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# Dietary Supplementation of *Lactobacillus brevis* Normalizes Metabolic Parameters in Mouse with Obesity and Hyperglycemia

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** Exploration of the ameliorative effects of *Lactobacillus brevis* on glycemic and obesity parameters in high fat diet (HFD) fed hyperglycemic Swiss albino mice.
**Study Design:** HFD with 58% fat, 25.6% carbohydrate, and 16.4% protein was fed to mice to develop metabolic syndrome. The experimental group of obese mice was fed with oral supplement of *L. brevis* for 28 days and the metabolic parameters of the mice are compared with control groups. The experiments were performed in triplicate.
**Place and Duration of Study:** The study was executed in the Laboratory of Immunology and Microbiology, Department of Zoology, University of North Bengal situated in the Darjeeling district of West Bengal, India in 2020-21.
**Methodology:** 'ND', fed with normal pellet-based diet; 'HFD', fed with HFD diet and 10% sucrose water and 'HFD + LB', fed with diet same as 'HFD' group along with $1\times 10^8$ CFU of *L. brevis* (for each animal/day) from 22$^{nd}$ day onwards. At the end of experiment (on 50$^{th}$ day) the body weights, Oral glucose tolerance test (OGTT), fasting blood glucose (FBG) levels and serum Lipid Profile were measured.
**Results:** the HFD group showed significantly high FBG levels (203.66 ± 8.73 mg/dL) when compared with the ND group (130± 2 mg/dL) but the FBG levels in HFD + LB group (163.66 ± 4.72

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mg/dL) were not as significantly higher as the HFD group. OGTT test revealed *L. brevis* helped to resist the persistent hyperglycemic effects. It was seen that the levels of lipids like Triglycerides and Total cholesterol in HFD + LB group were in normal range and similar like that of the ND groups (172.22 ± 3.854 mg/dL and 134.89 ± 4.705 mg/dL).

**Conclusion:** These findings indicate strong association for probiotic use of *L. brevis* as a potent preventative measure to combat the detrimental metabolic changes.

*Keywords: Metabolic syndrome; obesity; type 2 diabetes mellitus; white adipose tissue; short chain fatty acids; firmicutes; Lactobacillus brevis.*

## 1. INTRODUCTION

Metabolic syndrome is a group of medical conditions that raise the risk of acquiring diabetes. The metabolic syndrome, like many other disorders, is caused by a complex combination of hereditary and environmental factors. Sedentary lifestyle, overconsumption and intake of calorie enriched junk foods are factors contributing to rising number of people with metabolic syndromes like Obesity and Diabetes throughout the world [1-2]. Out of the two main types of diabetes the less common (approximately 5% of all diabetic patients) type 1 diabetes mellitus (T1DM) is an autoimmune disorder where pancreatic β cells are destroyed leading to insufficient production of insulin. On the other hand, the more common type 2 diabetes mellitus (T2DM) which is also known as adult-onset diabetes develops when the β cells either fail to secrete sufficient amount of insulin or the host tissue becomes insensitive to insulin (insulin resistance) [3]. Prolonged elevated levels of glucose in systemic circulation in diabetic patients can lead to a variety of other complications in renal, nervous, and cardiovascular system [4]. In T2DM, glucose access is blocked as membrane receptors are desensitized to promote hypoglycemic insulin-mediated cellular fuel-filling, which may change osmo-redox equilibrium and feed-behaviour/energy expenditure balance [5]. Despite the occurrence of genetic abnormalities in some cases, nearly 90% of patients with T2DM are overweight in spite of the fact that cell starvation is high, indicating that the disease is more acquired than innate [6]. There are numerous reasons for developing insulin resistance but the most important among them is the obesity associated chronic low grade systemic inflammation [7] Obesity is found to be linked to a low-grade inflammation of white adipose tissue (WAT). WAT has been discovered to play a role in a variety of physiological and pathological processes. Increased synthesis and release of inflammatory molecules like Tumor Necrosis Factor alpha (TNF-α) and interleukin-6 (IL-6) are associated with obesity-related WAT. These cytokines may not only have local impacts on WAT physiology but also have systemic implications [8]. Subcutaneous visceral adipose tissue depots differ in various respects, including cell size, metabolic activity, and potential function in insulin resistance. Visceral adiposity is the best predictor of insulin resistance and eventual T2DM [9]. Diet reach in saturated fat and simple sugar may be associated with metabolic changes like initiation of the inflammatory cascade via WATs or by inducing the dysbiosis of gut microbiota which also in turn initiates inflammatory cascades. These inflammatory processes can lead to insulin resistance and T2DM [10]. Human gut microbiota is reported to have six dominant phyla namely, Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Verucomicrobia and Proteobacteria [11]. There are several human and animal studies which reported that both High Fat Diet (HFD) and obesity may be positively associated with the composition of gut microbiota diversity and may have major impact on both immunological and metabolic functions of the host [12]. A decrease in the Bacteroidetes and increase in both the Firmicutes and Proteobacteria has been reported after persisting high fat diet (HFD) intake [12]. Some compositional studies reported that obese human and animal have gut microbiota with less phylogenetic diversity than lean controls. Moreover, fecal transplantation experiment of gut microbiota from obese to germfree mice has been shown to transfer the obese phenotype [13]. WAT proliferation due to high calorie consumption and HFD induced microbiome dysbiosis, can lead to chronic low-grade inflammation and eventual insulin resistance.

Obesity and eventual T2DM is a major health concern worldwide, and the traditional therapeutic options for treating T2DM are limited, due to the complex and interrelated pathophysiological scenario. Some limited therapeutic options for treating T2DM are

Sulfonylureas, Biguanides, Thiazolidinediones [14]. But these drugs have their own limitations and adverse contraindications like hepatic, renal and cardiovascular complications when used for longer durations [15]. Moreover, these drugs are used for regulation of glucose levels when T2DM is to an irreversible extent, including the systemic insulin resistance. At present there are no medically accepted options that are available as prophylactic measures to avoid the insulin resistance and eventual T2DM. Gut microbiome is treated as a distinct organ system in our body. Short Chain Fatty Acids (SCFAs) particularly acetate, butyrate, propionate are the most abundant microbial metabolites produced by the fermentation of non-digestible carbohydrates. The main producers of SCFAs are the ones that belong from the group Firmicutes [16]. Studies revealed that SCFAs have a diverse role in cell growth and differentiation, promotion of gut epithelial integrity, anti-inflammatory and immunomodulatory functions [16-17]. SCFA also regulates pancreatic beta cell proliferation and insulin biosynthesis [18] Moreover, it is also shown that intestinal inflammation is modulated by SCFA involving regulatory T cells [16]. SCFA has the ability to bind to G-protein coupled receptor-43 (GPR-43/FSA-2) that are expressed on enteroendocrine, intestinal epithelial cells and islets of Langerhans. Activated GPR-43 induces the synthesis of the gut hormone Glucagon Like Peptide (GLP-1). GLP-1, improves pancreatic beta cell function and insulin secretion [19]. So, activation of GPRs by SCFA improve insulin sensitivity, control blood glucose level and reduce systemic inflammation.

Recently the focus of scientific community is shifting towards microbiome research in different disease settings. *Lactobacillus brevis* is an obligately heterofermentative lactic acid bacterium (LAB), which is gram-positive and rod-shaped. It is a member of the Lactobacillaceae family under the order Lactobacillales and the Phylum Firmicute [20]. It can be found in a variety of places, including fermented foods, pickles and natural gut microbiota. Arabinose, fructose, glucose, gluconate, maltose, melibiose, and ribose are among the carbohydrates that are fermented by *L. brevis*. It is used worldwide for the production of various traditional fermented foods and used in the food preservation industry. *L. brevis* is regarded suitable for probiotic use since it grows well at pH levels of 4–5, which is the usual range for milk and yoghurt [21]. Many strains of *L. brevis* have been reported to have potent probiotic properties. For example, *L. brevis* KU200019 has been found effective against food borne pathogens [22]. The aim of the present study is to explore the ameliorative effects of *L. brevis* on hyperglycemia and obesity parameters in HFD fed, hyperglycemic Swiss albino mice.

## 2. MATERIALS AND METHODS

### 2.1 Procurement and Growth Curve Analysis of *L. brevis*

*L. brevis* strain LS-SR1 was procured as freeze-dried culture from Microbial Type Culture Collection, Chandigarh, India (MTCC Collection Acc. No 1750). The optimum growth kinetics for the strain was accessed according to standard protocol [23] with some modifications. Briefly, 100µL of inoculum from overnight liquid culture of *L. brevis* were transferred to 5 MRS broths (10 mL each) and were incubated at $37^0$C for 0, 60, 120, 180, 240 minutes. For each time frame, broths were taken, serially diluted and plated on MRS agar plates and then plates were incubated for 24 hours at $37^0$C. From the same broth optical density was taken at 600nm wavelength. On the next day, colony forming units (CFU) were calculated from the plates. Then the values of the $OD_{600}$ and Log CFU/mL were used to determine the growth kinetics for the specific strain.

### 2.2 Animal Procurement

For the study, 6 to 8 weeks old Swiss albino mice (20 ± 2 grams) were obtained from approved animal dealers (Chakraborty Enterprise, Kolkata, India; Regd. No. 1443/ PO/Bt/s/11/CPCSEA). The animals were housed in polypropylene cages (Tarsons, India) in the animal house of the Department of Zoology, University of North Bengal, Darjeeling, India. They were kept at a regular room temperature of 20°C to 22°C with a relative humidity of 30% to 70% and a light-dark cycle of 12:12 hours. The mice were given pellets as normal diet and were kept for acclimatization for 2 weeks.

### 2.3 Acute Oral Toxicity Test

In order to access the safety concern and for ensuring the safe feeding strategy for the bacterial strains acute oral toxicity test was done according to the Organization for Economic Co-operation and Development (OECD) Guideline for the Testing of Chemicals (No. 423, Acute Oral Toxicity, Acute Toxic Class Method, adopted

December 17, 2001).Six Swiss albino mice of both sexes were placed together in equal numbers (3 males and 3 females). After 2 weeks of acclimatization and a 16-hour fast, each group received a single dose *of L. brevis* (2× $10^8$ CFU) in 0.5 mL sterile water via oral gavage. Aggression, food intake, water consumption, sedation, diarrhea, fur loss, and lethargy symptoms were all observed constantly for the first half hour and then every 24 hours after the bacterial suspension was administered. For a period of two weeks, all of the mice were checked separately, with special attention paid to any late indicators of toxicological effects during the first four hours of each day.

## 2.4 Preparation of Diets and Feeding Suspensions of *L. brevis*

For the preparation of HFD, standard protocol [24] along with certain experimental modifications were followed. Commercial butter, Commercial ghee and vegetable oil along with 1% cholesterol were used as the source for fats (Saturated and unsaturated). Cornstarch, dextrose and sucrose were used as the source of carbohydrate. Casein and egg albumin was used for the source of proteins. All the ingredients were mixed in a ratio so that the overall nutritional profile of the HFD becomes 58% fat, 25.6% carbohydrate, and 16.4% protein. This preparation was mixed with standard pellet diet so that the energy content of the final diet becomes 8.64 kcal/g. Moreover, the animals that were feed HFD are also supplemented with 10% Sucrose solution for further aggravation of hyperglycemic state [25]. In order to feed the bacterial strain to experimental animals, the strains were cultured according to standard parameters for overnight. On the next day, inoculum from overnight grown cultures were given to fresh broths and incubated for 7 hours. Then OD at 600 nm was taken and plotted on the previously prepared growth curve to obtain the CFU present in that broth. Then accordingly the cultures were centrifuged and the pellets were resuspended in sterile water to obtain the desired CFU in 0.5mL sterile water solution. Then the experimental animals were orally administered the prepared 0.5ml sterile water solution containing 1× $10^8$CFU of *L. brevis* using micro gavage.

## 2.5 Animal Experimental Grouping and Duration

The experiments were performed in triplicate. In each set, there were total 9 male Swiss albino mice divided and grouped randomly according to the following heads:

ND: This group contained a total of three animals that were given normal pellet-based diet and normal water throughout the course of the experiment.

HFD: This group contained a total of three animals that were given experimental HFD diet and 10% sucrose water throughout the course of the experiment.

HFD + LB: This group contained a total of three animals that were given experimental HFD diet, 10% sucrose throughout the experiment. But from 22$^{nd}$ day onwards they were also administered *L. brevis* (1× $10^8$ CFU) suspended in 0.5ml of sterile water (for each animal/day) up to 49$^{th}$ day of the experiment.

The entire experimental duration was set for 50 days.

## 2.6. Tests Done for the Assessment of the Effects of *L. brevis* on Hyperglycemic and Lipidomic Parameters

### 2.6.1Determination of increment of body weight

Body weights were taken, at the starting of the experiment (Day 0) and at the end of the experiment (Day 50). Then for each mouse the body weight gain was determined by the following formula:

$$\%\ \text{of body weight gain} = \frac{(\text{Final Body Weight} - \text{Initial Body weight}) \times 100}{\text{Initial Body Weight}}$$

### 2.6.2 Fasting blood glucose (FBG)

FBG levels for experimental animals were determined on 49$^{th}$ Day of experiment. Briefly animals were fasted for 16 hours and after that one drop of blood was aseptically drawn from the tail vein using a sterile pricking needle and the blood glucose was measured using a handheld glucometer (Dr. Morepen BG-03, Morpen Laboratories Ltd., India).

### 2.6.3 Oral glucose tolerance test (OGTT)

On the last day of the experiment (day 50) the OGTT was performed. Glucose levels were measured from blood samples of overnight

fasted mouse (16 hours) by puncturing the tail vein with a sterile pricking needle. For OGTT the mice were administered a single oral dose of glucose (2 g/kg) and reading was taken just before the glucose administration (0 min) and after 30, 90, and 120 minutes of time interval using a handheld glucometer (Dr. Morepen BG-03, Morpen Laboratories Ltd., India) [26].

### 2.7 Sacrifice of Experimental Animals and Collection of Samples

At the end of the experimental duration (day 50) all of the experimental animals were euthanized with sodium pentobarbital (60 mg/kg; i.p.). Blood was drawn and centrifuged at 3000 rpm for 3 minutes. Serum was extracted and kept at -20 °C until the lipid profile was determined. Following blood collection, the perigonadal fat pads were isolated washed and weighed.

### 2.8 Assessment of Serum Lipid Profile

For the evaluation of serum lipid profile, standard colorimetric kits (No-1102040275; Coral clinical systems, India) were used. By following the manufactures instructions serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) (no-1102150040), and triglycerides (TG) (no-1102220025) were performed. TC, HDL-C, TG values were used for further calculation of low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and Non-HDL-C values according to Martin-Hopkin calculation [27].

### 2.9 Statistical Analysis and Graphical Representation

The results from different experimental parameters are expressed as mean ± SD. The statistical analysis was determined for all comparisons by using one-way analysis of variance (ANOVA). Whereas, in case of OGTT, two-way ANOVA is used. All the tests were further calculated for post hoc hypothesis testing using Dunnett's test for multiple comparison. For multiple comparisons the level of significance was set at $p < 0.05$. The significance values were plotted as 0.1234= ns, 0.0332= *, 0.0021= **, 0.0002= ***, <0.0001= ****. In every comparison testing the ND values of ND group was compared with the values of other two groups (HFD and HFD + LB), and only the p values <0.05 were plotted on the graphical representations. In case of the graphical representations of FBG results only the p values ≤0.0001 were plotted. The above-mentioned statistical analysis of all the experimental data was performed using GraphPad Prism 9 package (v 9.2.0; GraphPad Software Inc., California, USA). The graphs for the growth kinetics of *L. brevis* were prepared using Microsoft Excel.

## 3. RESULTS

### 3.1 Growth Curve Analysis of *L. brevis*

The rate of growth of *L. brevis* shows high rate of increase in OD during first 120 minutes ($OD_{600}$ = 0.0928, 0.2721, 0.749). The OD reached maximum and became stationary during in 180 and 240 mins ($OD_{600}$ = 1.5477, 1.7297) (Fig. 1A). The Log CFU/mL value also showed similar pattern where the values increased significantly until 120 mins (Log CFU/mL = 0, 2.741, 6.574) and became stationary in subsequent 180 and 240 mins (Log CFU/mL = 6.79, 6.95) (Fig. 1B). An empirical standard curve was plotted to relate the Log CFU/mL values with respective OD (at 600 nm) values (Fig. 1C).

### 3.2 Acute Oral Toxicity Test

After oral administration of fixed high dosage of bacterial suspension ($2\times 10^8$ CFU/animal) did not result in mortality or treatment-related toxicity symptoms. There was no difference in appearance or behavior, nor in weight or feed consumption. Based on the test feeding dosage has been selected ($1 \times 10^8$ CFU/Day/animal).

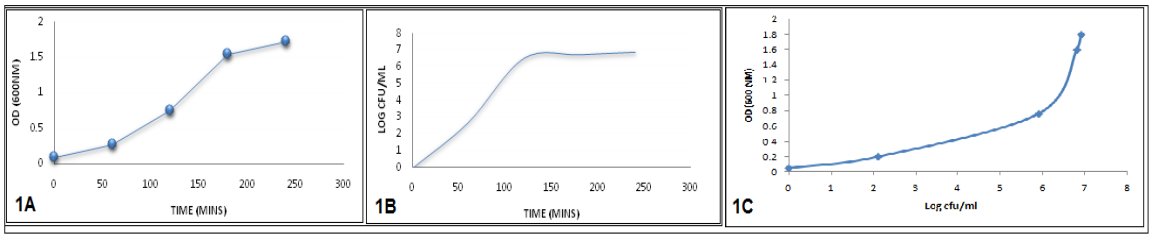


**Fig. 1. Growth kinetics of *L. brevis.* A. Measured in terms of $OD_{600}$ B. Measured in terms of Log CFU/mL C. Growth curve encompassing $OD_{600}$ Vs Log CFU/mL**

### 3.3 Effects of *L. brevis* on Body Weight Gain, Fat Accumulation (Weighed in g) in the Perigonadal Fat Pads, FBG and OGTT

At the end of the experimental duration (day 50), percentage of body weight gain was significantly higher in the HFD group (37± 8.22%; *P* = 0.0021) when compared with the ND group. Whereas, there was no significant difference in the percentage of the body weight gain between ND (8.01 ± 1.72%) and HFD + LB (18.64 ± 5.62%) groups (Fig. 2A). The extent of fat accumulation in the perigonadal fat pads were also higher in HFD group. The weight of perigonadal fat pads in HFD (0.973 ± 0.155 g; *P* = 0.0021) group were significantly high than both the ND (0.376 ± 0.83 g) and HFD + LB group (0.55 ± 0.11 g) (Fig. 2B).

One day before the end of the experimental duration (Day 49) FBG levels were measured. The HFD group showed significantly high FBG levels (203.66 ± 8.73 mg/dL) when compared with the ND group (130± 2 mg/dL). Whereas, in the HFD + LB the blood glucose levels were although on the higher side (163.66 ± 4.72 mg/dL) but not as significantly higher as the HFD group when compared with ND group (Fig. 3A).

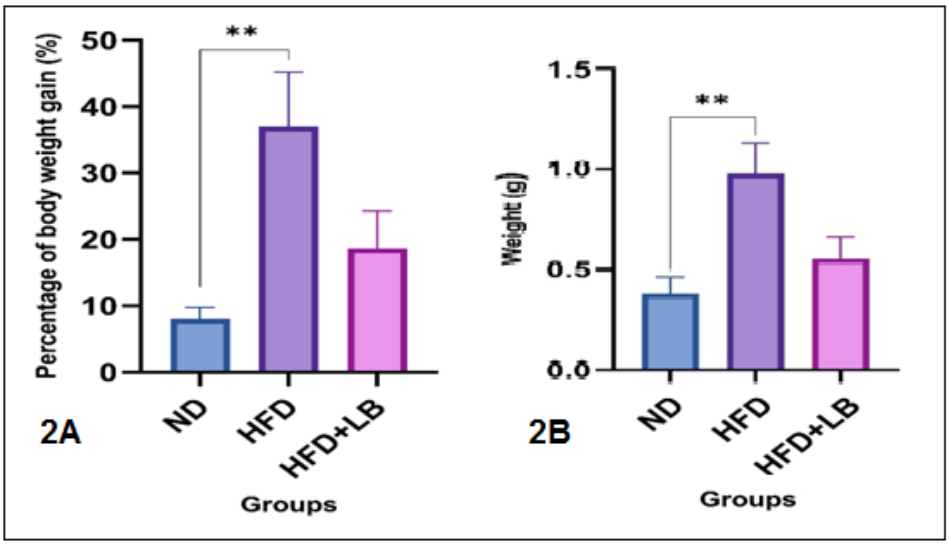


**Fig. 2. Comparison of percentage of body weight gain (2A) and weight of perigonadal fat tissues (2B) among experimental groups**

*The significance values were plotted as 0.1234= ns, 0.0332= *, 0.0021= ***

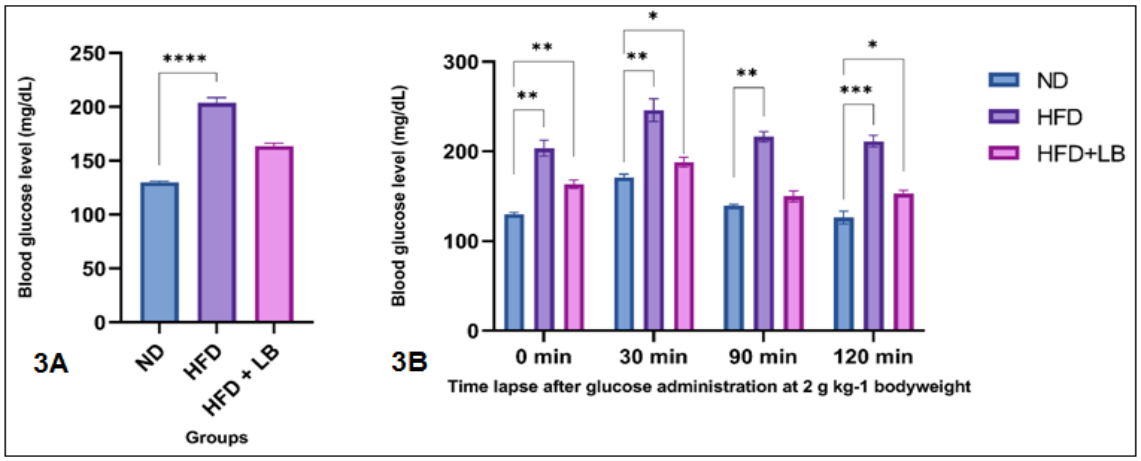


**Fig. 3. Comparison of Glycemic parameters among experimental groups. A. Results of Fasting blood glucose. B. Results of Oral Glucose Tolerance Test**

*The significance values were plotted as 0.1234= ns, 0.0332= *, 0.0021= **, 0.0002= ***, <0.0001= ****

The OGTT done at day 50, showed there was significantly high levels of blood glucose persisting throughout the OGTT duration (0, 30, 90, 120) in case of HFD group when compared with the ND group. Even at the end (120 mins) the glucose levels of HFD group were still elevated than the initial value (final value 211.33 ± 6.506 mg/dL as compared to initial value of 203.66 ± 8.736 mg/dL). It seems like the hyperglycemic state extended to such an extent that when challenged with high glucose load, the animals from the HFD group were unable to bring back the levels to normalcy. Whereas in case of the animals from the group HFD + LB, showed very less significant difference in blood glucose levels at 0, 30, and 120 mins, and no significant difference at 90 mins when compared with the ND group. Moreover, at 120 mins the blood glucose levels of HFD + LB group become even lower than its initial value (final value 153 ± 3.605 mg/dL as compared to initial value of 163.66 ± 4.725 mg/dL) (Fig. 3B). It showed that, feeding supplementation with *L. brevis* helped the animals from this group to overcome the initial glucose challenge (2g $kg^{-1}$), moreover it also infers that this feeding regime helped this group to resist the HFD associated detrimental metabolic changes to a greater extent.

### 3.4 Effects of *L. brevis* on Serum Lipid Profiles and Deposition of Fat in Perigonadal Fat Tissues

The levels of different species of serum lipid parameters like Serum HDL-C level, Total cholesterol, Triglyceride, LDL-C, VLDL-C and Non-HDL-C levels (mg/dL) were measured at the end of the experimental duration and after sacrificing the animals. The results showed that the levels of bad lipids like Triglycerides and Total cholesterol were significantly high in the HFD (189.91 ± 5.46 mg/dL; *P* = 0.0332 and 155.32 ± 13.87 mg/dL; *P* = 0.0332) groups when compared with the ND (172.22 ± 7.606 mg/dL and 126.90 ± 3.145 mg/dL) group. Whereas in case of HFD + LB groups the levels of the same lipids showed insignificant difference (172.22 ± 3.854 mg/dL and 134.89 ± 4.705 mg/dL) in comparison to ND group. Moreover, the good cholesterol, HDL-C levels were found to be significantly lower (29.01 ± 0.606 mg/dL; *P* = 0.0021) in HFD group in comparison to both ND (32.76 ± 1.049 mg/dL) and HFD + LB group (32.57 ± 0.912 mg/dL). The levels of other harmful lipids like LDL-C, VLDL-C and Non-HDL-C all were significantly high in HFD group than ND and HFD + LB group (Fig. 4).

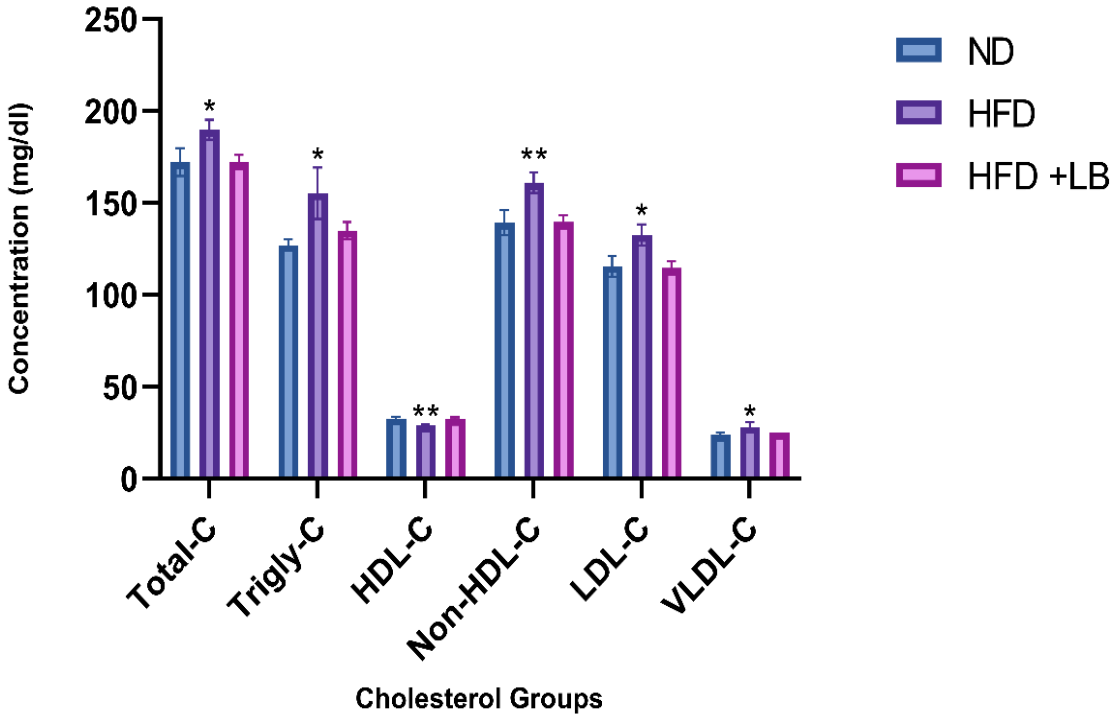


**Fig. 4. Comparison of circulating lipids among experimental groups**
*The significance values were plotted as 0.1234= ns, 0.0332= *, 0.0021= ***

## 4. DISCUSSION

HFD is reported to induce various metabolic problems in different experimental animals [28]. The sedentary lifestyle and calorie rich diet are also held responsible for the development of obesity related metabolic disorders like obesity and T2DM [29]. Probiotic bacteria have received a lot of attention in the recent times because they've been shown to provide a variety of health benefits when consumed. Supplementing with probiotics can help protect people from the bad effects of diabetes. On obese and diabetic mice, *L. plantarum* OLL2712 demonstrated stronger anti-inflammatory activity and anti-metabolic-disorder benefits in the exponential phase than its stationary-phase equivalent [30]. The preventive effects of a multispecies probiotic in reducing insulin resistance were studied using the probiotic-gut flora-butyrate-inflammatory pathway and proven to be effective. Other studies assessed the intervention effects of the probiotic *L. casei* CCFM419 against T2DM by integrating various putative pathways for the observed impact of gut microbiota [30].

In the present study, HFD induced mice replicated the state of hyperglycemia associated complications mimicking the human condition. It was seen that dietary supplementation with *L. brevis* has shown positive effects. The acute oral toxicity test revealed that there were no toxicological complications while administering *L. brevis* to experimental animals. Supplementation with *L. brevis* resisted the extreme body weight gain under the influence of HFD. We found the percentage weight gain in ND (8.01 ± 1.72 %) and HFD + LB (18.64 ± 5.62%) are non-significant but there was significant weight gain in the HFD group (37± 8.22%; $P$ = 0.0021). The results of FBG levels among experimental animals showed similar pattern where the HFD group showed significantly high FBG levels (203.66 ± 8.73 mg/dL) when compared with the ND group (130± 2 mg/dL) but the FBG levels in HFD + LB group (163.66 ± 4.72 mg/dL) were not as significantly higher as the HFD group. Moreover, the results of the OGTT revealed that dietary supplementation with *L. brevis* helped to resist the persistent hyperglycemic effects when the animals were initially challenged with glucose (2g $kg^{-1}$ body weight). The levels of the glucose remained significantly elevated in the HFD group throughout the observation (0, 30, 90, 120). Even at 120 mins the glucose levels were still greater than the initial value (final value 211.33 ± 6.506 mg/dL as compared to initial value of 203.66 ± 8.736 mg/dL). But in case of the HFD + LB group we observed that the systemic blood glucose elimination pattern of this group was more or less similar like that of the ND group. Even more, in the group HFD + LB, the end value at 120 mins was lower than that of the initial value (final value 153 ± 3.605 mg/dL as compared to initial value of 163.66 ± 4.725 mg/dL). These results indicate that *L. brevis* helped the animals to resist the phenomenon of insulin resistance and glucose tolerance under the context of prolonged HFD regime. The lipidomic analysis also revealed that dietary supplementation with *L. brevis* helped to regulate the levels of circulating lipids in a normal level. It was seen that the levels of lipids like Triglycerides and Total cholesterol in HFD + LB group were in normal range and similar like that of the ND groups (172.22 ± 3.854 mg/dL and 134.89 ± 4.705 mg/dL). But in HFD group the levels of the same are significantly higher (189.91 ± 5.46 mg/dL; $P$ = 0.0332 and 155.32 ± 13.87 mg/dL; $P$ = 0.0332). Moreover, in HFD + LB group, the levels of other harmful lipids like LDL-C, VLDL-C and Non-HDL-C were all in normal range and similar like ND group. In terms of fat disposition in the perigonadal fat tissues, the HFD + LD group showed less fat accumulation (0.55 ± 0.11 g). But the fat depositions in HFD group was significantly high (0.973 ± 0.155 g; $P$ = 0.0021) when compared with ND group (0.376 ± 0.83 g). All of these findings indicated a strong association of probiotic use of *L. brevis* as a potent preventative measure to combat the detrimental metabolic changes.

## 5. CONCLUSION

Our study demonstrated that dietary supplementation with *L. Brevis* can significantly protect the mice from the detrimental metabolic changes associated with obesity and hyperglycemia. Dietary supplementation of *L. brevis* shifted the metabolic parameters in a more or less normal range just like the ND group. The composition of the gut microbiota seems to be regulated, which may aid in the transformation of SCFAs which are beneficial to the metabolic activity. The research outcome of this work is a founding step towards further research leading to development of probiotic feed supplement incorporating *L. brevis* to protect from metabolic disorders.

## DISLAIMER

The standard drugs used for this research are commonly and predominantly used products in

the area of research. There is absolutely no conflict of interest between the authors and manufacturers of the products as they intended to use these products absolutely for the advancement of knowledge without any commercial interest.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Before the study, the University of North Bengal's Institutional Animal Ethical Committee (IAEC) of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) issued its clearance (IAEC/NBU/ 2018/03).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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