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ORIGINAL ARTICLE

Citri Unshius Pericarpium Improves Dexamethasone-Induced Muscle Atrophy in Mice

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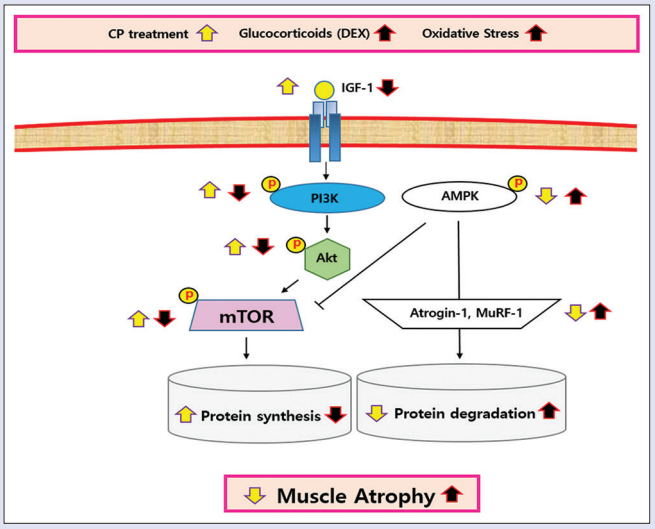
ABSTRACT

Background: Muscle atrophy means a progressive decrease in muscle mass, strength, and quality and has a lot of discomfort in daily life. High-dose or continuous use of glucocorticoids (GCs), which are negative muscle regulators, led to the risk of muscle atrophy and weakness. Up to now, the effect and the underlying mechanism of Citri unshius Pericarpium (CP) on muscle atrophy have not been fully elucidated. **Objectives:** Accordingly, the current study was performed to evaluate the effects and the underlying mechanisms of CP on dexamethasone (DEX)-provoked muscle atrophy in C57BL/6 mice. **Materials and Methods:** The 8-week-old mice were treated once a day (DEX 20 mg/kg body weight, i.p.), and CP was administrated orally for 10 days. Then, we measured the body weight, swimming time, and muscle weight, and histological evaluation and western blot were performed. **Results:** CP improved muscle function decline by bettering the swimming time and muscle weight to some extent. Moreover, histological muscle damage induced by DEX was enhanced through CP treatment. CP treatment induced the reduction of ROS-related factors. CP showed a decrease in the protein expressions such as myostatin, Atrogin-1, and MuRF1 via the down-regulation of the phosphorylation of AMPK. Besides, in the CP group, muscle protein synthesis was increased by the PI3K/Akt/mTOR signaling pathway. **Conclusion:** Taken together, CP could be highly commercialized as a commercial material for functional food for the prevention and improvement of muscle loss, which is induced by muscle atrophy.

Key words: AMPK, Citri unshius Pericarpium, dexamethasone, muscle atrophy, PI3K

SUMMARY

- The current study evaluated the effect of CP on dexamethasone-induced muscle atrophy. CP treatment also showed the reduction of the protein expressions such as Atrogin-1, MuRF1, and myostatin via the down-regulation of the AMPK activation. Meanwhile, in the CP-treated group, muscle protein synthesis was elevated by the activation of the PI3K/Akt/mTOR signaling pathway. We conclude that CP could reduce muscle loss, which is induced by muscle atrophy.



Abbreviations used: GCs: glucocorticoids; DEX: dexamethasone; CP: Citri unshius Pericarpium; AMPK: AMP-activated protein kinase; MuRF1: Muscle RING-finger protein-1; PI3K: phosphatidylinositol 4,5-bisphosphate; mTOR: Mechanistic target of rapamycin

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INTRODUCTION

The recent continuous increase in the elderly population possesses various medical problems.

Above all, muscle fibre size and skeletal muscle mass show different aspects according to various physiological and pathological factors. Herein, muscle atrophy usually occurs in situations such as when protein degradation rates exceed clearly protein synthesis rates. Moreover, this will be primarily provoked in the skeletal muscle in various conditions, including ageing, starvation, heart failure, cancer cachexia, and denervation.[1] Ultimately, it is being noted as the main cause of excessive medical and social costs.[2] Besides, it can be accelerated by a variety of agents including changes in poor nutrition, hormonal change, fatty infiltration, inactivity, chronic illness, and function loss in the central and peripheral nervous systems.[3,4]

Glucocorticoids (glucocorticoid steroids), which have been used in diseases such as auto-immune diseases, cancer, and allergic reactions for decades, are highly effective immuno-suppressant, anti-inflammatory, and decongestant agents.[5] Glucocorticoid steroids stimulate protein breakdown for the production of amino acids, and the amino acids produced at this time are utilized in hepatic gluconeogenesis (glucose synthesis) and the breakdown

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of glycogen (glycogenolysis).[6] Dexamethasone (DEX), which is developed as a strong synthetic glucocorticoid compound, has been well used clinically with regard to efficacy such as analgesic and immunomodulatory effects.[7] However, inappropriate and excessive usage can lead to systemic influence that provokes remodeling of adipose depots, muscle wasting, and bone remodeling.[8] Herein, the reduction in muscle mass by partial or complete muscle wasting can be defined as muscle atrophy. Muscle atrophy is ultimately characterized by a reduction in muscle protein content and strength.[9]

Citri unshius Pericarpium (CP, the dried and matured pericarps of *Citrus unshiu* Markovich) is usually applied for medicinal purposes to obtain anti-inflammatory properties, inhibit oxidative stress, improve blood circulation and bronchial, and treat dyspepsia and vomiting.[10-12] Besides, CP has been reported to exert anti-allergic and anti-bacterial activities and enhance the aging-induced demyelination diseases.[13] However, studies on the effect of CP on muscle atrophy induced by dexamethasone and its fundamental mechanism have not been fully explained.

In the present study, as mentioned above, C57BL/6 mice were injected with dexamethasone (DEX) (i.p.) to induce muscle atrophy *in vivo*. Our data suggested new knowledge about the effects of CP, both inhibition of protein degradation via the down-regulation of AMPK activation and the increase of protein synthesis by the activation of the PI3K/Akt/mTOR signaling pathway on muscle atrophy.

MATERIALS AND METHODS

Materials

Dexamethasone (Cat No, D2915), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Cat No. D9132), gallic acid (Cat No. G7384), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, Cat No. A1888), and potassium persulfate (216224) were provided by Sigma Aldrich Co. (St Louis, MO, USA). All antibodies were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA or Signaling Technology, Inc. (Danvers, MA, USA). PI3K (SC-365290), Atrogin-1 (SC-166806), MURF1 (SC-398608), NOX2 (SC-130543), p47phox (SC-17845), Rac1 (SC-217), mTOR (SC-517464), p-mTOR (SC-293133), S6K (SC-8418), and p-S6K (SC-8416) were also obtained. Myostatin was obtained from Abcam (Cambridge, USA). p-PI3K (#4228), Akt (#9272), and p-Akt (#9275) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, United States). Secondary antibodies were provided by GeneTex, Inc. (Irvine, LA, USA). The primary antibodies were equally diluted at 1:1000. However, the secondary antibodies were diluted at 1:5000.

Preparation of the plant material

CP was purchased from Bonchowon (Yeongcheon-si, Korea). CP (100 g) was extracted with distilled water (DW, 1 L) at 25°C for 2 hr. The powder of CP was a yield of 48% (extract powder g/raw material g) and stored at -80°C before use.

Analysis of narirutin and hesperidin

CP power (0.5 mg) was dissolved in 1.0 mL of 100% methanol. The solution was centrifuged at 13,500 rpm for 3 min, and the supernatant was collected for component analysis. We injected 1 µL of the sample into a reverse-phase ultra-high-performance liquid chromatography (UPLC) system. UPLC was performed with a Waters Acquity UPLC system (Waters®, Milford, MA, USA), and the chromatography system was used Phenomenex C_{18} (2.1 × 100 mm, particle size 2.6 µm, pore size 100Å). Mobile phase component A = 0.1% formic acid (aq.) and B = 100% acetonitrile with 0.1% formic acid. The gradient conditions were as follows: 0 min, 18% B; 1 min, 18% B; 15 min, 25% B; 20 min, 100% B; 25 min, 100% B; 26 min, 18% B; 32 min, 18% B. The flow rate was 0.2 mL/min, and the UV absorbance from 284 nm was monitored. The standard materials used were narirutin (5.0 µg/mL) and hesperidin (5.0 µg/mL). The peaks of standard materials were assigned by comparison of retention times (narirutin: 6.2 min, hesperidin: 8.0 min). The quantification of two components (narirutin and hesperidin) was confirmed by peak area measurements [Figure 1].

DPPH free and ABTS radical scavenging activity

The DPPH radical scavenging activity of CP was determined according to the Blois method.[14] 100 µL of 60 µM DPPH solution and 100 µL of the sample were mixed well and reacted for 30 min. After that, the absorbance was measured at 540 nm.

The ABTS radical scavenging activity of CP was measured as follows: [15] First, ABTS (7 mM) and potassium persulfate (2.45 mM) were mixed well to prepare the ABTS + solution. The ABTS + solution (95 µL of the prepared $ABTS^+$) solution and 5 µL of the sample were mixed well and allowed to react for 15 min. The absorbance was then measured at 415 nm.

The DPPH free radical and ABTS radical scavenging abilities are 50%, which are expressed as the IC_{50} value:

Radical scavenging activity (%)

= [1-(Sample OD ÷ Blank OD)] × 100

Experimental animals and induction of muscle atrophy

The experiment was approved by the Ethics Committee of the Daegu Haany University (No. DHU2021-088). Male C57BL/6 mice (8 weeks, 21–24 g) were purchased from DBL (Eumseong, Korea). After 1 week of adaptation (temperature, 24 ± 2°C; light/dark cycle, 12 hr; humidity, 50 ± 5%), the mice were divided into 4 groups (n = 7): Normal, control, CPL, and CPH. Mice in the normal group received saline by intraperitoneal (i.p.) injection and DW by oral gavage; the control group received DEX and DW; the CPL group received DEX and CP at 100 mg/kg/day; the CPH group received DEX and CP at 200 mg/kg/day. Muscular atrophy was induced by i.p. injection of DEX once a day for 10 days.[16] CP (100 or 200 mg/kg/day) was administrated orally for continuous 10 days. DW and CP were administered orally 1 hr 30 min prior to DEX treatment by oral gavage.

Forced swimming test

All mice except for the normal group were subjected to the forced swimming test 20 min after the last administration. The test was performed as previously described, with some modification.[16] In brief, the mice were placed in an acrylic plastic pool (120 x 70 x 50 cm) filled with fresh water (23 ± 1°C) to a depth of 25 cm. Exhaustion was defined as loss of coordinated movements and failure to rise to the surface within 5 s. The time before exhaustion was judged as swimming time.

Measurement of AST and ALT levels

Blood was centrifuged at 4,000 rpm for 10 min (at 4°C). The levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were measured with a Transaminase CII-Test (Wako Pure Chemical Industries Ltd., Osaka, Japan) according to the manufacturer's instructions.

Western blotting

For cytosol samples, Gast muscles were lysed with buffer A consisting of protease inhibitor solution (Wako), 0.1 mM EDTA, 10 mM HEPES (pH 7.8), 0.1 mM PMSF, 10 mM KCl, 1 mM DTT, and 2 mM $MgCl_2$.

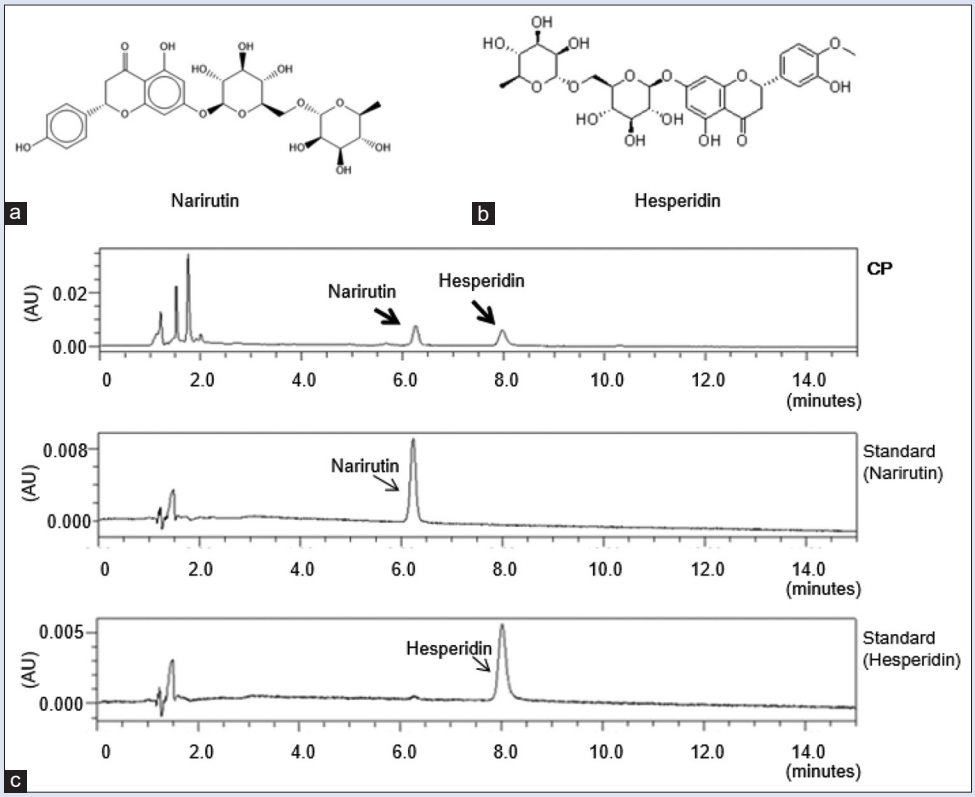


Figure 1: Analysis of narirutin and hesperidin in the extract of CP at 284 nm. (a) Chemical structure of narirutin; (b) Chemical structure of hesperidin; (c) UPLC chromatograms of CP and standard materials. CP, Citri unshius Pericarpium

12 µg of cytosol samples was electrophoresed through 8–15% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. The bands were visualized using ECL reagents of GE Healthcare (Chicago, IL, USA) and detected using a Sensi-Q 2000 Chemidoc (Lugen Sci Co., Ltd., Gyeonggi-do, Bucheon-si, Korea). We applied the methods of Mi-Rae Shin *et al.* (2021).[17]

Histological examination

Gast muscles samples were stained with hematoxylin and eosin (H&E). The muscles were fixed in 10% buffered formalin and then embedded in paraffin. These were sectioned with 3 µm thickness for the histological visualization. Each slide was observed and analyzed using the iSolution Lite (IMTechnology, Vancouver, Canada).

Statistical analysis

Experimental data are expressed as mean ± SEM. The significance of each data was indicated by assessment by the least significant difference (LSD) test of one-way analysis of variance (ANOVA) using SPSS 26 software (Chicago, IL, USA). The statistical significance (p values) was defined as less than 0.05.

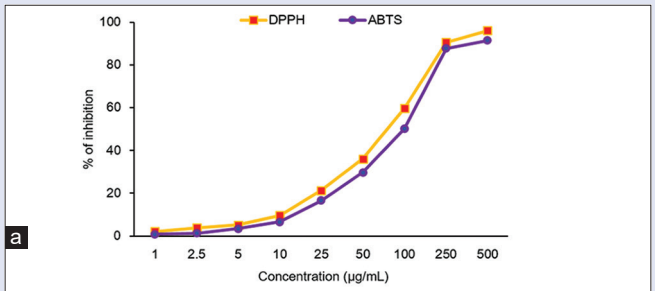
RESULTS

Phytochemical analysis of CP

The amounts of narirutin and hesperidin were analyzed using the UPLC chromatogram [Figure 1]. The amounts of the two components were analyzed to be 12.2 mg/g narirutin and 10.0 mg/g hesperidin.

Antioxidant activity of CP

The antioxidant activities of CP were evaluated based on the scavenging of DPPH free radicals and ABTS radicals [Figure 2]. DPPH IC_{50} was



Sample name	Total Polyphenol (GAE/g)	Total Flavonoid (QE/g)
CP	33.53 ± 0.02	7.22 ± 0.56

b

Figure 2: (a) DPPH and ABTS radical scavenging activities of CP. (b) Total polyphenolic and flavonoid contents. Each experiment was run in triplicate. CP, Citri unshius Pericarpium

79.48 ± 0.22 µg/mL, and ABTS IC_{50} was 99.56 ± 0.04 µg/mL. Moreover, total phenolic and flavonoid contents were also determined as other antioxidant agents. The total phenolic content was 33.53 ± 0.02 mg gallic acid equivalents (GAE)/g of CP extract. The flavonoid content was 7.22 ± 0.56 mg quercetin equivalent (QE)/g of CP extract. Accordingly, these potent anti-oxidant activities showed that CP could effectively prevent oxidative stress induced by muscle atrophy.

CP improved DEX-induced physiological and pathological changes

We first measured the effects of CP on the body weight and muscle weight of mice. A significant difference in body weight changes was

exhibited in the DEX control group ($p < 0.001$). However, CP treatment showed significant differences in the DEX control group (CPL, $P < 0.05$; CPH, $P < 0.01$). To investigate the effect of CP on liver dysfunction, serum liver function biomarkers such as AST and ALT were detected. The results are shown in Figure 3a. DEX-treated mice led to significant increases in both AST and ALT activities. Their levels in the DEX control group significantly increased by approximately 3.1-fold (AST) and 7.8-fold (ALT). However, CP administration dramatically decreased these levels ($p < 0.001$). Especially, the effects of AST and ALT reduction after CPH treatment were superior to those with CPL. In particular, a significant decrease in muscle weights was detected in the DEX control group (Quad, Gast, and EDL + TA, $P < 0.001$; Sol, $P < 0.01$), but the CPH-treated group effectively alleviated such a decrease [Figure 3b-3c]. To confirm the effect of CP on muscle damage, the Gast sections were evaluated by H and E. Representative images are shown in Figure 3d. Muscle fibers in the normal group were in close contact with muscle bundles. However, treatment with DEX led to an increase in perimysium and endomysium. Ultimately, these mean an increase of connective tissues surrounding the muscle fibers and bundles. These muscle damages were recovered by the administration of CP.

CP alleviated muscle dysfunction in DEX-induced muscle atrophy

The swimming time was measured as the exercise capacity for the muscle function. The swimming time was significantly decreased in the DEX control group compared with the normal group. However, after 10 days of CPH administration, the swimming time was significantly elevated compared with the DEX control group [Figure 4].

CP effectively regulated oxidative stress induced by NADPH oxidases

Many previous studies have demonstrated a close connection between NADPH oxidase (NOX) enzymes and oxidative stress. NOX enzymes produce an excessive amount of superoxide and ROS. As shown in Figure 5, NOX sub-units such as NOX2, p47phox, and Rac1 significantly increased in the DEX control group, whereas CP treatment drastically decreased such an increase. Herein, the levels of these factors by CP treatment decreased to the levels of the normal level. These results suggested that CP improved DEX-induced muscle atrophy by inhibiting ROS over-expression.

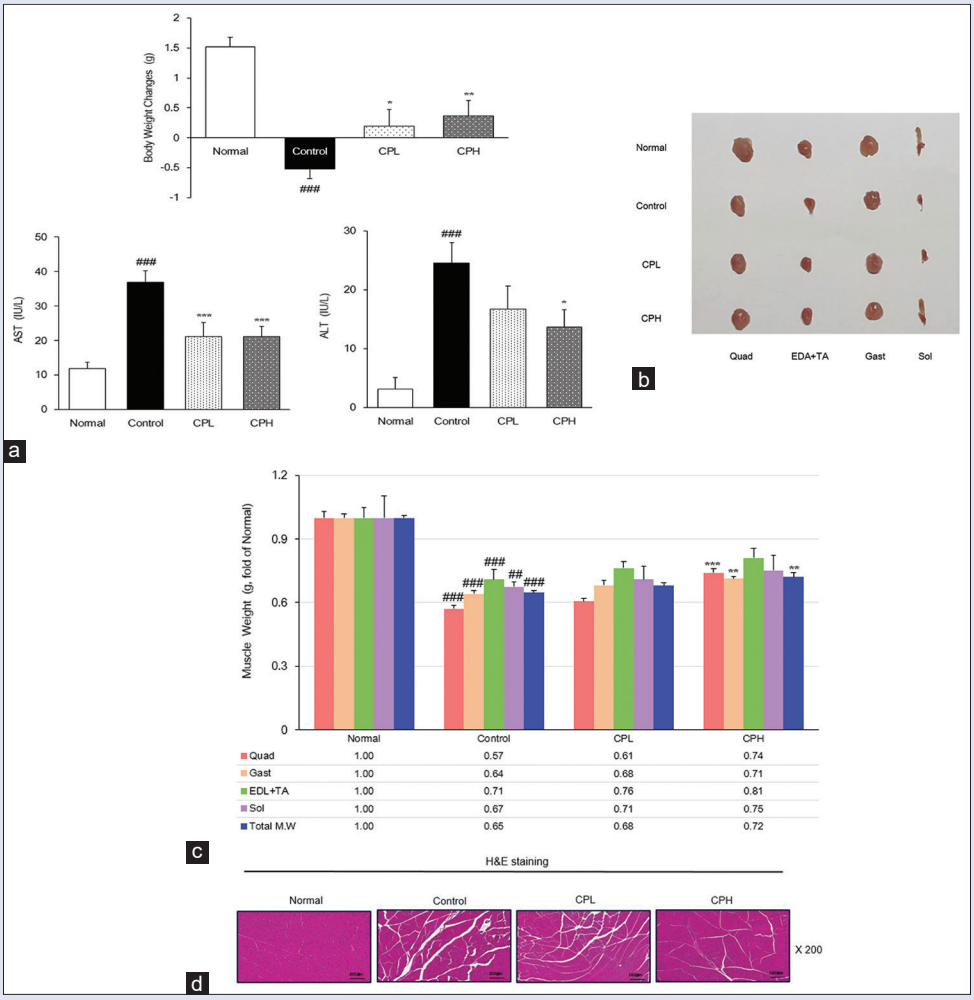


Figure 3: Inhibitory effects of CP on DEX-induced muscle atrophy. Mice were injected once a day with DEX for 10 days. CP (100 and 200 mg/kg daily) was orally administrated for 10 days. (a) Body weight changes, effect of CP on body weight changes, AST level in serum, ALT level in serum; (b) Representative gross appearance in muscles; (c) Muscle weight (g, fold of normal); (d) Representative macroscopical appearance in muscles. The Gast was stained with H and E. The scale bars represent 200 μm (magnification, ×200). Mean ± SEM (n = 7). Significance: $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. the normal group, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. the DEX control group. Quad, quadriceps; Gast, gastrocnemius; EDL, extensor digitorum longus; TA, tibialis anterior; Sol, soleus

CP negatively regulated the proteolysis-related pathway

Next, we confirmed the levels of proteins that are involved in muscle protein catabolic pathways. As shown in Figure 6, the data showed DEX treatment led to increased phosphorylation of AMPK, whereas CP treatment reversed such an increase. In addition, DEX treatment significantly up-regulated the protein expressions such as myostatin, Atrogin-1, and MuRF1. However, the protein expressions of myostatin, which is a negative regulator of muscle growth, and both Atrogin-1, and MuRF1, which are fundamental ubiquitin ligases involved in muscle protein degradation, were dramatically reduced by CP treatment. These results suggest that CP inhibits the activation of AMPK, thereby improving the growth inhibition of muscle protein and reducing the degradation of muscle protein.

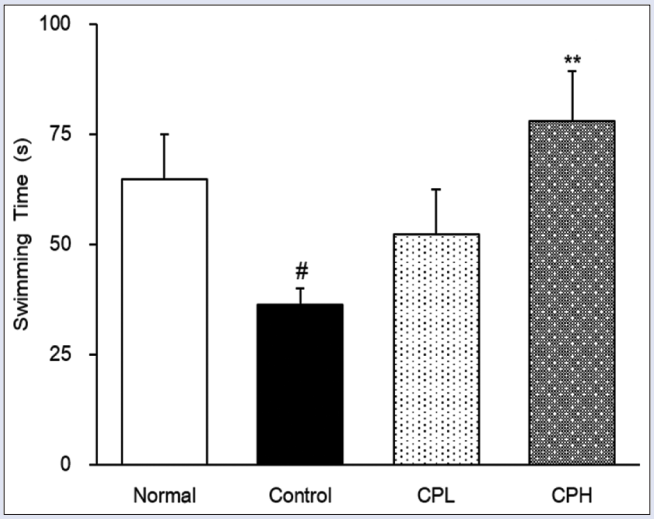


Figure 4: Improvement effect of swimming time by CP treatment on DEX-induced muscle atrophy. Mice were injected once a day with DEX for 10 days. CP (100 and 200 mg/kg daily) was orally administrated for 10 days. Mean ± SEM (n = 7). Significance: $^{\#}p < 0.05$ vs. the normal group and $^{**}p < 0.01$ vs. the DEX control group

CP supplementation increased the protein synthesis-related pathway

To explore whether CP controls protein synthesis, we examined the effect of CP on IGF-1 and PI3K/Akt/mTOR signaling, a key regulator of the muscle protein anabolic pathway. As shown in Figure 7, IGF and its down-stream factors including p-PI3K, p-Akt, and p-mTOR of the DEX control group were considerably decreased in response to DEX-induced muscle atrophy; however, the administration with CPH increased these proteins as compared with the DEX control group ($p < 0.05$ or 0.01). These results indicate that CP stimulates the activation of IGF-1, thereby improving the muscle protein synthesis.

DISCUSSION

Muscle atrophy is well known as a destructive symptom accompanied by cachexia, which is a devastating and irreversible syndrome in chronic diseases as well as aging.[18,19] Muscle atrophy characterizes the shrinkage of muscle fiber because of an actual loss of proteins, organelles, and the cytoplasm. The regulation of muscle mass and muscle fiber size fundamentally reflects protein turnover, that is, the balance between protein synthesis and protein degradation.[20] Accordingly, changes in protein turnover lead to muscle atrophy or hypertrophy, and muscle atrophy results from the decrease of protein synthesis and the elevation of protein degradation.[21] The persistent and excessive loss of muscle mass was judged to have a poor prognostic indicator and can abate the efficacy of

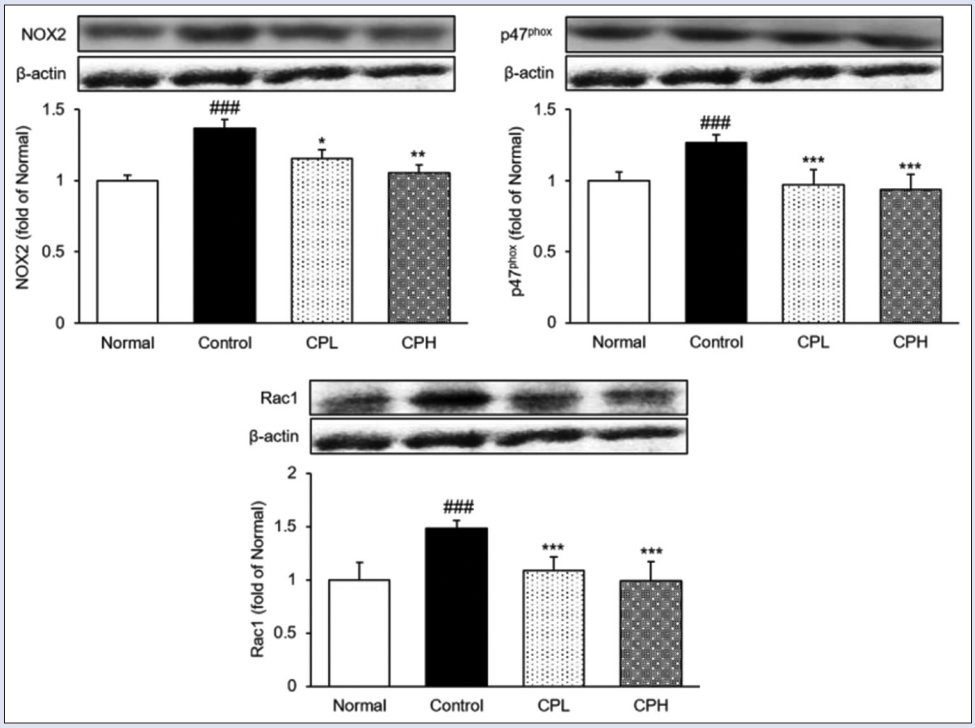


Figure 5: Effects of CP on NADPH oxidases related to ROS generation. The protein expressions were measured by western blotting. Bars are expressed as Mean ± SEM (n = 7). Significance: $^{\#\#\#}p < 0.001$ vs. the normal group and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. the DEX control group

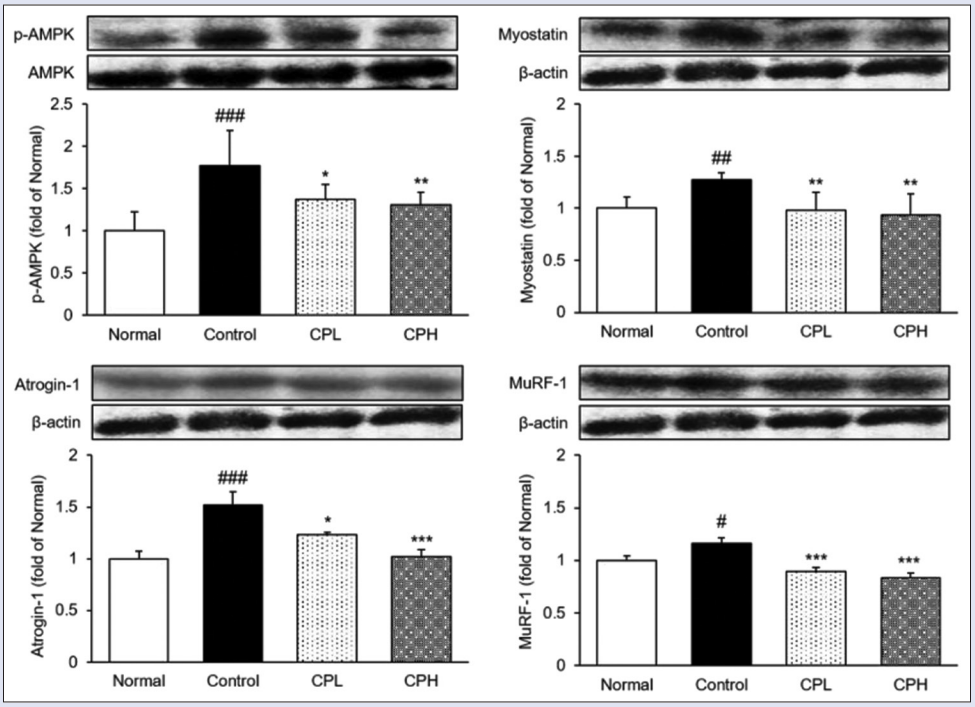


Figure 6: Effects of CP on the muscle protein catabolic pathway. The protein expressions of genes encoding p-AMPK, myostatin, Atrogin-1, and MuRF1 were measured by western blotting. Bars are expressed as mean ± SEM ($n = 7$). Significance: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. the normal group and $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. the DEX control group

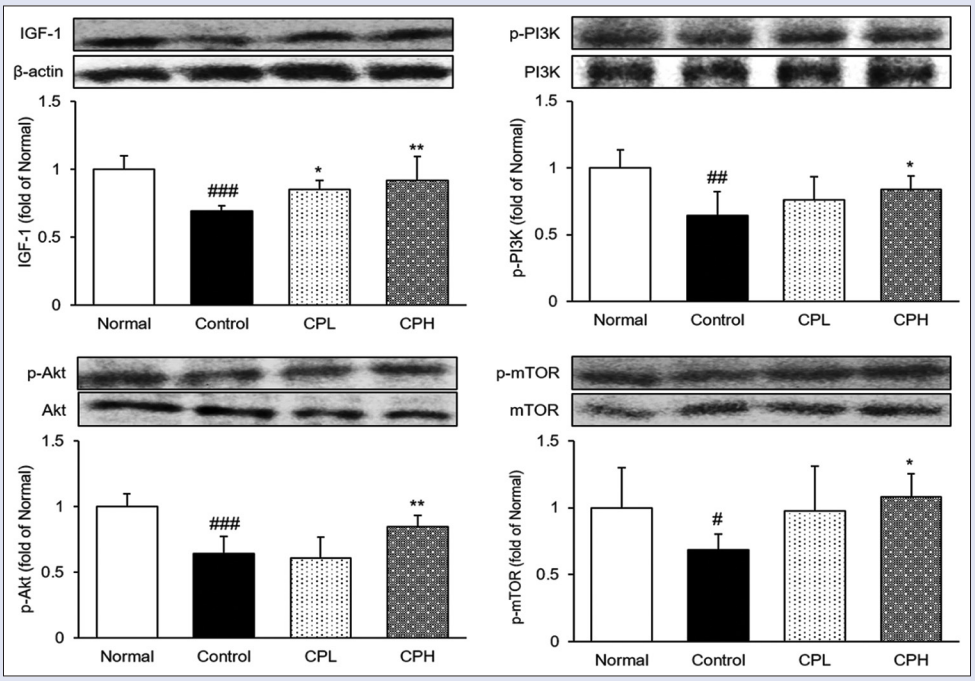


Figure 7: Effect of CP on the muscle protein anabolic pathway. The protein expressions such as IGF-1, p-PI3K, p-Akt, and p-mTOR were measured by western blotting. Bars are expressed as mean ± SEM ($n = 7$). Bars are expressed as mean ± SEM ($n = 7$). Significance: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. the normal group and $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the DEX control group

a number of different therapeutic treatments.[22] Most current treatments depend on protein supplementation or exercise,[23] and only a few drugs are prescribed in the clinical field for muscle atrophy conditions.[24] Thus, the development of effective herbal supplements with a few side effects for muscular atrophy is urged strongly. In the current study, we revealed the preventive effects of CP on muscle dysfunction using the DEX-induced muscle atrophy mice model. Moreover, we confirmed the underlying mechanisms through molecular biological analysis.

Prior to the evaluation of the effects on muscle atrophy, we supplied two different doses of CP (100 and 200 mg/kg) and measured the body weight of mice for 10 days. The UPLC analysis result of CP used in this experiment showed that CP contains 12.2 mg/g narirutin and 10.0 mg/g hesperidin [Figure 1]. Moreover, the potent antioxidant efficacies of CP on antioxidant analysis *in vitro* showed that CP could improve oxidative stress induced by muscle atrophy [Figure 2]. Therefore, when compared with the normal group, the DEX control group was considerably decreased in terms of body weight changes ($p < 0.001$), whereas the CP groups increased significantly in body weight changes (CPL, $P < 0.05$; CPH, $P < 0.01$). We also examined the improvement effect of muscle atrophy, such as changes through the measurement of muscle weight and H and E stain analysis. The oral administration of CP also increased the skeletal muscle weight including quadriceps, gastrocnemius, extensor digitorum longus plus tibialis anterior, and soleus [Figure 3]. Histological analysis of the Gast muscle also revealed that muscle fibers in the normal group were in close contact with muscle bundles. However, DEX injection led to an increase in perimysium and endomysium. Ultimately, these mean an elevation of connective tissues surrounding the muscle fibers and bundles. These muscle damages were recovered by CP. Especially, CPH significantly improves the DEX-induced impairment of muscle functions through the extension of swimming time. These data clearly indicate that CP has a preventive effect against DEX-induced muscle atrophy.

Muscle atrophy primarily caused by cachexia leads to energy imbalance and muscle protein degradation, consequently exacerbating the severity of the chronic disease. Recently, the regulation of reactive oxygen species (ROS) production has been reported as a promising strategy for controlling muscle atrophy.[25,26] In the skeletal muscle, ROS generation is a key mechanism in muscle atrophy progression.[27] The excessive ROS contents because of cellular stress are the key to triggering pathological disorders including muscle atrophy.[28] Many previous studies have demonstrated a close connection between NADPH oxidase (NOX) enzymes and oxidative stress. NOX enzymes are membrane-bounded proteins that transfer electrons to molecular oxygen. Finally, this results in the production of superoxide (O^{2-}) and subsequently ROS, including hydroxyl radicals (OH^{-}) and hydrogen peroxide (H_2O_2). NOX enzymes have unique distribution apparatus and expression levels through different tissues. Especially, NOX2 is highly present in phagocytes. Moreover, NOX2 interacts with cytosolic sub-units p47phox and Rac1.[29] Three factors related to ROS generation in this experiment were dramatically increased by DEX [Figure 5]. However, CP treatment decreased in a dose-dependent manner. Based on these results, CP treatment is considered to be effective in suppressing oxidative stress in DEX-induced muscle atrophy.

Various recent studies have been conducted to evaluate the essential mechanisms that contribute to the onset of muscle atrophy. AMPK is associated with metabolic regulation metabolism in the skeletal muscle and stimulates myostatin, which inhibits muscle growth and differentiation.[30] Besides, AMPK accelerates muscle protein degradation by promoting the expressions of two muscle-specific ubiquitin ligases such as Atrogin-1 and MuRF1.[31] Some reports demonstrated that ROS- or AMPK-mediated induction of Atrogin-1 and MuRF1 activates muscle protein breakdown and reduces muscle mass. Atrogin-1 and MuRF1 have been well known as the major factors causing muscle wasting, and these proteins are focused on as targets for therapeutic agents of muscle atrophy.[32] Moreover, previous studies reported that DEX, which is a synthetic glucocorticoid, accelerates Atrogin-1 and MuRF1-mediated proteolysis, leading to muscle wasting.[33] We analyzed the levels of proteins such as Atrogin-1 and MuRF1, which are involved in muscle protein catabolic pathways. Our result showed that DEX treatment significantly up-regulated the protein expressions of myostatin as well as both Atrogin-1 and MuRF1, whereas CP treatment notably inhibited such an increase by AMPK inactivation [Figure 6]. As a result, CP negatively regulated proteolysis-related proteins.

The available evidence indicates that increased ROS generation suppressed PI3K/Akt/mTOR signaling and reduced muscle protein synthesis. This ROS-mediated reduction in protein synthesis is likely because of down-regulation in Akt/mTOR signaling, and this causes a slower rate of translation.[34] On the other hand, active Akt promotes protein synthesis via activation of mTOR and its down-stream effectors.[20] As expected, DEX diminished PI3K/Akt/mTOR signaling and CP up-regulated p-mTOR levels by activating Akt in the CPH group [Figure 7]. Therefore, we speculated that activation of the PI3K/Akt/mTOR signaling contributed to the increase of protein synthesis by CP.

CONCLUSION

This study provides fundamental evidence for the effect of CP and its underlying mechanisms in preventing muscle atrophy. CPH treatment for 10 days dramatically increased the muscle mass and muscle function that were suppressed by DEX. These results indicate that CP has the ability to prevent muscle wasting. CP effectively regulated oxidative stress induced by NADPH oxidases. Moreover, CP inhibited proteolysis by AMPK inactivation. Moreover, CP showed an increase in protein synthesis through PI3K/Akt/mTOR signaling. As a result, CP could be used as a clinical remedy for patients with muscle atrophy in the future.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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