JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/43619
RESEARCH ARTICLE

Evaluation of *Lactobacillus acidophilus* and *Bacillus coagulans* against Simvastatin-induced Rhabdomyolysis in Hypercholesterolemic Rats

K. Dilip Raja[1], A. Shanta Kumari[2*] and A. Prameela Rani[1]

[1]Acharya Nagarjuna University College of Pharmaceutical Sciences, Guntur - 522510, Andhra Pradesh, India
[2]Department of Pharmaceutical Analysis, Rajarshi Shahu College of Pharmacy, Nanded - 443001, Maharashtra, India; skatakam9@gmail.com

Abstract

Background: This study investigates the possible synergistic influence of probiotics, specifically *Lactobacillus acidophilus* (LA) and *Bacillus coagulans* (BC), in conjunction with *Simvastatin* (SMV) for treating Rhabdomyolysis in hypercholesteremic rats. **Aim:** The study aims to evaluate the combined effects of SMV with LA or BC on various health parameters in hypercholesteremic rats induced with rhabdomyolysis. **Methods:** Hypercholesteremic rats with rhabdomyolysis were treated with SMV, LA, BC, and combinations of SMV with LA or BC. The study measured body weight, lipid profiles, renal function, skeletal muscle, and inflammation markers. **Results:** The combination treatments showed promise in reducing body weight gain. Regarding lipid profiles, SMV in tandem with LA demonstrated slightly enhanced efficacy in lipid regulation compared to other combinations. Both probiotic strains exhibited substantial potential in preserving nephron function alongside SMV. Probiotic combinations with SMV helped mitigate skeletal muscle dysfunction. Regarding inflammation, both combinations alleviated inflammation symptomatic of rhabdomyolysis. Notably, BC, combined with SMV, excelled in dampening inflammatory cytokines like TNF-α and IL-6, surpassing LA. **Conclusion:** The combined administration of SMV with LA or BC effectively mitigates rhabdomyolysis-induced issues, with varying impacts on diverse outcomes. Future investigations are imperative to comprehensively ascertain the optimal probiotic regimen for managing rhabdomyolysis. These positive outcomes underscore the multifaceted roles of probiotics in addressing rhabdomyolysis-related complications, emphasizing the necessity for further exploration into their mechanisms and clinical implications.

Keywords: *Bacillus coagulans*, Hyperlipidemia, *Lactobacillus acidophilus*, Probiotics, Rhabdomyolysis

Abbreviations: ANOVA - Analysis of Variance; BC - Bacillus coagulans; BUN - Blood Urea Nitrogen; CFU - Colony Forming Units; Cr - Creatinine; GS - Glutathione; HDL-C - High-Density Lipoprotein Cholesterol; HFD - High-Fat Diet; IL-6 - Interleukin 6 IL-β1 - Interleukin beta 1; LA - Lactobacillus acidophilus; LDL-C - Low-Density Lipoprotein Cholesterol; MBW - Mean Body Weight; NC - Normal Control; SMV - Simvastatin; SPSS - Statistical Package for the Social Sciences; TC - Total Cholesterol; TG - Triglycerides; TNF-α - Tumor Necrosis Factor Alpha; UA - Uric Acid; VLDL-C - Very Low-Density Lipoprotein Cholesterol

1. Introduction

The Centers for Disease Control and Prevention (CDCP) estimate that roughly 31.7% of Americans have raised LDL-C levels, resulting in a twofold increased risk of developing heart disease[1]. Studies in India reported hypercholesterolemia (≥200 mg/dl)in 25–30 % of urban and 15–20 % of rural subjects[2,3]. The ICMR-INDIAB study reported that the incidence of hypercholesterolemia varied from 4.6% to 50.3% across states in India[4]. Generally, Hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors, which are statins, serve with first-line therapy and have the potential to significantly impact the levels of LDL-C by as much as 60%[5–7] by inhibiting HMG-CoA reductase activity within the liver's cells, which efficiently reduces

*Author for correspondence

Article Received on: 29.05.2024 Revised on: 27.06.2024 Accepted on: 04.07.2024

cholesterol production within the body[8]. However, despite their effectiveness in reducing cholesterol levels, they pose notable concerns due to their association with adverse effects, including the debilitating condition of rhabdomyolysis[9]. Rhabdomyolysis is a condition that is characterized by the degeneration of skeletal muscle and the release of myoglobin into the circulatory system, ultimately affecting renal function. It stands as a significant complication of statin therapy, potentially leading to severe health implications and 10-40 % of Acute Kidney Failure (AKFs)[10], which is responsible for roughly seven to 10 % of the instances of all AKFs[11]. 3.4 cases of rhabdomyolysis for every 100,00 patient-years of treatment occur because of statin medication. It is widely known that this phenomenon is a consequence of pharmacological interactions[12]. In 1997, the Food and Drug Administration (FDA) granted permission for cerivastatin; however, in 2001, the approval was revoked due to severe cases of rhabdomyolysis, which included fatalities[13]. Among the statins, simvastatin is an effective medication responsible for approximately 17% of rhabdomyolysis, the highest percentage of any statin. This information comes from the Adverse Event Reporting System (FAERS) of the Food and Drug Administration[11,14]. Despite being well-recognized, rhabdomyolysis remains a concern, particularly considering its association with statin medications. This has prompted the exploration of alternative strategies to mitigate the risks associated with statin-induced rhabdomyolysis.

Probiotics, defined as living microorganisms conferring health benefits when administered adequately, have garnered considerable attention for their potential therapeutic applications across various health conditions. Emerging research has highlighted their role in multiple ailments[15], including ameliorating hypercholesteremia[16,17], sarcopenia[18], and also acts as an anti-inflammatory, anti-viral, and anti-cancer, immunomodulator[19], hepatoprotective and nephroprotective agents[20]. Probiotics have demonstrated diverse beneficial effects, including modulation of lipid metabolism, anti-inflammatory properties, and protection against renal damage[21]. *L. acidophilus*, a beneficial microbe, exhibits various biological functions, including lowering cholesterol levels and improving gastrointestinal health by regulating flora and reducing inflammation[22], alleviating cholesterol gallstone disease[23], uremia[24], ulcerative colitis[25] and acts as hepatoprotective, nephroprotective[20] anti-diabetic[26], and atherosclerotic[27]. The other probiotic strain, *B. coagulans*, was reported to alleviate metabolic diseases like dyslipidemia, several depressive disorders[28,29], skeletal muscle oxidative stress, inflammation[30,31], improve protein absorption and utilization[32], effective adjuvant treatment for rheumatoid arthritis symptoms[33] and acts as nephroprotective[34]. However, the specific role of probiotics in mitigating statin-induced rhabdomyolysis remains unexplored.

This study seeks to address this gap by evaluating the potential protective effects of two distinct probiotic strains, *L. acidophilus* and *B. coagulans* when administered in conjunction with simvastatin as an "adjunct therapy", there was a reduction in the incidence of rhabdomyolysis in experimentally hypercholesterolemic rats.

2. Materials and Methods

Sigma Aldrich India provided the reagents and chemicals used in the study. The Simvastatin gift sample was acquired from Dr Reddy Laboratories, Hyderabad, India. Casein and cholesterol are feed ingredients used in this investigation, obtained from Himedia laboratories, India. Furthermore, vitamins and minerals are from Sarabhai Chemicals, India, and dl-methionine is from Loba Chemie, India. total cholesterol kit from Erba Mannheim, Germany, TNF-α Elisa kit from Elab Science, USA,

3. Probiotic Culture

We obtained probiotic strains of *L. acidophilus* NCIM 5705 and *B. coagulans* 5648 from the National Collection of Industrial Microorganisms (NCIM), Pune, India. Man, Rogosa, and Sharpe (MRS) medium supplemented with 0.05% cysteine hydrochloride (MRS-C) cultivated the strains. Utilizing an Anaerogas pack (HiMedia), the Bifidobacteria cultures were maintained in anaerobic conditions. After passing through two phases of sub-culturing in an MRS-C medium, the cultures were initially brought back to life using frozen glycerol stock. This was done to get the cultures back to life. After that, the cultures were kept in an anaerobic atmosphere while they were incubated for twenty-four hours at 37 °C. A concentration of 10^8–10^9

cells per millilitre was produced by centrifuging the cultures and then resuspending them in skim milk. This concentration was intended to be administered to the study animals as an interventional dosage.

4. Animal Grouping and Induction of Hypercholesterolaemia

For the study, male adult Sprague-Dawley rats weighing an average of 180 and 200 grams were utilized. After being obtained from Mahaveer Enterprises in Hyderabad, they were stored at the Animal Facility of Nirmala College of Pharmacy (NCPA), Guntur, Andhra Pradesh, India, using the established protocols. These settings encompassed a consistent temperature of 22 ± 2 ºC, a humidity level of 50 ± 10 %, and a continual cycle of twelve hours of light and darkness every day. In addition to that, they were given enough food and drink. The Institutional Animal Ethics Committee (001/IAEC/Ph. D19-20) of the NCPA has approved all the procedures that have been carried out. After seven days of acclimatization, six male Sprague-Dawley rats were assigned to each group. They were assigned randomly using computerized randomization: the HFD group (HFD), which was given 28 days of a high-fat diet to cause hypercholesterolemia, or the Normal Control group (NC), which was given a Standard Pellet Diet (SPD). The hypercholesterolemic animals were divided into six groups according to their body weight: (a) the HFD, which received HFD ad libitum; (b) the SMV, which received Simvastatin (80 mg/kg) plus HFD; (c) the HFD+LA, which received *L. acidophilus* 1 mL of 1×10^9 CFU/mL plus HFD; (d) the HFD+BC, which received *B. coagulans* 1 mL of 1×10^9 CFU/mL plus HFD; (e) the HFD + SMV + LA, which received Simvastatin (80 mg/kg) plus *L. acidophilus* 1 mL of 1×10^9 CFU/mL plus HFD; (f) the HFD + SMV + BC, which received Simvastatin (80 mg/kg) plus *B. coagulans* 1 mL of 1×10^9 CFU/mL plus HFD. Casein (25 %), cholesterol (1 %), powdered NPD (36.5%), lard (31%), dl-methionine (0.3%), mineral and vitamin mix (6%), sodium chloride (0.1 %), and yeast powder (0.1 %) were the components of the HFD[35].

Throughout the induction period, weekly body weight records were made on days 1, 15, and 30. A retro-orbital puncture was used to collect serum samples, which were then kept at -20°C for subsequent analysis following a 10-minute centrifugation at 3000 rpm. Three kidney samples from each experimental group were surgically taken after the rats were euthanized for the duration of the investigation.

5. Muscle Strength

According to Bertelli and Mira[36] the grip strength meter was used to evaluate the muscular strength.

6. Biochemical Analysis

Lipid profile markers, which include triglyceride (TG)[37,38], serum Total Cholesterol (TC)[39], Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL)[40], and Very Low-Density Lipoprotein (VLDL) levels were determined based on the following formula[41]: LDL = TC − (HDL + TG/5) and VLDL = TC − (LDL + HDL). In addition to serum Creatinine (Cr)[42], Urea (U)[43], Uric acid (U), Blood Urea Nitrogen (BUN), serum Potassium (K^+), sodium, chloride (Na^+), Calcium (Ca^{+2}), and Chloride (Cl^-) electrolyte levels are also measured within the renal function tests[44]. Skeletal muscle indicators such as serum myoglobin and creatinine kinase are also assessed[45]. These cytokines included, TNF-α, IL-6, and IL-β1. The evaluation was conducted following the manufacturer's directions.

7. Histopathology

The kidney slices were cleaned with sterile saline and then fixed in neutral formalin at a concentration of 10% to prepare them for histological evaluation. To simplify the paraffin embedding process, the tissue samples were exposed to critical processes such as washing with xylene and dehydration with ethanol (from fifty % to one hundred %). After completing the embedding procedure, thin tissue sections were created. Hematoxylin and Eosin (HandE) dye, a standard histopathological method, were used to stain the slices above. This was done to enhance the contrast between the cells and to provide a more distinct image of the cell architecture. The coloured tissue slices were carefully examined and photographed using an Olympus light microscope with a digital camera. This ensured that the observations were correct and that

the microscopic analysis was comprehensive using the CellSens software solution[46].

8. Statistical Analysis

Calculating mean values, standard errors, and significance tests was accomplished with the help of SPSS software version 25. defining central tendencies, evaluating inaccuracy, and defining the statistical significance of the results were all achieved with the assistance of this software, which made data analysis much more straightforward. Following completing a one-way analysis of variance (ANOVA), Tukey carried out a post hoc test to facilitate subsequent analysis further. A p-value lower than 0.05 has been established as the standard for determining whether a statistical finding is significant. $^{***}p<0.001$ compared toNC, $^{\#}p<0.05$ $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$ contrastingwith HFD,$^{\wedge}p<0.05$; $^{\wedge\wedge}p<0.01$; $^{\wedge\wedge\wedge}p<0.001$ in contrast to HFD + SMV.

9. Results

9.1 Body Weight

As depicted in Figure 1, Rats that were given a High-Fat Diet (HFD) for 28 days displayed signs of hypercholesterolemia, as indicated by a significant increase in Mean Body Weight (MBW) and percentage of Body Weight Change (%BWC) on day 1 in comparison to the NC. Over thirty days, the following outcomes were noted after treatment with SMV, in conjunction with either LA or BC at a concentration of 1×10^9 CFU/mL or a coadministration of SMV with either LA or BC. HFD + SMV substantially dropped MBW on day 30. Similarly, HFD + SMV + LA and HFD + SMV + BC exhibited insignificant reductions in MBW on the 15th day. On the 30th day, HFD + SMV + BC exhibited a slight reduction in MBW, which was not statistically significant. Moreover, HFD + SMV, HFD + LA, HFD + BC, HFD + SMV + LA, and HFD + SMV + BC brought a notable drop in % BWC contrasted with the HFD. Notably, HFD + SMV + LA and HFD + SMV + BC demonstrated a noteworthy drop in % BWC in contrast to HFD + SMV alone on the 30th day.

9.2 Lipid Markers

Figure 2 illustrates a noteworthy elevation in the TC, TG, LDLC, and VLDLC in the HFD on days 15 and 30. However, contrasting with the NC, the HDL experienced a significant drop on these days. Compared to HFD, HFD + SMV demonstrated a substantial reduction in TC, TG, VLDLC, and LDLC on days 15 and 30 while simultaneously exhibiting an elevation in HDLC. Similarly, the HFD + LA exhibited dropped TC, TG, LDLC, and VLDLC levels on days 15 and 30, accompanied by raised HDL on these days, contrasting with HFD. In HFD + BC, TC and LDL-C were notably reduced, TG and VLDL-C exhibited insignificant decreases, while HDL-C levels rose on the 15th and 30th days, contrasting HFD. Additionally, in HFD + SMV + LA, TG, TC, LDL-C, and VLDL-C dropped in contrast to HFD, whereas HDL rose on the 15th and 30th day in contrast to the HFD. TC and LDL-C also dropped on days 15 as well as 30, and TG and VLDLC dropped

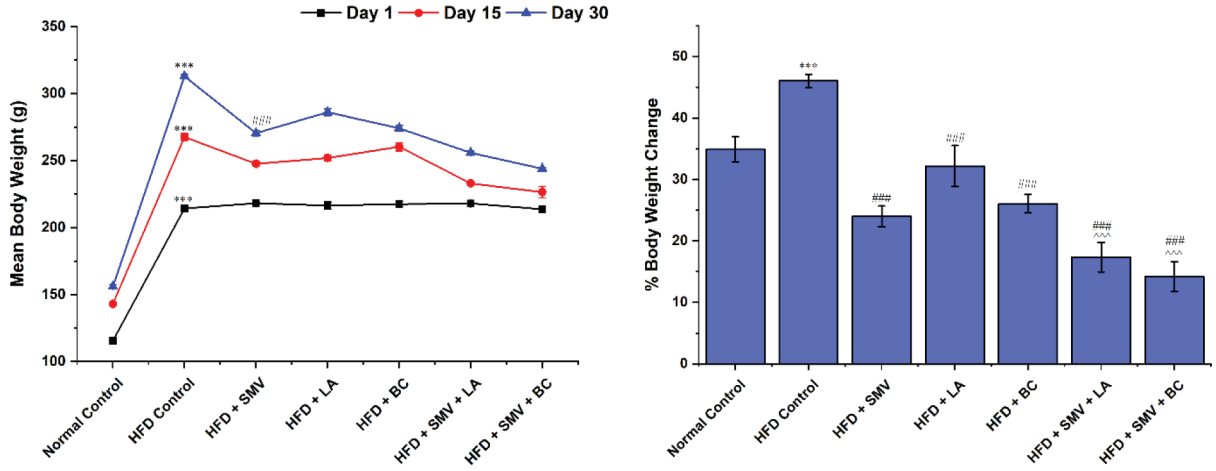

Figure 1. The MBW; and the %BWC from day 1 to 30.

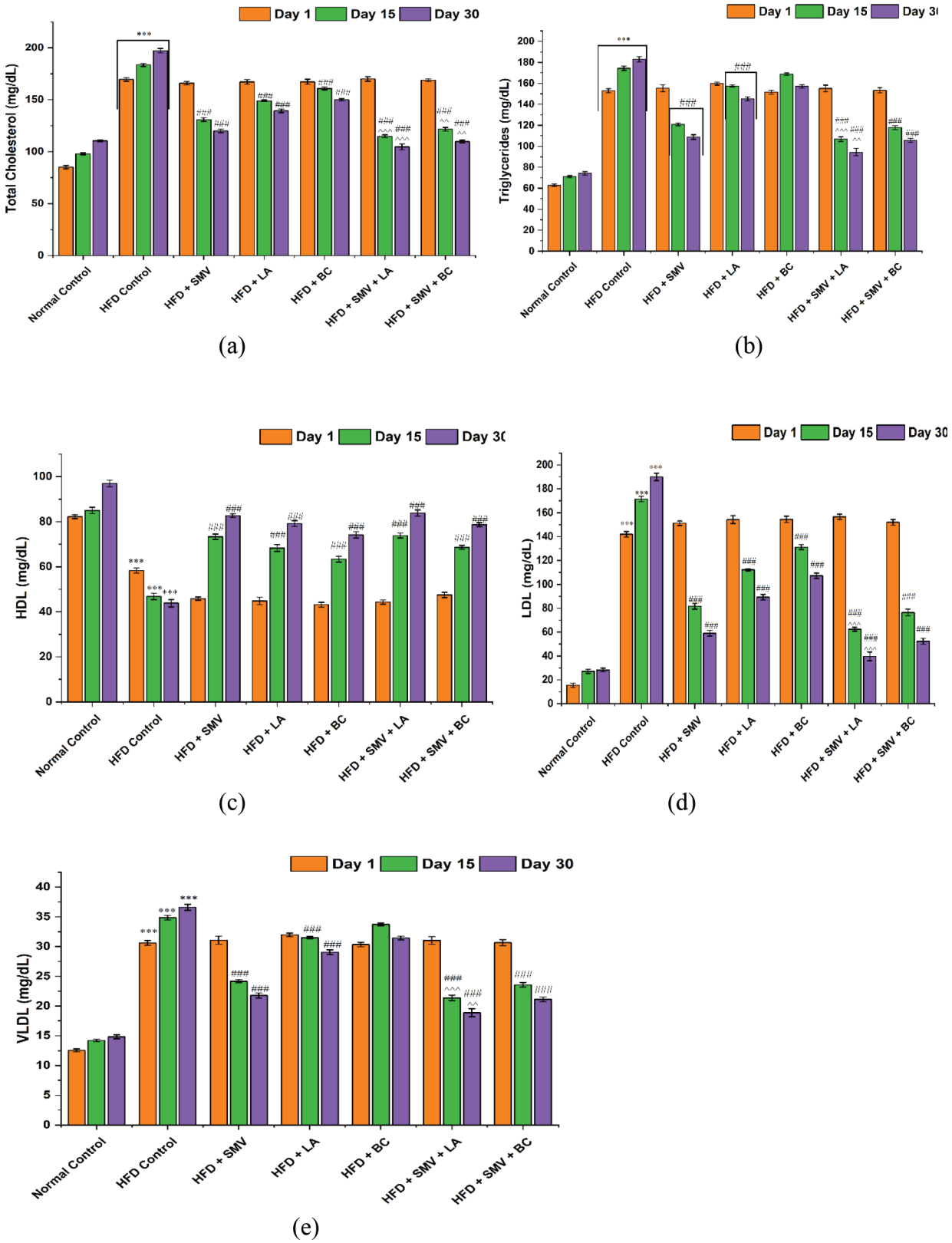

Figure 2. **(a).** TC; **(b).** TG; **(c).** HDLC; **(d).** LDLC and **(e).** VLDLC on 1, 15, and 30 days.

on the 15th and day 30. Finally, the HFD + SMV + BC exhibited diminutions in TC, TG, LDL-C, and VLDL-C levels at the 15th and 30th days, contrasting with the HFD, with only TC showing a reduction on days 15 as well as 30 contrasting with HFD + SMV, while the rest were insignificantly altered.Top of Form

9.3 Renal Markers

As depicted in Figure 3, various hyperlipidemia treatments on renal function indicators, including Cr, U, UA, and BUN, are shown in Figure 3. Noteworthy indicator changes were noted, with p-values indicating statistical significance. In HFD, substantial increases in Cr and UA at all three time points raised U on the 1st, 15th, and day 30. Aside from that, BUN rose 1st and on days 15 and 30. Contrasting with + SMV, Cr sharply rose. revealed a noteworthy on the 15th day, whereas U rose on the 15th and 30th day. UA also rose on the 15th and 30th days contrasting with HFD. Contrasting with HFD + LA demonstrated a drop in Cr on the 15th and 30th day, with a drop in U on the 15th day. UA notably dropped on days 15 and 30, and BUN dropped on the 30th day contrasting with HFD. Similarly, HFD + BC exhibited dropped Cr on the 15th and 30th, insignificantly reduced U on days 15 and 30, and a noteworthy drop in UA on the 15th and 30th day, along with dropped BUN on the 30th day, contrasting HFD. In HFD + SMV + LA, UA dropped on days 15 and 30, and BUN dropped on the 30th day, while Cr revealed a noteworthy drop on the 15th and 30th days in contrast to HFD. In addition, declines in these Cr, U, UA, and BUN were noted on the 15th and 30th days, contrasting HFD + SMV. Lastly, in HFD + SMV + BC, Cr dropped insignificantly on days 15 and 30, UA dropped substantially on the 15th and 30th days, and BUN non-significantly dropped on days 15 and 30 contrasting HFD. Moreover, declines in Cr, U, UA, and BUN were noted on the 15th and 30th days, contrasting HFD + SMV.

9.4 Serum Electrolytes

Data from Figure 4 depicts that, in HFD, no noteworthy changes in serum electrolytes were noticed. Likewise, HFD + LA and HFD + BC groups exhibited no substantial changes over the study period. On the other hand, HFD + SMV displayed significant alterations in the serum electrolytes. Remarkably, there was a considerable drop in Na, Cl^-, K, and Ca^{+2} on the 15th and 30th days contrasting HFD. In HFD + SMV + LA and HFD + SMV + BC increases in K, Na, $Cl^-$and Ca^{+2} on days 15 and 30, contrasting HFD + SMV.

9.5 Grip Strength and Skeletal Muscle Markers

Figure 5 illustrates that in HFD, GS remarkably dropped on days 15 and 30, while CK was exceptionally elevated compared to NC. In HFD + SMV, there was a substantial drop in GS on the 15th and 30th days, alongside an increase in M on these days. Additionally, CK substantially rose on days 15 and 30 relative to HFD. Likewise, in HFD + LA, GS dropped substantially on day 30, whereas M declined remarkably on days 15 and 30. CK also rose markedly, with significance noted on day 15 contrasting HFD. In HFD + BC, noteworthy declines were reported in GS on the 15th and 30th days, M on days 15 and 30, and day 15, contrasting HFD. In HFD + SMV + LA, GS exhibited a noteworthy drop on days 15 and 30, and CK dropped substantially on days 15 and 30, while M remained unaltered, contrasting HFD. On top of that, GS substantially rose on days 15 and 30, while M and CK dropped substantially on days 15 and 30, contrasting HFD + SMV. Lastly, in the HFD + SMV + BC, GS demonstrated a noteworthy drop on days 15 and 30, and CK also dropped substantially on the 15th and 30th day, contrasting HFD. Moreover, GS rose substantially on days 15 and 30, M dropped substantially on 15 and 30 days, and CK dropped on the 15th and 30th, contrasting HFD + SMV.

9.6 Inflammatory Cytokines

Figure 6 illustrates that in HFD, any of the inflammatory mediators demonstrate substantial variations contrasting NC. However, in HFD + SMV, there was a noteworthy rise of TNF-α, IL-6, and IL-β1 on the 15th and 30th days, contrasting HFD. In contrast, in HFD + LA, TNF-α rose significantly on the 15th and 30th days, IL-6 rose on day 15 and day 30, and IL-β1 rose on the 15th day and 30th day, contrasting HFD. Similarly, in HFD + BC, TNF-α rose insignificantly on the 15th and 30th days, IL-6 rose on the 15th day, and IL-β1 rose on the 30th day, contrasting HFD. In HFD + SMV + LA, TNF-α rose notably on the 30th day, IL-6 rose on both the 15th and 30th days and IL-β1 rose on the 15th and 30th days in contrast to HFD. Aside from that, TNF-α dropped on the 15th and 30th days, IL-6 dropped on the

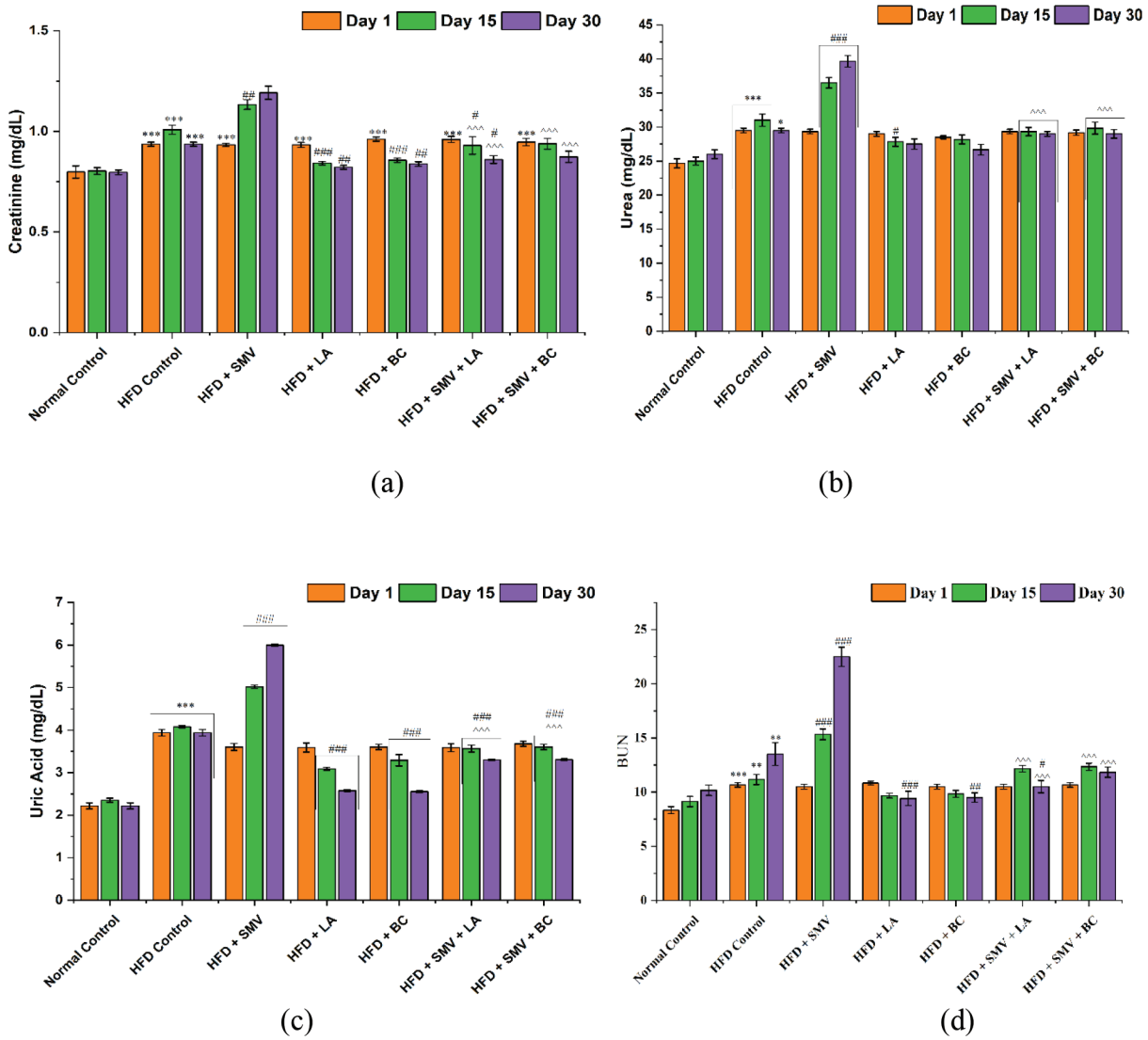

Figure 3. **(a).** Cr, **(b).** U, **(c).** UA and **(d).** BUN at days 1, 15, and 30.

15th and 30th days, and IL-β1 rose on the 15th and 30th days, contrasting HFD + SMV. And lastly, in HFD + SMV + BC, TNF-α rose on the 30th day, IL-6 rose on the 15th and 30th days, and IL-β1 rose on the 15th and 30th days, contrasting HFD. On top of it, TNF-α, IL-6, and IL-β1 dropped on the 15th and 30th days in contrast to HFD + SMV.

9.7 Histopathological Evaluation

As depicted in Figure 7, No anomalies were observed in the NC. HFD, on the other hand, displayed interstitial gaps and infiltration of adipocytes and inflammatory cells, which indicates unfavourable tissue alterations. It was shown that the HFD + SMV displayed equivalent alterations in tissue and death of epithelial cells, suggesting more significant damage. In contrast, the HFD + LA and HFD + BC groups had congestion of the glomeruli, but they kept their epithelial cells intact and had an essentially normal cell look. Normal cell formation was observed in HFD + SMV + LA and HFD + SMV + BC, suggesting that both combinations may have potential protective benefits against tissue changes. These histological examinations show that different interventions affect kidney tissues when an HFD is considered.

10. Discussion

The principal aim of the research was to find out if giving two probiotics, *L. acidophilus* (LA) and *B. coagulans*

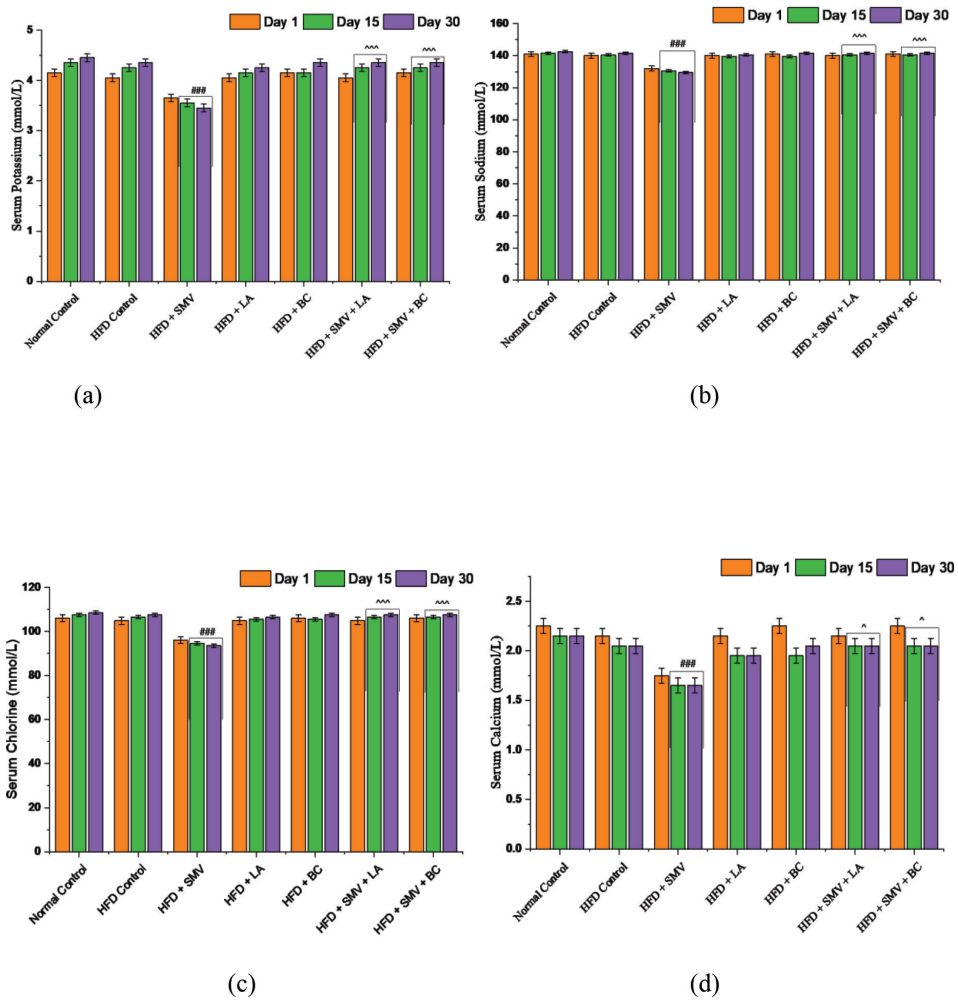

Figure 4. **(a).** K^+, **(b).** Na^+, **(c).** Cl^- and **(d).** Ca^{+2} at days 1, 15, and 30.

(BC), together with SMV as "adjunct therapy" would protect experimental rats with high cholesterol levels from SMV-induced rhabdomyolysis. A prominent statin drug used to treat high cholesterol, SMV, is correlated with a higher risk of rhabdomyolysis, a disorder marked by substantial muscular breakdown[35,40,47]. According to earlier research, probiotics may be able to prevent kidney and skeletal muscle diseases. Probiotics have been investigated as treatments for inflammation and muscular injury[48]. LA and BC were the probiotic strains examined in this investigation. Both strains have been acknowledged for their possible health benefits[49]. This trial's "adjunct therapy" method included probiotics and SMV. This strategy aimed to determine if probiotics could lessen the adverse effects of SMV. SMV use has well-documented dangers, including the possibility of rhabdomyolysis, which is what spurred our investigation. Probiotics' anti-inflammatory and immunomodulatory qualities present a viable strategy for mitigating these dangers. The study attempted to investigate the potential of LA and BC as an additional therapy in lowering the risk of SMV-induced rhabdomyolysis in high-cholesterol rats by delivering them in addition to SMV. The doses of LA and BC were chosen as 1×10^9 CFU/mL in the study based on prior research that has shown that dosages in the range of 1×10^8 to 1×10^9 CFU/mL are effective in ameliorating metabolic disorders, reducing inflammation, and improving gut health in animal models.

Firstly, it's notable that rats fed an HFD for 28 days exhibited obesity, as evidenced by substantial increases in MBW and %BWC compared to the NC on day 1. This indicates the successful establishment of the obesity model in the experimental setting. After 30 days of treatment, various effects were seen with SMV at 80 mg/kg, LA or BC at 1×10^9 CFU/mL, or a combination of

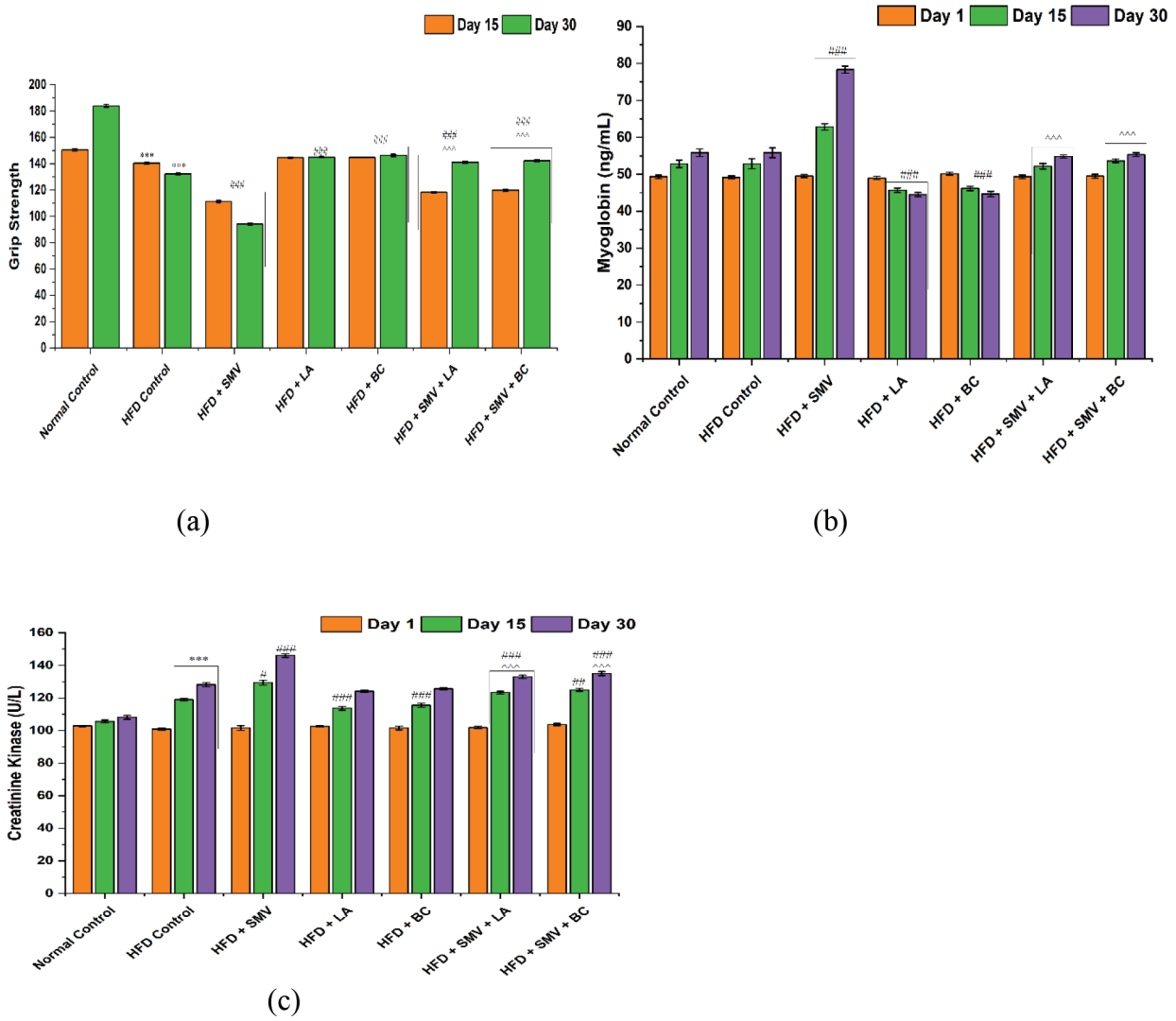

Figure 5. **(a).** GS, **(b).** M, and **(c).** CK on days 1, 15, and 30.

both. HFD + SMV alone substantially reduced MBW on day 30, showing SMV can reduce high-fat diet-induced weight gain. The 15th-day MBW reduction with HFD + SMV + LA or HFD + SMV + BC was negligible, suggesting that probiotics may not immediately reduce weight when taken with SMV. The combination therapies HFD + SMV + LA and HFD + SMV + BC exhibited substantial decreases in % BWC compared to the HFD g, demonstrating that probiotics plus SMV reduced body weight increase. The considerable fall in % BWC with HFD + SMV + LA and HFD + SMV + BC in contrast to HFD + SMV alone on the 30th day suggests a synergistic impact of probiotics and SMV in obesity reduction.

The lipid profile analysis demonstrates the effectiveness of SMV, LA, and BC in reducing hypercholesterolemia induced by an HFD in rats. SMV alone or in combination with LA and BC notably lowered total TC, TG, LDL-C, and VLDL-C levels even as they rose HDL-C in contrast to the HFD. These findings suggest a potential synergistic effect of SMV and probiotics in enhancing lipid profiles. However, it's worth noting that the HFD + SMV + LA combination exhibited slightly superior activity, showing more note-worthy decreases in TC, TG, LDL-C, and VLDL-C levels on days 15 and 30, contrasting HFD + SMV + BC. HDL-C levels rose substantially with both combinations, but the increase was particularly pronounced with HFD + SMV + LA. These results suggest that while both combination therapies effectively manage hypercholesterolemia, HFD + SMV

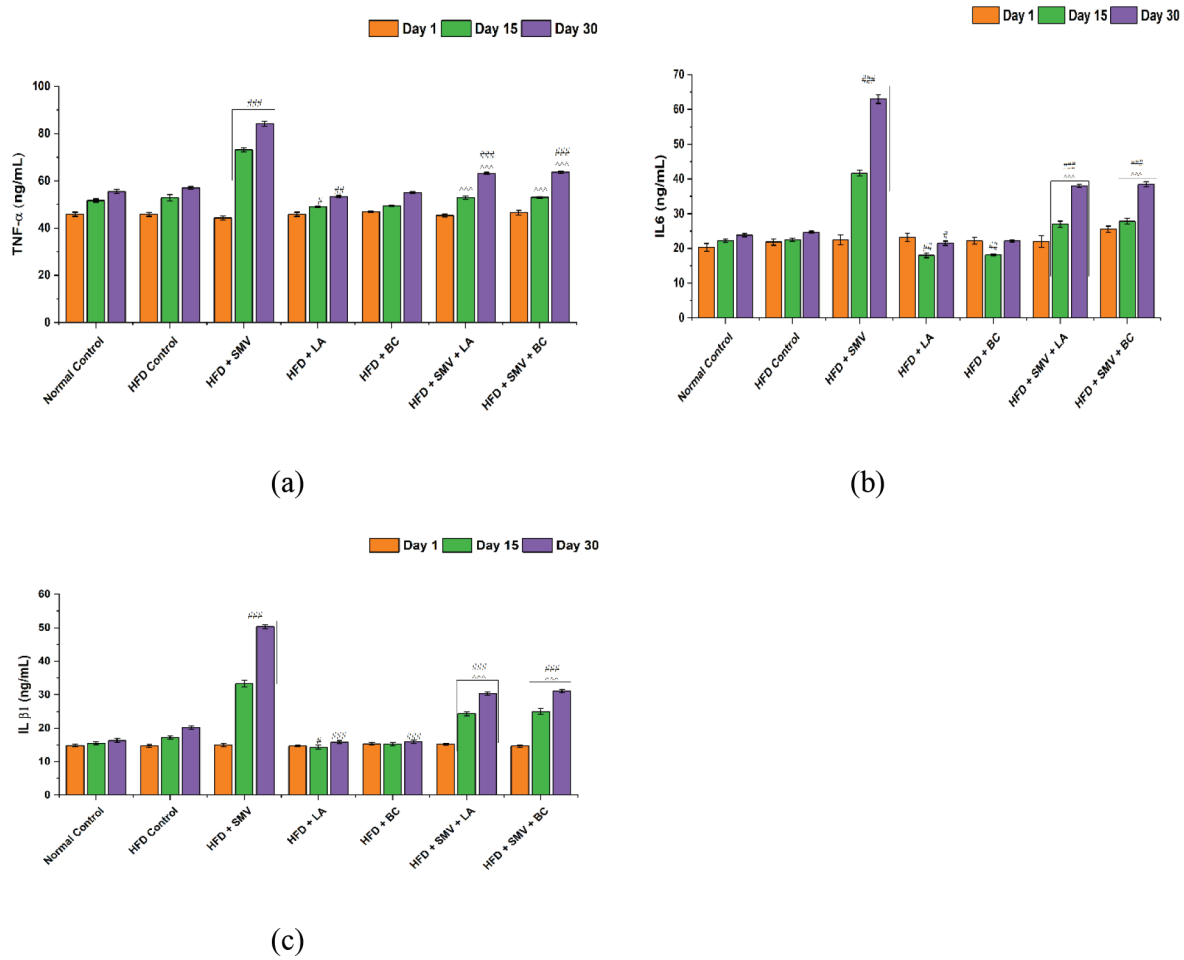

Figure 6. **(a).** TNF-α, **(b).** IL-6 and **(c).** IL β1 at days 1, 15, and 30.

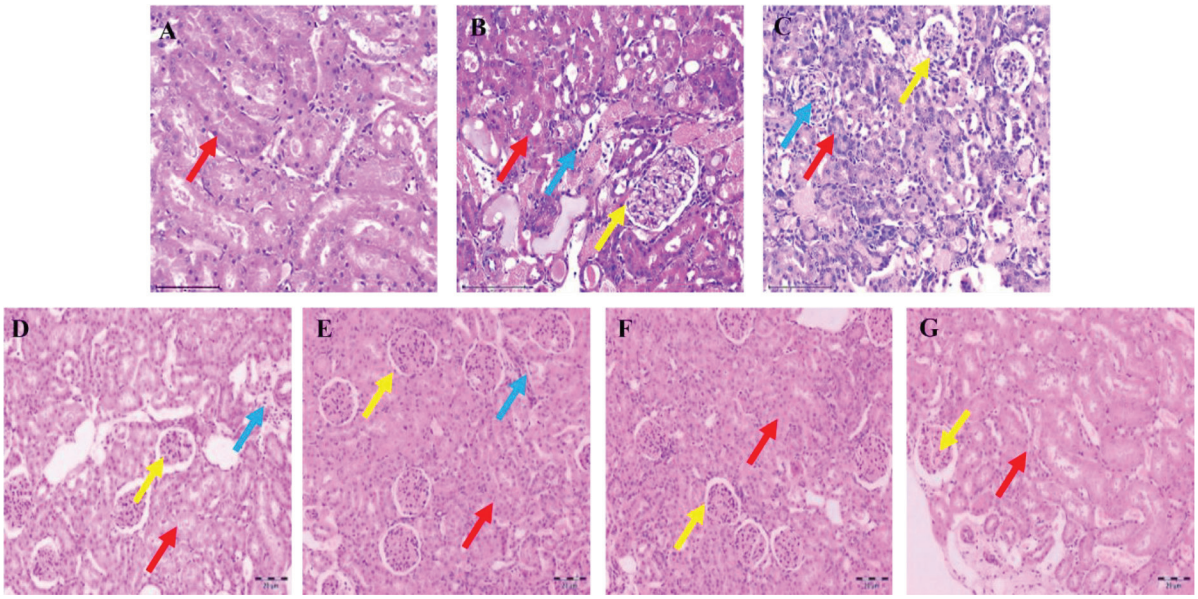

Figure 7. Histopathology after 30 days of treatment. **A.** NC, **B.** HFD, **C.** HFD + SMV, **D.** HFD + LA, **E.** HFD + BC, **F.** HFD + SMV + LA, **G.** HFD + SMV + BC, red arrow-epithelial cells, yellow arrow-glomerulus, blue arrow-infiltration of epithelial cells.

+ LA may offer slightly better lipid-lowering activity than HFD + SMV + BC.

The renal function indicators revealed noteworthy changes in Cr, U, UA, and BUN levels across various treatment groups. The HFD exhibits substantial increases in these indicators, suggesting impaired renal function. In contrast, SMV (HFD + SMV) treatment impacted renal function, substantially increasing Cr levels on day 15 and elevated U and UA levels on days 15 and 30. Treatment with LA demonstrated renal protective effects, evidenced by declines in Cr, UA, and BUN levels on days 15 and 30 in contrast to the HFD. Similarly, treatment with BC improved renal function, as indicated by decreases in Cr, UA, and BUN on days 15 and 30 in contrast to HFD. Combination therapies, mainly HFD + SMV + LA, exhibited promising renal protective effects, with substantial reductions in Cr, UA, and BUN levels noted on days 15 and 30 contrasting HFD and HFD + SMV groups. Overall, these findings suggest that combination therapies and specific lipid-lowering agents may offer benefits in preserving renal function in hyperlipidemic conditions.

The serum electrolytes, including Cl^-, Na^+, Ca^{+2}, and K^+, were monitored throughout the research to determine the effects of various treatments on electrolyte balance. Interestingly, while the HFD alone and combined with LA or BC did not significantly alter serum electrolyte levels, treatment with SMV led to substantial decreases in Na^+, Cl^-, K^+, and Ca^{+2} levels. These findings suggest that SMV administration may disrupt electrolyte balance. However, when SMV was combined with LA or BC, demonstrated increased electrolyte levels indicating that the addition of probiotics reverses the SMV induced electrolyte imbalance.

Grip strength and skeletal muscle indicators, including M and CK, were assessed to see how interventions affected skeletal muscle health. The HFD had significant GS declines and CK elevations, indicating muscular dysfunction. With or without LA or BC, SMV reduced these effects. SMV plus LA or BC improved GS and CK levels more than SMV alone. In contrast to HFD + SMV + BC, HFD + SMV + LA reduced GS levels on days 15 and 30, showing similar effects on skeletal muscle function. HFD + SMV + LA had a more significant drop in Creatine Kinase (CK) levels on both days than HFD + SMV + BC, showing that LA supplementation may protect muscles. This suggests that while both combinations reduced hyperlipidemia-induced skeletal muscle dysfunction, HFD + SMV + LA may be slightly more beneficial.

In contrast to the NC, inflammatory mediators were similar in the HFD. However, therapy with SMV alone significantly raised TNF-α, IL-6, and IL-β1 levels, indicating raised inflammation. SMV combined with LA or BC had different effects on inflammatory cytokines. Although both combinations reduced TNF-α, IL-6, and IL-β1in contrast to SMV alone, they also revealed cytokine increases relative to the HFD. These findings demonstrate that probiotics may control SMV-induced inflammation, suggesting adjunct probiotic therapy may reduce hyperlipidemia-related inflammation. LA and BC groups revealed similar inflammatory cytokine trends in contrast to the high-fat diet HFD, with TNF-α, IL-6, and IL-β1 increasing significantly. Higher TNF-α and IL-6 levels were seen in BC but not substantially higher than in LA on specific days. Both LA and BC groups demonstrated reduced inflammatory cytokine levels when coupled with SMV, suggesting probiotics may modulate SMV-induced inflammation. On some days, the combination with BC caused a more noteworthy drop in TNF-α and IL-6 than with LA. These findings imply that LA and BC may reduce inflammation when coupled with SMV, with BC perhaps having slightly higher effects. LA and BC affect inflammatory pathways differently and interact with SMV; Probiotics may achieve this by interacting with the Gut-Associated Lymphoid Tissue (GALT) to modulate systemic immune responses, leading to decreased inflammation in muscle tissues, hence more research is needed.

The histological examination of skeletal muscle tissues revealed distinct effects of different interventions in the context of an HFD. The HFD exhibited adverse tissue changes characterized by interstitial spaces, adipocyte infiltration, and inflammatory cell infiltration. Interestingly, SMV treatment exacerbated tissue damage, indicating more severe alterations, including epithelial cell apoptosis. In contrast, supplementation with LA and BC maintained relatively normal tissue architecture despite displaying glomerular congestion. Combining SMV with LA or BC demonstrated potential, as evidenced by histological results and protective effects against changes in tissue indicating normal cell formation. These results underscore the

importance of adjunct therapies, mainly probiotics, in mitigating HFD-induced kidney tissue damage and preserving tissue integrity.

The observed effects of LA and BC in combination with SMV in mitigating obesity, preserving skeletal muscle health, and reducing inflammation suggest potential mechanisms involving the gut-muscle-nephron axis. Gut-Muscle[30] probiotics may modulate gut microbiota composition, enhancing metabolic pathways that promote weight regulation, reduce inflammation, and exert nephroprotective effects. Probiotics could indirectly protect renal function by improving gut barrier function and reducing systemic inflammation. Additionally, probiotics might directly influence renal health through their metabolites or signalling molecules, enhancing renal antioxidant capacity and mitigating inflammatory responses, thus preserving nephron integrity. These findings underscore the interconnectedness between gut health, skeletal muscle function, and renal health, highlighting the potential of probiotics as multifaceted agents in managing rhabdomyolysis complications. Despite the promising findings, our study has a few limitations: Only two probiotic strains (LA and BC) were tested. Other strains may also have beneficial effects and should be investigated. The study suggests potential mechanisms and detailed mechanistic studies are required to understand how probiotics exert their protective effects entirely.

More research is required to clarify the mechanism behind these probiotics' overall protective effects within the gut-muscle-nephron axis framework, which treats the symptoms of rhabdomyolysis.

11. Conclusion

These results suggest that probiotics, particularly LA and BC, may assist SMV in treating obesity in rats caused by an HFD. SMV plus LA or BC synergistically reduces body weight growth, improves lipid profiles, preserves renal function, and reduces skeletal muscle dysfunction and inflammation, which are the significant symptoms of rhabdomyolysis. The gut-muscle-nephron axis is modulated by probiotics, which change gut microbiota, improve metabolic pathways, reduce inflammation, and protect the kidneys. These findings demonstrate probiotics' diverse role in rhabdomyolysis-related problems and the need further to study their mechanisms of action and clinical implications. When coupled with SMV, LA and BC reduced rhabdomyolysis-related problems. Each probiotic performed differently depending on the outcome. While SMV combined with LA appears to demonstrate slightly superior efficacy in contrast to SMV combined with BC in specific parameters such as lipid-lowering activity, both probiotic strains exhibit significant potential in preserving renal function, skeletal muscle health, and reducing inflammation when combined with SMV. At the same time, BC had a significantly more substantial effect on inflammatory cytokines, including TNF-α and IL-6. In addition, BC supplementation reduced TNF-α and IL-6 levels more than LA when combined with SMV. Further study is needed to find the best probiotic regimen for managing rhabdomyolysis.

12. References

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