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The effects of combined low-intensity exercise with naringenin on regenerating protein family *in vivo* and *in vitro* after sciatic nerve injury

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The purpose of this study was to investigate the effect of combining low-intensity treadmill exercise with naringenin treatment on the expression of axonal regrowth-related proteins following sciatic nerve injury (SNI). The extracts were evaluated for cytotoxicity and cell viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and the effects of the extracts were analyzed *in vitro* using primary cultured Schwann cells and dorsal root ganglion neurons (DRGs). *In vivo*, axonal regrowth-related protein expression levels and neurite outgrowth were assessed through Western blot and immunofluorescence staining, respectively. The results indicated that neither extract exhibited cytotoxicity. In primary cultured Schwann cells, 10 μM naringin and 10 μM/50 μM naringenin significantly increased growth associated protein-43 (GAP-43) expression, while in DRGs, both naringin and naringenin treatments resulted in increased neurite length. For *in vivo* experiment, all animals were divided into the vehicle group, the naringin-treated group post-SNI (Gin), the naringenin-treated group post-SNI (Genin), the naringin and exercise group post-SNI (Gin+Ex), and the naringenin and exercise group post-SNI (Genin+Ex). Naringenin treatment after early SNI enhanced GAP-43 expression. Following 14 days of exercise combined with treatment, both GAP-43 and phosphorylated extracellular signal-regulated kinase levels were significantly increased in the Genin and the Genin+Ex groups, whereas phosphorylated-protein kinase B significantly increased only in the Genin+Ex group. Our findings suggest that naringenin, when used in conjunction with low-intensity treadmill exercise, may effectively promote the expression of axonal growth-related proteins following SNI.

Keywords: Sciatic nerve injury, Dorsal root ganglion, Low-intensity exercise, Naringin, Naringenin, Axonal regrowth

INTRODUCTION

The sciatic nerve is the longest nerve in the human body and innervates the muscles of the lower limbs. Sciatic nerve injury (SNI) primarily occurs due to histological changes such as swelling of the piriformis muscle or impacts on the gluteal region during sports activities, leading to motor dysfunction with muscle atrophy in the lower limbs and neuropathic pain, which diminishes quality of life (Wong et al., 2022).

Various therapeutic approaches have been proposed to promote the regeneration of damaged peripheral nerves and alleviate allodynia, including autologous nerve grafting (Daradka et al., 2021), stem cell and bone marrow stromal cell transplantation (Seo et al., 2021), treatment with herbal/plant extracts (Wang et al., 2013), and regular exercise (Cho and Seo, 2021). Treatment with herbal/plant extracts involves using single components extracted from plants, fruits, and medicinal herbs, which have demonstrated anti-inflammatory, anti-cancer, and antioxidant effects, proving increasingly effective as therapeutics for various diseases (Ożarowski and Karpiński, 2021; Sharma et al., 2021). Among various extracts, naringin and naringenin are components classified as flavanones, flavonoids extracted from citrus fruits (Shen et al., 2022).

Naringin is in the form of a glycoside bound to sugar, while naringenin is an aglycone form converted from naringin through

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hydrolysis by glycosidase enzymes (Balestrieri et al., 2003; Cavia-Saiz et al., 2010). In animal and clinical studies, naringin and naringenin have been shown to have antibacterial, anti-inflammatory, antiapoptotic, antioxidant, and anticancer effects. Previous research has reported that naringin and naringenin effectively improve neuropathic pain by reducing several inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) (Rao et al., 2021). They are also reported to be neuroprotective and reduce neurotoxicity and oxidative stress (Atoki et al., 2024).

Apart from treatment with herbal/plant extracts, regular exercise has been considered a low-cost therapeutic method with minimal side effects, commonly applied during the rehabilitation phase following peripheral and central nervous system injuries (Liao et al., 2017). In terms of exercise intensity and form, low-intensity treadmill exercise has been reported to promote the diameter and number of axons through the proliferation, migration and differentiation of Schwann cells in injured sciatic nerves (Arbat-Plana et al., 2017), suggesting the possibility of promoting peripheral nerve regeneration (Bobinski et al., 2011; Jung et al., 2014).

Thus, treatment with herbal/plant extracts and low-intensity treadmill exercise must be important effectors for specific reinnervation within the injured sciatic nerve. However current studies on herbal extract treatments related to promoting nerve regeneration primarily focus on pain relief through single-component treatment, without presenting direct evidence for axonal regrowth of damaged nerves. Moreover, studies reporting the broad and specific synergistic effects on motor and sensory functions in peripheral and central nerves after the combined application of exercise with citrus extract treatment are scarce. To overcome these limitations, it is crucial to investigate how the integration of herbal extract and exercise approaches influences both Schwann cell proliferation and axonal regrowth in the injured sciatic nerve.

Therefore, the purpose of this study was firstly to determine the appropriate concentrations of naringin and naringenin on cytotoxicity and cell survival *in vitro*, and secondly to elucidate the protein mechanisms related to Schwann cell proliferation and axonal regrowth during sciatic nerve regeneration *in vivo* following combined exercise and extract treatment.

MATERIALS AND METHODS

Experimental animals

Six-week-old Sprague-Dawley rats (150–160 g) were divided into five groups: the control group (vehicle group, Veh, n = 6), the naringin-treated group post-SNI (Gin, n = 6), the naringenin-treated group post-SNI (Genin, n = 6), the naringin and exercise group post-SNI (Gin+Ex, n = 6), and the naringenin and exercise group post-SNI (Genin+Ex, n = 6). The experimental animals were provided with ad libitum access to food (Samyang Co., Seoul, Korea) and water, and they were housed in a specialized laboratory animal center with controlled temperature (22°C), humidity (60%), and light/dark cycle (12 hr/12 hr). This study was conducted with the approval of the Jeju National University Animal Care and Use Committee (2022-0056).

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

To analyze cell viability, human neuroblastoma cells (SH-SY5Y) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (Hi-FBS, Gibco Inc., Billings, MT, USA) and 1% Penicillin-Streptomycin (Gibco Inc.). Cells were seeded at a density of 2×10^4 in 96-well plates. After 16 hr of incubation, naringin and naringenin were applied to each well for 1 hr, followed by stress induction with 600 μM H_2O_2 for 23 hr. Cell viability was determined by adding 0.2 mg/mL MTT (Sigma Aldrich, St. Louis, MA, USA). Cells were further incubated for 4 hr at 37°C, then centrifuged at 2,000 rpm for 5 min to remove the medium. After adding 200 μL of dimethyl sulfoxide to each well, plates were shaken for 1 hr to dissolve the cells, and cell viability was measured at 570 nm using an enzyme-linked immunosorbent assay reader.

SNI and extract administration

Animals were anesthetized using an animal inhalation anesthesia device (Jeungdo Bio and Plant, Seoul, Korea), and the left sciatic nerve was exposed. Surgical forceps were used to apply compression injuries to the sciatic nerve twice for durations of 1 min and 1 min each (Seo et al., 2021). Naringin (Purity 90%, Molecular weight 580.53, Sigma-Aldrich, St. Louis, MA, USA) and naringenin (Purity 95%, Molecular weight 272.25, Sigma-Aldrich) were used in both *in vivo* and *in vitro* experiments. Upon administration to the sciatic nerve, extracts were injected locally at the injury site immediately after SNI using a microsyringe. Postsurgery, animals were allowed to recover on a heating pad before being transferred to their cages once they regained consciousness.

Low-intensity treadmill exercise

Animals subjected to exercise were given a 2-day rest period

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following SNI before participating in the exercise regimen. Low-intensity walking exercise was conducted on an animal treadmill (Jeungdo Bio and Plant) for 30 min per day at a 0° incline and a speed of 5–8 m/min for up to 14 days (Cho and Seo, 2022).

Dorsal root ganglion neuron and Schwann cell culture

Dorsal root ganglions (DRGs) and Schwann cells were extracted 3 days after SNI. DRG culture dishes were refreshed with DMEM 12 hr post primary culture, and then we treated with naringin and naringenin at concentrations of 10 μM and 50 μM per well. After 36 hr of incubation, immunofluorescence staining was performed. To observe biochemical changes in cultured Schwann cells, culture dishes were treated with naringin and naringenin at 10 μM and 50 μM 12 hr after primary culture. Cells were harvested after 72 hr for Western blot analysis.

Western blot analysis

For Western blot analysis, primary cultured Schwann cells was prepared as described elsewhere. The dissected sciatic nerves were rinsed with phosphate-buffered saline (PBS) and lysed in Triton lysis buffer. The nucleus and cytoplasm were separated using nuclear extraction buffer and cytosol extraction buffer. Denatured proteins were resolved by sodium dodecyl sulphate-polyacrylamide gel and then transferred onto a polyvinylidene difluoride membrane on ice at 200 mA for 2 hr. The membranes were blocked with 5% skim milk and 0.1% Tween 20 in tris buffered saline for 30 min at room temperature, followed by overnight incubation with primary antibodies at 4ºC. Primary antibodies used included anti-growth associated protein-43 (GAP-43, 43 kDa, mouse monoclonal antibody, 1:1,000, Santa Cruz Biotechnology, CA, USA), anti-phosphorylated extracellular signal-regulated protein kinase 1/2 (p-ERK1/2, 42-44 kDa, rabbit polyclonal antibody, 1:2,000, Cell Signaling Biotechnology, Danvers, MA, USA), anti-phosphorylated protein kinase B (p-AKT, 60 kDa, rabbit monoclonal antibody, 1:1,000, Cell Signaling), and anti-β-actin (43 kDa, 1:2,000, Santa Cruz). Secondary antibodies were goat anti-mouse or goat anti-rabbit horseradish peroxidase conjugated secondary antibodies (1:1,000, GeneTex Inc., Irvine, CA, USA). Reactions were developed using Westar ECL substrates (Cyanagen, Bologna, Italy) and analyzed with a Chemidoc system (Bio-Rad, Hercules, CA, USA).

Immunofluorescence staining

For analyzing neurite length of the cultured DRGs, culture dishes were fixed at room temperature for 40 min with 4% paraformaldehyde and 4% sucrose in PBS, permeabilized with 0.5% Nonidet P-40 in PBS, and incubated with 2.5% horse serum and 2.5% bovine serum albumin at room temperature for 4 hr. Neurons were reacted with the anti-neurofilament-200 (rabbit polyclonal antibody, NF-200, 1:700, Sigma-Aldrich) for 12 hr. Subsequently, cells were stained with rhodamine-goat anti-rabbit secondary antibody (1:600, Molecular Probes, Eugene, OR, USA) for 1 hr at room temperature. Stained samples were observed under a fluorescence microscope (Nikon model E-600, Nikon, Tokyo, Japan), and images were captured with a digital camera and analyzed using Adobe Photoshop Software (version CS6; Adobe, San Jose, CA, USA). The number and length of DRG neurites were evaluated using i-Solution software (Image and Microscope Technology, Irvine, CA, USA). The average neurite length of primary cultured DRG neurons was measured by analyzing at least 30 randomly selected sensory neurons per experiment. The number of neurite length number were analyzed by an examiner blinded to the experimental conditions to ensure reliability.

Statistical analysis

Statistical Analysis Data processing and graph preparation for this study were conducted using Prism 6 (GraphPad, La Jolla, CA, USA). One-way repeated measures analysis of variance was performed to analyze differences between groups, followed by Duncan *post hoc* test. The significance level was set at $P < 0.05$.

RESULTS

Naringin and naringenin prevent the loss of cell viability *in vitro*

To investigate the effect of naringin and naringenin on cell viability, we examined the cytotoxicity of two extracts in a concentration-dependent method using SH-SY5Y cells. As shown Fig. 1A and B, naringenin significantly increased cell population at concentrations of 6.25 μM, 25 μM, and 50 μM, but naringin did not bring about a significant difference at all concentration. To assess the toxicity of both compounds, cultured SH-SY5Y cells were exposed to H_2O_2. The number of SH-SY5Y cells was greatly decreased, but treatment of naringin and naringenin from low concentration to 62.5 μM significantly improved viability of SH-SY5Y cells (Fig. 1C and D).

Naringenin is more effective in activating regenerative responses *in vitro*

After SNI, Schwann cells and DRG were subjected to primary cell culture. Cultured Schwann cells were used for quantitative

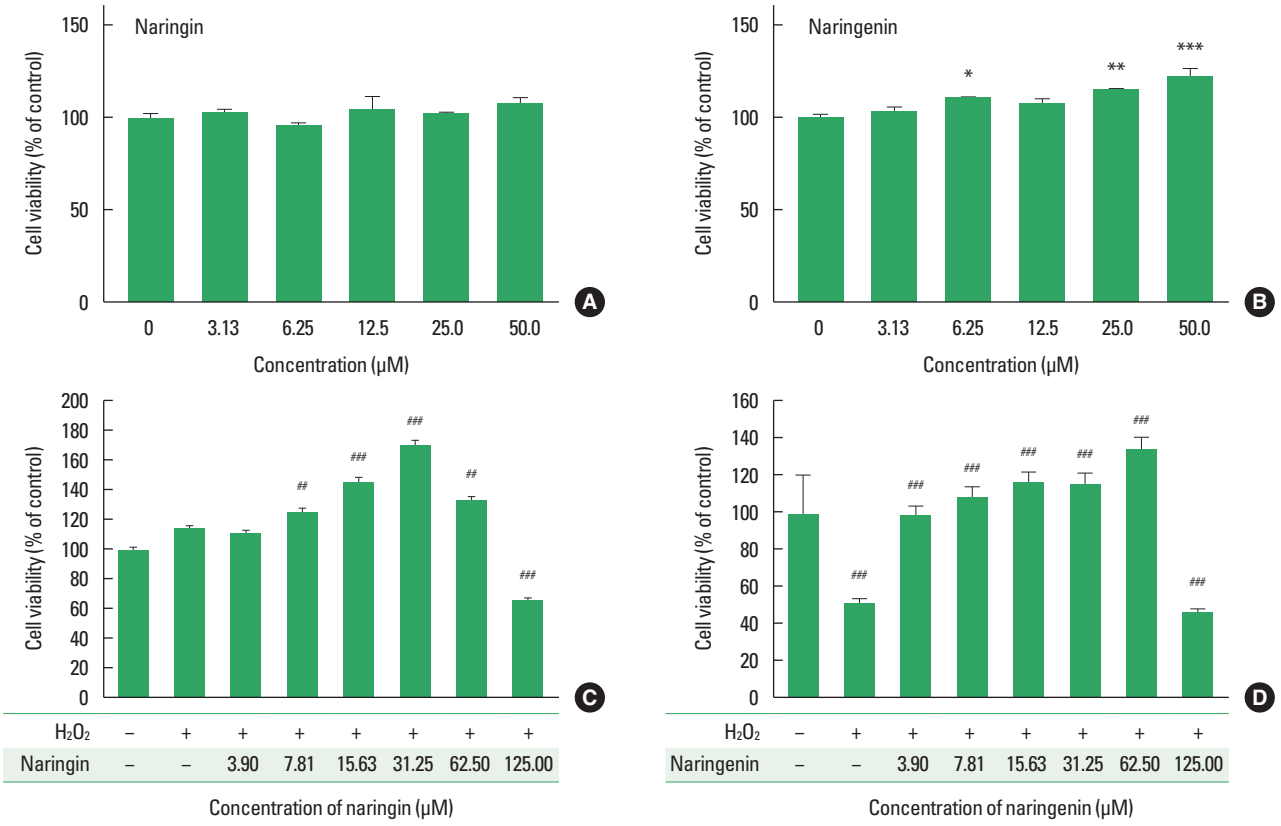

Fig. 1. Role of naringin and naringenin on cytotoxicity and cell viability in SH-SY5Y cells. (A, B) Quantitative graph of cell proliferation after treatment with naringin and naringenin. (C, D) Effect of naringin and naringenin on cytotoxicity in H_2O_2-exposed SH-SY5Y cell. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. nontreated group. $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs. only H_2O_2 treated group.

analysis of GAP-43 expression via Western blot technique, and DRGs were used for axonal sprouting assessment through immunofluorescence staining with anti-NF-200 antibody. Groups treated with Gin50 ($P<0.05$), and Genin10 ($P<0.05$) and Genin50 ($P<0.01$) significantly increased levels of GAP-43 expression in Schwann cells. In specific, the Genin treatment is more effective in inducing regeneration molecule *in vitro* compared to the Gin group (Fig. 2A). In primary DRGs culture, DRGs treated with the 50 μM of Gin ($P<0.05$) or Genin ($P<0.001$) showed longer neurite length compared to the Veh group (Fig. 2B), suggesting that the naringenin might be the most regulator for facilitating axonal elongation in the injured sciatic nerve.

Naringenin upregulates GAP-43 level *in vivo* during the early stage of the regeneration

To investigate the *in vivo* regulatory capacity of naringin and naringenin in the early stages of peripheral nerve regeneration, naringin or naringenin were injected into the injured sciatic nerve immediately after SNI and then sciatic nerves were prepared at 1 and 3 days after SNI. GAP-43 tended to increase over time after SNI to induce spontaneous axonal regeneration, and the Genin group ($P<0.001$ and $P<0.05$, respectively) showed a statistically significant increase in GAP-43 expression compared to the Gin group at both 1 and 3 days after injury (Fig. 3).

Combined exercise with naringenin activates *in vivo* protein expression for regeneration

It has been well known that GAP-43, p-AKT, and p-ERK1/2 are associated with Schwann cell proliferation, axonal elongation and pain relief after SNI. At 14 days after SNI, expression levels of regenerative proteins in the injured sciatic nerve were measured by Western blot analysis. The Genin+Ex group ($P<0.001$) significantly increased GAP-43, p-AKT, and p-ERK1/2 protein levels into the injured sciatic nerve compared to the Gin, Gin+Ex, and Veh groups. Additionally, the Genin group had a positive effect of GAP-43 ($P<0.001$) and p-ERK1/2 ($P<0.05$) expressions in the SNI (Fig. 4), confirming the synergistic effects of combined treatment with extracts and exercise for sciatic nerve regeneration.

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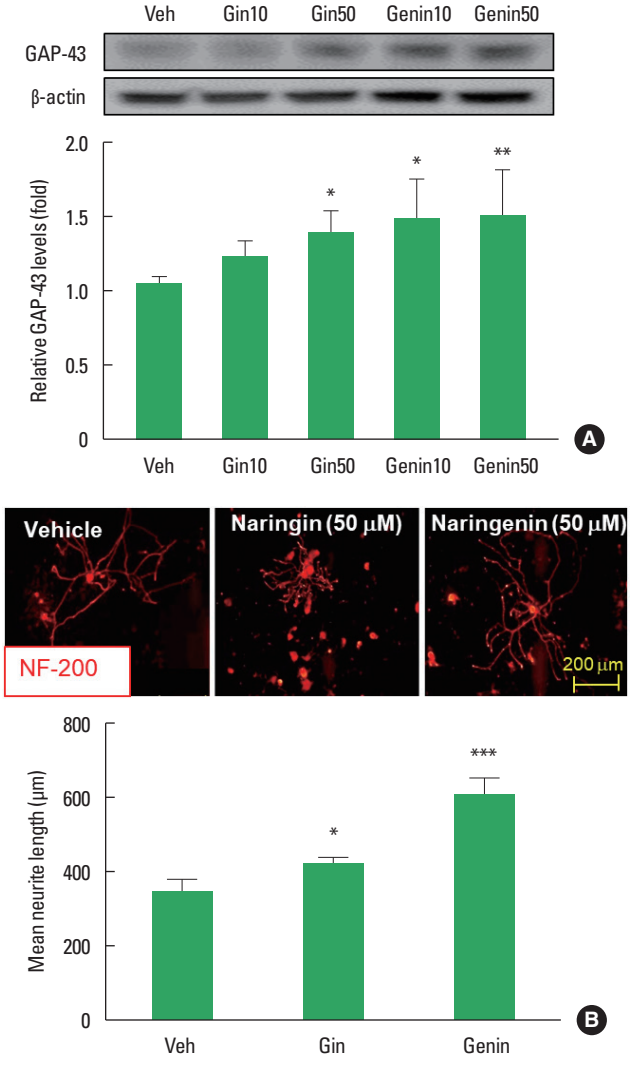

Fig. 2. Naringin and naringenin increased GAP-43 expression in Schwann cells and neurite outgrowth of DRG neurons *in vitro*. (A) Expression level of GAP-43 in the harvested Schwann cells. (B) Mean neurite length of cultured DRG neurons. GAP-43, growth associated protein-43; DRG, dorsal root ganglion; Veh, vehicle; Gin, naringin; Genin, naringenin. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. Veh.

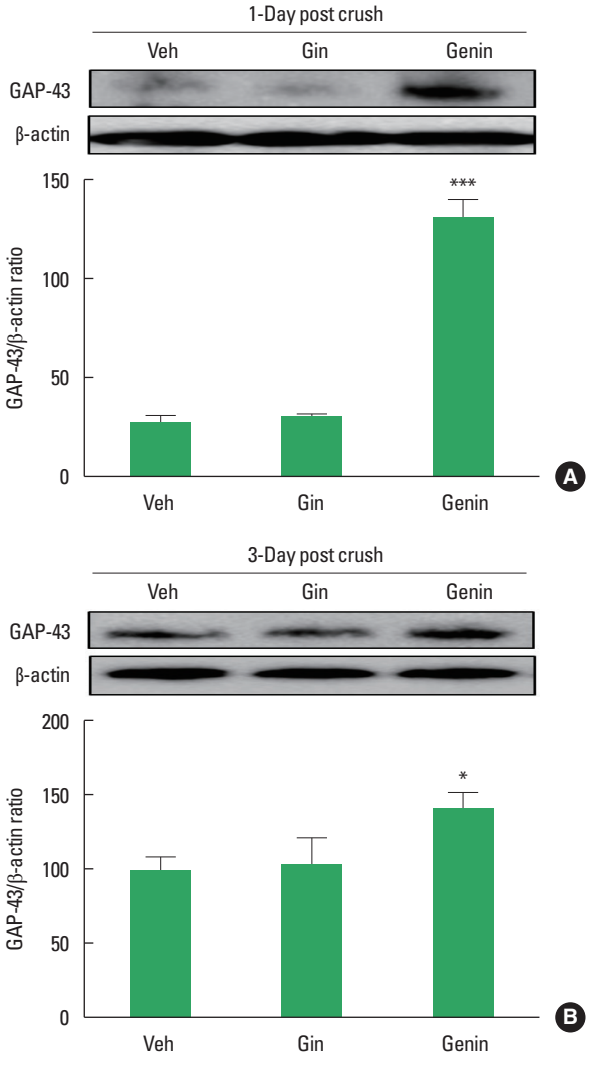

Fig. 3. Naringenin increased GAP-43 expression level in the injured sciatic nerve. (A) Quantitative analysis of GAP-43/actin at one day post crush. (B) Quantitative analysis of GAP-43/actin at 3 days post crush. GAP-43, growth associated protein-43; Veh, vehicle; Gin, naringin; Genin, naringenin. *$P<0.05$, ***$P<0.001$ vs. Veh.

DISCUSSION

This study first analyzed dose effect of naringin and naringenin, components of citrus extracts, on the cytotoxicity and cell viability using SH-SY5Y cells, and we found that both components were nontoxic. Notably, naringenin at concentrations of 6.25 μM and above significantly increased cell population following the induction of cell death. Previous research indicates that naringenin prevents cell death and promotes proliferation in SH-SY5Y cells by activating nuclear factor erythroid 2-related factor 2, which controls antioxidants (de Oliveira et al., 2017). Additionally, treatment with naringenin at concentrations of 10 μM or higher has been reported to favor anti-apoptotic effects and cell proliferation (Jin and Wang, 2019). Combining these findings with the results of our study suggests that naringenin may be more effective than naringin in inhibiting cell death and promoting cell proliferation, particularly at concentrations of 6.25 μM or higher.

Before conducting *in vivo* experiments, primarily cultured Schwann cells and DRGs were treated with naringin and naringenin at concentrations of 10 μM and 50 μM to determine the appropriate dosage of two components for Schwann cell proliferation and neurite outgrowth of DRGs. Our results showed a significant increase

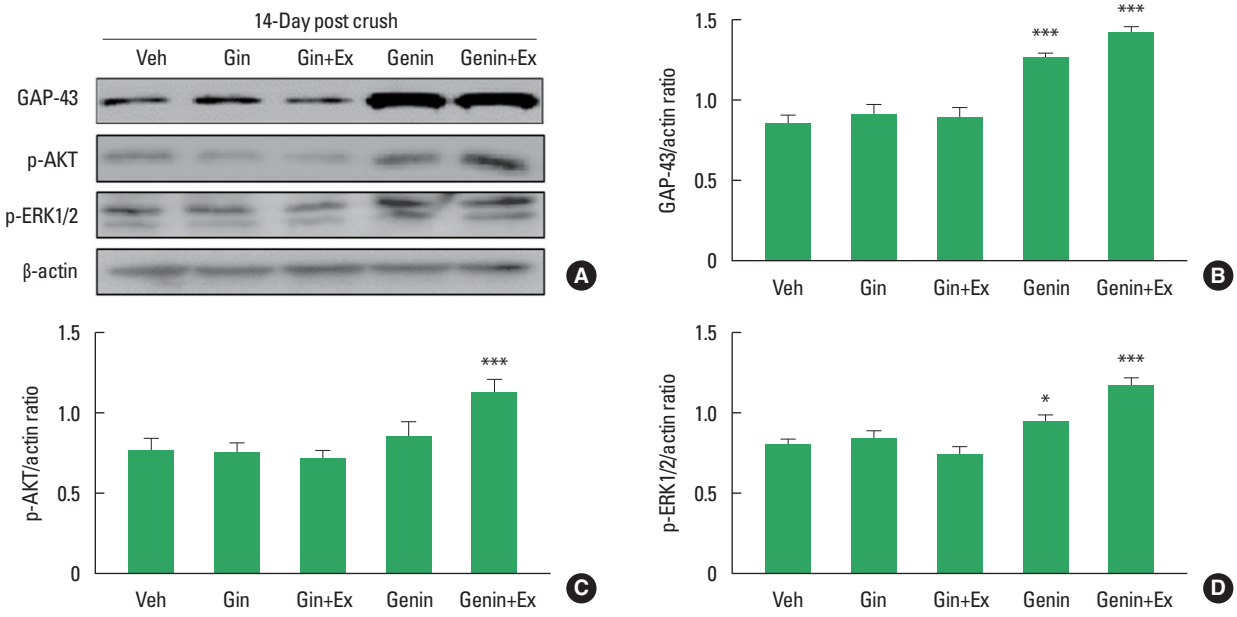

Fig. 4. Effects of combined exercise with naringin or naringenin on in vivo regenerative proteins. (A) Representative Western blot images of GAP-43 and p-AKT and p-ERK1/2 at 14 days after SNI. (B-D) Quantitative analysis of GAP-43/actin, p-AKT/actin and p-ERK1/2/actin ratio. GAP-43, growth associated protein-43; p-AKT, phosphorylated-protein kinase B; p-ERK1/2, phosphorylated extracellular signal-regulated kinase; Veh, vehicle; Gin, naringin; Genin, naringenin. *P<0.05, ***P<0.001 vs. Veh.

in GAP-43 expression with Gin50 and both Genin10 and Genin50. GAP-43 is a protein crucial in axonal regeneration post-nerve injury, which is expressed in the axonal membrane around growth cones (Arabzadeh et al., 2022). Based on these results, it was determined that treatment with 50 μM of naringin and naringenin would be effective for axonal regeneration. Consequently, primary cultured DRG were treated with naringin and naringenin at a 50 μM concentration. Both compounds increased the neurite outgrowth of DRG, with naringenin being more effective than naringin. We believed that 50 μM naringin and naringenin might accelerate axonal regeneration post-peripheral nerve injury.

This study also analyzed the effects of naringin and naringenin in the early stages (1 and 3 days) following SNI. The results showed that GAP-43 expression increased over time, with the Genin group exhibiting a significantly greater increase compared to the other two groups. Genin groups significantly increased GAP-43 expression on both 1 and 3 day-post injury. Previous studies have reported that naringenin, a flavonoid with anti-inflammatory and antioxidant effects, can reduce inflammatory markers and inhibit cell death, thereby accelerating regeneration (Manchope et al., 2017; Rong et al., 2012). These effects were corroborated in this study, suggesting that naringenin might enhance the expression of GAP-43 during the early stages of the sciatic nerve regeneration.

The study further examined the effects of the combined application of naringin and naringenin with exercise on sciatic nerve regeneration. Treadmill exercise not only promotes neurotrophic factors expression and Schwann cell activation but also enhances axonal regeneration by upregulating p-ERK1/2 and c-Jun in Schwann cells, thereby facilitating nerve repair. Moreover, the inhibition of p-ERK1/2 has been shown to suppresses Schwann cell proliferation and delays axonal regrowth, suggesting the critical role of treadmill exercise to mediate nerve regeneration (Seo et al., 2009). The results indicated that GAP-43 and p-ERK1/2 expression was significantly increased in both the Genin and Genin+Ex groups, while p-AKT showed significant improvement only in the Genin+Ex group. GAP-43 expression continuously increased from the early stages to day 14, indicating that the effects of naringenin may persist beyond the initial phase. The combined application with exercise further enhanced these effects, demonstrating the positive impact of combining naringenin treatment with exercise on expression of regenerative proteins until 14 days after SNI. p-ERK1/2 plays a role in improving cell survival and neuropathic pain after central and peripheral nerve injuries (Lu and Malemud, 2019). In this study, the combined application of naringenin treatment and exercise significantly increased p-ERK1/2 expressions, consistent with previous studies showing that naringenin inhibits the increase of TNF-α, IL-1 β, and monocyte che-

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moattractant protein-1 to improve mechanical allodynia after peripheral nerve injury as well as low-intensity treadmill exercise promotes p-ERK1/2 and p-c-Jun to increase Schwann cell population within the injured sciatic nerve (Hu and Zhao, 2014; Kim et al., 2018). p-AKT is a representative protein for cell survival and nerve regeneration in the nervous system, and in this study, it was significantly increased only with the combined treatment of naringenin and exercise, implying that the combined application of exercise and naringenin can amplify therapeutic effects during regeneration period.

In conclusion, the combined application of naringenin and exercise positively stimulated the regenerative capacity of Schwann cells and axonal regrowth from 1 to 14 days after SNI. Overall, we thought that naringenin is more effective than naringin in promoting the expression of axonal growth-related proteins after SNI, and its combined application with exercise accelerates axonal growth. Thus, these findings suggest that the combined use of citrus extracts and exercise may be an effective strategy for accelerating the regeneration of damaged sciatic nerves.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

Arabzadeh E, Rahimi A, Zargani M, Feyz Simorghi Z, Emami S, Sheikhi S, Zaeri Amirani Z, Yousefi P, Sarshin A, Aghaei F, Feizolahi F. Resistance exercise promotes functional test via sciatic nerve regeneration, and muscle atrophy improvement through GAP-43 regulation in animal model of traumatic nerve injuries. Neurosci Lett 2022;787:136812.

Arbat-Plana A, Cobianchi S, Herrando-Grabulosa M, Navarro X, Udina E. Endogenous modulation of TrkB signaling by treadmill exercise after peripheral nerve injury. Neuroscience 2017;340:188-200.

Atoki AV, Aja PM, Shinkafi TS, Ondari EN, Awuchi CG. Naringenin: its chemistry and roles in neuroprotection. Nutr Neurosci 2024;27:637-666.

Balestrieri ML, Castaldo D, Balestrieri C, Quagliuolo L, Giovane A, Servillo L. Modulation by flavonoids of PAF and related phospholipids in endothelial cells during oxidative stress. J Lipid Res 2003;44:380-387.

Bobinski F, Martins DF, Bratti T, Mazzardo-Martins L, Winkelmann-Duarte EC, Guglielmo LG, Santos AR. Neuroprotective and neuroregenerative effects of low-intensity aerobic exercise on sciatic nerve crush injury in mice. Neuroscience 2011;194:337-348.

Cavia-Saiz M, Busto MD, Pilar-Izquierdo MC, Ortega N, Perez-Mateos M, Muñiz P. Antioxidant properties, radical scavenging activity and biomolecule protection capacity of flavonoid naringenin and its glycoside naringin: a comparative study. J Sci Food Agric 2010;90:1238-1244.

Cho YH, Seo TB. Effect of treadmill exercise combined with bone marrow stromal cell transplantation on atrophy-related signaling pathway in the denervated soleus muscle. J Exerc Rehabil 2021;17:395-402.

Cho YH, Seo TB. The timing point of exercise intervention regulates neuropathic pain-related molecules in the ipsilateral dorsal root ganglion neurons after sciatic nerve injury. J Exerc Rehabil 2022;18:286-293.

Daradka MH, Bani Ismail ZA, Irsheid MA. Peripheral nerve regeneration: a comparative study of the effects of autologous bone marrow-derived mesenchymal stem cells, platelet-rich plasma, and lateral saphenous vein graft as a conduit in a dog model. Open Vet J 2021;11:686-694.

de Oliveira da Silva B, Ramos LF, Moraes KCM. Molecular interplays in hepatic stellate cells: apoptosis, senescence, and phenotype reversion as cellular connections that modulate liver fibrosis. Cell Biol Int 2017;41:946-959.

Hu CY, Zhao YT. Analgesic effects of naringenin in rats with spinal nerve ligation-induced neuropathic pain. Biomed Rep 2014;2:569-573.

Jin Y, Wang H. Naringenin inhibit the hydrogen peroxide-induced SH-SY5Y cells injury through Nrf2/HO-1 pathway. Neurotox Res 2019;36:796-805.

Jung SY, Kim DY, Yune TY, Shin DH, Baek SB, Kim CJ. Treadmill exercise reduces spinal cord injury-induced apoptosis by activating the PI3K/Akt pathway in rats. Exp Ther Med 2014;7:587-593.

Kim YM, Jin JJ, Lee SJ, Seo TB, Ji ES. Treadmill exercise with bone marrow stromal cells transplantation facilitates neuroprotective effect through BDNF-ERK1/2 pathway in spinal cord injury rats. J Exerc Rehabil 2018;14:335-340.

Liao CF, Yang TY, Chen YH, Yao CH, Way TD, Chen YS. Effects of swimming exercise on nerve regeneration in a rat sciatic nerve transection model. Biomedicine (Taipei) 2017;7:3.

Lu N, Malemud CJ. Extracellular signal-regulated kinase: a regulator of cell growth, inflammation, chondrocyte and bone cell receptor-mediated gene expression. Int J Mol Sci 2019;20:3792.

Manchope MF, Casagrande R, Verri WA Jr. Naringenin: an analgesic and anti-inflammatory citrus flavanone. Oncotarget 2017;8:3766-3767.

Ożarowski M, Karpiński TM. Extracts and flavonoids of passiflora spe-

cies as promising anti-inflammatory and antioxidant substances. Curr Pharm Des 2021;27:2582-2604.

Rao PN, Mainkar O, Bansal N, Rakesh N, Haffey P, Urits I, Orhurhu V, Kaye AD, Urman RD, Gulati A, Jones M. Flavonoids in the treatment of neuropathic pain. Curr Pain Headache Rep 2021;25:43.

Rong W, Wang J, Liu X, Jiang L, Wei F, Hu X, Han X, Liu Z. Naringin treatment improves functional recovery by increasing BDNF and VEGF expression, inhibiting neuronal apoptosis after spinal cord injury. Neurochem Res 2012;37:1615-1623.

Seo TB, Cho YH, Sakong H, Kim YP. Effect of treadmill exercise and bone marrow stromal cell engraftment on activation of BDNF-ERK-CREB signaling pathway in the crushed sciatic nerve. J Exerc Rehabil 2021; 17:403-409.

Seo TB, Oh MJ, You BG, Kwon KB, Chang IA, Yoon JH, Lee CY, Namgung U. ERK1/2-mediated Schwann cell proliferation in the regenerating sciatic nerve by treadmill training. J Neurotrauma 2009;26:1733-1744.

Sharma P, Tyagi A, Bhansali P, Pareek S, Singh V, Ilyas A, Mishra R, Poddar NK. Saponins: extraction, bio-medicinal properties and way forward to anti-viral representatives. Food Chem Toxicol 2021;150:112075.

Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B. Plant flavonoids: classification, distribution, biosynthesis, and antioxidant activity. Food Chem 2022;383:132531.

Wang Y, Xiang L, Wang C, Tang C, He X. Antidiabetic and antioxidant effects and phytochemicals of mulberry fruit (Morus alba L.) polyphenol enhanced extract. PLoS One 2013;8:e71144.

Wong CE, Hu CY, Lee PH, Huang CC, Huang HW, Huang CY, Lo HT, Liu W, Lee JS. Sciatic nerve stimulation alleviates acute neuropathic pain via modulation of neuroinflammation and descending pain inhibition in a rodent model. J Neuroinflammation 2022;19:153.

https://doi.org/10.12965/jer.2550124.062