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Catalpa bignonioides extract improves exercise performance through regulation of growth and metabolism in skeletal muscles

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ABSTRACT

Objective: To evaluate the effects of *Catalpa bignonioides* fruit extract on the promotion of muscle growth and muscular capacity *in vitro* and *in vivo*.

Methods: Cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cell proliferation was assessed using a 5-bromo-2'-deoxyuridine (BrdU) assay kit. Western blot analysis was performed to determine the protein expressions of related factors. The effects of *Catalpa bignonioides* extract were investigated in mice using the treadmill exhaustion test and whole-limb grip strength assay. Chemical composition analysis was performed using high-performance liquid chromatography (HPLC).

Results: *Catalpa bignonioides* extract increased the proliferation of C2C12 mouse myoblasts by activating the Akt/mTOR signaling pathway. It also induced metabolic changes, increasing the number of mitochondria and glucose metabolism by phosphorylating adenosine monophosphate-activated protein kinase. In an *in vivo* study, the extract-treated mice showed improved motor abilities, such as muscular endurance and grip strength. Additionally, HPLC analysis showed that vanillic acid may be the main component of the *Catalpa bignonioides* extract that enhanced muscle strength.

Conclusions: *Catalpa bignonioides* improves exercise performance through regulation of growth and metabolism in skeletal muscles, suggesting its potential as an effective natural agent for improving muscular strength.

KEYWORDS: *Catalpa bignonioides*; Skeletal muscle; Cell proliferation; Mitochondria; Energy metabolism; C2C12

Significance

Catalpa bignonioides Walt. is widely used to treat various diseases and has many biological activities. Our previous study proved that vanillic acid and pinoselinol from *Catalpa bignonioides* have muscle cell proliferation promoting activity. This study demonstrated that *Catalpa bignonioides* fruit extract improves myogenesis, the number of mitochondria, and glucose metabolism in C2C12 mouse myoblasts. It also improved motor abilities *in vivo*. Hence, the extract improves exercise performance by regulating growth and metabolism *in vitro* and *in vivo*.

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1. Introduction

The annual loss of skeletal muscles occurs at a rate of approximately 2% from the age of 40 to 80 years[1,2]. This condition may develop into sarcopenia (a muscle loss disease); therefore, preventing skeletal muscle loss and maintaining muscle mass in the elderly is crucial for an energetic life in old age[3]. The mechanism regulating skeletal muscle mass remains unclear. However, the insulin-like growth factor 1 (IGF-1)/Akt/mammalian target of rapamycin (mTOR) signaling pathway and myogenesis pathway are significantly linked to skeletal muscle maintenance[4–8]. The decrease in skeletal muscle mass is caused by various factors such as oxidative stress, mitochondrial dysfunction, apoptosis, excessive autophagy, and lack of hormone homeostasis in muscle cells[9,10]. Moreover, metabolic functions in muscle cells are dysregulated when the rate of myofibrillar protein degradation exceeds the rate of protein synthesis, thereby reducing skeletal muscle mass[11]. Therefore, it is necessary to stimulate muscle cell metabolism and maintain skeletal muscle mass for optimal performance.

Anabolic steroids and drugs are widely applied for muscle growth and maintenance; however, their prolonged or excessive use is associated with various side effects such as alopecia, Cushing's syndrome, osteoporosis, diabetes, myocardial infarction, and cancer[12–14]. Therefore, natural products and plant compounds are proposed as alternative medicines to compensate for the side effects of anabolic steroids. For instance, black ginseng improves myoblast differentiation and myotube growth by enhancing the Akt/mTOR/P70S6K signaling pathway[15]. Furthermore, *Cichorium intybus* root extract suppresses oxidative stress-induced apoptosis, and many other natural resources exert positive effects on muscle cells[16]. However, many reports are limited to studies at the cellular level, and further research on their *in vivo* applicability is still lacking.

Catalpa bignonioides (*C. bignonioides*) Walt. (Bignoniaceae) is a species widely used for medicinal purposes for treating gastric diseases, helminthic infections, oncological diseases, bronchial diseases, carbuncles, scabs, and abscesses in indigenous cultures of South America[17]. Phytochemical studies show that *C. bignonioides* extract contains phenols, terpenes, alkaloids, tannins, fats, and sugars[18]. In North American traditional medicine, *C. bignonioides* extract was used to treat bronchial diseases and has demonstrated antioxidant, anti-inflammatory, and antinociceptive effects[18]. In addition, *C. bignonioides* pods, seeds, leaves, and roots can treat many diseases such as ulcers, skin, respiratory diseases, scrofulous maladies, and helminthic infections[19,20]. In addition, other *Catalpa* species show anti-diabetic effects in mouse models, and their efficacy has been confirmed when the substance is incorporated into their diet[21]. Catalpic acid is abundant in the Bignoniaceae family including *C. bignonioides* and improves glucose homeostasis when fed to mice[22]. Our previous study demonstrated that vanillic acid and pinoresinol from *C. bignonioides* significantly increase the proliferation of C2C12 mouse myoblasts by regulating the IGF-1/Akt/mTOR signaling pathway[23]. However, it only included specific components of the *C. bignonioides* extract and did not explore the *in*

in vivo efficacy of the extract.

Therefore, this study aimed to evaluate whether oral administration of the *C. bignonioides* extract improves exercise performance in a mouse experimental model.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM) was purchased from Welgene (Gyeongsangbuk-do, Korea). Fetal bovine serum (FBS) was provided by Omega Scientific, Inc. (Tarzana, CA, USA). Penicillin and streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). Horse serum (HS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and radioimmunoprecipitation assay buffer were purchased from Sigma-Aldrich (St. Louis, MO, USA). The 5-bromo-2'-deoxyuridine (BrdU) assay kit was purchased from Millipore (Billerica, MA, USA). The NuPAGE 4%-12% Bis-Tris gel was purchased from Life Technologies (Carlsbad, CA, USA). Polyvinylidene fluoride membranes were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Rabbit anti-mouse phospho-Akt (Cat no. 4060; 1:1 000), rabbit anti-mouse phospho-mTOR (Ser2448, Cat no. 5536; 1:1 000), rabbit anti-mouse phospho-ribosomal protein S6 kinase (p70S6K, Cat no. 9204; 1:1 000), rabbit anti-mouse phospho-eukaryotic initiation factor 4E-binding protein 1 (4E-BP1, Cat no. 2855; 1:1 000), mouse anti-mouse glucose transporter type 4 (GLUT4, Cat no. 2213; 1:1 000), rabbit anti-mouse phospho-AMPK α (Cat no. 2535; 1:1 000), rabbit anti-human phospho-sirtuin 1 (Sirt1, Cat no. 2314; 1:1 000), rabbit anti-human peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α ; Cat no. 2178; 1:1 000), peroxisome proliferator-activated receptor gamma (PPAR γ ; Cat no. 2443; 1:1 000), goat anti-rabbit IgG (Cat no. 7074, 1:3 000), and goat anti-mouse IgG (Cat no. 7076; 1:3 000) were purchased from Cell Signaling Technology (Danvers, MA, USA). Mouse anti-human myoblast determination protein 1 (MyoD, Cat no. sc-377460; 1:1 000), mouse anti-human myogenin (Cat no. sc-12732; 1:1 000), and mouse anti-human β -actin (Cat no. sc-47778; 1:1 000) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). The SuperSignal West Femto Trial Kit and radioimmunoprecipitation assay (RIPA) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using colorimetric assay kits purchased from BioVision (Milpitas, CA, USA).

2.2. Preparation of *C. bignonioides* extract

The fruits of *C. bignonioides* collected from the Medicinal Herb Garden, College of Pharmacy, Seoul National University in Goyang, Gyeonggi-do, Korea, in 2021 were certified by Sang Il Han, the general manager of the Medicinal Herb Garden. A voucher sample

(CB202106) was deposited with the Korea Basic Science Institute (Chuncheon, Korea). The samples were washed with fresh water to remove sand and dust, and dried at room temperature for 1 week. Dried samples (500 g) were extracted using 70% ethanol (5 L) with continuous agitation at 37 °C. The solvent was removed using a vacuum rotary evaporator, and the extract was stored at -20 °C until use.

2.3. Cell culture and differentiation

C2C12 cells, belonging to a murine myoblast cell line, were maintained in DMEM containing 10% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL). For differentiation, the medium was replaced with DMEM supplemented with 2% HS when the cell confluence reached approximately 80%, which was subsequently replaced every alternate day, and differentiation was performed for 6 d. All cell cultures were maintained at 37 °C in a 5% CO₂ incubator.

2.4. Cytotoxicity assay

C2C12 cells (1×10^5 cells/mL) were seeded in 96-well plates and incubated with *C. bignonioides* extracts (6.25-50 µg/mL) for 24 h. Next, 100 µg/mL MTT was added to each well and incubated for 2.5 h at 37 °C. The supernatant was removed, and cells were treated with DMSO to dissolve formazan crystals. The absorbance of the colored solution was determined at 540 nm using a SpectraMax M2/M2e spectrophotometer (Molecular Devices, San Jose, CA, USA).

2.5. Cell proliferation activity

Skeletal muscle cell proliferation activity was assessed using a colorimetric assay. C2C12 cells (5×10^4 cells/mL) were seeded in a 96-well plate, and the medium was replaced with DMEM supplemented with 2% HS 2 days later for differentiation. The *C. bignonioides* extract (6.25-50 µg/mL) was added whenever the medium (2% HS-containing DMEM) was changed every alternate day. To evaluate the effect of *C. bignonioides* extract on the proliferation of skeletal muscle cells, a BrdU cell proliferation assay was conducted during the differentiation period, as reported previously[23–26]. Cell proliferation was measured using the BrdU assay kit 6 days after inducing differentiation, according to the manufacturer's instructions.

2.6. Western blot analysis

Cell lysates were prepared using RIPA buffer[23]. Quantified protein lysates were loaded onto NuPAGE 4%-12% Bis-Tris gels, which were subsequently blotted onto a polyvinylidene fluoride membrane. Primary antibodies (each diluted 1:1 000, including rabbit anti-mouse phospho-Akt, rabbit anti-mouse phospho-mTOR, rabbit anti-mouse phospho-p70S6K, rabbit anti-mouse phospho-4E-BP1, mouse anti-mouse MyoD, mouse anti-mouse myogenin, mouse anti-mouse GLUT4, rabbit anti-mouse phospho-AMPK α , rabbit anti-mouse phospho-Sirt1, rabbit anti-human PGC-1 α , rabbit

anti-mouse PPAR γ , and mouse anti-mouse β -actin) were added to cover the membrane and incubated overnight at 4 °C. Subsequently, secondary antibodies (diluted 1:3 000, including goat anti-rabbit IgG and goat anti-mouse IgG) were added, followed by incubation for 1.5 h at 25 °C. Signals were developed using the SuperSignal West Femto Trial Kit, and images were acquired using Fusion FX (Vilber Lourmat Ste, Collegien, France).

2.7. Treadmill exhaustion test and whole-limb grip strength assay

Six-week-old ICR male mice (Nara-Biotec, Korea) were maintained under controlled conditions of temperature [(23 ± 2) °C], humidity [(55 ± 5)%], and light (12-h light/dark cycle) at the Korea Basic Science Institute (KBSI); and they had access to food and water *ad libitum*.

To evaluate the *in vivo* efficacy of the *C. bignonioides* extract, mice were randomized into four groups with at least 8 mice in each group, and orally administered distilled water, 200 mg/kg of creatine, 50 mg/kg of *C. bignonioides* extract, or 200 mg/kg of *C. bignonioides* extract daily for 10 weeks. To confirm the biotoxicity or liver damage of *C. bignonioides* extract in mice, ALT and AST levels were determined using colorimetric assay kits. Mice were acclimatized to treadmill running through training for 5 min at a speed of 10 m/min on the Exer 3/6 treadmill (Columbus Instruments; Columbus, OH, USA) with a 10% uphill slope for 3 d before the test. The mice were subjected to an endurance test until exhaustion. The treadmill was initially run at a speed of 10 m/min for 5 min, and the speed was increased by 2 m/min every 2 min. The running time, distance, and maximum speed until exhaustion were recorded for each mouse. Exhaustion was defined as when a mouse could not run on the treadmill for over 10 s despite mechanical prodding[27]. In the whole-limb grip strength assay, the tail of each mouse was horizontally pulled when they grabbed a grid, and the maximum force was recorded using a grip strength meter (Ametek; Paoli, PA, USA)[20]. Each mouse was tested at least twice for 3 d. The thigh thickness of mice was measured using a caliper.

2.8. High-performance liquid chromatography (HPLC) analysis

Chemical (vanillic acid and pinoreosinol) composition was analyzed using UFLC LC-20A (Shimadzu, Kyoto, Japan) with a UV detector at 254 nm using the PRONTOSIL 120-5-C18 ACE-EPS column (5 µm, 250 mm × 4.6 mm). A flow rate of 1.0 mL/min was maintained for elution using a mobile phase solvent system containing 0.1% (*v/v*) formic acid in water (A) and acetonitrile (B) (30% B; 3 min, 50% B; 3-20 min, 90% B; 20-25 min, 90% B; 25-27 min, 30% B; 27-28 min, 30% B; 28-35 min).

2.9. Statistical analysis

Statistical analyses of data were performed using a two-tailed one-way analysis of variance with Tukey's *post-hoc* test using Prism

(Version 4.00; GraphPad Inc.; La Jolla, CA, USA). Data were considered statistically significant at $P < 0.05$.

2.10. Ethical statement

All animal experiments were approved by the Institutional Animal Care and Use Committee at KBSI (KBSI-IACUC-21-30).

3. Results

3.1. *C. bignonioides* extract enhances skeletal muscle cell proliferation

Before examining the effect of *C. bignonioides* fruit extract on the proliferation of muscle cells, its cytotoxicity to C2C12 mouse myoblasts was evaluated using the MTT assay. No cytotoxic effect was observed up to 50 $\mu\text{g/mL}$ ($P > 0.05$) (Figure 1A).

No effect on cell proliferation was detected in skeletal muscle cells

treated with 6.25 $\mu\text{g/mL}$ *C. bignonioides* fruit extract; however, 12.5 and 25 $\mu\text{g/mL}$ extracts significantly increased cell proliferation ($P < 0.01$). Elevated cell proliferation was also observed at 50 $\mu\text{g/mL}$ ($P < 0.05$), although the level was reduced compared with that observed at 25 $\mu\text{g/mL}$ (Figure 1B).

3.2. *C. bignonioides* extract stimulates the Akt/mTOR signaling in skeletal muscle cells

Western blot analyses were conducted to elucidate the molecular mechanism by which *C. bignonioides* extract enhances cell proliferation. We observed significantly elevated levels of phosphorylated Akt, mTOR, and p70S6K and downregulation of phosphorylated 4E-BP1 (a translational repressor) in C2C12 cells treated with *C. bignonioides* extract ($P < 0.001$) (Figure 2). In addition, Myo D was activated during muscle differentiation and considerably increased following treatment with the extracts ($P < 0.01$). *C. bignonioides* extract at 12.5 $\mu\text{g/mL}$ significantly upregulated the expression of myogenin ($P < 0.01$), but no significant change was

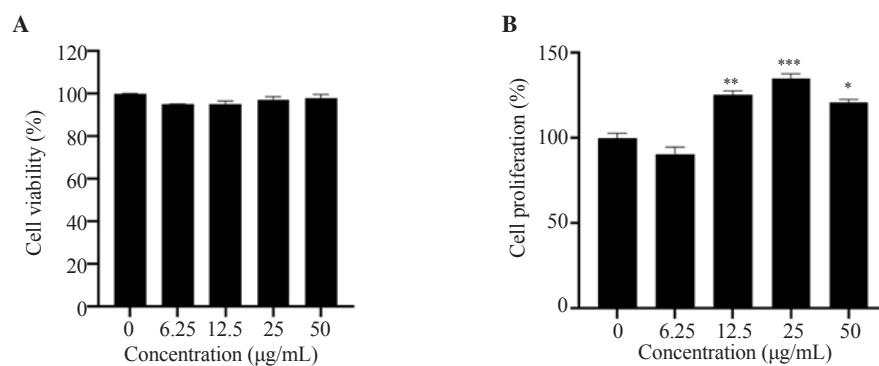


Figure 1. Effect of *Catalpa bignonioides* fruit extract on mouse myoblast proliferation. (A) Cytotoxic effects of various concentrations of *Catalpa bignonioides* extract on C2C12 cells by MTT assay. (B) Effects of various concentrations of *Catalpa bignonioides* extract on the differentiation of C2C12 cells. Data are represented as mean \pm SEM of measurements in at least triplicate. *, **, and *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$ compared with the control, respectively.

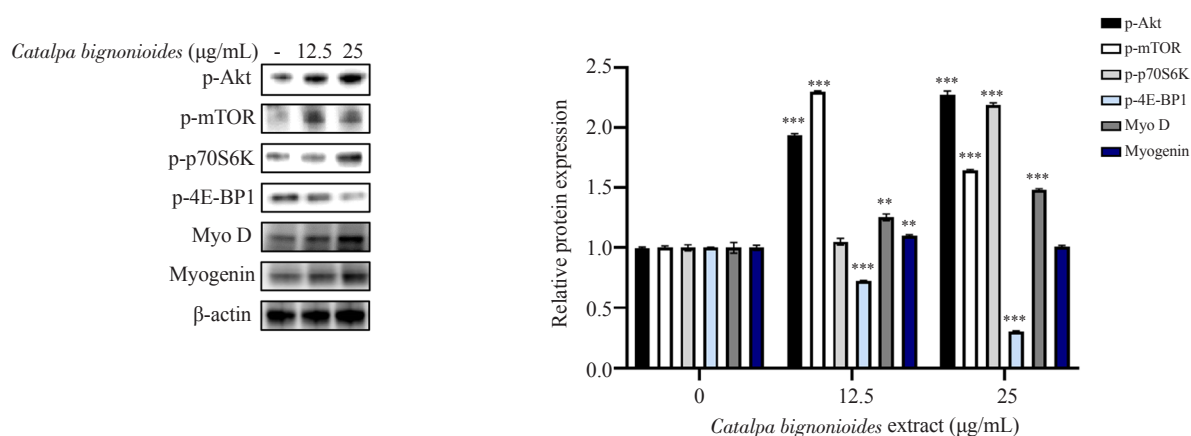


Figure 2. Effect of *Catalpa bignonioides* extract on the expression of p-Akt, p-mTOR, p-p70S6K, and p-4E-BP1, Myo D, and myogenin in C2C12 mouse myoblasts. The levels of IGF-1 signaling (p-Akt, p-mTOR, p-p70S6K, and p-4E-BP1), Myo D, and myogenin were determined using Western blot analysis. β -actin was used as the loading control. Data are represented as mean \pm SEM of measurements in at least triplicate. **, and *** indicate $P < 0.01$, and $P < 0.001$ compared with the control, respectively.

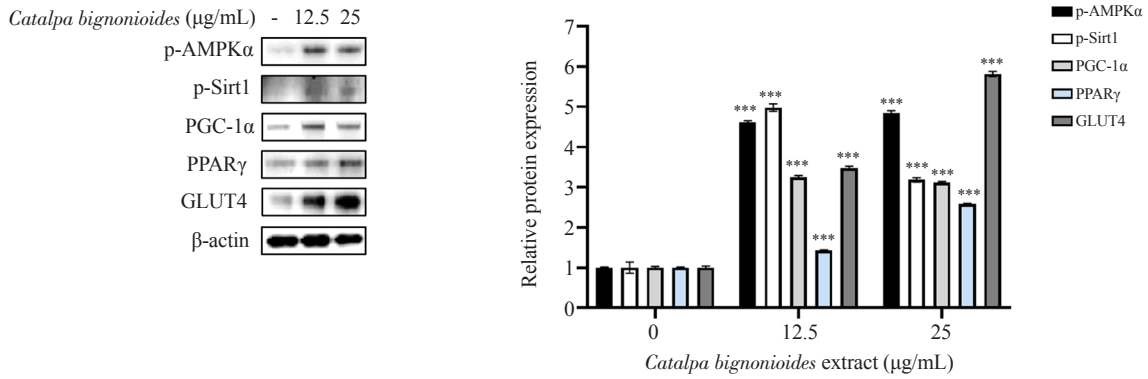


Figure 3. Mitochondrial metabolism is stimulated by *Catalpa bignonioides* extract in C2C12 cells. The levels of mitochondrial biosynthesis (p-AMPKα, p-Sirt1, and PGC-1α) and glucose metabolism (PPARγ and GLUT4) were determined using Western blot analysis. β-actin was used as the loading control. Data are represented as mean ± SEM of measurements in at least triplicate. ****P* < 0.001 compared with the control.

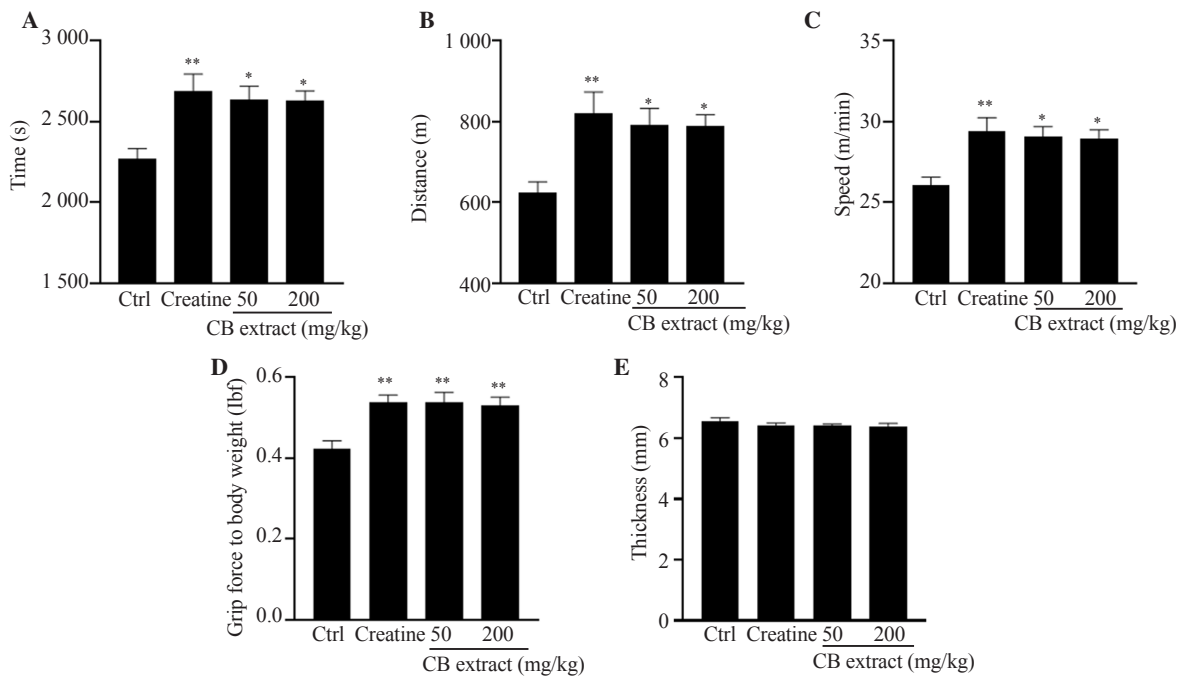


Figure 4. Improved motor performance of mice treated with *Catalpa bignonioides* extract. Time (A), distance (B), and maximum speed (C) before exhaustion on the treadmill in mice supplemented with distilled water (Ctrl), creatine (200 mg/kg), or *Catalpa bignonioides* extract (50 and 200 mg/kg). Grip strength (D) and leg muscle thickness (E) were measured in mice. Data are expressed as mean ± SEM (*n* = 8 mice per *Catalpa bignonioides* extract 200 mg/kg group and *n* = 9 mice per the other groups). * and ** indicate *P* < 0.05 and *P* < 0.01 compared with the control, respectively. CB: *Catalpa bignonioides*.

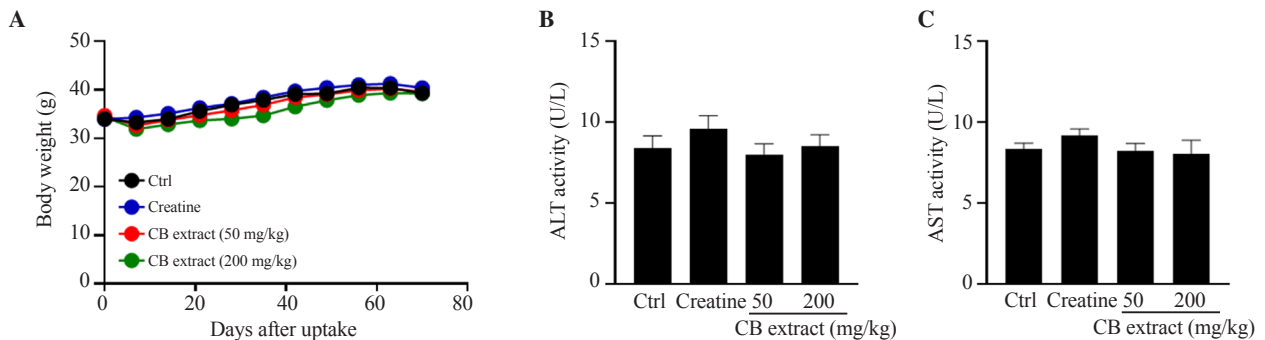


Figure 5. Biotoxicity assay in mice treated with *Catalpa bignonioides* extract. Changes in body weight over 10 weeks (A) and serum levels of ALT (B) and AST (C) were determined in mice supplemented with distilled water (Ctrl), creatine (200 mg/kg), or *Catalpa bignonioides* extract (50 and 200 mg/kg). Data are expressed as mean ± SEM (*n* = 8 mice per *Catalpa bignonioides* extract 200 mg/kg group and *n* = 9 mice per the other groups). ALT: alanine aminotransferase; AST: aspartate aminotransferase.

observed after treatment with 25 µg/mL of the extract ($P>0.05$).

3.3. Energy metabolism is modulated in *C. bignonioides*-treated skeletal muscle cells

To explore the mitochondrial energy metabolism induced by the *C. bignonioides* extract, Western blot analyses were performed to determine the factors related to mitochondrial biogenesis and glucose metabolism in C2C12 skeletal muscle cells. We found that treatment with *C. bignonioides* extracts markedly increased the protein expression of PGC-1 α , promoting mitochondrial biogenesis and the phosphorylation of AMPK α and Sirt1, which are regulators of PGC-1 α , in C2C12 ($P<0.001$) (Figure 3). Furthermore, the expressions of PPAR γ (which regulates glucose uptake in skeletal muscle) and GLUT4 (a downstream target of PPAR γ and AMPK) were pronouncedly increased in C2C12 cells treated with both concentrations of *C. bignonioides* extract ($P<0.001$).

3.4. Exercise performance is enhanced in *C. bignonioides* extract-treated mice

To analyze the *in vivo* effect of *C. bignonioides* extract on proliferation and energy metabolism in muscle cells, a muscular endurance test was conducted using the motorized treadmill following administration of the *C. bignonioides* extract for 10 weeks. Extract-treated mice showed significantly increased running time, distance, and maximum speed to exhaustion compared to those recorded in the control group ($P<0.05$); moreover, they showed improved exercise capacity, similar to that of the creatine-supplemented group used as a positive control (Figure 4A-C). The whole-limb grip strength assay revealed enhanced muscular strength in mice treated with *C. bignonioides* extract (Figure 4D). No change in thigh thickness was noted at the end of the experiment (Figure 4E). The exposure to the extract for 10 weeks neither altered the body weight of the mice (Figure 5A) nor increased the levels of ALT and AST in serum (Figure 5B and 5C), which suggested that *C. bignonioides* extract improved exercise performance without causing significant biotoxicity or liver damage to mice.

3.5. Analysis of vanillic acid and pinoresinol in *C. bignonioides*

The presence of vanillic acid and pinoresinol in *C. bignonioides* was confirmed by comparing their retention time with that of standard compounds. The retention time of vanillic acid was found to be 7.154 min. *C. bignonioides* contained 9.222 mg/g vanillic acid, but pinoresinol was not detected (Figure 6).

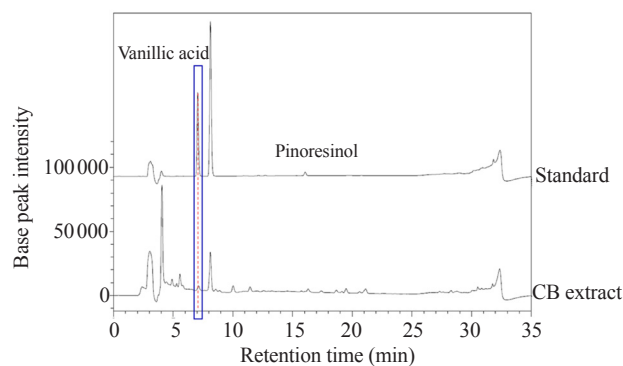


Figure 6. High-performance liquid chromatography chromatogram of vanillic acid and pinoresinol. Vanillic acid is marked with a blue box.

4. Discussion

During aging, various changes in biochemical signaling pathways and breakdown of muscular homeostasis result in a natural decline in skeletal muscle mass and strength[28]. The amount of muscle and exercise capacity needs to be maintained using supplements with biosafety to overcome muscle loss. This study demonstrated that the extract collected from *C. bignonioides* (an indigenous medicinal plant) induces muscle growth and improves muscle strength. The extract promoted proliferation and energy metabolism in C2C12 mouse myoblasts and muscular endurance and strip strength significantly increased when administered orally. These results suggest the efficacy of *C. bignonioides* extract as an adjuvant for enhancing muscle strength function.

Proliferation and differentiation of muscle cells are promoted through the activation of the IGF-1 signaling pathway[29,30]. Regulation of skeletal muscle growth is associated with the Akt/mTOR signaling. Akt is activated *via* phosphorylation, plays an important role in muscle cell proliferation, and induces muscle hypertrophy when sufficient energy sources (such as growth factors and sugars) are available[5,31]. Activated Akt triggers phosphorylation cascades in the mTOR and p70S6K pathways, and the expression levels of regulatory factors related to proliferation and protein synthesis are elevated. MyoD and myogenin are myogenic regulatory factors required for myogenesis in proliferating myoblasts[32]. We previously demonstrated that specific compounds isolated from *C. bignonioides* fruits regulate the proliferation of C2C12 cells *via* the IGF-1 signaling[23]. We demonstrated that *C. bignonioides* extract promoted the proliferation of differentiating C2C12 myoblasts *via* activation of the Akt/mTOR signaling and increased Myo D and myogenin expression.

Skeletal muscles consume energy generated in the mitochondria for their contraction; therefore, an increase in the number of mitochondria and activation of mitochondrial metabolism are associated with increased muscle strength[33]. Adenosine triphosphate (ATP) is produced in the mitochondria, and mitochondrial biosynthesis is regulated by PGC-1 α activation

through energy sensors, AMPK, and Sirt1[34]. Phosphorylation of AMPK α (the catalytic subunit of AMPK complex) occurs when the adenosine monophosphate (AMP)/ATP ratio increases after ATP consumption, and its function is activated. Sirt1 is an NAD⁺-dependent deacetylase, activated by phosphorylation when the NAD⁺/NADH ratio increases[34]. AMPK and Sirt1 facilitate the activity of PGC-1 α (a master regulator of mitochondrial biosynthesis and function) through phosphorylation and deacetylation, respectively[35]. Therefore, activation of AMPK, Sirt1, and PGC-1 α that stimulate mitochondrial biosynthesis is required to enhance metabolic efficacy to improve muscle performance. Activated AMPK is also recognized as a key regulator of energy metabolism in skeletal muscle, facilitating glucose uptake and increasing insulin sensitivity[36]. It regulates PPAR γ , improving glucose and fatty acid metabolism in skeletal muscle[37]. GLUT4 is an insulin-dependent glucose transporter mainly distributed in skeletal muscle and adipose tissue that is expressed by AMPK and PPAR γ to move glucose from the outside to the inside of the cell[38,39]. In this study, *C. bignonioides* extract increased the phosphorylation of AMPK α and Sirt1 in C2C12 cells and the expression of their target molecule, PGC-1 α , which is expected to increase the number of mitochondria. In addition, it increased the protein levels of PPAR γ and GLUT4, which are downstream targets of AMPK; this in turn promoted glucose uptake, which is essential for glycolysis required for muscle contraction and exercise. Furthermore, *C. bignonioides* extract-treated mice significantly increased running time, distance, speed, and grip force to body weight compared with control mice. These data suggest that the *C. bignonioides* extract may modulate metabolism in muscle cells, induce their proliferation, increase mitochondrial numbers *in vitro*, and improve exercise performance *in vivo*.

C. bignonioides extract contains several compounds such as isolariciresinol, pinoresinol, catalposide, vanillic acid, and minecoside. Kim *et al.* demonstrated that many compounds of the *C. bignonioides* extract promote the proliferation of C2C12 cells, with vanillic acid and pinoresinol showing the best efficacy[23]. Moreover, vanillic acid improves the proliferation of myoblasts through the IGF-1R/Akt/mTOR signaling pathway[23]. Vanillic acid was detected in the *C. bignonioides* extract, which improved the motor performance in mice. Therefore, it is indicated that vanillic acid is the main active compound of *C. bignonioides* extract that enhances exercise performance *in vitro*.

The main limitation of this study is that we did not demonstrate additional mechanisms in mice. Moreover, the effect of vanillic acid *in vivo* was not proved. Therefore, further studies are needed to demonstrate the mechanistic studies of *C. bignonioides* extract and the effect of vanillic acid *in vivo*.

In summary, this study demonstrated the potential effect of *C. bignonioides* on muscle growth and strength improvement. *C. bignonioides* extract increased muscle cell proliferation *via* the Akt/mTOR signaling and improved energy metabolism, increasing the number of mitochondria, and promoting glucose oxidation. Furthermore, a significantly enhanced exercise capacity was detected in mice. Hence, all these results indicate that *C. bignonioides* extract could help prevent muscle loss and maintain muscular strength.

Conflict of interest statement

The authors declare no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

SHC and KNK supervised and designed the study. HJ, DJL, SPK, SRK, JWP, BY, JIP, and SHC performed experimental analysis. HJ, DCL, KMC, and WWL performed the analytic calculation. SP and SHK provided the resources. HJ, DJL, SHC, KNK contributed to the final version of the manuscript. KNK supervised the project.

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