We investigated the effects of a *Eupatorium chinense* var. *simplicifolium* (EUC) root extract on muscle disorders and explored the underlying mechanism for oxidative stress-induced C2C12 myoblast damage. An EUC pre-treatment reduced the decreased cell viability after an H2O2 treatment. The heat shock protein (HSP) 70 level increased, and the phosphorylation of Jun amino-terminal kinases (JNKs) decreased in the EUC-pre-treated C2C12 myoblasts. The results of the present study demonstrate the potential benefit of a herbal medicine in treating oxidative stress-related muscle disorders.

**Key words:** *Eupatorium chinense* var. *simplicifolium*; muscle atrophy; HSP70

Such muscle disorders as muscular dystrophy are associated with an increase in oxidative stress. Proposed treatments for muscular dystrophy include gene therapy and muscle cell transplantation, some of which are under clinical trial. Skeletal muscle cells are susceptible to oxidative stress due to electron transport and oxygen flux during normal contraction, and this stress may increase with exercise intensity. In addition to functional defects in dystrophin, studies have shown increased oxidative stress in patients with muscular dystrophy.

Natural products have been the starting point for the discovery of many important modern drugs. *Eupatorium chinense* var. *simplicifolium* (EUC) is a perennial herb widely distributed in Korea, Japan and China that has anti-palsy and anti-hypertension effects; EUC extracts have also been reported to have anti-tumor effects. However, the effect of an EUC extract on muscle atrophy remains unknown. The aim of the present study was therefore to evaluate the effects of EUC on the signal transduction mechanisms involving muscle cell damage induced by oxidative stress.

C2C12 myoblasts (ATCC, USA) were treated for 24 h with EUC at concentrations from 0 to 50 μg/mL, and the cell viability was measured with an EZ-Cytox kit (Daeilab, Korea) to determine the cytotoxicity of EUC. EUC showed no evidence of cytotoxicity toward C2C12 myoblasts within this dose range (Fig. 1A). Cell injury associated with ROS may contribute to a variety of muscle diseases and pathological conditions. C2C12 myoblasts were exposed to H2O2 to clarify the effect of EUC on the oxidative stress response in myoblasts. The cell viability at a 1 mM H2O2 concentration recovered significantly as the EUC concentration was increased from 0 to 50 μg/mL (Fig. 1B). These findings suggest that EUC enhanced the inherent oxidative stress defenses, counteracting the H2O2-induced loss of cell viability in a dose-dependent manner.

We investigated the effects of EUC on muscle disorders and the underlying mechanism for H2O2-induced C2C12 myoblast damage. It is important to enhance the defense against antioxidants in myoblasts or stem cells as they repopulate muscle compartments and assume functional loads, as with exercise and disease. Heat shock protein (HSP) expression increases in response to stressors. HSPs are a family that includes HSP90, HSP70, HSP27, and other small HSPs. HSP70 protects cells from a number of apoptotic stimuli, including heat shock, tumor necrosis factor, growth factor withdrawal, oxidative stress, chemotherapeutic agents, ceramide, and radiation. HSP70 has been significantly down-regulated in various models of skeletal muscle atrophy. HSP70 has prevented caspase-3 and stress-activated protein kinase (SAPK)/Jun amino-terminal kinase (JNK) activation in heat shock-induced apoptosis.

We hypothesized that EUC would protect C2C12 myoblasts through modulation of HSP70 signaling in the cellular response to stress, and tested the effects of EUC on HSP70 expression in H2O2-treated myoblasts by Western blotting. Following culturing for 24 h in the presence or absence of EUC (25 and 50 μg/mL), C2C12

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Abbreviations: JNK, Jun amino-terminal kinase; SAPK, stress-activated protein kinase; DAPI, 4,6-diamidino-2-phenylindole
myoblasts were treated for 1 h with 1 mM H$_2$O$_2$. The EZ-Cytox reagent was then added to the medium, and the medium was replaced for 24 h with a serum-free medium with or without EUC (0–50 µg/mL). The EZ-Cytox reagent was then added to the medium, and the medium was then replaced with a serum-free medium with or without EUC (0–50 µg/mL). After pre-incubating for 24 h, H$_2$O$_2$ (1 mM) was added for 1 h. The EZ-Cytox reagent was then added to the medium, and the C$_2$C$_{12}$ myoblasts were incubated for an additional 1 h. The optical density was determined at 450 nm by using a microplate reader. Cell viability was calculated by using the equation: cell viability (%) = [(absorbance of the H$_2$O$_2$-treated sample/absorbance of the untreated control) × 100]. Values represent the mean (±SD) from six experiments, each performed in triplicate (**p < 0.01).

Changes in the chromatin morphology of H$_2$O$_2$-treated C$_2$C$_{12}$ myoblasts were evaluated by using 4′,6-diamidino-2-phenylindole (DAPI) staining to test the effect of EUC on apoptotic cell death. Treating with H$_2$O$_2$ for 1 h caused apoptosis characterized by chromatin condensation, small membrane-bound bodies (apoptotic bodies), cytoplasmic condensation, and cell shrinkage. Pre-treating for 24 h with EUC reduced apoptosis in the H$_2$O$_2$-treated C$_2$C$_{12}$ myoblasts (Fig. 3). The cell-protective effects of HSP70 are closely linked to apoptosis inhibition, and evidence suggests that HSP70 prevents apoptosis by inhibiting the SAPK/JNK signaling cascade. However, the direct effect of HSP70 on JNK remains to be clarified. Although we focused on HSP70 and JNK in C$_2$C$_{12}$ myoblasts and linked its protective function to defense against antioxidants, the full role of EUC in this function is still unclear. EUC may participate in other pathways involving the antioxidant enzymes and cellular components that generate free radicals as an antioxidant molecule, which in turn engage in complex signal transduction reactions in a stress response.

In conclusion, EUC increased intracellular HSP70 and decreased the JNK phosphorylation caused by H$_2$O$_2$-induced oxidative stress in C$_2$C$_{12}$ myoblasts. Through its ability to induce HSP70 and down-regulate JNK phosphorylation, an EUC treatment may be used to delay or prevent skeletal muscle atrophy. There generally seem to be many potential benefits of herbal
medicines in treating oxidative stress-related muscle disorders.

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