Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.12.03.Art007

Comparative Anti-Obesity Activity of Probiotics with Short-Chain Fatty Acids (SCFAs) Through GLP 1 and PYY Activity in High Fat Diet Induces Obesity in Rats

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Abstract

Microbiota in the intestine produces SCFAs like Butyrate and Propionate through undigested fiber, which plays an important role in metabolic disorders, especially in obesity and type II diabetes. To compare the anti-obesity activity of probiotics with SCFAs through GLP-1 and PYY activity in HFD induced obesity in rats. For the study, rats weighing around 150-200g were chosen, separated into multiple groups, and given Fat-enriched diet for 12 weeks to trigger obesity. SCFAs like Butyrate, propionate (400mg/kg, P.O), and probiotics (10^8 CFU P.O) were continuously administered to their groups along with the HFD. Weekly once the animal's BW & daily food intake by the animal, and every 15 days once lipid profile, GLP, PYY, insulin, and leptin parameters were measured. After the completion of the study blood was collected through retro-orbital puncture for evaluation of biochemical parameters and animals were anesthetized and sacrificed for organ isolation for histopathological studies. Administration of SCFAs along with Probiotics in HFD-induced Overweight rats led to a notable drop in BW, food intake, fat accumulation, fasting insulin, leptin, and lipids throughout 12 weeks as compared with rats fed with HFD and standard. SCFAs along with probiotics effectively increase HDL levels and reduce LDL. Histopathology examination reveals that fat tissue accumulation was absent in treated groups when compared with only the HFD feed group. Results show that SCFAs are showing a notable decrease in lipid profile, food intake, and body weight than probiotics-treated group possesses significant anti-obesity activity

Keywords: *HFD, Leptin, Obesity, Probiotics, SCFAs, Sprague Dawley Rats.*

Introduction

A disparity in energy intake and burn is the primary cause of overweight, also known as metabolic syndrome. However, obesity is also a result of a complex interplay between a person's nutrition, lifestyle choices, genetic predisposition, environment, and reduced physical activity. Excessive fat storage and high plasma lipid levels are markers of obesity. Both the quantity and size of fat cells increase the body's overall fat mass. Due to its correlation with metabolic disorders and some forms of cancer, obesity is one of the biggest global health concerns. Obesity is now considered a global epidemic by the WHO. Obesity results from an unjustified increase in adipose tissue

Received: 24.06.2024 ***Accepted: 04.08.2024*** ***Published on: 30.09.2024***
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caused by excess energy intake over energy expenditure [1, 2]. Has turned into a noteworthy public medical challenge in both industrialized & other territories because of its rising incidence and connections to chronic conditions such Non-alcoholic liver disease, osteoarthritis, Insulin intolerance, hyperlipidemia, hypertension, and cardiovascular diseases [3, 4]. When prescribed medications for obesity are stopped, there is frequently a rebound in weight gain and unfavorable aftereffects. Therefore, there is a dire need for better obesity prevention and control techniques. A high dietary fibre consumption lowers the risk of several chronic diseases, including diabetes, obesity, gastrointestinal problems, and cardiovascular diseases [5, 7, 8]. Experimental and epidemiological investigations have demonstrated this. Giving rodents a high-fibre meal helps prevent their body weight and fat mass from increasing due to a high-fat diet. Moreover, increasing dietary fiber intake can help people lose weight and curb their appetite [9, 10, 11]. Recent research suggests that the gut microbiota may create SCFAs as a byproduct of fermenting insoluble fiber, mainly resistant starch and dietary fiber [3, 6]. This could potentially act as a partial mediating mechanism for these effects. Large dietary fibre intakes, however, are typically not well tolerated due to gastrointestinal side effects. Clinical trials using SCFAs have thus been conducted, and the outcomes have demonstrated the positive impact of SCFAs for alleviating UC, Crohn's disease, and obesity [12, 13, 14]. Putting greater effort into understanding the processes by which SCFAs lower body weight could lead to more successful weight control. The amounts of SCFAs, which have a major impact on the host's energy balance, may be determined by the makeup and relative abundance of the gut microbiota [15, 16]. After passing through the portal vein and the intestinal barrier, SCFAs can directly enter the circulation and have a significant impact on metabolism.

GPCRs, such as FFAR, are bound by SCFAs. FFAR1, formerly known as GPR40, FFAR4 (GPR120), FFAR2 (GPR43), and FFAR3 (GPR41) are the four members of this receptor family. SCFAs have a stronger affinity for binding to FFAR2 and FFAR3. Through GPCRs, SCFAs raise GLP-1 and PYY in the stomach and lower leptin, one of the tissue markers associated with obesity, in adipose tissue [15, 16]. In this work, we look at and evaluate how probiotics and SCFAs prevent obesity in Sprague Dawley rats by examining and comparing their effects on amount of food consumption, body weight gain, glucose metabolism, leptin, and enteric hormones (GLP-1 and PYY). We demonstrate that probiotics and SCFA`s inhibit food intake, guard against weight gain and glucose intolerance brought on by high-fat diets, and primarily promote the release of gut hormones through FFAR3-independent processes.

Material and Methods

SCFAs

SCFAs like Butyrate and Propionate are organic monocarboxylic compounds characterized by an aliphatic chain containing 1 to 6 carbons [17, 18]. Sodium salts of these SCFAs are procured from UV Scientifics, Habsiguda, Hyderabad, Telangana. These are highly soluble in water.

Probiotics

Selected probiotics like Lactobacillus and Bifidobacterium were procured from INLIFE Health Care, Himayat nagar, Hyderabad, Telangana.

Experimental Animals and Diet

We bought our animals 8-12 weeks 150–200 gram Sprague Dawley rats in Mahaveer Enterprises in Hyderabad. We got the HFD and the normal diet from the NIN in Tarnaka, India. The animals were held in a routine lab setting

with a 12-hour photoperiod, a temperature of 25 ± 2 °C, and 65% RH. Over the span of a week, the animals were permitted unrestricted food and water consumption before their evaluation in the lab animal facility [18, 19, 20]. HFD composition mentioned in Table 1:

Table 1. Composition of High-Fat Diet

Sr. No	Ingredient Name	Quantity(g/kg)
1	Casein	25.85
2	L-Cystein	0.39
3	Maltodextin	16.15
4	Sucrose	8.89
5	Soyabean Oil	6.46
6	Lard	3.23
7	Cellulose	31.66
8	Di-calcium Phosphate	1.68
9	Calcium Carbonate	0.71
10	Potassium Citrate	2.13
11	Vitamin Mix	1.29
12	Mineral Mix	1.29
13	Choline bitartarate	0.26

Acute Toxicity Studies

IAEC of Sarojini Naidu Vanitha Pharmacy Maha Vidyalaya, located in Tarnaka, Secunderabad, Telangana, India (registered under CCSEA no.: 287/R/S/2000/CPCSEA, IAEC No: SNV/10/2023/PC/23) approved every experimental procedure carried out in compliance with guidelines. Throughout the investigations, the protocols for handling and providing care for laboratory animals were adhered to. This study was conducted using wistar rats, adhering to the guidelines outlined in OECD 423. Through acute toxicity studies results $1/3^{th}$ and $1/5^{th}$ doses were selected as low and high doses of SCFAs, high dose was selected for Antiobesity activity i.e 400mg/kg [20].

Experimental Design

Six groups of six rats each were randomly selected from among 150–200 g of rats (n = 6) and G1 was fed a regular pellet that met AIN-93 recommendations for vitamins and minerals. Over the course of the 12-week experiment, the rats in the distinct group were fed a high-fat diet. Initially, rats were treated with Antibiotics for three days to clear gut microbiota. The faecal inoculum was prepared and cultured in culture plates and kept for incubation for 24 hrs to confirm the absence of microbiota.

G1: Normal Control group (NC)
G2: HFD
G3: HFD + Atorvastatin 10 mg/kg p.o
G4: HFD + Probiotics 10^6 CFU p.o
G5: HFD + Butyrate 400 mg/kg p.o
G6: HFD + Propionate 400 mg/kg p.o

OGTT

An OGTT was carried out following the 12^{th} week trial's conclusion. Following an overnight fast, blood samples were taken from the supraorbital sinus at 0, 30, 60, 90, and 120 minutes, and an oral glucose dose of 2.0 g/kg body weight was given. At every interval, glucose levels were assessed [22]. Using a glucose kit and a semi-auto analyzer, blood glucose was estimated.

Evaluation of Biochemical Parameters

Using the retro-orbital puncture technique, blood was drawn from animals that had fasted

for the whole night while under 2% anesthetic. To get plasma or serum, blood was drawn into two separate vials and centrifuged at 2500 rpm for 15 minutes [21]. To estimate the biochemical parameters, Serum was extracted and kept at -20°C in a refrigerator. Lipid profile including LDL, total cholesterol, HDL, and VLDL cholesterol, SGPT, SGOT, total protein, ALP and Leptin were among the biochemical markers that were examined [23, 24,25]. The formula was used to compute the AI.

Measurement of Serum GLP-1 & PYY

ELISA method was used to measure serum GLP-1 & PYY levels in Osmania Medical College (Dept. Microbiology) ELISA kits were procured from TMR Scientific, Secunderabad. GLP 1- ELK8502, 48T, 4.92 pg/ml (sensitivity) PYY - ELKS5984, 48T, 4.71 pg/ml (sensitivity) [26].

Measurement of Liver markers

Using a semi-auto analyzer, AST, ALT, and ALP levels were gauged using analytical kit techniques by the manufacturer's protocol.

Assessment of Fasting Insulin and Serum Leptin Levels

Rats were allowed to fast for the entire night to evaluate the amount of leptin & fasting insulin. Blood was then extracted using the retro-orbital puncture procedure. Samples were sent Micron Life Sciences for the estimation.

Histopathological Studies

Histopathological examinations were conducted in organs like liver and pancreas. The principal organs surgically removed & immersed in 10% buffered formalin solution. Following fixation, tissue samples underwent dehydration using a series of ethanol solutions followed by washing in toluene, and subsequent embedding in liquid paraffin. Next, tissue sections with a thickness of 5µm were acquired, followed by staining with haematoxylin-eosin (HE). The tissue sections were subsequently examined for pathological analysis, and photomicrographs were captured.

Statistical Analysis

The results of the computation were shown as Mean ± Standard Deviation. One-way ANOVA was used to evaluate the collected data to find significant differences. Significant changes in between groups were assessed using Dunnett's t-test. There was a significant $p < 0.05$. For statistical analysis and data display, Microsoft Excel 2013 Standard (Microsoft Corp., Redmond, WA, USA) and Graph Pad in Stat Version 3.06 (Graph Pad Software, Inc., La Jolla, CA, USA) were used.

Results

SCFAs Inhibit Food Intake & Body Weight Gain

As can be seen in Figure 1. after the study period, rats given the HFD increased in BW & food intake more than the group fed the control diet ($p< 0.05$). The rats also gained weight gradually over time. Probiotics and SCFAs were administered, and their effects significantly inhibited the HFD-induced weight gain ($p< 0.05$) (Figure 1). The HFD-fed rats showed an increase in food consumption two weeks following the feeding intervention ($p< 0.05$). After that, compared to the control group, energy intake continued to be higher ($p< 0.05$), and there were food consumption did not change at 10 or 12 weeks. Administration of probiotics & SCFAs showed significant reduction in food intake & body weight at the nutrition intervention ($p<0.05$).

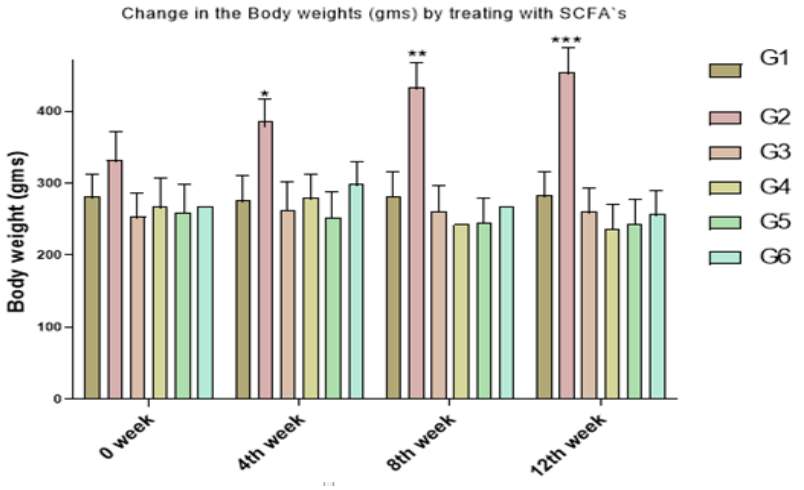

Figure 1. Impact of SCFAs and Probiotics on Animal Body Weight in Control and Treated groups

Each value represents mean ±SD. Data were analyzed by using ANOVA followed by Dunnett's multiple comparison test. Each group was compared standard *represents p<0.05, **indicates p<0.01**,** ***indicates p<0.001.

Effect of Probiotics & SCFA on Glucose

After the glucose load, the blood glucose level in rats fed a regular diet peaked 60 minutes later and then fell to nearly basal values after 120 minutes. In rats on an HFD-induced obesity diet, on the other hand, the blood glucose level peaked 60 minutes later and stayed high for the following 60 minutes. It is noted that, in comparison to HFD treated rats, the administration of SCFAs & Probiotics resulted in a considerable drop in blood glucose levels at 60 minutes and beyond.

Effect of Probiotics & SCFA on Lipid Profile

As depicted in Figure 2, 3, 4, 5. the high-fat diet feed group had higher serum levels of triglycerides, CHL, LDL (P< 0.05), and reduced HDL levels. Probiotic and SCFA administration resulted in a lower lipid profile and mitigated the HFD-induced increases in TGs, CHL, and LDL. Treated groups showed significant elevation in HDL levels.

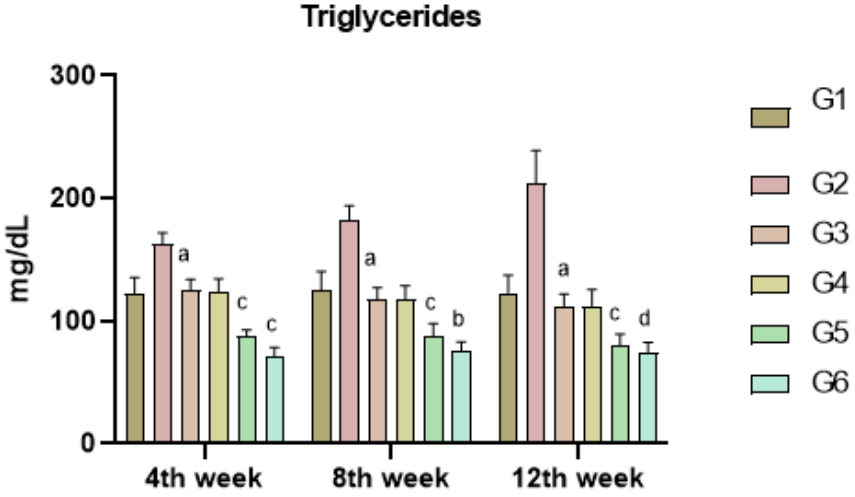

Figure 2. Impact of SCFAs and Probiotics on Triglycerides in Various Groups

Each value represents mean ±SD. Data were analyzed by using ANOVA followed by Dunnett's multiple comparison test. Each group was compared disease control a represents p<0.05, b indicates p<0.01**,** c indicates p<0.001.

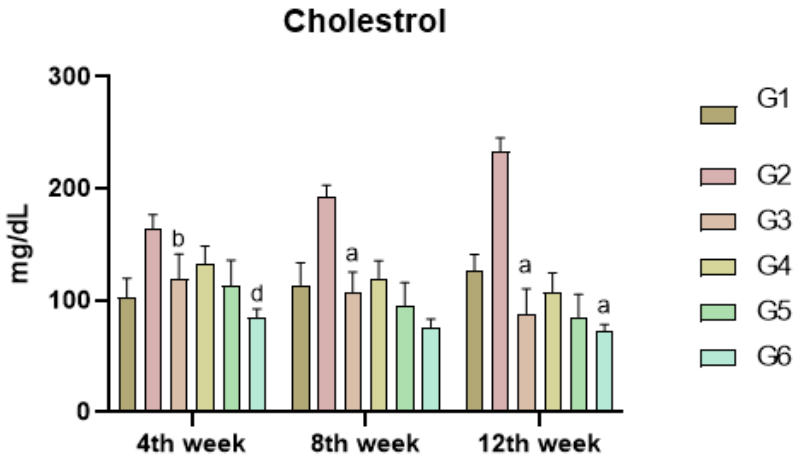

Figure 3. Impact of SCFAs and Probiotics on Cholesterol in Various Groups

Each value represents mean ±SD. Data were analyzed by using ANOVA followed by Dunnett's multiple comparison test. Each group was compared disease control a represents $p<0.05$, d indicates $p<0.0001$.

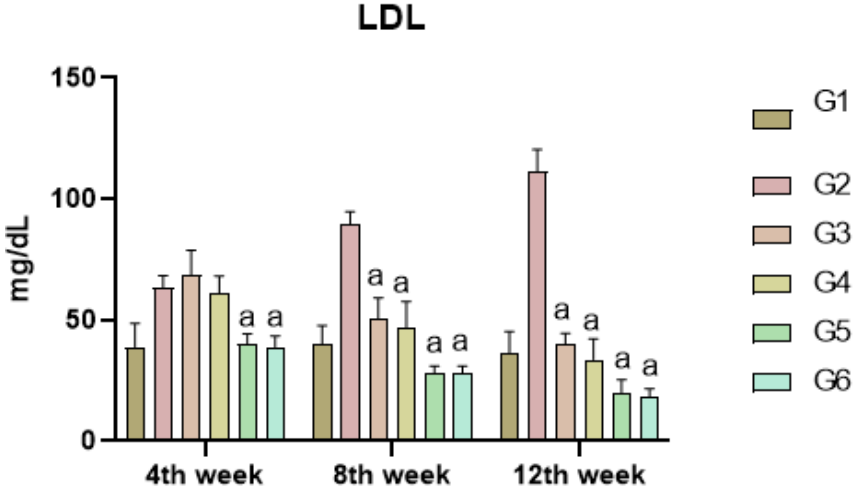

Figure 4. Impact of SCFAs and Probiotics on Low Density Lipoprotein Levels in Various Groups

Each value represents mean ±SD. Data were analyzed by using ANOVA followed by Dunnett's multiple comparison test. Each group was compared standard a represents $p<0.05$.

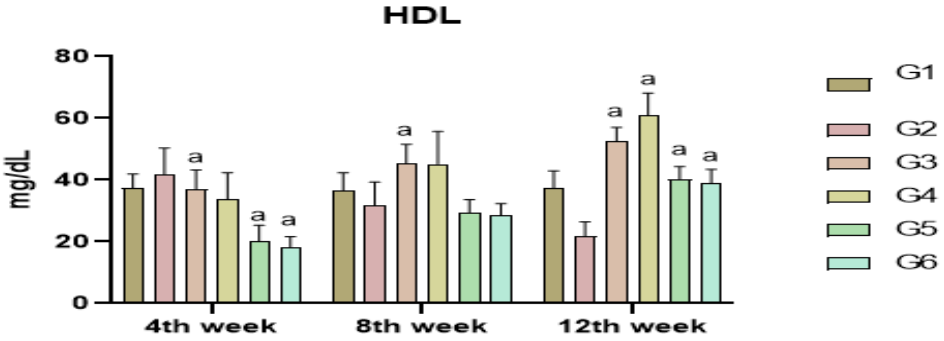

Figure 5. Impact of SCFAs and Probiotics on High Density Lipoproteins in Various Groups

Each value represents mean ±SD. Data were analyzed by using ANOVA followed by Dunnett's multiple comparison test. Each group was compared disease control a represents $p<0.05$.

Effect of Probiotics & SCFAs on GLP 1 & PYY

Figures 6 & 7 shown significantly decreased serum levels of GLP 1 & PYY in rats given HFD. However, 400 mg/kg of SCFAs and oral probiotics (10^6 CFU) have significantly increased these levels.

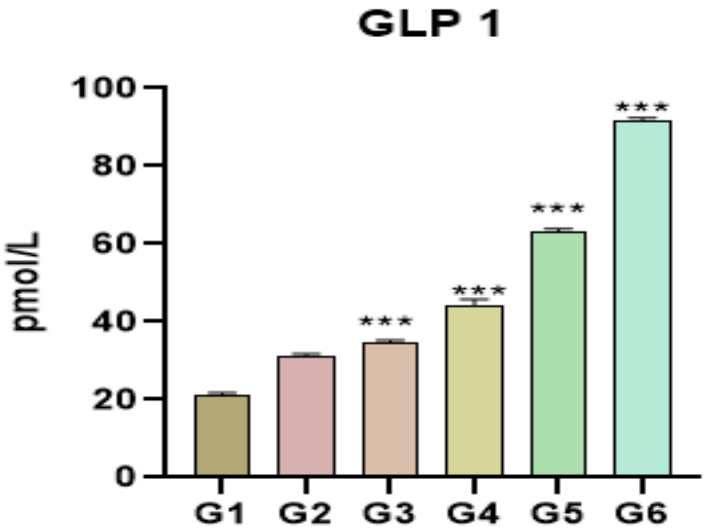

Figure 6. Impact of SCFAs and Probiotics on GLP 1 in Control and Treated groups

Each value represents mean ±SD. Data were analyzed by using ANOVA followed by Dunnett's multiple comparison test. Each group was compared disease control ***indicates p<0.001.

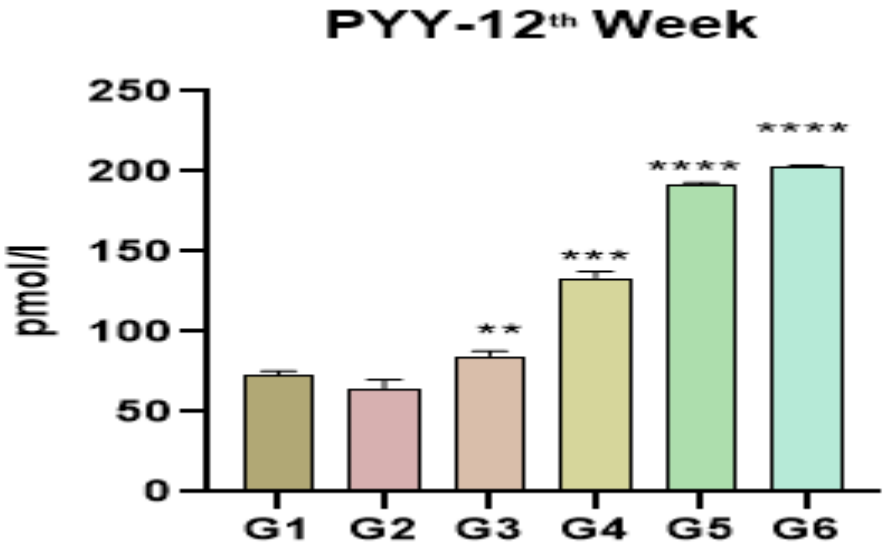

Figure 7. Impact of SCFAs and Probiotics on PYY in Control and Treated Groups

Each value represents mean ±SD. Data were analyzed by using ANOVA followed by Dunnett's multiple comparison test. Each group was compared disease control **indicates p<0.01**,** ***indicates p<0.001, ****indicates p<0.0001.

Impact of Probiotics & SCFAs on Liver Enzyme Markers

The blood levels of liver-specific enzymes are displayed in Figure 8, 9, 10. Rats given a HFD had significantly increased serum values of liver markers. However, 400 mg/kg of

SCFAs and oral probiotics (10^6 CFU) have significantly reduced these levels.

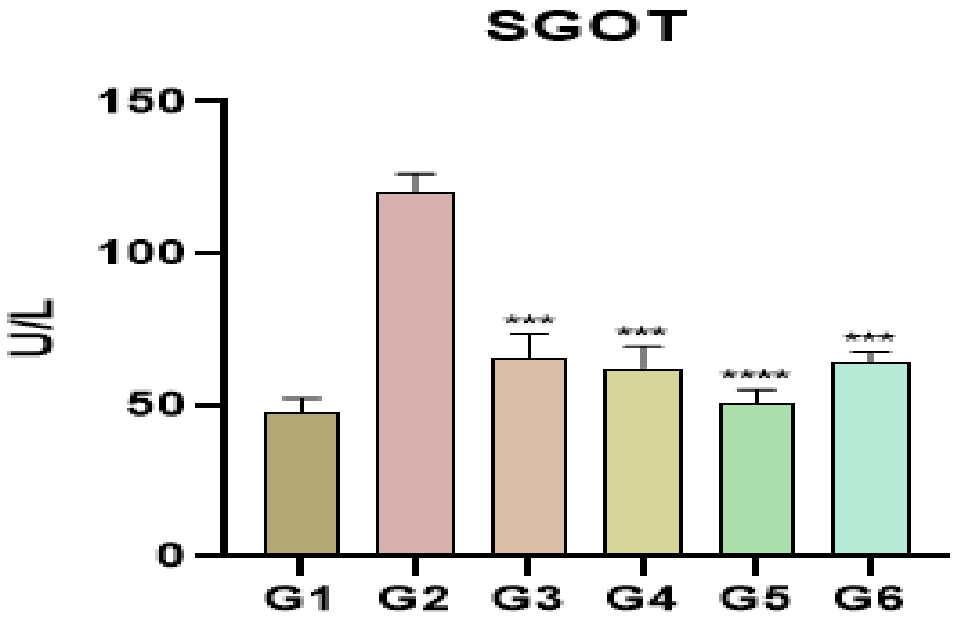

Figure 8. Impact of SCFAs and Probiotics on SGOT

Each value represents mean ±SD. Data were analyzed by using ANOVA followed by Dunnett's multiple comparison test. Each group was compared disease control ** indicates p<0.01**,** *** indicates p<0.001, ****indicates p<0.0001.

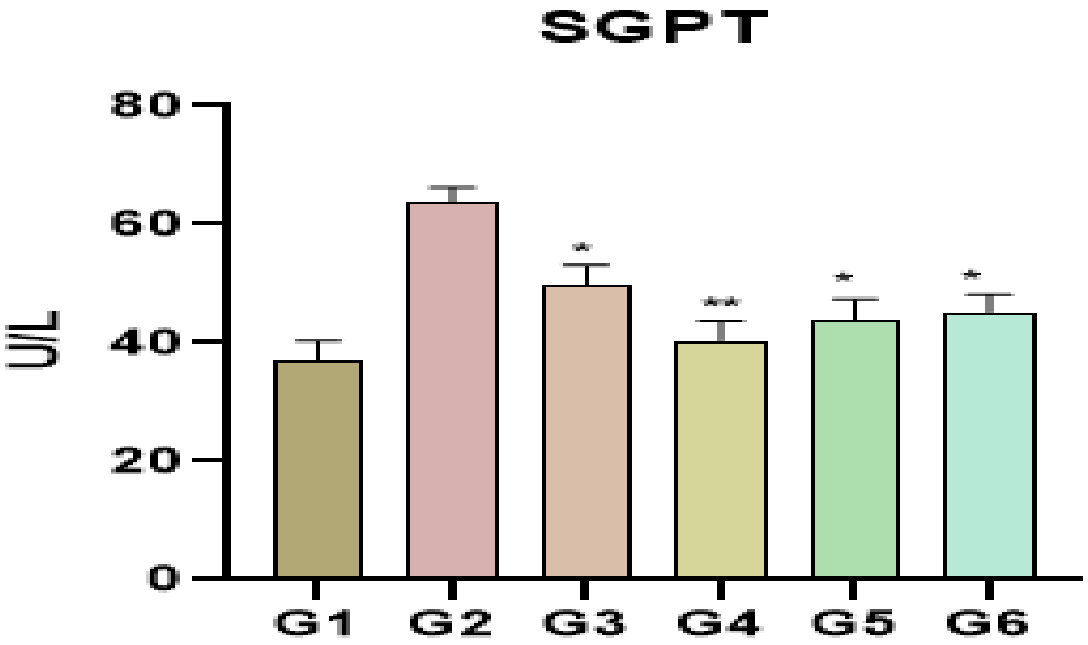

Figure 9. Impact of SCFAs & Probiotics on SGPT

Each value represents mean ±SD. Data were analyzed by using ANOVA followed by Dunnett's multiple comparison test. Each group was compared disease control **indicates p<0.01.

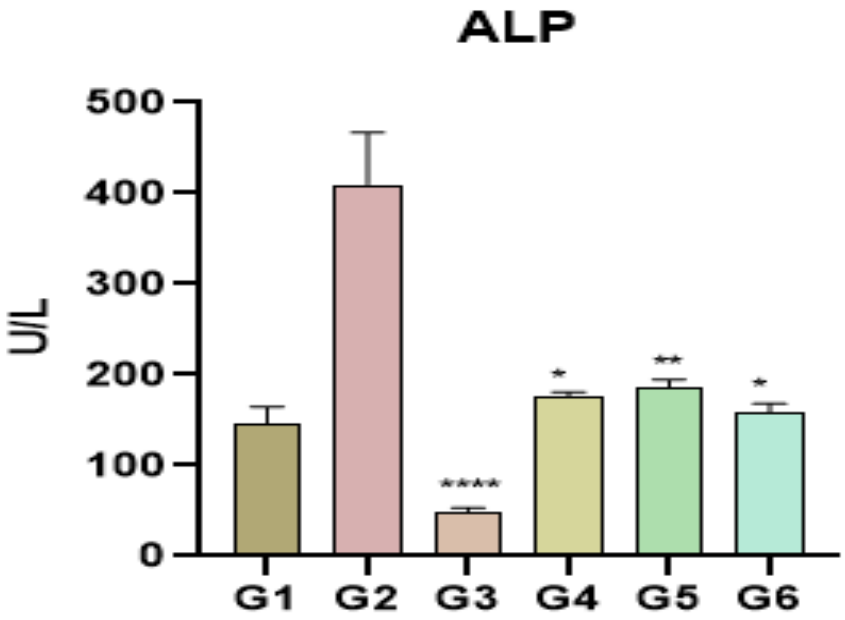

Figure 10. Impact of SCFAs & Probiotics on ALP

Each value represents mean ±SD. Data were analyzed by using ANOVA followed by Dunnett's multiple comparison test. Each group was compared disease control **indicates $p<0.01$**,** ****indicates $p<0.0001$.

Effect of Probiotics & SCFAs on Leptin & Fasting Insulin

When comparing the G1, G2 animals showed a significant increase in leptin & Fasting Insulin levels. It's interesting to note that animals treated with oral 400 mg/kg of SCFAs & probiotics has a considerable decrease in leptin & Fasting Insulin levels when comparing with G2 to the normal control group [21]. Surprisingly, oral SCFA 400 mg/kg more distinctively ($p < 0.05$) decreased in these values than probiotics in HFD-fed rats depicted in Figure 11 & 12.

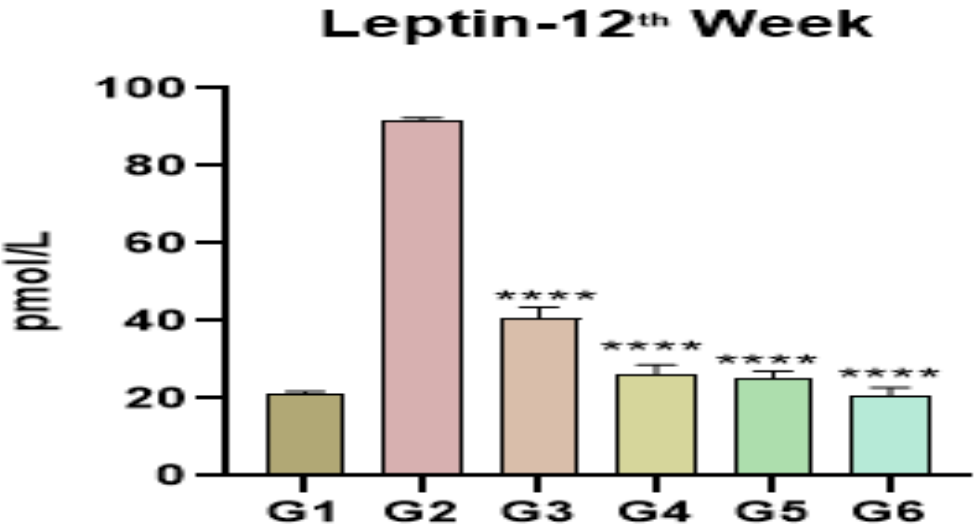

Figure 11. SCFAs & Probiotics on Leptin Levels

Each value represents mean ±SD. Data were analyzed by using ANOVA followed by Dunnett's multiple comparison test. Each group was compared disease control ****indicates $p<0.0001$.

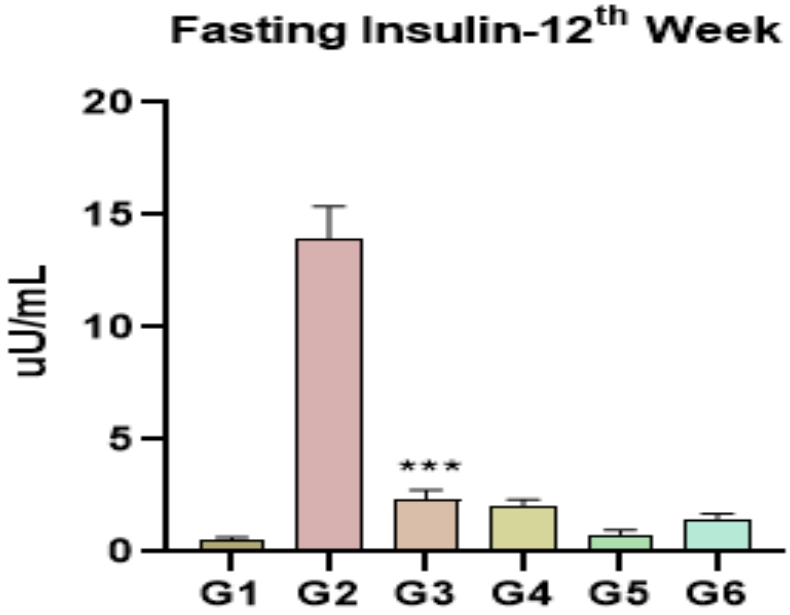

Figure 12. SCFAs & Probiotics on GLP-1

Each value represents mean ±SD. Data were analyzed by using ANOVA followed by Dunnett's multiple comparison test. Each group was compared disease control ***indicates $p<0.001$.

Histopathology

Adipocytes in HFD groups significantly enlarged as a result of consuming high-fat diets. Figure 13 illustrates increased fat deposition and the corresponding notable adipocyte size expansion with steatosis in positive conrtro group (G2). We compare the G1 animals with the HFD-fed animals showed larger adipocytes and greater fat deposits. It's shown that the administration of Probiotics & SCFAs (400 mg/kg) has significantly decreased adipocyte size, fat accumulation illustrated in Figure 13 & 14 respectively comparable to atorvastatin.

In the control group, the hepatocytes were observed to be normal with an intact portal vein and centrilobular region. In the positive control group, steatosis along with ballooning of hepatocytes was noted [21]. The probiotics group exhibited a focus of inflammatory cells in the centrilobular region. In the butyrate treated group, there was multifocal periportal infiltration of immune cells. Conversely, the propionate treated group showed normal morphology of hepatocytes, portal, periportal, and centrilobular regions with no abnormalities observed.

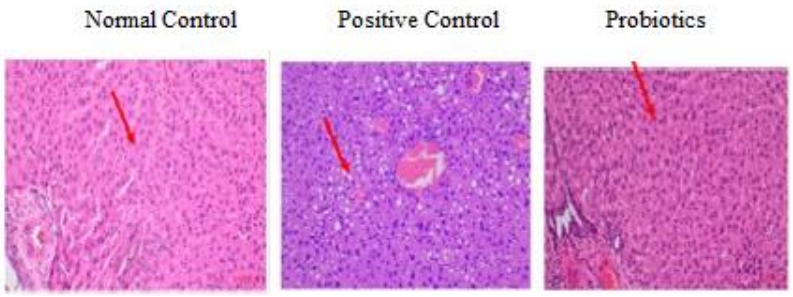

Figure 13. Liver Histopathology in Animals in Normal Control, Positive Control & Probiotics

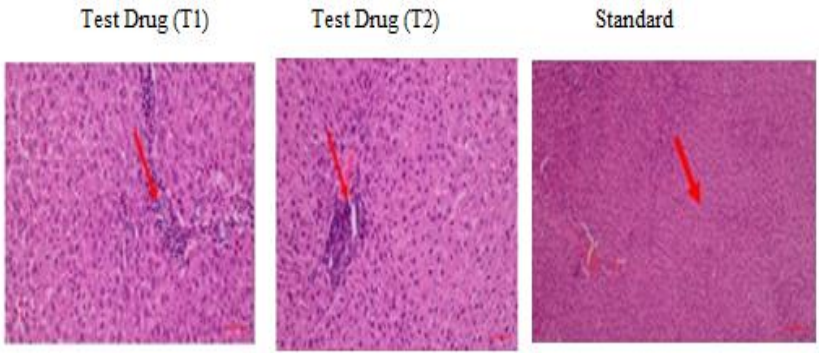

Figure 14. Liver Histopathology in Animals Treated with Butyrate (T1), Propionate (T2) and Rosuvastatin (SD)

Discussion

There has been much discussion on the vital role that gut bacteria play an important role in host metabolism. Various studies established the significant alterations in gut microbiota are linked to metabolic diseases [27]. Mechanistic understanding is, however, inadequate, and it is unclear if microbial dysfunction affects the pathophysiology of metabolic disorders. Specifically, little is known about the interactions between host food sensing mechanisms and metabolites produced by the microbiota, including SCFAs, that influence energy metabolism. In this investigation, we closely looked at the impacts on gut hormones and energy metabolism of probiotics & SCFAs occurring naturally in the colon. We found that probiotics and SCFAs prevented diet-induced obesity. Because SCFAs stimulate anorexigenic gut hormones, they also reduce food intake, which helps to manage body weight. Probiotics, on the other hand, inhibited weight gain even in the absence of dietary restriction and had an instantaneous effect on gut hormones [26]. To ascertain the effects of these pathways concerning energy balance in chronic situations, more investigation will be required. Food intake, lipid profiles, and body weight all significantly decreased after Administration of probiotics and SCFAs. Probiotics and SCFAs significantly decreased fasting insulin and leptin levels while significantly increasing GLP-1 and PYY levels. Rats on (HFD) acquired visceral obesity, insulin resistance, hyperglycemia, dyslipidemia, hepatocyte enlargement or ballooning, and hepatic steatosis all of which are clearly associated with obesity [27]. The GLP-1 and PYY plasma levels of rats administered oral SCFA for ten minutes and an hour increased significantly. When it comes to diet-induced obesity, SCFAs have a major anti-obesity effect by raising GLP-1 and PYY levels while lowering leptin levels. When consumed with dietary fiber, probiotics will raise SCFAs and have a notable anti-obesity effect [18, 19, 20]. It is demonstrated that SCFAs have greater anti-obesity activity than usual. When hypocholesterolemic and anti-obesity medications lower serum lipid concentrations, the clinical consequences of atherosclerosis and obesity may be lessened. Probiotics and SCFAs were administered to HFD-fed rats in the current investigation, and this resulted in a considerable improvement in their lipid profiles, as shown reduced CHL, TGs, and LDL & elevated HDL, all leading to a healthy atherogenic index. According to histopathology studies, both probiotics and SCFAs have anti-obesity effect by avoiding hepatocyte ballooning, which is shown in the positive control group, and by reducing hepatic steatosis [18, 19, 20]. Liver function tests are helpful instruments for ascertaining the liver's present level of operation because the liver is an essential organ involved in both general metabolism and drug detoxification [27].

Conclusion

In summary, our results demonstrate that SCFAs significantly reduce the levels of leptin

and increase GLP-1 and PYY in diet-induced obesity. When consumed with dietary fiber, probiotics will raise SCFAs and have a notable anti-obesity effect. It is demonstrated that SCFAs have greater anti-obesity activity than usual. Through GPCR, SCFAs lower body weight and food intake, which is the main way they prevent diet-induced obesity. Lastly, we propose that the chosen SCFAs and probiotics may be utilized as a possible therapeutic substitute for the oversight of diet-induced obesity & hyperlipidemia.

Conflict of Interest

The authors of this study confirm that there are no competing interests

Acknowledgement

Authors convey thanks to Sarojini Naidu Vanitha Pharmacy Maha Vidyalaya, GITAM School of Pharmacy, and Centre for Global Health Research, Saveetha Medical College, Saveetha Institute of Medical and Technical Sciences, Saveetha University for providing necessary facilities to complete the works.

Source of Funding

None

Author Contribution

Planning the research, carrying out the project, and writing the manuscript: Rajyaakshmi Devi. P; Designing the study, statistical analysis, manuscript drafting: Vinod kumar Nelson, M.Vinyas; Biochemical evaluation and ELISA testing: Mote Srinath.

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