Korean Mint (*Agastache rugosa*) Extract and Its Bioactive Compound Tilianin Alleviate Muscle Atrophy via the PI3K/Akt/FoxO3 Pathway in C2C12 Myotubes

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ABSTRACT: Skeletal muscle atrophy, which is characterized by diminished muscle mass, strength, and function, is caused by malnutrition, physical inactivity, aging, and diseases. Korean mint (*Agastache rugosa* Kuntze) possesses various biological functions, including anti-inflammatory, antioxidant, anticancer, and antiosteoporosis activities. Moreover, it contains tilianin, which is a glycosylated flavone that exerts antioxidant, anti-inflammatory, antidiabetic, and neuroprotective activities. However, no studies have analyzed the inhibitory activity of *A. rugosa* extract (ARE) and tilianin on muscle atrophy. Thus, the present study investigated the potential of ARE and tilianin on muscle atrophy and their underlying mechanisms of action in C2C12 myotubes treated with tumor necrosis factor-α (TNF-α). The results showed that ARE and tilianin promoted the phosphatidylinositol 3-kinase/protein kinase B pathway, thereby activating mammalian target of rapamycin (a protein anabolism-related factor) and its downstream factors. Moreover, ARE and tilianin inhibited the mRNA expression of muscle RING-finger protein-1 and atrogin-1 (protein catabolism-related factors) by blocking Forkhead box class O$_3$ translocation. ARE and tilianin also mitigated inflammatory responses by downregulating nuclear factor-kappa B expression levels, thereby diminishing the expression levels of inflammatory cytokines, including TNF-α and interleukin-6. Additionally, ARE and tilianin enhanced the expression levels of antioxidant enzymes, including catalase, superoxide dismutase, and glutathione peroxidase. Overall, these results suggest that ARE and tilianin are potential functional ingredients for preventing or improving muscle atrophy.

Keywords: *Agastache rugosa*, C2C12, Korean mint, muscular atrophy, tumor necrosis factor-alpha

INTRODUCTION

The skeletal muscle comprises 40% of body weight and is a major organ responsible for performing physical activities and metabolizing carbohydrates, fats, and proteins (Frontera and Ochala, 2015). In addition, it stores 50%~75% of proteins in an individual's body, indicating that the role of the skeletal muscle is intricately linked to protein quality and quantity (Frontera and Ochala, 2015; Sartori et al., 2021). However, skeletal muscle abnormalities lead to a progressive decline in protein content, which is a primary contributor to muscle atrophy (Moro et al., 2016). This condition is typified by reductions in muscle fiber, density, and mass. Muscle atrophy has been consistently linked to a decline in overall quality of life. Furthermore, the reduction in muscle mass resulting from muscle atrophy is associated with the increased occurrence of chronic conditions, including type 2 diabetes, cardiovascular disease, and depression, and increased mortality rate in patients (Nunes et al., 2022). Thus, maintaining protein quality and quantity in skeletal muscle is crucial for overall health.

In the normal state, the balance between protein anabolism and catabolism, known as protein turnover, is maintained. However, if protein breakdown surpasses protein synthesis, it results in muscle atrophy. The activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway induces mammalian target of rapamycin (mTOR) phosphorylation to stimulate protein anabolism, which leads to increased protein content in the skeletal muscle (Egerman and Glass, 2014). The phosphorylation of mTOR also stimulates its downstream factors, thereby accelerating the process of translation for protein synthesis in the central dogma (Egerman and Glass, 2014; Yoon, 2017). Forkhead box class O$_3$ (FoxO3) serves as a transcription factor that regulates genes associated with the

ubiquitin-proteasome system (UPS), including muscle RING-finger protein-1 (MuRF1) and atrogin-1 (Jackman and Kandarian, 2004; Egerman and Glass, 2014). FoxO3 stimulation through inflammatory response and oxidative stress triggers protein breakdown, contributing to skeletal muscle atrophy.

Among the inflammatory cytokines, tumor necrosis factor-α (TNF-α) is known as a potent inducer of inflammatory responses. It stimulates nuclear factor-kappa B (NF-κB), a major regulator of inflammatory cytokine transcription (Li et al., 2008). Activated NF-κB increases the expression of inflammatory cytokines and E3 ubiquitin ligases, leading to protein degradation via the UPS (Li et al., 2008; Sa et al., 2017). The upregulated inflammatory cytokines work as autocrine and/or paracrine factors, triggering the abovementioned signaling in muscle cells (Kim and Hwang, 2020). Aside from inflammatory responses, TNF-α also triggers oxidative stress by activating reactive oxygen species (ROS) and decreasing the levels of antioxidant enzymes, including catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx), which trigger apoptosis or programmed cell death (Reid and Li, 2001). Moreover, TNF-α inhibits the PI3K/Akt pathway, thereby inhibiting protein synthesis and stimulating protein catabolism in atrophied muscles (Sa et al., 2017). Thus, blocking TNF-α-related signaling pathways not only ameliorates inflammatory responses and oxidative stress but also increases protein content in atrophied muscles by stimulating the PI3K/Akt pathway.

Korean mint (*Agastache rugosa* Kuntze) has traditionally been used in food and medicines to treat vomiting, nausea, and fungal infection (Nam et al., 2020). It possesses various pharmacological activities, including anti-inflammatory, antioxidant, and antiatherosclerosis properties (Yun et al., 2019). A previous study demonstrated that *A. rugosa* exhibits antioxidant activity by reducing ROS and activating antioxidant enzymes, and anti-inflammatory activity by downregulating inflammatory cytokines (Yun et al., 2019). Moreover, *A. rugosa* contains tilianin, a prominent flavonoid compound that exerts anti-inflammatory, antioxidant, antidiabetic, and antihypertensive effects (Akanda et al., 2019). Although *A. rugosa* and tilianin have demonstrated beneficial effects on various organs, including the skin, liver, brain, and smooth muscle (Akanda et al., 2019), their protective effects against skeletal muscle damage remain unknown. Therefore, in the present study, we examined the suppressive effects of *A. rugosa* and tilianin on skeletal muscle atrophy and dysfunction in TNF-α-treated C2C12 myotubes.

MATERIALS AND METHODS

Preparation of *A. rugosa* extract (ARE)
Dried aerial parts of *A. rugosa* were ground and extracted using water at 95°C for 4 h. The extract was filtered and evaporated by using a vacuum rotary evaporator to obtain ARE with a yield of 10% (w/w). Tilianin was purchased from Sigma-Aldrich, and its purity was verified to be ≥98%.

Cell culture
C2C12 myoblasts, which originated from *Mus musculus*, were purchased from the American Type Culture Collection. They were grown in Dulbecco's modified Eagle's medium (DMEM; Hyclone) supplemented with 10% fetal bovine serum (Hyclone) at 37°C and 5% CO$_2$. For differentiation, the cells were cultured in DMEM (Hyclone) supplemented with 2% horse serum (Gibco). The medium was replaced every alternate day for four days. Fully differentiated C2C12 myotubes were cotreated with 50 ng/mL of TNF-α (PeproTech) and 40 and 80 μg/mL of ARE or 20 and 40 μM tilianin for 24 h.

Cell viability
The cytotoxic effects of ARE and tilianin on C2C12 myotubes were analyzed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Sigma-Aldrich). The cells were treated with ARE or tilianin for 24 h and incubated with 0.5 mg/mL of MTT solution at 37°C for 4 h. The MTT solution was removed after the formation of MTT-formazan product. Then, formazan crystals were dissolved using dimethyl sulfoxide, and the absorbance was measured using a VersaMax tunable microplate reader (Molecular Devices, Inc.) at 540 nm.

Western blot analysis
The proteins were extracted from C2C12 myotubes using NP-40 lysis buffer (Elpis Biotech) containing 0.2% protease inhibitor cocktail (Sigma-Aldrich). The protein lysate concentration was assessed using Bradford solution (Bio-Rad). The lysate was boiled at 95°C for 5 min and separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis at 85 V for 30 min and then at 110 V for 90 min. The separated proteins were transferred onto a nitrocellulose membrane (GE Healthcare) at 110 V for 70 min. Thereafter, the membrane was incubated with 5% skim milk (Difco) in Tris-buffered saline and Tween 20 (Dyne Bio) for 30 min to block non-specific binding. Next, the membrane was incubated overnight at 4°C with the primary antibodies against α-tubulin, NF-κB (Santa Cruz Biotechnology Inc.), PI3K, phosphorylated-PI3K (p-PI3K), Akt, phosphorylated-Akt (p-Akt), 70-kDa ribosomal protein S6 kinase (p70S6K), phosphorylated-p70S6K (p-p70S6K), eukaryotic initiation factor 4E bind-

ing protein 1 (4EBP1), phosphorylated-4EBP1 (p-4EBP1), mTOR, phosphorylated-mTOR (p-mTOR), FoxO3, and phosphorylated-FoxO3 (p-FoxO3) (Cell Signaling Technology). After washing, the membrane was incubated with secondary antibodies at 4°C for 2 h. The specific proteins on the membrane were developed using enhanced chemiluminescence solution (Dyne Bio) and detected using the G:BOX EF imaging system and GeneSys software (Syngene). The band intensity was visualized using the ImageJ software (National Institutes of Health).

Reverse transcription polymerase chain reaction (RT-PCR)

RNA was isolated from C2C12 myotubes using TRIzol reagent (Takara) and the GeneAmp PCR System 2700 (Applied Biosystems). The RNA concentration was quantified using the NanoDrop 1,000 Spectrophotometer (Thermo Fisher Scientific Inc.). Reverse Transcriptase Premix (Elpis Biotech) and total RNA were reacted at 42°C for 1 h and then at 95°C for 5 min to synthesize cDNA. PCR amplification was conducted using SafeDry Taq PCR Premix (CellSafe) and specific primers (Bioneer) shown in Table 1. The target gene amplification conditions were as follows: enzyme activation at 95°C for 10 min, denaturation at 95°C for 30 s, annealing at 58°C~60°C for 30 s, elongation at 72°C for 45 s, and final extension step at 72°C for 5 min. The amplified products were stained with 5× Loading STAR dye (Dyne Bio) and separated via 1.5% agarose gel electrophoresis. PCR product bands were identified using the G:BOX imaging analysis system and GeneSys software (Syngene). The band intensity was visualized using the ImageJ software.

Statistical analysis

Data are expressed as the mean±standard deviation. Differences among groups were evaluated using one-way ANOVA and Duncan's multiple range test in IBM SPSS (version 25.0, IBM Corp.). Significant differences between each group were considered at $P<0.05$ and $P<0.01$.

RESULTS

Cytotoxic effects of ARE and tilianin on C2C12 myotubes

MTT assay was used to evaluate the cytotoxic effects of ARE and tilianin on C2C12 myotubes. ARE exerted a significant cytotoxic effect on C2C12 myotubes at concentrations greater than 100 μg/mL. Meanwhile, tilianin exhibited cytotoxic effect on C2C12 myotubes at concentrations greater than 80 μM (data not shown). Therefore, further experiments were conducted using 40 and 80 μg/mL of ARE and 20 and 40 μM tilianin.

Effects of ARE and tilianin on protein synthesis

TNF-α treatment markedly diminished p-PI3K and p-Akt expression compared with the control group, whereas ARE dose-dependently upregulated p-PI3K and p-Akt expression (Fig. 1A). In addition, tilianin restored p-PI3K and p-Akt protein expression in TNF-α-treated C2C12 myotubes (Fig. 1B). Located downstream of the PI3K/Akt pathway, the mTOR pathway is essential for muscle hypertrophy. TNF-α significantly downregulated p-mTOR, thereby decreasing p-p70S6K and p-4EBP1 expression in TNF-α-treated C2C12 myotubes (Fig. 2). However, treatment with 80 μg/mL of ARE significantly recovered p-mTOR, p-p70S6K, and p-4EBP1 protein expression. Moreover, treatment with 40 μg/mL of ARE enhanced p-p70S6K and p-4EBP1 expression, but it did not affect p-mTOR expression (Fig. 2A). Meanwhile, treatment with 40 μM tilianin increased p-mTOR, p-p70S6K, and p-4EBP1 expression. However, treatment with 20 μM tilianin increased p-mTOR expression only (Fig. 2B).

Effects of ARE and tilianin on protein degradation

p-FoxO3 protein expression was downregulated in TNF-α-treated C2C12 myotubes (Fig. 3A and 3B). By contrast, MuRF1 and atrogin-1 expression exhibited significant upregulation compared with that in the control group (Fig. 3C and 3D). However, treatment with 40 and 80 μg/mL of ARE dose-dependently increased p-FoxO3 expression (Fig. 3A). In response to the elevated p-FoxO3 protein expression, its downstream factors MuRF1 and atrogin-1 were markedly reduced by high ARE doses (Fig. 3C). Similarly, tilianin restored p-FoxO3 protein expression and concurrently diminished MuRF1 and atrogin-1 mRNA expression at a concentration of 40 μM, but not 20 μM (Fig. 3B and 3D).

Table 1. Primer sequences used in reverse transcription polymerase chain reaction analysis

| Gene	Direction	Sequence (5′–3′)
MuRF1	Forward	AGATGAGTGAGACACGCTCTG
	Reverse	CCACTTTGTGACAATCGCCAG
Atrogin-1	Forward	TCTCAGTTGAACGGGAGGGA
	Reverse	GCCGAGAACCATACACTGCT
TNF-α	Forward	CCCTCACACTCACAAACCAC
	Reverse	ACAAGGTACAACCCATCGGC
IL-6	Forward	GTCCTTCCTACCCCAATTTCCA
	Reverse	TAACGCACTAGGTTTGCCGA
Catalase	Forward	AACGCTGGATGGATTCTCCC
	Reverse	TCCCTTCAGGAAACGGCATC
SOD	Forward	GAGAAGATAGGCGACACGCA
	Reverse	CTGGTTGCCACCTTTACCCA
GPx	Forward	ACCACAGTCAGCAACGTCAA
	Reverse	AGTTGTGCCAGGCTTGTCTT
β-Actin	Forward	GCTTCTAGGCGGACTGTTACT
	Reverse	AGGGTGAGGGACTTCCTGTA

A

B
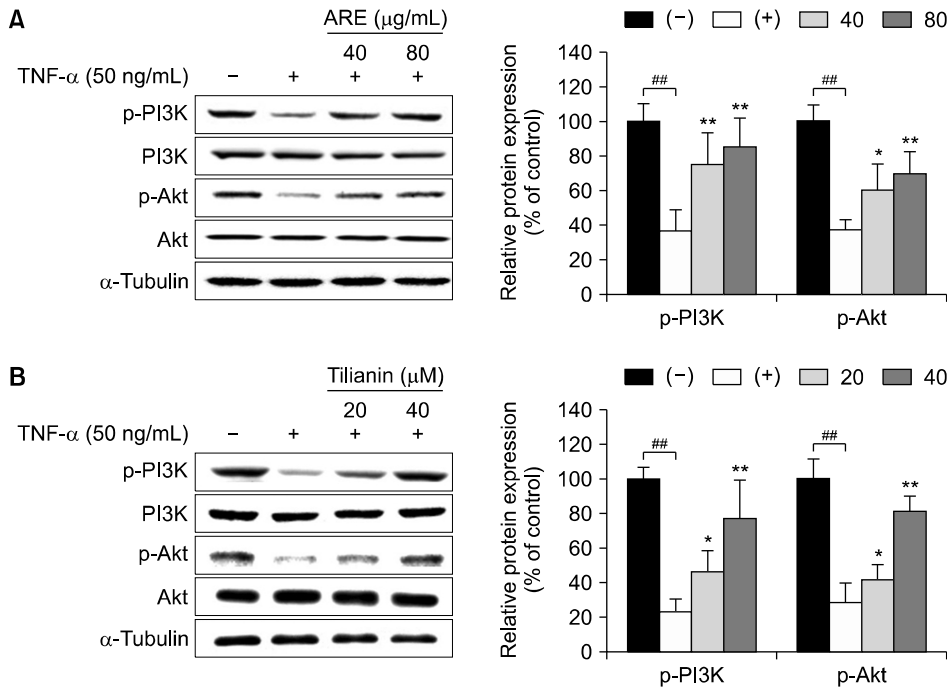
Fig. 1. Effects of *Agastache rugosa* extract (ARE) and tilianin on the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway in C2C12 myotubes. C2C12 myotubes were simultaneously exposed to tumor necrosis factor-α (TNF-α, 50 ng/mL) along with ARE (40 and 80 µg/mL) or tilianin (20 and 40 µM). Western blot analysis was used to assess the protein expression levels of phosphorylated-PI3K (p-PI3K), PI3K, phosphorylated-Akt (p-Akt), and Akt in response to (A) ARE and (B) tilianin. α-Tubulin served as the internal control. Experimental results were presented as the mean± SD. Significant differences were assessed using Duncan's multiple range test. $^{\#\#}P<0.01$ (control group vs. TNF-α group); $^{*}P<0.05$, $^{**}P<0.01$ (TNF-α group vs. ARE or tilianin group).
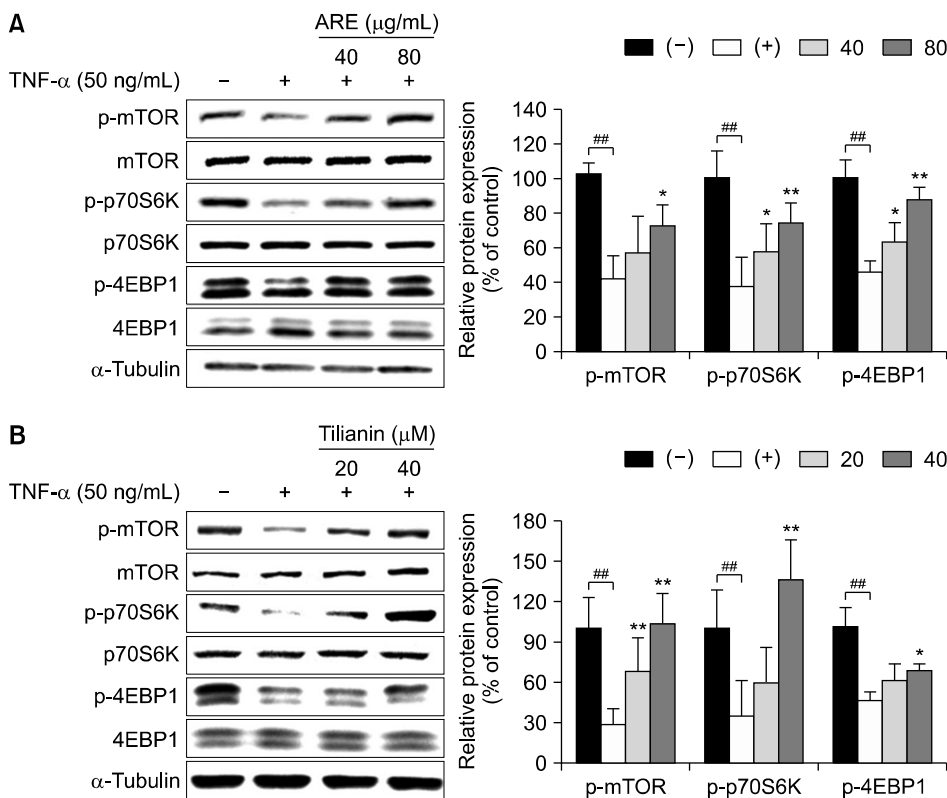
A

B

Fig. 2. Effects of *Agastache rugosa* extract (ARE) and tilianin on the mammalian target of rapamycin (mTOR) pathway in C2C12 myotubes. C2C12 myotubes were simultaneously exposed to tumor necrosis factor-α (TNF-α, 50 ng/mL) along with ARE (40 and 80 µg/mL) or tilianin (20 and 40 µM). Western blot analysis was used to assess the protein expression levels of phosphorylated-mTOR (p-mTOR), mTOR, phosphorylated-70-kDa ribosomal protein S6 kinase (p-p70S6K), p70S6K, phosphorylated-eukaryotic initiation factor 4E binding protein 1 (p-4EBP1), and 4EBP1 in response to (A) ARE and (B) tilianin. α-Tubulin served as the internal control. Experimental results were presented as the mean±SD. Significant differences were assessed using Duncan's multiple range test. $^{\#\#}P<0.01$ (control group vs. TNF-α group); $^{*}P<0.05$, $^{**}P<0.01$ (TNF-α group vs. ARE or tilianin group).

Effects of ARE and tilianin on inflammatory response and oxidative stress

ARE and tilianin markedly reduced NF-κB protein expression increased by TNF-α in C2C12 myotubes (Fig. 4A and 4B). TNF-α treatment substantially increased the expression of inflammatory cytokines such as interleukin-6 (IL-6) and TNF-α. However, treatment with 80 µg/mL of ARE and 40 µM tilianin drastically reduced the levels of these inflammatory cytokines (Fig. 4C and 4D). ARE substantially restored the decreased mRNA expression of SOD, GPx, and catalase induced by TNF-α (Fig. 5A). Moreover, tilianin dose-dependently recovered their expression, except for SOD in the 20 µM tilianin group (Fig. 5B).

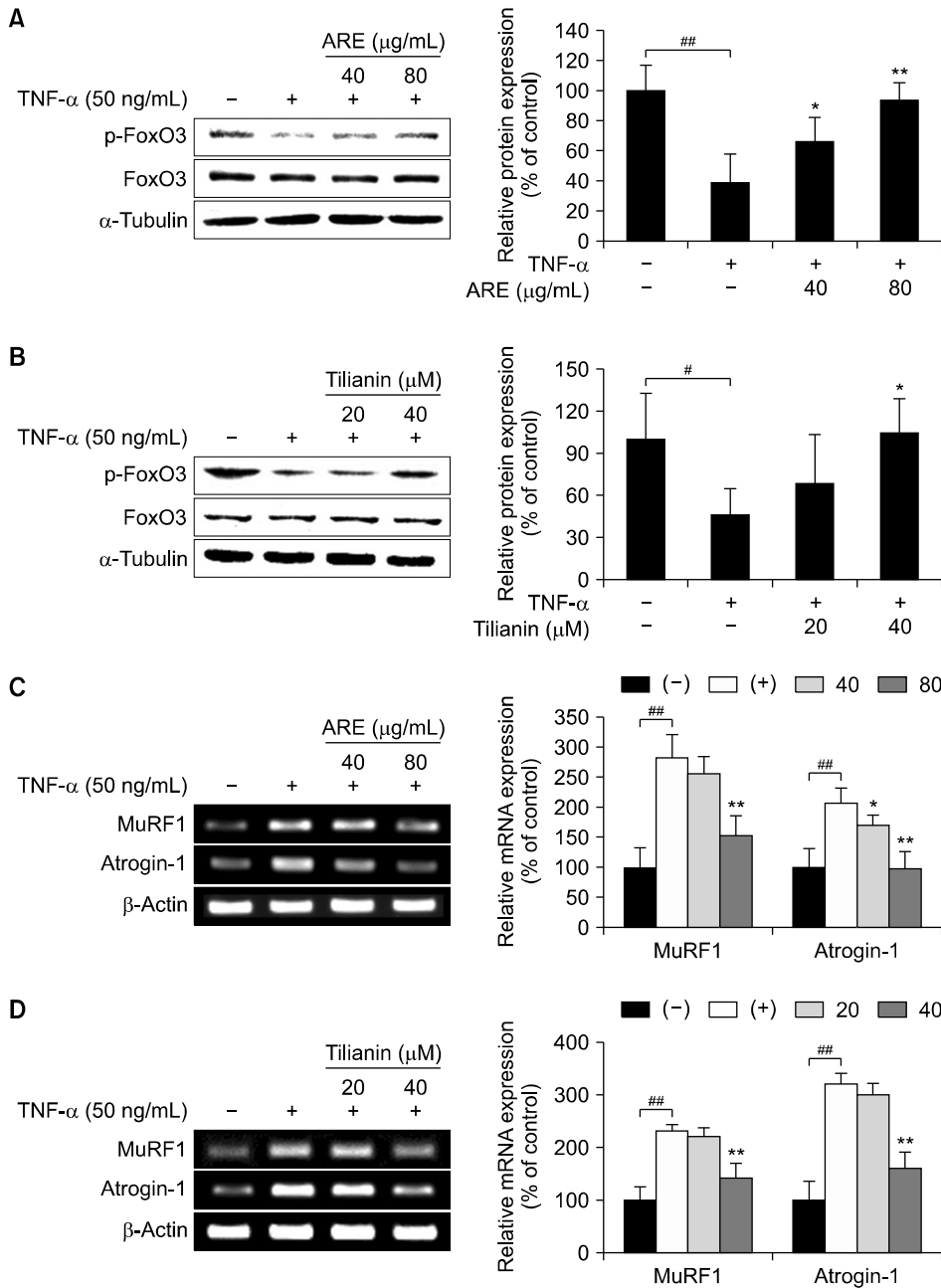**A**

B

C

D

Fig. 3. Effects of *Agastache rugosa* extract (ARE) and tilianin on protein-degradation-related pathways in C2C12 myotubes. C2C12 myotubes were simultaneously exposed to tumor necrosis factor-α (TNF-α, 50 ng/mL) along with ARE (40 and 80 μg/mL) or tilianin (20 and 40 μM). Western blot analysis was used to assess the protein expression levels of phosphorylated-forkhead box class O_3 (p-FoxO3) and FoxO3 in response to (A) ARE and (B) tilianin. α-Tubulin served as the internal control. Reverse transcription polymerase chain reaction was used to determine the mRNA expression levels of muscle RING-finger protein-1 (MuRF1) and atrogin-1 in response to (C) ARE and (D) tilianin. β-Actin served as the internal control. Experimental results were presented as the mean±SD. Significant differences were assessed using Duncan's multiple range test. [#]$P<$0.05, [##]$P<$0.01 (control group vs. TNF-α group); [*]$P<$0.05, [**]$P<$0.01 (TNF-α group vs. ARE or tilianin group).

DISCUSSION

Muscle atrophy is usually caused by aging, metabolic diseases, cancer, and physical inactivity (Jackman and Kandarian, 2004). Despite occurring in different manners, muscle atrophy shows common symptoms, including decreased muscle weight, strength, and physical performance (Shiota et al., 2015). The balance between protein synthesis and degradation in the skeletal muscle is important. Muscle atrophy occurs when proteolysis surpasses protein synthesis (Sartori et al., 2021). The PI3K/Akt pathway plays a crucial role in regulating protein synthesis and degradation (Yoon, 2017). Akt is involved in the downstream mTOR pathway for muscle hypertrophy and inhibits FoxO3 nuclear translocation, there-

by downregulating ubiquitin E3 ligases such as MuRF1 and atrogin-1 (Egerman and Glass, 2014). In the present study, ARE and tilianin effectively recovered the decreased protein expression of phosphorylated PI3K, Akt, mTOR, p70S6K, and 4EBP1 induced by TNF-α treatment (Fig. 1 and 2). Furthermore, they inhibited the nuclear translocation of FoxO3 through phosphorylation, thereby decreasing the mRNA expression of MuRF1 and atrogin-1 (Fig. 3).

In the molecular aspect, TNF-α plays a major role in inducing muscle atrophy. It also induces inflammatory responses and excessive ROS accumulation in muscle cells. Moreover, it disrupts the balance between protein anabolism and catabolism, leading to abnormal protein turnover and decreased protein content in muscle cells
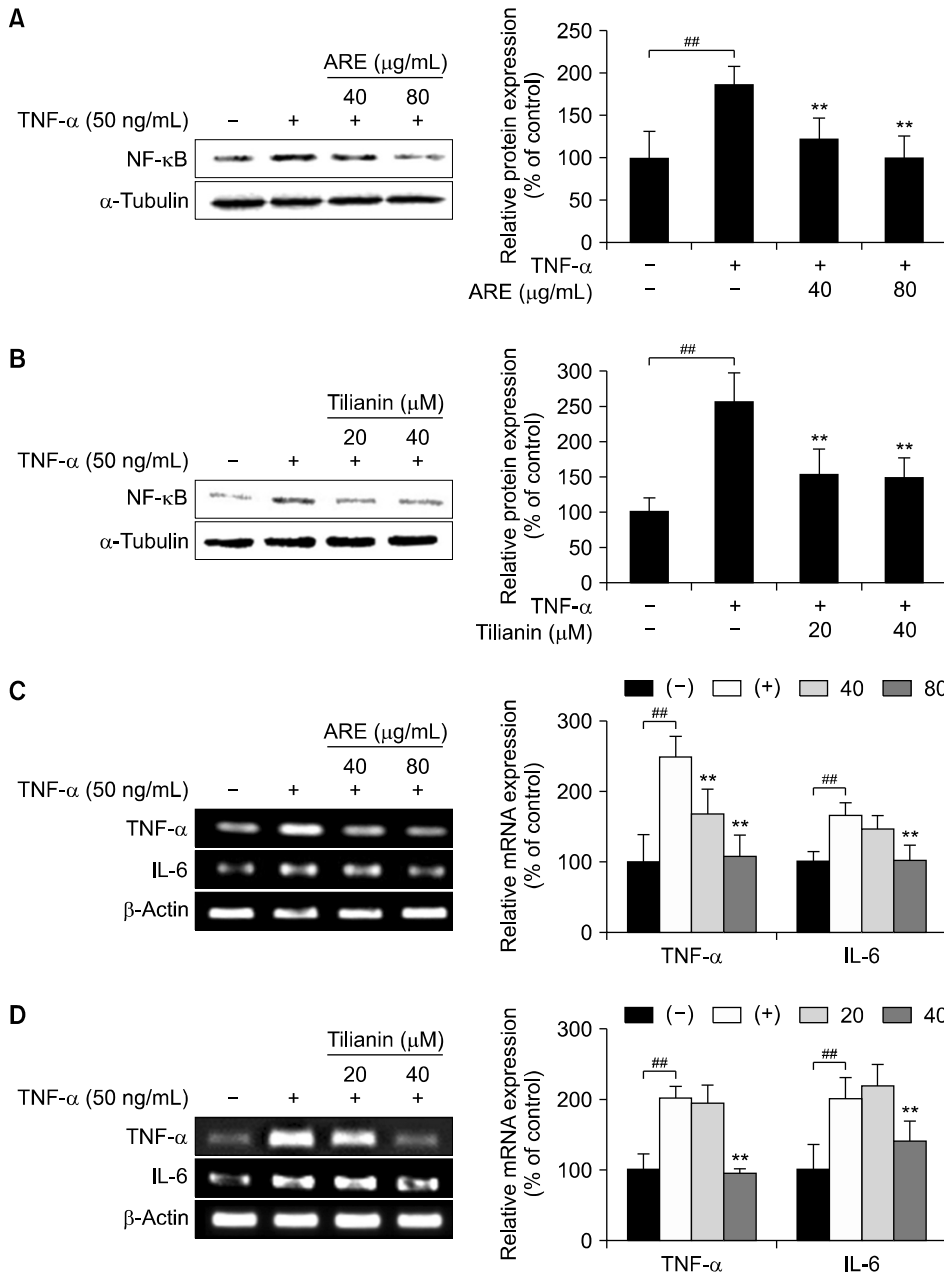
Fig. 4. Effects of *Agastache rugosa* extract (ARE) and tilianin on inflammatory response in C2C12 myotubes. C2C12 myotubes were simultaneously exposed to tumor necrosis factor-α (TNF-α, 50 ng/mL) along with ARE (40 and 80 μg/mL) or tilianin (20 and 40 μM). Western blot analysis was used to assess the protein expression levels of nuclear factor-kappa B (NF-κB) in response to (A) ARE and (B) tilianin. α-Tubulin served as the internal control. Reverse transcription polymerase chain reaction was used to determine the mRNA expression levels of TNF-α and interleukin-6 (IL-6) in response to (C) ARE and (D) tilianin. β-Actin served as the internal control. Experimental results were presented as the mean±SD. Significant differences were assessed using Duncan's multiple range test. $^{\#\#}P<0.01$ (control group vs. TNF-α group); $^{**}P<0.01$ (TNF-α group vs. ARE or tilianin group).

(Zoico and Roubenoff, 2002). Several studies have reported that *A. rugosa* exerts anti-inflammatory effects in many diseases and organs. For example, ARE decreased IL-6 and TNF-α expression in ultraviolet B (UVB)-treated hairless mice and cells (Yun et al., 2019). Moreover, *A. rugosa* essential oil decreased the expression of inducible nitric oxide synthase by inhibiting NF-κB activation (Hong et al., 2012). In addition, tilianin (a major bioactive compound responsible for the anti-inflammatory effect of ARE) attenuated the mRNA expression of IL-6 in UVB-treated Hs68 skin dermal fibroblasts (Seo et al., 2019). It also effectively mitigated atherosclerosis by suppressing TNF-α and IL-1β expression triggered by IκB kinase activation in a hyperlipidemic mouse model (Nam et al., 2005). In the present study, ARE and tilianin reduced the mRNA expression of TNF-α and IL-6 and downreg-

ulated the protein expression of NF-κB, a transcription factor regulating inflammatory cytokines, in TNF-α-treated C2C12 myotubes (Fig. 4). These results suggest that ARE and tilianin can suppress NF-κB activity, thereby leading to the downregulation of inflammatory cytokine expression.

Several plant extracts that exhibit antioxidant and anti-inflammatory effects have been considered as pharmacological agents for muscle atrophy prevention (Nam et al., 2005; Sa et al., 2017). Oxidative stress usually occurs because of an imbalance between oxidant and antioxidant species. Normally, ROS act as signaling molecules for muscle regeneration and repair, promoting mitochondrial biogenesis during exercise. However, excessive ROS levels induce muscle atrophy through mitochondrial dysfunction, UPS activation, downregulation of protein syn-
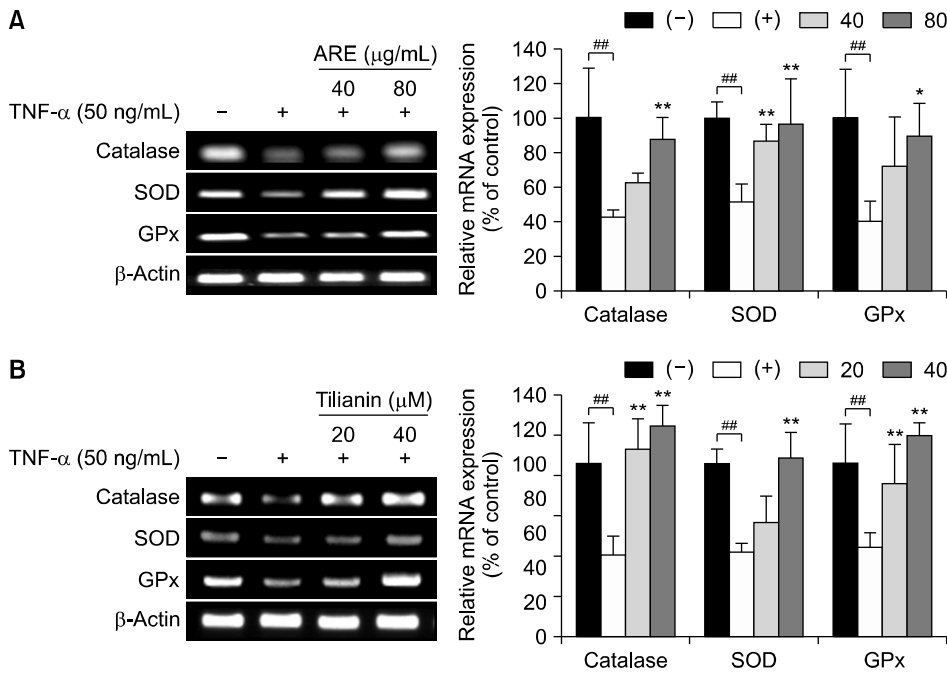
A

B

Fig. 5. Effects of *Agastache rugosa* extract (ARE) and tilianin on oxidative stress in C2C12 myotubes. C2C12 myotubes were simultaneously exposed to tumor necrosis factor-α (TNF-α, 50 ng/mL) along with ARE (40 and 80 μg/mL) or tilianin (20 and 40 μM). Reverse transcription polymerase chain reaction was used to determine the mRNA expression levels of catalase, SOD, and GPx in response to (A) ARE and (B) tilianin. β-Actin served as the internal control. Experimental results were presented as the mean±SD. Significant differences were assessed using Duncan's multiple range test. $^{\#\#}P<0.01$ (control group vs. TNF-α group); $^{*}P<0.05$, $^{**}P<0.01$ (TNF-α group vs. ARE or tilianin group).

thesis pathways, and autophagy deregulation (Powers et al., 2007). Therefore, antioxidant enzymes, including SOD, catalase, and GPx, are crucial for preserving cellular oxidative homeostasis (Ábrigo et al., 2018). Increased levels of inflammatory cytokines induce ROS production and accelerate muscle atrophy via the NF-κB and FoxO3 signaling pathways (Jackman and Kandarian, 2004; Li et al., 2008). In the present study, ARE and tilianin conferred anti-inflammatory and antioxidation activities by downregulating the NF-κB signaling pathway and upregulating SOD, catalase, and GPx (Fig. 4 and 5). On the basis of these findings, the antioxidant activities of ARE and tilianin are partly involved in muscle atrophy prevention.

Tilianin is a glycosylated flavone that possesses phenyl, glycosyl, and hydroxyl groups at the 2-, 7-, and 5-position, respectively. Flavonoids can ameliorate muscle atrophy; however, their efficacy differ on the basis of their structure (Kim and Hwang, 2020). Previous studies have compared the efficacy of quercetin (3,3',4',5,7-pentahydroxyflavone), which is categorized as a flavonol, and flavone on muscle atrophy. Quercetin increased the weight of the gastrocnemius muscle in mice that underwent tail-suspension-induced muscle atrophy, a representative animal model of unloading-induced muscle atrophy, whereas flavone did not affect the weight (Mukai et al., 2010; Kim and Hwang, 2020). At the molecular level, only quercetin downregulated the mRNA expression of MuRF1 and atrogin-1 in atrophic gastrocnemius muscle. These results suggest that the hydroxyl groups present in flavonol's structure play an important role in ameliorating muscle atrophy by downregulating the UPS (Mukai et

al., 2010; Le et al., 2014). In a previous study, luteolin (3',4',5,7-tetrahydroxyflavone) and apigenin (4',5,7-trihydroxyflavone) reduced atrogin-1 expression and increased myotube diameter in lipopolysaccharide-treated C2C12 cells (Shiota et al., 2015). However, 5,7-dihydroxychromone, a compound wherein the phenyl group at the 2-position of apigenin and luteolin is replaced by a hydrogen group, had no impact on atrogin-1 mRNA expression (Shiota et al., 2015). This result suggests that the presence of the phenyl group in flavone's structure is closely associated with atrogin-1 downregulation. Collectively, tilianin's inhibitory effect on TNF-α-induced muscle atrophy in C2C12 cells might be because of its specific structure: a hydroxyl group at the 5-position and a phenyl group at the 2-position. However, the role of the glycosyl group at the 7-position in tilianin's structure remains unknown and should be further studied. Previous studies have revealed that *A. rugosa* contains quercetin, apigenin, and luteolin (Desta et al., 2016; Nam et al., 2020). Thus, the presence of various flavonoid compounds in ARE, including tilianin, may be responsible for its inhibitory effect on muscle atrophy.

This study revealed that ARE and its active compound tilianin can prevent muscle atrophy by restoring protein turnover. They not only upregulated the protein synthesis pathways but also downregulated the protein-degradation-related pathways by blocking FoxO3 nuclear translocation. Moreover, ARE and tilianin exhibited anti-inflammatory and antioxidant activities by downregulating the NF-κB pathway and upregulating antioxidant enzyme expression. Overall, ARE and tilianin are potential nutraceutical agents for treating skeletal muscle atrophy.

FUNDING

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: JKH. Analysis and interpretation: YKW, CK, MK. Data collection: YKW, CK. Writing the article: YKW, CK, MK. Critical revision of the article: JKH. Final approval of the article: all authors. Statistical analysis: YKW, CK. Obtained funding: JKH. Overall responsibility: JKH.

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