

Publisher: J-INSTITUTE
ISSN: 2435-0702

Website: www.j-institute.jp/kinesiology/
Editor: kinesiology@j-institute.jp

Corresponding author
E-mail: smkmana@naver.com

dx.doi.org/10.22471/kinesiology.2020.5.2.65

© 2020 J-INSTITUTE

Eucommiae Cortex(Eucommia Ulmoides Oliver) Modulates MUSCLE Atrophy In Aged Mice

Sanghyun Ahn

Semyung University, Jecheon, Republic of Korea

Abstract

Purpose: Although exercise is the only way to suppress sarcopenia, using it for treating sarcopenia in the elderly is very difficult. Sports supplements used for the purpose of maximizing the benefits of exercise may include drugs that directly modulate sarcopenia. Therefore, the intake of sports supplements can be expected to have a synergistic effect of strengthening muscles in combination with exercise.

Methods: In this study, we attempted to find a candidate adjuvant that might have a synergistic effect in the treatment of sarcopenia. To test the effect of Eucommiae(Eucommia ulmoides Oliver) cortex extract, which is used in Korean herbal medicines, in the treatment of sarcopenia, mice were divided into 3 groups: a control group(CON), a sarcopenia model group (age-elicited group; AEG), and a group consisting of sarcopenia model mice being administered with Eucommiae cortex extract(ECTG).

Results: We identified muscle loss in aged mice and applied this mouse model to evaluate the efficacy of sports supplements. Muscle loss was reduced as a result of treatment with Eucommiae cortex extract to aging mice. The expression of Caspase-3 was measured in the femur using specific antibodies. Muscle mass was analyzed using dual-energy X-ray absorptiometry. In the AGE group, protein expression of Caspase-3 was increased; however, in the ECTG group, Caspase-3 expression was significantly reduced.

Conclusion: These findings suggested that Eucommiae cortex extract decreased muscle loss and muscle cell death by regulating Caspase-3.

[Keywords] Sarcopenia, Muscle Atrophy, Caspase-3, Eucommiae Cortex, Eucommia Ulmoides Oliver

1. Introduction

World Health Organization, in 2017, formally classified and defined sarcopenia as a disease characterized by less than normal muscle mass. Furthermore, the main cause of sarcopenia is decreased number of muscle cells because of aging and lack of physical activity[1]. Sarcopenia can be exacerbated by a nutritional imbalance in the muscles. After the onset of sarcopenia, rapid muscle loss is observed. Eventually, the ability to store energy also decreases, leading to fatigue. In addition, because basal metabolism decreases, weight can change frequently. In the case of diabetic patients, it is difficult to control blood sugar, leading to increased mortality[2]. Thus, one of the greatest challenges in treatment of sarcopenia lies in controlling the gradual loss of skeletal muscle mass and function because of aging.

To the best of our knowledge, exercise therapy is considered to be the only treatment for sarcopenia at present. Furthermore, there have been no reports of sarcopenia treatments approved by the U.

S. Food and Drug Administration. Exercise therapy is based on the study of the dynamics of body movements, to provide information on the health of all organs and systems, and it is considered to play a central role in the treatment of sarcopenia[3]. Therefore, exercise program development must be appropriately designed, accounting for the differences in individual's ability to exercise. Moreover, the importance of the intake of sports supplements to support the exercise program must also be emphasized.

In this study, we tried to demonstrate the effects of a candidate adjuvant mentioned in traditional Korean medicine that can modulate sarcopenia. *Eucommiae cortex*(*Eucommia ulmoides* Oliver) extract exhibits various effects in vivo, such as antioxidant, anti-osteoporosis, anti-diabetic, and anti-cancer[4]. In addition, it has been reported to be effective in treating depression caused by aging. There has been no in vivo study on *Eucommiae cortex*(*E. ulmoides* Oliver) extract to see if its effects, including antioxidant and anti-osteoporosis, can be effective against sarcopenia-induced muscle loss. Therefore, this study investigated whether *Eucommiae cortex* extract could be as effective as a prescription drug for sarcopenia, a senile disease.

2. Materials

2.1. Chemicals

Vectastain ABC kits and DAB kit were obtained from Vector Laboratories(Burlingame, CA, USA). Trichome stain kit and caspase antibody were purchased from Abcam(Cambridge, UK).

2.2. Water extraction of eucommiae cortex extract

The aqueous extract from the plant material was obtained by slightly modifying the methods described previously[5][6]. *Eucommiae cortex*(EC)(400 gm) was placed in 3,000 mL of distilled water and heated for 2 h at 100 °C. The water with the extract was filtered using a rotary evaporator. Using this method, we obtained 45.1 g of extract(yield: 11.3%).

2.3. In vivo study

The ICR mice(male; Jung-a bio, Gyeonggi-do, Korea) were maintained at 23 °C ± 2 °C with a 12 h light/dark cycle. All animal experiments and care were performed in accordance with institutional guidelines(SEMCARE 18-12-04). The mice were divided into 3 groups: control group(CON;8-week-old mice),; Aging-elicited group(AEG;50-week-old mice treated with normal saline),; AEG mice treated with EC(ECTG; 50-week-old mice treated with 0.51 g/kg/daily of EC extract).

2.4. Muscle mass analysis

This analysis was performed as described in a previous study[7]. The mice were anesthetized using sodium pentobarbital, and the muscle mass was analyzed using X-ray absorptiometry(Medikors Inc., Seoul, Korea). The bone mass was analyzed using dual-energy X-ray absorptiometry(DXA, Medikors Inc., Seoul, Korea).

2.5. Immunohistochemistry analysis

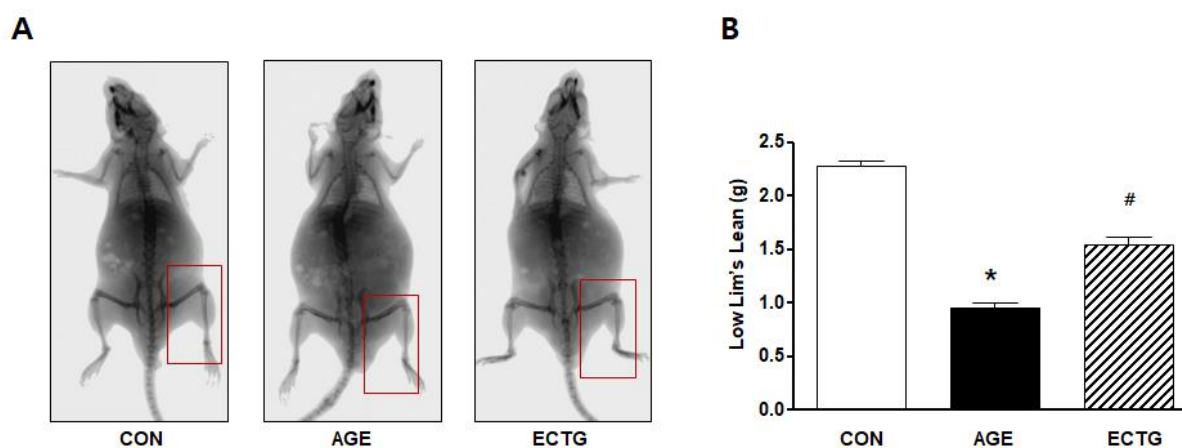
Immuno-histochemistry analysis was performed as described in a previous study[8][9]. Immunohistochemical results were quantified(means ± standard deviation) by image analysis using Image-Pro Plus(Media Cybernetics, USA). In the positive spots, randomly selected from each group, were imaged at a 400× magnification and at positive pixels/50,000,000 pixels. Statistical analysis was performed using SPSS ver. 23.0(IBM Corp., Armonk, NY, USA). One-way ANOVA was performed to verify significance (P <0.05), followed by the least significant difference test.

3. Results and Discussion

3.1. Eucommiae cortex extract regulates muscle atrophy in aged mice

The double-energy X-ray absorptiometry used in this study can determine the degree of bone density and muscle atrophy. To investigate the effect of EC extract on muscle mass and muscle atrophy in an in vivo model, we analyzed muscle mass around the femur. As shown in <Figure 1>, the level of muscle mass in the CON group was 2.28 ± 0.05 g. In AGE (sarcopenia-induced muscle loss group) muscle mass was 0.96 ± 0.04 g, whereas ECTG had a muscle mass of 1.54 ± 0.08 g. These results suggested that muscle loss in aged mice was consistent, and the administration of EC extract suppressed this muscle loss.

Figure 1. Effects of eucommiae cortex extract on muscle atrophy in mice.

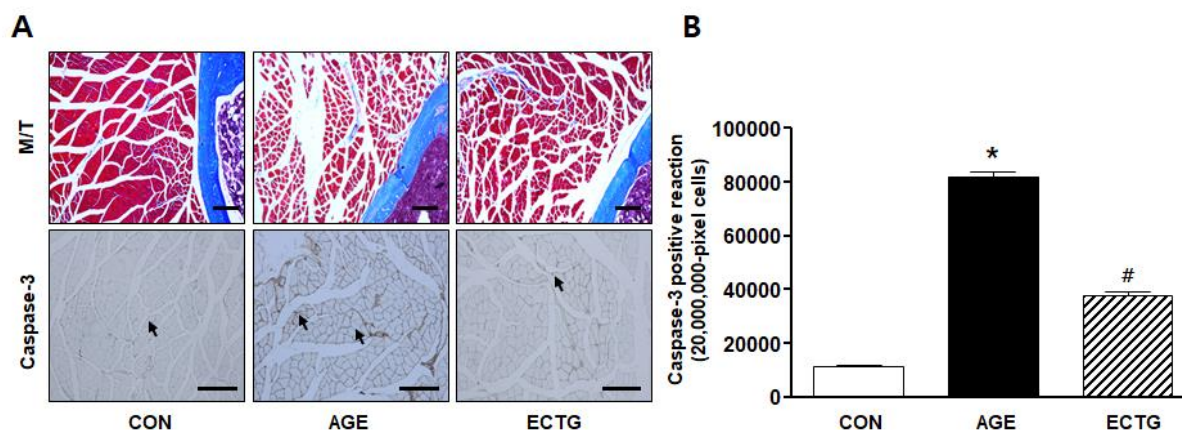


Note: (A) Representative photographs of DAX image; the red colored squares indicate muscle area. (B) Bar graphs indicate the muscle mass of each mouse in panel A. Data are expressed as means \pm standard deviation. * $p < 0.05$ vs. CON. CON, control group; AGE, aging-elicited group; ECTG, Eucommiae cortex treatment group.

3.2. Eucommiae cortex extract modulates muscle atrophy in aged mice by inhibiting caspase-3

Apoptosis performs important biological functions during regeneration and proliferation. However, apoptosis in muscle cells can induce muscle atrophy[10]. Furthermore, Siu et al. reported that inhibition of apoptosis attenuated muscle atrophy[11]. Therefore, controlling apoptosis of muscle cells may be one of the methods to control sarcopenia. The main signals of apoptosis pathway lead to the activation of caspases such as caspase-8, caspase-9, and caspase-12. Consequently, the upstream signals of these activated caspases stimulate the downstream caspase-3 to trigger apoptosis[12]. To determine whether EC extract can regulate aged-muscle death, we performed masson's trichrome stain (M/T) staining and immunohistochemistry using specific antibodies. As shown in <Figure 2A>, AGE showed significantly reduced muscle mass compared to that in CON. In contrast, muscle loss in ECTG was reduced as compared to that in AGE. As shown in <Figure 2B>, Caspase-3 expression in CON, AGE, and ECTG was $11,523 \pm 466$ pixels, $81,787 \pm 2022$ pixels, and $37,651 \pm 1,460$ pixels, respectively.

Figure 2. Effect of eucommiae cortex extract on caspase-3 expression.



Note: (A) Representative photographs of masson's trichrome stain (M/T) and Caspase-3 immunohistochemical staining in rat skeletal muscle. The arrows indicate OPC positive regions. (B) Bar graphs indicate the brown intensity of each caspase-3 photograph in panel (A). Data are expressed as means \pm standard deviation. * $p < 0.05$ vs. CON. CON, control group; AGE, aging-elicited group; ECTG, Eucommiae cortex treatment group.

Although exercise is a treatment modality for sarcopenia, vigorous exercise for patients with senile diseases or degenerative diseases is harmful. Therefore, we propose that the standard treatment of sarcopenia should be a combination of sports supplements and exercise. We suggest that *EC* extract might be a sports supplement for preventing sarcopenia.

3. References

- [1] Tournadre A & Vial G & Capel F & Soubrier M & Boirie Y. Sarcopenia. *Joint Bone Spine*, 86(3), 309-314 (2019).
- [2] Dhillon RJ & Hasni S. Pathogenesis and Management of Sarcopenia. *Clinics in Geriatric Medicine*, 33(1), 17-26 (2017).
- [3] Marzetti E & Calvani R & Tosato M & Cesari M & Di Bari M & Cherubini A & Collamati A & D'Angelo E & Pahor M & Bernabei R & Landi F & Sprintt Consortium. Sarcopenia: An Overview. *Aging Clinical and Experimental Research*, 29(1), 11-17 (2017).
- [4] Wang CY & Tang L & He JW & Li J & Wang YZ. Ethnobotany, Phytochemistry, and Pharmacological Properties of Eucommia Ulmoides: A Review. *The American Journal of Chinese Medicine*, 47(2), 259-300 (2019).
- [5] Seo IB & Ahn SH. A Korean Poly-herbal Medicine, SMO16, Regulates the RANKL/OPG Bone Remodeling in OVX Rat Model. *Kinesiology*, 5(1), 58-64 (2020). [Article]
- [6] Kang HR & Yang HJ. A Sports Nutrition Supplement, Albizia Julibrissin Extract Fermented with Kefir Yogurt Regulates Lipopolysaccharide Induced Inflammatory Signal. *Kinesiology*, 4(2), 1-6 (2019). [Article]
- [7] Seo IB & Ahn SH. Effect of SMO16, a Korean Poly-herbal Medicine, on Bone Mass in Ovariectomized Rat. *International Journal of Sport*, 2(2), 18-22 (2017).
- [8] Yoon TG & Hwang BY & Ahn SH & Kim KB. A Candidate Sports Nutrition Supplement, Lonicerae Flos, Attenuates Collagen-induced Arthritis in Mice. *International Journal of Sport*, 1(2), 1-5 (2016).
- [9] Choi NH & Jhun HJ & Lee DW & Chang HR & Lee KP. Protective Effect of Cynanchum Wilfordii Root Crude Extract on Acetaminophen-induced Liver Toxicity. *International Journal of Sport*, 2(1), 1-6 (2017). [Article]

- [10] Dupont-Versteegden EE. Apoptosis in Skeletal Muscle and Its Relevance to Atrophy. *World Journal of Gastroenterology*, 12(46), 7463-7466 (2006).
- [11] Siu PM & Alway SE. Mitochondria-associated Apoptotic Signalling in Denervated Rat Skeletal Muscle. *The Journal of Physiology*, 565(1), 309-323 (2005).
- [12] Fischer U & Jänicke RU & Schulze-Osthoff K. Many Cuts to Ruin: A Comprehensive Update of Caspase Substrates. *Cell Death Differentiation*, 10(1), 76-100 (2003).

4. Appendix

4.1. Authors contribution

	Initial name	Contribution
Author	SHA	<ul style="list-style-type: none"> -Set of concepts <input checked="" type="checkbox"/> -Design <input checked="" type="checkbox"/> -Getting results <input checked="" type="checkbox"/> -Analysis <input checked="" type="checkbox"/> -Make a significant contribution to collection <input checked="" type="checkbox"/> -Final approval of the paper <input checked="" type="checkbox"/> -Corresponding <input checked="" type="checkbox"/> -Play a decisive role in modification <input checked="" type="checkbox"/> -Significant contributions to concepts, designs, practices, analysis and interpretation of data <input checked="" type="checkbox"/> -Participants in Drafting and Revising Papers <input checked="" type="checkbox"/> -Someone who can explain all aspects of the paper <input checked="" type="checkbox"/>

4.2. Funding agency

This Research was Supported by the Basic Science Research Program through the National Research Foundation of Korea(NRF) Funded by the Ministry of Education(NRF-2019R1F1A105841412).