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Schizonepeta tenuifolia alleviated muscle weakness in dexamethasone-induced muscle atrophy mouse model

Young Sook Kim^{1,2*} , Heung Joo Yuk¹, Jin Hong Jang^{1,2} and Dong-Seon Kim^{1,2*} 

Abstract

Sarcopenia starts around age 40, with individuals losing more than 30% of muscle mass by age 70. Due to the absence of approved pharmacological treatments, current management primarily relies on exercise and protein supplementations. *Schizonepeta tenuifolia* is a medicinal plant commonly found in Korea, China, Japan and is traditionally used for headaches, colds and allergies. However, its preventive effect against sarcopenia have not yet been explored. This study to investigate whether the ethanol extract of *Schizonepeta tenuifolia* (DKB138) enhances muscle strength in a dexamethasone (DEX)-muscle atrophy mice model. The preventive effects of DKB138 against muscle atrophy were evaluated in DEX-induced models both in vitro and in vivo. In vitro, DEX-induced myotubes were assessed for cell viability to determine cytoprotective effects. In vivo, a DEX-induced muscle atrophy mouse model was used evaluate muscle strength and functional performance. Using UPLC and comparison with reference standards, seven components of DKB138 were identified. DKB138 effectively prevented DEX-induced death in C2C12 skeletal muscle cells. In vivo, mice treated with DKB138 exhibited 28.5% increase in grip strength ($***p < 0.001$) and a threefold significant increase in running distance ($**p < 0.01$) compared to the DEX-induced mice. Additionally, DKB138 treatment resulted in downregulation of Murf-1 and Atrogin mRNA while upregulating of MyoD mRNA expression. These finding demonstrate that DKB138 protects against DEX-induced muscle atrophy by suppressing muscle degradation factors and enhancing muscle strength. These results suggest that DKB138 has potential as functional food against muscle dysfunction and atrophy.

Keywords *Schizonepeta tenuifolia*, Sarcopenia, C2C12, Dexamethasone, Grip force, Muscle atrophy

Introduction

Sarcopenia is the age-related loss of muscle mass, strength and functions, is becoming an increasing concern, particularly as the global population ages. It is considered as a specific form of skeletal muscle atrophy and can be caused by a variety of factors, including prolonged inactivity, malnutrition, aging, long-term infections, and chronic disease. Muscle atrophy is known to be caused by mechanisms such as oxidative stress, inflammation, and dysregulation of signaling pathways such as the ubiquitin-proteasome system, autophagy-lysosome system, and mammalian target of rapamycin (mTOR) [1–4].

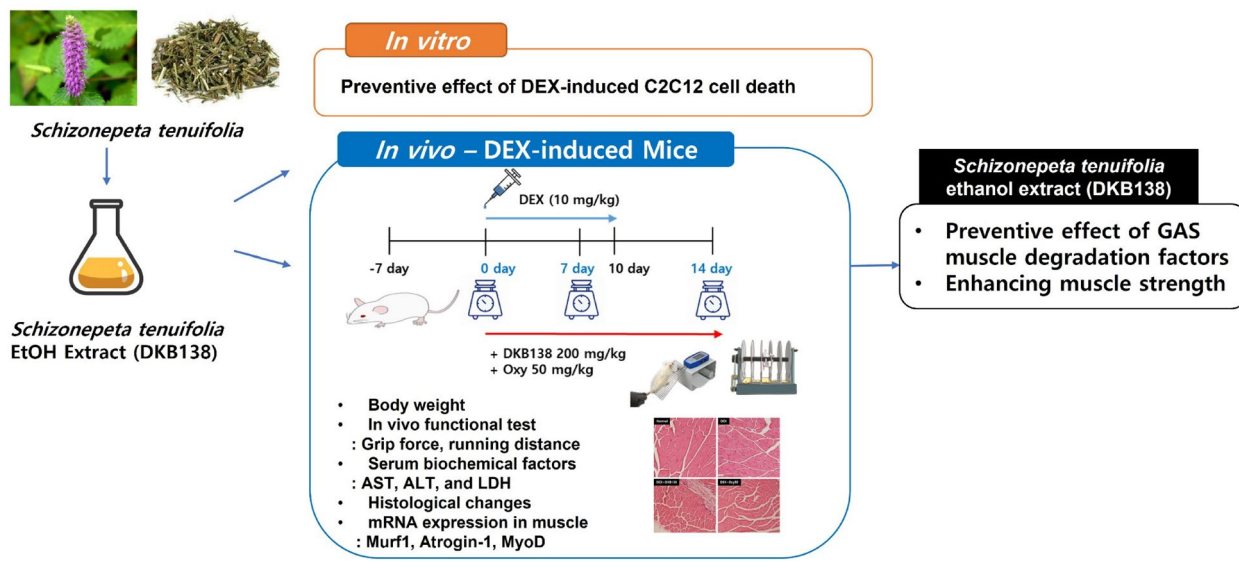
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Graphical Abstract

Protective effects of DKB138 against dexamethasone-induced muscle atrophy



Glucocorticoids are an important regulatory molecule in the body, as well as the most widely used and most effective anti-inflammatory and immunosuppressant drug. However, one of the major side effects of high-dose glucocorticoid use is skeletal muscle atrophy [5]. Dexamethasone (DEX), a potent synthetic glucocorticoid, has been extensively used in experimental models to induce muscle atrophy both in vivo and in vitro [6, 7]. Skeletal muscle atrophy is a progressive and a debilitating condition commonly observed in various chronic disease such as cancer, diabetes and aging. It is characterized by an imbalance between protein synthesis and degradation, favoring catabolism. At the molecular level, muscle atrophy is primarily driven by the upregulation of proteolytic systems, particularly the ubiquitin-proteasome and autophagy-lysosome pathways. Two key E3 ubiquitin ligases, Muscle ring finger protein-1 (Murf1) and atrogin-1, play a central role in mediating muscle protein degradation through the ubiquitin-proteasome pathway [8, 9]. Both gene are transcriptionally upregulated during glucocorticoid-induced and disuse-related muscle atrophy, and are considered reliable molecular markers of muscle-wasting condition [8].

Schizonepeta tenuifolia Briquet extract is a medicinal plant widely used in traditional East Asian medicine, particularly in Korea, China, and Japan [10]. The extract contains many chemical components including monoterpenes, sesquiterpenes, aldehydes, ketones, quinones, alcohols, phenols, carboxylic acids, and esters [11]. It has demonstrated anti-inflammatory, antioxidant,

anti-pyretic, and immunomodulatory properties [12]. However, it has also been associated with side effects such as muscle wasting and weakness. In a previous study, over 800 natural products were screened to discover potential therapeutic agents for sarcopenia, and *Schizonepeta tenuifolia* Briquet extract emerged as one of the candidates [13]. Here, we evaluated the muscle strength-enhancing effects of *Schizonepeta tenuifolia* Briquet ethanol extract (DKB138) using DEX-induced muscle atrophy mouse model and investigated the underlying mechanisms by assessing related factors.

Materials and methods

Preparation of *Schizonepeta tenuifolia* ethanol extract

Schizonepeta tenuifolia ethanol extract (DKB138) used in this study was provided by Dongkook Pharmaceutical Co. Ltd (Seoul, Republic of Korea). The manufacturing process was as follows: The air-dried spikes of *Schizonepeta tenuifolia* were extracted twice using 70% ethanol (20-fold volume) at 80 °C, followed by filtration through a 10 µm filter and concentration under reduced pressure at 60 °C.

Identification of seven compounds in DKB 138 using UPLC analysis

An Ultra Performance Liquid Chromatography (UPLC) analysis was conducted using a system (Waters, Milford, MA, USA) equipped with a binary solvent delivery system, an auto-sampler, and a UV detector, as previously described [13]. An aliquot (2.0 µL) of the sample was

injected into a BEH C18 column and eluted at a flow rate of 0.4 mL/min using a binary gradient of two mobile phases: A (water containing 0.1% formic acid) and B (acetonitrile containing 0.1% formic acid). A linear gradient was optimized as follows: 0–1 min, 5% B; 1–16 min, 5–8% B; 16–17 min, 85–100% B; 17–18.3 min, 100% B; 18.3–18.4 min, 100–5% B; and 18.4–20 min, back to 5% B. Seven standards for identification of the samples were purchased from ChemFaces (Wuhan, Hubei, China).

C2C12 myoblasts culture and differentiation

Mouse skeletal muscle-derived C2C12 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). C2C12 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Sigma-Aldrich, Santa Clara, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Australia), 100 units/mL penicillin, and 100 µg/mL streptomycin (Gibco) in humidified air at 37 °C with 5% CO₂. To induce the differentiation of myoblasts into myotubes, C2C12 cells at 90% confluence were cultured in DMEM containing 2% FBS for 7 days. After the induction of differentiation, the cells were treated with sample for assay.

Cell proliferation

Cell proliferation was detected using CCK-8 (Cell Counting Kit-8, Dojindo, Japan) assay, previously described [13]. Briefly, C2C12 cells were seeded in 94-well plates at a density of 1 × 10⁴ cells per well and cultured for 7 days in DMEM containing 2% FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin (Gibco). After the induction of differentiation, the cells were cultured in a serum-free medium for 12 h. The C2C12 cells were then incubated for 24 h with DKB138 at concentrations of 50–100 µg/mL and 10 µM DEX treated. Cells were pre-treated with seven individual compounds (1 µM) from DKB138 for 24 h, followed by exposure to 250 µM H₂O₂ for 3 h. The assay was performed according to the manufacturer’s instructions, 10 µL of CCK-8 solution was added in the each well and absorbance (Abs) was measured at 450 nm using a spectrophotometer (Synergy HT;

BioTek, Winooski, VT, USA). The formula used to calculate cytotoxicity is as follows:

Cytotoxicity (%)
= (Abs of medium) / (Abs of medium + Abs of cells) × 100

Total RNA extraction and quantitative real-time polymerize chain reaction

The effect of DKB138 on the expression of genes linked to muscle atrophy was evaluated using quantitative real-time PCR (qRT-PCR). Table 1 presents the genes associated with muscle atrophy. Total RNA extraction from the gastrocnemius (GAS) and tibialis anterior (TA) muscles was performed using the TRIzol™ reagent (Invitrogen, Thermo Fisher Scientific Inc., MA, USA). The total RNA was converted into cDNA using a cDNA synthesis kit (iScript, Bio-Rad, Laboratories, USA) according to the manufacturer’s instructions. Total RNA was reverse-transcribed into cDNA using a cDNA synthesis kit (iScript, Bio-Rad Laboratories, USA). The SYBR® (iTaQ Universal, Bio-Rad Laboratories, USA) was used for analyzing DNA amplification. The reaction cycling conditions were configured as follows: an initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing at 60 °C for 1 min. Amplified mRNA levels were analyzed using the delta-delta Ct method, with GAPDH serving as the reference gene.

Animals and treatments

Seven-week-old-male BALB/c mice were purchased from RaonBio (Yongin-si, Korea). The mice received standard diets, were housed in an air-conditioned room with a 12:12 light/dark cycle (light off at 8:00 PM) and were allowed ad libitum access to food and tap water. All experiments were approved by the Animal Care and Use Committee of Korea Institute of Oriental Medicine (IACUC no. 22–034) and were performed in accordance with the institute’s animal care guidelines.

Dexamethasone (DEX)-induced muscle atrophy mouse model

After a one-week adaption period, the mice were randomly divided into four groups (n = 8 animals per group) as follows: (1) the normal group, which was administered 0.9% saline (N group); (2) the dexamethasone (DEX) group, which was intraperitoneally injected DEX at 10 mg/kg, and then administrated 0.9% saline once a day for 10 days (DEX group); (3) one group was injected DEX and administered 200 mg/kg of DKB138 (DKB138 group); (4) one group was injected DEX and administered 50 mg/kg of Oxymetholone (Oxy50 group) as a positive control [14]. During the treatment period, the body weights of mice were measured once a week.

Table 1 Primers used for quantitative real-time PCR

Gene		Sequence (5'–3')
Atrogin-1	(Sense)	CTTCAACAGACTGGACTTCTCGA
	(Antisense)	CAGCTCCAACAGCCTTACTACGT
Murf-1	(Sense)	ACGAGAAGAAGAGCGAGCTG
	(Antisense)	CTTGGCACTTGAGAGGAAGG
MyoD	(Sense)	AGTGAATGAGGCCTTCGAGA
	(Antisense)	GCATCTGAGTCGCCACTGTA
GAPDH	(Sense)	GGCCATCTCTGTGTCCA
	(Antisense)	TGAGCTGAGGCCTTCTCAAT

Atrogin-1, atrophy gene-1; Murf-1, muscle RING-finger protein-1; MyoD, myoblast determination protein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase

Forelimb grip strength test

Forelimb grip strength was assessed using a Grip Strength Meter (GPM-100; Melquest, Toyama, Japan). Mice were allowed to grasp a horizontal metal bar connected to a digital force transducer, which recorded the peak pull force (in grams) as the mouse was gently pulled by the tail. The gauge was reset to 0 g after stabilization before each trial. In the conventional test, the bar was positioned horizontally, and the tail was pulled straight back by the examiner. For the modified test, the force gauge was rotated vertically and fixed to a metal stand to stabilize the system; all other procedures remained identical, except for the altered pulling direction. Trials were excluded if the mouse used only one forepaw or hindlimbs, turned during the pull, or released the bar without resistance. To minimize variability, tail pulls were performed at a constant, slow speed to allow mice to generate adequate resistance. Each mouse underwent six consecutive trials per day at one-minute intervals. When both test types were conducted on the same day, mice were allowed a minimum 30 min rest between tests. Testing order was randomized daily, and the examiner was blinded to prior results. All measurements were conducted between 11:00 AM and 5:00 PM during the animals' light cycle. Equipment calibration was regularly performed by the manufacturer.

Histological analysis

Gastrocnemius muscle tissues were fixed in 4% paraformaldehyde at room temperature for 24 h, embedded in paraffin, and sectioned at a thickness of 3 μ m. For hematoxylin and eosin (H&E) staining, paraffin sections were deparaffinized, rehydrated, and processed using standard protocols to visualize muscle fiber morphology. Images were captured at 10 $\times$ magnification using a bright-field microscope (Olympus BX53) equipped with a digital camera. Cross-sectional area (CSA) of individual muscle fibers was measured using ImageJ software (NIH, USA). At least 100 muscle fibers per sample were manually outlined and analyzed. The average CSA per mouse was calculated and used for statistical comparisons between control, DEX-treated groups and DKB138 or Oxy50 treated groups.

Statistical analysis

Data are expressed as mean $\pm$ SE or mean $\pm$ SD. ANOVA with Tukey's test or t- test were used for multiple comparisons using the Prism 7.0 software (GraphPad software, San Diego, CA, USA).

Results

Identification of seven compounds in DKB138 using UPLC

As shown in Fig. 1A, seven individual components of the 70% ethanol extract of *Schizonepeta tenuifolia* (DKB138)

were identified using UPLC. Identification was performed by comparing the retention times (RT) and UV absorption maxima ($\lambda_{\max}$) of each peak with those of authentic reference standards and previously reported data (Fig. 1B). To determine the optimal extraction yield of marker and active compounds, extracts were prepared using various concentrations (water, 30%, 50%, 70%, and 99.5~100%), and the yields were quantified. The extraction yields were 18.2% for water, 12.9% for 30% ethanol, 13.9% for 50% ethanol, 12.9% for 70% ethanol, and 4.4% for 99.5~100% ethanol, indicating that moderate ethanol concentrations resulted in higher recovery of extractable compounds compared to absolute ethanol.

DKB138 attenuated DEX-induced cell death and muscle

Atrophy-related gene expression

To evaluate the cytopreventive effect of DKB138 extracted at different ethanol concentrations, C2C12 myotubes were treated with DKB138 prepared using water, 30%, 50%, 70%, 99.5 ~ 100% ethanol at concentrations of 50 and 100 μ g/mL, in cotreated with DEX (10 μ M) for 24 h. Cell viability was assessed using the CCK-8 assay. DEX treatment reduced cell viability by approximately 25% compared to control cells. Treatment with DKB138 extracted with 100% ethanol restored cell viability in a dose-dependent manner at both of 50 and 100 μ g/mL (red box). DKB138 extracted with 70% ethanol and 50% ethanol at 100 μ g/mL significantly reduced DEX-induced cell death by approximately 25% and 20%, respectively. Although a similar decreasing trend in cell death was observed at lower concentrations and with the water extract, the differences were not statistically significant (Fig. 2A). Among the tested fractions, the 70% ethanol extract exhibited the strongest cytoprotective activity. Due to the limited extraction yield of the 100% ethanol extract, subsequent experiments were therefore performed using DKB138 prepared with 70% ethanol. Collectively, these results indicate that DKB138 inhibits DEX-induced muscle atrophy and exerts a protective effect on muscle cells (Fig. 2A). To further investigate the effect of DKB138 on muscle atrophy, C2C12 myotubes were treated with DKB138 (50 and 100 μ g/mL) and isolated mRNA to check the atrophy-related genes expression. Muscle ring-finger protein-1 (Murf-1) is an E3 ubiquitin ligase expressed in skeletal muscle and plays a critical role in muscle remodeling. Transcriptional upregulation of Murf-1 gene is closely associated with the progression of skeletal muscle atrophy [8]. Atrogin-1, another muscle-specific E3 ubiquitin ligase, is widely recognized as a key molecular marker of muscle atrophy, with its mRNA levels consistently upregulated in various atrophic conditions [9]. As shown in Fig. 2B C, the expression of muscle atrophy-related factors was

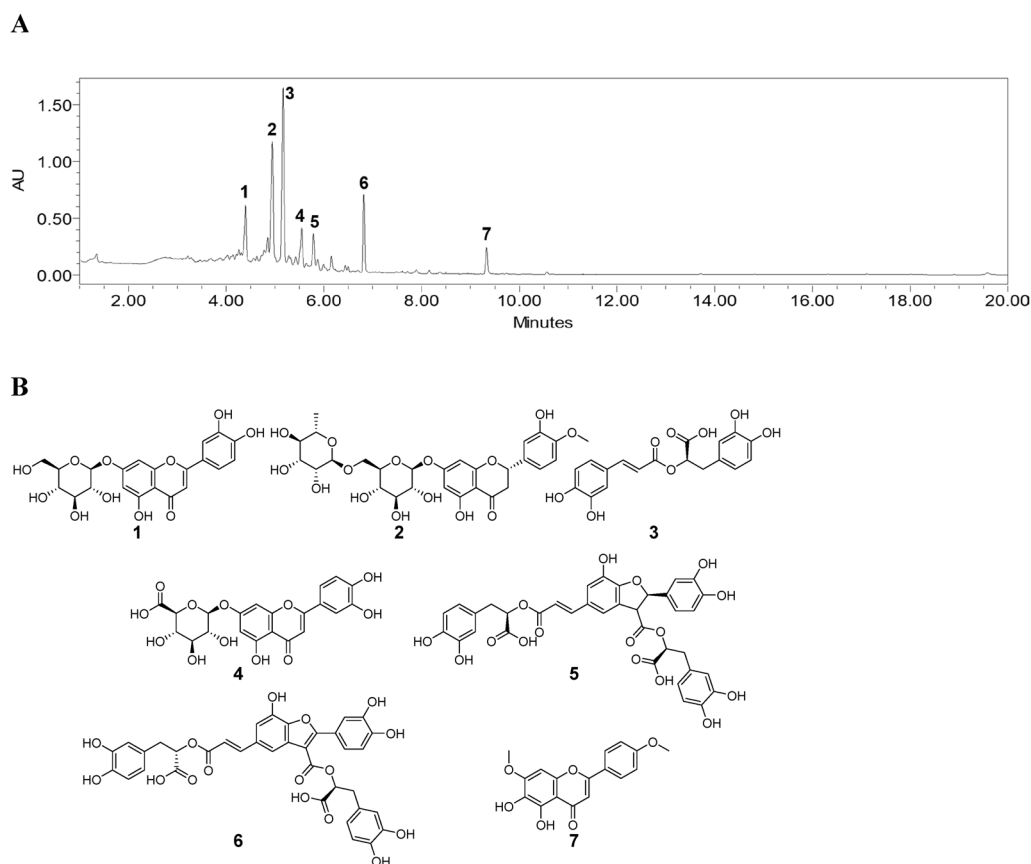


Fig. 1 Representative UPLC-PDA chromatographic profiles of DKB138, showing the presence of seven identified compounds (A): cynaroside (1), hesperidin (2), rosmarinic acid (3), luteolin-7-O-glucuronide (4), salvianolic acid B (5), schizotenuin A (6), and pulegone (7), (B) Chemical structure of compounds

significantly increased in DEX-treated muscle cells and markedly decreased by DKB138 treatment.

Effects of DKB138 on body weight in DEX-induced muscle atrophy mouse model

A schematic representation of the experimental design is shown in Fig. 3A. During the 2 weeks of feed treatment, all groups showed an increase in body weight (Fig. 3B). However, the body weight was markedly decreased upon DEX treatment at 2 weeks. No differences in body weight were found between the DEX-treated groups. To evaluate potential hepatotoxicity and tissue damage, serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were measured in mice after treatment. Dexamethasone (DEX)-treated mice showed significantly elevated levels of AST, ALT, and LDH compared to the control group, indicating liver and tissue damage. In contrast, mice administered with DKB138 exhibited a reduction in these enzyme levels. Notably, co-treatment with DKB138 significantly attenuated DEX-induced increases in AST, ALT, and LDH levels, suggesting a protective effect against DEX-induced hepatic and muscular injury.

DKB138 increased grip force in DEX-induced muscle atrophy mouse model

To evaluate the functional effects of DKB138 on muscle strength, forelimb grip strength was measured to assess muscle force, and a treadmill running test was performed to assess endurance capacity. To directly assess its contribution to improvements in endurance and strength, grip force and running distance were measured. To ensure accurate grip strength measurements, practice trials were conducted the day before testing, and on test day, grip force was measured seven times with the average value calculated. As shown in the Fig. 4, the DKB138-treated group exhibited a 28.5% increase in grip force compared to DEX-induced group. In addition, running distance was significantly increased by threefold in DKB138-treated group compared to the DEX-induced group. Both DKB138-treated group and Oxy50-treated group showed a significantly improvement in muscle functions.

DKB138 prevented muscle atrophy in the GAS of DEX-induced mouse model

The preventive effect of DKB138 on muscle function loss was demonstrated in the previous result. To further assess its preventive effects at the tissue level, structural

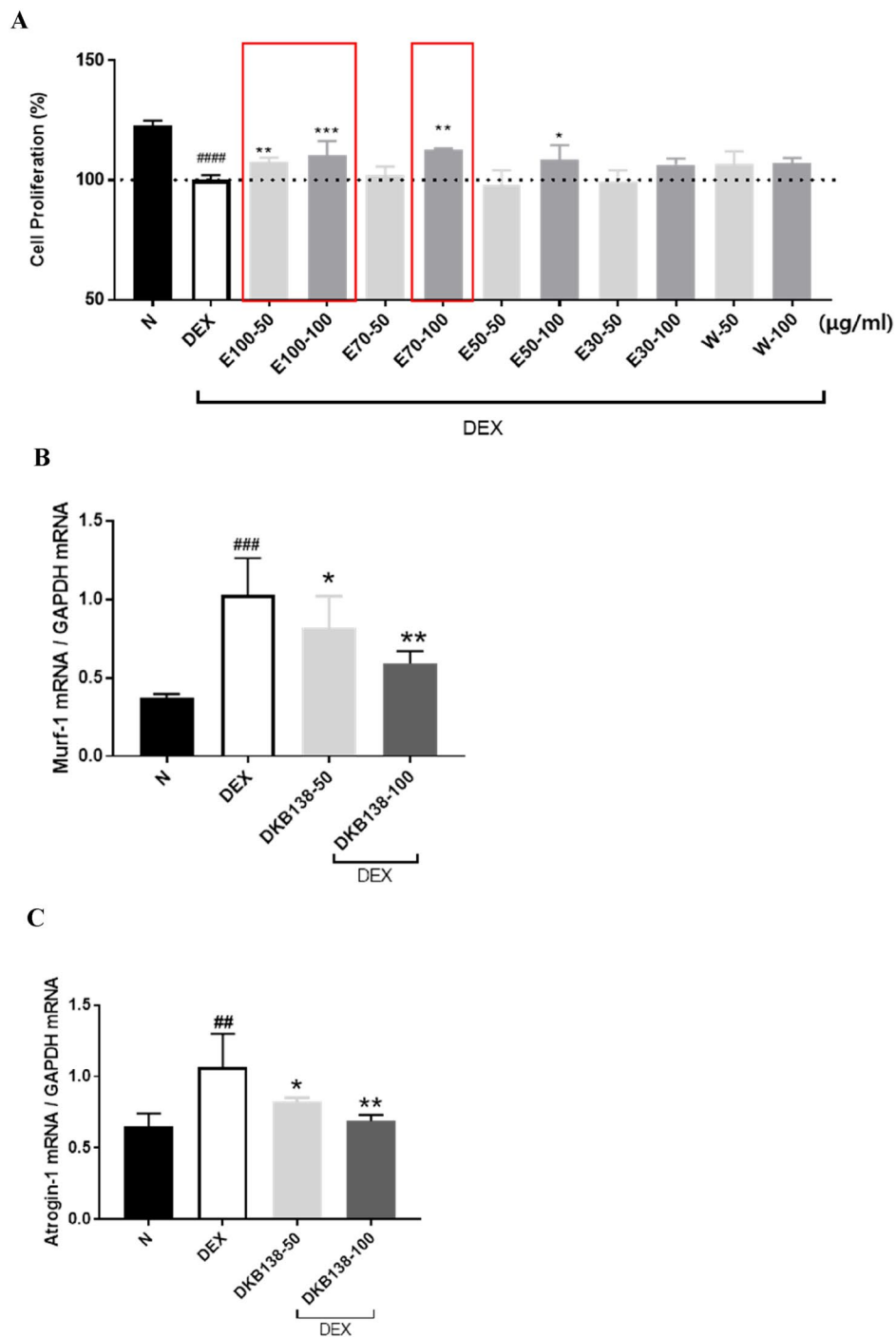


Fig. 2 Effects of DKB138 on cell proliferation and expression of Murf-1 and Arogin-1 mRNA. Murf-1 and Arogin-1 mRNA in dexamethasone (DEX)-induced C2C12. **A** C2C12 cells were co-treated with the indicated concentrations of DKB138 (50 and 100 μg/mL) and DEX (10 μM). To investigate cell viability, CCK-8 (Cell Counting Kit-8) assay was performed. Expression levels of the genes of interest in each treated group were determined after normalizing levels to GAPDH expression as an internal control using comparative cycle threshold (Ct) analysis. Expression of mRNA was determined using qPCR. **B** Murf-1 mRNA and **C** Arogin-1 mRNA. Results are representative of three independent experiments and are expressed as mean ± SE ($n = 3$). #### $p < 0.0001$, ### $p < 0.001$ vs. N, * $p < 0.05$, ** $p < 0.01$ vs. DEX-treated cells, respectively

changes in the GAS of a DEX-induced atrophy mouse model were examined through hematoxylin and eosin (H&E) staining. The effect of DKB138 on DEX-induced muscle atrophy were investigated in mice. The GAS

muscle tissues stained with H&E observed under microscope at 100x magnification. The analysis revealed that DEX treatment led to significant muscle fiber atrophy, characterized by a reduction in the cross-sectional area

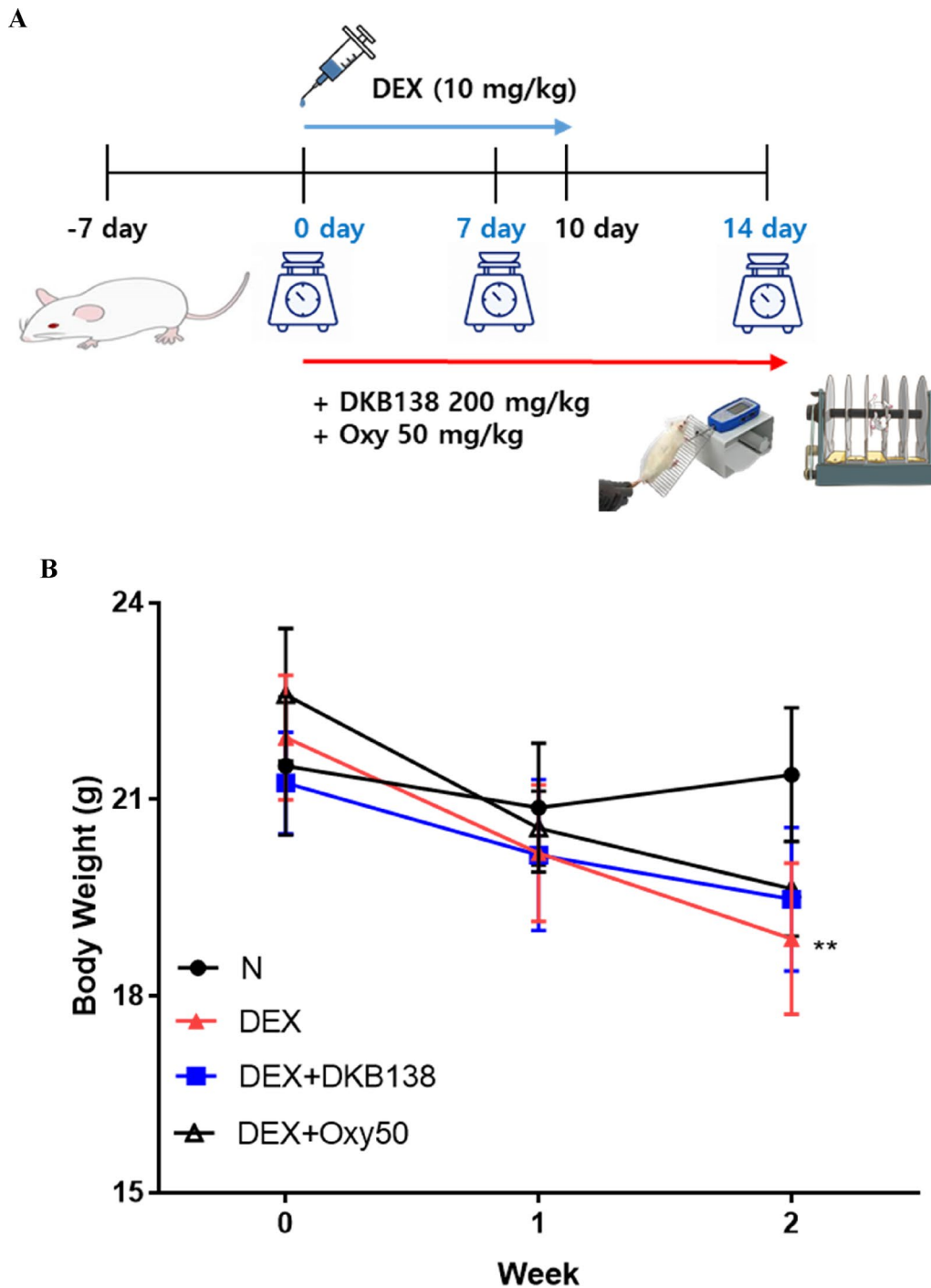
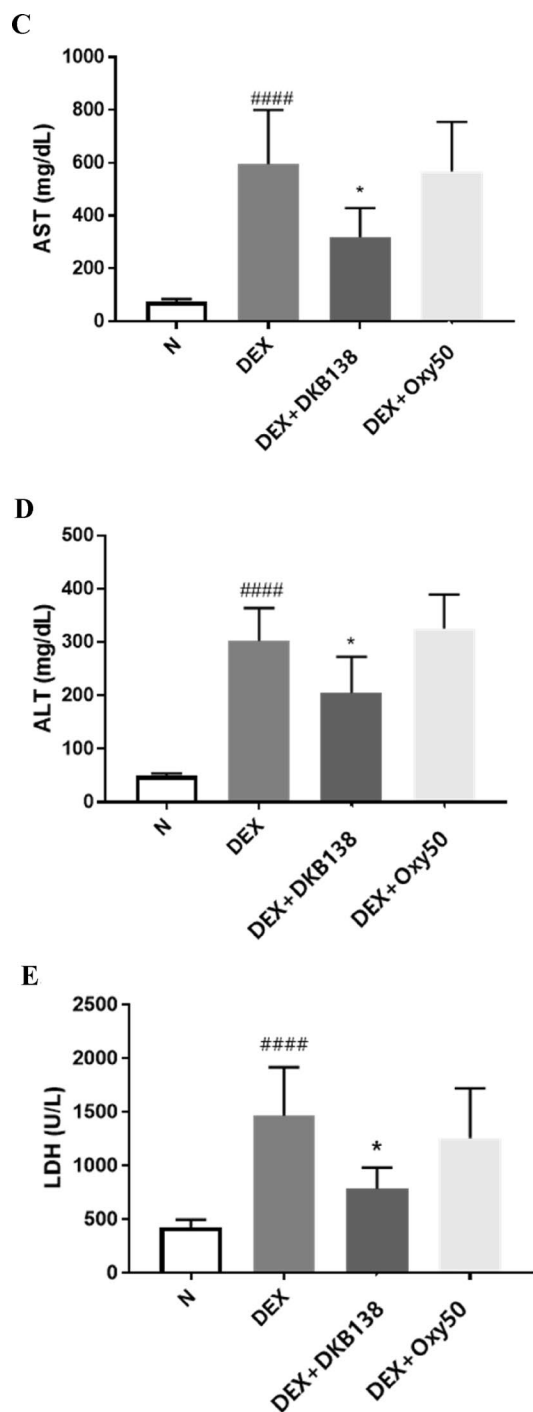


Fig. 3 Effects of DKB138 on body weight and serum biomarkers in DEX-induced skeletal muscle atrophy mouse model. **(A)** Schematic representation of the animal experiment design. **(B)** Body weight during the experiment. **(C-E)** Effects of DKB138 on serum AST, ALT, and LDH levels in DEX-induced skeletal muscle atrophy mouse model. DEX significantly increased the levels of all three markers compared to the Normal group. Data are shown as the mean $\pm$ SE ($n=8$). Statistical significance was evaluated by Tukey's test. ** $p < 0.01$ vs. Normal group (N)

(CSA) of muscle fibers (Fig. 5). However, administration of DKB138 (200 mg/kg) or Oxy (50 mg/kg) ameliorated these histological changes, indicating its preventive effect of DEX-induced muscle atrophy.

Effect of DKB138 on mRNA levels of atrophy biomarkers in mouse GAS muscle

Functional and morphological changes in the GAS muscle of DKB138-treated mice were confirmed (Figs. 4 and 5). To further investigate the underlying molecular

**Fig. 3** (continued)

mechanisms, mRNA expression levels of muscle atrophy-related biomarkers were analyzed in the GAS muscle tissue. Murf-1 and Atrogin-1 are key E3 ubiquitin ligases that play critical roles in regulating ubiquitin-mediated degradation in skeletal muscle [15]. MyoD is a key biomarker associated with muscle atrophy, as it plays a pivotal role in the regulating of myogenic differentiation and muscle regeneration [16]. The gene expression of atrophy

markers, such as Murf-1 and Atrogin-1 were increased after DEX-induced group and significantly decreased in DKB138-treated group or Oxy 50-treated group (Fig. 6A and B). MyoD mRNA levels were decreased after DEX-induced group and increased in DKB138-treated group (Fig. 6C). These results demonstrated that DKB138 effectively ameliorated DEX-induced muscle atrophy, likely through the inhibition of protein degradation pathways.

Discussion

The present study investigated the protective effects of DKB138, an ethanol extract from *Schizonepeta tenuifolia*, against DEX-induced muscle atrophy through a comprehensive evaluation of biochemical, histological, and biomarkers. Our findings provide compelling evidence that DKB138 ameliorates muscle atrophy by preserving muscle structure, function, and reducing markers associated with protein degradation.

Natural product research has long emphasized the importance of extraction parameters in modulating the phytochemical profile and subsequent bioactivity [17]. First, we identified seven marker compounds in DKB138 using UPLC, and determined that the optimal extraction yield was obtained with 70% ethanol. In vitro, DKB138 significantly prevented on cell death in a dose-dependent manner following DEX-induced cytotoxicity. Treatment with 100 $\mu\text{g/mL}$ DKB138 restored viability nearly to control levels, indicating a cytoprotective effect. In vivo, the DEX-induced muscle atrophy mouse model exhibited characteristic reductions in body weight and muscle function. DKB138 did not prevent weight loss associated with DEX, however, significantly reduced serum levels of AST, ALT, and LDH—markers of liver and muscle damage [18]. Although liver histopathological assessments were not included in this study, it is deemed necessary to conduct pathological analyses of liver tissue following treatment with DKB138 to clearly elucidate its hepatoprotective effects.

One of the most striking findings was the enhancement of physical performance metrics in the DKB138-treated mice. Compared to the DEX-treated group, mice treated with DKB138 showed a 28.5% increase in grip strength and a threefold increase in treadmill running distance.

These improvements in muscular endurance and force suggest that DKB138 may contribute to both neuromuscular function and mitochondrial energy metabolism. Similar functional recoveries have been reported in studies utilizing natural compounds targeting oxidative stress and mitochondrial biogenesis [19]. Histological analysis further supported these findings. H&E staining revealed

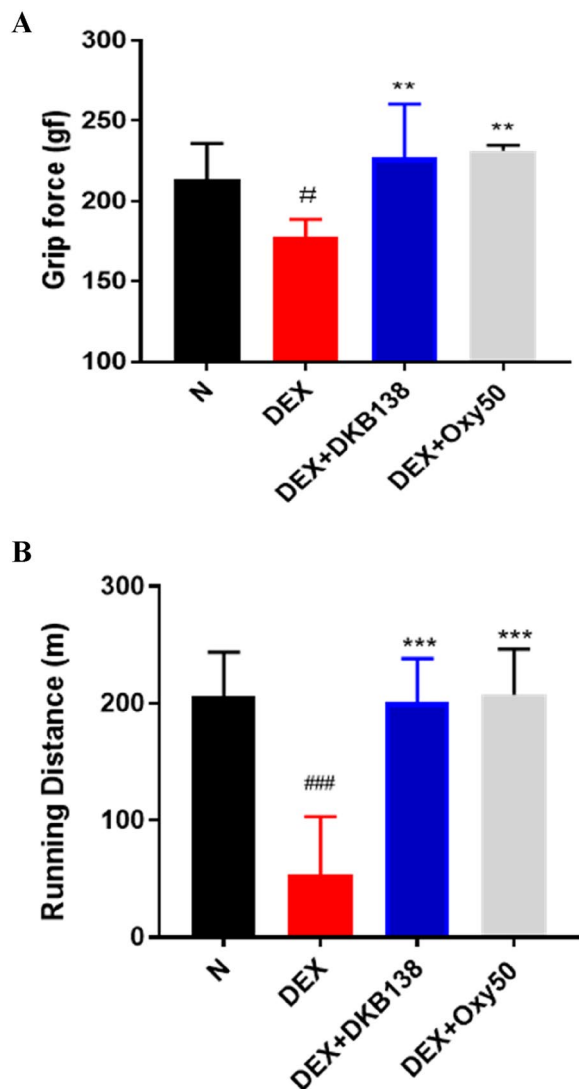


Fig. 4 Effects of DKB138 on grip strength and treadmill tests in DEX-induced skeletal muscle atrophy mouse model. **(A)** Grip strength test **(B)** Treadmill test. Data are shown as the mean $\pm$ SE ($n=8$). Statistical significance was evaluated by Tukey's test. ### $p < 0.001$, # $p < 0.05$ vs. Normal group (N). *** $p < 0.001$, ** $p < 0.01$ vs. DEX group

that DKB138 treatment preserved the integrity of muscle fiber architecture in the GAS muscle, in contrast to the pronounced atrophy observed in the DEX group. The reduction in CSA in the DEX-treated group was consistent with classic atrophy pathology, which was significantly ameliorated in both the DKB138- and Oxy-treated groups. These structural improvements indicated that DKB138 not only supported muscle function but also attenuated the morphological deterioration characteristic of glucocorticoid-induced muscle loss. At the molecular level, DKB138 significantly modulated the expression of key muscle atrophy biomarkers. DEX treatment elevated the expression of Murf-1 and Atrogin-1, two E3 ubiquitin ligases that mediate protein degradation via

the ubiquitin-proteasome system [20]. Treatment with DKB138 significantly reduced the expression of these genes, suggesting that DKB138 suppresses muscle protein catabolism. Interestingly, MyoD expression, which was suppressed by DEX, was upregulated following DKB138 treatment. As a master regulator of myogenic differentiation and regeneration [16], increased MyoD expression may indicate a shift toward muscle repair and regeneration under DKB138 influence. In addition to the observed *in vitro* and *in vivo* effects of DKB138, the bioactivity of its constituent compounds further supports its therapeutic potential in muscle health. Among the seven marker compounds identified in DKB138, several have been reported to exert protective effects on musculoskeletal tissues. Cynoroside, one of seven marker compounds, exhibits chondroprotective effect by inhibiting inflammatory mediators and oxidative stress in IL-1 β -induced chondrocytes, suggesting potential benefits in musculoskeletal health [21]. Hesperidin, a citrus flavonoid, ameliorates sarcopenia by regulating inflammation, thereby preserving muscle mass and function in aging population [22]. Rosmarinic acid demonstrates protective effects against DEX-induced muscle atrophy by inhibiting protein degradation pathways and oxidative stress and by promoting mitochondrial function [23]. Luteolin-7-*o*-glucuronide, although specific studies on its role in muscle atrophy are scarce, its parent compound, Lutolin alleviate muscle atrophy and mitochondrial dysfunction, indicating potential similar benefits [24]. Salvianolic acid B prevents bone loss in glucocorticoid treated model by stimulating osteogenesis and inhibiting adipogenesis, which may indirectly support to support muscle loss [25].

This study demonstrates that DKB138 effectively prevents muscle atrophy through multiple converging mechanisms, including inhibition of protein degradation, protection against cell death, preservation of muscle fiber integrity, and enhancement of muscle function. These multi-modal actions position DKB138 as a promising therapeutic or preventive candidate against glucocorticoid-induced muscle atrophy. The primary objective was to assess DKB138's potential to improve muscle strength in both *in vivo* and *in vitro* models. Our findings indicate that DKB138 mitigates muscle loss by modulating key molecular pathways related to muscle degradation, inflammation, and myogenesis. However, several limitations should be acknowledged. This study assessed only mRNA expression of key muscle atrophy markers without corresponding protein-level validation such as Western blot or immunohistochemistry. Moreover, important signaling pathways involved in muscle homeostasis and atrophy—including mTOR, FoxO3a, autophagy markers (LC3/p62), and inflammatory cytokines (TNF- α , IL-6)—were not investigated, limiting mechanistic insight. These pathways play critical roles in

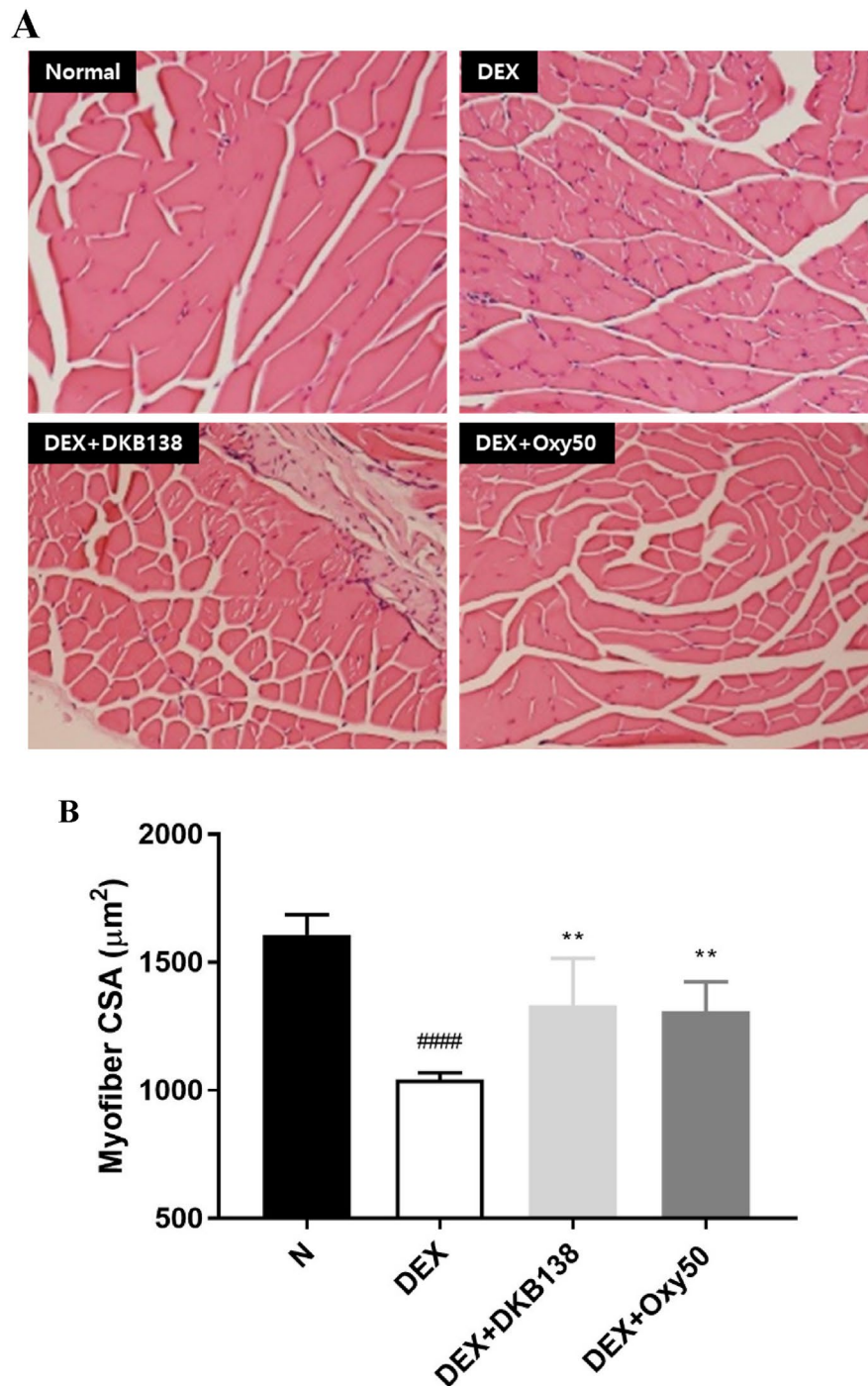


Fig. 5 Histological effects of DKB138 on the GAS in DEX-induced skeletal muscle atrophy mouse model. **(A)** Hematoxylin and eosin (H&E) staining **(B)** Schematic representation of the animal experiment design. **(B)** Cross-sectional area (CSA) of muscle fibers. Data are shown as the mean $\pm$ SE ($n=8$). Statistical significance was evaluated by Tukey's test. #### $p < 0.0001$ vs. Normal group (N); ** $p < 0.01$ vs. DEX treated group

regulating muscle protein synthesis, degradation, inflammation, and autophagy, which are essential for maintaining muscle mass and function [26–29]. Additionally, the DEX-induced acute catabolic model used differs pathophysiologically from age-related sarcopenia, suggesting cautious interpretation when extrapolating these

results to chronic muscle-wasting conditions. Validation in aged or diet-induced sarcopenia models is warranted to establish broader applicability. Notably, the muscle strength-enhancing and sarcopenia-preventive effects of *Schizonepeta tenuifolia* ethanol extract have been recognized through patent registration in Korea

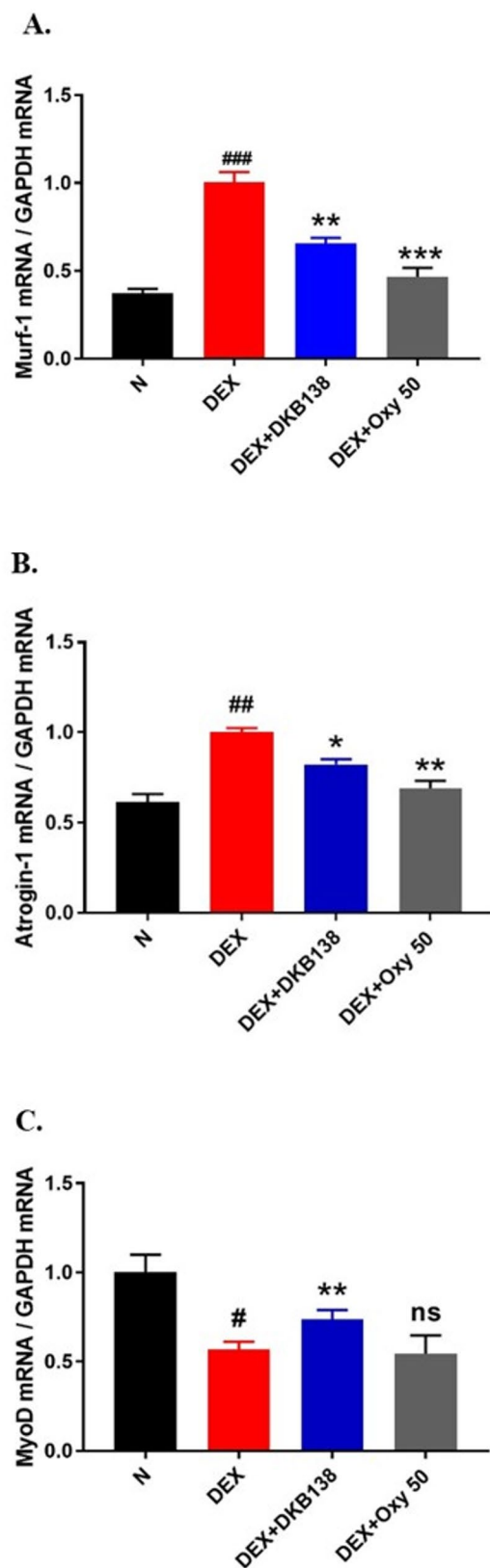


Fig. 6 Effects of DKB138 on Murf-1, Atrogin-1 and MyoD mRNA expression in DEX-induced skeletal muscle atrophy mouse model. Murf-1 (A), Atrogin-1 (B), and MyoD (C) mRNA in GAS were analyzed using qPCR. N, normal control group; DEX, DEX-treated group; DEX + DKB138, DEX treated with DKB138 (200 mg/kg); DEX + Oxy50, DEX treated with Oxymetholone (50 mg/kg); DEX treated with oxymetholone (50 mg/kg) as a positive control. Results expressed as mean $\pm$ SE ($n=8$). ### $p < 0.01$, # $p < 0.05$ vs. N; * $p < 0.05$, ** $p < 0.01$ vs. DEX treated group, respectively

(#10-2684311) and patent application in the United States (US18/278,227), underscoring its potential as a natural supplement to combat muscle wasting.

In conclusion, this study provides foundational evidence supporting the development of DKB138 as a safe and effective natural intervention for glucocorticoid-induced muscle atrophy and potentially other muscle-wasting disorders. Future investigations incorporating comprehensive protein-level analyses, functional assessments of key signaling pathways, and evaluation in chronic sarcopenia models will strengthen mechanistic understanding and clinical relevance.

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Author contributions

This study was carried out with collaboration among all authors. D.S.K. and Y.S.K. designed and supervised. Y.H.J. prepared DKB138. Y.S.K. and J.H.J. performed the experiments and analyzed the data and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability

The data generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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