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Protective Effect of *Bifidobacterium longum* and *Streptococcus thermophilus* against Simvastatin-Induced Rhabdomyolysis in Hypercholesteraemic Rats

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Abstract

Simvastatin (SMV), a commonly prescribed drug for lowering lipid levels, is linked to the serious side effect of rhabdomyolysis. This study explores the potential of probiotics, specifically *Bifidobacterium longum (BL)* and *Streptococcus thermophilus (ST)*, as supplementary treatments to alleviate simvastatin-induced rhabdomyolysis in rats with high cholesterol levels. This study assesses the effects of combining simvastatin with probiotics on parameters such as lipid profiles, renal function, skeletal muscle markers, inflammatory cytokines, and histological characteristics. Rats with elevated cholesterol levels were exposed to SMV treatment alone and in conjunction with probiotics. This study compared the effects of combining simvastatin with *BL* and *ST*, focusing on their potential to ameliorate SMV-induced rhabdomyolysis. Combining simvastatin with *BL* and *ST* yielded notable outcomes. The supplementation significantly improved lipid profiles by reducing atherogenic lipids and increasing cardioprotective HDL-C levels. Additionally, the probiotics, particularly *ST* and *BL*, showed indications of preserving renal function and mitigating the adverse effects of simvastatin on muscle health. Analysis of inflammatory cytokines suggested that probiotics may modulate inflammation. Histological assessments confirmed the protective effects of probiotics by maintaining tissue integrity and normal cell appearance. While *BL* exhibited a slight advantage over *ST*, both probiotics demonstrated similar potential as adjunction therapies. This study's findings highlight the promising role of probiotics, specifically *BL* and *ST*, in ameliorating simvastatin-induced rhabdomyolysis. These probiotics show the potential to improve lipid profiles, safeguard renal function, preserve muscle health, modulate inflammation, and maintain tissue integrity. These results provide a hopeful basis for potential therapeutic interventions in individuals experiencing adverse effects associated with SMV treatment.

Keywords: *Bifidobacterium longum*, Probiotics, Rhabdomyolysis, *Streptococcus thermophilus*, Simvastatin

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1. Introduction

Hypercholesterolemia is a medical condition characterized by high cholesterol (Low density lipoprotein cholesterol) levels (LDL-C)) in blood, is responsible for 5.71 million years of life lost globally, ranking as the third highest contributor to cardiovascular disease DALYs (Disability-Adjusted Life Years) in 2019[1]. 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors, more often referred to as statins, serve as the first line of treatment for cholesterol reduction. They substantially affect the LDL-C levels by up to 60%[2-4] by blocking HMG-CoA reductase activity in the liver cells. This enzymatic inhibition effectively curtails cholesterol synthesis within the body[5]. Despite being the most effective drugs, statins have side effects, including the development of Type-II diabetes mellitus, neurological and neurocognitive implications, and Statin-Associated Muscle Symptoms (SAMS), such as myalgia, myopathy, and rhabdomyolysis[6]. Rhabdomyolysis causes muscle tissue breakdown and myoglobin release into the bloodstream, thus affecting lipid metabolism, skeletal muscle health, and renal function. This condition usually requires hospitalization and can lead to Acute Kidney Failure (AKF). According to estimates, around 10-40 % of instances involving rhabdomyolysis result in the development of AKF[7]. Globally, its occurrence remains uncertain; however, rhabdomyolysis contributes to approximately 7-10 % of all cases of AKF[8]. Rhabdomyolysis from statin therapy occurs 3.4 times per 100,000 patient-years of treatment and is well publicized to be secondary to pharmacological interactions[9]. In 1997, the FDA approved cerivastatin. Still, due to severe cases of rhabdomyolysis, including fatalities, its approval was withdrawn in 2001[10]. Simvastatin (SMV) is an efficacious drug that contributes around 17% to rhabdomyolysis, the highest among all the statins, according to the FDA's Adverse Event Reporting System (FAERS)[8,11]. Statins are the first line of treatment for managing hyperlipidemia and require prolonged usage. Considering the potentially detrimental effects of statin medication, there has been growing interest in exploring alternative approaches to mitigate these risks.

Probiotics may be described as living microorganisms that have a beneficial effect on the well-being of the host organism when supplied in sufficient quantities. Multiple studies have proven probiotics in various health conditions and diseases[12], including hypercholesteremia[13,14], sarcopenia[15], Anti-cancer, anti-viral, anti-inflammatory, immunomodulatory[16], hepatoprotective and nephro protective[17]. Previous studies showed that they could help with hypercholesterolemia, muscle-related conditions, and nephrotoxicity, thus making them good candidates for studying their protective role against statin-induced rhabdomyolysis. Lipid-lowering activity was reported for *Bifidobacterium longum (BL)*[18-22] and *Streptococcus thermophilus (ST)*[23-27]. Despite the individual effects of probiotics being reported in a few studies, no study has documented the relationship between probiotics and rhabdomyolysis nor explored the combination of probiotics with statins. Taking all of these things into account, the primary objective of our study was to assess if two different probiotic strains, *Bifidobacterium longum,* and *Streptococcus thermophilus*, could help protect against simvastatin-induced rhabdomyolysis in experimentally hypercholesterolemic rats when given alongside simvastatin as an "adjunct therapy".

2. Materials and Methods

The chemicals and reagents utilized in the study were obtained from Sigma Aldrich India. The gift sample of Simvastatin was obtained from Dr. Reddy Labs, Hyderabad, India. The feed ingredients utilized in this study include casein and cholesterol, both sourced from Himedia laboratories, India. Additionally, dl-methionine from Loba Chemie, India, is a vitamin and mineral mix from Sarabhai Chemicals, India. Total Cholesterol Kit, Erba Mannheim from Germany, TNF -α Elisa kit, Elab Science, USA.

3. Probiotic Culture

Bifidobacterium longum NCIM 5672 and *Streptococcus thermophilus* 2412 probiotic strains were obtained from India's National Collection of Industrial Microorganisms (NCIM), Pune. The strains were cultivated on a medium called Man, Rogosa, and Sharpe (MRS) combined with 0.05% cysteine hydrochloride supplement. An Anaerogas pack (HiMedia) created anaerobic conditions for the Bifidobacteria cultures. The cultures were first revived from frozen glycerol stock by subjecting them to two successive sub-culturing phases in an MRS-C medium. Subsequently, the cultures were incubated under anaerobic conditions at 37°C for 24 hours. Subsequently, the cultures were centrifuged and resuspended in skim

milk to achieve a concentration of 10^8-10^9 cells per 1 ml, intended for administration to rats as a probiotic dose.

4. Animal Grouping and Feeding Experiments

This study employed adult male Sprague-Dawley rats weighing an average range of 180-200 g, sourced from Mahaveer Enterprises, Hyderabad and housed in standard conditions at the Animal Facility of Nirmala College of Pharmacy (temperature: 22 ± 2°C, humidity: 50 ± 10 % and a continuous alternation between Light and dark for twelve hours each, along with adequate access of food and water. The Institutional Animal Ethics Committee of Nirmala College of Pharmacy, Guntur, Andhra Pradesh, India (001/IAEC/Ph. D19-20) has authorized all procedures. After an acclimatization period of seven days, six male Sprague-Dawley rats were randomly assigned using computerized randomization to a Control group receiving a Normal Pellet Diet (NPD) or an HFD group exposed to a High-Fat Diet (HFD) for 28 days to induce obesity. Among the obese animals, six groups were formed based on body weight: (a) HFD fed group receiving HFD *ad libitum*, (b) SMV group receiving Simvastatin (80 mg/kg) + HFD, (c) *BL* group receiving *Bifidobacterium longum* 1 mL of 1×10^9 CFU/mL + HFD, (d) *ST* group receiving *Streptococcus thermophilus* 1 mL of 1×10^9 CFU/mL + HFD, (e) SMV+*BL* group receiving SMV (80 mg/kg) + *Bifidobacterium longum* 1 mL of 1×10^9 CFU/mL + HFD, (f) SMV+*ST* group receiving SMV (80 mg/kg) + *Streptococcus thermophilus* 1 mL of 1×10^9 CFU/mL + HFD. The HFD consisted of lard (31%), powdered NPD (36.5%), cholesterol (1%), casein (25%), dl-methionine (0.3%), mineral and vitamin mix (6%), sodium chloride (0.1%) and yeast powder (0.1%)[28].

Body weight was documented weekly throughout the induction period. Serum samples were obtained *via* retro-orbital puncture on days 1, 15, and 30, kept at -20°C for further analysis after being centrifuged for 10 minutes at 3000 rpm. At the study's conclusion, the rats were euthanized, and kidney samples were surgically obtained, with three samples collected from each experimental group.

5. Muscle Strength

Bertelli and Mira[29] reported that the grip strength meter assessed muscular strength.

6. Biochemical Analysis

Lipid profile markers, including Serum Total Cholesterol (TC),[30] Triglyceride (TG),[31,32] Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL),[33] and Very Low-Density Lipoprotein (VLDL) levels were calculated using the formula as follows[34]: LDL = TC − (HDL + TG/5) and VLDL = TC − (LDL + HDL). Renal function tests include Serum Creatinine,[35] Urea,[36] Uric acid, Blood Urea Nitrogen and electrolyte levels, including Serum Potassium, Sodium, Chloride, Calcium and Chlorine[37]. Additionally, Skeletal muscle markers such as Serum Myoglobin, Creatinine Kinase[38], TNF-α, IL-6, and IL β1 were among the proinflammatory cytokines that were assessed using ELISA kits (Cusabio, USA) following the manufacturer's instructions.

7. Histopathology Examination

Kidney sections were rinsed with sterile saline and preserved in 10% neutral formalin for histological examination. The tissue samples underwent a series of necessary steps: gradual dehydration with ethanol (ranging from 50% to 99%) and clearing with xylene to enable embedding in paraffin. After successful embedding, thin tissue sections were prepared. Subsequently, the sections above have been subjected to staining using Hematoxylin and Eosin (H&E) dye, a standard technique in histopathology, to enhance cellular contrast for improved visualization of cell structures. A digital camera-equipped Olympus light microscope was utilized to carefully inspect and capture the stained tissue sections, ensuring precise observation and comprehensive microscopic analysis using CellSens software[39].

8. Statistical Analysis

SPSS software version 25 was used to calculate mean values, standard errors, and significance tests. This software facilitated data analysis, including determining central tendencies, measuring error, and assessing the statistical significance of the results. Tukey's post hoc

test was conducted after a one-way Analysis of Variance (ANOVA) to facilitate further analysis. A p-value of less than 0.05 has been set as the criterion for statistical significance.

9. Results

9.1 Mean Body Weight (MBW) and %Change in Body Weight (%BWC)

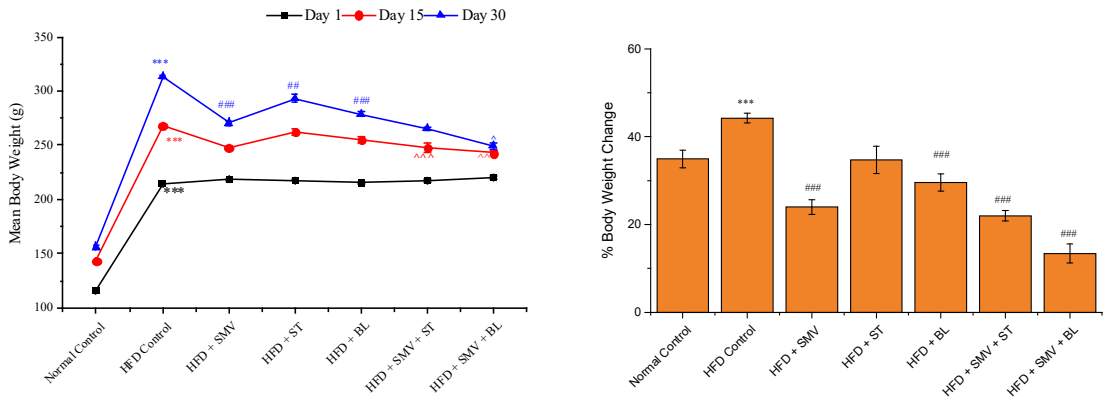
High-fat diet-fed rats for 28 days developed obesity, evident in significantly increased Mean Body Weight (MBW) and %Body Weight Change (%BWC) changes in contrast to the Normal Control group (NCg) on day 1 ($p < 0.001$). After 30 days of treatment with SMV at 80 mg/kg, with *ST* or *BL* at 1×10^9 CFU/mL and a combination of SMV with *ST* or *BL*, SMV+*ST* significantly reduced MBW on the 15th day ($p < 0.001$). SMV+*BL* decreased MBW on the 30th day ($p < 0.05$) and reduced % BWC ($p < 0.001$) in contrast with HFD control. HFD+*BL* also exhibited a notable decline in both MBW and %BWC in contrast to HFD control ($p < 0.001$).

9.2 Lipid Profile Markers

The lipid profile, encompassing TC, HDL-C, TG, LDL-C and VLDL-C, was evaluated to assess the effects of different treatments on hypercholesterolemia. Figure 2 reveals that levels substantially rose in the HFDg, TG, TC, LDL-C and VLDL-C. On 15 and 30 days ($p < 0.001$), HDL levels decreased ($p < 0.001$) on these same days compared to NCg. In contrast, the HFD+SMV group exhibited a notable decline in TG, TC, VLDL-C and LDL-C levels on days 15 and 30 ($p < 0.001$), a concurrent rise in HDL levels ($p < 0.001$) seen on the specified days as compared to the NCg that followed an HFDg. Similarly, the HFD+*ST* group saw decreased TC and LDL-C levels on days 15 and 30 ($p < 0.001$), as well as reduced TG and VLDL-C levels on days 15 and 30 ($p < 0.05$), along with elevated HDL levels ($p < 0.001$) on these days in contrast to the HFDg. In the HFD+*BL* group, TG, TC, LDL-C, and VLDL-C levels were lowered ($p < 0.001$), while HDL-C levels increased ($p < 0.001$) on 15th and 30th days compared to the HFDg. Furthermore, in the HFD+SMV+*ST* group, TG, TC, LDL-C, and VLDL-C levels reduced ($p < 0.001$) compared to the HFDg, while HDL levels increased ($p < 0.001$) on days 15 and 30 in contrast to the HFDg. TC levels also decreased ($p < 0.001$) on days 15 and 30, TG and VLDL-C levels decreased ($p < 0.01$) on day 15, and HDL-C levels increased ($p < 0.01$) on day 15 in contrast to the HFD+SMVg. Lastly, the HFD+SMV+*BL*g, TC, TG, LDL-C, and VLDL-C levels showed reductions ($p < 0.001$) at 15 and 30 days compared to the HFDg and HFD+SMVg.

9.3 Renal Function Markers

Cr, U, UA, and BUN were assessed as indicators of renal function. This study aimed to ascertain the effects of various hyperlipidemia treatments, as shown in Figure 3. Notable parameter alterations were noted, supported by p-values denoting statistical significance on different



(a) **(b)**

Figure 1. **(a)** Mean Body weights on Days 1, 15, and 30, in contrast to the normal control group (NCg). **(b)** % BWC from day 1 to day 30, $^{###}p<0.001$ in contrast to HFDg $^{***}p<0.001$, $^{##}p<0.01$; $^{###}p<0.001$ in contrast to HFD control, $^{\wedge}p<0.05$; $^{\wedge\wedge}p<0.01$; $^{\wedge\wedge\wedge}p<0.001$ compared to HFD + SMV.

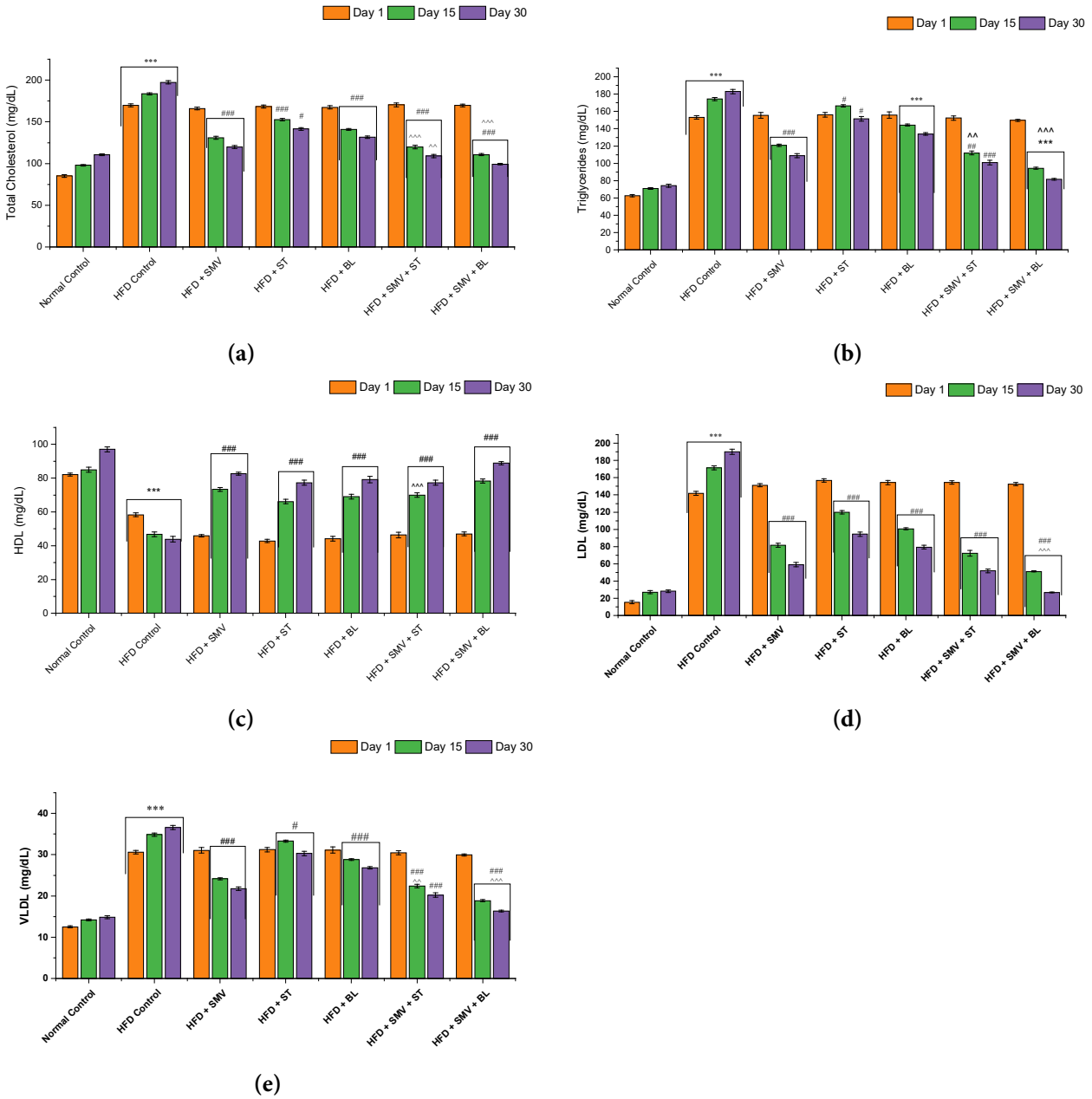


Figure 2. **(a)** TC mg/ dL on 1, 15, and 30 days **(b)** TG mg/ dL on 1,15 and 30 days **(c)** HDL-C mg/ dL on 1,15, and 30 days; **(d)** LDL-C mg/ dL on 1,15, and 30 days **(e)** VLDL-C mg/ dL on 1,15, and 30 days. $^{***}p<0.001$ in contrast to NCg, $^{\#}p<0.05$ $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$ in contrast to HFDg, $^{\wedge}p<0.05$; $^{\wedge\wedge}p<0.01$; $^{\wedge\wedge\wedge}p<0.001$ in contrast to HFD + SMVg

study days. In the HFDg, there were notable increases in Cr and UA levels ($p < 0.001$) on days 1, 15, and 30, elevated U levels ($p < 0.001$) on days 1 and 15, and a significant increase ($p < 0.05$) on day 30. Additionally, BUN levels increased ($p < 0.001$) on 1st day and ($p < 0.01$) on 15 and 30 days. Comparatively, in HFD+SMVg, Cr levels significantly increased ($p < 0.01$) on the 15th day and ($p < 0.001$) on the 30th day, while U levels increased ($p < 0.001$) on days 15 and 30. UA and BUN levels also rose ($p < 0.001$) on the 15th and 30th day in contrast to the HFDg. In contrast, the HFD+*ST*g witnessed a decline in Cr levels ($p < 0.001$) on the 15th and ($p < 0.01$) on the 30th day, with unchanged U levels. UA levels notably decreased ($p < 0.001$) on days 15 and 30, and BUN levels decreased ($p < 0.001$) on the 30th day compared to the HFDg. Similarly, the HFD+*BL*g showed decreased Cr levels ($p < 0.001$) on

days 15 and 30, reduced U levels ($p < 0.01$) on days 15 and ($p < 0.05$) on 30th day, and a notable decline in UA ($p < 0.001$) on 15th and 30th day, along with decreased BUN levels ($p < 0.01$) on 15th day and ($p < 0.001$) on 30th day, in contrast to the HFDg. In the HFD+SMV+*ST* group, UA levels decreased ($p < 0.001$) on days 15 and 30, and BUN levels declined ($p < 0.05$) on day 30 in contrast to the HFDg. Furthermore, there were reductions in Cr, U, UA, and BUN levels ($p < 0.001$) on the 15th and 30th days in contrast to the HFD+SMVg. Lastly, in the HFD+SMV+*BL*g, Cr levels decreased ($p < 0.05$) on day 15, UA levels decreased substantially ($p < 0.001$) on 15th and 30th day, and BUN levels decreased ($p < 0.01$) on day 30 compared to the HFDg. Moreover, reductions in Cr, U, UA, and BUN levels ($p < 0.001$) were observed on the 15th and 30th days in contrast to the HFD+SMVg.

9.4 Serum Electrolytes

Figure 4 presents data on serum electrolyte levels, including Cl^-, Na, Ca^{+2} and K over the study's duration, with associated p-values indicating statistical significance on different days. In the HFDg, no notable alterations in serum electrolyte levels were noted throughout the study. Similarly, the HFD+*ST* and HFD+*BL* groups showed no significant alterations in these levels during the study period. Conversely, the HFD+SMV group exhibited substantial shifts in serum electrolyte levels. Notably, Na, Cl-, and K levels decreased notably ($p < 0.001$) on the 15th and 30th day. Additionally, Ca^{+2} levels decreased, with statistical significance reached on the 15th and 30th day ($p < 0.05$) in contrast to the HFDg. In the HFD+SMV+*ST* and HFD+SMV+*BL* groups, a noticeable decline in K, Na, and Cl^- levels ($p < 0.001$) on days 15 and 30 was observed, along with decreased Ca^{+2} levels, reaching statistical

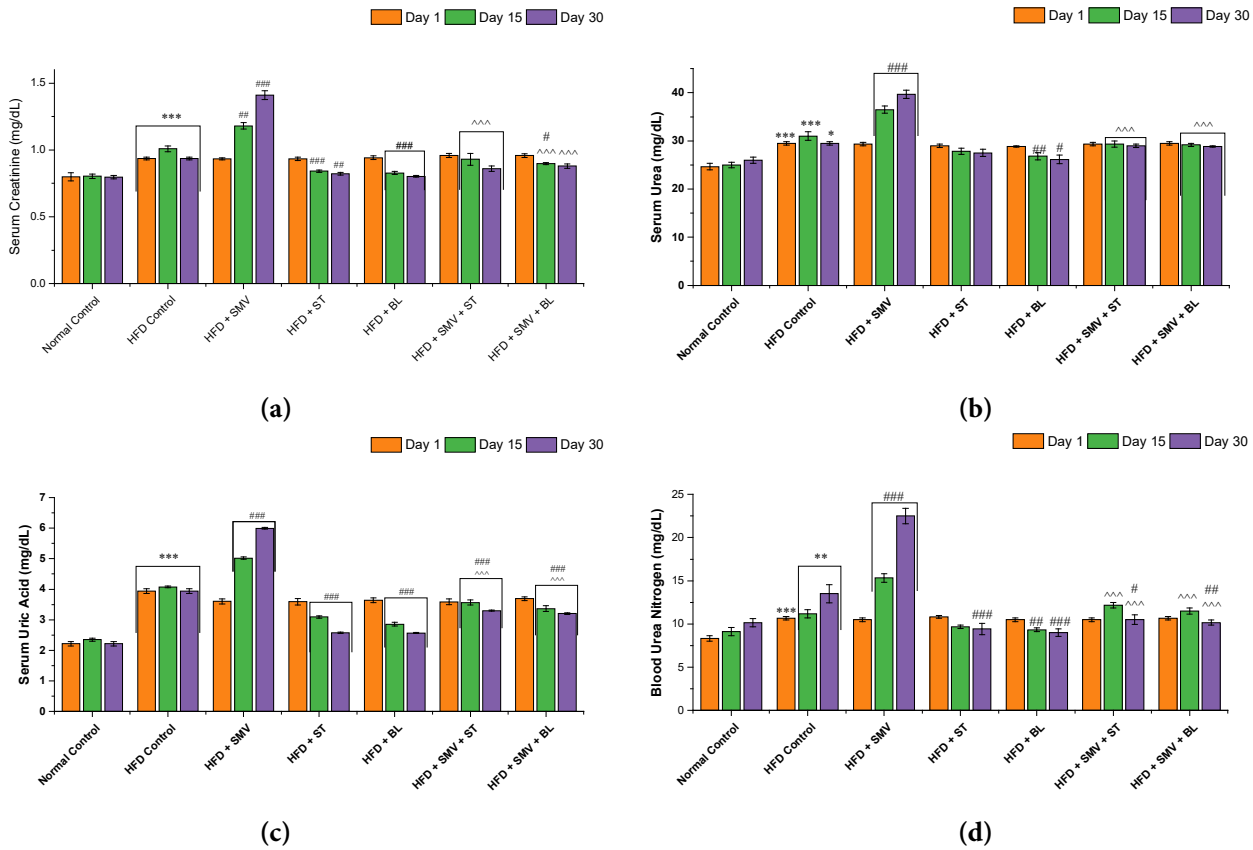


Figure 3. **(a)** Serum Creatinine (Cr) mg/ dL on days 1, 15 and 30, **(b)** Serum urea (U) mg/ dL on days 1, 15, and 30, **(c)** Serum Uric acid (UA) mg/ dL at the days 1, 15, and 30, **(d)** Serum Blood Urea Nitrogen (BUN) mg/ dL at the days 1,15, and 30. ***p<0.001 in contrast to NCg, #p<0.05 ##p<0.01; ###p<0.001 in contrast to HFDg, ^p<0.05; ^^p<0.01; ^^^p<0.001 in contrast to HFD+SMVg

significance on days 15 and 30 ($p < 0.05$) in contrast to the HFD+SMVg.

9.5 Skeletal Muscle Markers

Figure 5 depicts alterations in skeletal muscle markers throughout the study, encompassing GS on days 15 and 30 and M and CK on days 1, 15, and 30. These parameters exhibited significant variations, as substantiated by the accompanying p-values indicating their statistical significance at different time points during the study. In the HFDg, GS notably declined ($p < 0.001$) on day 15, although CK levels rose significantly ($p < 0.001$) on days 15 and 30 compared to the NCg. In the HFD+SMV group, there was a substantial GS decline ($p < 0.001$) on both days 15 and 30, accompanied by a rise in M ($p < 0.001$) on these days. In addition, CK levels increased significantly on the 15th day ($p < 0.05$) and 30th day ($p< 0.001$) relative to the HFDg. Similarly, in the HFD+*ST*g, GS decreased ($p < 0.01$) on the 15th day and significantly on the 30th day ($p < 0.001$), while M levels dropped substantially on both the 15th and 30th day ($p < 0.001$). CK levels also increased, with significance observed on day 15 ($p < 0.05$) in contrast to the HFDg. In the HFD+*BL*g, notable reductions were seen in GS ($p < 0.001$) on the 30th day, M levels ($p < 0.001$) on the 15th and 30th days, and CK levels ($p < 0.01$) on the 15th and significantly 30th day ($p < 0.001$) relative to HFDg. In the HFD+SMV+*ST*g, GS exhibited notable decline ($p < 0.001$) on the 15th and 30th day, and CK levels decreased significantly on the 15th ($p < 0.05$) and 30th day ($p < 0.001$) in contrast to the HFDg. Furthermore, GS significantly increased ($p < 0.001$) on the 15th and 30th, M decreased significantly ($p < 0.001$), and CK decreased significantly on the 15th ($p < 0.01$) and 30th day ($p < 0.001$) relative to HFD+SMVg. Finally, in HFD+SMV+*BL*g, GS exhibited a notable decline ($p < 0.001$) on both days 15 and 30, and CK levels also decreased significantly on day 15 ($p < 0.001$) compared to the HFDg. Moreover, GS significantly increased ($p < 0.001$) on the 15th and 30th day, while M and CK decreased significantly ($p < 0.001$) on both the 15th and 30th day in contrast to the HFD+SMVg.

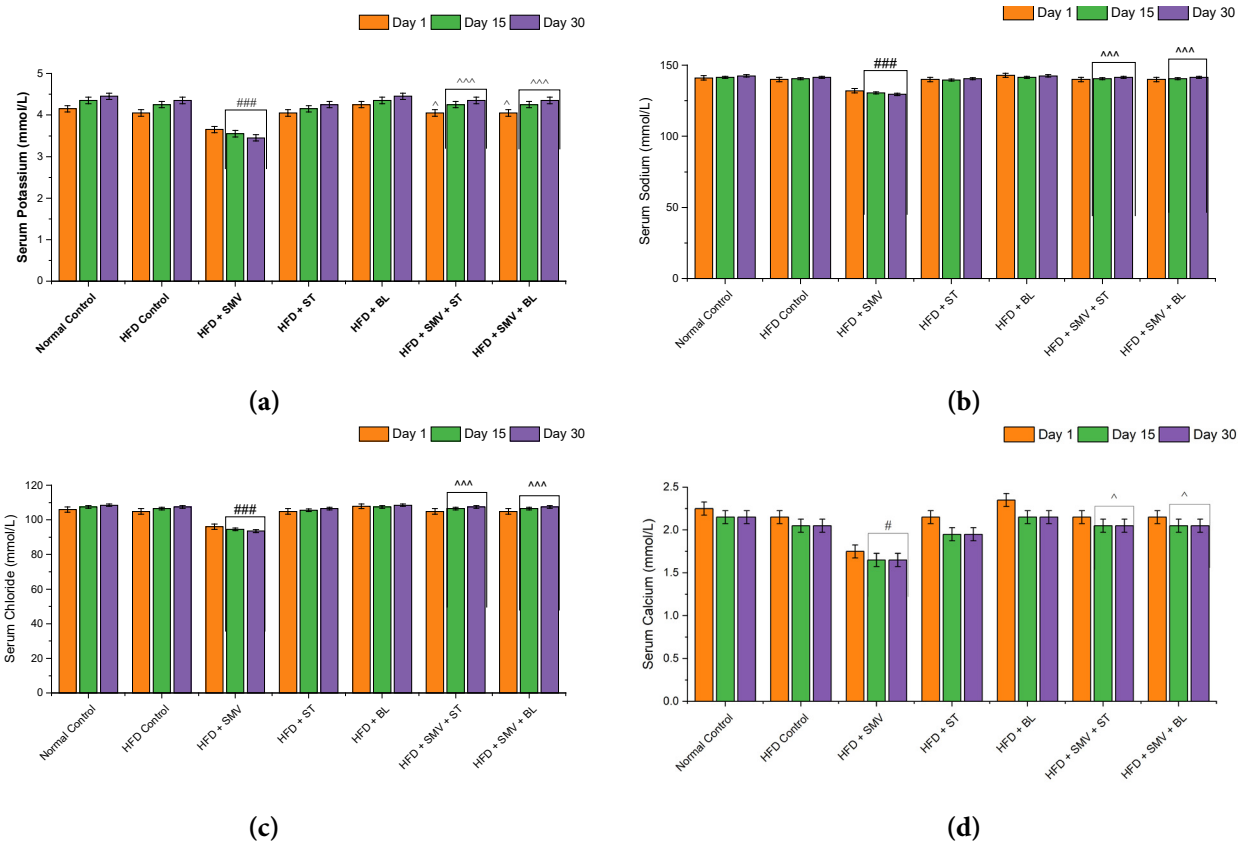


Figure 4. **(a)** K mmol/ dL on days 1,15, and 30, **(b)** Na mmol/ dL on days 1, 15, and 30, **(c)** Cl- mmol/dL on days 1, 15, and 30, **(d)** Ca^{+2} mmol/ dL at the days 1,15, and 30. $^{***}p<0.001$ in contrast to NCg, $^{\#}p<0.05$ $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$ in contrast to HFDg, ^p<0.05; ^^p<0.01; ^^^p<0.001 in contrast to HFD + SMVg

These findings provide valuable insights into the impact of interventions on skeletal muscle markers within the context of the study.

9.6 Inflammatory Cytokines

Figure 6 depicts the changes in inflammatory cytokines throughout the study, encompassing TNF-α on days 15 and 30, IL-6 on 1, 15, and 30 days, and IL-β on 1, 15, and 30 days. The data reveals significant alterations in these parameters, and the corresponding p-values confirming their statistical significance during the study provide support. In the HFDg, none of the inflammatory mediators exhibited significant differences relative to the NCg. In HFD+SMVg, a substantial rise in TNF-α, IL-6, & IL-β ($p < 0.001$) on the 15th and 30th days relative to HFDg. However, in the HFD+*ST*g, TNF-α increased on the 15th ($p < 0.05$), on the 30th ($p < 0.01$) days, and IL-6 levels increased ($p < 0.01$) on day 15. IL-β levels increased ($p < 0.05$) on the 15th day and 30th day in contrast to the HFDg. In the HFD+*BL*g, TNF-α rose ($p < 0.01$) on the 15th and 30th day, and IL-6 rose ($p < 0.001$) on the 15th and ($p < 0.01$) on day 30. IL-β levels increased ($p < 0.05$) on the 15th ($p < 0.001$) on the 30th relative to the HFDg. In the HFD+SMV+*ST*g, TNF-α increased to ($p < 0.001$) during the 30th day, and IL-6 increased ($p < 0.001$) on both the 15th and 30th days. IL-β levels increased ($p < 0.001$) during the 15th and 30th in contrast to the HFDg. Additionally, TNF-α decreased ($p < 0.001$) on 15th and 30th day, and IL-6 decreased ($p < 0.001$) on 15th and 30th day. IL-β rose ($p < 0.001$) on 15th and 30th in contrast to the HFD+SMVg. Finally, in the HFD+SMV+*BL* group, TNF-α increased ($p < 0.001$) on the 30th day, and IL-6 levels increased ($p < 0.001$) on the 30th day. IL-β levels increased ($p < 0.001$) on the 15th and 30th days relative to the HFDg. Additionally, TNF-α decreased ($p < 0.001$) on the 15th and 30th days, and IL-6 decreased ($p < 0.001$) on the 15th and 30th days. IL-β decreased ($p < 0.001$) on the 15th and 30th days relative to the HFD+SMVg.

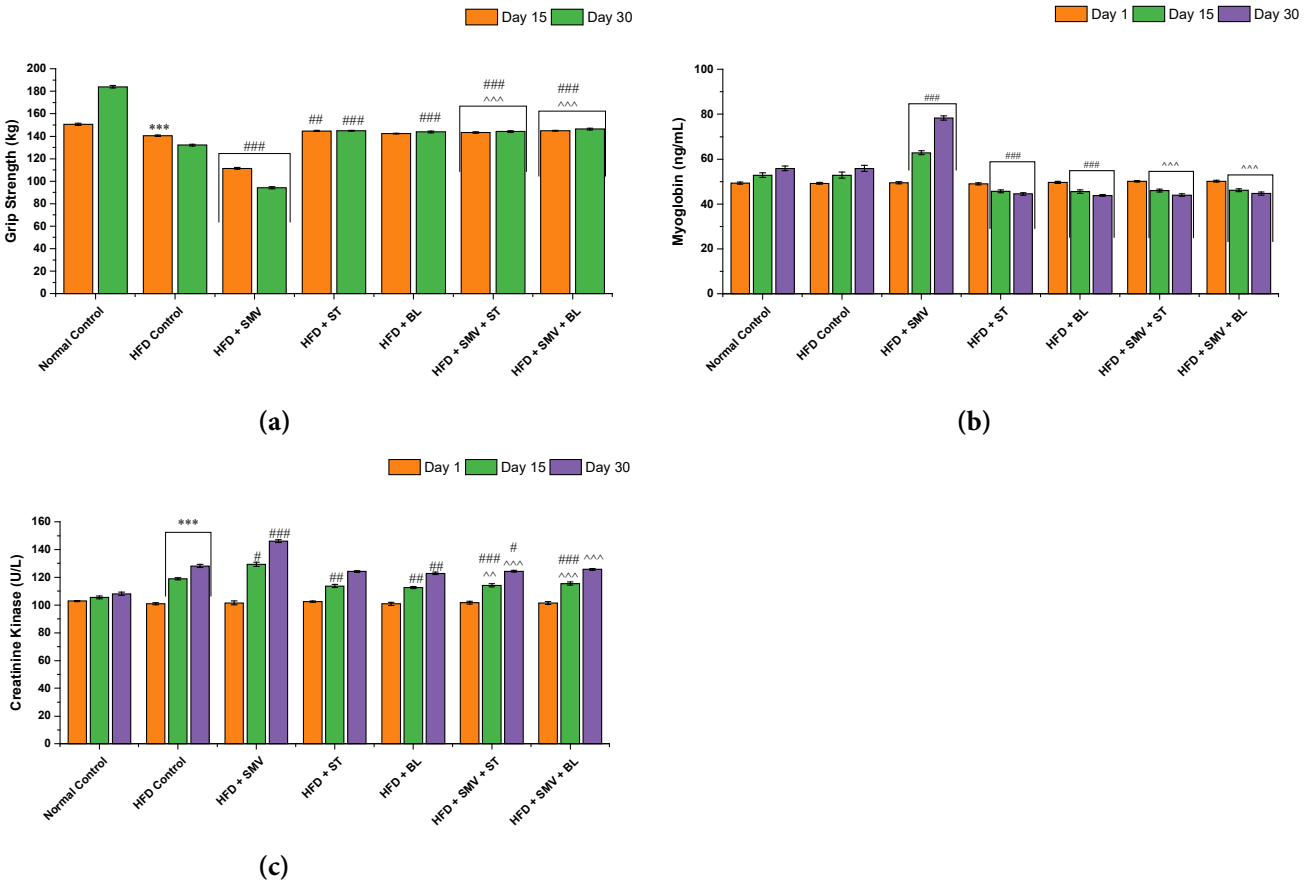


Figure 5. **(a)** GS kg on days 15 and 30, **(b)** M ng/ ml on days 1,15, and 30, and **(c)** CK U/L on days 1,15, and 30. ***p<0.001 in contrast to NCg, #p<0.05 ##p<0.01; ###p<0.001 in contrast to HFDg, ^p<0.05; ^^p<0.01; ^^^p<0.001 in contrast to HFD + SMV

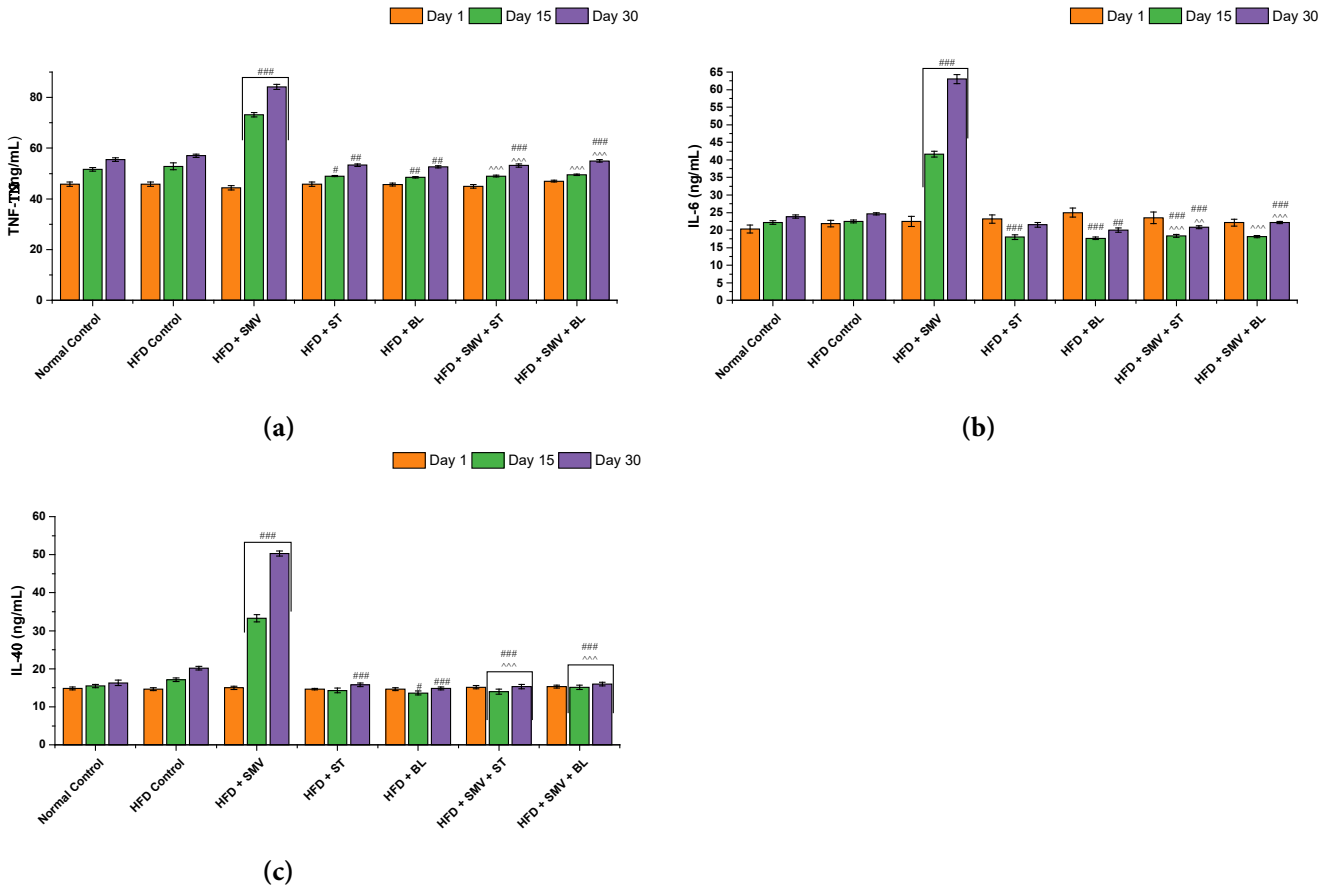


Figure 6. **(a)** Serum TNF-α ng/ mL on days 1,15, and 30, **(b)** Serum IL-6 ng/ mL on days 1, 15, and 30, **(c)** IL β1 ng/mL on days 1, 15, and 30. $^{***}p<0.001$ in contrast to NCg, $^{\#}p<0.05$ $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$ in contrast to HFDg, $^{\wedge}p<0.05$; $^{\wedge\wedge}p<0.01$; $^{\wedge\wedge\wedge}p<0.001$ in contrast to HFD + SMVg

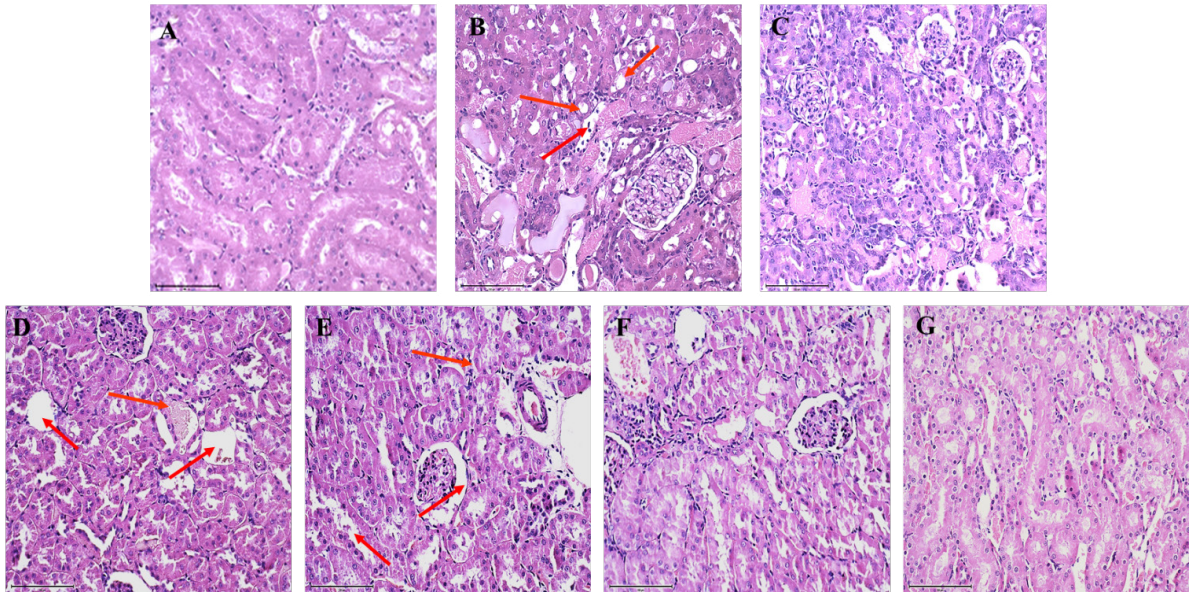


Figure 7. Histopathology of the groups after 30 days of treatment. (**A**) NCg; (**B**) HFDg; (**C**) HFD+SMVg; (**D**) HFD+*ST*g; (**E**) HFD+*BL*g; (**F**) HFD+SMV+*ST*g; (**G**) HFD+SMV+*BL*g Red arrow-vacuolization; brown arrow-necrosis.

9.7 Histopathological Evaluation

This study examined different experimental groups for histological changes in skeletal muscle tissues. The Normal control group showed no abnormalities. In contrast, the HFDg exhibited interstitial spaces and infiltration of adipocytes and inflammatory cells, indicating adverse tissue changes. The HFD+SMV group showed similar tissue alterations and epithelial cell apoptosis, indicating more severe damage. On the other hand, the HFD+*ST* and HFD+*BL* groups displayed glomerular congestion but maintained intact epithelial cells and a generally normal cell appearance. The HFD+SMV+*ST* and HFD+SMV+*BL* groups demonstrated normal cell formation, suggesting potential protective effects against tissue alterations. These histological findings reveal varying impacts of interventions on skeletal muscle tissues in the context of an HFD.

10. Discussion

This study investigated whether *Bifidobacterium longum (BL)* and *Streptococcus thermophilus (ST)* probiotics, given concurrently with SMV as "adjunct therapy" in rats with high cholesterol levels, could prevent SMV-induced rhabdomyolysis. SMV, a typical statin for high cholesterol, increases the risk of rhabdomyolysis, which causes muscular breakdown[40,41]. Pasternak *et al.* found a dose-dependent increase in SMV-induced rhabdomyolysis[42]. Anti-inflammatory and immune-modulating probiotics are well-known[43,44]. *BL* and *ST* probiotics have been studied for improving exercise performance, muscle protein synthesis, and tiredness[45]. *Lactobacillus plantarum* TWK10 supplementation increased mice's exercise performance, perhaps through anti-inflammatory mechanisms[46]. Probiotics have been demonstrated to protect against renal and skeletal muscle problems. Probiotics may reduce muscle injury and inflammation[47]. This study examined *BL* and *ST* probiotics, and health benefits have been linked to both strains[48]. This study used "adjunct therapy" with SMV and probiotics and investigated if probiotics could reduce SMV's adverse effects. The well-known dangers of SMV use, including rhabdomyolysis, prompted this study. Probiotics' anti-inflammatory and immunomodulatory abilities may mitigate these hazards. *BL* and *ST* were given with SMV to see if they may reduce SMV-induced rhabdomyolysis in high-cholesterol rats.

In hypercholesterolemia, evaluating the lipid profile is critical for assessing cardiovascular health, and the effects of SMV, *ST*, and *BL* on hypercholesterolemia lipid profile parameters were studied here. HFDg lipid profiles were significantly altered. A significant increase was observed in LDL-C, TC, TG, and VLDL-C values. However, HDL-C fell. This group developed hypercholesterolemia, as expected, with an HFD. Lipid profile values improved significantly in HFD+SMVg. Statins lower lipids, such as TG, TC, VLDL-C, and LDL-C, decreased significantly. This reduction was backed by a significant HDL-C increase, indicating cardiovascular wellness. These findings support SMV's long-standing involvement in hypercholesterolemia management[42]. HFD+*ST*g and HFD+*BL*g improved lipid profiles. TC and LDL-C values dropped dramatically on days 15 and 30, suggesting lipid-lowering. TG and VLDL-C levels also decreased, but less significantly. HDL-C levels rose considerably, improving cardiovascular risk factors[49,50]. The HFD+SMV+*ST*g and HFD+SMV+*BL*g groups combined SMV with *ST* or *BL*. These groups showed significant decreases in TG, TC, LDL-C, and VLDL-C, suggesting that SMV and probiotics may work together to treat hypercholesterolemia. Furthermore, HDL-C levels rose considerably, improving the lipid profile. These findings suggest probiotics, particularly *ST* and *BL*, may help treat hypercholesterolemia. When given with SMV, these probiotics improved lipid profiles, especially atherogenic lipids (TG, TC, LDL-C, & VLDL-C). Increased cardioprotective HDL-C has been shown. Probiotics' mechanisms and long-term effects on hypercholesterolemia therapy need further study. These preclinical findings must be confirmed in human clinical trials to ensure probiotic adjunct therapy safety and efficacy.

The HFDg showed significant serum Cr and UA increases on days 1, 15, and 30. This supports the idea that HFDs cause hyperlipidemia and renal impairment[51]. The HFD+SMVg had a substantial Cr rise on day 30, suggesting renal damage after SMV therapy. High U and UA levels indicated renal stress. This supports prior findings that SMV may harm kidney function[52]. The HFD+*ST*g reduced Cr levels on days 15 and 30, suggesting renal protection. The significant decrease in UA levels supports the idea that probiotics like *ST* may improve uric acid metabolism[53]. The HFD+*BL*g had lower Cr, U, and UA levels on days 15 and 30. *BL* may be reactive. Similar investigations have shown Bifidobacterium strains may

reduce kidney damage[47]. The HFD+SMV+*ST* group had lower BUN and UA on day 30 than the HFDg. SMV and *ST* as adjuvant therapy may protect renal function, as shown by lower UA and BUN[54]. Cr levels dropped on day 15 in the HFD+SMV+*BL* group, suggesting renoprotection. BUN and UA decreased significantly on the 15th and 30th day compared to HFD+SMVg, suggesting that *BL* and SMV improve renal function[55]. This study found that HFD caused renal function alterations, and SMV alone worsened them. With SMV as an adjuvant therapy, *ST* and *BL* probiotics appeared to protect renal function. Existing data suggests probiotics may preserve renal function.

About skeletal muscle indicators (GS, M, CK). The HFDg significantly dropped GS on day 15. This shows the HFD weakened muscles. CK levels dramatically increased on the 15th and 30th days ($p < 0.001$), suggesting hyperlipidemia-related muscle injury[56]. The HFD+SMV group experienced a significant decrease in GS on days 15 and 30, indicating that SMV treatment worsened the muscular weakness in the HFDg group. Moreover, M levels increased significantly on days 15 and 30, indicating muscle injury. This was corroborated by a significant increase in CK levels on days 15 and 30 compared to HFDg[57]. The HFD+*ST*g showed decreased GS on day 15 and significantly decreased on day 30, indicating a harmful impact on muscle strength. M levels decreased substantially on days 15 and 30, suggesting a possible muscular tissue protection effect. Additionally, CK levels increased significantly on day 15, indicating muscle tension, but did not differ considerably from HFDg on day 30. A significant decrease in GS on day 30in the HFD+*BL*g group suggests a negative impact on muscle strength. M levels considerably decreased on days 15 and 30, suggesting muscle protection. CK levels increased substantially on days 15 and 30, indicating muscle stress compared to HFDg. The HFD+SMV+*ST*g group showed a substantial decrease in GS on days 15 and 30, indicating a harmful impact on muscle strength. CK levels considerably lowered on days 15 and 30 and compared to HFDg. This shows that SMV and *ST* may protect muscles[46]. The HFD+SMV+*BL* group showed a substantial drop in GS on days 15 and 30, affecting muscle strength. CK levels decreased considerably on day 15, suggesting muscle protection relative to HFDg. Additionally, M levels significantly decreased on days 15 and 30 ($p < 0.001$), indicating muscle protection. According to this study, the HFD and SMV therapy decreased muscular strength and injury markers. In addition to SMV, probiotics like *ST* and *BL* may protect muscles by improving muscular strength and damage markers.

The changes in inflammatory cytokines used in the study, including TNF-α, IL-6, and IL-β, contribute to the inflammatory response. In the HFDg, no inflammatory mediators differed from the NCg, demonstrating that the HFD alone did not cause considerable inflammation. When SMV was introduced to the HFD+SMV group, IL-6, IL-β, and TNF-α significantly increased on days 15 and 30, demonstrating a strong inflammatory response to SMV administration during an HFD. Compared to HFDg, HFD+probiotics groups, notably HFD+*ST* and HFD+*BL*, showed differences in inflammatory markers. In conjunction with SMV treatment, probiotics significantly reduced TNF-α and IL-6 levels on days 15 and 30. Additionally, IL-β showed unique patterns in response to *ST* and *BL*, showing probiotics may modulate inflammatory cytokines. As demonstrated by cytokine levels, inflammation and treatment regimens interact intricately.

Skeletal muscle tissue abnormalities are absent in the Normal Control group, which has normal physiological and biochemical indicators. In contrast, the HFDg had interstitial gaps, adipocyte infiltration, and inflammatory cells. These changes match biological markers, including higher lipid profiles, renal function measures, skeletal muscle markers, and inflammatory cytokine levels. These data show that an HFD damages skeletal muscle tissues, causing hypercholesterolemia and accompanying consequences. The HFD+SMVg had similar tissue changes and epithelial cell death, indicating higher muscle tissue injury. This matches the worsening of biochemical markers in this group, highlighting the hazards of SMV therapy in an HFD. While HFD+*ST*g and HFD+*BL*g showed glomerular congestion, they had intact epithelial cells and a normal cell appearance. *ST* and *BL* may protect tissue from changes by improving biochemical markers, including renal function measures and inflammatory cytokines. These probiotics may reduce HFD and SMV side effects. Finally, HFD+SMV+*ST*g and HFD+SMV+*BL*g showed normal skeletal muscle cell production, suggesting tissue protection. These findings are consistent with improved biochemical markers such as lipid profiles, renal function, inflammatory cytokine levels, and skeletal muscle markers. Probiotics and SMV may work synergistically to prevent hypercholesterolemia-related tissue damage.

In conclusion, histological evaluation of skeletal muscle tissues complements biochemical markers to show how treatments affect tissue morphology. Probiotics, specifically *ST* and *BL*, utilized as adjunct therapy with SMV may protect against hypercholesterolemia-induced tissue changes, suggesting candidates for further study in controlling related problems.

11. Summary and Conclusion

This study explored the potential of probiotics, namely *Bifidobacterium longum* and *Streptococcus thermophilus*, as adjunct therapies to mitigate the adverse effects of SMV-induced Rhabdomyolysis in rats with high cholesterol levels. Combining SMV with these probiotics showed promise in enhancing lipid profiles by reducing atherogenic lipids and increasing cardioprotective HDL-C levels. This suggests that probiotics, as adjunct therapies, hold the potential for effectively managing hypercholesterolemia. Renal function analysis revealed that probiotics, particularly *Streptococcus thermophilus* and *Bifidobacterium longum*, appeared to protect renal function in this context. Skeletal muscle markers indicated that SMV's adverse effects on muscle were alleviated when administered with probiotics, suggesting a protective role for probiotics. Inflammatory cytokine analysis showed that Probiotics administered with SMV exhibited variations in cytokine levels, indicating a potential modulatory effect on inflammation. Histological examination of skeletal muscle tissues provided further evidence of probiotics' protective effects, maintaining tissue integrity and normal cell appearance.

In conclusion, this study suggested that *Bifidobacterium longum* showed a slight overall benefit in mitigating Rhabdomyolysis induced by statins compared to *Streptococcus thermophilus*; however, both have almost equal potential as adjunct therapies. They showed promise in improving lipid profiles, protecting renal function, preserving muscle health, modulating inflammation, and maintaining tissue integrity. Additional research and rigorous Clinical trials must be conducted to substantiate and confirm the findings above and assess probiotics' safety and efficacy in managing hypercholesterolemia and related complications.

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