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다양한 인삼종의 분획물에 대한 C2C12 세포에서의 보호 및 분화 활성 평가

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Differentiation-promoting and Protective Effects of the Fractions of Various Ginseng Species in C2C12 Cells

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ABSTRACT

Background: The effects of *Panax ginseng* C. A. Meyer in protecting and promoting the differentiation of muscle cells have been reported. However, the influences of various ginseng species and various fractions were not investigated.
Methods and Results: In this study, these effects on muscle cells were confirmed using butanol and water fractions of various ginseng species. The fractions of *P. ginseng* were confirmed to have a cytoprotective effect on C2C12 cells. The myogenin expression level was significantly higher by 1.5 times or more, in the *P. ginseng* fraction groups than in the other groups, confirming that the *P. ginseng* fractions promoted cell differentiation. Both fractions of *P. ginseng* significantly reduced the rate of production of reactive oxygen species when compared with that of the control group. In the mechanism study, *P. ginseng* fractions tended to decrease muscle RING-finger protein-1 (MuRF-1), AMP-activated protein kinase (AMPK), forkhead box O3α (Foxo3α), and the expression of the BCL2-associated agonist of cell death (BAD). In particular, AMPK was significantly reduced in the *P. ginseng* water fraction group when compared to that in the butanol fraction and control groups.
Conclusions: The results of this study confirmed that *P. ginseng* has superior cell differentiation-promoting and protective effects on muscle cells when compared with the effects of *Panax quinquefolium* and *Panax notoginseng*.

Key Words: *Panax Ginseng* C. A. Meyer, *Panax quinquefolium*, *Panax notoginseng*, Saponin, Non-saponin, C2C12 cell

INTRODUCTION

Panax ginseng C. A. Meyer, *Panax quinquefolium* L., and *Panax notoginseng* Burk. are three major perennial herbal plants in the genus *Panax* of the Araliaceae family (Lee *et al*., 2017). These ginsengs have been used as foods or herbal medicines worldwide for thousands of years. Ginseng has mostly been used as a tonic, and it has been confirmed to improve bodily function, increase vitality, increase resistance to stress and aging, and have immunomodulatory activities (Kim, 2012).

P. ginseng is a essential herbal medicine resource in East Asia owing to its pharmacological properties and physiological activities. *P. quinquefolium* (American ginseng), which is

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distributed in North America, is another important ginseng species. *P. notoginseng* (Sanchi) is available in Japan and the southern area of China (Jee *et al*., 2014).

Ginseng-related effects include antioxidant (Lee *et al*., 2004; Doh *et al*., 2010; Moon *et al.*, 2019), anticancer (Zhang *et al*., 2012), antidiabetic (Jung and Kang, 2013; Seo *et al*., 2016), antihypertensive (Choi *et al*., 2006), anti-stress (Rai *et al*., 2003), immunity-promoting (Ha *et al*., 2009), and memory-enhancing (Kim *et al*., 2018; Sohn *et al*., 2008; Lee *et al.*, 2020) effects. Lau *et al*. (2009) evaluated the antithrombotic efficacy of three ginseng species and found that *P. notoginseng* showed the highest antithrombotic effect; moreover, it was confirmed that the efficacy was increased when the ginseng was steamed.

Recent studies on the functionality of ginseng have focused on its efficacy in protecting and promoting the production of muscle cells. In rat models of muscle injury due to eccentric exercise, rats treated with dammarane steroids showed decreased muscle cell necrosis and significantly increased distribution of $CD68^+$ M1 macrophages and 3-nitrotyrosine (Yu *et al*., 2014). The administration of red ginseng extract resulted in the relaxation of rabbit corpus cavernosal smooth muscle following contraction induced by phenylephrine (Choi *et al*., 1998).

In addition, rats that ingested *P. ginseng* extract showed significant improvement in exercise time in treadmill test, and the *P. ginseng* intake group showed a significant increase in serum IGF-1 levels (Sohn *et al*., 2012). Furthermore, ingestion of ginseng extract in rats significantly protected the muscles from oxidative stress damage caused by exercise (Voces *et al*., 2004).

G115, a standardized ginseng extract, was also confirmed to decrease lipid peroxidation and inflammatory markers in the muscle (Oliveira *et al*., 2001). Similar results were confirmed in a clinical trial. The group that ingested red ginseng extract showed significantly reduced exercise-induced production of inflammatory markers, such as IL-6, in the muscles (Jung *et al*., 2011). Studies on ginseng at the component level confirmed that ginsenoside Rg1, a major constituent of ginseng, improves glycogen supplementation after exercise and decreases the mRNA levels of inflammatory factors, such as TNF-α and IL-10 (Hou *et al*., 2015).

In this study, to identify the components or fractions responsible for the protective and regeneration-promoting effects of ginseng, the efficacy of saponin and non-saponin fractions of ginseng using liquid-liquid extraction was investigated. C2C12 muscle cells, developed in 1977, are myoblasts in the pre-differentiation stage into muscle cells and often used as a control cell line in studies of *in vitro* muscular dystrophy (Denes *et al*., 2019). The muscle cell-protective effects of the fractions of various ginseng species, namely *P. ginseng*, *P. quinquefolium,* and *P. notoginseng*, which are representatives of the *Panax* genus, were compared. In this study, we aimed to confirm the protective and regeneration-promoting effects of *P. ginseng* in muscle cells compared with those of other ginseng genera and to provide basic research results to elucidate the active components or fractions.

MATERIALS AND METHODS

1. Samples and preparation

Four-year-old *P. ginseng* grown in Geumsan, Republic of Korea, was purchased in 2019 at Geumsan Ginseng Market, Republic of Korea. *P. quinquefolium* grown in the United States was purchased in Seongwan Market, Hong Kong. *P. notoginseng* grown in the Changbai Mountain area was purchased in 2019 at Changbai Mountain Market, Jilin, China. Each of the three ginseng species was verified by experts to be suitable for research.

For each ginseng species, 2 g of ginseng powder and 20 ㎖ of 50% methanol (JT Baker, Center Valley, PA, USA) were added to a 50 ㎖ conical tube. The first extraction was then performed in a shaking water bath at 250 rpm and 60℃ for 4 h (MaXturdy 45; DAIHAN Scientific Co., Ltd., Wonju, Korea). After the extraction was completed, only the supernatant was recovered by centrifugation at 4,000 rpm for 10 min using a centrifuge (VARISPIN 4, Novapro Co., Ltd., Bucheon, Korea), and 20 ㎖ of 50% methanol was added to the precipitate, which was then used for the second extraction in the same manner. Similarly, only the supernatant was recovered, mixed with the first extract, and then used for analysis.

The mixed extract was mixed with volatilizing methanol in a vacuum evaporator (N-1100V, Eyela Co., Ltd., Tokyo, Japan) to obtain a solution of approximately 60 ㎖, and then the same volume of *n*-butanol (Daejung Chemicals and Metals Co., Ltd., Siheung, Korea) was added to separate the layers. As a result, the solution was separated into approximately 30 ㎖ of aqueous layer and approximately 90 ㎖ of saturated butanol layer. The water-saturated butanol layer was separated using a solvent

through a vacuum evaporator, yielding 596 ㎎ of *P. ginseng* C butanol fraction [G(B)], 610 ㎎ of *P. notoginseng* butanol fraction [N(B)], and 650 ㎎ of *P. quinquefolium* butanol fraction [Q(B)] powders.

The water layer of each ginseng extract was sublimated with water using a freeze dryer (FDS8508, ilShinBioBase, Dongducheon, Korea), yielding 1,921 ㎎ of *P. ginseng* water fraction [G(W)], 2,330 ㎎ of *P. notoginseng* water fraction [N(W)], and 2,569 ㎎ of *P. quinquefolium* water fraction [Q(W)], which were used in the subsequent tests.

2. Cell culture and differentiation methods

Mice-derived C2C12 cells were obtained from the American Type Culture Collection (CRL-1772, ATCC, Manassas, VA, USA) and cultured in a 5% CO_2 incubator at 37 ℃. The cells were incubated in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) in the cell proliferation phase before differentiation, and the 10% FBS was changed to 2% horse serum to induce differentiation. To eliminate the over-density phenomenon caused by cell proliferation, the appropriate number of cells was maintained by subculturing every 48 h.

During differentiation, C2C12 myoblast cells were seeded at a concentration of 1×10^5 cells/㎖ in a six-well plate and cultured until 70% - 80% confluence. The differentiation medium was collected once a day to confirm whether the cells had differentiated into a myotube shape under a microscope.

3. Confirmation of the cytotoxic and cell-protective effects of ginseng extract

To investigate the cytotoxicity of the ginseng extracts, the viability of C2C12 cells was evaluated using Cell Counting Kit-8 (CK04-20, MedChemExpress, Monmouth Junction, NJ, USA) according to the manufacturer's instruction. C2C12 cells were seeded in a 96-well plate at a concentration of 1×10^4 cells/㎖ day before the experiment.

Subsequently, the cells were treated with 12.5, 25.0, 50.0, or 100.0 ㎍/㎖ of ginseng extract and incubated for 24 h. After that, CCK-8 solution was dispensed at 10 ㎕, which corresponded to 10% of the total volume, and the cells were incubated for 2 h. Absorbance was measured at 450 ㎚ using a microplate reader (Infinite M200 PRO NanoQuant, TECAN Ltd., Zurich, Switzerland).

To confirm the cytoprotective effect of the ginseng extracts, C2C12 cells were divided into a control group (treated with 600 μM H_2O_2 only) and ginseng fraction groups [treated with G(B), G(W), Q(B), Q(W), N(B), and N(W) at concentrations of 3.125, 6.250, and 12.500 ㎍/㎖, followed by 600 μM H_2O_2).

To confirm the cell-protective efficacy of the ginseng extracts, C2C12 cells were seeded in a 96-well plate at a concentration of 1×10^4 cells/㎖ day before the experiment. Next, the ginseng-treated groups were treated with each concentration of the sample. After 1 h of incubation, all groups except the control group were treated with 600 μM H_2O_2. After that, the cells were further incubated for 24 h. Next, 10 ㎕ of CCK-8 solution was added to the cells, which were then incubated for another 2 h. Absorbance was measured at 460 ㎚ using a microplate reader (Infinite M200 PRO NanoQuant; TECAN Ltd., Zurich, Switzerland).

4. Protein separation and western blotting analysis

Western blotting analysis was performed to confirm the differentiation of myoblasts into myotubes and confirm changes in the factors indicating the cytoprotective effect of each fraction. After appropriate cell culture and treatment in 100 ㎜ dishes, intracellular protein was isolated using 1 mM phenylmethylsulfonyl fluoride (MedChemExpress, Mnmouth Junction, NJ, USA), 1% protease inhibitor cocktail, and NP40 cell lysis buffer (Invitrogen, Carlsbad, CA, USA).

The separated protein was quantified using a Pierce™ BCA Protein Assay Kit (Invitrogen, Calesbad, CA, USA). Next, 30 μg of protein was electrophoresed using Bolt™ 4% - 12% Bis-Tris Plus Gels, and the protein was then transferred to a dry iBlot® Transfer Stack (Invitrogen), a nitrocellulose membrane of a gel substrate, using an iBlot Gel Transfer Device (Invitrogen, Calesbad, CA, USA).

Each membrane was blocked at room temperature for 1 h using 5% skim milk and subsequently washed three times with 0.1% Tris-buffered saline in 0.1% Tween 20 buffer. The membrane was incubated with primary antibodies, including anti-myogenin [F5D] (ab1835) (1 : 250, Abcam, Cambridge, England), anti-MyoD1 [5.2F] (ab16148) (1 : 1000, Abcam, Cambridge, England), anti-MuRF1 (ab96857) (1 : 1000, Abcam, Cambridge, England), anti-Foxo3α (phospho S253) (ab47285) (1 : 1000, Abcam, Cambridge, England), and anti-Foxo3α ab121620 (1 : 2500, Abcam, Cambridge, England), overnight in a refrigerator at 4℃. The membrane was then incubated with HRP-conjugated IgG secondary antibody (1 : 30000, Abcam, Cambridge, England) for 1 h at room temperature.

After washing the membrane three times, the developed protein bands were analyzed using a C-DiGit® Blot Scanner (LI-COR Inc., Lincoln, NE, USA) to determine the protein expression levels. The band was quantified using the Image J Program (National Institutes of Health, Bethesda, MD, USA), and protein expression level was expressed as a fold change relative to the value of the control group.

5. ROS generation measurement

Intracellular ROS levels were measured using the DCF-DA method. C2C12 myoblasts were dispensed into a 6-well plate at 1×10^5 cells/㎖ and cultured for 24 h, and then the ginseng extract sample [G(B), G(W), Q(B), Q(W), N(B), N(W)] was added to serum-free DMEM at a concentration of 12.5 ㎍/㎖ for 24 h. After 1 h, the cells were washed with PBS, and treated with 600 μM H_2O_2 for 24 h. After that, it was washed with PBS, and 10 μM DCF-DA was dispensed into each well and cultured in an incubator at 37 ℃ in a 5% CO_2 atmosphere for 30 min.

After 30 min, it was washed with PBS, and 1 ㎖ of PBS was dispensed into each well, and fluorescence was measured at excitation 485/20 and emission 528/20 using a microplate reader (Infinite M200 PRO NanoQuant, TECAN Ltd., Zurich, Switzerland).

6. Statistical analysis

Statistical analysis was performed using analysis of variance in the SPSS program (PASW Statics 18, IBM Co., Armonk, NY, USA). Significant differences in the parameters, such as ginsenoside content, were analyzed by Duncan's Multiple Range Test (DMRT) at a significance level of 5% ($p < 0.05$).

RESULTS AND DISCUSSION

1. Cytotoxicity of ginseng extract in C2C12 cells

In the butanol fraction of all ginseng species, as the concentration increased, cytotoxicity increased. On the contrary, there was no significant change in cell viability in the water fraction group. Therefore, the water fractions of the three ginseng species did not appear to cause cytotoxicity, showing similar cell viability to that of the control group (Fig. 1).

2. Muscle cell differentiation-promoting effect of ginseng extract

To determine whether the ginseng extracts affect myoblast differentiation into myotubes, C2C12 myoblasts were induced to differentiate into myotubes. After differentiation induction, the myoblasts were confirmed to have differentiated into myotubes morphologically. After H_2O_2 was added to the differentiated cells, the length of the tube was shortened, and the cells were killed (data not shown). Each extract sample was used to induce differentiation, and the expression of myogenin, a marker gene that induces skeletal muscle

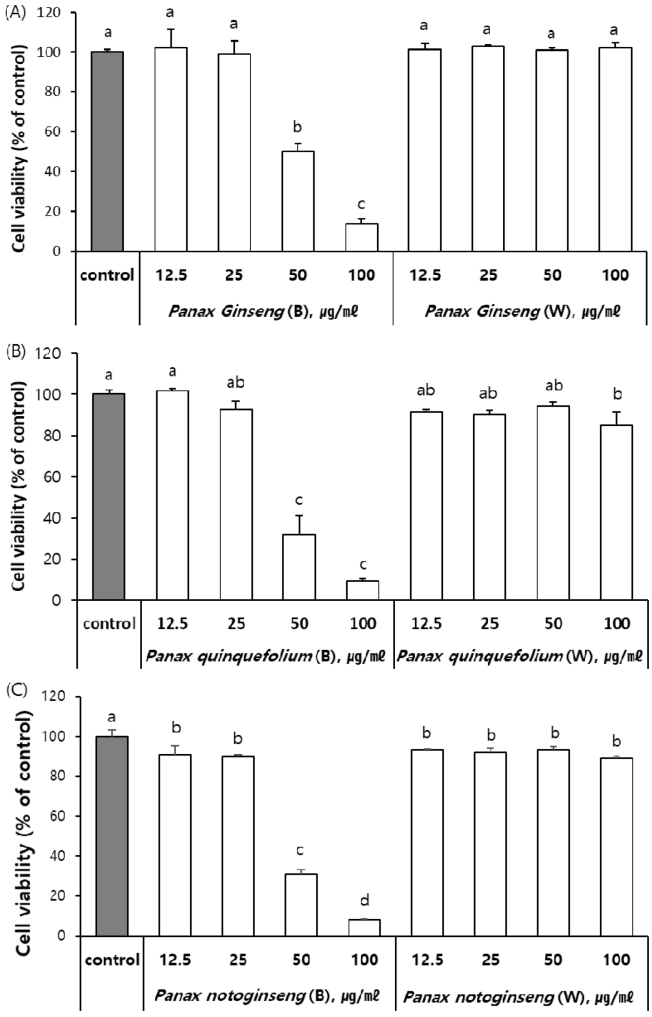

Fig. 1. C2C12 cell viabilities of Butanol or water fraction of various *Panax* species. All data are shown as the means ± SD (n = 3). *Panax ginseng* (B); Butanol fraction of *Panax ginseng* C. A. Meyer, *Panax ginseng* (W); Water fraction of *Panax ginseng* C. A. Meyer, *Panax quinquefolium* (B); Butanol fraction of *Panax quinquefolium*, *Panax quinquefolium* (W); Water fraction of *Panax quinquefolium*, *Panax notoginseng* (B); Butanol fraction of *Panax notoginseng*, *Panax notoginseng* (W); Water fraction of *Panax notoginseng*. *Different superscript letters show significant differences at 5% by Duncan's Multiple Range Test (DMRT, $p < 0.05$).

differentiation, was confirmed by western blotting analysis.

Our results showed that the butanol fraction and water fraction of each ginseng species, excluding the water fraction of *P. ginseng* C, showed similar levels of myogenin expression as Q(B); 0.64 ± 0.05, Q(W); 0.57 ± 0.03, N(B); 0.58 ± 0.07, N(W); 0.54 ± 0.02, and G(B); 0.65 ± 0.01.

Moreover, in the group treated with the non-fraction of *P. ginseng* C, myogenin expression level was 0.96 ± 0.06, which was a significantly increased ($p < 0.05$) compared with in the groups treated with other test materials, further confirming that the water fraction of *P. ginseng* promoted the differentiation of muscle cells (Fig. 2).

3. Cytoprotective effects of ginseng extract against oxidative stress

Oxidative cell damage in C2C12 cells was induced by H_2O_2 treatment, and the protective effect of ginseng extract was confirmed. Following treatment with H_2O_2 at various concentrations, the cell viability was approximately 50% after treatment with 600 μM H_2O_2. Thus, this concentration of H_2O_2 was used in this experiment.

The cells were pretreated with each ginseng extract for 1 h and then treated with H_2O_2, a toxic substance, for 24 h. Subsequently, cell viability was examined. The results showed

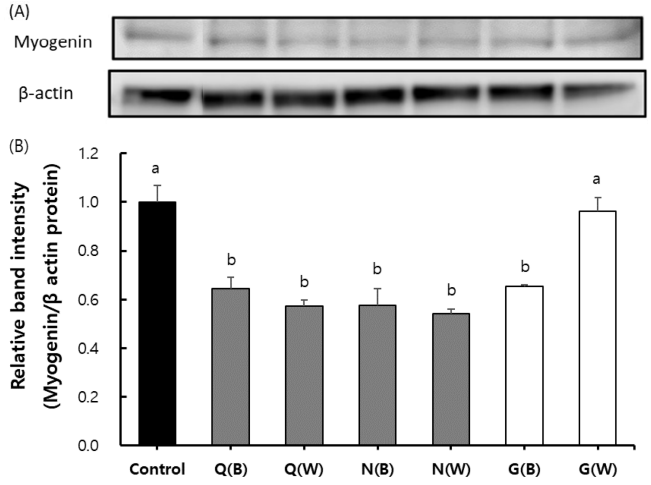

Fig. 2. Myogenin Expression of Butanol or water fraction of various *Panax* species in C2C12 cells. (A) Expression of myogenin in C2C12 cell, (B) Graph presents relative intensity compared with the beta-actin band intensity. All data are shown as the means ± SD (n = 3). Q(B); Butanol fraction of *Panax quinquefolium*, Q(W); Water fraction of *Panax quinquefolium*, N(B); Butanol fraction of *Panax notoginseng*, N(W); Water fraction of *Panax notoginseng*, G(B); Butanol fraction of *Panax ginseng* C. A. Meyer, G(W); Water fraction of *Panax ginseng* C. A. Meyer. *Different superscript letters show significant differences at 5% by Duncan's Multiple Range Test (DMRT, $p < 0.05$).

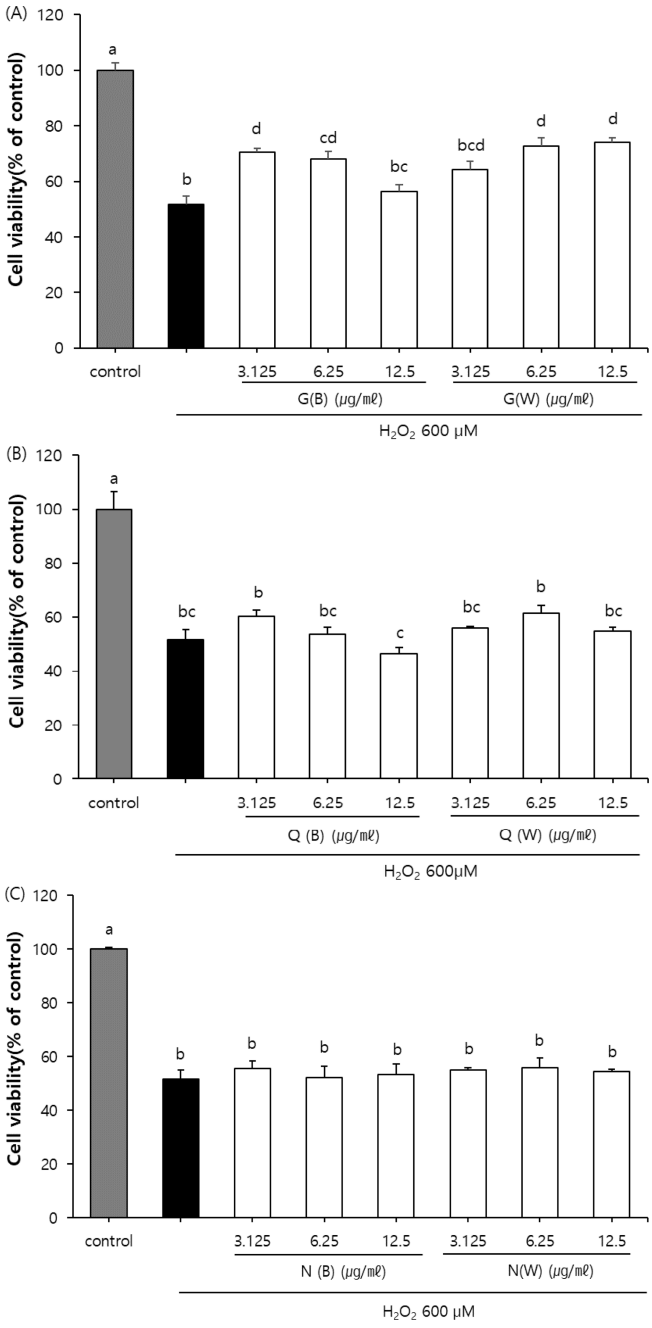

Fig. 3. Protective effects of fractions of *Panax ginseng* on the viability of H_2O_2-treated C2C12 cells. All data are shown as the means ± SD (n = 3). G(B); butanol fraction of *P. ginseng*, G(W); water fraction of *P. ginseng*, Q(B); butanol fraction of *Panax quinquefolium*, Q(W); water fraction of *P. quinquefolium*, N(B); butanol fraction of *Panax notoginseng*, N(W); water fraction of *P. notoginseng*. *Different superscript letters show significant differences at 5% as analyzed by Duncan's Multiple Range Test (DMRT, $p < 0.05$).

that there was no significant difference in cell viability in the groups treated with extracts of *P. quinquefolium* and *P. notoginseng* compared with that in the group treated with H_2O_2 alone. However, cell viability was significantly higher ($p < 0.05$) in the groups treated with *P. ginseng* extracts. Thus, the ginseng extracts were confirmed to have cell-protective effect against oxidative stress caused by H_2O_2.

In particular, the water fraction of *P. ginseng* significantly protected cells from oxidative damage, compared with the butanol fraction, in a concentration-dependent manner ($p < 0.05$, Fig. 3).

4. Effect of each ginseng extract on ROS production and oxidative stress

Cell damage caused by H_2O_2 is related to impaired mitochondrial function due to abnormal ROS production in the mitochondria. The protective effect of the ginseng extracts against ROS production was thus assessed.

Intracellular ROS levels were determined using the DCF-DA method. Fluorescence analysis showed that there was no significant difference in fluorescence between the extract groups and the H_2O_2 group (169.17 ± 6.50%). However, ROS levels were 140.11 ± 4.30% in the *P. ginseng* butanol fraction group and 124.98% ± 3.73% in the *P. ginseng* water fraction group, showing a significant reduction compared with that in the group treated with only H_2O_2 ($p < 0.05$).

These findings confirmed that among the ginseng extracts, *P. ginseng* extract had protective effect against oxidative stress. In particular, the water fraction of *P. ginseng* effectively reduced ROS levels compared with the other extracts (Fig. 4)

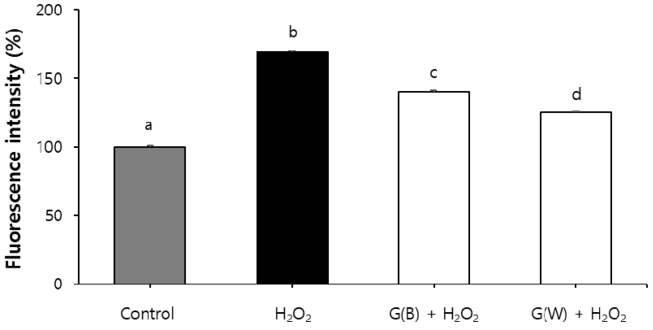

Fig. 4. Effect of fractions of various *Panax* species on intracellular ROS levels in C2C12 cells as analyzed by DCF-DA assay. All data are shown as the means ± SD (n = 3). G(B); Butanol fraction of *Panax ginseng*, G(W); water fraction of *P. ginseng*. *Different superscript letters show significant differences at 5% as analyzed by Duncan's Multiple Range Test (DMRT, $p < 0.05$).

5. Mechanism of the cytoprotective effect of ginseng extract against oxidative stress in C2C12 cells

The expression of muscle damage indicator AMP-activated protein kinase (AMPK) and proteolytic signaling transducers muscle RING-finger-1 (MuRF1) and forkhead box O3α (Foxo3α), which are activated by an increase in AMP level due to ATP depletion, was confirmed using western blotting. The efficacy of the extracts in inhibiting apoptosis due to oxidative stress-induced cell damage was examined.

The results of protein expression levels showed that the *P. ginseng* fraction had protective effect on cell viability. Compared with those in the control group, the expression levels of the protein degradation markers AMPK and MuRF1 in the group treated with H_2O_2 were increased, confirming the presence of muscle damage.

The MuRF-1 expression level in the *P. ginseng* butanol fraction group was 0.85 ± 0.06, showing a decreasing trend compared with that in the H_2O_2 group (1.16 ± 0.09), but the decrease was not significant. The MuRF-1 expression in the *P. ginseng* water fraction group was 0.67 ± 0.08, showing a significant decrease compared with that in the H_2O_2 group ($p < 0.05$, Fig. 5B).

AMPK expression level showed a decreasing tendency in the H_2O_2 alone group (1.17 ± 0.06). The expression level of AMPK was 1.13 ± 0.05 and 0.84 ± 0.07 in the *P. ginseng* butanol and water fraction groups, respectively, showing significant decreases compared with that in the H_2O_2 alone group ($p < 0.05$, Fig. 5C).

BAD expression in the *P. ginseng* butanol and water fraction groups were 0.53 ± 0.04 and 0.43 ± 0.02, respectively, showing significant decreases compared with that in the H_2O_2 group ($p < 0.05$, Fig. 5D).

Foxo3α expression in the *P. ginseng* butanol fraction group was 0.79 ± 0.04, which showed a decreasing trend compared with that in the H_2O_2 alone group (1.02 ± 0.07), but the decrease was not significant. Foxo3α expression in the *P. ginseng* water fraction group was 0.66 ± 0.03, which was significantly decreased compared with that in the group treated with H_2O_2 alone ($p < 0.05$, Fig. 5E).

Myogenin, which belongs to the MyoD family, plays an important role in the regulation of the differentiation of single nucleated myoblasts into multinucleated myofibers (Lee *et al*., 2017). Scavenging of ROS from muscle cells is a possible approach to prevent muscle cell damage (Choi *et al*., 2017). Abnormally high levels of ROS cause dysfunction of muscle

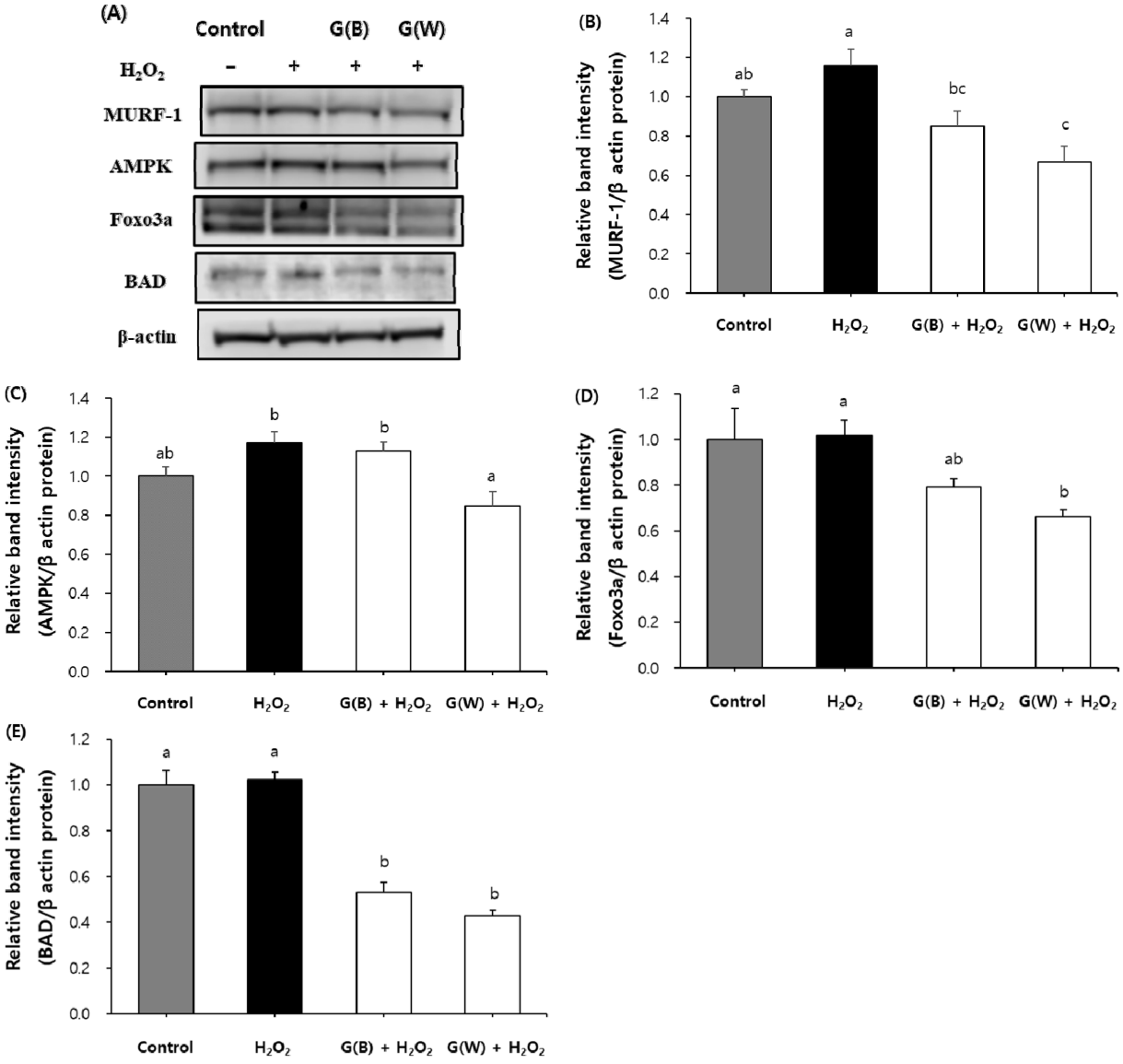

Fig. 5. Effects of *Panax ginseng* fractions on MuRF-1, AMPK, Foxo3a and BAD expression in H_2O_2-treated C2C12 cells as analyzed by western blotting. (A) Expression of MuRF-1, AMPK, Foxo3a and BAD. (B) The graph presents the relative intensity of MuRF-1 compared with that of beta-actin. (C) The graph presents the relative intensity of AMPK compared with that of beta-actin. (D) The graph presents the relative intensity of Foxo3a compared with that of beta-actin. (E) The graph presents the relative intensity of BAD compared with that of beta-actin. All data are shown as the means ± SD (n = 3). G(B); Butanol fraction of *P. ginseng*, G(W); water fraction of *P. ginseng*. *Different superscript letters show significant differences at 5% as analyzed by Duncan's Multiple Range Test (DMRT, $p < 0.05$).

cells and damage to intracellular macromolecules such as proteins, lipids, and nucleic acids in muscle cells; they act as a causative factor for cell death (Choi, 2015). Abnormal ROS production by H_2O_2 impairs mitochondrial function. The production of BAD, the pro-apoptotic factor, is one of the intrinsic mechanisms of action involved in apoptosis; BAD plays a role in aggregating several mitochondrial signals involved in cell death and blood glucose regulation (Sung *et al.,* 2006).

AMPK is an important regulator of intracellular energy balance. When AMPK is activated, the ubiquitin-proteasome pathway is activated through increases in muscle-specific ubiquitin ligases, muscle atrophy F-box (MAFbx)/atrogin-1, and MuRF1 through the transcription factor Foxo3α, resulting in muscle atrophy due to muscle protein degradation (Choi *et al*., 2017; Yeo *et al*., 2019; Chen *et al*., 2020; Yoshikawa *et*

al., 2020).

This study was aimed to evaluate the efficacy of each ginseng and provide basic research results to track the active components or fractions in the ginseng genus for differentiation-promoting and protective effects on muscle cells. There was an evaluation report on each ginseng genus, but not reported about comparison of fractions using each ginseng genus.

Butanol fractions and water fractions of various ginseng species were prepared, and the cytoprotective effect of these fractions against H_2O_2-induced oxidative stress in C2C12 cells was evaluated by measuring myogenin expression and ROS levels. The expression levels of MuRF-1, AMPK, Foxo3α, and BAD were evaluated in the mechanism study.

The *P. ginseng* fraction showed the highest cytoprotective effect in C2C12 cells subjected to oxidative damage caused by H_2O_2. Myogenin activity, a marker of muscle cell differentiation, was confirmed to increase by 1.5 times or higher after treatment with the water fraction of *P. ginseng* compared with that after treatment with the butanol fraction of *P. ginseng* and other ginseng fractions. This trend was also observed in ROS production, but no significant difference was found between the *P. ginseng* fractions.

These results were thought to be due to the suppression of the expression of MuRF-1, AMPK, Foxo3α, and BAD. In particular, the expression level of AMPK was significantly suppressed by the water fraction of *P. ginseng,* compared with its butanol fraction.

The findings of this study confirmed that *P. ginseng* has superior muscle cell-protective effect to that of *P. quinquefolium* and *P. notoginseng*. Furthermore, the water fraction of *P. ginseng* exhibited the most potent muscle cell-protective effect.

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