Oxidative stress and nitrosative stress are the result of an imbalance between excessive production of free radicals and depletion of antioxidant defense systems to neutralize and eliminate reactive oxygen species (ROS) and reactive nitrogen species (RNS). These stressors are considered important mediators of cell injuries including DNA damage, lipid oxidation, protein modification, and alterations of cellular receptor functions. Therefore, the excessive accumulation of ROS and RNS is associated with many chronic degenerative diseases such as cardiovascular disease, arteriosclerosis, diabetes, cancer, Alzheimer’s, and Parkinson’s disease, as well as the aging process. In biological systems, the inactivation and removal of free radicals depends on the activity of antioxidants. Antioxidants that prevent cellular damage induced by ROS and RNS are considered effective therapeutic antioxidants. Antioxidants showed preventive effects against oxidative damage under a cellular system.

In the present study, we investigated the radical scavenging activity of KML in vitro and its protective effects against oxidative stress of Korean mistletoe (Viscum album coloratum) lectin were investigated in vitro and with a cellular system using LLC-PK, renal epithelial cells. The Korean mistletoe lectin (KML) showed 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity with an IC₅₀ value of 42.6 µg/ml. It also exerted nitric oxide (NO), superoxide anion (O₂⁻), and hydroxyl radical scavenging activities in concentration-dependent manners. These results suggest that KML is a promising antioxidant by scavenging free radicals. Furthermore, under the LLC-PK, cellular model, the cells showed declines in viability and increases in lipid peroxidation through oxidative stress induced by sodium nitroprusside (SNP) and pyrogallol, generators of NO and O₂⁻, respectively. However, KML significantly and dose-dependently inhibited cell cytotoxicity and lipid peroxidation. In addition, 3-morpholinosydnonimnie (SIN-1), a generator of peroxynitrite (ONOO⁻) formed by simultaneously releases of NO and O₂⁻, caused cytotoxicity, lipid peroxidation, and NO overproduction in the LLC-PK, cells while KML ameliorated ONOO⁻-induced oxidative damage. Furthermore, overexpressions of cyclooxygenase-2 and inducible NO synthase induced by SIN-1 were observed, but KML down-regulated the expression levels of both genes. KML also reduced SIN-1-induced nuclear factor kappa B expression and the phosphorylation of inhibitor kappa B alpha in LLC-PK, cells. These results indicate that KML has protective activities against oxidative damage induced by free radicals.

**Key words** Korean mistletoe lectin; LLC-PK, cell; antioxidative effect; cyclooxygenase-2; inducible nitric oxide synthase; nuclear factor kappa B

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**MATERIALS AND METHODS**

**Reagents** The KML was obtained from Mistlebiotech (Pohang, Korea), and was isolated from Korean mistletoe as previously described. Sodium nitroprusside (SNP), a metabolic generator of NO, was purchased from Wako Pure Chemical Industries (Osaka, Japan). 3-Morpholinosydnonimine (SIN-1), pyrogallol, and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). LLC-PK, porcine renal epithelial cells were provided by ATCC (Manassas, VA, U.S.A.). Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 (DMEM/F-12) and fetal bovine serum (FBS) were purchased from Hyclone (Grand Island, NY, U.S.A.) and GIBCO (Cleveland, OH, U.S.A.), respectively. Antibodies for cyclooxygenase (COX)-1, COX-2, inducible nitric oxide synthase (iNOS), nuclear factor kappa B (NF-κB), I kappa B (IκBα), phosphorylated IκBα (pIκBα), and actin were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, U.S.A.). All other chemicals and reagents used were of analytical grade and obtained from commercial sources.

**1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity** In a microwell plate, 100 µl of KML (con-
trol: 100 μl of distilled water) was added to an ethanolic solution of DPPH (60 μM) according to the method of Hatano et al. After being mixed gently and left for 30 min at room temperature, the DPPH radicals were determined using a microplate reader (model SPECTRAMax 340PC, Molecular Devices, Sunnyvale, CA, U.S.A.).

**NO Scavenging Activity** NO was generated from SNP and measured by the Griess reaction according to the method of Sreejayan and Rao. SNP (5 mM) in phosphate buffered saline was mixed with different concentrations of KML and incubated at 25 °C for 150 min. The amount of NO produced by SNP was assayed by measuring the accumulation of nitrite, using a microplate assay method based on the Griess reaction.

**O₂⁻ Scavenging Activity** O₂⁻ levels were measured following the method described by Ewing and Janero. For the assay, KML was added to microplate wells containing 200 μl of freshly prepared 0.125 mM ethylenediaminetetraacetic acid (EDTA), 62 μM nitro blue tetrazolium (NBT), and 98 μM reduced nicotinamide adenine dinucleotide phosphate (NADH) in 50 mM phosphate buffer (pH 7.4). The reaction was initiated with the addition of 25 μl of freshly prepared 33 μM 5-methylphenazinium methyl sulfate (PMS) in 50 mM phosphate buffer (pH 7.4). The absorbance was continuously monitored at 540 nm over 5 min as an index of NBT reduction using a microplate reader.

**Hydroxyl Radical (·OH) Scavenging Activity** The reaction mixture contained 0.45 ml of 0.2 mM sodium phosphate buffer (pH 7.0), 0.15 ml of 10 mM 2-deoxyribose, 0.15 ml of 10 mM FeSO⁴·EDTA, 0.15 ml of 10 mM H₂O₂, 0.525 ml of H₂O, and 0.075 ml of sample solution. The reaction was started by the addition of H₂O₂. After incubation at 37 °C for 4 h, the reaction was stopped by adding 0.75 ml of 2.8% trichloroacetic acid (TCA) and 0.75 ml of 1.0% of 2-thiobarbituric acid in 50 mM of NaOH. The solution was boiled for 10 min, and then cooled in water. The absorbance of the solution was measured at 520 nm. Hydroxyl radical scavenging activity was evaluated as the inhibition rate of 2-deoxyribose oxidation by ·OH.

**Cell Culture** Commercially available LLC-PK1 cells were maintained in a culture flask containing 5% FBS-supplemented DMEM/F-12 medium (pH 7.2) at 37 °C in a humidified atmosphere of 5% CO₂ in air. All subsequent procedures were carried out under these conditions. The cells were sub-cultured 5 d with 0.05% trypsin–EDTA in PBS.

**Radical Generation** LLC-PK1 renal tubular epithelial cells are susceptible to oxidative stress. A generator-induced cellular oxidative model was employed to investigate the protective effects of the KML against oxidative damage. After confluence had been reached, the cells were plated into 96-well culture plates and incubated for 4 h at 37 °C, and then the medium containing MTT was removed. The incorporated formazan crystals in the viable cells were solubilized with 100 μl of dimethyl sulfoxide (DMSO) and the absorbance of each well was read at 540 nm using a microplate reader.

**Thiobarbituric Acid Reactive Substances (TBARS)** The level of lipid peroxidant released from the cultured cells was estimated as TBARS according to the methods of Yagi and Yokode et al. with slight modifications. One aliquot of medium was mixed with 1.5 ml of 0.67% TBA aqueous solution and 1.5 ml of 20% TCA, and boiled at 95—100 °C for 45 min. The mixture was cooled with water and shaken vigorously with 3.0 ml of n-butanol. After the mixture was centrifuged at 4000 × g for 10 min, the n-butanol layer was removed, and the absorbance was measured at 520 nm on a fluorescence spectrophotometer (Model FR-550, Shimadzu, Kyoto, Japan).

**Assay of NO Levels** The amount of NO production was assayed by measuring the accumulation of nitrite, using a microplate assay method based on the Griess reaction. Briefly, 100 μl of culture supernatant was allowed to react with 100 μl of Griess reagent, and was then incubated at room temperature for 5 min. The optical density of the samples was measured at 540 nm using a Microplate Reader.

**Protein Extraction, Gel Electrophoresis, and Western Blot Analysis** The total cell lysates were lysed in an extraction buffer [25 mM Tris–Cl (pH 7.5), 250 mM NaCl, 5 mM EDTA, 1% nonidet P-40 (NP-40), 0.1 mM sodium orthovanadate, 2 ml leupeptin, and 100 ml phenylmethylsulfonyl fluoride]. For the determination of NF-κB translocation to nucleus, nuclear protein was extracted using a nuclear extraction kit (Sigma, St. Louis, MO, U.S.A.) by following the manufacturer’s methods. In brief, the cells were lysed with ice-cold buffer containing 10 mM N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (HEPES) (pH 7.9), 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM dithiothreitol (DTT), and 0.05% NP-40. After centrifugation, the supernatants were used as the cytoplasmic extract and the pellets were resuspended with nuclear extraction buffer [5 mM HEPES (pH 7.9), 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM DTT, and 26% glycerol] and centrifuged. The supernatants from centrifugation were used as the nuclear proteins. Protein concentration was determined with a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, U.S.A.). For western blot analysis, the proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and then electrotransferred to nitrocellulose membranes. The membranes were subjected to immuno blot analysis with desired antibodies and the proteins were visualized by the enhanced chemiluminescence method.

**RNA Extraction and Reverse Transcription (RT)-Polymerase Chain Reaction (PCR)** Total RNA was isolated using Trizol reagent (Invitrogen Co., Carlsbad, CA, U.S.A.) following the manufacturer’s methods. The total RNA was digested with RNase-free DNase (Roche, Indianapolis, IN, U.S.A.) for 15 min at 37 °C and repurified using an RNAse kit according to the manufacturer’s protocol (Qiagen, La Jolla, CA, U.S.A.). cDNA was synthesized from 2 μg of total RNA by incubation at 37 °C for 1 h with AMV reverse transcriptase (Amersham, U.S.A.) with random hexanucleotides according to the manufacturer’s instructions. The primers to specifically amplify the genes of interest are shown in Table 1. The reaction mixture was subjected to PCR to amplify the sequences of the desired primers. Amplification was performed in a Mastercycler (Eppendorf, Hamburg, Germany).
with cycles of denaturation at 94 °C, annealing at 58 °C, and extension at 72 °C for 30 s, respectively. The amplified PCR products were run on 1.5% agarose gels and visualized by ethidium bromide (Sigma, U.S.A.).

**Statistical Analysis** Significance was verified by performing Duncan’s multiple range tests using SAS software (version 6.0, SAS Institute, Cary, NC, U.S.A.).

**RESULTS**

**Radical Scavenging Activity** Table 2 represents the DPPH radical scavenging activity of the KML, showing that it inhibited DPPH formation by 50% at a concentration of 42.60 µg/ml (IC$_{50}$). Table 3 shows the scavenging effects of the KML on NO, O$_2^-$ and ·OH radicals in vitro. KML exerted significant inhibitory activity against NO radicals with dose dependence. At concentrations of 10, 50, and 500 µg/ml, KML scavenged NO by 26.0%, 49.6%, and 57.2%, respectively. Similar to its NO scavenging activity, KML significantly and dose-dependently scavenged O$_2^-$ radicals. At the high concentration of 500 µg/ml, it scavenged 49.3% of O$_2^-$. KML also scavenged ·OH effectively in a dose-dependent manner, and its protective effects against ·OH were markedly stronger than those against NO and O$_2^-$. 

**Protective Effects against SNP-Induced Oxidative Stress** The protective effects of KML against NO under a cellular system are shown in Fig. 1. Cell viability through generation of NO by SNP was markedly decreased to 25.1% compared to cells not treated with SNP. KML, however, exerted an increase in cell viability in a dose-dependent manner. Cell viability was elevated to 57.9 and 75.8% when 50 and 500 µg/ml of KML was treated after NO generation. The cellular lipid peroxidation levels in SNP-treated LLC-PK$_1$ cells were increased by 2-fold from 0.198 nmol malondialdehyde (MDA)/mg protein to 0.801 nmol MDA/mg protein. However, KML decreased MDA levels dose-dependently. At the concentrations of 50 and 500 µg/ml, lipid peroxidation levels were decreased by 36% (0.512 nmol/mg protein) and 71% (0.227 nmol/mg protein), respectively.

**Protective Effects against Pyrogallol-Induced Oxidative Stress** The generation of O$_2^-$ by pyrogallol decreased cell viability to 13.3% of normal cells, as shown in Fig. 2. However, treatment with KML recovered pyrogallol-induced cellular damage in a concentration dependent manner. At the concentration of 500 µg/ml, 44.9% of cell viability was observed. KML also showed protective effects against lipid peroxidation induced by O$_2^-$. Superoxide anion increased the formation of MDA from 0.216 to 0.901 nmol MDA/mg protein. The treatment of KML significantly inhibited lipid peroxidation, particularly at 500 µg/ml, and MDA level was decreased to 0.525 nmol MDA/mg protein (41% decrease).

**Protective Effects against SIN-1-Induced Oxidative Stress** The treatment of KML ameliorated SIN-1-induced cytotoxicity in the LLC-PK$_1$ cells. Cell viability declined to

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### Table 1. Gene-Specific Primers for RT-PCR

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence of primers</th>
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<tr>
<td>iNOS</td>
<td>5′-AGA-GAG-ATC-CGG-TTC-ACA-3′</td>
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<td></td>
<td>antisense 5′-CAC-AGA-GCT-GAG-GTA-ACA-3′</td>
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<tr>
<td>COX-1</td>
<td>5′-TGC-CCA-GCT-CTC-GGC-CGG-CGG-CFT-3′</td>
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<td></td>
<td>antisense 5′-GTG-CAT-CAA-CAC-AGG-GCC-CTC-TTC-3′</td>
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<tr>
<td>COX-2</td>
<td>5′-TTC-AAA-TGA-GAT-TGT-GGG-AAA-AT-3′</td>
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<tr>
<td></td>
<td>antisense 5′-AGA-TCA-TCT-CTG-GAG-TAC-CTT-3′</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5′-CGG-AGT-CAA-CGG-ATT-TGG-TCT-TAT-3′</td>
</tr>
<tr>
<td></td>
<td>antisense 5′-AGC-CTT-CTC-CAT-GGT-GTT-GAA-GAC-3′</td>
</tr>
</tbody>
</table>

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### Table 2. DPPH Radical Scavenging Effects of KML

<table>
<thead>
<tr>
<th>Material</th>
<th>IC$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Korean mistletoe lectin</td>
<td>42.60±0.76</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.97±0.12</td>
</tr>
</tbody>
</table>

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**Table 3. NO, O$_2^-$ and ·OH Radical Scavenging Effects of KML**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>NO Scavenging effect (%)</th>
<th>O$_2^-$ Scavenging effect (%)</th>
<th>·OH Scavenging effect (%)</th>
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<tr>
<td>10</td>
<td>26.0±1.0$^a$</td>
<td>5.6±0.3$^b$</td>
<td>25.2±0.8$^c$</td>
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<td>50</td>
<td>49.6±0.7$^b$</td>
<td>12.7±1.1$^c$</td>
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<tr>
<td>250</td>
<td>57.2±0.6$^b$</td>
<td>36.8±2.1$^c$</td>
<td>62.3±0.3$^c$</td>
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<tr>
<td>500</td>
<td>60.5±1.1$^b$</td>
<td>49.3±0.6$^d$</td>
<td>66.9±0.7$^d$</td>
</tr>
</tbody>
</table>

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Fig. 1. Protective Effects of KML on Cell Viability and TBARS Generation in SNP-Treated LLC-PK$_1$ Cells
18% by the addition of SIN-1 (Fig. 3). After treatment of SIN-1, which concomitantly generates NO with O$_2^-$ leading to ONOO$^-$ formation, KML was added at various concentrations. KML significantly and dose-dependently inhibited reductions of cell viability from damage by ONOO$^-$. The cells exposed to KML recovered to 61.6% viability at 500 $\mu$g/ml. Furthermore, the SIN-1-treated cells (0.189 nmol MDA/mg protein) had higher MDA levels than the non-treated cells (0.821 nmol MDA/mg protein), as shown in Fig. 3. However, the treatment of KML exerted protective activity against cellular damage by ONOO$^-$. When KML was treated at doses of 50 and 500 $\mu$g/ml, the formation of MDA declined to 0.534 nmol MDA/mg protein (35% decrease) and 0.278 nmol MDA/mg protein (66% decrease), respectively.

KML showed protective effects against SIN-1-induced NO production in LLC-PK$_1$ cells, as shown in Table 4. NO production was increased in the LLC-PK$_1$ cells; however, NO products were decreased in a dose-dependent manner by KML treatment. At the concentration of 1000 $\mu$g/ml, nitrite was decreased up to approximately 56% in the SIN-1-treated LLC-PK$_1$ cells.

Effects on the mRNA and Protein Expressions of COX-2 and iNOS Figures 4 and 5 show the effects of KML on the expressions of protein and mRNA related to oxidative stress, respectively. The mRNA and protein expressions of COX-2 were elevated in SIN-1 treated LLC-PK$_1$ cells without the up-regulation of COX-1 expression. In addition, iNOS expression was increased by ONOO$^-$. However, KML treatment markedly decreased the expressions of both genes.

Regulation of NF-$\kappa$B and I$\kappa$B$\alpha$ SIN-1 also induced elevated NF-$\kappa$B protein expression in the nucleus, while KML treatment markedly decreased this expression. Furthermore, I$\kappa$B$\alpha$ protein levels in cytoplasmic extract decreased with oxidative stress. KML treatment, however, resulted in higher levels compared to SIN-1-treated cells. The expression of p-I$\kappa$B$\alpha$ was increased by ONOO$^-$, indicating SIN-1 induced the phosphorylation of I$\kappa$B$\alpha$ while KML down-regulated p-I$\kappa$B$\alpha$ expression (Fig. 6).
DISCUSSION

ROS and RNS are by-products of cellular metabolism and have controversial dual effects in living organisms. They are necessary to mature cellular structures, and at moderate concentrations, work as part of the body’s defense system to destroy pathogens and induce mitogenic responses. However, free radical overproduction induces both oxidative and nitrosative stress, which are closely related to degenerative diseases. Oxidative and nitrosative stress are the result of an imbalance between the generation of free radicals and the antioxidant protective defense system. Antioxidants are important to alleviate damage induced by oxidative stress.

Mistletoe is a semi-parasitic plant that grows on many different kinds of deciduous trees all over the world. It has been used as a complementary treatment for many diseases including cancer, hypertension, cardiac vascular disease, epilepsy, arthritis, menopausal symptoms, and rheumatism. Research on the activity of Korean mistletoe has focused on its anti-

Fig. 4. Effects of KML on Protein Expressions of iNOS and COXs in SIN-1-Treated LLC-PK₁ Cells

(A) Western blot

(B)

(C)

Fig. 5. Effects of KML on mRNA Expressions of iNOS and COXs in SIN-1-Treated LLC-PK₁ Cells

(A) RT-PCR
cancer effects. Among the components of Korean mistletoe, lectin, in particular, has been reported to have anticancer activity by the stimulation of immune function. However, its direct radical scavenging activity and protective effects against oxidative stress are not known.

Therefore, the direct radical scavenging effects of KML were evaluated against free radicals in vitro. In the present study, we demonstrated that KML showed strong DPPH, NO, O\textsubscript{2} and ·OH radical scavenging activities. The DPPH radical scavenging activities of KML and ascorbic acid, a well known antioxidant compound as a positive control, were IC\textsubscript{50} value of 42.60 μg/ml, and 0.97 μg/ml (5.5 μM), respectively. Sharma and Bhat have been reported that IC\textsubscript{50} value against DPPH depends on the reaction solvent. Several other reports demonstrated the similar value of IC\textsubscript{50} to our study.\textsuperscript{19,20)} KML showed NO, O\textsubscript{2} and ·OH radical scavenging activities in dose dependent manners. In particular, KML showed the strongest activity against ·OH production and relatively weak scavenging activity against O\textsubscript{2}. The present results for KML’s radical scavenging effects support that it has antioxidative effects by the direct scavenging of NO, O\textsubscript{2} and ·OH.

We employed a cellular model system to investigate the protective effects of KML using LLC-PK\textsubscript{1} renal epithelial cells, which are susceptible to free radicals.\textsuperscript{21)} LLC-PK\textsubscript{1} cells are vulnerable to oxidative stress induced by free radicals. Therefore, the experimental model of oxidative damage, in which LLC-PK\textsubscript{1} cells are exposed to free radicals, would be useful for searching for agents that can provide effective protection from free radicals. Consistent with the present results, several studies demonstrated that cellular oxidative stress in LLC-PK\textsubscript{1} cell was induced by free radical generators such as SNP, pyrogallol and SIN-1.\textsuperscript{22,23)}

Our results show that SNP led to oxidative damage in the LLC-PK\textsubscript{1} cells through decreases in viability and elevations of lipid peroxidation. However, treatment of KML resulted in increases of cell viability with dose dependence. Lipid peroxidation is initiated by free radicals attacking unsaturated fatty acids in cellular membranes. KML treatment also showed decreases in SNP-induced lipid peroxidation. Peroxynitrite derived from NO and O\textsubscript{2} is a strong oxidant and nitrating agent and can lead to the most toxic ·OH radical.\textsuperscript{24)} SIN-1 simultaneously generates both NO and O\textsubscript{2}, which then combine rapidly to form ONOO\textsuperscript{−}. Therefore, it is widely used as a generator of NO and O\textsubscript{2} in experimental models.\textsuperscript{25)} KML showed cytoprotective effects against ONOO\textsuperscript{−}-induced toxicity and declines in MDA levels.

NO is involved in many physiological processes and has
both beneficial and detrimental effects. Although appropriate levels of NO production are necessary to protect body systems, sustained levels of NO cause tissue damage and various physiological conditions including neuronal degeneration, neurotransmission, and macrophage cytotoxicity. Furthermore, it works as a main precursor of ONOO\(^-\), which is cytotoxic and decomposes into \(\cdot OH\).\(^1\) NO is formed from the conversion of l-arginine to l-citrulline by nitric oxide synthase (NOS), which exists in three isoforms: endothelial NOS (eNOS), neural NOS (nNOS), and inducible NOS (iNOS). NO produced by eNOS and nNOS regulates physiological reactions, whereas NO produced by iNOS has been associated with degenerative diseases.\(^26\) Lee et al. reported that KML had immunoregulatory effects by modulating the production of NO in lipopolysaccharide (LPS)-treated macrophages.\(^7\)

COX, the key enzyme of prostaglandin biosynthesis, exists in two isoforms as COX-1 and COX-2.\(^22\) COX-1 is constitutive and detectable under normal physiological conditions, while COX-2 is almost undetectable. COX-2 is induced in various stimuli including many cytokines related to inflammation. The overexpression of COX-2 is related to elevations of prostaglandin levels in the inflammatory response.\(^7,28\) Therefore, to investigate the protective mechanisms of KML against free radicals, SIN-1-induced NO production and iNOS expression were investigated in LLC-PK\(_1\) cells. NO generation was decreased by mistletoe treatment. In addition, the mRNA and protein expressions of iNOS were down-regulated by KML treatment. Several studies have reported that KML and mistletoe itself clearly blocked LPS-induced NO production in macrophages. The present study also demonstrated that KML down-regulated SIN-1-induced iNOS expression at both the transcriptional and translational levels. COX-2 is regulated by NO directly. COX-2 mRNA and protein expressions were dramatically increased without alterations of COX-1 expression via ONOO\(^-\) generation. However, KML treatment inhibited the mRNA and protein expressions of COX-2.

NF-\(\kappa B\) is an oxidative-stress-responsive transcription factor. It is well known to mediate the expression of inducible genes such as of iNOS, COX-2, VCAM-1, and ICAM-1 in immune and inflammatory responses. The inactive form of NF-\(\kappa B\) is present in the cytosol as an NF\(\kappa B/\kappa B\) complex. The complex is phosphorylated by I\(\kappa B\) kinase through activation by stimulators such as potent endogenous inducers and endogenous inducers. This stimulation facilitates the translocation of free NF-\(\kappa B\) from the cytosol to the nucleus and this translocation of free NF-\(\kappa B\) is important in regulating NO production by iNOS and COX-2. Peroxynitrite is known to increase iNOS through NF-\(\kappa B\) in endothelial cells.\(^29\) Therefore, SIN-1-induced NF-\(\kappa B\) expression was investigated in the LLC-PK\(_1\) cells. The generation of ONOO\(^-\) markedly increased NF-\(\kappa B\) protein expression in the cells. However the treatment of KML markedly decreased this expression. The phosphorylation of I\(\kappa B\)\(\alpha\) is required to activate cytoplasmic NF-\(\kappa B\) so it can translocate into the nucleus. The p-I\(\kappa B\)\(\alpha\) protein levels in cytoplasmic extract were increased by ONOO\(^-\). However, KML markedly down-regulated p-I\(\kappa B\)\(\alpha\) protein expression indicating an inhibition effect of KML on ONOO\(^-\)-induced nuclear translocation of NF-\(\kappa B\). The degradation and phosphorylation of I\(\kappa B\)\(\alpha\) in ONOO\(^-\)-induced oxidative stressed cells were inhibited by the treatment of KML, as was nuclear translocation of cytoplasmic NF-\(\kappa B\) to the nucleus.

In conclusion, KML showed strong direct scavenging activities against DPPH, NO, and \(O_2^-\) in concentration-dependent manners. In addition, it attenuated the oxidative stress induced by NO, \(O_2^-\), and ONOO\(^-\) through elevations of cell viability and the inhibition of lipid peroxidation. Furthermore, the present results also implicate that its protective effects against oxidative stress were related to down-regulations of mRNA and protein expressions of COX-2 and iNOS through NF-\(\kappa B\) regulation. Therefore, this study suggests that Korean mistletoe, especially its lectin component, is a promising agent with protective activities against oxidative stress induced by free radicals.

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