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Original Article

Anti-aging Effects of *Angelica keiskei* Leaf Extract on Skeletal Muscle: Associations with NF-κB p65 and PGC-1α in a D-galactose Model

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Abstract

Aging-associated skeletal muscle dysfunction is highly interlinked to chronic inflammation and mitochondrial dysregulation, with the suppression of PGC-1α representing a key molecular event. The use of natural phytochemicals to prevent aging-related pathological conditions has received increasing attention, including *Angelica keiskei.* This study examined the effects of *A. keiskei* leaf extract on skeletal muscle in a D-galactose (D-gal)-induced aging rat model, focusing on NF-κB p65 protein levels and PGC-1α-associated mitochondrial regulatory markers. Male rats were administered D-gal to induce accelerated aging and subsequently treated with *A. keiskei* extract. NF-κB p65 levels were assessed by ELISA, while the mRNA expression of *Ppargc1a* and its target gene *Tfam,* and mitochondrial antioxidants (*Sod2*, *Txn1*, and *Prdx1*) in skeletal muscle tissue was quantified using real-time quantitative PCR. Administration of D-gal markedly increased NF-κB p65 protein levels, reduced the *Ppargc1a* and *Tfam* expression, and inhibited the mRNA expression of *Sod2*, *Txn1*, and *Prdx1*, suggesting that inflammation impairs mitochondrial biogenesis and antioxidant defenses. Treatment with *A. keiskei* extract reduced NF-κB p65 protein levels, restored *Ppargc1a* mRNA expression, and partially restored antioxidant-related gene expression. These data indicate that *A. keiskei* extract attenuates the molecular characteristics of skeletal muscle aging through the suppression of inflammaging and the preservation of the PGC-1α-dependent mitochondrial regulatory pathways. This study collectively provides preliminary preclinical evidence that *A. keiskei* leaf extract may attenuate selected molecular and functional features of D-gal-induced skeletal muscle aging through changes consistent with reduced inflammatory signaling and preservation of PGC-1α-associated mitochondrial regulation.

Keywords

Aging, *Angelica keiskei*, atrophy, inflammation, mitochondrial biogenesis, skeletal muscle

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Introduction

Aging is accompanied by profound physiological changes across multiple organ systems, with skeletal muscle being particularly vulnerable. Loss of muscle mass, strength, and function, or sarcopenia, is a hallmark of aging and represents a major public health challenge worldwide. Sarcopenia affects a substantial portion of the elderly population, with estimates suggesting that approximately 10% of adults aged 60–70 years and up to 50% of those over 80 years are affected.[1] Moreover, systematic reviews indicate that the pooled prevalence of sarcopenia and frailty may approach

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27% (95% CI: 19%–35%) in older adults, reflecting the widespread nature of this condition.[2] The consequences of sarcopenia extend beyond muscle loss to impair mobility, independence, and quality of life. Age-related muscle weakness contributes significantly to falls.[3,4] Falls and related injuries are major drivers of disability, hospitalization, and mortality in older populations. Sarcopenia also contributes to frailty, which in turn is associated with greater healthcare utilization and reduced resilience.[5,6]

Despite its clinical importance, the molecular basis of skeletal muscle aging remains incompletely understood. Chronic low-grade inflammation, oxidative stress, and dysregulated mitochondrial function are key drivers of age-related muscle decline.[7] Among these mechanisms, the nuclear factor-κB (NF-κB) pathway was prioritized in the present study because it represents a central transcriptional regulator of inflammaging and provides a direct mechanistic link between inflammatory activation and mitochondrial impairment.[8] In skeletal muscle, persistent NF-κB activation promotes the expression of pro-inflammatory cytokines and catabolic mediators, thereby contributing to muscle wasting and metabolic dysfunction.[9,10] Importantly, NF-κB–mediated inflammatory signaling that disrupts the regenerative satellite-cell niche, whereas enhanced PGC-1α activity preserves mitochondrial, metabolic, and regenerative features associated with a younger muscle phenotype.[11,12] Although other pathways, such as AMPK, SIRT1, and Nrf2, are also involved in mitochondrial adaptation and antioxidant defense,[13] these pathways primarily regulate PGC-1α through energy sensing, deacetylation, or redox-responsive mechanisms. In contrast, NF-κB directly reflects the inflammatory component of skeletal muscle aging and may transcriptionally repress PGC-1α, making it particularly relevant to the D-galactose (D-gal) model, which is characterized by oxidative stress and inflammation. PGC-1α is a master regulator of mitochondrial biogenesis and antioxidant defense.[14] Therefore, suppression of PGC-1α may contribute to mitochondrial dysfunction, reduced oxidative capacity, and reactive oxygen species accumulation in aging skeletal muscle.[15,16]

Because of its ability to induce oxidative damage, D-gal has been widely employed to mimic accelerated aging by triggering advanced glycation end-product buildup, persistent oxidative stress, and chronic inflammation.[17] In skeletal muscle, D-gal administration has been shown to activate NF-κB signaling, impair mitochondrial function, and suppress antioxidant defense systems, thereby recapitulating key molecular features of age-related muscle degeneration.[18] However, the precise transcriptional mechanisms linking D-gal–induced inflammation to mitochondrial biogenesis failure in skeletal muscle require further investigation.

Natural phytochemicals have received increasing attention due to their properties related to anti-aging activities such as anti-inflammatory, antioxidant, and mitochondrial-protective properties.[19] *Angelica keiskei*, an edible medicinal plant rich in chalcones and flavonoids, has shown anti-inflammatory and cytoprotective activity in various models of oxidative stress and aging.[20–23] Previous studies have reported skeletal-muscle-related effects using different *A. keiskei*-based preparations, including a mixture containing *A. keiskei* and *Morus alba* leaf extracts,[24] an isolated chalcone from *A. keiskei*,[25] and an *A. keiskei* water extract in naturally aged mice.[26]

However, these studies have primarily focused on the anabolic, phenotypic, or general antioxidant effects of *A. keiskei*, while its role in regulating the transcriptional crosstalk between chronic inflammation and mitochondrial dysfunction during skeletal muscle aging remains unclear. Because the NF-κB–PGC-1α axis represents a key molecular link between inflammaging and impaired mitochondrial regulation, we hypothesized that *A. keiskei* leaf extract could attenuate D-gal–induced skeletal muscle aging by reducing NF-κB protein levels and preserving PGC-1α–dependent mitochondrial transcriptional programs.

To test this hypothesis, we evaluated skeletal muscle function, atrophy-related markers, NF-κB p65 protein levels, inflammatory cytokine expression, and the transcript levels of PGC-1α-associated mitochondrial biogenesis and antioxidant genes in D-gal-treated rats with or without *A. keiskei* intervention. By addressing this specific inflammation–mitochondria regulatory pathway, the present study provides mechanistic insight into the anti-aging potential of *A. keiskei* in skeletal muscle. This focus is also aligned with the United Nations Sustainable Development Goal 3 (Good Health and Well-being), particularly in supporting healthy aging, reducing disability risk, and improving quality of life in older populations.

Materials and Methods

Production of A. keiskei Leaf Extract

Fresh *A. keiskei* leaves were collected from the farm located in Poncokusumo, Malang Regency, East Java, Indonesia. Immediately after harvesting, the leaves were cleaned thoroughly with running water to eliminate surface contaminants. The plant material was subsequently dried naturally under shaded, well-ventilated conditions to prevent degradation of thermolabile compounds. Once fully dried, the leaves were pulverized into coarse particles and sieved using a 40-mesh screen to ensure uniform particle size. Extraction was performed by soaking the powdered material in 70% ethanol at ambient temperature for 48 hours with occasional agitation. The extract obtained was filtered, and the solvent was eliminated using a rotary evaporator under reduced pressure. The extract was kept at −20 °C and freshly diluted in distilled water prior to oral administration.[23]

Metabolomic Profiling

Metabolomic profiling was performed using LC-HRMS. Briefly, 50 mg of sample was diluted in 1,000 µL of 70% HPLC-grade methanol, vortex-homogenized for 1 min, ultrasonicated for 30 min, centrifuged at 1,400 × *g* for 5 min, and filtered through a 0.2 µm nylon membrane filter before injection. Chromatographic separation was conducted using a Thermo Scientific™ Vanquish™ Horizon UHPLC system with a binary pump (Germering, Germany) and a Thermo Scientific™ Accucore™ Phenyl-Hexyl analytical column (100 × 2.1 mm, 2.6 µm; Lithuania). The mobile phase consisted of MS-grade water with 0.1% formic acid (A) and MS-grade acetonitrile with 0.1% formic acid (B), delivered at 0.3 mL/min with a 25-min gradient from 5% B to 90% B over 16 min, held for 4 min, and returned to 5% B until the end of the run. The column temperature was maintained at 40 °C, and the injection volume was 5 µL. HRMS analysis was performed using a Thermo Scientific™ Orbitrap™ Exploris 240 HRMS system (Bremen, Germany) equipped with an OptaMax™ NG heated electrospray ionization source in Full MS/dd-MS² mode with positive/negative polarity switching. Full MS spectra were acquired at 60,000 FWHM over *m/z* 70–800, with a maximum injection time of 100 ms, intensity threshold of 5,000, charge state of 1, and mass tolerance of 5 ppm. Data-dependent MS² spectra were acquired at 22,500 FWHM using normalized collision energies of 30, 50, and 70, with nitrogen as the collision gas. Ion source parameters were set as follows: Spray voltage, 3,500 V positive and 2,500 V negative; sheath gas, 35 AU; auxiliary gas, 7 AU; sweep gas, 1 AU; ion transfer tube temperature, 300 °C; and vaporizer temperature, 320 °C.[27,28]

Experimental Animals

Fifteen male Wistar rats, aged between 6 and 8 weeks and weighing 150–200 g, were obtained from a licensed laboratory animal supplier located in Bogor, Indonesia. The number of animals required per group was estimated using the resource equation approach.[29] With 15 animals allocated into three experimental groups, the error degrees of freedom were E = total number of animals − total number of groups = 15 − 3 = 12, which lies within the commonly accepted range of 10–20 for exploratory animal experiments. Therefore, five animals per group were considered appropriate for this controlled study, while also adhering to the ethical principle of reduction in animal experimentation. The animals were kept in a controlled environment that included a 12-hour light/dark cycle, temperatures of 22 °C–24 °C, and relative humidity levels of 45%–65%. Standard laboratory food and water were made available *ad libitum*. Prior to the initiation of experimental procedures, all animals were permitted to acclimatize to the housing environment for 1 week. All protocols were carried out in compliance with internationally accepted guidelines for animal welfare and approved by the Ethics Committee of the School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia (Approval No. 23/05/KEP-FKIKUAJ/2025).

Experimental Design and Treatment Regimen

Rats were randomly and equally divided into three groups. The control group (N = 5) received an equivalent volume of water administered orally and an equivalent volume of phosphate-buffered saline (PBS) solution administered intraperitoneally once daily. The aging model group (N = 5) was treated with an equivalent volume of water administered orally and intraperitoneal D-gal (300 mg/kg body weight/day; Merck, Darmstadt, Germany) to induce accelerated aging. The treatment group (N = 5) received the same dose of intraperitoneal D-gal in combination with *A. keiskei* leaf extract at 200 mg/kg body weight/day, administered orally by orogastric gavage. All interventions were continued daily for a period of 6 consecutive weeks. The selected doses and treatment duration were determined based on preliminary, unpublished studies assessing efficacy and safety.

Functional Assessment of Skeletal Muscle Performance

Functional assessments were performed at the end of the 6-week treatment period before blood and tissue collection. Animals were transferred to the testing room and allowed to acclimatize for at least 30 min before testing. Handgrip strength was assessed using a grip strength meter (76-1065, Harvard Apparatus, Holliston, MA, USA). Each rat was allowed to grasp the metal grid with its forelimbs and was gently pulled backward in a horizontal direction until grip release; the peak force was recorded in grams. Three trials were performed for each rat with a rest interval of at least 5 min between trials, and the mean value was recorded. Muscle endurance was assessed using the wire-hanging test. Each rat was placed on a wire grid, which was then inverted above a padded surface, and the latency to fall was recorded in seconds. Three trials were performed with 5-min rest intervals, with a maximum cutoff time of 60 s per trial, and the mean latency was used for analysis. Functional testing was performed by an investigator blinded to group allocation.

Skeletal Muscle Sampling

At the end of the experimental period, rats were anesthetized using intraperitoneal ketamine 300 mg/kg and xylazine 40 mg/kg according to AVMA Guidelines on Euthanasia:

Table 1. Primers Used for RT-qPCR.

Genes	GenBank Acc. No.	Forward	Reverse
Fbxo32	NM_133521	5′-GCCTTCAAAGGCCTCACGAT-3′	5′-CCACAGTAGCCGGTCTTCAC-3′
Trim63	NM_080903	5′-CATAGCAGAGGCCTTGAGGG-3′	5′-CATTGGTGTCCCTCTGTGG-3′
Tnf	NM_012675	5′-CAGCAGATGGGCTGTACCTT-3′	5′-AAATGGCAAATCGGCTGACG-3′
Il1b	NM_031512	5′-CCTATGTCTTGCCCGTGGAG-3′	5′-CACACACTAGCAGGTCGTCA-3′
Ppargc1a	NM_031347	5′-GGACATGTGCAGCCAAGACT-3′	5′-GGGTGTGGTTTGCATGGTTC-3′
Tfam	NM_031326	5′-GCTTCCAGGAGGCTAAGGAT-3′	5′-CCCAATCCCAATGACAACTC-3′
Sod2	NM_017051	5′-AGGGCCTGTCCCATGATGTC-3′	5′-AGAAACCCGTTTGCCTCTACT-3′
Txn1	NM_053800	5′-GGTAGTGGACTTCTCTGCCAC-3′	5′-AGGTCGGCATGCATTTGACT-3′
Prdx1	NM_057114	5′-AAATTGGGCATCCTGCTCCC-3′	5′-AGCAATGATCTCCGTGGGAC-3′
Gapdh	NM_017008	5′-CACCTAGACAAGGATGCAGA-3′	5′-GCATGACTGGAGGAATCACA-3′

2020 Edition. After deep anesthesia was confirmed by the absence of pedal withdrawal and corneal reflexes, blood was collected by terminal cardiac puncture, resulting in euthanasia by exsanguination under deep anesthesia. Death was confirmed before gastrocnemius muscles were rapidly excised. Tissue samples were washed in ice-cold PBS to remove residual blood and subsequently preserved either in RNAlater solution or PBS. All samples were stored at −20 °C until further biochemical and molecular analyses were performed.

Measurement of Serum Creatine Kinase Isoenzyme (CK-MB)

Blood was collected from the cardiac puncture at the end of the experiment and centrifuged at 3,000 g for 10 min at 4 °C, and the serum was stored at −80 °C until further use. The levels of creatine kinase–MB (CK-MB) were measured using the ELISA kits (Elabscience Biotechnology Co., Ltd, Wuhan, China) in adherence to the manufacturer's instructions. Because CK-MB can originate from both cardiac and skeletal muscle, it was interpreted as a non-specific marker of muscle tissue injury rather than as an independent skeletal-muscle-specific marker.

Determination of NF-κB p65 Protein Levels

Frozen muscle tissues were homogenized on ice and centrifuged for 20 min at 3,000 rpm to obtain tissue lysates. The supernatants were collected, and total protein concentrations were measured using standard Biuret assays. The NF-κB p65 protein levels were measured using a commercial ELISA kit (Elabscience, Wuhan, China; Cat. No. E-EL-R0674) according to the manufacturer's protocol.

Examination of mRNA Expression

Total RNA was isolated from gastrocnemius muscle tissue using the SV Total RNA Isolation System (Promega, Madison, WI, USA), following the manufacturer's guidelines. The extracted RNA was reverse-transcribed into complementary DNA using the AccessQuick™ RT-PCR System (Promega). Quantitative real-time PCR was subsequently performed using GeneAce SYBR® qPCR Mix α (Nippon Gene, Tokyo, Japan) and gene-specific primers (Table 1) on a Thermal Cycler Dice Real-time PCR platform (Takara Bio Inc., Shiga, Japan). Relative gene expression levels were calculated using the comparative $2^{-}\Delta\Delta Ct$ method, with *Gapdh* serving as the endogenous control gene.

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test. Each experimental group consisted of five rats ($n = 5$ per group). A p value of less than .05 was considered statistically significant.

Results

Metabolomic Profiling of A. keiskei Extract

Qualitative LC-HRMS profiling of *A. keiskei* leaf extract revealed a chemically diverse metabolite profile comprising phenolic acids, flavonoids, flavonoid glycosides, a prenylated chalcone, organic acids, and a sugar derivative (Table 2). The major putatively identified compounds included chlorogenic acid, caffeic acid, cynaroside, quercetin, quercetin-3β-D-glucoside, trifolin, apigenin, baicalein, delphinidin, xanthoangelol F, citric acid, malic acid, quinic acid, and trehalose. These findings provide a qualitative phytochemical profile of the extract. Because authenticated reference standards were not used for targeted quantification, the identified compounds should be interpreted as putative annotations rather than validated quantitative markers. Overall, these findings indicate that *A. keiskei* leaf extract is enriched with compounds with potential antioxidant and cytoprotective relevance.

Table 2. Putatively Identified Major Phytochemicals in *Angelica keiskei* Leaf Extract by LC-HRMS.

No.	Compound	Molecular Formula	RT (min)	Mass (Da)	Compound Class
1.	Chlorogenic acid	$C_{16}H_{18}O_9$	0.81	354.09426	Phenolic acid
2.	Caffeic acid	$C_9H_8O_4$	3.09	180.04197	Phenolic acid
3.	Cynaroside	$C_{21}H_{20}O_{11}$	4.30	448.10000	Flavonoid glycoside
4.	Quercetin	$C_{15}H_{10}O_7$	4.12	302.04248	Flavonol
5.	Quercetin-3β-D-glucoside	$C_{21}H_{20}O_{12}$	4.11	464.09491	Flavonol glycoside
6.	Trifolin	$C_{21}H_{20}O_{11}$	5.04	448.10008	Flavonoid glycoside
7.	Apigenin/baicalein-like isomer	$C_{15}H_{10}O_5$	6.69	270.05254	Flavone isomer
8.	Delphinidin	$C_{15}H_{11}O_7$	4.12	303.05030	Anthocyanidin
9.	Xanthoangelol F	$C_{26}H_{30}O_4$	13.65	406.21407	Prenylated chalcone
10.	Citric acid	$C_6H_8O_7$	1.04	192.02669	Organic acid
11.	D-(+)-malic acid	$C_4H_6O_5$	0.76	134.02122	Organic acid
12.	D-(−)-quinic acid	$C_7H_{12}O_6$	2.70	192.06302	Organic acid
13.	α,α-trehalose	$C_{12}H_{22}O_{11}$	0.73	342.11507	Sugar derivative

Profile of Muscle Atrophy in D-gal- and A. keiskei-treated Rat

To determine the effects of D-gal and *A. keiskei* extract on skeletal muscle function and atrophy-related markers, handgrip strength, wire-hanging latency, serum CK-MB levels, and the mRNA expression of the muscle-specific ubiquitin ligases *Fbxo32* (atrogin-1) and *Trim63* (MuRF1) were assessed. D-gal-treated rats exhibited reduced handgrip strength (Figure 1A) and shorter wire-hanging latency (Figure 1B) compared with the control group. Treatment with *A. keiskei* tended to improve handgrip strength relative to the D-gal group, although the difference did not reach statistical significance (p = .067). In contrast, the improvement in wire-hanging latency was statistically significant. D-gal administration also significantly increased serum CK-MB levels (Figure 1C) and upregulated *Fbxo32* and *Trim63* expression in gastrocnemius muscle (Figure 1D and 1E). Treatment with *A. keiskei* significantly attenuated these changes. Because CK-MB is not skeletal-muscle-specific, these serum data were interpreted together with functional and atrophy-related mRNA findings rather than as independent evidence of skeletal muscle injury. These findings indicate that *A. keiskei* improves selected functional and molecular features associated with D-gal-induced skeletal muscle deterioration.

Effect of D-gal and A. keiskei Extract on Inflammatory Markers in Skeletal Muscle

To evaluate the involvement of inflammatory signaling in D-gal-induced skeletal muscle aging, NF-κB p65 protein levels were measured in gastrocnemius muscle tissue. Rats exposed to D-gal showed significantly increased NF-κB p65 levels compared with the control group. Treatment with *A. keiskei* significantly reduced NF-κB p65 protein levels relative to the D-gal group; the treatment group was not significantly different from the control group (Figure 2A). D-gal administration also increased *Tnf* (Figure 2B) and *Il1b* (Figure 2C) mRNA expression. *A. keiskei* treatment showed a tendency to reduce *Tnf* and *Il1b* expression relative to the D-gal group; however, these differences did not reach statistical significance (p = .063 and p = .104, respectively). These directional changes were consistent with the reduction in NF-κB p65 protein levels.

Regulation of Mitochondrial Biogenesis by A. keiskei Extract

To evaluate the impact of *A. keiskei* on mitochondrial biogenesis, *Ppargc1a* mRNA expression was assessed in skeletal muscle. D-gal treatment significantly reduced *Ppargc1a* transcript levels compared with controls, indicating suppression of mitochondrial regulatory signaling under aging. In contrast, *A. keiskei* administration significantly restored *Ppargc1a* mRNA expression relative to the D-gal group (Figure 3A). Consistent with changes in *Ppargc1a*, D-gal markedly downregulated the expression of mitochondrial transcription factor A (*Tfam*), reflecting impaired mitochondrial transcriptional capacity. Treatment with *A. keiskei* significantly increased *Tfam* mRNA levels compared with D-gal alone, indicating partial recovery of mitochondrial biogenesis-related transcriptional programs (Figure 3B). Collectively, these findings demonstrate that *A. keiskei* preserves PGC-1α–dependent mitochondrial regulation in D-gal–induced skeletal muscle aging.

Modulation of Mitochondrial Antioxidant Defense by A. keiskei

To examine mitochondrial redox regulation, the mRNA expression of antioxidant enzymes, including superoxide dismutase 2 (*Sod2*), thioredoxin 1 (*Txn1*), and peroxiredoxin

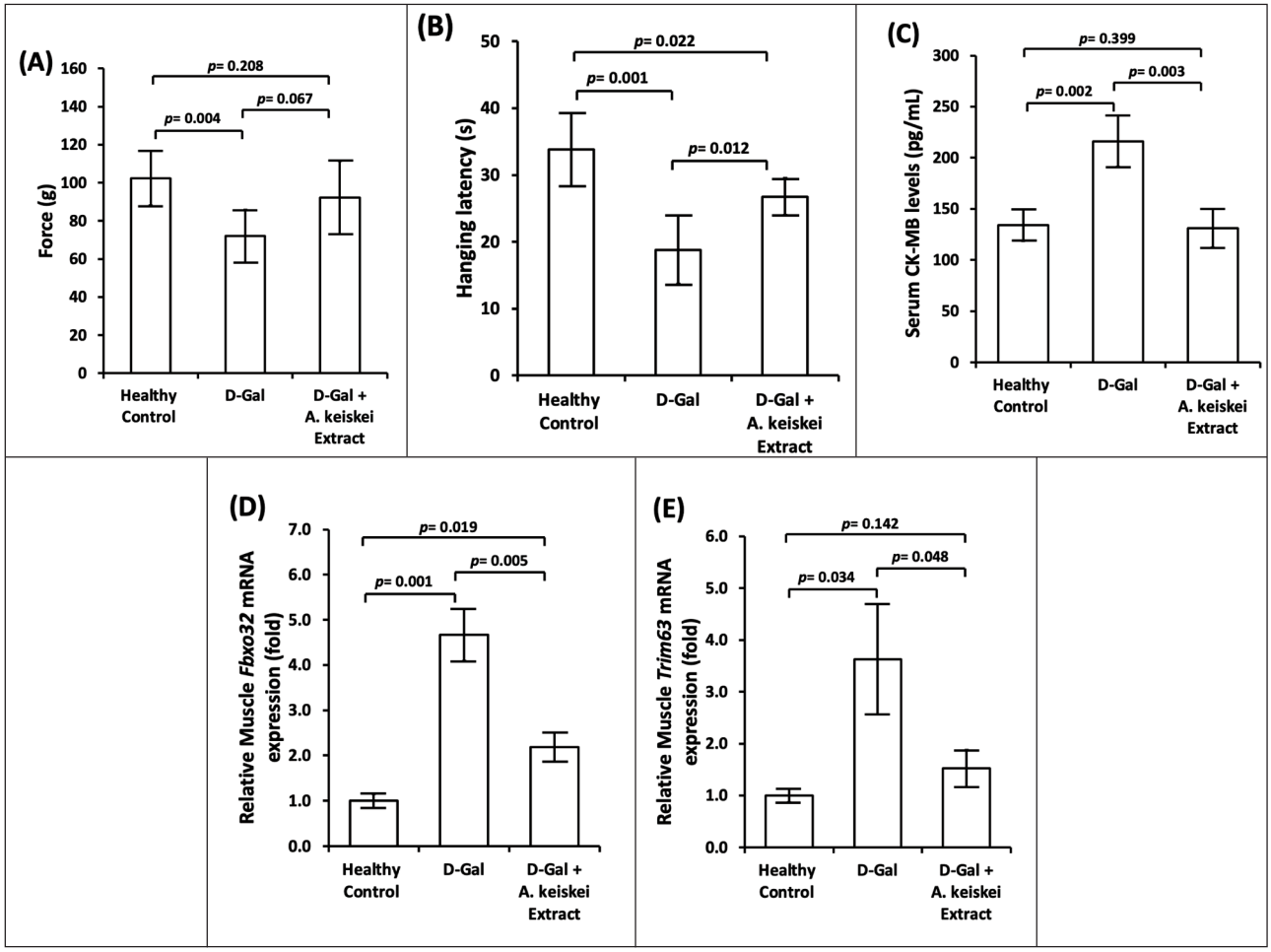

Figure 1. Effects of D-galactose and *Angelica keiskei* Extract on Skeletal Muscle Function and Atrophy-related Biomarkers. (A) Handgrip Strength, (B) Wire-hanging Latency, (C) Serum Creatine Kinase–MB (CK-MB) Levels, (D) *Fbxo32* (Atrogin-1) mRNA expression, and (E) *Trim63* (MuRF1) mRNA Expression in Gastrocnemius Muscle Tissue. Data are Presented as Mean ± SEM (n = 5 Per Group). Statistical Significance Was Determined Using One-way ANOVA Followed by Tukey's Post Hoc Test.

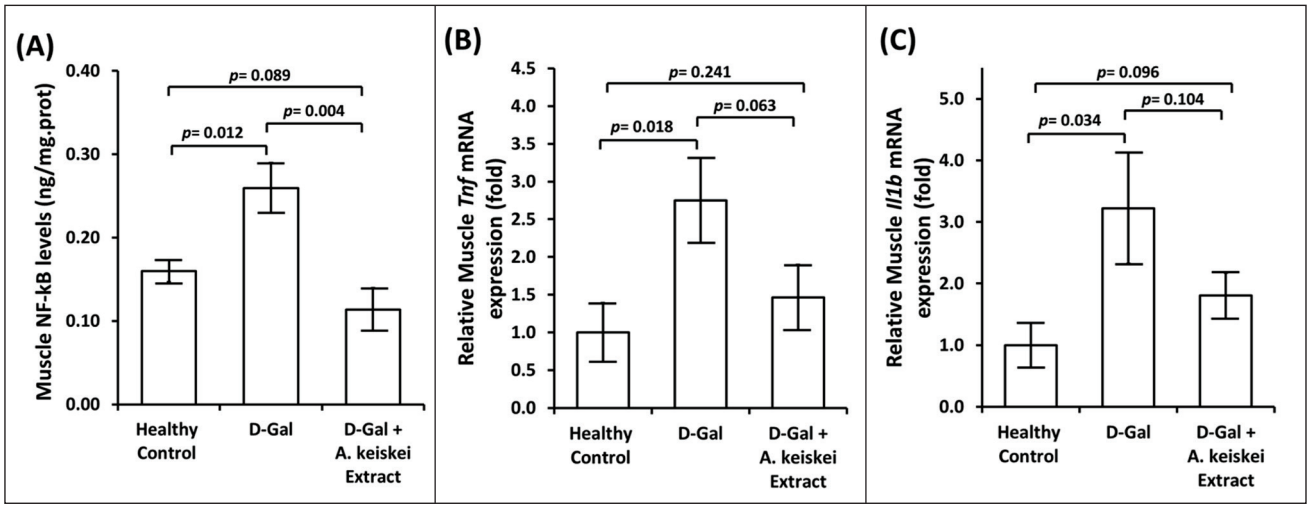

Figure 2. Effects of D-galactose and *Angelica keiskei* Extract on Inflammatory Markers in Skeletal Muscle. (A) NF-κB p65 Protein Levels in Gastrocnemius Muscle Tissue. (B) *Tnf* mRNA Expression and (C) *Il1b* mRNA Expression in Skeletal Muscle. Data are Presented as Mean ± SEM (n = 5 Per Group). Statistical Analysis Was Performed Using One-way ANOVA Followed by Tukey's Post Hoc Test.

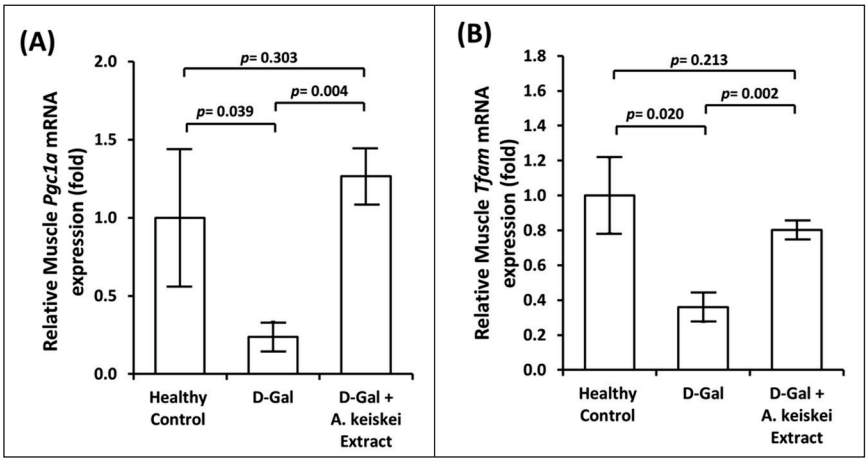

Figure 3. Effects of D-galactose and *Angelica keiskei* Extract on Mitochondrial Biogenesis-related Gene Expression in Skeletal Muscle. (A) Ppargc1a mRNA Expression and (B) *Tfam* mRNA Expression in Gastrocnemius Muscle Tissue. Data are Presented as Mean ± SEM (*n* = 5 Per Group). Statistical Analysis Was Performed Using One-way ANOVA Followed by Tukey's Post Hoc Test.

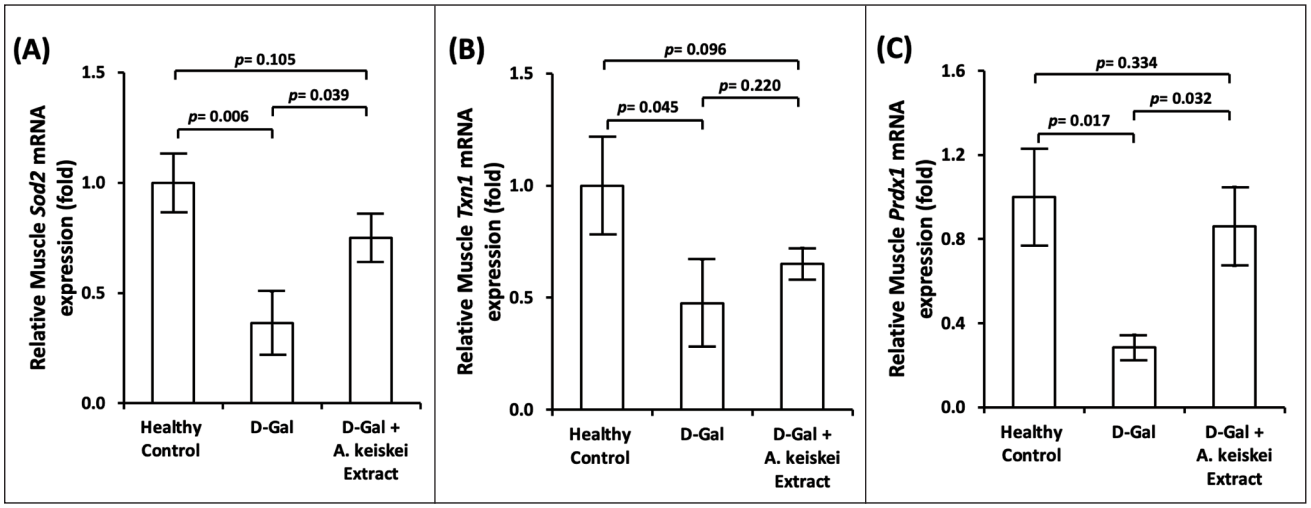

Figure 4. Effects of D-galactose and *Angelica keiskei* Extract on Mitochondrial Antioxidant Gene Expression in Skeletal Muscle. (A) *Sod2*, (B) *Txn1,* (C) *Prdx1* mRNA Expression in Gastrocnemius Muscle Tissue. Data are Presented as Mean ± SEM (*n* = 5 Per Group). Statistical Analysis Was Performed Using One-way ANOVA Followed by Tukey's Post Hoc Test.

1 (*Prdx1*), was analyzed in skeletal muscle. D-gal treatment significantly reduced *Sod2* (Figure 4A), *Txn1* (Figure 4B), and *Prdx1* (Figure 4C) expression compared with the control group. Treatment with *A. keiskei* significantly restored *Sod2* and *Prdx1* expression relative to the D-gal group. Although *Txn1* expression showed a tendency toward recovery, the difference did not reach statistical significance ($p = .220$). These findings indicate partial restoration of antioxidant defense pathways by *A. keiskei* treatment.

Discussion

The present study provides preliminary evidence that *A. keiskei* leaf extract attenuates selected functional, biochemical, and molecular features of D-gal-induced skeletal muscle aging. D-gal increased NF-κB p65 protein levels and reduced *Ppargc1a*, *Tfam*, and antioxidant-related gene expression in skeletal muscle. Treatment with *A. keiskei* reduced NF-κB p65 protein levels and was associated with restoration of PGC-1α-related mitochondrial regulatory markers (*Sod2*, *Txn1*, and *Prdx1*). Because the ELISA assay measured total NF-κB p65 protein concentration rather than phosphorylated p65, nuclear translocation, DNA-binding activity, or transcriptional activity, the present findings should be interpreted as changes in NF-κB p65 protein levels that are consistent with altered inflammatory signaling, not as direct evidence of NF-κB pathway activation or inhibition.

D-gal is a well-established experimental model of accelerated aging, characterized by excessive production

of free radicals, chronic inflammation, and mitochondrial dysfunction across multiple tissues,[30] including skeletal muscle.[19,31] Previous studies have shown that prolonged D-gal exposure activates NF-κB signaling through oxidative stress–mediated mechanisms,[32] resulting in the transcriptional activation of several pro-inflammatory cytokines and transcriptional repression of mitochondrial regulators.[33,34] Canonically, NF-κB p65 promotes the transcription of pro-inflammatory genes, including *Tnf* and *Il1b*, thereby amplifying inflammatory signaling in skeletal muscle.[35,36] Therefore, the concurrent increase in NF-κB p65 protein levels and *Tnf*/*Il1b* expression in the present study supports activation of NF-κB–dependent inflammatory signaling following D-gal exposure. In skeletal muscle, NF-κB has been strongly implicated in age-related muscle dysfunction, as it promotes catabolic signaling, suppresses mitochondrial biogenesis, and interferes with redox homeostasis.[37,38]

One of the most critical downstream consequences of NF-κB is repression of PGC-1α. PGC-1α is a transcriptional coactivator that plays a central role in mitochondrial biogenesis, oxidative metabolism, and antioxidant defense.[39] NF-κB has been shown to suppress PGC-1α transcription directly or indirectly via inflammatory mediators, thereby uncoupling mitochondrial maintenance from energetic demand.[11] Mechanistically, the p65 subunit of NF-κB can interfere with PGC-1α gene transcription by binding to regulatory regions of the PGC-1α promoter or by recruiting transcriptional repressors that limit PGC-1α expression under inflammatory conditions.[11,12] In addition to this direct transcriptional repression, NF-κB leads to the synthesis of TNF-α and IL-1β, notable pro-inflammatory cytokines, which further suppress PGC-1α expression through downstream signaling cascades involving stress-activated kinases and redox-sensitive pathways.[40] Consistent with this mechanism, D-gal administration in the present study significantly reduced PGC-1α mRNA expression, followed by reduced expression of *Tfam* and *SOD2*, two canonical PGC-1α target genes essential for mitochondrial DNA transcription and mitochondrial antioxidant capacity, respectively.[33,41] This coordinated suppression is consistent with previous in vitro studies of the cochlear basilar membrane and age-related hearing loss demonstrating that chronic D-gal exposure induces mitochondrial dysfunction through oxidative stress.[42] Together, our findings reinforce the concept that inflammation-driven repression of PGC-1α is a central molecular event linking aging-related inflammation to mitochondrial dysfunction in skeletal muscle.

Treatment with *A. keiskei* extract effectively counteracted these D-gal–induced changes. The observed reduction in NF-κB p65 levels suggests that *A. keiskei* suppresses pro-inflammatory signaling, thereby alleviating transcriptional repression of PGC-1α. Restoration of PGC-1α expression by *A. keiskei* was accompanied by increased *Tfam* and *SOD2* mRNA levels, indicating reactivation of mitochondrial biogenesis and antioxidant defense programs. This interpretation is supported by accumulating evidence demonstrating potent anti-inflammatory properties of *A. keiskei* and its bioactive chalcones, particularly 4-hydroxyderricin and xanthoangelol, which have been shown to suppress inflammatory signaling in multiple experimental systems.[43] Several studies have reported that *A. keiskei* extracts enforce suppression of IκBα degradation and NF-κB nuclear translocation, thereby reducing transcription of proinflammatory genes. By dampening inflammatory signaling, *A. keiskei* may sustain mitochondrial biogenesis and antioxidant defense gene expression.[39] This mechanism is particularly relevant in skeletal muscle, where chronic low-grade inflammation disrupts mitochondrial homeostasis and accelerates aging-related functional decline.[44,45] Nevertheless, because upstream NF-κB regulatory events and direct mitochondrial functional assays were not measured, the present data should be interpreted as associative molecular evidence rather than definitive proof of pathway-level suppression or mitochondrial functional restoration.

The protective effects of *A. keiskei* are biologically plausible given its phytochemical composition, which is rich in chalcones and flavonoids. These compounds have been reported to exert potent anti-inflammatory and antioxidant activities.[46] Chalcones from *A. keiskei* (such as 4-hydroxyderricin and xanthoangelol) have been shown to prevent dexamethasone-induced muscle wasting by antagonizing glucocorticoid receptor signaling, suppressing FoxO3a-dependent ubiquitin–proteasome activation, and thereby limiting muscle protein degradation in both myotube and murine models.[24,47] Previous studies investigating *A. keiskei* in skeletal muscle contexts have primarily focused on anabolic signaling pathways, muscle atrophy, and myogenesis, demonstrating improvements in muscle mass, differentiation, and functional outcomes.[24]

Notably, the present study included limited functional assessments using handgrip strength and wire-hanging tests but did not include histological validation, muscle-fiber morphometry, protein-level confirmation, or direct measurements of mitochondrial function. While fiber composition and mitochondrial morphology are important features of skeletal muscle aging, molecular dysregulation often precedes overt phenotypic decline.[48] From a translational perspective, the protective effects of *A. keiskei* observed in this study suggest its potential as a phytochemical strategy for preventing and/or treating aging skeletal muscle. However, extrapolation to humans should be made cautiously, as effective dosage, bioavailability, metabolism, and long-term safety may differ substantially between experimental animals and older adults. Ultimately, well-designed clinical studies are required to determine whether *A. keiskei* supplementation can improve muscle function, inflammatory status, and mitochondrial health in aging human populations.

Although this study included functional assessments of skeletal muscle performance using handgrip strength and wire-hanging tests, muscle atrophy was not validated

histologically or by immunohistochemistry. In addition, while changes in PGC-1α-associated mitochondrial biogenesis and antioxidant defense markers were observed, direct measurements of mitochondrial function, including ATP production, mitochondrial respiration, membrane potential, and ROS generation, were not performed. NF-κB signaling was assessed through p65 protein levels and downstream inflammatory gene expression, whereas upstream regulatory events, such as IκB phosphorylation, IκB degradation, IKK activation, and p65 nuclear translocation, were not examined. Furthermore, LC-HRMS profiling provided qualitative putative identification of extract constituents, but validated quantitative standardization of characteristic bioactive markers was not performed.

Conclusion

In conclusion, this study provides preliminary preclinical evidence that *A. keiskei* leaf extract may attenuate selected features of D-gal-induced skeletal muscle aging. These effects were associated with reduced NF-κB p65 protein levels, increased *Ppargc1a* and *Tfam* mRNA expression, partial restoration of antioxidant-related gene expression, and improvement in selected functional and atrophy-associated outcomes. Further studies incorporating larger sample sizes, dose-response analysis, standardized extract quantification, histological validation, protein-level confirmation, and direct mitochondrial functional assays are required before the translational potential of *A. keiskei* can be established.

Authors' Contribution

All authors made substantial contributions to conception and design, acquisition of data, or analysis, and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors requirements/guidelines.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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Supplemental Material

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