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Nutritional composition of shiitake mushrooms and their antioxidant and anti-inflammatory effects in LPS-stimulated C2C12 myoblasts

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ABSTRACT

Background: Shiitake mushrooms (*Lentinula edodes*) are valued for their nutritional and medicinal properties, including antioxidant and anti-inflammatory effects. However, their impact on skeletal muscle health at the cellular level remains underexplored. Investigating their bioactive potential may reveal natural therapeutic strategies for reducing muscle inflammation and oxidative stress while promoting cell viability.

Objective: This study aims to investigate the nutritional composition, bioactive compounds, and therapeutic potential of shiitake extract on muscle cells.

Methods: Shiitake mushrooms were freeze-dried, powdered, and extracted with 70% ethanol. Nutritional and amino acid profiles were analyzed using standard methods and LC-MS/MS. identified by HPLC, and antioxidant activity was evaluated by measuring the radical scavenging activity using the DPPH assay. C2C12 myoblast cells were treated with the extract at concentrations of 125–1000 µg/mL. Cellular responses, including cell viability (MTS assay), IL-6 secretion (ELISA), ROS production, apoptosis, and cell cycle distribution (flow cytometry), were subsequently evaluated.

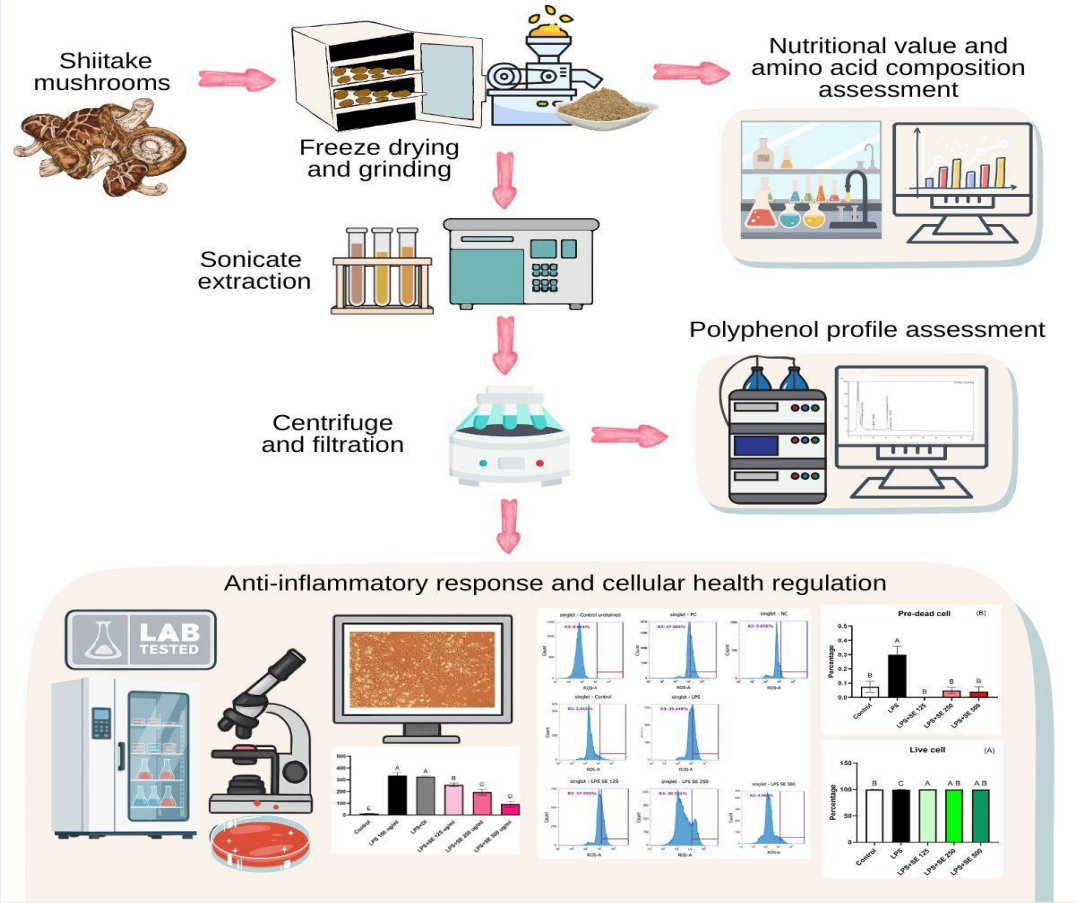
Results: Nutritional analysis revealed that shiitake mushrooms provide 356 calories per 100 grams, with a high protein content (27.43 g), low fat (1.5 g), and abundant carbohydrates (58.2 g), including significant dietary fiber

(34.98 g). The amino acid profile was dominated by glycine, L-Aspartic acid, and L-Leucine, along with various essential and non-essential amino acids. Bioactive compounds, such as gallic acid, p-Coumaric acid, and methyl gallate, were identified in the extract, which also exhibited potent antioxidant activity (314.04 mg Trolox/100g). In vitro experiments demonstrated that the extract reduced oxidative stress and inflammation in LPS-stimulated myoblast cells, as evidenced by decreased IL-6 secretion and ROS production. Moreover, the extract enhanced cell viability and reduced apoptosis at concentrations of 125–500 µg/mL, while concentrations above 1000 µg/mL showed toxicity.

Conclusion: The findings suggest that shiitake extract has favorable potential as a therapeutic agent for promoting muscle health, and cell survival in muscle cells, as well as reducing inflammation and oxidative stress.

Novelty of the Study: This study is unique in that investigates the therapeutic potential of Lentinula edodes (shiitake) extract on muscle cells, focusing on its protective effects against inflammation and oxidative stress in LPS-stimulated myoblasts. Unlike prior studies, it highlights specific bioactive compounds and demonstrates improved cell viability and reduced apoptosis, suggesting its potential as a natural agent for muscle health support.

Keywords: Anti-inflammation, Antioxidant activity, Bioactive compounds, C2C12 cells, Nutritional composition, Shiitake mushroom



Graphical Abstract: Nutritional composition of shiitake mushrooms and their antioxidant and anti-inflammatory effects in LPS-stimulated C2C12 myoblasts

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INTRODUCTION

Muscle mass and strength begin to decline around the age of 25, with the rate of decline accelerating as individuals age. Research shows that, the rate of loss for muscle mass increases to 8% starting at approximately age 40, and it can rise to as much as 15% by the age of 70 [1-2]. Studies also report that the prevalence of sarcopenia increases markedly with age, with the highest rates observed among individuals aged 80 years and older. The overall prevalence of sarcopenia was 6.8%. Among men, the prevalence was 5.5% in those aged ≥60 years, 9.6% in those aged ≥70 years, and 21.5% in those aged ≥80 years. Among women, the prevalence was slightly higher, at 7.9%, 10.5%, and 25.9% in the ≥60, ≥70, and ≥80 age groups, respectively [3-4]. In Thailand, the prevalence of low muscle mass is reported to be 33.9%–35.33% in men and 29.3%–34.74% in women [5-6]. The decline in muscle mass can be attributed to several factors, including the reduction of certain hormones such as growth hormone, testosterone, and estrogen. Additionally, the accumulation of free radicals leads to oxidative stress, which damages various biomolecules within cells, including DNA, proteins, and lipids. This damage contributes to increased muscle breakdown. Other factors influencing mass loss include lifestyle behaviors, the use of certain medications, and the natural process of aging [7-9].

Currently, there are no pharmaceutical treatments reported for low muscle mass, though it can be prevented through exercise and nutrition. Studies have shown that resistance exercises, such as weightlifting or strength training, help increase muscle mass and improve muscle strength. In contrast, aerobic exercises, which rely on oxygen to metabolize carbohydrates, fats, and proteins, such as running, swimming, and cycling, contribute to the increase in muscle size and cross-sectional area. Furthermore, a combination of resistance and aerobic exercises can enhance both muscle strength and functionality, providing a more effective strategy for preventing the loss of muscle mass [10-11].

Dietary intake plays an important role in preventing muscle mass loss. Adequate protein consumption has been associated with a reduced risk of sarcopenia, an age-related condition characterized by the progressive decline in skeletal muscle mass, strength, and physical performance. The Food and Nutrition Board of the Institute of Medicine recommends a daily protein intake of 0.8 g per kilogram of body weight for adults with higher recommendations of 1.5 grams per kilogram for older adults Dietary intake plays [12-13]. In addition to protein, other nutrients have been identified as influential in muscle mass preservation. As an example, vitamin D is known to improve muscle strength, balance, and fall prevention, while low levels of vitamin D are associated with reduced muscle mass. Antioxidants, including flavonoids, carotenoids, vitamin E, and other polyphenols, have also been reported to reduce muscle breakdown by counteracting the damage caused by free radicals [14-16].identified and quantified to confirm their health benefits through specific biological mechanisms Within the Functional Food Science (FFS) framework, developing functional foods requires strong scientific evidence beyond basic nutrition. Bioactive compounds are key components and must be clearly [17].

While the health benefits of shiitake mushrooms have been studied extensively, their effects on muscle function have not been thoroughly explored. Therefore, this study aims to investigate the nutritional profile of shiitake mushrooms, with a particular focus on their amino acid composition, mineral content, and other bioactive compounds. Additionally, we assess the antioxidant and anti-inflammatory properties of shiitake extract in vitro, specifically in myoblast cells.

MATERIALS AND METHODS

Materials and Chemicals: Material were purchased from Sigma-Aldrich (St. Louis, MO, USA). The C2C12 mouse myoblast cell line was obtained from ATCC

(Rockville, MD, USA). Cell culture reagents were supplied by Gibco (Grand Island, NY, USA). The MTS assay kit was obtained from Promega (Madison, WI, USA), while ELISA kits and flow cytometry reagents were purchased from Thermo Fisher Scientific (Waltham, MA, USA). All reagents were of analytical or biological grade. Fresh shiitake mushrooms (*Lentinula edodes*) were obtained from a local market in Ayutthaya, Thailand. Key chemicals, including lipopolysaccharide (LPS, E. coli O111), Trolox, DPPH, and several polyphenol standards, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sample preparation: Fresh shiitake mushrooms were washed, frozen, freeze-dried, and ground into powder. The samples were stored in a humidity-controlled chamber at room temperature until further analysis. For extraction, the mushroom powder was treated with 70% ethanol and subjected to ultrasonic-assisted extraction at room temperature for 30 min. The mixture was then centrifuged at 5000 rpm for 10 min and filtered. The resulting supernatant was collected and stored at −20°C for subsequent analyses.

Nutritional Composition and Amino Acid Profiles: Nutritional composition was analyzed using in-house analytical methods certified under ISO/IEC 17025 by SGS (Thailand) Limited, an accredited laboratory service provider in Thailand. Energy and carbohydrate contents were determined according to established procedures for nutrition labeling analysis. Other nutrients, including protein, fat, dietary fiber, vitamins, and minerals, were measured using in-house analytical methods developed based on AOAC Official Methods.

For total amino acid analysis, 0.1 g of freeze-dried mushroom powder was hydrolyzed with 10 mL of 6 M hydrochloric acid and incubated in a heating bath at 110°C for 24 h. Following hydrolysis, the sample was diluted with 0.1 M ammonium formate, filtered, and transferred into vials for analysis. For tryptophan determination, alkaline hydrolysis was performed using 4.2 M sodium hydroxide instead of acid. Amino acid profiling was subsequently carried out using an LCMS-8060 system (Shimadzu, Japan) equipped with an Intrada amino acid column (50 × 3 mm, 3 µm). A 1 µL sample was injected into the system, and chromatographic separation was achieved using gradient elution with two mobile phases: solvent A (acetonitrile containing 0.1% formic acid) and solvent B (0.1 M ammonium formate). The gradient program was set as follows: 0–3 min (14% B), 3–10 min (100% B), and 10–15 min (14% B) at a flow rate of 0.6 mL/min. Amino acid concentrations were quantified by comparing the chromatographic peaks of the samples with calibration curves generated from amino acid standards.

Determination of bioactive compounds (phenolic acid and flavonoid) and antioxidant activity: The polyphenol content of the shiitake extract was determined by High-Performance Liquid Chromatography (HPLC; Model Prominence LC-20 series, Shimadzu, Kyoto, Japan) with a UV-Vis detector set to 272 nm. The method was adapted from previous studies on the quantitative analysis of phenolic acids and flavonoids [18]. A 10 µL injection volume was used, and separation was carried out on an Ascentis Express C18 column (5.0 µm, 15 cm × 4.6 mm) with a mobile phase consisting of 1% acetic acid in water (Phase A) and 100% acetonitrile (Phase B), with a flow rate of 0.4 mL/min and a column temperature of 28°C. A gradient elution profile was applied: 10% B (0-28 min), 40% B (28-39 min), 60% B (39-45 min), and 90% B (45-50 min). Phenolic acids and flavonoids were identified based on retention times of corresponding standards. Linearity was confirmed using a six-point calibration curve with concentrations ranging from 2.5 to 40 µg/mL, with an R^2 value greater than 0.99. The results were reported with a convergence limit.

Antioxidant activity was assessed using the DPPH radical scavenging assay following a previously

reported method with minor modifications [19]. The extract was reacted with DPPH solution at 25°C for 30 min, and absorbance was measured at 525 nm. Results were expressed as milligrams of Trolox equivalents per milliliter of sample.

Cell Culture and Testing: C2C12 myoblast cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and maintained at 37°C in a 5% CO_2 atmosphere. Cells were used for experiments after reaching approximately 80% confluence [20].

Cell viability and anti-inflammatory activity assessment: Cell viability and anti-inflammatory activity were evaluated following previously reported methods with minor modifications [19]. For the MTS assay, C2C12 cells were treated with shiitake extract (125–1000 µg/mL), while untreated cells were used as the negative control and distilled water (DI) was used as the positive control.

For anti-inflammatory assessment, cells were pre-stimulated with LPS for 24 h and subsequently treated with shiitake extract (125–500 µg/mL). Untreated cells were used as negative control, while LPS-treated cells and LPS plus DI were used as positive controls. Following incubation, the culture medium was collected and centrifuged at 4000 rpm for 10 min to separate the supernatant. The IL-6 cytokine level was determined using an ELISA kit, and cytokine concentrations were calculated based on standard curves.

Cell Cycle Assessment, ROS Detection, and Apoptosis Analysis: C2C12 cells were seeded at a density of 5 × 10^5 cells per well in a 6-well plate and treated with the shiitake extract for 24 hours. The cells were washed and treated with 0.5% Trypsin-EDTA for approximately 5 minutes to detach the cells. After trypsinization, cells were collected and centrifuged at 4000 rpm. To fix the cells, 70% ethanol was added, and the samples were stored at -20°C until further analysis. Cell cycle distribution, ROS levels, and apoptosis were analyzed following previously reported methods [19].

Statistical Analysis: A completely randomized design (CRD) was applied, with each treatment performed in triplicate. Treatment effects were assessed across several parameters, including cell viability, cytokine expression, cell cycle distribution, ROS levels, and apoptosis. Statistical differences among groups were evaluated using one-way ANOVA followed by Tukey's multiple comparison test. A p-value of less than 0.05 was considered statistically significant at the 95% confidence level. Data analysis was conducted using GraphPad Prism software (version 10.2.2; GraphPad Software, San Diego, CA, USA).

RESULTS

Shiitake mushrooms are a highly nutritious food source, as shown in Table 1. They provide 356 calories per 100 grams, with a significant protein content of 27.43 grams and a low-fat content of 1.5 grams, of which 0.28 grams are saturated fats. The mushrooms are predominantly composed of carbohydrates (58.2 grams), including a notable amount of dietary fiber (34.98 grams). The mineral content is also impressive, with 2606 mg of potassium, 3.09 mg of iron, and 117.98 mg of magnesium per 100 grams. Additionally, shiitake mushrooms are rich in vitamins, providing 0.42 micrograms of vitamin B1, 1.79 mg of vitamin B2, and 1.12 mg of vitamin D per 100 grams.

Table 1. Nutritional composition of freeze-dried shiitake mushroom powder

Nutrition (per 100g)	Shiitake mushroom
Energy (kcal)	356
Protein (g)	27.43
Fat (g)	1.50
Saturated fat (g)	0.28
Carbohydrate (g)	58.2
Dietary fiber (g)	34.98A
K (mg)	2606
Fe (mg)	3.09
Mg (mg)	117.98
Vitamin B1 (mg)	0.42
Vitamin B2 (mg)	1.79
Vitamin D (ug)	1.12

The amino acid profile of shiitake mushroom powder is presented in Table 2, which shows, Glycine being the most abundant at 2432.45 mg, followed by L-Aspartic acid (1516.98 mg), L-Threonine (916.98 mg), L-Leucine (954.72 mg), L-Lysine (849.06 mg), L-Glutamic acid (833.96 mg), L-Serine (695.47 mg), L-Arginine (720.75 mg), L-Alanine (537.17 mg), and L-Ornithine (545.28 mg). Other amino acids include L-Phenylalanine (632.08 mg), L-Proline (365.28 mg), L-Valine (367.36 mg), L-Histidine (323.77 mg), L-Isoleucine (294.34 mg), L-Tryptophan (320.46 mg), L-Cystine (300.19 mg), and 4-Aminobutyric acid (148.87 mg).

Table 2. Amino acid profile of freeze-dried shiitake mushroom powder

Amino acid profile	Total Amino acid (mg/100g)
L-Aspartic acid	1516.98
L-Glutamic acid	833.96
L-Threonine	916.98
L-Serine	695.47
L-Alanine	537.17
L-Proline	365.28
Glycine	2432.45
L-Valine	367.36
L-Methionine	76.79
L-Leucine	954.72
L-Isoleucine	294.34
L-Lysine	849.06
L-Phenylalanine	632.08
L-Histidine	323.77
L-Tryptophan	320.46
L-Tyrosine	259.62
L-Arginine	720.75
L-Cystine	300.19
Sacrosine	13.77
LCitrulline	5.85
L-Ornithine	545.28
4-Aminobutyric acid	148.87
Total	13111.22

Trace amounts of Sacrosine (13.77 mg) and L-Citrulline (5.85 mg) were also detected, while L-Methionine was present at 76.79 mg. However, L-Asparagine, Beta-Alanine, Taurine, and L-Hydroxyproline were not detected (ND) using this method.

The analysis of bioactive compounds in the shiitake extract focused on phenolics and flavonoids. All measurements were performed in triplicate (n = 3). Polyphenol compounds were quantified using HPLC. A chromatogram with a high R^2 value (>0.99) recorded at 272 nm of the standard and shitake extract is presented in Figures 2A and 2B. The results indicated that the extract contained gallic acid, p-Coumaric acid, and methyl gallate at concentrations of 4.41, 71.17, and 22.26 mg/100g, respectively, as detailed in Table 3. Additionally, the antioxidant activity of shiitake extract was assessed using the DPPH assay. The results revealed that the extract had an antioxidant capacity of 314.04 mg Trolox/100g

Table 3. Bioactive compound and antioxidant activity of shiitake mushroom powder

Bioactive compounds	Shitake extract (mg/100 g)
Phenolic and Flavonoid content	97.84±36.39
Gallic acid	4.41±0.03
acidp-Coumaric	71.17±38.13
Methyl gallate	22.26±3.28
Antioxidant activity (mg Trolox/100g)	314.04±34.89

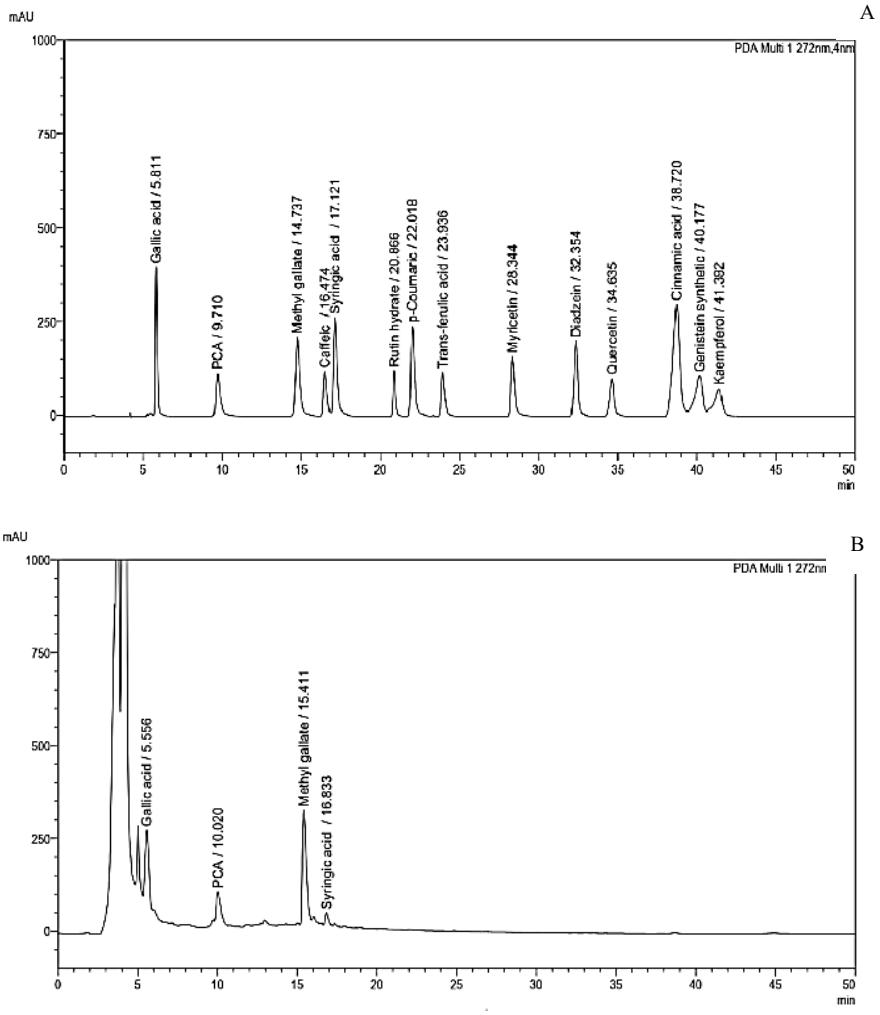

Figure 2. Chromatogram of 14 standards phenolic acid and flavonoid(A) and shiitake extract(B).

The results of the experiment investigating the effect of shiitake extract on muscle cell toxicity indicated that concentrations of 125–500 µg/mL did not induce toxicity in muscle cells. However, the extract significantly reduced cell viability to below 80% at a concentration of 1000 µg/mL, as shown in Figure 3A, suggesting a toxic effect at this higher concentration.

Furthermore, when the anti-inflammatory properties of the shiitake extract were evaluated in muscle cells, the extract at concentrations of 125, 250, and 500 µg/mL significantly reduced inflammation in LPS-stimulated muscle cells (Figure 3B). This was evidenced by a marked decrease in the secretion of the inflammatory cytokine IL-6.

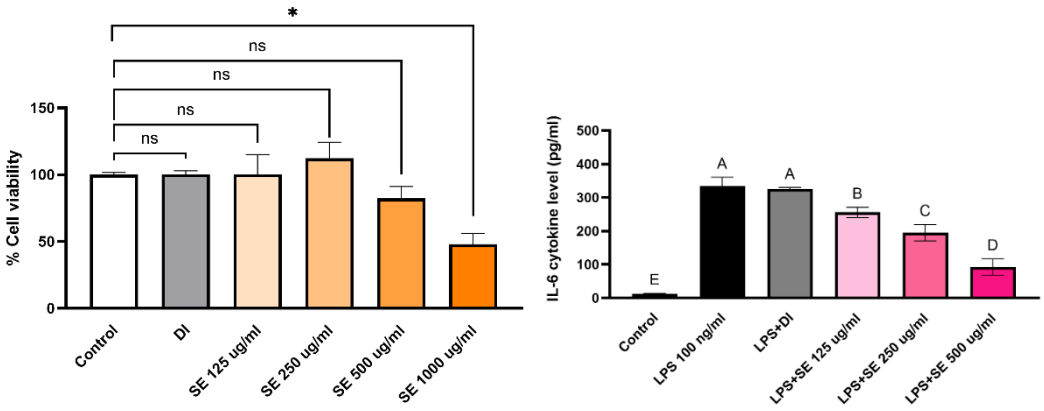

Figure 3. Cell viability (A) and IL-6 levels (B) were measured in LPS (100 ng/mL)-stimulated myoblasts treated with shiitake extract. Data are presented as mean ± SD (n = 3). In (A), ns indicates no significant difference and * indicates $p < 0.05$ vs. control. In (B), different lowercase letters denote significant differences ($p < 0.05$).

The evaluation of ROS production in muscle cells were stimulated with 100 ng/mL LPS and treated with shiitake extract at concentrations of 125–500 µg/mL for 24 hours revealed that LPS stimulation alone induced a significant increase in ROS production, approximately 40%, similar to the positive control group, which was treated with 200 µM tert-butyl hydroperoxide (TBHP). In contrast, the negative control group, treated with 1000 µM N-acetylcysteine (NAC), showed a marked reduction in ROS production, comparable to the control group without any stimulation ($p < 0.05$). In cells treated with both LPS (100 ng/mL) and shiitake extract at concentrations of 125–500 µg/mL, a significant concentration-dependent reduction in ROS production was observed, when compared to the LPS-only stimulated group (Figure 4). These findings suggest that shiitake extract at concentrations of 125–500 µg/mL effectively reduces ROS generation in LPS-stimulated muscle cells.

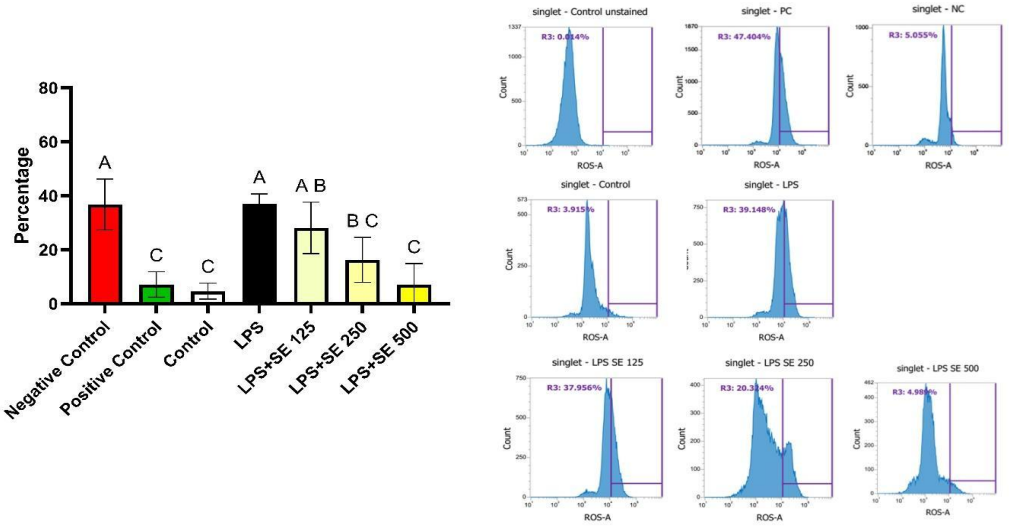

Figure 4. ROS of C2C12 stimulated with LPS and shiitake extracts. Values are expressed as mean ± SD (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$).

The effect of shiitake extract on the cell cycle was investigated. Figure 5 shows that, at concentrations of 125–500 µg/mL, the extract did not induce any changes in the cell cycle during the G0/G1 phase , or the S phase, during which DNA replication occurs.. However, the shiitake extract caused a decrease in the G2/M phase (the phase where cells check DNA replication and proceed to mitosis, ensuring that replicated chromosomes are equally divided into two daughter cells). This suggests a potential influence on cell cycle regulation, possibly promoting differentiation or enhancing cellular responses to stress.

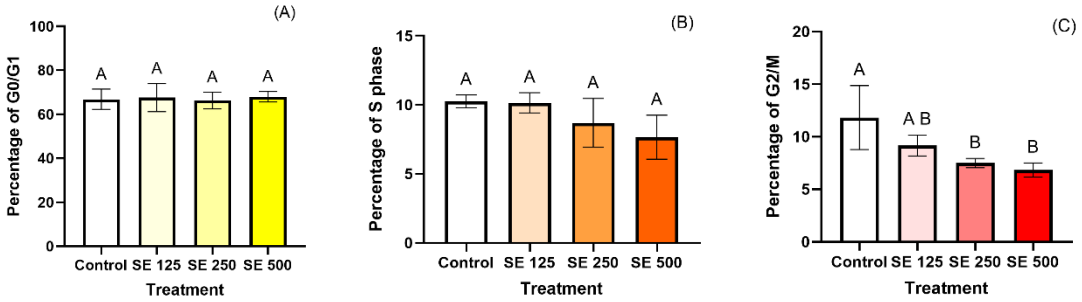

Figure 5. Cell cycle of C2C12 incubated with shiitake extracts. Values are expressed as mean± SD (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$).

Apoptosis detection was performed on muscle cells stimulated with 100 ng/mL LPS and treated with shiitake extract (125–500 µg/mL) for 24 hours. As shown in Figure 6, LPS stimulation alone significantly decreased the proportion of viable cells and increased apoptotic cells compared with the non-stimulated control group. However, cells co-treated with shiitake extract at concentrations of 125–250 µg/mL, significantly increased cell viability and reduced apoptosis relative to the LPS-only stimulated group. These results suggest that shiitake extract can effectively reduce LPS-induced cell death and enhance cell survival.

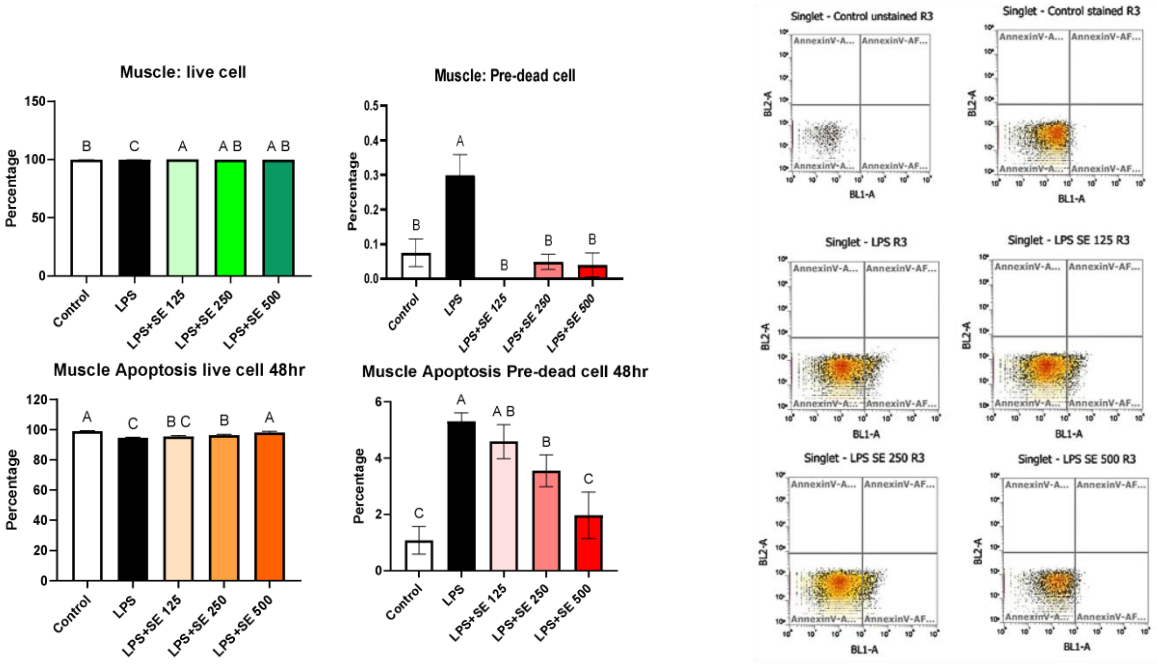

Figure 6. Apoptosis detection in myoblast muscle cells treated with shiitake extract for 24 and 48 hours was performed. Values are expressed as mean ± SD (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$).

DISCUSSION

Shiitake mushrooms are not only widely consumed as an edible fungus but also have significant nutritional and therapeutic potential [21]. This study comprehensively examined the nutritional content, amino acid profile, and bioactive compounds of shiitake mushrooms, as well as their potential effects on muscle cell health through antioxidant and anti-inflammatory properties. The findings demonstrate that shiitake mushrooms are a rich source of essential nutrients, including protein, carbohydrates, fiber, vitamins, and minerals, making them a valuable dietary component for health promotion.

Shiitake also has one of the highest fiber contents among edible mushrooms. This high fiber content plays an essential role in promoting gut health, regulating blood sugar levels, and improving satiety, which may help prevent metabolic diseases such as obesity and diabetes [22]. In addition to their macronutrient composition, shiitake mushrooms are rich in essential minerals such as potassium, iron and magnesium, which contribute to various physiological functions including electrolyte balance, oxygen transport, and muscle function [23, 24]. The presence of vitamins such as vitamin B1 (0.42 µg/100g), vitamin B2 (1.79 mg/100g), and vitamin D (1.12 mg/100g) further enhances the nutritional value of shiitake mushrooms, supporting energy metabolism, antioxidant defenses, and muscle health [15, 25].

The amino acid profile of shiitake mushroom powder, analyzed in this study, reveals that these amino acids, as the building blocks of proteins, are essential for muscle protein synthesis, immune function, and cellular repair. The most abundant amino acids in shiitake mushrooms include glycine (2432.45 mg/100g), L-aspartic acid (1516.98 mg/100g), and L-glutamic acid (833.96 mg/100g), all of which offer significant benefits for muscle tissue. For example, glycine is known to promote muscle protein synthesis, enhance physical performance by supporting collagen production in muscles and connective tissues, improve endurance, and reduce fatigue. It also aids recovery by supporting muscle repair, reducing inflammation, and alleviating post-exercise soreness [26]L-aspartic acid, critical for muscle health, plays a key role in supporting muscle protein synthesis, improving exercise performance, and aiding muscle recovery. It helps generate energy for muscle function, reduces fatigue, and protects muscles from damage and degeneration [27]. Other amino acids found in shiitake mushrooms, such as leucine, isoleucine, glutamine, and arginine, further support muscle growth and recovery by activating key metabolic pathways. Optimal supplementation, particularly with leucine, has been shown to enhance muscle protein synthesis and exercise performance [28]. These findings suggest that shiitake mushrooms could serve as a valuable dietary supplement for promoting muscle health.

The bioactive compounds in shiitake extract have also been shown to possess antioxidant and anti-inflammatory properties. The DPPH assay revealed a strong antioxidant activity of 314.04 mg Trolox/100g, indicating that shiitake mushrooms could effectively neutralize free radicals. Oxidative stress, caused by an imbalance between ROS and antioxidant defenses, is a key contributor to the pathogenesis of muscle aging and various chronic diseases. By reducing oxidative damage, shiitake mushrooms could help protect muscle cells from degeneration and enhance muscle recovery [29]. The anti-inflammatory effects of shiitake extract were evaluated in vitro using LPS-stimulated muscle cells. LPS, a bacterial endotoxin, induces inflammation by triggering the release of pro-inflammatory cytokines such as IL-6. In this study, shiitake extract at concentrations of 125–500 µg/mL significantly reduced IL-6 secretion, indicating its potential to modulate inflammation. Chronic inflammation is closely linked to muscle wasting, and the ability of shiitake mushrooms to suppress inflammatory cytokine production could offer a promising strategy to counteract muscle loss and sarcopenia in aging individuals [30]. In addition, the study revealed that shiitake extract reduced ROS production in muscle cells. LPS stimulation led to a

significant increase in ROS, but treatment with shiitake extract at concentrations of 125–500 µg/mL reduced ROS generation in a dose-dependent manner. This finding suggests that shiitake mushrooms may possess a protective effect against oxidative damage, which is crucial for maintaining muscle integrity and function. Previous research has demonstrated that antioxidant-rich foods can effectively reduce oxidative stress and improve muscle function [31-32].

The cytoprotective effects of shiitake extract were also assessed by examining apoptosis in muscle cells. LPS stimulation induced significant cell death, as evidenced by increased apoptosis, whereas co-treatment with shiitake extract at 125–250 µg/mL significantly reduced the proportion of apoptotic cells, thereby enhancing cell viability. Similar protective effects have been observed in other mushroom species, where polysaccharides and other bioactive compounds exert cytoprotective effects by modulating apoptosis [33-34]. Beyond their role in muscle preservation, various mushroom species have demonstrated broad therapeutic potential in managing other chronic conditions [35-36]. These results suggest that shiitake extract may contribute to the prevention or management of muscle wasting, particularly by modulating oxidative stress and inflammation, which are key contributors to muscle aging and degeneration. The experiments were conducted in vitro using undifferentiated C2C12 myoblasts, which are suitable for evaluating early cellular responses such as oxidative stress and inflammation. However, this model may not fully represent mature muscle physiology. Therefore, further studies using differentiated myotubes, as well as in vivo investigations, are needed to confirm the efficacy and optimal dosage of shiitake extract in more physiologically relevant systems.

Importantly, these findings align with the Functional Food Center (FFC) 17-step framework by addressing key early-stage components of functional food development [17]. The study explores health-related targets, including muscle cell inflammation and oxidative stress, and considers potential bioactive compounds such as gallic acid and p-coumaric acid. The use of biomarkers, including IL-6 and ROS, provides mechanistic insight and supports dose-related effects. Collectively, these results offer essential pre-clinical evidence for the functional food potential of shiitake mushrooms and support future clinical validation and product development.

CONCLUSION

As shown in this study, shiitake mushrooms are a highly nutritious food source, rich in protein, carbohydrates, fiber, essential minerals, and amino acids. The bioactive compounds in shiitake extract, including phenolics and flavonoids, contribute to its strong antioxidant activity (314.04 mg Trolox/100g) and anti-inflammatory effects. The extract significantly reduced the secretion of IL-6 cytokine and ROS production in LPS-stimulated muscle cells, without causing toxicity at concentrations of 125–500 µg/mL. Additionally, the extract decreased apoptosis and enhanced cell survival in LPS-stimulated cells. These findings suggest that shiitake extract may have potential therapeutic benefits for reducing inflammation and oxidative stress, while promoting muscle health.

Abbreviations: ANOVA: Analysis of variance, AOAC: Association of Official Analytical Chemists, C2C12: Mouse myoblast cell line, DNA: Deoxyribonucleic acid, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ELISA: Enzyme-linked immunosorbent assay, HPLC: High-performance liquid chromatography, IL-6: Interleukin-6, LC-MS/MS: Liquid chromatography-tandem mass spectrometry, LPS: Lipopolysaccharide, ROS: Reactive oxygen species, UV-Vis: Ultraviolet-visible

Conflict of Interest: The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Authors' Contributions: K.H. conceptualized and designed the study, conducted the experiments, performed data analysis and interpretation, and drafted the original manuscript. S.B. contributed to the

conceptualization, provided supervision, and critically reviewed and revised the manuscript.

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