropylene cages with standard controlled conditions were employed to keep the animals (humidity: 50 ± 5%, temperature: 23 ± 2 °C, and 12 h light/dark cycle). They also had unlimited access to water ad libitium and standard pellet diets. At the Ramanbhai Patel College of Pharmacy in Changa, animal study was carried out in accordance with CPCSEA norms and with IAEC permission.

Induction of obesity After 1 week of acclimatization, for continuous 6 weeks feeding, 24 rats were divided into

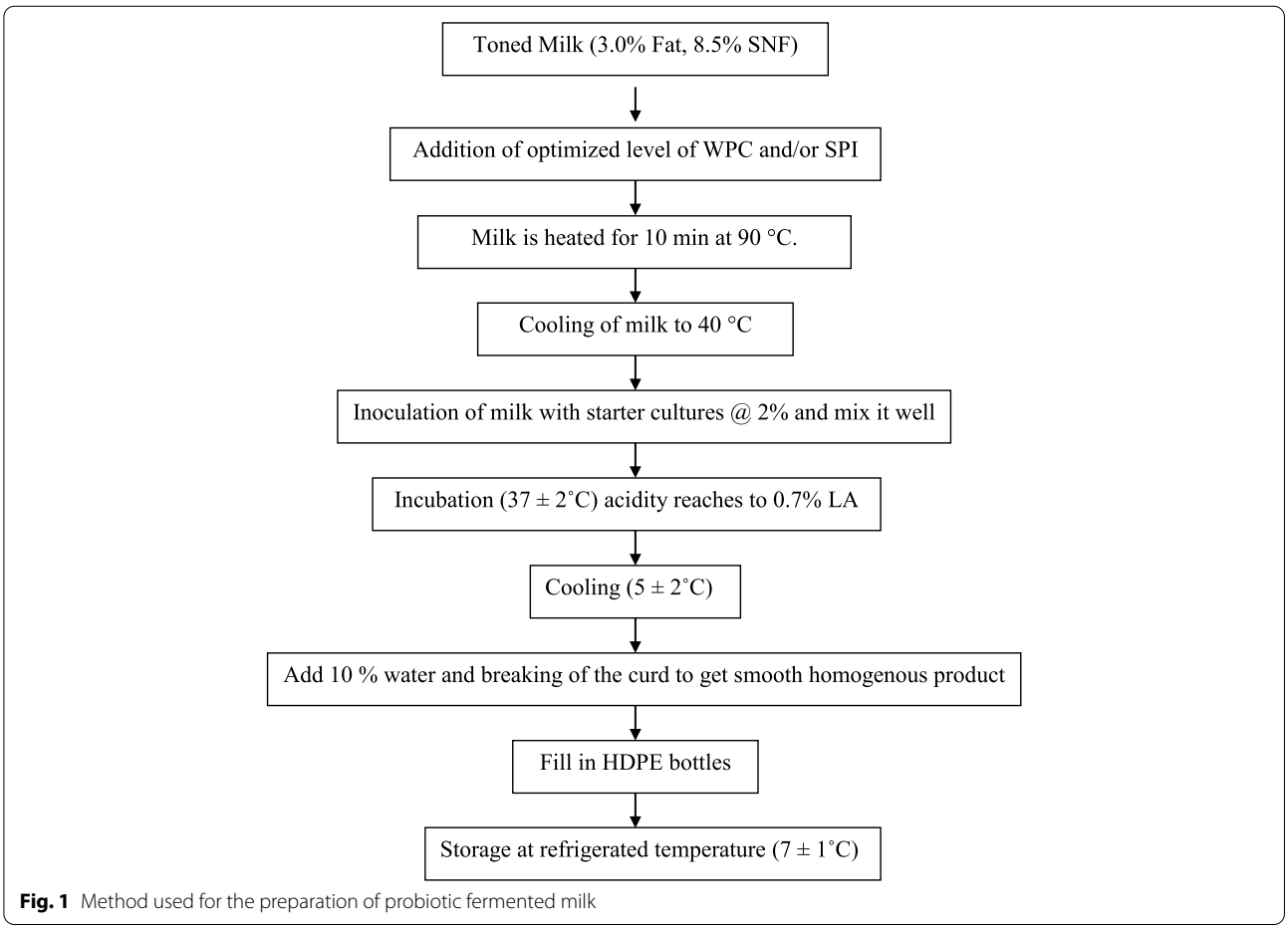

Fig. 1 Method used for the preparation of probiotic fermented milk

two separate groups and given either a normal pellet diet (NPD; $n=6$) or a high-fat diet (HFD; $n=18$). After the 7th week, disease model animals were divided into one control group and two treated groups. As an outcome, the following 4 groups were kept alive for 10 weeks:

Group I: Normal – diet fed rats.

Group II: HFD – fed rats.

Group III: Products T1 – HFD fed rats treated with product-C (2 ml/day; per oral).

Group IV: Products T2 – HFD fed rats treated with product-P (2 ml/day; per oral).

There were six rats ($n=6$) in each group. All groups of rats received a dose of both products (2 ml/day), with the exception of normal control (NC) and disease control (DC). Except for the NC and DC groups, all the rats received the products via oral administration. Every morning, each group's body weight was noted. After the 10-week period, the animals were fasted and sacrificed the day after that. Serum was collected from blood samples and also collected from the retro-orbital vein and then centrifuged at 4 °C, 8000 rpm for 10 minutes for various biochemical assays. The animals were killed with over 90% diethyl ether, and the kidney, liver, and body fat pads were removed, and the weights were taken. Liver tissues were preserved in 10% formalin solution at room temperature for 24 h in order to conduct histological investigation (Altunkaynak 2005).

List of parameters investigated

The following parameters were investigated during the study.

Serum biochemical parameters The levels of the aspartate transferase (AST), serum alanine transferase (ALT), triglyceride (TG), alkaline phosphate (ALP), total cholesterol (TC), HDL-cholesterol, and low-density cholesterol (LDL-C) were measured using commercially available kits with an auto-analyzer (Tulip diagnostic kits Pvt. Ltd.). Serum leptin levels were also measured using a rat LEP ELISA kit (Evirone life sciences, New Delhi), (Munshi et al. 2014).

Histopathological study Fixed liver tissue was divided into thin (5 m) sections and embedded in paraffin for histologic examination. The sections were stained with eosin and hematoxylin dye, and their histological analysis was evaluated under a light microscope (Chidambaram & Carani 2010).

Microbial counts in the fecal matter

To create a 1:10 dilution, aseptically weighed 11 g of faecal sample were then added to 99 ml of phosphate buffer. Then, 1 ml of the above dilution was used to make another dilution in 9 ml phosphate buffer tubes. After preparation, the appropriate dilutions were added to a set of duplicate sterile Petri plates. The counts for lactobacilli, streptococci, and coliforms are listed below.

Preparation of fermented milk samples for microbial analysis To create a 1:10 dilution, aseptically weighed 11 g of faecal sample were then added to 99 ml of phosphate buffer. Then, 1 ml of the above dilution was used to make another dilution in 9 ml phosphate buffer tubes. After preparation, the appropriate dilutions were added to a set of duplicate sterile Petri plates.

Probiotic/lactobacilli count The samples were diluted in series, as described in section 2.6.4.1. Selected 1 ml dilutions were added, when the sterile MRS agar was cooled. After the poured agar had set, a second layer (5–7 ml) of the same medium was added. After that, the plates were incubated for 72 hours at 37 ± 2 °C. The total amount of typical lactobacilli colonies in the plates were enumerated and given as log cfu/g (IS: 1479, Part III, 1962).

Streptococcal count The samples were diluted in series, as described in section 2.6.4.1. Selected 1 ml dilutions were added, when the sterile M17 agar was cooled. After the poured agar had set, a second layer (5–7 ml) of the same medium was added. After that, the plates were incubated for 72 hours at 37 ± 2 °C. The total number of streptococcal colonies in the plates was enumerated and given as log cfu/g (IS: 1479, Part III, 1962).

Coliform count As already stated above, serial dilutions of the samples were prepared. One ml selected dilutions were poured into duplicate plates of sterile, cooled VRBA agar. A second layer (5–7 ml) of the same medium was added. After that, the plates were incubated for 18–24 h at 37 ± 2 °C. The total amount of typical coliform colonies in the plates were enumerated and represented as log cfu/g (BIS, 1964).

Evaluation of SCFA of the cecal contents

With just a few minor modifications, Asano et al. (2007) method was used to measure the short chain fatty acids in the cecal contents. The cecal contents were added to

1.8 ml of 0.05 [N] sulfuric acid and thoroughly homogenized. The mixture was then centrifuged at 40 °C for 10 minutes at 5000 rpm. A 0.45 m membrane filter has been used in the process, the supernatant was collected and used for HPLC analysis. A Shimadzu LC-20 (Japan) instrument was used for the HPLC process. Anion exchange chromatography was carried out using an analytical column [C 18] and 0.012 N H2S04 elution at 45 °C. The flow was kept at 0.4 ml per minute. Each sample was examined three times.

Statistical analysis using dunnet's test

The in vivo studies were statistically analyzed using one-way ANOVA and Dunnet's Test in Graph Pad Prism V6.0. Statistical significance was maintained at $P < 0.05$ and $P < 0.01$.

Results and discussion

Obesity is a significant concern in the fields of preventive medicine and public health. In both humans and animals, it is characterized by an increase in adipocyte size and number at the cellular level. The aim of the present study was to examine the impact of probiotic fermented milk on obesity in male rats fed a high-fat diet. Wistar rats were used in this study to examine the in vivo antiobesity activity, with enriched whey protein and soy protein of probiotic fermented milk (T1) or without enriched whey protein and soy protein of probiotic fermented milk (T2).

Experimental animals and its maintenance

From the Zydus Research Centre in Changodar, Gujarat, India, adult male wistar rats weighing between 250 to 350 g were obtained. Polypropylene cages with standard controlled conditions were employed to keep the animals (humidity: 50 ± 5%, temperature: 23 ± 2 °C, and 12 h light/dark cycle). They also had unlimited access to water ad libitium and standard pellet diets (Barrière et al. 2018).

Experimental design

After a week of acclimation, for 6 weeks continuously, 24 Wistar rats were divided into 2 groups and given a high-fat diet (HFD; $n = 18$) or a normal pellet diet (NPD; $n = 6$). The disease model animals were further divided into two treated groups (each with six animals) and one disease control group ($n = 6$) after the seventh week. As a result, four groups of rats were kept alive for 10 weeks under regular and regulated conditions. Experimental design fed for Wistar rats displayed in Fig. 2.

Establishment of obesity model

Obesity was induced by feeding the animals ($n = 6$) either a high-fat diet ($n = 18$) or a normal pellet diet (NPD), which is made by combining powdered NPD (37 g/100 g), vegetable ghee (25 g/100 g), casein (10 g/100 g), fructose (20 g/100 g), cholesterol (5 g/100 g), and a vitamins and minerals mix (3 g/100 g), for the next 6 weeks.

Probiotic fermented milks

Probiotic fermented milk with WPC and SPI (T1) and probiotic fermented milk enriched without WPC and SPI (T2) were made fresh each week using the method. Products were given orally to the rats at a rate of 2 ml/day (10^8 to 10^9 log cfu/ml) during the trial, which lasted for 4 weeks after the model's development for 6 weeks.

The following variables were examined in the study: body weight, organ weight, lipid profile, liver enzymes, liver histology, and serum leptin level.

Effect of probiotic fermented milk products on body weight

Table 1 depicted the impact of probiotic fermented milk products on the rats' body weights during the experiment. In comparison to normal control rats (NC) (374.1 ± 10.48 g), over a period of 10 weeks, obese-induced rats displayed a significant ($P < 0.05$) increase in

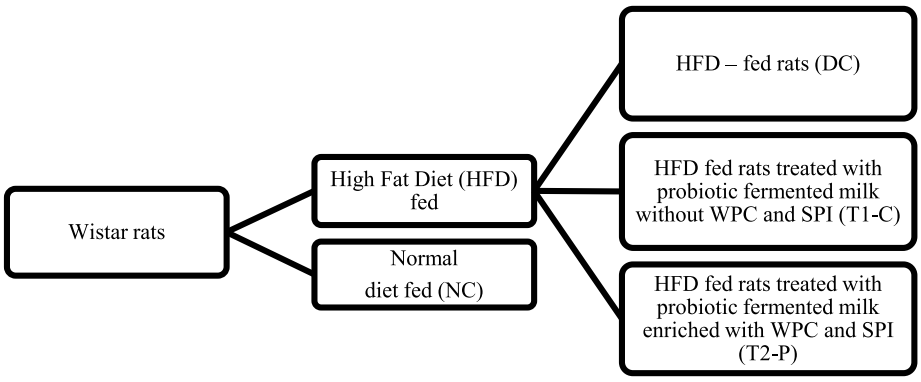

Fig. 2 Experimental design fed for Wistar rats

Table 1 Effect of probiotic fermented milk products on body weight (g) of experimental animals

Experimental group	Body weight (g) of rats			% increase in body wt. in 10 weeks
	At the start of experiment	After 10 weeks	Average increase in body wt. in 10 weeks	
NC	266.1 ± 19.20	374.1 ± 10.48	108.0	40.50
DC	323.1 ± 05.60	551.9 ± 18.09[#]	228.8	70.81
T1	304.1 ± 10.60	445.3 ± 25.40[*]	141.2	46.43
T2	320.6 ± 14.86	496.1 ± 40.77[*]	175.5	55.03

Values are expressed as mean ± SD ($n = 6$). [#]Significantly ($p < 0.05$) different from Normal Control. [*]Significantly ($P < 0.05$) different from Disease Control. Statistical analysis was performed by applying one-way ANOVA followed by Dunnet's Test using Graph Pad Prism V6.0. Statistical significance was kept at $P < 0.05$.

NC Normal control, *DC* Disease control, *T1* Probiotic fermented milk with WPC and SPI, *T2* Probiotic fermented milk without WPC and SPI

body weight (551.9 ± 18.09 g). Treatment groups, where probiotic fermented milk was administered in the HFD model after 6 weeks, compared to the control group, showed a significantly ($P < 0.05$) lower body weight gain. T2 without whey protein and soy protein showed body weight of 496.1 ± 40.77 g and T1 with whey protein and soy protein showed 445.3 ± 25.40 g after 10 weeks, which was at par. In terms of percent increase in body weight, the HFD group showed 71% increase, while 4 weeks of feeding probiotic fermented milk helped it to restrict to 46–55%. This was a significant achievement. The average body weight of Wistar rats had been increased within 10 weeks for the test groups, i.e., NC, DC, T1, T2 and were showed 108.0 g, 228.8 g, 141.2 g and, 175.2 g, respectively and percentage rise value of body weights were 40.50, 70.81, 46.43, and 55.03%, sequentially.

Obesity is typically indicated by an accumulation of excess body fat in the adipose tissue, which raises the body weight (WHO 2015). Due to its close similarity to the development of obesity in humans, the most often studied animal model by researchers has been high-fat diet-induced obesity in rats. It is generally indicated that eating a high-fat diet over a long period of time causes mammal abdominal fat weight to significantly increase (Barrière et al. 2018). According to this study, an animals abdominal fat weight and body weight significantly increases after being fed a high-fat diet for 10 weeks, which suggests that obesity in animals has been induced. We found no noticeable differences in the amount of food consumed daily by the groups, indicating that dietary caloric content was independent of the quantity of food consumed by the animals. The obese control animals consumed more calories than the normal control animals as a result. As obese animals were treated with products T1 and T2, their body weight and abdominal fat content significantly decreased when compared to the disease control group.

Through the gastrointestinal system and modulation of the gastrointestinal bacterial community, probiotics are thought to be one of the most essential tools for modifying the composition of the intestinal microbiota, which in turn affects intake of food, metabolic activities and body weight composition (Sanchez et al. 2014). Additionally, several probiotic strains have been implicated to the diminution of obesity (Yin et al. 2010). The effective reduction of body weight growth is achieved by supplemented by using *Lactobacillus curvatus* HY7601 with *Lactobacillus plantarum* KY1032. For up to 9 weeks, it effectively reduced adipose tissue weight in rats fed a high-fat, high-cholesterol diet (Yoo et al. 2013).

Lactobacillus paracasei CNCMI-4270, or *Lactobacillus rhamnosus* I-3690, *Bifidobacterium animalis* subsp. lactis I-2494, effectively reduced HFD-induced weight gain in mice over a 12-week period despite no changes in dietary intake (Wang et al. 2015).

Hong et al. (2015) examined the antiobesity effects of fermented whey beverages prepared with lactic acid bacteria in rats that had been induced obese through diet. A normal diet, an HFD, as well as fermented whey beverage were given to the three groups, respectively, for a four-week periods. In comparison to the groups receiving a normal diet and the HFD plus fermented whey beverage, it was shown that the rats in the HFD group had significantly ($P < 0.05$) increased their body weight after the experiment. The final status body weights of the HFD with fermented whey beverage sample (259.28 ± 19.23 g) and the usual diet group (274.78 ± 10.54 g) were not considerably different ($P > 0.05$), even though the HFD with fermented whey beverage used group gained less body weight than the normal diet group. In a similar way, the food intake of the high-fat dietary group was considerably ($P < 0.05$) greater compared to the other two groups.

Most of the studies support the anti-obesity benefits of probiotics such as Lactobacillus (*L. casei* strain *Shirota* (LAB13), *L. gasseri*, *L. rhamnosus*, and *L. plantarum*, among others) and *Bifidobacterium* (mainly *B. infantis*, *B. longum*, and *B. breve* B3) during 4 to 6 week periods (Ejtahed et al. 2019).

According to novel finding by Yoda et al. (2015), the anti-obesity impact of dairy products and dietary calcium during the nutritional dietary changes in P2-agouti mice may be enhanced by fermentation of milk with probiotics including milk supplementation of whey proteins. This might be accomplished by controlling the metabolism of lipids and glucose as well as by reducing oxidative and inflammatory stress. According to Rouxinol-Dias et al. (2016), certain probiotics may be important tools in the fight against obesity because the probiotic effect on bodyweight is species- and strain-specific.

Lee et al. (2021) demonstrated that *Lactobacillus johnsonni* 3121 and *Lactobacillus rhamnosus* 86 could act as novel probiotic strains and reduced the cholesterol levels. *L. johnsonni* 3121 and *L. rhamnosus* 86 were selected for in vivo evaluation of their anti-obesity effects using a high-fat diet-induced obese mouse model. Daily oral administration of *L. johnsonni* 3121 and *L. rhamnosus* 86 for 12 weeks significantly improved serum lipid profile and downregulated the expression of genes related to adipogenesis and lipogenesis in epididymal white adipose tissue of high-fat diet fed obese mice ($p < 0.05$). Fecal analysis also suggested that the two probiotic strains could normalize the altered obesity–related gut microbiota in high-fat diet–fed obese mice. These results collectively demonstrated that oral administration of *L. johnsonni* 3121 and *L. rhamnosus* 86 could prevent obesity, thereby improving metabolic health.

Food addiction (FA) is an important contributor to obesity. This study aimed to assess the effects of probiotic supplementation on the anthropometric indices, eating behavior, and hormone levels of obese women with FA. Multi-probiotic supplementation may have beneficial effects on anthropometric indices, eating behavior, and some appetite-regulating hormones in obese women with FA (Bagarolli et al. 2017).

Effect of probiotic fermented milk products on organ weights

In obese rats caused by the HFD, Table 2 displays that fermented milk products with probiotics affected the weight of the kidneys, liver, and abdominal fat.

As indicated in Table 2, the weight of the liver (23.53 ± 0.59 g) increased significantly ($P < 0.05$) in DC rats upon obesity induction (10.67 ± 0.11 g) in NC rats. While the liver weight in treatments T1 and T2 was considerably ($P < 0.05$) reduced to 15.53 ± 0.40 g and 19.99 ± 0.74 g, respectively, by probiotic fermented milk products. In comparison to the normal control group, this was higher. The kidney weight of DC rats was higher (1.67 ± 0.30 g) than that of NC rats (1.20 ± 0.12 g), but the difference was not significant statistically. T1 and T2 group showed not much difference in kidney weights. As illustrated in Table 2. there was increased in abdominal fat weight in DC rats (17.36 ± 0.59 g) in comparison to NC rats (2.36 ± 0.11 g), which was statistically significant ($P < 0.05$). The probiotic-fermented milk given to groups T1 and T2 resulted in a significant ($P < 0.05$) decrease in the weight of abdominal fat in each group (9.87 ± 0.40 g and 13.55 ± 0.74 g, respectively). The T1 group demonstrated a fairly significant ($P < 0.01$) effect in comparison to the T2 group.

Table 2 Effect of probiotic treatment on organ weights after 10 weeks in high fat diet induced obesity

Organs	Normal	DC	T1	T2
Liver wt (g)	10.67 ± 0.11	23.53 ± 0.59$^{\#}$	15.53 ± 0.40*	19.99 ± 0.74
Kidney wt (g)	1.20 ± 0.12	1.67 ± 0.30	1.51 ± 0.071	1.52 ± 0.06
Abdominal fat (g)	2.36 ± 0.11	17.36 ± 0.59$^{\#}$	9.87 ± 0.40*	13.55 ± 0.74

Values are expressed as mean ± SD ($n = 6$). $^{\#}$Significantly ($p < 0.05$) different from Normal Control. *Significantly ($P < 0.05$) different from Disease Control. Statistical analysis was performed by applying one-way ANOVA followed by Dunnet's Test using Graph Pad Prism V6.0. Statistical significance was kept at $P < 0.05$

NC Normal control, *DC* Disease control, *T1* Probiotic fermented milk with WPC and SPI, *T2* Probiotic fermented milk without WPC and SPI

Karimi et al. (2015) tested the antiobesity effect of high fat diet-induced obese rats using *Lactobacillus casei* strain *Shirota* versus Orlistat. The test mice were grouped into 4 categories: standard, HFD, HFD supplemented with LcS (108–109 CFU units), and HFD treated using orlistat (10 mg/kg body weight). They found that the HFD-orlistat and HFD-LcS groups had high levels of high-density adiponectin and lipoprotein while having lower levels of body mass index, body weight, fat mass, leptin, and glucose when ccomparedwith the HFD group. Furthermore, there was a significant difference in body fat mass between the HFD LcS groups and the HFD orlistat group (19.19 ± 95.76 g vs. 30.19 ± 97.98 g). While interleukin-6 levels were significantly lower in the HFD-LcS and HFD-orlistat groups compared to the HFD group, other inflammatory biomarkers did not alter noticeably.

Body fat mass was also significantly different between both the HFD LcS groups and the HFD orlistat groups (19.19 ± 95.76 g vs. 30.19 ± 97.98 g). When compared to the HFD group, interleukin-6 values were significantly reduced in the HFD-LcS and HFD-orlistat groups, but other inflammatory biomarkers did not change appreciably.

In diet-induced obese mice, Park et al. (2016) examined the antiobesity effects of yoghurt that has been fermented by *Lactobacillus plantarum* Q180. Male Sprague-Dawley rats were fed six diet groups to investigate their effects. A typical diet and saline solution were administered to

Group A. Both oral saline and HFD solution were given to Group B. Oral yoghurt and HFD fermented using ABT-3 and *L. plantarum* Q180 were given to Group C. Oral yoghurt and HFD fermented using ABT-3 and *L. plantarum* Q180 were given to Group D. For 8 weeks, GHFD and yoghurt with Garcinia cambogia extract and *L. plantarum* Q180-fermented were given to Group F, while Group E received both HFD and oral yoghurt made by *L. plantarum* Q180. After 8 weeks, groups C, D, E, as well as F had lower rates of body weight growth than group B, with respective rates of 5.14, 6.5, 3.35, and 10.8%. Groups C, D, E, and F showed much lower levels of triglycerides and leptin than group B, and groups E and F both had significantly lower epididymal fat weights. Additionally, group C had lower levels of AST than the other groups. In comparison to group B, groups A, C, D, E, and F had a less distribution amount of large-size adipose tissue.

The effects of fermented milk, nutritional supplements, and the *Lactobacillus plantarum* (LP625) singly and in combination with the medicinal herb's aloe-vera and *Gymnema sylvestre* on rats fed a high-fat diet (60 kcal fat) for up to 12 weeks were studied by Pothuraju et al. (2016). The administration of *Lactobacillus plantarum* was observed to reduce the final body weight, alone or even in combination with both herbs. However, a significant difference was noted between *Lactobacillus plantarum* fed with *Gymnema sylvestre* solely compared to the HFD-fed group (25.06 0.18 vs. 27.29 0.72 g, $P < 0.05$). Fasting blood glucose, serum insulin levels, and epididymal fat mass all decreased in all different treatment groups at considerable amounts ($P < 0.05$). Furthermore, it was discovered that consumption of *Lactobacillus plantarum* either alone or along with herbs had a protective function against the increase in serum and liver triglyceride levels together with liver total cholesterol levels. Furthermore, administration treatment of *Lactobacillus plantarum* alone or in combined with herbs produced a significant ($P < 0.05$) decreases in the size of the epididymal fat cells, in contrast with the high-fat diet-fed mice, which demonstrated a noticeable increase in the size of these cells.

Fortified yoghurt was given to overweight rats, it was (prepared with *S. thermophiles* and *L. bulgaricus* as starter culture and enriched with 107 CFU of *B. lactis* Bb 12 per gram, inulin, whey protein, vitamin D3, and calcium) considerably improved the animal's body composition (reduced the waist circumference, increased the HDL level and metabolic profile, body fat percentage, body fat, TG level). In the fortified yoghurt enriched group, the threshold of free fat mass was not considerably decreased. The study found that regular use of probiotic-enriched fortified yoghurt along with a strict diet can aid in weight loss and enhance metabolic health in obese people (Mohammadi-Sartang et al. 2018).

In a study on DIO mice, *Lactobacillus rhamnosus, L. acidophilus*, and *Bifidobacterium bifidumi* were administered to modulate gut microbiota. An improvement in insulin sensitivity and therefore obesity had resulted through the hypothalamic control of food intake, and insulin and leptin signaling besides equilibrium of the flora (Bagarolli et al. 2017).

Effect of probiotic fermented milk products on lipid profile

Table 3 displays the effects of the oral administration of probiotic fermented milk on the triglyceride, total cholesterol level, HDL level and LDL level of HFD-induced obese rats.

In comparison to NC rats (105.36 5.58 mg/dl and 90.46 7.17 mg/dl respectively), a considerable increase ($P < 0.05$) in triglyceride (TG) level and total cholesterol (TC) level was seen in DC rats (159.06 ± 16.36 mg/dl and 175.15 ± 21.34 mg/dl, respectively). While the TC and TG levels in T1 and T2 were both considerably ($P < 0.05$) less than in DC. However, there were no noticeable variations between T1 and T2.

Table 3 shows that there was a notable ($P < 0.05$) rise in LDL (120.70 ± 8.94 mg/dl) and a significantly ($P < 0.05$) reduction in HDL (13.25 ± 1.39 mg/dl) levels in DC rats compared to NC rats (70.86 ± 10.29 mg/dl and 24.36

Table 3 Effect of probiotic treatment on lipid profile

Lipid Profile	NC	DC	T1	T2
TG (mg/dl)	90.46 ± 7.17	159.06 ± 16.36[#]	107.38 ± 18.71*	92.41 ± 11.99*
TC (mg/dl)	105.36 ± 5.58	175.15 ± 21.34[#]	149.5 ± 24.18*	153.7 ± 11.94*
HDL (mg/dl)	24.36 ± 7.81	13.25 ± 1.39[#]	14.11 ± 2.95	17.26 ± 3.95*
LDL-C (mg/dl)	70.86 ± 10.29	120.7 ± 8.94[#]	112.25 ± 20.47	116.33 16.87

Values are expressed as mean ± SD ($n = 6$). [#]Significantly ($p < 0.05$) different from Normal Control. [*]Significantly ($P < 0.05$) different from Disease Control. Statistical analysis was performed by applying one-way ANOVA followed by Dunnet's Test using Graph Pad Prism V6.0. Statistical significance was kept at $P < 0.05$

NC Normal control, *DC* Disease control, *T1* Probiotic fermented milk with WPC and SPI, *T2* Probiotic fermented milk without WPC and SPI) *TG* Triglyceride level, *TC* Total cholesterol, *HDL* High-density lipoprotein, and *LDL-C* low-density lipoprotein- cholesterol

7.81 mg/dl, respectively). Between T1 and T2, LDL levels considerably ($P < 0.05$) increased (112.25 and 116.33 mg/dl, respectively). When compared to DC (13.25 mg/dl), the T2 group HDL level (17.26 mg/dl) increased significantly ($P < 0.05$), while T1 was on par with DC.

Hyperlipidemia was a serious risk factor for non-alcoholic cardiovascular disease and fatty liver disease. Visceral adiposity is associated with hyperlipidemia which is manifested as hypertriglyceridemia and associated with metabolic syndrome. Increased serum triglyceridemia in high fat-induced obese rats is represented by de novo lipid biosynthesis. Moreover, the Accumulation of fat in adipocytes helps in the formation and release of fatty acids, which are then transformed into triglycerides in the liver where hepatocytes are re-esterified to form triglycerides, packed into VLDL and secreted into the blood circulation. Further, the loss of diacylglycerol to convert into LDL is further transported to peripheral tissue and may produce atherosclerotic disease conditions (Munshi et al. 2014).

In the present investigation, apart from weight induction by high-fat diet, it caused a notable increase of lipid profiles as evidenced by a rise in serum triglycerides, cholesterol, VLDLlevel and LDL level as well as a fall in HDL. Treatment with product T1 and product T2 produced a significant decline in TG, cholesterol but failed to alter levels of LDL to a significant extent. However, T2 improvised the serum HDL at significant levels but T1 failed to improve serum HDL a significant extent. Many of the research reports suggested circumventing hypertriglyceridemia and hypercholesterolemia without altering the levels of HDL and LDL.

According to Kiessling et al. (2002), probiotic-containing yoghurts (*L. acidophilus, Enterococcus faecium*, *Bifidobacterium longum, Streptococcus thermophilus, L. plantarum*, and/or *B. lactis*) considerably reduced LDL cholesterol and total serum cholesterol as well as improved the LDL: HDL cholesterol ratio when consumed as part of a diet.

Nagasawa et al. (2002) studied the effect of a calorie-restricted diet using soy protein isolate. Assess the impact on the genes, lipids, plasma glucose, and adiponectin involved in glucose metabolism and fatty acid in obese male KK-Ay mice. As a result of the SPI diet, epididymal, mesenteric, and brown fat adipose tissue weights decreased. Additionally, the SPI diet led to reduced plasma concentrations of glucose, cholesterol, triglycerides, and FFA. These researchers came to the conclusion that the soy protein diet reduced the amount of body fat in obese mice.

Lee et al. (2013) conducted studies on the antiobesity characteristics of soy milk products fermented by Lactobacillus culture. In HFD Wistar rats, they discovered that unfermented soy milk, *Lactobacillus paracasei subsp. paracasei* NTU 101 and NTU 102 were found to be able to considerably decrease increases in serum TG, TC, and LDL in a dose-dependent way in HFD Wistar rats, with NTU 101 and NTU 102 having a greater effect than unfermented soy milk, While in the unfermented soy milk, NTU 101, and NTU 102 treatment groups, The serum HDL level was unaffected.

Lactic acid bacteria were used in obese rats induced by a diet, Hong et al. (2015) investigated the antiobesity effects of fermented whey beverages. The diets of the three groups varied; For a period of 4 weeks, three groups received different diets: a standard diet, a high-fat diet (HD), and a high-fat diet plus fermented whey beverage (HDFWB). The study's results showed that while there was no significant difference in blood HDL cholesterol levels across the experimental groups, there was a significant ($P < 0.05$) decrease in triglycerides, total cholesterol, and LDL cholesterol in the HDFWB group in comparison to the HD group.

Effect of probiotic fermented milk products on liver enzymes (AST, ALT, ALP and CRP)

The most frequently tested indicator of liver disease is elevated serum levels of the three aminotransferases alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). More frequently than in the population at large, obesity is associated with higher levels of some hepatic enzymes. Elevated serum levels of ALT and AST, which speed up the conversion of amino groups to generate products in amino acid metabolism and gluconeogenesis, indicate either acute or long-term liver damage.

Figs 3 and 4 show that serum levels of ALP and ALT were significantly greater in high-fat diet-fed (DC) to rats than in the normal control group. Although there was no notable difference between the levels of C-reactive protein (CRP) and AST from the normal control group. T1 display the significant decline in the levels of ALT (88.66 IU/L) and ALP (209.16 IU/L) whereas T2 failed to demonstrate significant variations in the level of ALP (276.83 IU/L), AST (184.66 IU/L) and CRP (1.88 mg/L) as compared to HFD group, which showed ALP value of 252.00 IU/L, AST 185.0 IU/L and CRP value 1.74 mg/L after 10 weeks of experiment.

The liver capability to transport fatty acids across biological membranes may be compromised by hyperlipidemia, which is frequently accompanied by liver dysfunction. Furthermore, liver inflammation is also linked to obesity. To know the effects on the liver of both T1 and T2 high-fat diet, we investigated AST, ALT, ALP and C-reactive protein. Liver enzymes, AST, ALT and ALP are released from hepatocytes in response to

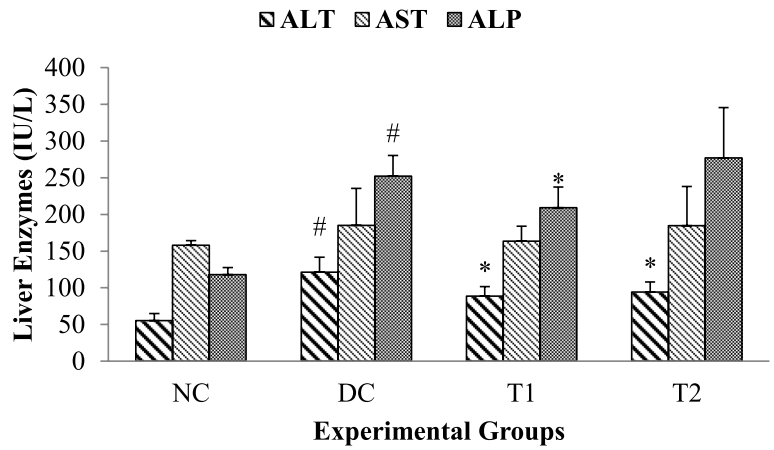

Fig. 3 Effect of probiotic fermented milk products on liver enzymes

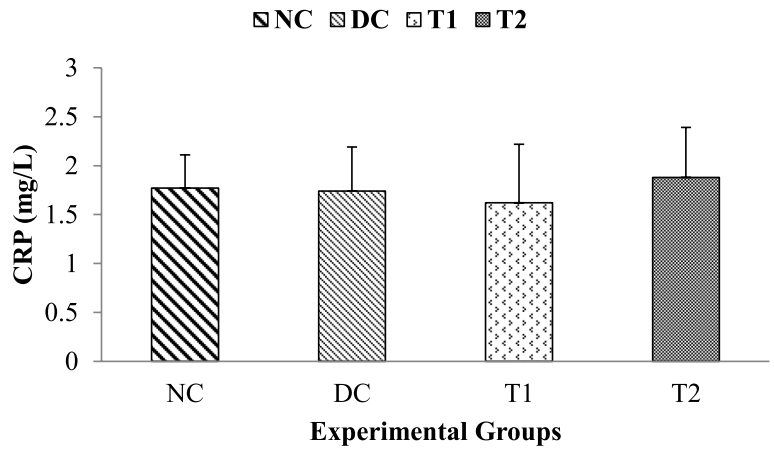

Fig. 4 Effect of probiotic fermented milk products on CRP. (NC = normal control, DC = disease control, T1 = probiotic fermented milk withWPC and SPI, T2 = probiotic fermented milk without WPC and SPI) Statistical analysis was performed by applying one-way ANOVA followed by Dunnet's Test using Graph Pad Prism V6.0. Statistical significance was kept at $P < 0.05$ and $P < 0.01$. #-Indicate significant difference from Normal control group. *-Indicate significant difference from Disease control group

hepatocellular damage. Our findings showed that T1 and T2 reduced liver damage in obese rats fed a high-fat diet showed a reduction in ALT, but that neither the high-fat diet nor T1 or T2 changed AST. Moreover, in obese animals, we observed an elevation of ALP compared to normal control animals. T1 reduced elevated ALP levels in obese animals but T2 failed to alter levels of ALP. We investigated C-reactive protein as a measure of liver inflammation which was not altered significantly in any group.

Effect of probiotic fermented milk products on serum leptin level

Fat cells (adipocytes) release leptin, which was formerly believed to communicate with the brain to reduce appetite and weight (Ahima, 2008). Figure 5 depicts how probiotic fermented milk affects serum leptin levels. In comparison to normal control (DC) rats (4.93 ng/ml), serum leptin levels increased significantly ($P < 0.05$) in disease control (DC) rats (8.41 ng/ml). T1 and T2 notably ($P < 0.05$) delayed the rise in blood leptin levels in comparison to DC and NC (6.24 and 5.88 ng/ml, respectively).

It is generally known that leptin is a fat-derived regulator of food intake and energy utilization. Additionally, obesity caused by a high-fat diet is associated with hyperleptinemia, and TG production in adipose tissue is significantly correlated with leptin. The current study's finding that T1 and T2 reduced serum leptin levels suggests that there was less fat accumulation in

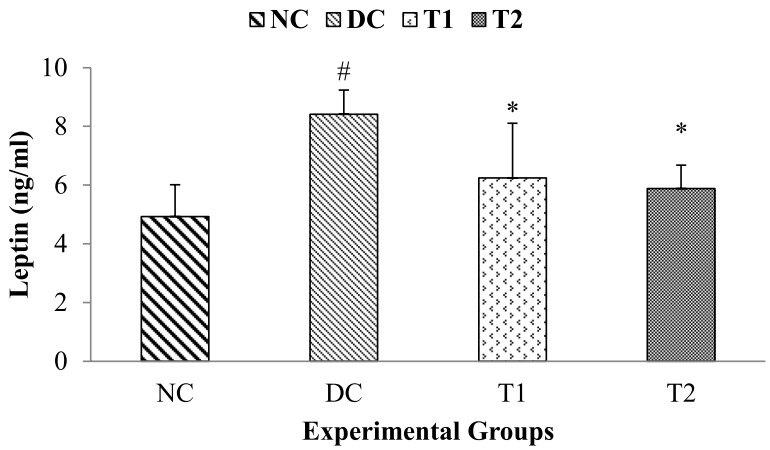

Fig. 5 Effect of probiotic fermented milk products on serum leptin level**.** (NC = normal control, DC = disease control, T1 = probiotic fermented milk with WPC and SPI, T2 = probiotic fermented milk without WPC and SPI) Statistical analysis was performed by applying one-way ANOVA followed by Dunnet's Test using Graph Pad Prism V6.0. Statistical significance was kept at $P < 0.05$ and $P < 0.01$. #-Indicate significant difference from Normal control group. *-Indicate significant difference from Disease control group

adipocytes, which is further supported by the finding that there was less abdominal fat mass.

The effect of fermentation of milk using *Lactobacillus gasseri* SBT2055 on the size of rat adipocytes was investigated by Sato et al. (2008). Male Sprague-Dawley rats were given either skim milk (the control diet) or skim milk (the LGSP diet) for 4 weeks. They found that the serum leptin levels in the *Lactobacillus gasseri* SBT2055 treated groups had decreased by 32% compared to the control group.

Using Lactobacillus fermented soy milk products, Lee et al. (2013) investigated the antiobesity effects. They found that serum leptin levels were increased in the *Lactobacillus paracasei subsp. paracasei* NTU 102 and *Lactobacillus paracasei subsp. paracasei* NTU 101 groups as compared to normal control and high-fat diet groups (10^6–10^{10} CFU administration). The high-fat diet group serum leptin levels remained constant when compared to the normal control group.

Yogurt that has been fermented using *Lactobacillus plantarum* Q180 was tested by Park et al. (2016) to determine whether it had any anti-obesity effects on rats that had been made obese through diet. In groups fed with yoghurt containing Garcinia cambogia extract, they found that leptin was significantly reduced.

Simon et al. (2015) and Mobini et al. (2017) administered two different strains of *Lactobacillus reuteri* to target different patient populations, and they found diverse results for the same clinical parameters. Brahe et al. (2015) studied the administration of one strain of *L. paracasei* and they did not find any changes in the clinical parameters of the patients (obese postmenopausal women). Finally, Sanchez et al. (2017) administered a *Lactobacillus rhamnosus* strain and found significant differences between genders. While a decrease in the *Subdoligranulum genus*, coupled to weight loss and decreasing leptin levels were found in women, no significant differences were found in microbiota or any clinical biomarker in men treated.

Histopathological study of liver

Histopathology of Liver tissues of NC. There were no histological abnormalities observed in liver tissue such as pentagonal or hexagonal lobules with peripheral hepatic triads embedded in connective tissue and central veins. Rats on a normal diet show a Portal vein at the periphery of a classic hepatic lobule, which is surrounded by flattened endothelial cells, Kupffer cells, and blood sinusoids, which are present in the areas between hepatocyte cords.

Histopathology of Liver tissues of DC indicates lipid accumulation in liver hepatocytes, and mild inflammation**.** There were histological abnormalities observed in liver tissue such as showing the elements of the portal tract have infiltrations around them and between them, Infiltrations between the hepatocytes, Dilated and congested portal vein, hepatocytes with variable cytoplasmic vacuolation observed such as Tiny cells with ovoid nuclei and numerous small hepatocytes with vacuoles are placed in rows between the hepatocytes, Dilated sinusoids, Nuclei fragmented, Lysed nuclei, Hepatocytes containing vacuoles in a rat fed a high-fat diet.

The liver histopathology in obese rats that were fed a high-fat diet with T1 shows mild histological abnormalities that were were seen in the liver tissue, such as infiltrations between the mild hepatocytes, a normal portal

vein, a normal central vein, normal sinusoids, and mildly small hepatocytes with vacuoles in rats that were given a high-fat diet.

The liver histopathology in obese rats that were fed a high-fat diet with T1 shows mild histological abnormalities that were were seen in the liver tissue, such as infiltrations between the mild hepatocytes, a normal portal vein, a normal central vein, normal sinusoids, and mildly small hepatocytes with vacuoles in rats that were given a high-fat diet (McGill, 2016).

Fig. 6a to d show sections that have been stained with eosin and hematoxylin (E and H). For the animals within normal group, the liver histology was normal (Fig. 6a), while the liver in the disease model group had large lipid vacuoles formed inside the parenchyma cells (Fig. 6b). T1 appeared better than T2, and T2 showed fewer micro vesicular fatty alterations. (Fig. 6c and Fig. 6d).

The antiobesity properties of *Lactobacillus casei* strain *Shirota* versus *Orlistat* in obese rats produced by a high-fat diet were evaluated by Karimi et al. (2015). Histological examinations were conducted on the adipose tissues and liver of rats fed the *Lactobacillus casei* strain *Shirota*, and the researchers found no signs of inflammation, necrosis, or bleeding in hepatocytes (LCs). This was almost similar to our findings.

According to Muzafar and Amin's (2017) findings, the probiotic mixture reduces the symptoms of fatty liver disease by influencing lipid profiles, leptin levels, and inflammatory biomarkers. They examined at the histological analysis of hepatocyte structure, inflammatory cell presence or absence, fat globule presence or absence between and within the hepatocytes, and any hepatocyte degenerative alterations. According to the data from the high-fat and sucrose diet (HFSD) group, there were numerous fat globules within and between hepatocytes as well as associated degenerative changes in hepatic cells. Furthermore, the group studying non-alcoholic fatty liver disease recognized macrovesicular steatosis (bold line arrow) also as existence of major fat droplets in microvesicular steatosis (dotted arrow) and hepatocytes as the presence of small fat droplets in hepatocytes.

Overall, the study revealed that feeding probiotic fermented milk with or without whey protein and soy protein has an anti-obesity effect in Wistar rats.

Microbial count in the faecal matter

One of the most popular probiotics that are beneficial to human health is lactic acid bacteria. The impact of the gut microbiota on obesity-related metabolic disorders and problems brought on by a high-fat diet may have a major impact on how energy is used. Therefore, it is essential that the harmful effects of HFD feeding and complications brought along by obesity can be managed by changing the gut flora (Cani et al. 2009).

We collected faecal matter of the experimental rats weekly after the start of feeding probiotic fermented milk and analyzed for *Lactobacillus, Streptococcus* and coliform count.

Data presented in Table 4 is displayed the average faecal viable counts of *Lactobacillus*. During the feeding phase, the average viable counts increased significantly ($P < 0.05$). The fecal viable count of T1 was higher (7.51 log cfu/ml), followed by T2 (7.41 log cfu/ml), DC (7.35 log cfu/ml) and NC (7.32 log cfu/ml). The counts in DC and NC were at par. The counts in DC and NC were at par. The count also increased progressively during feeding period, which indicated that the probiotic lactobacilli from our product is stable in the intestinal tract of the rats. An increase in count in NC and DC also indicated that native lactobacilli also get promoted during growth.

Table 5 shows the average fecal viable counts of *Streptococcal.* The fecal viable count in T1 was higher (7.49 log cfu/ml) followed by T2 (7.37 log cfu/ml), DC (7.32 log cfu/ml) and NC (7.31 log cfu/ml). All samples showed progressively increasing counts during the feeding period. It had been observed that in all the products with minimum value showed at 0 week (T1–6.79 log cfu/ml, T2–6.72 log cfu/ml, NC-6.68 log cfu/ml, DC-6.35 log cfu/ml) and as week passed the value increased to 7.91 log cfu/ml, 7.83 log cfu/ml, 7.71 log cfu/ml, 7.89 log cfu/ml, respectively on fourth week.

As indicated in Table 6, the average coliform count increased significantly after one week of feeding from all samples. Counts in NC and DC (6.54 log cfu/ml and 6.46 log cfu/ml) was marginally higher as compared to T2 (6.38 log cfu/ml) and T1 (6.34 log cfu/ml), which was non-significant. It had been showed that in all the products with minimum value showed at 0 week (T1–5.91 log cfu/ml, T2–5.95 log cfu/ml, NC-6.18 log cfu/ml, DC-6.37 log cfu/ml) and as week passed, the value progressed to 6.39 log cfu/ml,6.39 log cfu/ml,6.66 log cfu/ml, 6.53 log cfu/ml, respectively on fourth week.

Data presented in Table 7, the results showed higher acetate production in T1 (251 ± 0.009 µg/g) followed by NC (206 ± 0.004 µg/g), T2 (151 ± 0.008 µg/g) and DC (16 ± 0.003 µg/g). T1 showed higher propionate production with 56.06 ± 0.008 µg/g of cecum followed by T2 (18.09 ± 0.005 µg/g), DC (18.06 ± 0.005 µg/g) and NC (17.73 ± 0.003 µg/g). overall comparison between the two short chain fatty acids, the production of acetic acid was higher than propionic acid (Table 7).

L. acidophilus NCDC 13 containing probiotic dahi to mice on a high-fat diet increased the number of feacal and caecal bifidobacteria (Arora et al. 2012). In order to favour the health-promoting bifidobacteria and reduce

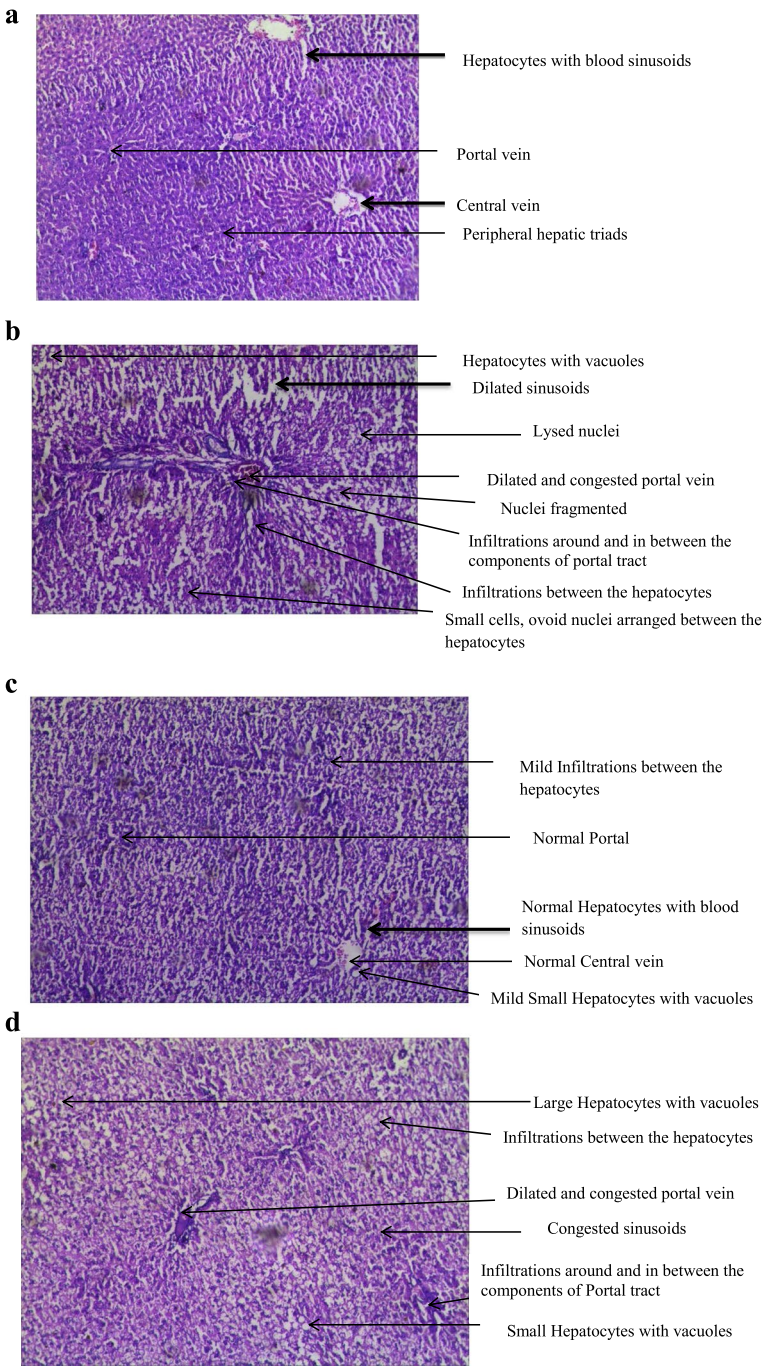

Fig. 6 **a** Light photomicrographs of liver sections from NC experimental group (H and E staining, Magnification: 10X). **b** Light photomicrographs of liver sections from DC experimental group (H and E staining, Magnification: 10X). **c** Light photomicrographs of liver sections from T1 experimental group (H and E staining, Magnification: 10X). **d** Light photomicrographs of liver sections from T2 experimental group (H and E staining, Magnification: 10X)

the unpleasant effects of obesity caused by a high-fat diet, dietary methods must be modelled using probiotics. After drinking fermented milk with *L. gasseri* SBT2055, rats also had reduced adipocyte size (Cariou et al. 2006; Sato et al. 2008).

The antiobesity effects of feeding probiotic dahi containing *Lactobacillus casei* NCDC 19 to obese mice produced by HFD were studied by Rather et al. (2014). They found no statistically significant differences between groups in the total number of microorganisms.

Table 4 Evaluation of *Lactobacillus* count (log cfu/ml) of fecal matter

Product (T)	Feeding Period (P)					Treatment Mean
	0 week (Before feeding PFM)	1 week	2 week	3 week	4 week	
T1	6.82 ± 0.02	7.40 ± 0.03	7.52 ± 0.02	7.90 ± 0.04	7.92 ± 0.01	**7.51**[c]
T2	6.79 ± 0.02	7.15 ± 0.10	7.34 ± 0.12	7.88 ± 0.06	7.90 ± 0.06	**7.41**[b]
NC	6.68 ± 0.02	7.15 ± 0.10	7.36 ± 0.06	7.70 ± 0.05	7.72 ± 0.09	**7.32**[a]
DC	6.74 ± 0.05	7.06 ± 0.06	7.37 ± 0.04	7.78 ± 0.10	7.78 ± 0.12	**7.35**[a]
Period Mean	**6.76**[a]	**7.19**[b]	**7.40**[c]	**7.82**[de]	**7.84**[e]	
Source	**SEm**		**CD(0.05)**		**CV%**	
T	0.02		0.05		0.89	
P	0.02		0.05			
T*P	0.04		0.11			

PFM Probiotic fermented milk

T1: PFM + WPC + SPI@ 2%

T2: PFM without WPC + SPI

NC Normal control

DC Disease control

Each observation is a mean ± SD of three replications

Table 5 Evaluation of *Streptococcal* count (log cfu/ml) of fecal matter

Product (T)	Feeding Period (P)					Treatment Mean
	0 week (Before feeding PFM)	1 week	2 week	3 week	4 week	
T1	6.79 ± 0.03	7.41 ± 0.02	7.47 ± 0.01	7.89 ± 0.03	7.91 ± 0.06	**7.49**[c]
T2	6.72 ± 0.05	7.04 ± 0.07	7.39 ± 0.07	7.84 ± 0.04	7.83 ± 0.04	**7.37**[b]
NC	6.68 ± 0.03	7.19 ± 0.09	7.30 ± 0.08	7.70 ± 0.04	7.71 ± 0.04	**7.31**[a]
DC	6.35 ± 0.11	7.16 ± 0.11	7.31 ± 0.13	7.88 ± 0.04	7.89 ± 0.03	**7.32**[a]
Period Mean	**6.63**[a]	**7.20**[b]	**7.37**[c]	**7.83**[de]	**7.84**[e]	
Source	**SEm**		**CD (0.05)**		**CV%**	
T	0.02		0.05		0.87	
P	0.02		0.05			
T*P	0.04		0.11			

PFM Probiotic fermented milk

T1: PFM + WPC + SPI@ 2%

T2: PFM without WPC + SPI

NC Normal control

DC Disease control

Each observation is a mean ± SD of three replications

In comparison to the animals given a normal diet, the HFD group's bifidobacteria levels seem to be much lower. Bifidobacteria counts considerably increased (7.99 0.05 log cfu/ml) when *L. casei* NCDC 19 was provided a high-fat diet combined with probiotic dahi as compared with the HFD group (6.91 0.09 log cfu/ml) ($P < 0.05$). Additionally, there were noticeably more Bifidobacterium counts in the HFD with added probiotic dahi group compared with the group of normal dahi-fed.

Karimi et al. (2017) studied the impacts of single-species versus dual-species probiotic supplementation as a new therapeutic strategy for obesity. To conduct the study, 40 male Sprague-Dawley rats were subdivided into five groups: high-fat diet fed; standard diet given; HFD with *Lactobacillus casei* strain Shirota; HFD supplied with *Bifidobacterium longum*; and HFD with a mixture of these two bacterial strains. The results of their study found that the total number of microorganisms in the faecal microbiota was not significantly affected by probiotic administration and the HFD diet.

Table 6 Evaluation of Coliform count (log cfu/ml) of fecal matter

Product (T)	Feeding Period (P)					Treatment Mean
	0 week (Before feeding PFM)	1 week	2 week	3 week	4 week	
T1	5.91 ± 0.56	6.48 ± 0.36	6.47 ± 0.37	6.45 ± 0.21	6.39 ± 0.21	**6.34**
T2	5.95 ± 0.20	6.53 ± 0.35	6.50 ± 0.17	6.42 ± 0.32	6.39 ± 0.31	**6.38**
NC	6.18 ± 0.18	6.52 ± 0.44	6.62 ± 0.46	6.63 ± 0.44	6.66 ± 0.37	**6.54**
DC	6.37 ± 0.19	6.42 ± 0.37	6.43 ± 0.22	6.52 ± 0.37	6.53 ± 0.34	**6.46**
Period Mean	**6.10**^{a}	**6.43**^{bc}	**6.47**^{c}	**6.51**^{c}	**6.55**^{c}	
Source	**SEm**		**CD (0.05)**		**CV%**	
T	0.09		NS		5.27	
P	0.10		0.28			
T*P	0.20		NS			

PFM Probiotic fermented milk

T1: PFM + WPC + SPI@ 2%

T2: PFM without WPC + SPI

NC Normal control

DC Disease control

Each observation is a mean ± SD of three replications

Table 7 Short chain fatty acid content of the cecal contents

Product (T)	Acetic acid (µg/g)	Propionic acid (µg/g)
T1	251 ± 0.009^{d}	56.06 ± 0.008^{d}
T2	151 ± 0.008^{b}	18.09 ± 0.005^{c}
NC	206 ± 0.004^{c}	17.73 ± 0.003^{a}
DC	16 ± 0.003^{a}	18.06 ± 0.010^{b}
SEm	0.004	0.004
CD (0.05)	0.013	0.014
CV %	4.40	26.84

PFM Probiotic fermented milk

T1: PFM + WPC + SPI@ 2%

T2: PFM without WPC + SPI

NC Normal control

DC Disease control

Each observation is a mean ± SD of three replications

SCFAs of cecal contents

The results showed higher acetate production in T1 (251 ± 0.009 µg/g) followed by NC (206 ± 0.004 µg/g), T2 (151 ± 0.008 µg/g) and DC (16 ± 0.003 µg/g). T1 showed higher propionate production with 56.06 ± 0.008 µg/g of cecum followed by T2 (18.09 ± 0.005 µg/g), DC (18.06 ± 0.005 µg/g) and NC (17.73 ± 0.003 µg/g).

After administering a small amount of Swiss-type cheese prepared with *P. acidipropionici* CRL 1198 to mice, Chaia & Zárate (2005) examined the roles played by different four propionibacteria strains in the production of lactic acid and SCFA in the mouse cecum both in vitro as well as in vivo as well as the metabolic activity and viability of propionibacteria inside this cecum sample. It was found that feeding milk and propionibacteria (PAB) and lactose fermentation in cecal content treated with PAB increased propionic acid production. The intestinal microflora's production of butyric acid and Propioni bacteria production of propionic acid both increased.

Huazano-García et al. (2017) analysed the variations in the mice's cecal microbiota and the connection between body weight and short-chain fatty acids. With the use of soy protein agavins, whey protein, and/or oligofructose with HFD, the concentration of butyric acetic, and propionic acids throughout the cecal content was significantly greater when compared to non-supplementation. Overall, the result of the fecal matter indicated that T1 had higher *Lactobacillus* and *streptococcal* count but did not change coliform count. Cecal content of probiotic fermented milk enriched with WPC and SPI exerted better SCFA production compared to probiotic fermented milk without whey protein and soy protein, NC and DC.

Conclusions

Overall, the in vivo study results indicated that, oral administration of probiotic fermented products with or without WPC and SPI for 4 weeks exerted a beneficial effect against HFD induced obesity in rats by improving the organ weights and serum biochemical markers.

The probiotic fermented milk exerted better anti-obesity activity compared to the control. The result of fecal matter indicated that T1 (Probiotic fermented milk enriched with WPC and SPI) had higher *Lactobacillus* and *streptococcal* count and lower coliform count compared to T2, NC and DC. Cecal content of Probiotic fermented milk enriched with WPC and SPI exerted better SCFA production compared to probiotic fermented milk without WPC and SPI, NC and DC. The extremely promising antiobesity activity that both products demonstrated in vivo in a high-fat diet-fed Wistar rat model suggests that both LAB strains, as well as WPC and SPI, may play a part in preventing obesity. Hence, it can be concluded that Probiotic fermented milk enriched with WPC and SPI as well as the probiotic product without WPC and SPI, can be very promising functional foods for preventing obesity.

Abbreviations

HFD: High fat diet; NC: Normal pellet diet fed; DC: HFD fed; WPC: Whey protein concentrate; SPI: Soy protein isolate; T1: Probiotic fermented milk with WPC and SPI; T2: Probiotic fermented milk without WPC and SPI.

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Authors' contributions

JBP, SH: conceptualized the study, designed experiments, supervised data collection and the study; SM, RP: analyzed data, prepared the draft manuscript; JBP, SH: read and edited the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participation

All animal experiments were undertaken with the approval from the Ramanbhai Patel College of Pharmacy's Institutional Animal Ethics Committee (IAEC), Changa, Gujarat, India on 11/06/2019 in accordance with suggestions provided by the Ministry of Social Justice and Empowerment of India Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Protocol No. RPCP/IAEC/2019–20/R6).

Consent to publication

Not applicable.

Competing interests

Not applicable.

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