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Polysaccharides from split gill mushrooms (*Schizophyllum commune* Fr.) improve glucose metabolism and reduce muscle inflammation in a rat model of type 2 diabetes

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Submission Date: July 8th, 2025, **Acceptance Date:** July 29th, 2025, **Publication Date:** August 7th, 2025

Please cite this article as: Khimmaktong W., Matsathit U., Nuinamwong S., Sermwittayawong D., Chubuathong P., Sukpondma Y., Radenahmad N., Komolkriengkrai M. Polysaccharides from split gill mushrooms (Schizophyllum commune Fr.) improve glucose metabolism and reduce muscle inflammation in a rat model of type 2 diabetes. *Functional Foods in Health and Disease* 2025; 15(8): 519 – 539. DOI: https://doi.org/10.31989/ffhd.v15i7.1705

ABSTRACT

Background: Chronic hyperglycemia in type 2 diabetes mellitus (T2DM) contributes to muscle inflammation and impaired glucose metabolism. This study aimed to evaluate the therapeutic effects of polysaccharides extracted from *Schizophyllum commune* Fr. (split gill mushroom) on glucose metabolism and muscle inflammation in a rat model of insulin resistance.

Methods: Male Wistar rats were divided into five groups: normal diet (ND), ND with 240 mg/kg body weight (BW) *S. commune* polysaccharides (ND240), high-fat diet-induced diabetic rats (HFD+DM), HFD+DM treated with *S. commune* polysaccharides (HFD+S240), and HFD+DM treated with metformin (HFD+Met). Blood glucose levels were monitored weekly for eight weeks. Muscle tissues were then collected for histological staining, ELISA, immunohistochemistry, and

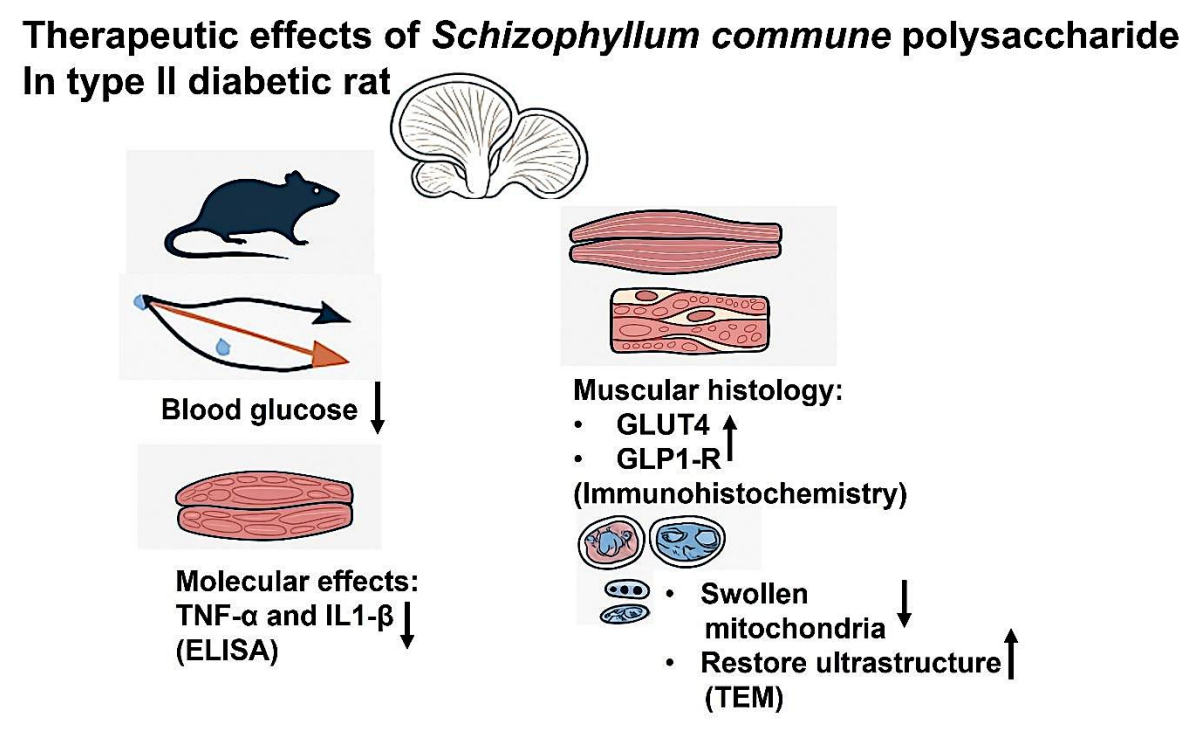
transmission electron microscopy to assess inflammation markers (TNF-α, IL-1β) and expression of glucose transporter 4 (GLUT4) and glucagon-like peptide-1 receptor (GLP-1R).

Results: Rats in the HFD+DM group exhibited elevated blood glucose levels, increased inflammation, and reduced GLUT4 and GLP-1R expression. Treatment with *S. commune* polysaccharides significantly lowered blood glucose levels, reduced inflammatory markers, and restored the expression of GLUT4 and GLP-1R, with results comparable to metformin-treated rats.

Conclusions: These findings suggest that *S. commune* polysaccharides may ameliorate hyperglycemia-induced muscle inflammation and enhance insulin sensitivity, supporting their potential as a natural therapeutic candidate for managing T2DM.

Novelty: This study demonstrates, for the first time, that polysaccharides extracted from *Schizophyllum commune* Fr. (split gill mushroom) significantly reduce muscle inflammation and improve glucose metabolism in a high-fat diet and streptozotocin-induced diabetic rat model. The crude extract restored GLUT4 and GLP-1 receptor expression, decreased TNF-α and IL-1β levels, and preserved muscle fiber architecture, as confirmed by immunohistochemistry and electron microscopy. These findings suggest a novel therapeutic role of S. commune polysaccharides in ameliorating diabetes-related muscle dysfunction.

Keywords: Diabetes, muscle, polysaccharide, *Schizophyllum commune* Fr., GLUT4, GLP-1R



INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major public health concern characterized by hyperglycemia resulting from insulin resistance and β-cell dysfunction. Inadequate glucose regulation can lead to systemic complications, affecting multiple organs and tissues [1]. Insulin is synthesized by pancreatic β-cells and regulates glucose uptake primarily through the activation of phosphoinositide 3-kinase (PI3K)/Akt signaling, promoting translocation of glucose transporter 4 (GLUT4) to the plasma membrane [2]. In addition to facilitating glucose uptake, insulin signaling suppresses gluconeogenesis and enhances glycogen and protein synthesis by modulating downstream targets such as glycogen synthase kinase 3 (GSK3) and forkhead box O1 (FOXO1) [3].

Skeletal muscle plays a pivotal role in systemic glucose homeostasis. However, diabetes accelerates the loss of muscle mass and strength, contributing to sarcopenia and functional decline. Recent studies demonstrated that *Schizophyllum commune* polysaccharides not only improve glycemic control but also help preserve pancreatic function and muscle integrity in diabetic rats, suggesting a potential role in delaying diabetes-induced sarcopenia [4]. Incretins, including glucagon-like peptide-1 (GLP-1), enhance glucose-dependent insulin secretion and provide cytoprotective effects on pancreatic β-cells [5]. GLP-1 may also promote β-cell proliferation and modulate peripheral glucose uptake. Notably, muscle loss occurs 26% faster and strength declines 33% faster in individuals with diabetes compared to non-diabetic counterparts [6]. Current therapeutic options for diabetes-related muscle atrophy remain limited and include glycemic control, nutritional support, physical exercise, and hormonal therapies [7].

Natural compounds with anti-inflammatory and insulin-sensitizing properties are of increasing interest as complementary treatments for diabetic complications. Mushrooms have long been consumed for their nutritional and medicinal properties, with species such as *Schizophyllum commune* Fr. (split gill mushroom) attracting attention for their bioactive β-glucans and antioxidant constituents [8]. Schizophyllan, a β-glucan from S. commune, exhibits immunomodulatory, anti-tumor, and antioxidant activities. Additionally, S. commune contains other beneficial compounds such as schizocommunin, riboflavin (vitamin B2), and essential amino acids, contributing to its therapeutic potential [9,10]

Despite its promising bioactive profile, the effects of S. commune polysaccharides on skeletal muscle structure and glucose metabolism in diabetes have not been fully elucidated. This study aimed to investigate whether polysaccharide extracts from S. commune could ameliorate hyperglycemia-induced muscle inflammation, enhance GLUT4 and GLP-1R expression, and reduce inflammatory cytokines (TNF-α and IL-1β) in a rat model of type 2 diabetes.

MATERIALS AND METHODS

Animal Model: This study was conducted using male Wistar rats, aged eight weeks and weighing between 180 and 200 grams. The animals were obtained from the Southern Laboratory Animal Facility at Prince of Songkla University, Thailand. Upon arrival, all rats were housed in a controlled environment with a temperature of 25 ± 2 °C, 55 ± 5% humidity, and a 12-hour light/dark cycle. Standard rodent chows and clean drinking water were provided ad libitum. The experimental procedures were approved by the Animal Ethics Committee of Prince of Songkla University (Protocol No. AR027/2022) and

followed institutional animal care guidelines in accordance with the ARRIVE reporting standards.

After a one-week acclimatization period, the rats were randomly assigned into five experimental groups, with ten animals in each. Two groups were fed a standard diet throughout the study. One served as the normal control (ND), and the other received 240 mg/kg body weight of *Schizophyllum commune* polysaccharide extract orally (ND240). The remaining three groups were given a high-fat diet (HFD) for four weeks to induce insulin resistance. After this period, diabetes was induced in these groups with a single intraperitoneal injection of streptozotocin (STZ) at 35 mg/kg, dissolved in 0.1 M citrate buffer (pH 4.5). One week later, fasting blood glucose levels were measured, and rats with glucose concentrations exceeding 200 mg/dL were considered diabetic and included in the study.

Among the diabetic groups, one group remained untreated and served as the diabetic control (HFD+DM). The other two groups received either *S. commune* polysaccharide extract (240 mg/kg body weight, HFD+S240) or metformin (20 mg/kg body weight, HFD+Met). Both treatments were administered orally once daily for eight weeks. During the treatment period, all animals were closely monitored, and fasting blood glucose was recorded weekly to assess the progression of hyperglycemia and the effects of treatment.

Preparation of *Schizophyllum commune* Polysaccharide Extract

Crude Polysaccharide Extraction: Crude polysaccharides were extracted from fruiting bodies of *Schizophyllum commune* (split gill mushroom), kindly provided by Inno4 (Ban Hedkrang, Thailand), using a modified hot water extraction protocol based on previous work [8]. Briefly, the mushrooms were thoroughly cleaned, shredded, and dried in a hot-air oven at 70 °C for 48 hours. The dried material was ground into a fine powder and suspended in distilled water at a ratio of 1 g to 8 ml. This mixture was heated at 90–95 °C for 4 hours and then cooled to room temperature. The extract was centrifuged at 5,000 rpm for 10 minutes to separate the supernatant from the solid residue.

The solid residue was subjected to a second round of extraction under the same conditions, and the supernatants from both extractions were pooled. The combined extract was concentrated using a rotary evaporator (60 °C, 100 rpm, 100 mbar, cooling temperature 10 °C) and then precipitated by the addition of three volumes of absolute ethanol. After overnight incubation at 4 °C, the precipitated polysaccharides were collected by centrifugation and dissolved in distilled water. Residual ethanol was removed through dialysis against distilled water, and the final product was lyophilized. The resulting polysaccharide powder was stored in a desiccator until further use [8].

Total Carbohydrate Content: The total carbohydrate content of the extract was determined using the phenol-sulfuric acid method, as described by [10], with glucose used to generate a standard curve. The assay was conducted according to previously established procedures [8].

Total Protein Content: Total protein content was measured using the Bradford assay [11], based on dye binding to protein molecules. Bovine serum albumin was used to prepare the standard curve. The method followed the protocol reported previously in similar polysaccharide studies [8]

β-Glucan Content: Total β-glucan content was analyzed using a commercial β-glucan assay kit (Megazyme®, Ireland), following the manufacturer's instructions. This method involves enzymatic digestion to quantify both total glucans and α-glucans in the sample. Endoglucanase and exoglucanase enzymes were used to hydrolyze β-(1→3) and β-(1→6) linkages, thereby determining total

glucans, while α-amylase was used to digest α-glycosidic bonds. The β-glucan content was calculated by subtracting the α-glucan concentration from the total glucan concentration and then adjusted based on the standard β-glucan reference, which contains 49% β-glucan by weight.

Monosaccharide Composition Analysis: The monosaccharide composition of the polysaccharide extract was analyzed using a modified two-step acid hydrolysis method [12]. Initially, 15 mg of sample was treated with 2 mL of 0.1 M trifluoroacetic acid (TFA) and heated at 80 °C for 48 hours with constant stirring. After evaporation at 60 °C, the residue was hydrolyzed further using 2 mL of 2 M sulfuric acid (H_2SO_4) at 100 °C for 4 hours. The resulting hydrolysate was filtered and analyzed using ion chromatography (ICS-3000, Dionex, USA) equipped with a CarboPac PA1 column. Monosaccharide peaks were identified by comparison with retention times of known standards, and concentrations were calculated from peak areas using standard calibration curves.

Amino Acid Composition Analysis: Amino acid composition was analyzed using an L-8900 Amino Acid Analyzer (Hitachi, Japan). For hydrolysis, 15 mg of the polysaccharide sample was treated with 6 M hydrochloric acid in sealed tubes under vacuum and incubated at 110 °C for 22 hours. After hydrolysis, the sample was dried using a SpeedVac concentrator and redissolved in 0.02 M HCl. The solution was filtered through a 0.2 µm syringe filter before being injected into the analyzer. Amino acids were identified by matching retention times with known standards, and their concentrations were quantified based on peak area integration.

Histological Preparation and Staining: Gastrocnemius muscle tissues were collected from all experimental groups and fixed in 10% neutral-buffered formalin for 24 hours at room temperature. After fixation, tissues were dehydrated through a graded ethanol series (70%, 80%, 90%, 95%, and 100%), with each concentration applied twice for one hour per step. Tissues were then cleared in three successive changes of xylene (30 minutes each) and embedded in paraffin wax.

Paraffin-embedded tissues were sectioned at a thickness of 5 µm using a microtome. Sections were mounted on slides and stained with hematoxylin and eosin (H&E) to assess general morphology, and with Masson's trichrome to evaluate collagen deposition and fibrosis, following standard histological protocols. Stained tissues were examined and photographed using a light microscope (BX-50, Olympus, Japan).

Transmission Electron Microscopy (TEM): For ultrastructural analysis, approximately 1 mm^3 of fresh muscle tissue was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4 °C overnight. Samples were then post-fixed with 1% osmium tetroxide, dehydrated in graded ethanol, treated with propylene oxide, and embedded in epoxy resin. Semi-thin sections (0.5–1.0 µm) were stained with Toluidine blue to identify regions of interest.

Ultrathin sections (~60 nm) were obtained using an ultramicrotome and mounted on copper grids (200–300 mesh). Sections were stained with uranyl acetate and lead citrate before examination under a transmission electron microscope (JEM-2010, JEOL, Japan). Images were captured for analysis of mitochondrial morphology, sarcomere structure, and nuclear integrity.

Immunofluorescence for GLUT4 Expression: Immunofluorescence was performed to localize and quantify GLUT4 expression in muscle tissue. Paraffin sections were deparaffinized in xylene, rehydrated through a descending ethanol series, and permeabilized with 0.1% Triton X-100 in phosphate-buffered saline (PBS) for 30 minutes. Non-specific binding was blocked

by incubating sections with 10% horse serum in PBS for 1 hour at room temperature.

Sections were then incubated overnight at 4 °C with rabbit anti-GLUT4 antibody (1:100, Abcam, UK) diluted in blocking solution. After three PBS washes, sections were incubated with a Texas Red–conjugated goat anti-rabbit IgG secondary antibody (1:200, Vector Laboratories, USA) for 2 hours in the dark at room temperature. Nuclei were counterstained with DAPI. Fluorescence images were captured using a fluorescence microscope (BX-50, Olympus), and signal intensity was quantified using ImageJ software (NIH, version 1.52).

Immunohistochemistry for GLP-1R: To detect GLP-1 receptor (GLP-1R) expression, tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval using 0.02% hyaluronidase in PBS at 37 °C for 20 minutes. Endogenous peroxidase activity was blocked by incubating sections in 0.3% hydrogen peroxide in methanol for 30 minutes. Non-specific binding was blocked with 10% normal goat serum and 1% bovine serum albumin (BSA) in PBS for 30 minutes at room temperature.

Sections were incubated overnight at 4 °C with rabbit polyclonal anti-GLP-1R antibody (1:400, Santa Cruz Biotechnology, USA) diluted in PBS with 1% BSA. After washing, sections were incubated with HRP-conjugated goat anti-rabbit IgG secondary antibody (1:1000, Vector Laboratories) for 2 hours. Immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) in 0.05 M PBS containing 0.01% hydrogen peroxide for 10 minutes. Slides were counterstained with Mayer's hematoxylin, dehydrated in graded alcohols, cleared in xylene, and mounted for light microscopy.

Quantification of Inflammatory Cytokines by ELISA: To assess inflammatory responses, muscle tissues were homogenized in 500 µl of radioimmunoprecipitation assay (RIPA) buffer containing 1% protease inhibitor cocktail (Sigma-Aldrich, USA) on ice for 90 seconds. Homogenates were centrifuged at 12,000 × g for 15 minutes at 4 °C. The supernatant was collected, and levels of tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) were measured using commercially available ELISA kits (RAB0480, Sigma-Aldrich, USA), according to the manufacturer's instructions.

Briefly, 100 µl of each sample was added to well pre-coated with rat TNF-α antibody and incubated at room temperature for 2.5 hours with gentle shaking. After washing, biotinylated detection antibody was added, followed by streptavidin-HRP and TMB substrate. Plates were incubated at room temperature and protected from light. The reaction was stopped by adding stop solution, and absorbance was read at 450 nm using a microplate reader (Multiskan, Thermo Fisher Scientific, Finland). All samples were analyzed in duplicate.

Statistical Analysis: Data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were conducted using one-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant. Analyses were performed using GraphPad Prism software, version 9.0 (GraphPad Software, USA).

RESULTS

Extraction and characterization of crude polysaccharides: Polysaccharides were extracted from *Schizophyllum commune* fruiting bodies using a hot water extraction technique. The resulting crude extract was precipitated with ethanol, dialyzed, and lyophilized for use in subsequent experiments. No additional purification steps were performed, and the sample was referred to as crude polysaccharide. A summary of its physicochemical composition is presented in Table 1.

Table 1: Characterization of crude polysaccharides

Total carbohydrate (percent of dry mass ± S.D.)	Total protein (percent of dry mass ± S.D.)	Glucans		
		Total glucans (percent of dry mass)	α- glucans (percent of dry mass)	b- glucans (percent of dry mass)
52.51 ± 1.50	5.25 ± 0.08	43.52	0.48	43.04

To further characterize the extract, we analyzed its monosaccharide and amino acid composition. Due to limited availability of standard monosaccharides, we focused on five sugars: arabinose, galactose, glucose, mannose, and fructose. Chromatograms comparing sample peaks with monosaccharide standards are shown in Fig. 1. Six major peaks were detected in the sample chromatogram (Fig. 1A), but only peaks 2, 3, 4, and 6 could be matched to arabinose, galactose, glucose, and mannose, respectively. Peaks 1 and 5 could not be identified due to the absence of corresponding standards. Among the detectable monosaccharides, galactose, glucose, and mannose were present in a ratio of 1.00:8.40:1.10, confirming glucose as the dominant monosaccharide (Table 2). Both arabinose and fructose were below the detection threshold (0.5 ppm) and therefore reported as undetected.

Table 2: monosaccharide composition of the crude polysaccharides

Monosaccharides	Arabinose	Galactose	Glucose	Mannose	Fructose
Amount (ppm)	N/D*	262.67	2,207.30	288.83	N/D*

*N/D = not detected, detection limit is 0.5 ppm

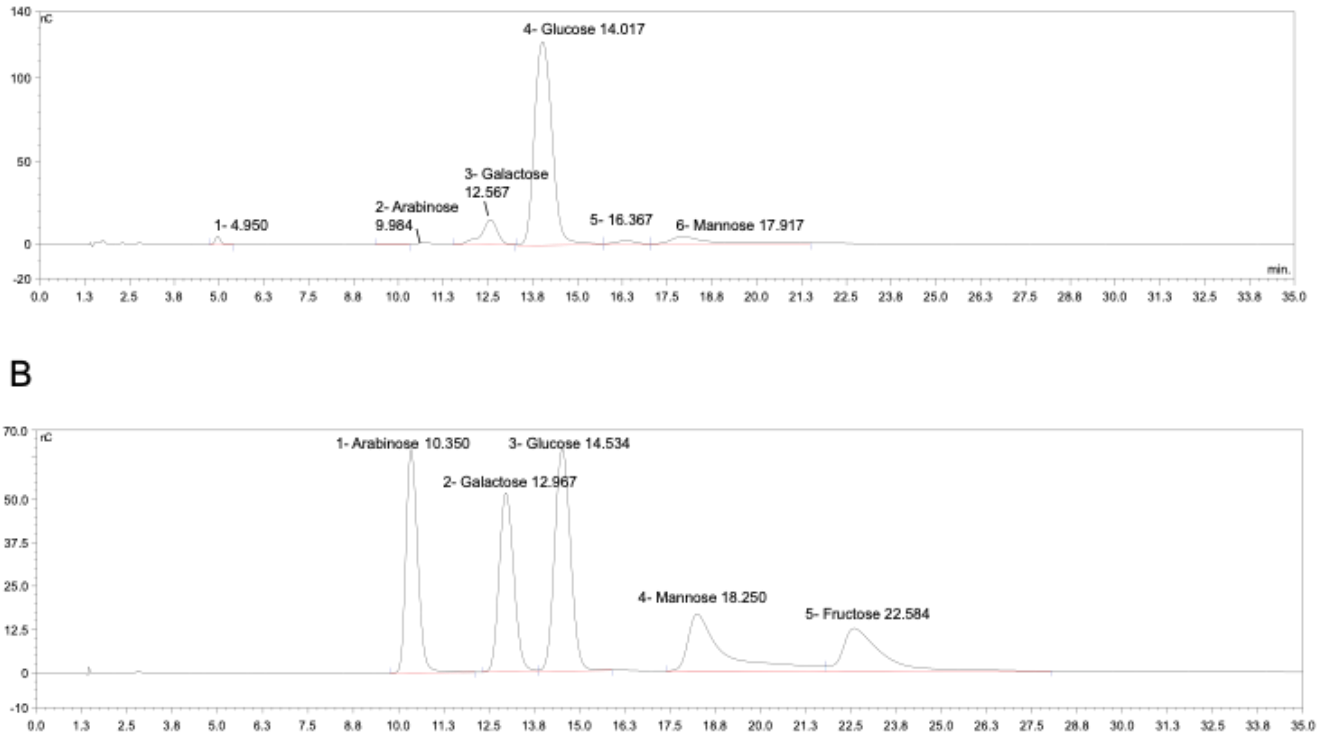


Figure 1 Monosaccharide composition analysis of the crude polysaccharides. (A) shows a chromatogram of monosaccharides in the crude polysaccharides. (B) shows a chromatogram of 5 standard monosaccharides

Consistent with the glucose dominance, β-glucan analysis revealed that the extract contained 43.04% β-glucose, while α-glucose content was only 0.48%, indicating that the majority of glucose residues were in the β-configuration.

Amino acid composition analysis was also performed to identify trace proteinaceous components within the crude extract. The five most abundant amino acids were glutamate, aspartate, methionine, serine, and alanine (Table 3). Notably, cysteine was not detected.

Few studies have comprehensively characterized polysaccharides extracted from the fruiting bodies of *S. commune*. One study compared polysaccharides obtained using various extraction methods including hot water, microwave-assisted, ultrasound-assisted, and high-pressure extraction and reported a total carbohydrate yield of 67.96% using the hot water method, which is higher than the 52.51% yield observed in our study [13]. Interestingly, while we found glucose to be the major monosaccharide in our extract, Chen and colleagues reported galacturonic acid as the predominant sugar in all extraction methods [13].

Table 3: amino acid composition in the crude polysaccharides

Number	Amino acid	Amount (mg/mg) ± S.D.
1	Aspartate	0.0044 ± 0.0003
2	Threonine	0.0022 ± 0.0004
3	Serine	0.0039 ± 0.0003
4	Glutamate	0.0046 ± 0.0003
5	Proline	0.0004 ± 0.0000
6	Glycine	0.0016 ± 0.0000
7	Alanine	0.0024 ± 0.0001
8	Cysteine	0.0000 ± 0.0000
9	Valine	0.0008 ± 0.0001
10	Methionine	0.0033 ± 0.0002
11	Isoleucine	0.0006 ± 0.0000
12	Leucine	0.0007 ± 0.0000
13	Tyrosine	0.0004 ± 0.0000
14	Phenylalanine	0.0005 ± 0.0000
15	Lysine	0.0005 ± 0.0001
16	Histidine	0.0004 ± 0.0000
17	Arginine	0.0006 ± 0.0000

Another study assessed β-glucan content in five different strains of *S. commune* collected in Thailand. Their analysis revealed β-glucan levels ranging from 48.51–49.76% (w/w), slightly higher than the 43.52% we observed [14]. This difference is likely due to methodological differences: whereas our β-glucan measurement was based on extracted polysaccharides, their quantification was performed directly on mushroom powder.

Together, these results support the presence of glucose-rich, β-linked polysaccharides in our extract and provide important context for comparing extraction yields and composition across different *S. commune* strains and protocols.

Blood Glucose Levels: Fasting blood glucose levels were monitored weekly over the eight-week treatment period. At baseline and throughout the study, rats in the HFD+DM, HFD+S240, and HFD+Met groups maintained significantly higher glucose levels compared to the ND and ND240 groups ($p < 0.0001$). However, treatment with either *Schizophyllum commune* extract (HFD+S240) or metformin (HFD+Met) led to a progressive reduction in blood glucose levels over time.

In the HFD+S240 group, blood glucose levels began to decline significantly by week 6 and remained lower through weeks 7 and 8 compared to the untreated diabetic group ($p < 0.05$). Similarly, the HFD+Met group exhibited a marked decrease in glucose levels beginning at week 6 ($p < 0.001$), with further reductions at weeks 7 and 8 ($p < 0.0001$). These findings indicate that both treatments were effective in improving glycemic control, with metformin demonstrating a slightly earlier and more pronounced effect (Fig 2).

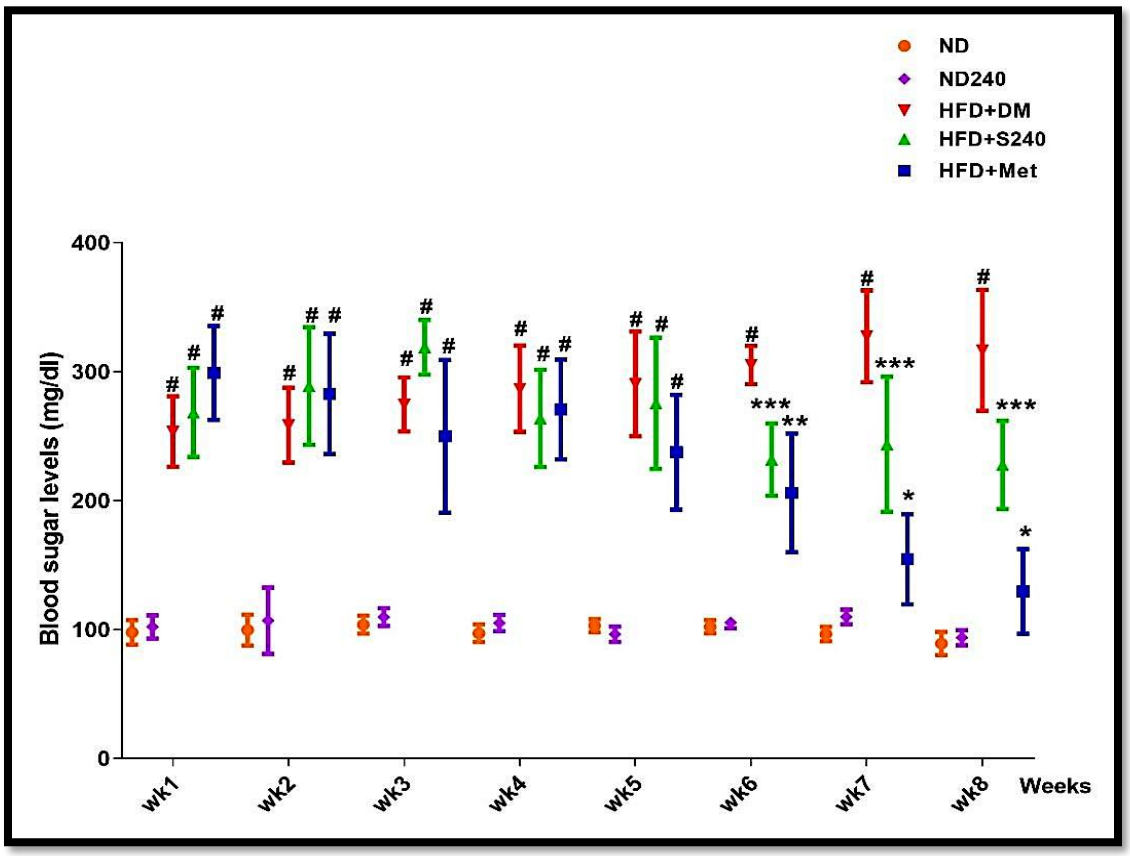
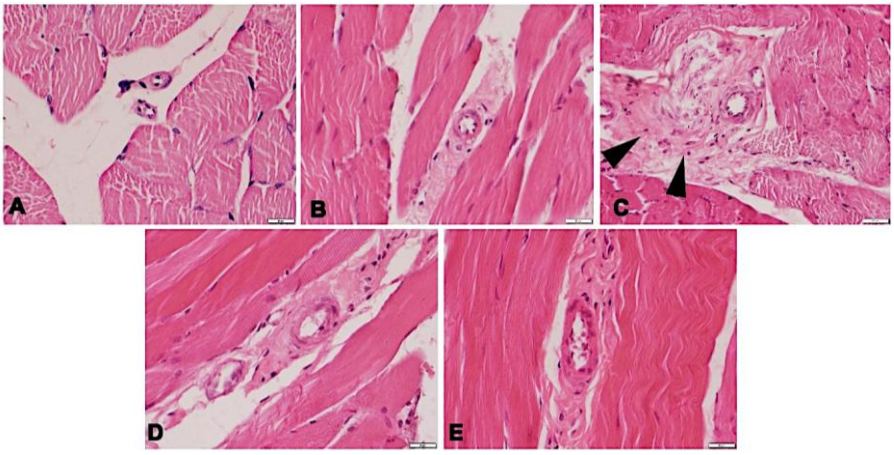


Figure 2 Blood glucose levels of ND, ND240, HFD+DM, HFD+S240, and HFD+Met groups for a period of 8 weeks. Values are mean ± SE, #($p < 0.00001$) in HFD+DM, HFD+S240, and HFD+Met compared with the ND rats. *** ($p < 0.05$) in HFD+SD240, ** ($p < 0.001$) and * ($p < 0.0001$) in HFD+Met compared with the HFD+DM rats.

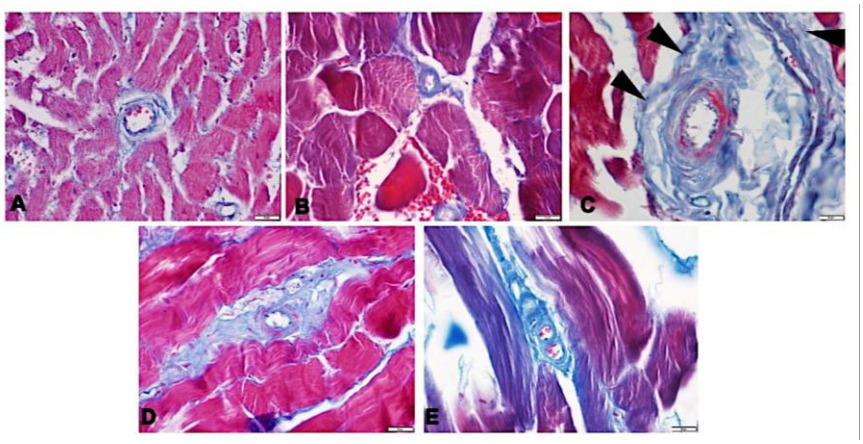
Histological Examination: Histological analysis of gastrocnemius muscle sections was performed using H&E and Masson's trichrome staining to evaluate structural integrity and collagen deposition, respectively. Muscle tissues from the ND (Fig. 3A, 4A) and ND240 (Fig. 3B, 4B) groups exhibited normal histoarchitecture, with well-organized muscle fibers and no signs of inflammation or vascular abnormalities.



Figures 3 Light micrograph form H&E staining of the skeletal muscle tissue in the following groups: control group (ND) (A), control group treated with polysaccharide extract from split gill (ND240) (B), high-fat diet-induced diabetes group (HFD+DM) (C), high-fat diet-induced diabetes group treated with polysaccharide extract from split gill 240 mg/kgBW (HFD+S240) (D), and high-fat diet-induced diabetes group treated with Metformin (HFD+Met) (E). The black arrows indicate collagen fibers surrounding the blood vessels (arteries). (scale bar = 2 µm.).

In contrast, the diabetic control group (HFD+DM) showed marked pathological changes. H&E staining revealed thickening of the muscle arterial walls and fragmentation of muscle fibers (Fig. 3C). Masson's trichrome staining demonstrated a substantial accumulation of collagen fibers, particularly around blood vessels, as indicated by the intense blue staining (Fig. 4C). These findings reflect increased connective tissue deposition and muscle tissue damage in response to chronic hyperglycemia.

Treatment with *S. commune* extract (HFD+S240) led to notable improvements in muscle histology. Arterial wall thickness was visibly reduced (Fig. 3D), and the muscle fibers appeared more aligned and less fragmented compared to the untreated diabetic group. Similarly, collagen deposition was decreased, as evidenced by the reduced intensity of blue staining in Masson's trichrome sections (Fig. 4D).



Figures 4 Light micrograph form Masson's trichrome staining of the skeletal muscle tissue in the following groups: control group (ND) (A), control group treated with polysaccharide extract from split gill (ND240) (B), high-fat diet-induced diabetes group (HFD+DM) (C), high-fat diet-induced diabetes group treated with polysaccharide extract from split gill 120 (HFD+S240) and 240 mg/kgBW (HFD+S240) (D), and high-fat diet-induced diabetes group treated with Metformin (HFD+Met) (E). The black arrows indicate collagen fibers surrounding the blood vessels (arteries). (scale bar = 2 µm.).

Rats treated with metformin (HFD+Met) showed similar improvements (Figs. 3E and 4E). Muscle fiber organization was largely restored, and collagen accumulation was minimized, closely resembling the histological appearance of non-diabetic controls.

Quantitative comparison of arterial wall thickness and collagen density between groups confirmed that both treatments significantly mitigated diabetes-induced histopathological changes. These results suggest that *S. commune* polysaccharide extract may help protect muscle tissue integrity in diabetic conditions, comparable to standard treatment with metformin.

Immunofluorescence Analysis of GLUT4 Expression: We used immunofluorescence staining to examine the expression of glucose transporter 4 (GLUT4) in skeletal muscle fibers. As shown in Fig. 5, rats in the high-fat diet-induced diabetic group (HFD+DM) displayed noticeably weaker red fluorescence compared to the normal diet control (ND) and the ND240 groups, indicating reduced GLUT4 protein levels.

Interestingly, rats in the HFD+S240 group, which were treated with *Schizophyllum commune* polysaccharides, showed a clear increase in red fluorescence around the muscle fibers and nuclei. This stronger staining suggests that GLUT4 expression was restored. Quantitative analysis of fluorescence intensity (Fig. 6) confirmed that GLUT4 levels were significantly higher in the HFD+S240 group compared to the diabetic control group ($p < 0.05$). A similar increase was observed in the metformin-treated group (HFD+Met), where GLUT4 expression was significantly higher than in the HFD+DM group ($p < 0.01$). In the ND240 group, which received polysaccharide extract without diabetes induction, the fluorescence pattern was similar to that of the ND group. This suggests that the extract did not alter GLUT4 levels under normal conditions. Together, these results indicate that treatment with *S. commune* polysaccharides can help restore GLUT4 expression in skeletal muscle, which may contribute to improved glucose regulation in diabetic rats.

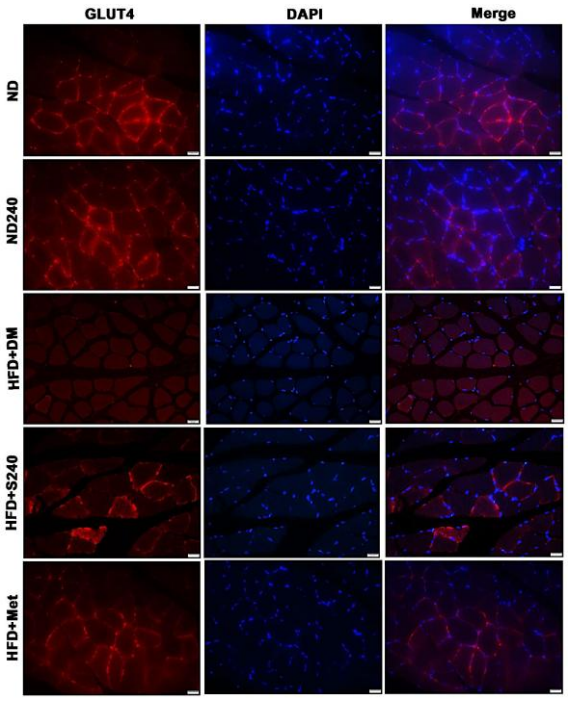


Figure 5 Photomicrograph of the immunofluorescence reaction of GLUT 4 in the muscle tissue. The illustration includes different groups: the control group (ND), the control group treated with polysaccharide extract from split gill (ND240), the diabetic group (HFD+DM), the diabetic group treated with polysaccharide extract from split gill 240 mg/kg BW (HFD+S240), and the diabetic group treated with Metformin (HFD+Met). (scale bar = 2 µm.).

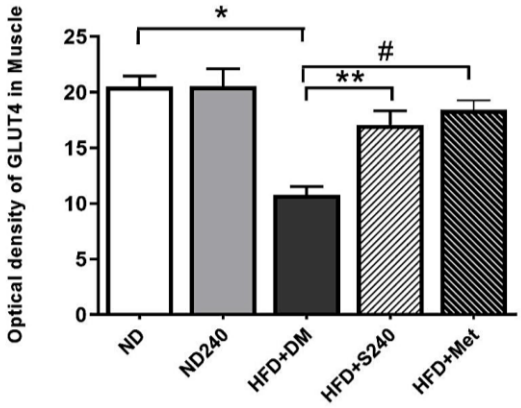


Figure 6 Optical densities were analyzed and illustrated the comparative analysis of GLUT4 protein expression in muscle tissue. Values are mean ± SE, A statistically significant difference ($p < 0.001$) was seen when comparing the experimental group to the control group. ($p < 0.05$) was observed when comparing the specified group to the HFD+DM group. Additionally, a very significant difference ($p < 0.01$) was also observed.

Immunohistochemical Analysis of GLP-1R Expression: Immunohistochemical staining was used to examine the expression of glucagon-like peptide-1 receptor (GLP-1R) in skeletal muscle tissue. As shown in Fig. 7, GLP-1R was localized to the muscle fibers (muscle cells) in all groups. In the diabetic group (HFD+DM, Figure 7C), GLP-1R expression was significantly reduced compared to the normal diet control (ND, Figure 7A) and ND240 (Figure 7B) groups, as indicated by weaker brown staining. Quantitative analysis (Fig. 8) confirmed that GLP-1R levels were significantly lower in the HFD+DM group ($p < 0.001$). Treatment with *Schizophyllum commune* polysaccharides (HFD+S240, Fig. 7D) or metformin (HFD+Met, Fig. 7E) markedly restored GLP-1R expression. Muscle tissues from these groups exhibited stronger brown staining, particularly around the muscle fibers and nuclei. Quantitative analysis showed that GLP-1R expression was significantly higher in both the HFD+S240 and HFD+Met groups compared to the HFD+DM group ($p < 0.001$ for both) (Fig. 8).

These findings suggest that treatment with *S. commune* polysaccharides can enhance GLP-1R expression in skeletal muscle, similar to the effects observed with metformin, potentially contributing to improved insulin signaling in diabetic rats.

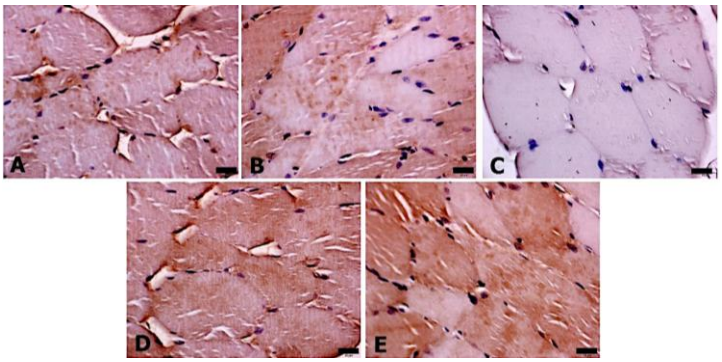


Figure 7. Immunohistochemistry micrographs that depicted the expression of GLP-1 protein at the muscle cells in each of the five experimental groups revealed that the HFD+DM group had a low pronounced expression of the protein at the muscle tissue: the control group (ND), the control group treated with polysaccharide extract from split gill (ND240), the diabetic group (HFD+DM), the diabetic group treated with polysaccharide extract from split gill 240 mg/kgBW (HFD+S240), and the diabetic group treated with Metformin (HFD+Met). (scale bar = 2 µm.).

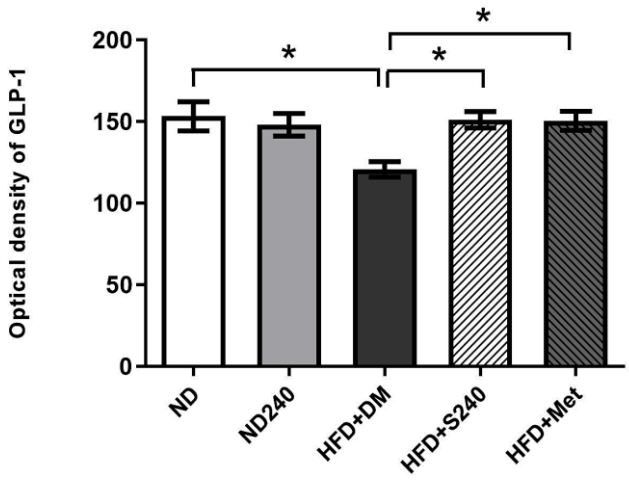


Figure 8 Optical densities were analyzed and illustrated the comparative analysis of GLP-1 protein expression in muscle tissue. Values are mean ± SE, A statistically significant difference ($p < 0.001$) was seen when comparing the experimental group to the control group. ($p < 0.001$) was observed when comparing the specified group to the HFD+DM group.

The results of Quantification of Inflammatory Cytokines by ELISA: The concentration of tumor necrosis factor-alpha (TNF-α) in skeletal muscle tissue was measured to assess the inflammatory status of each group. As shown in Fig. 9, TNF-α levels in the ND and ND240 groups were low, measuring 28.16 ± 0.76 pg/mg and 25.76 ± 1.28 pg/mg of muscle tissue, respectively.

In contrast, rats in the diabetic group (HFD+DM) exhibited significantly elevated TNF-α levels, reaching 39.63 ± 6.56 pg/mg, indicating heightened muscle inflammation compared to the control groups. Treatment with *Schizophyllum commune* polysaccharides (HFD+S240) resulted in a substantial reduction in TNF-α levels to 23.67 ± 5.25 pg/mg, bringing values closer to those observed in healthy controls. Similarly, rats treated with metformin (HFD+Met) showed TNF-α levels of 17.05 ± 6.63 pg/mg, suggesting an even greater anti-inflammatory effect. These findings indicate that both *S. commune* extract and metformin were effective in lowering muscle inflammation in diabetic rats.

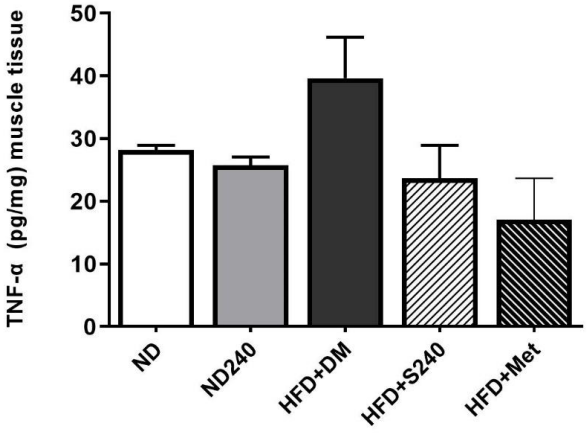


Figure 9. Levels of pro-inflammatory cytokines TNF-α in muscle tissue in different groups: control group (ND), control group receiving polysaccharide extract from split gill mushrooms (ND240), diabetic group (HFD+DM), diabetic group receiving polysaccharide extract from split gill mushrooms (HFD+S240), and diabetic group receiving metformin (HFD+Met).

Levels of interleukin-1 beta (IL-1β) in skeletal muscle tissue were measured to further assess inflammatory responses across the groups. As shown in Fig. 10, IL-1β levels were low in the ND and ND240 groups, measuring 186.23 ± 10.63 pg/mg and 180.06 ± 11.90 pg/mg of muscle tissue, respectively. In the diabetic group (HFD+DM), IL-1β levels were significantly elevated compared to the control groups ($p < 0.01$), reaching 327.5 ± 5.85 pg/mg, indicating a strong inflammatory response. Treatment with *Schizophyllum commune* polysaccharides (HFD+S240) significantly reduced IL-1β levels to 244.5 ± 58.51 pg/mg compared to the untreated diabetic group. Similarly, rats treated with metformin (HFD+Met) also exhibited reduced IL-1β levels at 237.53 ± 17.23 pg/mg, which was significantly lower than in the HFD+DM group ($p < 0.05$).

These findings suggest that both *S. commune* polysaccharides and metformin were effective in lowering IL-1β-mediated inflammation in the skeletal muscle of diabetic rats.

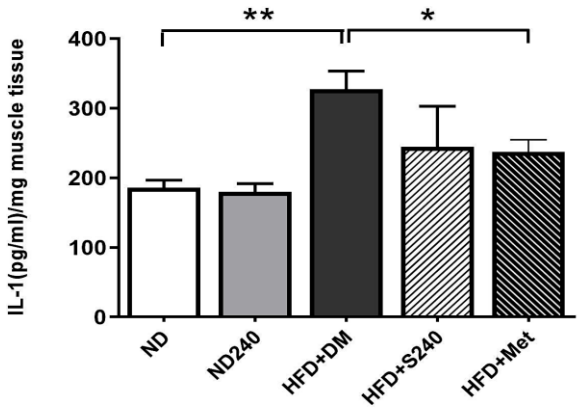


Figure 10. Levels of pro-inflammatory cytokines IL-1β in muscle tissue in different groups: control group (ND), control group receiving polysaccharide extract from split gill mushroom (ND240), diabetic group (HFD+ DM), diabetic group that received polysaccharide extract from split gill (HFD+S240), and diabetic group that received metformin (HFD+Met). ** There was a significant difference ($p < 0.01$) compared to the ND group. * There was a significant difference ($p < 0.05$) compared to the HFD+DM group.

Comparative Study of Histological Changes in Muscle Structure by Transmission Electron Microscopy: Transmission electron microscopy (TEM) was used to examine ultrastructural changes in the gastrocnemius muscle fibers across the experimental groups (Fig. 11).

In the control group (ND, Fig. 11A) and the group receiving *Schizophyllum commune* extract without diabetes induction (ND240, Fig. 11B), muscle fibers displayed normal ultrastructure. Cell nuclei were round and intact, and cytoplasmic organelles, including mitochondria, appeared normal and well-organized.

In contrast, the diabetic group (HFD+DM) exhibited pronounced pathological alterations (Fig. 11C). Muscle fibers showed signs of disorganization, swollen mitochondria, and features consistent with cellular apoptosis. Additionally, collagen fiber accumulation was observed around the arteries supplying the muscle fibers, indicating fibrosis.

Importantly, treatment with S. commune polysaccharides (HFD+S240, Fig. 11D) or metformin (HFD+Met, Fig. 11E) improved muscle ultrastructure. Muscle cells in both treated groups displayed more organized fibers, healthier nuclei, and better-preserved cytoplasmic organelles, closely resembling the ultrastructure observed in the control group. These findings suggest that both treatments helped restore muscle fiber integrity at the ultrastructural level.

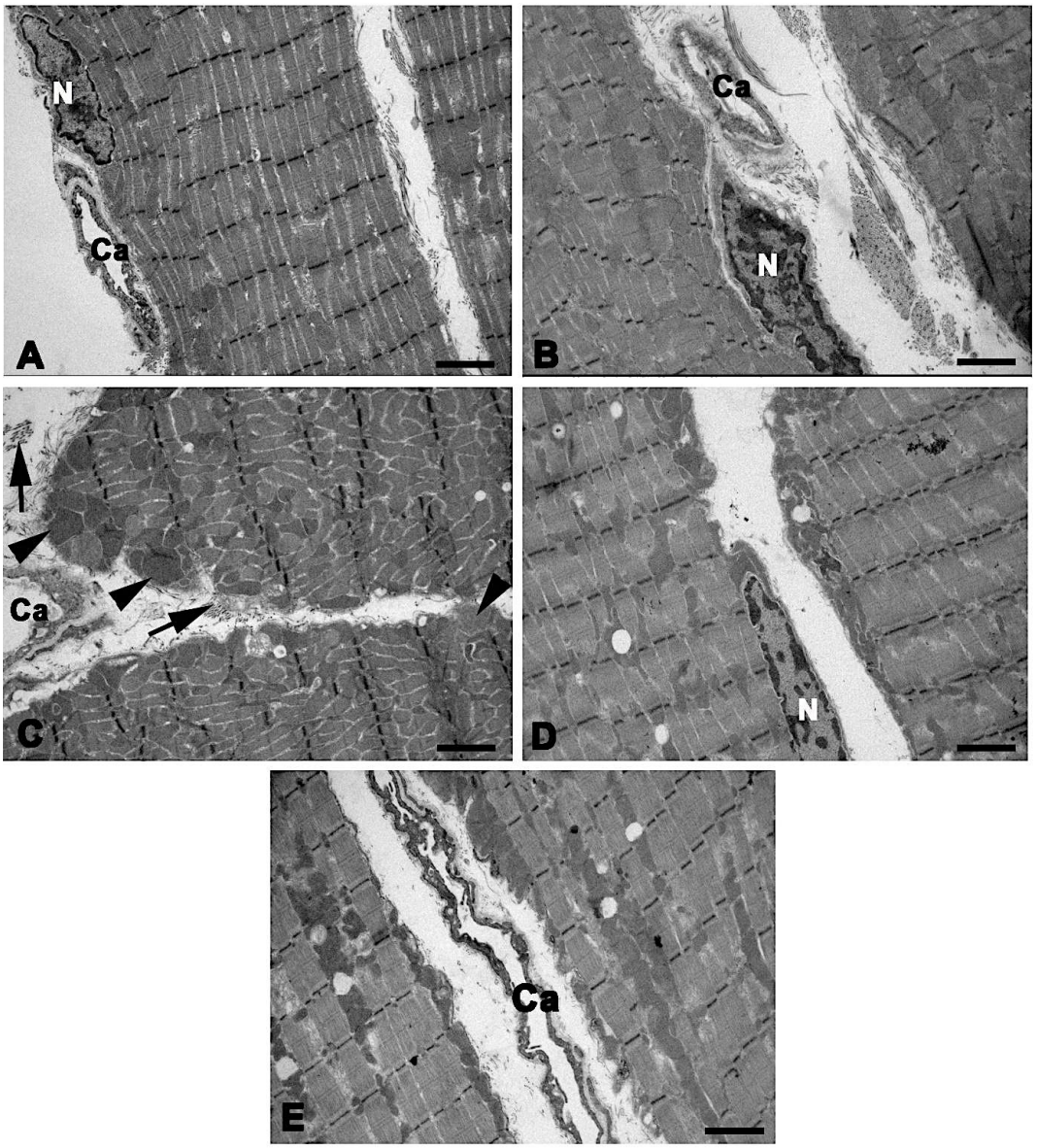


Figure 11. The transmission electron micrograph illustrates the characteristics of muscle fibers in the gastrocnemius muscle. Each group of rats includes the ND group (A), the ND240 group (B), the HFD+DM group (C), the HFD+S240 group (D), and the HFD+Met group (E). These groups show the cell parts, like the nucleus (N) and other structures in the cytoplasm. In contrast, the diabetic group (HFD+DM) has pathological conditions. The results reveal cells initiating apoptosis, characterized by tangled muscle fibers, swollen mitochondria, and a lack of organization (black arrow). There is also the buildup of collagen fibers (red arrows) around the arteries supplying the muscle fibers. Magnification 3000x (scale bar = 2 μm.).

DISCUSSION

In this study, type 2 diabetes was induced in male Wistar rats by combining a high-fat diet with a low dose of streptozotocin (STZ), a method known to elevate blood glucose levels. STZ activates the sorbitol pathway and protein kinase C (PKC), promoting the release of cytokines and growth factors that worsen hyperglycemia [15]. Additionally, increased blood glucose alters the activities of mitogen-activated protein kinase (MAPK) and phosphoenolpyruvate carboxykinase (PCK) [16].

Over the eight-week period, diabetic rats exhibited progressive hyperglycemia, as confirmed by weekly fasting blood glucose measurements. Histological analysis of muscle tissues revealed that diabetic rats

(HFD+DM) developed distinct vascular abnormalities compared to the normal control (ND) and polysaccharide-treated (ND240) groups. Diabetic rats showed thickening of arterial walls, fat vacuole accumulation in the tunica media, and collagen fiber deposition narrowing the vascular lumen. These features are indicative of accelerated atherosclerosis, resulting from persistent hyperglycemia [17]. Vascular stiffening and lumen narrowing impair blood flow and oxygen delivery, leading to organ dysfunction and contributing to diabetic complications.

Skeletal muscle tissues in diabetic rats exhibited connective tissue thickening and reduced vascular flexibility, consistent with chronic hyperglycemia-induced damage. Prolonged high blood sugar levels lead to excessive connective tissue deposition, particularly in the vasculature, impairing the elasticity and function of both small and large arteries [18]. Reduced blood supply contributes to muscle cell injury through a cascade involving insulin resistance, mitochondrial oxidative stress, and chronic inflammation. These pathological conditions collectively activate apoptotic pathways and promote degradation of myofibrillar proteins, resulting in muscle fiber fragmentation. Recent studies have shown that mushroom-derived polysaccharides can attenuate these effects by downregulating TNF-α and NF-κB signaling while preserving mitochondrial function and reducing the expression of muscle atrophy genes such as Atrogin-1 and MuRF1 [19]. Chronic hyperglycemia enhances mitochondrial ROS production, disrupting mitochondrial membranes, DNA, and proteins, and resulting in mitochondrial swelling and muscle fiber degeneration [20]. In this context, the pathological cascade involving inflammation and oxidative stress may serve as a therapeutic target for bioactive agents capable of modulating vascular integrity and cellular metabolism.

Insulin signaling is crucial for maintaining skeletal muscle function, particularly glucose metabolism. Insulin activates phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt), facilitating glucose uptake [21]. Activation of Akt through phosphorylation at Thr308 and Ser473 promotes GLUT4 translocation to the plasma membrane, enhancing glucose uptake [22]. Studies have shown that insulin mainly promotes glucose absorption by facilitating GLUT4 translocation rather than directly increasing GLUT4 activity [23]. Therefore, the restoration of PI3K/Akt signaling is a key target in reversing insulin resistance, especially in skeletal muscle where glucose uptake accounts for the majority of whole-body glucose disposal.

Our immunohistochemical findings revealed that GLP-1 receptor (GLP-1R) expression was significantly reduced in diabetic rats. This reduction is primarily attributed to β-cell dysfunction, which impairs GLP-1 secretion [24]. Similarly, GLUT4 expression was diminished due to insulin resistance and disrupted signaling pathways. GLP-1, secreted by intestinal L-cells, plays a crucial role in optimizing glucose metabolism and insulin secretion. Reduced GLP-1 levels in diabetic rodents exacerbate glucose dysregulation. Emerging evidence suggests that certain mushroom-derived polysaccharides and peptides may modulate GLP-1 secretion or protect GLP-1R expression by inhibiting dipeptidyl peptidase-4 (DPP-4), the enzyme responsible for GLP-1 degradation [6,24]. These actions potentially contribute to the preservation of GLP-1 signaling observed in our treated groups.

GLUT4 is the major glucose transporter in insulin-sensitive tissues such as skeletal muscle and adipose tissue. Insulin resistance in type 2 diabetes reduces GLUT4 expression and translocation to the membrane, impairing glucose uptake [25]. Muscle inflammation, driven by oxidative stress and elevated pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, further disrupts GLUT4 function [26]. Elevated pro-inflammatory cytokines activate NF-κB signaling, which impairs GLUT4

translocation and worsens insulin resistance [27]. This cytokine-driven feedback loop exacerbates metabolic dysfunction by both directly interfering with insulin signaling and sustaining chronic inflammation at the tissue level.

The observed reduction in TNF-α and IL-1β levels in polysaccharide-treated groups suggests that the bioactive components in split gill mushrooms may suppress inflammatory signaling, particularly by modulating NF-κB activation. β-glucans have been shown to bind to pattern recognition receptors such as Dectin-1 and TLRs, leading to downstream inhibition of NF-κB and MAPK pathways [33,35]. This results in reduced transcription of pro-inflammatory cytokines and improved GLUT4 dynamics. Furthermore, amino acids present in the extract—such as methionine, serine, and glutamate—contribute to antioxidant defense via glutathione biosynthesis, thereby attenuating ROS-induced cytokine upregulation [34]. These complementary actions may explain the concurrent restoration of GLUT4 expression and reduction in tissue inflammation observed in our study.

Chronic inflammation is a common problem in diabetes that disrupts insulin signaling, slows down glucose transport, and throws off the body's overall metabolic balance. When blood sugar stays high for too long, glucose starts sticking to proteins inside cells in a process called non-enzymatic glycation, eventually forming advanced glycation end-products (AGEs) [28]. These AGEs cause type I and type IV collagen to build up and thicken the basement membrane of blood vessels, making them less flexible [29]. At the same time, blood vessels lose their ability to respond properly to nitric oxide because the enzymes eNOS and guanylyl cyclase aren't working as well as they should. This leads to more free radicals being produced both through enzyme-independent and enzyme-dependent pathways and increased activation of protein kinase C (PKC) [30]. In hyperglycemia, glycolysis produces more diacylglycerol (DAG), which switches on PKC. In particular, the PKC-β type boosts the production of vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-β) [31]. Meanwhile, NADPH oxidase raises the levels of reactive oxygen species (ROS) and nitric oxide (NO), adding even more damage to blood vessel linings in diabetes [32]. Our findings suggest that split gill polysaccharides exert multi-faceted protective effects against this pathogenic cascade. By simultaneously reducing systemic inflammation, enhancing antioxidant defense, and restoring insulin and incretin signaling, these compounds help reverse several key features of diabetic muscle pathology. Although polysaccharides tend to have only modest antioxidant activity in directly scavenging free radicals in vitro, mushrooms contain a variety of bioactive compounds, including flavonoids, polysaccharides, phenols, tocopherols, ascorbic acid, and terpenes [33]. Research has shown that mushroom polysaccharides can support the antioxidant defenses of key enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), which are important for protecting the body against reactive oxygen species (ROS) [34]. For example, ABMP, a β-glucan found in the fruiting body of Agaricus blazei Murill, helped protect mice from organ dysfunction syndrome (MODS) caused by high doses of zymosan by reducing levels of inflammatory cytokines like TNF-α, IL-1β, IL-6, COX-2, and PGE-2 after modulating the NF-κB signaling pathway [35].

Similarly, Ganoderma lucidum spore polysaccharides (BSGLP) have been shown to reduce levels of TNF-α, IL-1β, and monocyte chemoattractant protein-1 (MCP-1) in a mouse model of diet-induced obesity. BSGLP also prevented macrophage infiltration into white adipose tissue (WAT), largely by suppressing the TLR4/Myd88/NF-κB signaling pathway [36]. To improve the therapeutic potential of active

polysaccharides in diabetes, researchers are exploring strategies like molecular modifications, nanoparticle delivery, and enhancing intestinal absorption [37]. For instance, exopolysaccharides from split gill mushrooms were found to lower iNOS mRNA expression and reduce nitric oxide (NO) and 5-LOX production in RAW 264.7 macrophages, demonstrating clear anti-inflammatory properties [38]. Beyond these effects, *Schizophyllum commune* also shows immunomodulating, antiviral, antitumor, and antioxidant activities [39]. In diabetic rats, treatment with S. commune extract helped correct severe oxidative stress imbalances, including abnormal lipid peroxidation and excessive superoxide anion production, suggesting a potential role for these compounds in diabetes management [40]. At the molecular level, split gill polysaccharides promote insulin signaling by enhancing phosphorylation pathways, which contributes to their blood sugar-lowering effects. In diabetic rodents, activation of PI3K/Akt improves glucose transport by boosting GLUT4 expression in adipose tissue. The polysaccharides also appear to enhance GLP-1 secretion by regulating lipoprotein concentrations and lowering blood glucose levels [41]. Additionally, they help mitigate insulin resistance by reducing serum cholesterol and lipid peroxides, potentially delaying the onset of diabetes [40]. Improvements in hyperglycemia, insulin resistance, and glycogen storage, particularly through phosphorylation of GSK-3 and GLUT4 in liver and muscle tissues, have been observed after polysaccharide treatment in STZ-induced diabetic rodents [38]. Taken together, these mechanisms highlight the therapeutic relevance of *Schizophyllum commune* polysaccharides as functional bioactive agents with systemic effects on inflammation, oxidative stress, insulin resistance, and glucose metabolism.

Bridging to Functional Food Science: The findings of this study align closely with the foundational principles of functional food science as defined by the Functional Food Center (FFC), which describes functional foods as natural or processed foods that contain bioactive compounds capable of providing documented health benefits beyond basic nutrition—specifically in the prevention, management, or treatment of chronic diseases [41].

The crude polysaccharide extract derived from *Schizophyllum commune* fulfills these criteria through its demonstrable biological effects on multiple pathological features of type 2 diabetes, including insulin resistance, chronic inflammation, mitochondrial dysfunction, and impaired glucose metabolism. These effects were achieved not via isolated pharmacological action but through the synergistic interaction of naturally occurring β-glucans, amino acids, and potentially bioactive peptides—an approach consistent with the multi-compound, multi-target paradigm embraced in functional food science. Mechanistically, these compounds modulate key signaling pathways such as PI3K/Akt and NF-κB, restore GLUT4 and GLP-1R expression, and suppress pro-inflammatory cytokines including TNF-α and IL-1β, thereby addressing both metabolic and immunological disruptions at their root [21-22,24,26,33–35].

Furthermore, FFC emphasizes the importance of molecular mechanisms and target specificity in functional food development, underscoring the need for mechanistically validated evidence to support structure–function claims [42]. The present study contributes to this requirement by demonstrating both phenotypic outcomes and pathway-level changes that can be directly attributed to the administered polysaccharide extract.

From a translational perspective, this work also resonates with international public health strategies that promote functional foods as sustainable interventions for non-communicable diseases. Comparative frameworks between the U.S. and Japan have demonstrated the utility of bioactive-rich foods in glycemic control, lipid

modulation, and vascular protection [43]. As such, the bioactive components characterized in this study not only qualify under the FFC's definition of functional foods but also offer realistic potential for integration into future dietary therapeutic programs.

CONCLUSIONS

In this study, we demonstrated that crude polysaccharides extracted from *Schizophyllum commune* improved glucose metabolism and reduced muscle inflammation in a rat model of type 2 diabetes. Treatment with the polysaccharide extract led to a significant decrease in fasting blood glucose levels, restoration of skeletal muscle structure, reduction in collagen deposition, and normalization of GLUT4 and GLP-1R expression. The polysaccharide extracts effectively attenuated oxidative stress and inflammation, as indicated by lower TNF-α and IL-1β levels in skeletal muscle. Moreover, our findings align with previous studies demonstrating the antioxidant and anti-inflammatory properties of mushroom-derived polysaccharides, which protect tissues from hyperglycemia-induced damage. By preserving mitochondrial integrity and enhancing GLUT4 translocation, *S. commune* polysaccharides show potential as a complementary therapeutic approach for managing type 2 diabetes and preventing its complications. Further studies are warranted to clarify the molecular mechanisms underlying these effects and to explore the clinical potential of *S. commune* polysaccharides in human diabetes management.

Abbreviation: HFD: high fat diet, GLUT4: glucose transporter 4, GLP-1R: glucagon-like peptide-1 receptor, TNF-α: tumor necrosis factor-alpha, IL-1β: interleukin 1 beta, ELISA: enzyme-linked immunosorbent assay, STZ: streptozotocin, HCL: hydrochloric acid, PBS: phosphate-buffered saline, H&E: hematoxylin and eosin, TEM: transmission electron microscopy, PKC: protein kinase C, MAPK: mitogen-activated protein kinase, PCK: phosphoenolpyruvate carboxykinase, AGEs: advanced glycosylated end-products.

Acknowledgements and funding: This study was supported by the National Science, Research and Innovation Fund (NSRF) and Prince of Songkla University (Grant No. AGR6505051M and AGR6505051d).

Authors contributions: WK and MK designed and conducted the research. DS, SN, PC and YS prepared and analyzed the polysaccharide of *Schizophyllum commune* Fr. WK and UM prepared the tissue and performed staining. UM performed ELISA. WK and NR performed the TEM method. WK and MK wrote the manuscript and performed the statistical analysis. All authors read and approved the final version of the manuscript.

Competing interests: The authors declare no competing interests.

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