Differential protective effects of *Radix astragali*, herbal medicine, on immobilization-induced atrophy of slow-twitch and fast-twitch muscles

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ABSTRACT

*Radix astragali* is a popular traditional herbal medicine that provides significant protection against tissue injury in various models of oxidative stress-related diseases. In this study, we aimed to investigate whether administration of *Radix astragali* prevented atrophy in both slow- and fast-twitch muscles following cast immobilization. Twenty-seven 12-week-old male F344 rats were divided into three experimental groups: control (CON), immobilized (IM), and immobilized with *Radix astragali* administration (IM+AR). Rats in the IM and IM+AR groups were subjected to immobilization of both lower extremities using casting-tape for 14 days. Rats in the IM+AR group were orally administered a decoction of *Radix astragali* daily for 21 days beginning 7 days before cast immobilization. As expected, rats in the IM group showed significant decreases (\(P < 0.05\)) in soleus and plantaris muscle-to-body weight ratios by 74.3% and 70.5%, respectively, compared with those in the CON group. Administration of *Radix astragali* significantly reversed (+35.5%) the weight reduction observed in soleus muscle, but not in the plantaris muscle, compared with that in the IM group. Furthermore, administration of *Radix astragali* inhibited MuRF1 mRNA expression only in the soleus muscle during cast immobilization. Our results demonstrated that administration of *Radix astragali* suppressed the immobilization-induced reductions in skeletal muscle mass and expression of MuRF1 mRNA in slow-twitch soleus muscles, but not in fast-twitch plantaris muscles.

INTRODUCTION

Loss of skeletal muscle mass and function occurs in cases of prolonged physical inactivity, such as bed rest and cast immobilization. Although this is a common clinical problem, effective therapies to prevent physical inactivity-induced muscle wasting are lacking, in part, due to a lack of understanding of the molecular mechanisms responsible for the induction and maintenance of muscle atrophy (Bodine 2013). Muscle regrowth is diminished during aging after cast immobilization, and restoration of fast-twitch (type II) fibers is significantly reduced by aging (Suetta et al. 2013). Moreover, physical inactivity-induced muscle wasting increases the morbidity of sarcopenia during aging (Machida and Booth 2004). Cast immobilization is usually used for the treatment of injuries or fracture, but leads to significant muscle atrophy. Muscle atrophy is triggered by an imbalance between protein synthesis and protein degradation. Notably, cast immobilization-induced muscle atrophy depends on protein degradation, but is independent of protein synthesis (Krawiec et al. 2013).
One major signaling pathway involved in protein degradation is the ubiquitin proteasome pathway, and the muscle-specific E3 ligases—muscle atrophy F-box (MAFbx)/atrogin-1 and muscle-specific RING finger 1 (MuRF1)—are critical contributors in this signaling pathway. A previous study demonstrated that MAFbx/atrogin-1 and MuRF1 may contribute more to the atrophy of fast-twitch muscles than to that of slow-twitch muscles in cast immobilization. In contrast, calpain and caspase-3 may contribute more to the atrophy of slow-twitch muscles than to that of fast-twitch muscles during cast immobilization. 

Importantly, traditional herbal medicines have been shown to have potential applications for alleviation of muscle atrophy (Zhang et al. 2014; Kishida et al. 2015). However, the effects of traditional herbal medicines on cast immobilization-induced muscle atrophy are unknown. Radix astragali is an important medicinal plant used in Asian countries and has been shown to exhibit medicinal properties for the treatment of various diseases. The biological functions of Radix astragali include anti-oxidative, anti-inflammatory, and anti-tumor effects (Gong et al. 2018). In particular, administration of Radix astragali has been shown to alleviate atrophy of slow-twitch soleus muscles in a hindlimb unloading model (Gao et al. 2005; Zhang et al. 2007) and fast-twitch tibial muscles in a denervation model (Zhou and Mei 2014).

In hindlimb unloading models, slow-twitch muscles exhibit more atrophy than fast-twitch muscles, whereas fast-twitch muscles show more atrophy than slow-twitch muscles in denervation models. In contrast, cast immobilization leads to the same levels of atrophy in both slow- and fast-twitch muscles (Machida and Booth 2005). Similarly, long-term bed rest in human results in atrophy of both slow- and fast-twitch muscles (Salanova et al. 2008). Accordingly, it will be important to develop appropriate muscle-specific treatments to inhibit immobilization-induced atrophy in both slow- and fast-twitch muscles. Furthermore, the mechanisms mediating the effects of Radix astragali on the prevention of muscle inactivity-induced muscle atrophy have not yet been elucidated. Thus, in the present study, we evaluated whether Radix astragali similarly suppressed atrophy in slow- and fast-twitch muscles during cast immobilization.

**MATERIALS AND METHODS**

**Experimental animals.** Twenty-seven male F344 rats (12 weeks old) were obtained from Japan SLC (Shizuoka, Japan). The animals were allowed to acclimate to the environment for 1 week before the study. The animals were randomly assigned to one of three experimental groups: 1) control (CON; 192.0 ± 7.0 g body weight, n = 9), 2) hindlimb immobilized (IM; 192.4 ± 6.4 g body weight, n = 9), and 3) immobilized and treated with Radix astragali (IM+AR; 193.9 ± 8.0 g body weight, n = 9). All animal experiments were conducted in compliance with the ethical requirements of the Animal Committee at Juntendo University (#15-10).

**Hindlimb immobilization.** Hindlimb immobilization of the rats was performed according to previously described procedures (Okamoto et al. 2011). Both the right and left hindlimbs of the animals were fixed in a shortened position with casting tape. Immobilization was imposed for 0 (as the control) or 14 days.

**Decoction and administration of herbal medicine.** The decoction was made by boiling 80 g of Radix astragali (chopped; Uchidawakanyaku, Tokyo, Japan) in 200 mL water over low heat for 30 min, followed by filtering. The concentration of the decoction corresponded to 8-times the typical dose in human adults. The decoction was administered to rats in the IM+AR group, and water was administered to rats in the CON and IM groups for 21 days beginning 7 days before casting to the end of plaster fixation. For administration, a disposable feeding needle (needle length: 85 mm; Fuchigami Kikai, Kyoto, Japan) was used, and 1.5 mL was administered directly into the stomach of rats by oral gavage after fasting for 12 h.

**Muscle sampling.** At the end of the cast immobilization, hindlimb skeletal muscles (soleus and plantaris) from both legs were carefully dissected, weighed, and frozen in liquid nitrogen for biochemical analysis or in isopentane cooled by liquid nitrogen for immunohistochemical analysis. Muscle samples were stored at −80°C until analysis.
Immunohistochemistry. Cross-sectional area (CSA) analysis of individual muscle fibers was carried out using the midbelly region of the soleus and plantaris muscles. Frozen cross-sections (10 μm) were immunostained using mouse monoclonal antibodies against MHC types I, Ila, and Iib. Briefly, muscle sections were fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS) and permeabilized with 0.5% Triton X-100/PBS. The sections were blocked in 5% normal goat serum (NGS)/PBS at room temperature and then incubated with primary antibodies at 4°C for 2 h. The primary antibodies, *i.e.*, anti-MHC type I antibodies (clone BA-F8), anti-MHC type Ila antibodies (clone SC-71), anti-MHC type Iib antibodies (clone BF-3; Developmental Studies Hybridoma Bank, Iowa, IA, USA), and anti-laminin antibodies (Sigma Aldrich, St. Louis, MO, USA), were diluted 1:300 in 5% NGS/PBS. After washing with PBS, the sections were incubated for 1 h at 4°C with secondary antibodies (goat anti-mouse IgG [Invitrogen, Carlsbad, CA, USA] for BA-F8 and SC-71, goat antimouse IgM [Invitrogen] for BF-3, and goat anti-rabbit IgG [Invitrogen] for laminin). The secondary antibodies were diluted 1:1000 in 5% NGS/PBS. Finally, sections were cover-slipped. Images of the stained sections were captured from a light microscope (ECLIPSE Ti; Nikon). CSA of approximately 150 fibers/muscle section was calculated using image analysis software (NIS-ElementsD; Nikon). For analysis of muscle fiber composition, the number of muscle fibers was counted for each muscle fiber type from a total of approximately 500 fibers.

Real-time reverse transcription polymerase chain reaction (RT-qPCR). Total RNA was isolated from soleus and plantaris muscle tissues using TRIzol Reagent (Invitrogen Japan, Tokyo, Japan) following the manufacturer’s protocol. Each pair of muscles was homogenized in 1 mL TRIzol reagent with a handheld homogenizer. Next, 200 μL chloroform was added to the homogenate, which was shaken vigorously for 15 s and then kept at room temperature for 2–3 min. The mixture was then centrifuged at 14,000 × g for 15 min at 4°C, and the aqueous phase (approximately 0.5 mL) was transferred to a fresh tube. Next, 2 μL glycogen (Nacalai Tesque, Kyoto, Japan) was added to improve RNA precipitation. Isopropanol (500 μL) was added to this aqueous solution to precipitate the RNA. After standing at room temperature for 10 min, the tube was centrifuged at 20,000 × g for 5 min at 4°C. The RNA pellet was air-dried for 5 min, and the RNA was dissolved in RNase-free water. RNA extracts were treated with DNase (Invitrogen Japan) to remove any residual genomic DNA. The purity of the RNA was determined by calculating the absorbance ratio at 260 and 280 nm. RNA concentrations were determined by measuring absorbance at 260 nm. For each sample, cDNA was synthesized from 1 μg total RNA using components from a SuperScript VILO Master Mix (Invitrogen Japan). RT was performed at 25°C for 10 min, 37°C for 120 min, and 85°C for 5 s.

Real-time PCR was performed to analyze the mRNA expression of *MAFbx/atrogen-1, MuRF1*, calpain, and caspase-3 using an Applied Biosystems 7300 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). PCR was performed in a total volume of 20 μL, containing 10 μL TaqMan Fast Universal PCR Master Mix (Applied Biosystems), 1 μL primer and probe mix, and 9 μL cDNA. PCR analyses were carried out using the following cycle parameters: one cycle at 95°C for 10 min, 40 cycles of 95°C for 15 s, and a final cycle of 60°C for 1 min.

The results were expressed using the comparative cycle threshold (Ct) method, as described in User Bulletin No. 2 (Applied Biosystems). The Ct or threshold cycle represents the polymerase PCR cycle at which an increase in reporter fluorescence above a baseline signal can initially be detected. The ΔCt values were calculated for each gene of interest as follows: Ct (gene of interest) – Ct (internal control gene). The housekeeping gene 18S rRNA was used as the internal control gene. Relative changes in the expression levels of one specific gene (ΔΔCt) were calculated by subtracting the ΔCt of the CON group (used as the calibrator) from that of the IM group or IM+A group. Quantification of mRNA expression was performed using the comparative Ct method and normalized by 18s rRNA expression. The expression of mRNA was determined by arbitrarily setting the value of the CON group to 1. The primer and probe sets for *MAFbx/atrogen-1* (Rn00591730) and *MuRF1* (Rn00590197) were purchased from Applied Biosystems.

Statistics. The data are shown as means ± standard deviation (SD). Muscle weight, CSA of muscle fibers, composition ratio of muscle fiber type, and expression levels of muscle atrophy-related genes were assessed by one-way analysis of variance with Tukey’s post-hoc tests. Results with *P* values of less than 0.05 were considered significant.
RESULTS

Muscle weights
Two weeks after hindlimb immobilization, rats in the IM group showed significant decreases ($P < 0.05$) in soleus and plantaris muscle-to-body weight ratios by 25.7% and 29.5%, respectively, compared with those in the CON group. In contrast, rats in the IM+AR group, administered *Radix astragali*, exhibited mild decreases in soleus, and plantaris muscle-to-body weight ratios of 16.6%, and 27.1% compared with those in the CON group; that is, IM+AR treatment significantly reversed (+35.5%) the weight reduction in the soleus muscle compared with that in the IM group ($P < 0.05$). The reversal rate for the weight reduction in the plantaris muscle was 8.1%, although this difference was not significant (Fig. 1).

Muscle fiber CSA
The CSAs of soleus muscle are shown in Fig. 2. In the IM group, there was a significant decrease ($P < 0.05$) in all muscle fiber CSA by 37.2% compared with that in the CON group (Fig. 3A). This decrease was reversed by 12.3% in the IM+AR group, although this difference was not significant. CSAs of type I and IIa muscle fibers of the soleus muscle in the IM group were significantly reduced ($P < 0.05$) by 36.3% and 33.7%, respectively, compared with those in the CON group (Fig.3B, C). These decreases were reversed by 12.3% and 11.8% in the IM+AR group, although no significant differences were observed.

The CSAs of plantaris muscle are shown in Fig. 4. Rats in the IM and IM+AR groups showed a significant decrease ($P < 0.05$) in all muscle fiber CSA by 51.2% and 54.4%, respectively, compared with that in the CON group (Fig. 5A). In the IM group, CSAs of type I, IIa, and IIb+IId/x muscle fibers of the plantaris muscles were significantly reduced ($P < 0.05$) by 52.8%, 46.3%, and 52.9%, respectively, compared with those in the CON group. These decreases were reversed by 18.5%, 34.2%, and 11.5% in the IM+AR group, although no significant differences were observed between the IM group and the IM+AR group (Fig. 5B, C, D).

Muscle fiber composition
In the soleus muscles of the CON group, the proportion occupied by type I muscle fibers was 90.3%, which decreased ($P < 0.05$) to 81.3% in the IM group (Table I). Thus, a shift in muscle fiber composition toward fast muscle fibers was observed in the IM group, although the shift was not observed in the IM+AR group. In the plantaris muscle, the composi-
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Expression of MAFbx/atrogin-1, and MuRF1 mRNA

As shown in Fig. 6, the expression level of MAFbx/atrogin-1 mRNA in the soleus muscle was about 2.9-fold higher ($P < 0.05$) in the IM group than in the CON group. In the IM+AR group, the expression of this gene was approximately 2.3-fold higher than that in the CON group, indicating a tendency for suppression of the effects of immobilization; no significant difference was observed between the IM and IM+AR groups (Fig. 6A). The expression level of MuRF1 mRNA in the IM group was about 1.8-

Fig. 2  Cross-sectional areas and myosin heavy chain (MHC) labeling of soleus muscles. (A) CON group, (B) IM group, (C) IM+AR group. Cryostat sections were immunolabeled with several specific anti-MHC antibodies. The reactivities for major fiber types, including type I (red) and type IIA (green), and laminin (pink) are shown for soleus muscles. Scale bar: 100 μm.

Fig. 3  Effects of immobilization on cross-sectional areas in the soleus muscle. (A) Total fiber type, (B) fiber type I, (C) fiber type IIA. Bars represent means ± standard deviations ($n = 9$/group). $^*P < 0.05$ versus the CON group.
fold higher ($P < 0.05$) than that in the CON group in the soleus muscle, but was approximately 1.3-fold higher in the IM+AR group; this value was significantly lower than that in the IM group ($P < 0.05$; Fig. 6B).

In the plantaris muscle, the expression level of MAFbx/atrogen-1 mRNA in the IM group was about 6.1-fold higher ($P < 0.05$) than that in the CON group, and this increase was not significantly reversed by IM+AR treatment (Fig. 6C). The expression level of MuRF1 mRNA in the IM group was about 3.0-fold higher ($P < 0.05$) than that in the CON group in plantaris muscle, whereas that in the IM+AR group was about 2.8-fold higher ($P < 0.05$) than that in the CON group; the difference between the IM and IM+AR groups was not significant (Fig. 6D).

**Expression of calpain mRNA**

The expression level of calpain mRNA in the IM group was about 2.5-folds higher ($P < 0.05$) than that in the CON group in the soleus muscle, and this increase was not significantly reversed in the IM+AR group (Fig. 7A). In contrast, the expression of calpain mRNA did not change in the IM and IM+AR groups compared with that in the CON group in the plantaris muscle (Fig. 7C).

**Expression of caspase-3 mRNA**

The expression level of caspase-3 mRNA in the soleus muscle was about 4.0-fold higher ($P < 0.05$) in the IM group than in the CON group, and this increase was not significantly reversed in the IM+AR group (Fig. 7B). In the plantaris muscle, the expression level of caspase-3 mRNA in the IM group was approximately 3.0-fold higher ($P < 0.05$) than that in the CON group, and this increase was not significantly reversed in the IM+AR group (Fig. 7D).

**DISCUSSION**

In this study, we aimed to investigate whether administration of *Radix astragali* prevented atrophy in both slow- and fast-twitch muscles following cast immobilization. Our hypothesis was that *Radix astragali* would similarly suppress atrophy of slow- and fast-twitch muscles during cast immobilization. Our results demonstrated that administration of *Radix astragali* suppressed the immobilization-induced reductions in skeletal muscle mass and the elevated expression of MuRF1 in slow-twitch soleus muscles, but not in fast-twitch plantaris muscles.

Cast immobilization leads to atrophy of both
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During cast immobilization. Thus, we concluded that this cast immobilization model was effective for investigating the effects of administration of Radix astragali on slow- and fast-twitch muscle atrophy.

In a previous study evaluating slow-twitch muscles, Zhang et al. (2007) demonstrated that the administration of Radix astragali increased the soleus muscle-to-body weight ratio by 33% compared with hindlimb unloading plus intragastric water instillation (vehicle control). The experimental results in the present study also indicated that Radix astragali inhibited the reduction in soleus muscle-to-body

Table 1  Effect of immobilization on muscle fiber type distribution in soleus and plantaris muscle

|          | Soleus        | Plantaris     |
|----------|---------------|---------------|
|          | TypeⅠ (%)     | TypeⅠ (%)     | TypeⅠ (%)     | TypeⅠ (%)     | TypeⅠ (%)     |
| CON      | 90.3 ± 6.8    | 9.7 ± 6.8     | 7.3 ± 2.9     | 30.3 ± 6.1    | 62.4 ± 8.1    |
| IM       | 81.3 ± 7.8*   | 18.7 ± 7.8*   | 8.6 ± 1.5     | 33.9 ± 5.0    | 57.5 ± 5.5    |
| IM+AR    | 83.5 ± 4.4    | 16.5 ± 4.4    | 6.4 ± 4.3     | 31.2 ± 10.2   | 62.4 ± 14.0   |

Values are means ± SD. *P < 0.05 vs CON.

Fig. 5  Effects of immobilization on cross-sectional areas in the plantaris muscle. (A) Total fiber type, (B) fiber type I, (C) fiber type IIA, (D) fiber type IIB+IId/x. Bars represent means ± standard deviations (n = 9/group). *P < 0.05 versus the CON group.

slow- and fast-twitch muscles (Machida and Booth 2005), similar to long-term bed rest-induced muscle atrophy (Salanova et al. 2008). Cast immobilization is thought to be an ideal animal model for investigation of physical inactivity-induced slow- and fast-twitch muscle atrophy. In a previous study, Okamoto et al. (2011) indicated that soleus and plantaris muscle masses of mice were decreased by 31.2% and 28.3%, respectively, after cast immobilization for 7 days. In the present study, soleus and plantaris muscle-to-body weight ratios were reduced by 25.7% and 51.2%, during cast immobilization. Thus, we concluded that this cast immobilization model was effective for investigating the effects of administration of Radix astragali on slow- and fast-twitch muscle atrophy.

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Fig. 6 Effects of immobilization on E3 ubiquitin ligase mRNA expression in soleus and plantaris muscles. (A, C) MAFbx/atrogin-1 mRNA expression. (B, D) MuRF1 mRNA expression. Bars represent means ± standard deviations (n = 9/group). *P < 0.05 versus the CON group, †P < 0.05 versus the IM group.

Fig. 7 Effects of immobilization on calpain and caspase-3 mRNA expression in soleus and plantaris muscles. (A, C) Calpain mRNA expression. (B, D) Caspase-3 mRNA expression. Bars represent means ± standard deviations (n = 9/group). *P < 0.05 versus the CON group.
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weight ratio by 35.5% compared with cast immobilization. In fast-twitch muscles, Zhou and Mei (2014) reported that Radix astragali reversed the reduction in tibial muscle-to-body weight ratio compared with denervated control. In our study, administration of Radix astragali reversed the reduction of plantaris muscle muscle-to-body weight ratio by 8.1% compared with cast immobilization, although this result was not significantly different. Our findings suggested that administration of Radix astragali exhibited differential protective effects on cast immobilization-induced atrophy of slow- and fast-twitch muscles.

Administration of Radix astragali in the present study reversed the reduction of soleus muscle-to-body weight ratio compared with cast immobilization. We also observed decreased CSAs in soleus muscle during cast immobilization. Unexpectedly, administration of Radix astragali did not suppress the decreases in CSAs of soleus muscle during cast immobilization. The CSAs of type I and type IIa soleus muscle fibers were not reversed by administration of Radix astragali, suggesting that administration of Radix astragali reversed the reduction of soleus muscle-to-body weight ratios without inhibiting the reduction in the CSA of soleus muscle. Shah et al. (2001) indicated that fiber CSAs and lengths were both decreased by cast immobilization; they observed a significant 26% reduction in sarcomere number and 25% decrease in CSA in soleus muscle. In the present study, administration of Radix astragali inhibited the reduction in soleus muscle-to-body weight ratio, but did not improve the CSAs of soleus muscles. Inhibition of the reduction in soleus muscle-to-body weight ratio may be associated with the regulation of fiber length and sarcomere number. Furthermore, muscle fiber type showed a shift from slow to fast in soleus muscles following cast immobilization, but did not shift significantly in plantaris muscles following cast immobilization (Table 1). Administration of Radix astragali did not significantly alter muscle fiber type distribution in soleus muscles compared with that in the CON group. Similarly, in a previous report, Gao et al. (2005) indicated that administration of Radix astragali reduced the percentage of type II fibers in the soleus muscles in tail-suspended rats.

Administration of Radix astragali has been shown to suppress soleus muscle atrophy in a hindlimb unloading model (Zhang et al. 2007); however, the mechanism remains unclear. The effects of cast immobilization are not related to suppression of protein synthesis, e.g., involving the mammalian target of rapamycin/S6K/4E binding protein-1 pathway, but were related to muscle-specific E3 ligases, e.g., MAFbx/atrogin-1 and MuRF1 (Krawiec et al. 2005). Thus, we evaluated the effects of Radix astragali administration on MAFbx/atrogin-1 and MuRF1. Our results indicated that administration of Radix astragali suppressed cast immobilization-induced MuRF1 mRNA expression in soleus muscles, but did not affect MAFbx/atrogin-1 expression. MAFbx/atrogin-1 targets eukaryotic initiation factor 3 subunit 5 (eIF3f) which regulates protein synthesis and skeletal muscle mass (Lagirand-Cantaloube et al. 2008), whereas MuRF1 targets myofibrillar proteins, such as myosin light chains 1 and 2 and actin (Cohen et al. 2009; Polge et al. 2011). MuRF1 contributes to degradation of myofibrillar proteins. Moreover, cast immobilization-induced muscle atrophy was attributed to increased protein degradation, and not suppression of protein synthesis (Krawiec et al. 2005). Taken together, previous studies suggested that MuRF1 had a greater effect than MAFbx/atrogin-1 on cast immobilization-induced muscle atrophy. Our experimental results also suggested that the inhibitory effects of Radix astragali on immobilization-induced muscle atrophy in the soleus muscle may be related to MuRF1 expression.

Previous studies demonstrated that Radix astragali alleviated atrophy of fast-twitch tibial muscles in a denervation model (Zhou and Mei 2014) and that the polysaccharide composition of Radix astragali decreased atrophy of fast-twitch tibial muscles in renal failure model (Lu et al. 2016). However, the experimental data in the present study indicated that Radix astragali did not improve cast immobilization-induced atrophy of plantaris muscle. The tibial muscle fiber CSA was decreased by 29.9% in a denervation model (Peretti et al. 2017), and was reduced by approximately 27.2% in a renal failure model (Lu et al. 2016). It is worthy to note that the reduction of plantaris muscle fiber CSA in cast immobilization was 51.2% in our study. Thus, we assumed that the effect of Radix astragali may be not be sufficient for alleviating plantaris atrophy by cast immobilization. On the other hand, Radix astragali has antioxidative effect (Gong et al. 2018). Previous studies reported that antioxidants attenuate inactivity-induced soleus muscle atrophy via their anti-oxidative effect (Servais et al. 2007; Yoshihara et al. 2017) and that antioxidants alleviate soleus muscle atrophy more effectively than plantaris atrophy (Min et al. 2011). Thus, Radix astragali may have differential protective effects on cast immobilization-induced atrophy of slow-twitch and fast-twitch muscles.
In summary, our findings demonstrated that administration of *Radix astragali* suppressed the reductions in skeletal muscle mass and cast immobilization-induced MuRF1 expression in slow-twitch soleus muscles, but not fast-twitch plantaris muscles. *Radix astragali* could be an effective therapy candidate to prevent cast immobilization-induced slow-twitch muscle atrophy.

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