

Protective Effect of Oak Extracts on Oxidative Stress Induced by Hydrogen Peroxide¹

Jeong Bin Nam² · Hyung Bin Park³ · Ji Young Jung² · Jae-Kyung Yang^{2,†}

ABSTRACT

This study was done to evaluate the antioxidant effect of oak hot water extracts on the oxidative stress induced by reactive oxygen species (ROS). The cytotoxicity of H₂O₂-induced oxidative stress was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay for the cell viability according to the dose-dependent treatment. Oak extracts demonstrated a dose-dependent ability to inhibit H₂O₂-induced apoptosis in cultured tenofibroblasts, as assessed by MTT assay and FACS analysis. H₂O₂ increased the phosphorylation of extracellular regulated kinase1/2 (ERK1/2) and of c-Jun N-terminal kinase (JNK) and the production of reactive oxygen species (ROS). In contrast, treatment with oak extracts was decreased this activation of ERK1/2 and JNK, as confirmed by western blot analysis, and reduced the production of ROS, as verified by fluorescent microscopic and flow cytometry (FACS) analyses. These findings suggest that oak extracts, by suppressing JNK, ERK1/2, and intracellular ROS production, have a concentration-dependent antiapoptotic effect on achilles tenofibroblasts exposed to an oxidative stressor, and may have therapeutic potential.

Keywords : achilles tenofibroblast, oak, steam explosion, apoptosis, ROS

1. INTRODUCTION

Oaks (*Quercus variabilis*), the temperate-zone forest tree species and the most commercially important hardwood genus are widely distributed across Asia, Europe, North America and Africa. Oak is a deciduous broadleaf tree, common in East Asia (24° to 42° N and 96° to 140° E); it not only supplies raw material for the timber industry (e.g., cork, fodder, and bioethanol) but also serves an ecological func-

tion in China, Korea, and Japan (Kang *et al.* 2011; Wang *et al.* 2009). Most of the oak stands, occupying about 26% of the total forest area in South Korea, originated through natural regeneration, in particular from sprouting. Their growth rates are comparable to those of red pine (Lee *et al.* 2004). Some oak species are used as sources of antifungal and antidiarrheal agents, and astringents, as well as in the treatment of hemorrhoids, tonsillitis, and inflammation of the oral and anal mucosa. Moreover,

¹ Date Received February 13, 2014, Date Accepted March 17, 2014

² Department of Environmental Materials Science, Institute of Agriculture & Life Science, Gyeongsang National University, Jinju, 660-701, Korea

³ Department of Orthopaedic Surgery, School of Medicine, Gyeongsang National University, Jinju, 660-251, Korea

[†] Corresponding author : Jae-Kyung Yang (e-mail: jkyang@gnu.ac.kr)

the decoctions of these plants are used to treat burns and added to ointments for wound healing. Oak species have been previously shown to possess antimicrobial, anti-inflammatory, gastro-protective, antioxidant, and antitumoral properties. They are a rich source of saponins and polyphenols, particularly tannins, flavonoids, and proanthocyanidins. Oak extracts exhibited strong radical-scavenging activity (Şöhretoğlu *et al.* 2012; Şöhretoğlu *et al.* 2007). The main chemical constituents of the aqueous oak extracts are low molecular weight polyphenols, being ellagitannins the most abundant compounds (Pardo-Garcia *et al.* 2014; Puech *et al.* 1988). The oak wood shows high levels of monomer ellagitannins, such as castalagin, roburin E, vescalagin, and grandinin, and low molecular weight (LMW) phenolic compounds, ellagic and gallic acids, besides lignin derivatives, especially vanillin that can vary greatly depending on the species and geographical origin of the wood as well as the processing that undergoes in cooperation (Fernández de Simón *et al.* 2014; Panchal and Brown 2011).

Polyphenols are well recognized for their antioxidant activities. These compounds scavenge free radicals and disrupt the free-radical chain reaction of lipid peroxidation. Oxygen free radicals are continuously formed as intermediates of enzymatic reactions during normal cellular functions and some may be involved in growth regulation and intercellular signaling (Rahman *et al.* 2006). As antioxidants, polyphenols may protect cell constituents against oxidative damage and therefore, limit the risk of various degenerative diseases associated with oxidative stress (Rocha-Guzmán *et al.* 2009). Recently, attention has been focused on a wide array of non-vitamin antioxidants, such as plant polyphenolic components that are able to scavenge ROS and protect cells from oxidative damage (Jang and Surh 2001).

The achilles tendon connects the calf muscle (gastrocnemius) to the heel bone (calcaneus). The achilles tendon, formed by the merging of the tendons of the gastrocnemius and soleus, is the thickest and strongest tendon in the human body. Apoptosis is important in the development of tendon degeneration in achilles, patellar and rotator cuff tendons. Likewise, excessive autophagic cell death is involved in many degenerative diseases such as Parkinson's disease, Alzheimer's disease, and aortic valve disease (Chen *et al.* 2010; Scott *et al.* 2005).

Antioxidants have a strong antiapoptotic effect on cells exposed to intracellular oxidative stress. However, the specific types of antioxidants that are useful in protecting achilles tendon fibroblasts from apoptosis remain unknown. Therefore, we investigated the role of oak extracts in oxidative stress-mediated tenofibroblast cell death. Additionally, the current study investigated whether the underlying mechanism of action of oak hot water extracts on oxidative stress-mediated tenofibroblast cell death. Cell death is related to mitogen-activated protein kinases (MAPKs) activation and intracellular reactive oxygen species (ROS) production.

2. MATERIALS and METHODS

2.1. Plant Material and Extracts Preparation

Oak (*Quercus variabilis*) were obtained from Gyeongsang National University Research Forest (Sancheong, Korea). The steam explosion device is described in detail in Ballesteros *et al.* (2004). The reactor was charged with 20 kg (dry matter) of oak wood chip per batch and heated to the desired temperature (25 kg/cm², 250°C) with saturated steam for 10 minutes. The solid fraction was used extracts obtained from steam treatment. Hot water extraction

was carried out compound to distilled water (solids: liquid ratio, 1:20) using an autoclave at 121°C for 15 minutes. Hot water extracts were filtered using Whatman filter paper No. 2 to remove particulate matter, evaporated in a rotary evaporator at 50°C under reduced pressure and freeze-dried as a powder. Then, oak hot water extracts were dissolved in DMSO.

2.2. Achilles Tendon Primary Cell Culture

The achilles tendons from 6weeks Sprague-Dawley rats (weighing 200-250 g) were excised. The excised tendon was washed twice in phosphate-buffered saline (PBS). Each tendon was then cut into small pieces of approximately 1.5 - 2.0 mm (six pieces in total) and these pieces were individually placed in six-well culture plates. After 5 minutes of air-drying for better adherence 0.5 ml of DMEM (Dulbecco's modified Eagle's medium), with 30% fetal bovine serum (FBS) (Cansera, Rexdale, Ontario, Canada), 100 IU/ml penicillin, and 100 mg/ml streptomycin in a humidified 5% CO₂ atmosphere at 37°C. After 2 weeks, the cells reached 90% confluence. The cells were then trypsinized (0.02% trypsin, 0.02% EDTA in PBS) for 5 minutes, centrifuged for 5 minutes at 1,500 rpm, and expanded in a second passage. The cells were then harvested with trypsin/EDTA and cryopreserved. These cryopreserved six-passage cells were then thawed and used for all experiments in the current study.

2.3. Cell Viability Analysis

MTT assay was used to detect cell survival and proliferation after rat achilles tenofibroblast cells were treated with H₂O₂. Cells were first seeded into 24-well flat-bottomed culture plate. Oak extracts was then added to the culture dish at dosages of 0

(control group), 1.25, 2.5, 5 and 10 µg/ml. One hour later, 0.5 mM H₂O₂ was added to the plate and incubated at 37°C for an additional 24 hours. Cells were observed under microscope and then MTT assay (Hansen *et al.* 1989) was performed. DMEM, containing 50 µg/ml MTT, was added to each well and the plate was incubated at 37°C in an incubator for one hour. Then, the MTT solution was discarded and the 0.2 ml dimethyl sulfoxide (DMSO) was added to each well. After the formazan crystals were dissolved by mixing with micropipette, the colorless DMSO turned purple. Aliquots were transferred to the wells of the 96-well plates and then read immediately at 570 nm in a scanning multiwell spectrophotometer. Cell viability was expressed as a percentage of cytoprotection, versus controls set at 100%. The experiments were performed in triplicate.

2.4. Analysis of Apoptosis Rates

A total of 3×10^5 cells/well was plated onto 60-mm culture dishes and incubated overnight at 37°C, 5% CO₂. Oak extracts was then added to the culture dish at dosages of 0 (control group), 1.25, 2.5, 5 and 10 µg/ml. Twenty-four hours later, 0.5 mM H₂O₂ was added to cells. Cells were trypsinized thereafter, washed twice with cold PBS, pelleted, and fixed using cold ethanol (70%) for 3 hours. After that, cells were incubated with 50 µg/ml RNase A (Sigma- Aldrich Ltd., Gillingham, Dorset, UK) and incubated at 37°C for 1 hour. The cells were chilled over ice for 10 minutes and then stained with 50 µg/ml propidium iodide (Sigma-Aldrich Ltd., Gillingham, Dorset, UK). The percentage of the sub-G1 population was determined by flow cytometry, using a FACSCalibur (BD Biosciences, San Jose, CA).

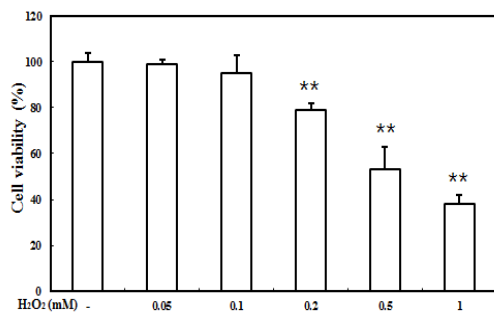


Fig. 1. Dose-dependent cell death induced by H₂O₂ in rat achilles tenofibroblast cells. Cultured cells were exposed to H₂O₂ (0.05 – 1 mM), and cell viability assays (MTT) were performed at 24 hours. Values are the means ± SD of three separate experiments performed in triplicate (**p < 0.01, compared to the control group).

2.5. Reactive Oxygen Species (ROS) Measurement

The ROS measurement is described in detail in Park *et al.* (2010). ROS generation in the tenofibroblasts was assessed using the probe 2,7-dichloro-fluorescein (DCF). Preconfluent cells were treated with the membrane-permeable diacetate form of the dye (DCFH-diacetate) at a final concentration of 5 mM, for 10 minutes. The dye that integrated into the cells was deacetylated by intracellular esterases. After incubation, the dye-integrated cells were washed with serum-free DMEM. The DCF-induced fluorescence was assessed with a laser-scanning confocal imaging system (OLYMPUS IX70, OLYMPUS, Tokyo, Japan). To quantify the production rates of ROS, the cells were stained with DCF for 10 minutes, removed from the plate with trypsin-EDTA (GibcoBRL, Grand Island, NY), and collected on the FACSCalibur (BD Biosciences). Data were analyzed using Cell Quest software (BD Biosciences).

2.6. Analysis for Intracellular MAPKs Activation

Cells were lysed in radioimmunoprecipitation assay buffer (RIPA buffer, Cell Signaling Technology) containing protease inhibitors (Protease Inhibitor Cocktail Set I, Calbiochem) and centrifuged at 13,000 rpm at 4°C for 15 minutes. Equal amounts of protein (30 μg) was separated with sodium dodecyl sulphate (SDS)-polyacrylamide gels and transferred onto a nitrocellulose membrane (Schleicher & Schuell, Postfach, Germany). After blocking with 5% skim milk, membranes were probed with primary anti-bodies against p-ERK (p42/44), p-JNK (p46/54) (Cell Signaling Technology, Beverly, MA, USA) overnight at 4°C. Peroxidase-conjugate anti-rabbit IgG (Serotec, Oxford, UK) were used for enhanced chemiluminescence (ECL) detection (Amersham Pharmacia Biotech, Arlington Heights, IL, USA).

2.7. Statistical Analysis

All experiments were performed using triplicate cultures, with the results expressed in each case as the mean standard deviation (SD) of the triplicate cultures. Each experiment was also performed at least three times, and representative data were reported. All statistical analyses were performed via one-way ANOVA, followed by Tukey's multiple-comparison tests.

3. RESULTS and DISCUSSION

3.1. Cytotoxicity of Oak Extracts in Rat Achilles Tenofibroblasts

To investigate the cytotoxic effects concerning the external addition of H₂O₂, we performed MTT assays in cultured rat achilles tenofibroblast. Cells

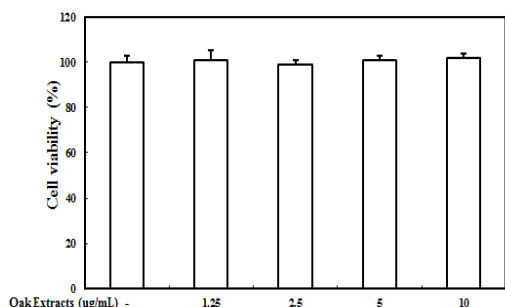


Fig. 2. Cell viability of rat achilles tenofibroblast cells after treatment with different extracts. Cells were cultured and treated by extract. Viability was measured 24 hours after treatment by extract. Values are the means \pm SD of three separate experiments performed in triplicate.

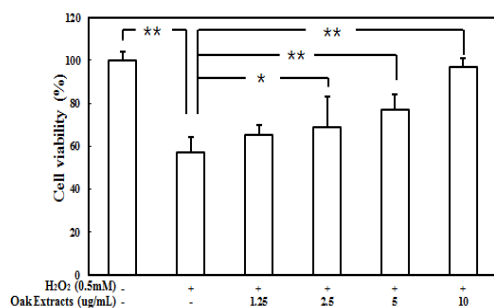


Fig. 3. Cell viability protective effect of oak extracts on H₂O₂ induced cytotoxicity in rat Achilles tenofibroblast cells. Cell viability was determined by MTT assay. Cells were treated with the indicated concentrations of co-treated with oak extracts and 0.5 mM H₂O₂ for 24 hours. Values are the means \pm SD of three separate experiments performed in triplicate (*p < 0.05, **p < 0.01).

were incubated with H₂O₂ at the indicated concentration for 24 hours, and then cell viability was measured using the MTT assay (Fig. 1). As a result, cell viability was significantly decreased by greater than 0.2 mM H₂O₂ in a concentration-dependent manner. Moreover, cell viability after exposure to 0.5 mM H₂O₂ was reduced to 50% of the control.

To study the cytotoxic effect of oak extracts, we employed the MTT assay (Fig. 2). We treated rat achilles tenofibroblast with various concentrations of oak extracts for 24 hours. There were no significant changes in cell viability until a dose of 10 $\mu\text{g}/\text{mL}$ of oak extracts was reached. To study the cytoprotective effect of oak extracts against H₂O₂-induced cell death, cells were pre-incubated with 1.25 - 10 $\mu\text{g}/\text{mL}$ oak extracts for 1 hours and then exposed to 0.5 mM H₂O₂ for 24 hours (Fig. 3). H₂O₂ treatment alone significantly decreased cell viability to about 50%. However, when cells were pretreated with 1.25 - 10 $\mu\text{g}/\text{mL}$ oak extracts for 1 hour, the cell viability was restored to roughly 97% of the control at a concentration of 10 $\mu\text{g}/\text{mL}$. Park *et al.* (2010) reported that anthocyanins exerted a significant cytoprotective effect on H₂O₂- mediated tenofibroblast cell death, in a somewhat concentration-dependent manner. The cell viability was restored to roughly 90.89% of the control at a concentration of 200 $\mu\text{g}/\text{mL}$.

3.2. Oak Extracts Protected Rat Achilles Tenofibroblast Cells against H₂O₂-induced Apoptosis

In order to analyze the protective effect of oak extracts on H₂O₂-induced apoptosis, the rat Achilles tendon fibroblasts were stained with propidium iodide dye to enable flow cytometric detection. The induction of a prolonged G₁ arrest by H₂O₂ may require the cells to be in the early G₁ phase before the treatment (Chen *et al.* 1998). H₂O₂ increased in the sub-G₁ phase cells about 53.79% at 0.5 mM H₂O₂, which was reduced to 17.62% by oak extracts at 10 $\mu\text{g}/\text{mL}$ (Fig. 4A). An additional finding was that H₂O₂-induced sub-G₁ phase cells were reduced by oak extracts in a dose-dependent manner (1.25, 2.5, 5 and 10 $\mu\text{g}/\text{mL}$). The rates of apoptosis were 1.10%

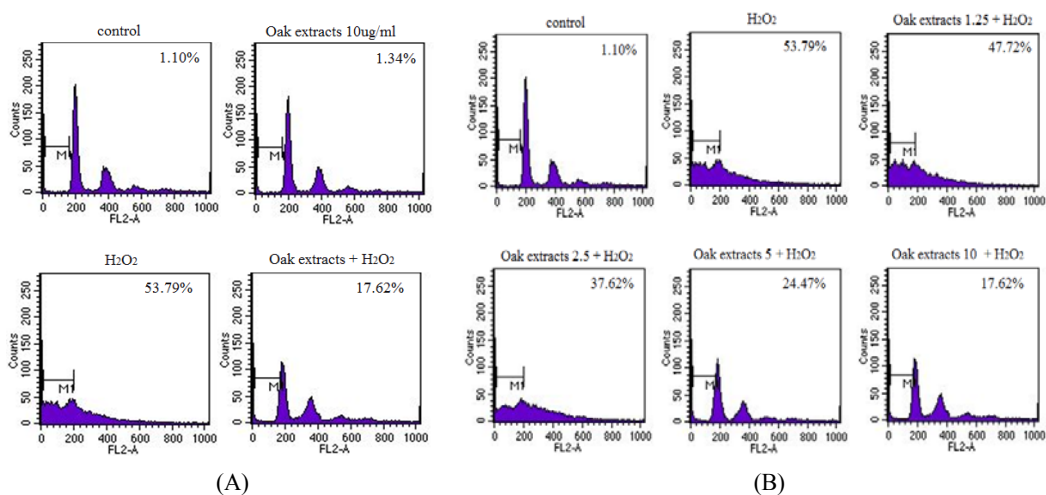


Fig. 4. Effects of pretreatment with oak extracts on H₂O₂-mediated apoptosis. (A) Tenofibroblasts were pretreated with oak extracts (10 µg/ml) for 1 hour, followed by a media change, then exposure to H₂O₂ for 24 hours. Tenofibroblast apoptosis was analyzed using PI staining, followed by flow cytometry analysis. (B) Tenofibroblasts were stressed with H₂O₂ in the absence or presence of different concentrations of oak extracts, as indicated. After 24 hours, apoptotic cells were analyzed by FACS for DNA fragmentation. (A) and (B) show representative results of three independent experiments; the numbers indicate the percentages of cells in sub-G₁ phase.

in the control group and 53.79% in the H₂O₂-treated group. The rates of apoptosis in each of the oak extracts-H₂O₂-treated subgroups were as follows: 47.72% in 1.25 µg/ml, 37.62% in 2.5 µg/ml, 24.47% in 5 µg/ml, and 17.62% in 10 µg/ml (Fig. 4B). These results demonstrated that oak extracts had a dose-dependent antiapoptotic effect on H₂O₂-mediated apoptosis.

3.3. Oak Extracts Inhibited The H₂O₂-induced Intracellular ROS

To elucidate the anti-oxidative mechanism of the oak extracts, we chose reactive oxygen species (ROS) for the investigation because ROS promote the oxidation of lipid, protein and DNA, thereby affecting the normal cell physiology, leading to neuronal demise. Reactive oxygen species (ROS), includ-

ing H₂O₂, activate an array of intracellular signaling cascades (Chang and Karin, 2001). According to the results of fluorescence microscopic analysis using the fluorescent probe DCF-DA, H₂O₂-induced ROS production was reduced by pretreatment with 10 µg/ml of oak extracts (Fig. 5A). The level of intracellular ROS production was assumed to be 1.00 in the control group. In the study groups, the levels were 2.04 in the H₂O₂-treated group and 1.48 in the 10 µg/ml-oak extracts-H₂O₂-treated group, according to the results of the FACS analysis (Fig. 5B). The level of intracellular ROS production in the H₂O₂-treated group was significantly higher than the level in the control group (p<0.01). The level of intracellular ROS production in the 10 µg/ml-oak extracts-H₂O₂-treated group was significantly lower than the levels in the H₂O₂-treated group (p<0.01). These results demonstrated that oak extracts have

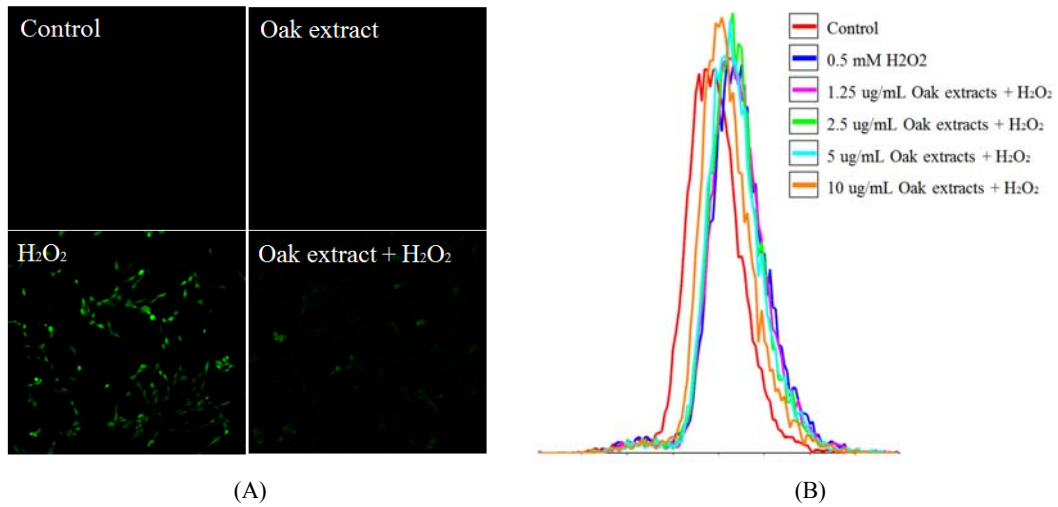


Fig. 5. Effects of oak extracts and scavengers against oxidative stress in rat achilles tenofibroblast cells. (A) Concentration data for oak extracts-induced ROS levels in rat achilles tenofibroblast cells. Examination of the effect of oak extracts on ROS level by the DCF-DA assay. Cells were exposed to oak extracts at concentrations of 10 $\mu\text{g}/\text{ml}$ for 24 hours. (B) Protection of cells against oak extracts-induced ROS levels by H₂O₂. Rat achilles tenofibroblast cells were exposed to 0 - 10 $\mu\text{g}/\text{ml}$ of oak extracts at 1 hour after 0.5 mM H₂O₂ treatment. Values are the means \pm SD of three separate experiments performed in triplicate (* $p < 0.05$, ** $p < 0.01$).

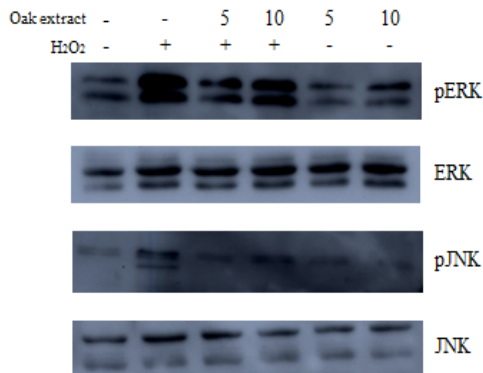


Fig. 6. Effects of oak extracts on the expression of ERK1/2, and JNK in rat achilles tenofibroblast cells in response to H₂O₂. Cells were pretreated with 5 $\mu\text{g}/\text{ml}$, 10 $\mu\text{g}/\text{ml}$ oak extracts for 30 minutes and then exposed to H₂O₂ (0.5 mM) for 12 hours. The cells were lysed, and the expression levels of ERK1/2, and JNK were determined by western blot analysis.

the ability to reduce H₂O₂-mediated intracellular ROS production.

3.4. Effects of Oak Extracts on MAPKs Activation

ERK and JNK are the main members of the MAPK family. ERK is an important protein that controls the cellular response to both proliferation and stress signals. JNK is mainly involved in the cell apoptosis and cell growth. Oxidative stress can stimulate MAPK signaling pathways that are closely associated with cell-death and cell-survival pathways (Chang and Karin, 2001). We investigated the effects of oak extracts on the expression levels of ERK1/2 and JNK in rat achilles tenofibroblasts cells in response to H₂O₂ treatment. The effects of oak extracts on the expression levels of ERK1/2 and JNK were

observed after exposure to H₂O₂ for 1 hour. The expression of ERK1/2 and JNK was increased in rat achilles tenofibroblasts cells exposed to H₂O₂ for 1 hour. After pretreatment with oak extracts, the expression of ERK1/2 and JNK proteins was inhibited significantly. These results suggest that the protective effects of oak extracts against H₂O₂-induced apoptosis involve blocking the activation of ERK1/2 and JNK.

4. CONCLUSION

This study demonstrates the possibility that oak extracts can play an important antiapoptotic role in the prevention of achilles tendon degeneration. Apoptosis induced by exposure to H₂O₂, although the involvement of H₂O₂ in the development of achilles tendon apoptosis has not yet been confirmed. Therefore, we postulated that H₂O₂ has a high probability of being involved in the apoptosis process of achilles tenofibroblasts. Because H₂O₂ is also routinely used in experiments to induce apoptosis of cells, we chose it to induce apoptosis of achilles tenofibroblast. To investigate the cytotoxic effects concerning the external addition of H₂O₂, we performed MTT assays in cultured rat achilles tenofibroblast. H₂O₂ treatment alone significantly decreased cell viability to about 50%. However, when cells were pretreated with 1.25 - 10 $\mu\text{g/ml}$ oak extracts for 1 hour, the cell viability was restored to roughly 97% of the control at a concentration of 10 $\mu\text{g/ml}$. In order to analyze the protective effect of the oak extracts on H₂O₂-induced apoptosis, the rat achilles tenofibroblast cells were stained with propidium iodide dye for flow cytometric detection. Oak extracts had a dose-dependent antiapoptotic effect on H₂O₂-mediated apoptosis. DCF-DA, H₂O₂- induced ROS production was reduced by pretreatment with 10 $\mu\text{g/ml}$ of oak extracts. These results demonstrated that oak extracts have the ability to reduce H₂O₂-mediated intracellular

ROS production. The effects of oak extracts on the expression levels of ERK1/2 and JNK in rat achilles tenofibroblasts cells in response to H₂O₂ treatment. In this study, the authors observed that the protective effects of oak extracts against H₂O₂-induced apoptosis involve the blocking of the activation of JNK and ERK1/2. The cytoprotective effect of oak extracts may, therefore, be the antiapoptotic result of the inhibition of the production of the ROS necessary to the activation of JNK and ERK1/2.

In conclusion, we focused on the inhibitory effect of oak extracts on H₂O₂-induced apoptosis. The data imply that oak extracts inhibit H₂O₂-induced apoptosis by suppressing both the intracellular ROS production and activation of JNK and ERK1/2. These findings suggest the possibility of the therapeutic use of oak extracts in patients with tendon degeneration.

ACKNOWLEDGEMENT

This study was carried out with the support of "Forest Science & Technology projects (project No. S211313L010140)" provide by Korea Forest Service.

REFERENCES

- Ballesteros, M., Oliva, J.M., Negro, M.J., Manzanares, P., Ballesteros, I. 2004. Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with *Kluyveromyces marxianus* CECT 10875. *Process Biochemistry*. 39: 1843~1848.
- Chang, L., Karin, M. 2001. Mammalian MAP kinase signalling cascades. *Nature*. 410: 37~40.
- Chen, J., Wang, A., Xu, J., Zheng, M. 2010. In chronic lateral epicondylitis, apoptosis and autophagic cell death occur in the extensor carpi radialisbrevis tendon. *Journal of Shoulder and Elbow Surgery*. 19: 355~362.

- Chen, Q.M., Bartholomew, J.C., Campisi, J., Acosta, M., Reagan, J.D., Ames, B.N. 1998. Molecular analysis of H₂O₂-induced senescent-like growth arrest in normal human fibroblasts: p53 and Rb control G₁ arrest but not cell replication. *Biochemical Journal*. 332: 43~50.
- Fernández de Simón, B., Sanz, M., Cadahía, E., Martínez, J., Esteruelas, E., Muñoz, A.M. 2014. Polyphenolic compounds as chemical markers of wine ageing in contact with cherry, chestnut, false acacia, ash and oak wood. *Food Chemistry*. 143: 66~76.
- Hansen, M.B., Nielsen, S.E., Berg, K. 1989. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *Journal of Immunological Methods*. 119: 203~210.
- Jang, J.H., Surh, Y.J. 2001. Protective effects of resveratrol on hydrogen peroxide-induced apoptosis in rat pheochromocytoma (PC12) cells. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 496: 181~190.
- Kang, H.Z., Liu, C.J., Yu, W.J., Wu, L.L., Chen, D.M., Sun, X., Ma, X.P., Hu, H.B., Zhu, X.L. 2011. Variation in foliar $\delta^{15}\text{N}$ among oriental oak (*Quercus variabilis*) stands over eastern China: patterns and interactions. *Journal of Geochemical Exploration*. 110: 8~14.
- Lee, W.K., Gadow, K.V., Chung, D.J., Lee, J.K., Shin, M.Y. 2004. DBH growth model for *Pinus densiflora* and *Quercus variabilis* mixed forests in central Korea. *Ecological Modelling*. 176:187~200.
- Panchal, S.K., Brown, L. 2011. Cardioprotective and hepato protective effects of ellagitannins from European oak bark (*Quercus petraea* L.) extract in rats. *European Journal of Nutrition*. doi: 101007/s00394-011-0277-1.
- Pardo-García, A.I., Martínez-Gila, A.M., Cadahiac, E., Pardob, F., Alonso, G.L., Salinas, M.R. 2014. Oak extract application to grapevines as a plant biostimulant to increase wine polyphenols. *Food Research International*. 55: 150~160.
- Park, H.B., Hah, Y.S., Yang, J.W., Nam, J.B., Cho, S.H., Jeong, S.T. 2010. Antiapoptotic effects of anthocyanins on rotator cuff tenofibroblasts. *Journal of Orthopaedic Research*. 28: 1162~1169.
- Puech, J.L., Rabier, P., Moutounet, M. 1988. Preparative separation by high-performance liquid chromatography of an extract of oak wood and determination of the composition of each fraction. *Journal of chromatography*. 457: 431~436.
- Rahman, I., Biswas, S.K., Kirkham, P.A. 2006. Regulation of inflammation and redox signaling by dietary polyphenols. *Biochemical Pharmacology*. 72: 1439~1452.
- Rocha-Guzmán, N.E., Gallegos-Infante, J.A., González-Laredo, R.F., Reynoso-Camacho, R., Ramos-Gómez, M., Garcia-Gasca, T., Rodríguez-Muñoz, M.E., Guzmán-Maldonado, S.H., Medina-Torres, L., Lujan-García, B.A. 2009. Antioxidant activity and genotoxic effect on HeLa cells of phenolic compounds from infusions of *Quercus resinosa* leaves. *Food Chemistry*. 115: 1320~1325.
- Scott, A., Khan, K.M., Duronio, V. 2005. IGF-I activates PKB and prevents anoxic apoptosis in Achilles tendon cells. *Journal of Orthopaedic Research*. 23: 1219~1225.
- Şöhretoğlu, D., Ekizoğlu, M., Kılıç, E., Sakar, M.K. 2007. Antibacterial and antifungal activities of some *Quercus* species growing in Turkey. *Fabad Journal of Pharmaceutical Sciences*. 3 2: 127~130.
- Şöhretoğlu, D., abuncuoğlu, S.S., Harput, Ü.Ş. 2012. Evaluation of antioxidative, protective effect against H₂O₂ induced cytotoxicity, and cytotoxic

activities of three different *Quercus* species.
Food and Chemical Toxicology. 50: 141~146.
Wang, J., Wang, S., Kang, H., Xin, Z., Qian, Z.,
Liu, C. Distribution pattern of oriental oak

(*Quercus variabilis* Blume) and the character-
istics of climate in East Asia. Journal of
Shanghai Jiao Tong University (Agriculture
Science). 27: 235~241.