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**Master's Thesis of Science in Biomodulation**

**Identification of Inhibitory effects of Gomisin N from Omija (*Schisandra chinensis*) on Early Stage of Atherosclerosis**

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# ABSTRACT

Omija *(Schisandra chinensis)* has long been used as a food material for its unique color and taste, and also as herbal medicine for its abundant bioactive substances. Omija is known to have the protective effect against cardiovascular diseases, but its bioactive compounds and underlying mechanisms remain unclear. In this study, I compared the preventive effect of the dibenzocyclooctadiene lignans which are specific compounds in Omija on early atherosclerosis. Among the lignans rich in Omija, gomisin N most effectively attenuated adhesion of THP-1 monocytes to Human umbilical vein endothelial cells (HUVECs). Western blot, ELISA and RT-qPCR results showed that gomisin N suppressed tumor necrosis factor-alpha (TNF-α)-induced expression of vascular adhesion molecule (VCAM)-1 and monocyte chemoattractant protein (MCP)-1 related to monocyte adhesion. In addition, gomisin N downregulated the phosphorylation of proteins involved in Nuclear Factor-Kappa B (NF-κB) pathway in a concentration-dependent manner.

Using a kinase profiling and IC50 assays, we elucidated that gomisin N significantly inhibited the activity of mammalian sterile-20 3 (MST3/STK24). Gomisin N also suppressed TNF-α-induced phosphorylation of ezrin/radixin/moesin (ERM), the substrate of MST3/STK24, in HUVECs. Taken together, gomisin N markedly inhibited THP-1 monocyte adherence to vascular endothelial cells by targeting MST3/STK24. Our findings suggest that gomisin N is a potential functional material for preventing early atherosclerosis.

# CONTENTS

# Ⅰ. INTRODUCTION

Atherosclerosis is a typical aspect of cardiovascular diseases that has been a major cause of death[1, 2] all over the world. It can develop for many years without symptoms and lead to sudden death or disability[3]. Therefore, it is recommended to maintain vascular health through prevention. Atherosclerosis is a chronic inflammatory disease with a condition in which the elasticity of the arteries decreases and the blood vessel width narrows. Circulating leukocytes, such as monocytes, transmigrate into the subendothelial layer due to the effect of overexpressed chemokines and adhesion molecules from endothelial cells, and differentiate into macrophages[4, 5]. Uptake of oxidized low-density lipoprotein (LDL) forms a lipid plaque[6] and abnormal proliferation of vascular smooth muscle cells thickens the arterial wall. As a result, the above procedures narrow the blood vessels and block blood flow. In order to prevent early stage of atherosclerosis, it is important to inhibit the adhesion of monocyte to vascular endothelial

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cells.

Omija (*Schisandra chinensis*) is a small red fruit that grows in Asian countries. In Korea, Omija was named after five tastes like sweetness, saltiness, bitterness, sourness and pungency. Omija has long been used as not only a food for its unique color and taste but also an herbal medicine because it contains abundant bioactive substances such as organic acids, vitamins and essential oils[7]. In a Korean traditional medical book, Donguibogam, it is said that Omija has the effect of improving circulation. Previous studies presented that extract of Omija has protective effects against cardiovascular diseases such as myocardial infraction and hypertension[8, 9]. However, such studies have not investigated its bioactive components and the underlying mechanism.

The specific bioactive ingredients of Omija are lignans with a dibenzocyclooctadiene structure. Although they have similar structures, each lignan has been reported to have different therapeutic effects of cardiovascular disease such as vasorelaxation, anti-oxidative stress and inflammation[10-12]. Among them, schisandrin, gomisin N and gomisin

A are used as marker components for herbal medicine standards because of their high contents in Omija. However, the preventive effects of those lignans on monocyte-endothelial cell adhesion have not been reported.

In this study, I identified the most effective lignan rich in Omija for preventing THP-1 monocyte-endothelial adhesion in HUVECs at first. In addition, I elucidated the molecular mechanism and target of its bioactive compound.

# Ⅱ. MATERIALS AND METHODS

## 1. Chemicals and reagents

Gomisin N and gomisin A were purchased from ALB Technology (Hong Kong, China, ≥98%, HPLC). Schisandrin was purchased from Chemfaces (Hubei, China, ≥98.9%, HPLC). Medium199 (M199), hydrocortisone, 2-mercaptoethanol, puromycin, fetal bovine serum (FBS) and calcein AM dye were purchased from Sigma-Aldrich (St. Louis, MO, USA). RPMI 1640 medium was purchased from Welgene (Daegu, Republic of Korea). L-glutamine, fetal bovine serum (FBS), basic fibroblast growth factor (bFGF), and recombinant human epidermal growth factor (hEGF) were purchased from Gibco (Grand Island, NY, USA). Penicillin (10,000 units/ml)-streptomycin (10,000 μg/ml) (P/S) was purchased from Corning (Corning, NY, USA). Recombinant human tumor necrosis factor-alpha (TNF-α) was purchased from PeproTech Korea (Seoul, Republic of Korea). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium salt (MTT) solution was

purchased from USB Corporation (Cleveland, OH, USA). Dimethylsulfoxide (DMSO) was purchased from Duksan Pure Chemicals (Ansan, Republic of Korea). Antibodies against vascular cell adhesion molecule-1 (VCAM-1), β-actin and basal IκB kinase (IKK)α/β were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Phosphorylated IKKα/β and IκBα, basal IκBα, phosphorylated ERM and basal ERM were purchased from Cell Signaling Biotechnology (Danvers, MA, USA).

## 2. Cell culture

Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza (Walkersville, MD, USA) and cultured in M199 with 25 mM HEPES containing 3 growth factors (1 ng/ml hydrocortisone, 1 ng/ml hEGF and 2 ng/ml bFGF), 1% (v/v) P/S, 2 mM L-glutamine and 10% (v/v) FBS (Gibco). Usage of HUVECs was limited between passages 7 and 14. To culture THP-1 monocyte-like leukemia derived cells purchased from the Korean Cell Line Bank, RPMI1640 supplemented with 50 μM 2-mercaptoethanol, 1% (v/v) P/S, and 10% (v/v) FBS (Sigma-Aldrich) were

used. Density of THP-1 was maintained between 2 x $10^5$ and 1 x $10^6$ cells/ml.

## 3. Cell viability assay

Confluent HUVECs in 96-well plates were serum-starved with M199 containing 1% (v/v) P/S and 2 mM L-glutamine for 4 h and incubated with various concentrations of gomisin N and lignan standards for 22 h. MTT solution was added to each well to 0.5 mg/ml of medium. After 2 h of incubation, the medium was removed and 200 µl DMSO was added. After 30min of incubation, the absorbance was measured at wavelength of 570 nm.

## 4. THP-1 monocyte adhesion assay

Confluent HUVECs in 96-well plates were serum-starved for 4 h and treated with gomisin N and lignan standards for 1 h. After stimulation with 10 ng/ml TNF-α for 5 h, stained THP-1 cells with calcein AM were added to HUVECs at density of 5 x $10^5$ cells/well in M199 and incubated for 1h. The plates were washed off with phosphate-buffered saline (PBS) to remove non-adhered THP-1. Florescence of adhered THP-1 was

measured with Infinite 200 PRO system (Tecan group Ltd., Männedorf, Switzerland) at excitation and emission wavelengths of 485 nm and 538 nm, respectively.

## 5. Western blot assay

Confluent HUVECs in 6-well plates or 6 cm dishes were serum-starved for 4h and treated with 10, 20 or 40 µM gomisin N for 1 h. The cells were stimulated with 10 ng/ml TNF-α for 5-15 min (NF-κB signaling and ERM) or 5 h (VCAM-1). To harvest cell lysates, the cells were washed with cold PBS and scraped with lysis buffer. The extracts containing equal amount of proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% fat-free dry milk and incubated with specific primary antibodies at 4 ℃ overnight. After incubation with horseradish peroxidase-conjugated secondary antibodies for 1h, protein bands were detected using an enhanced chemiluminescence detection kit (GE Healthcare, London, UK).

## 6. Enzyme-linked immunosorbent assay

Levels of MCP-1 in culture supernatant were determined using Human MCP-1/CCL2 ELISA MAX Deluxe Sets (BioLegend, San Diego, CA, USA) according to the manufacturer's protocol. Briefly, 100µl of diluted standards and culture supernatants were added to each well of 96-well plates pre-coated with diluted capture antibody and incubated for 2 h at room temperature. After each well was washed 4 times, incubated with detection antibody and Avidin-HRP solution for 1 h and 30 min, respectively. After washing the plates 5 times, TMB substrate solution was added and incubated 20 min in the dark. Stop solution was added and optical density of each well was measured using a microplate reader at wavelengths of 450 and 570 nm. A standard curve was generated and linear regression analysis was performed.

## 7. Real-time quantitative PCR

Total RNA was extracted from HUVECs using Trizol, RNA iso Plus (Takara Bio Inc., Shiga, Japan). RNA was quantified using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA,

USA). Reverse transcription was conducted with 1 μg/μl RNA using a PrimeScript™ 1st strand cDNA Synthesis Kit (Takara Bio, Inc., Shiga, Japan). Primers of human VCAM-1 forward (5'-CCC TCC CAG GCA CAC ACA-3'), human VCAM-1 reverse (5'-GAT CAC GAC CAT CTT CCC AGG-3'), human MCP-1 forward (5'-TCG CCT CCA GCA TGA AAG TC-3'), human MCP-1 reverse (5'-GGC ATT GAT TGC ATC TGG CT-3'), human GAPDH forward (5'-CAG GGC TGC TTT TAA CTC TGG TAA A-3'), and human GAPDH reverse (5'-GGG TGG AAT CAT ATT GGA ACA TGT AA-3') were purchased from Bioneer (Daejeon, Republic of Korea). For quantitative real-time PCR, iQ™ SYBR Green® Supermix and CFX Connect™ Real-time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) were used. The amount of target gene expression was calculated as a ratio of target gene relative to GAPDH in each sample.

## 8. Luciferase assay

The lentiviral expression vector pGF1-NF-kB-EF1-Puro (System Biosciences, Palo Alto, CA, USA) was transfected into HEK293T cells

with the packaging vectors pMD2.G and psPAX2 (Addgene, Cambridge, MA, USA). For transfection, JetPEI DNA transfection reagent (Polyplus-transfection, New York, NY, USA) was used. After the HEK293T cell culture was harvested and filtered, HUVECs were transfected using the medium and 10 μg/ml polybrene (EMD Millipore, Billerica, MA, USA). Stably-transfected HUVECs selected by 1 μg/ml puromycin (InvivoGen, San Diego, CA, USA) were seeded at density of $1 \times 10^4$ cells/well in 96-well plates. HUVECs were serum-starved for 4 h and treated with gomisin N 10, 20, 40 μM for 1 h before stimulation with 10 ng/ml TNF-α. After 14h of incubation, the cells were disrupted using lysis buffer (0.1 M pH 7.8 PBS, 1% Triton X-100, 1 mM DTT and 2 mM EDTA) and luciferase activity was measured by a Luminoskan Ascent (Thermo Electron, Helsinki, Finland).

## 9. Kinase assay

Kinase profiling was conducted by kinase assay service (Reaction Biology Corporation, Malvern, PA). Kinases were incubated with substrate and required cofactor. The reaction was initiated by the

addition of the compound in dimethyl sulfoxide (DMSO) and $^{33}$P-ATP (specific activity 10 µCi/µl). After incubation for 120 min at room temperature, reaction was spotted onto P81 ion exchange paper (GE healthcare) and washed extensively in 0.75% phosphoric acid. Kinase activity data was expressed as the percent remaining kinase activity in test samples compared to vehicle (DMSO) reactions.

## 10.  Statistical analysis

Statistical analyses were performed using SPSS (Statistical Analysis System Institute, 2017). All data were expressed as mean ± standard error of the mean (SEM) and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference test. $P < 0.05$ was used for statistical significance.

# Ⅲ. RESULTS

## 1. Gomisin N most effectively inhibits TNF-α-induced THP-1 monocyte-endothelial adhesion compared to other lignans rich in Omija (*Schisandra chinensis*)

To compare preventive effect of lignans rich in Omija (*schisandra chinensis*) on THP-1 monocyte-endothelial adhesion, HUVECs were pretreated with 40 μM of three compounds (gomisin N, gomisin A and schisandrin) for 1 h and induced with 10 ng/ml of TNF-α. Those compounds are abundant in Omija and have been used as marker components for herbal medicine standards. Among them, gomisin N showed superior inhibitory effect against TNF-α-induced adhesion of THP-1 to HUVECs compared to gomisin A and schisandrin (Fig. 1B). There is no effect on cell viability up to 40 μM within 24 h (Fig. 1C). Considering the contents in Omija and inhibitory effects on monocyte adhesion, I carried out this research with gomisin N.
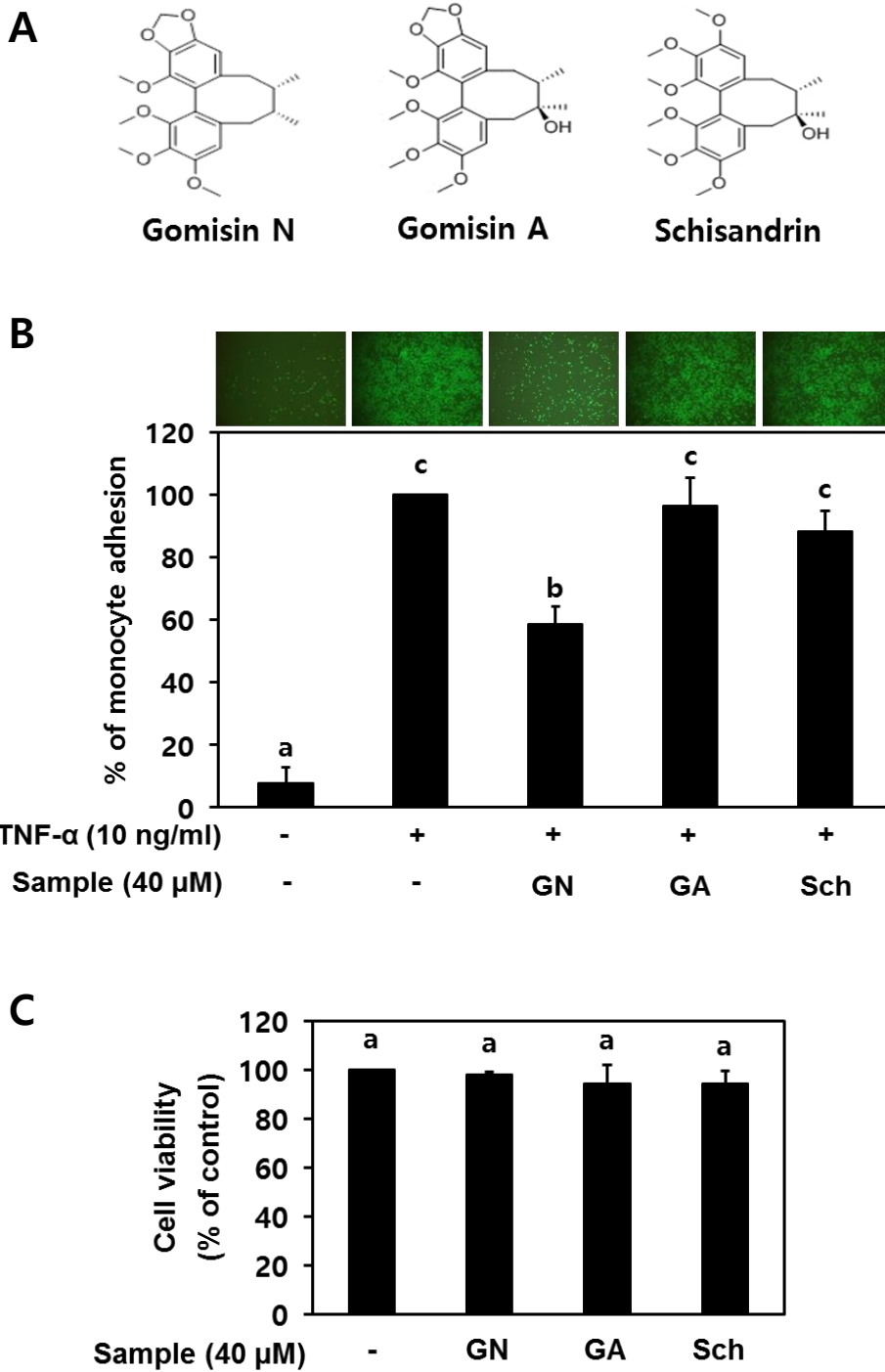
# Figure 1

**A**



Gomisin N          Gomisin A          Schisandrin

**B**



| TNF-α (10 ng/ml) | - | + | + | + | + |
| Sample (40 μM) | - | - | GN | GA | Sch |

**C**



| Sample (40 μM) | - | GN | GA | Sch |

**Figure 1. Effect of dibenzocyclooctadiene lignans rich in Omija on THP-1 monocyte-endothelial adhesion**

**A.** Structure of gomisin N, gomsin A and schisandrin. **B.** Representing images of TNF-α -stimulated THP-1 adhesion to HUVECs in treatment with 40 μM of gomisin N, gomisin A and schisandrin. Quantification of adhered THP-1 cells was conducted as described in the Materials and Methods. **C.** Cell cytotoxicity of all above compounds was tested by MTT assay. Data (n = 3) represent the means ± SD. Means with different letters are significantly different from each other at *P* < 0.05. GN, gomisin N; GA, gomisin A; Sch, schisandrin.

## 2. Gomisin N attenuates TNF-α-induced THP-1 monocyte-endothelial adhesion in a concentration-dependent manner

To identify the inhibitory effect of gomisin N, which was the most effective lignan, on interaction between THP-1 monocytes and endothelial cells, HUVECs were treated with various concentrations of gomisin N for 1h and induced with 10 ng/ml of TNF-α. From 20 μM treated group, the interaction was significantly attenuated. In addition, 40 μM of gomisin N showed about 50% of inhibitory effects on monocyte adhesion compared to inducer group. Therefore, gomisin N inhibited TNF-α-induced THP-1 monocyte adhesion to HUVECs in a concentration-dependent manner (2A and B).
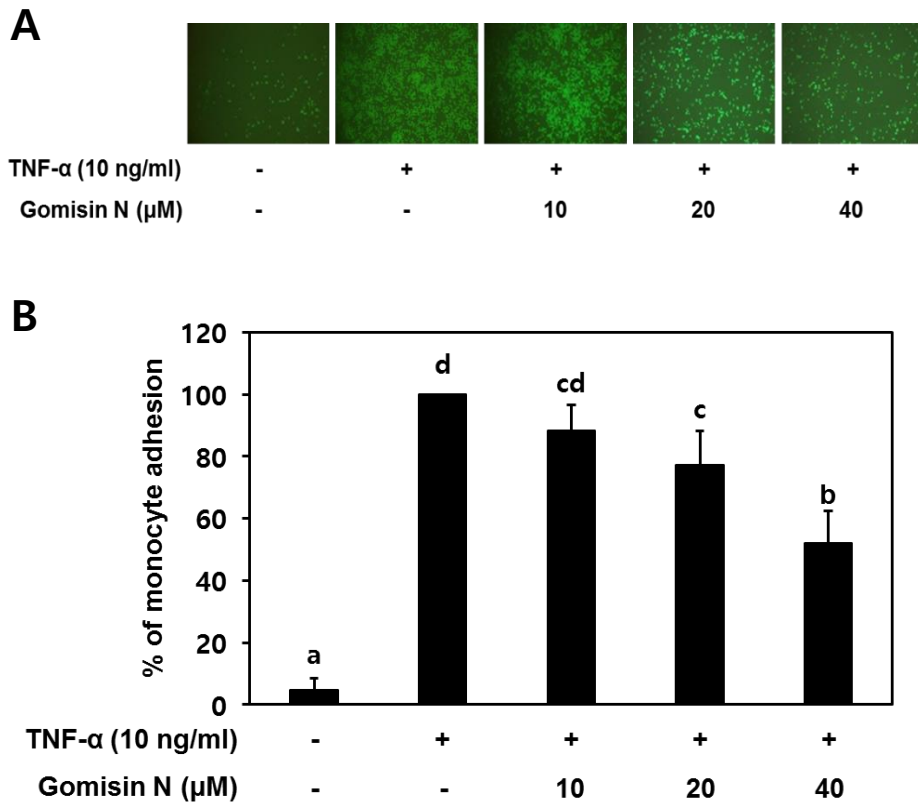
# Figure 2

**A**



| TNF-α (10 ng/ml) | - | + | + | + | + |
| Gomisin N (μM) | - | - | 10 | 20 | 40 |

**B**



| TNF-α (10 ng/ml) | - | + | + | + | + |
| Gomisin N (μM) | - | - | 10 | 20 | 40 |

**Figure 2. Effect of gomisin N on THP-1 monocyte-endothelial adhesion**

**A.** Representing images of TNF-α-stimulated THP-1 adhesion to HUVECs in treatment with 10, 20, 40 μM of gomisin N. **B.** Quantification of adhered THP-1 cells was conducted as described in the Materials and Methods. Data (n = 3) represent the means ± SD. Means with different letters are significantly different from each other at $P < 0.05$.

## 3. Gomisin N suppresses TNF-α-induced VCAM-1 expression in HUVECs

I next investigated the effect of gomisin N on the expression of VCAM-1 which is a key adhesion molecule to regulate vascular inflammation and binding of monocytes to endothelial cells[13]. As shown in western blot analysis results, 10 – 40 μM of gomisin N significantly attenuated TNF-α-induced protein expression levels of VCAM-1 in HUVECs. In particular, the group treated with 20, 40 μM of gomisin N inhibited VCAM-1 expression to a level similar to that of the control group. (Fig. 3A). Similarly, RT-qPCR results showed that gomisin N suppressed TNF-α-induced mRNA expression levels of VCAM-1 in a concentration-dependent manner (Fig. 3B).
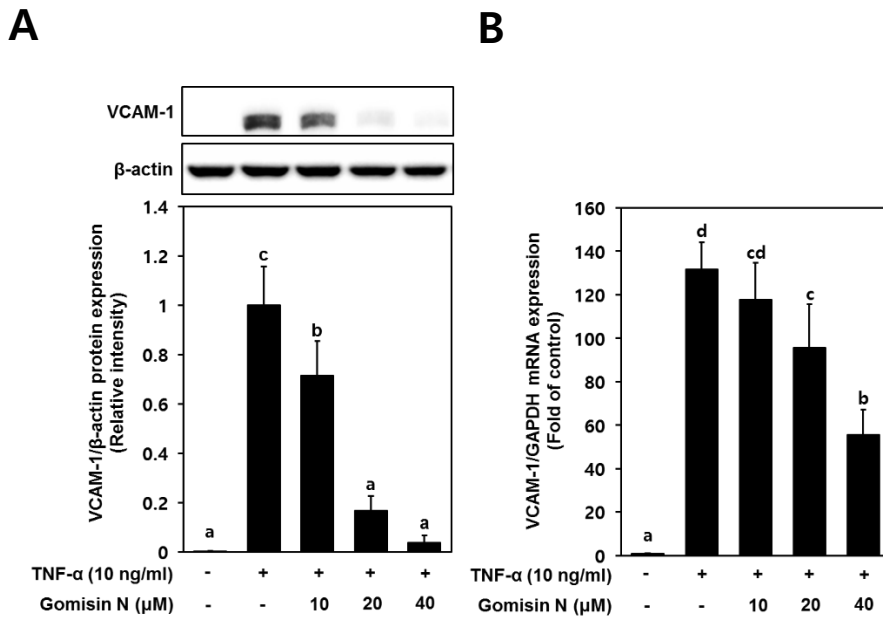
**Figure 3**

**A**



**B**



**Figure 3. Effect of gomisin N on TNF-α-stimulated VCAM-1 expression levels in HUVECs**

**A.** Western blot analysis shows inhibitory effect of gomisin N on TNF-α-stimulated VCAM-1 protein expression. **B.** VCAM-1 mRNA expression was investigated using RT-qPCR as described in the Materials and Methods. Data (n = 3) represent the means ± SD. Means with different letters are significantly different from each other at $P < 0.05$.

## 4. Gomisin N suppresses TNF-α-induced MCP-1 expression in HUVECs

We assessed the effect of gomisin N on expression of MCP-1 which is a chemokine that plays an important role in transmigration of monocytes into vascular intima[14]. As shown in ELISA results, gomisin N reduced TNF-α-induced secretion of MCP-1 protein in HUVECs (Fig. 4A). Similarly, RT-qPCR results showed that gomisin N suppressed TNF-α-induced mRNA expression levels of MCP-1 (Fig. 4B) in a concentration-dependent manner.

**Figure 4**



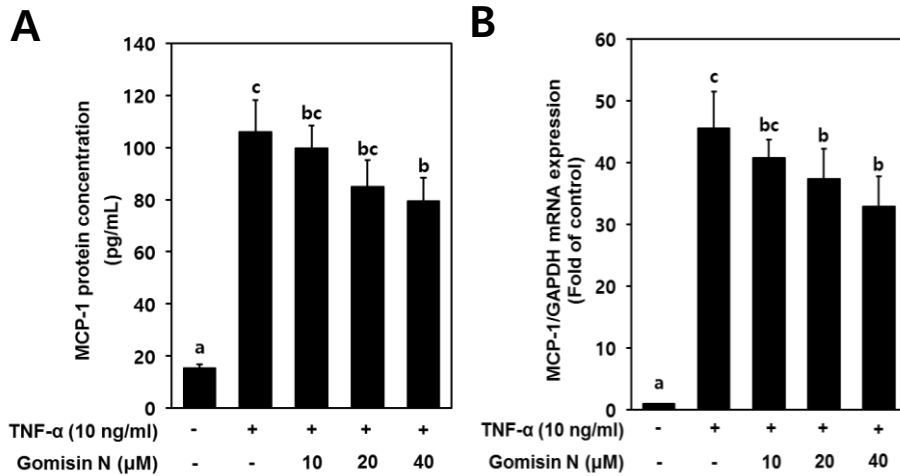**Figure 4. Effect of gomisin N on TNF-α-stimulated MCP-1 expression levels in HUVECs**

**A.** ELISA analysis shows preventive effect of gomisin N on TNF-α-stimulated MCP-1 protein expression **B.** MCP-1 mRNA expression was investigated using RT-qPCR as described in the Materials and Methods. Data (n = 3) represent the means ± SD. Means with different letters are significantly different from each other at $P < 0.05$.

## 5. Gomisin N downregulates TNF-α-induced transactivation of NF-κB in HUVECs

I examined the preventive effect of gomisin N on NF-κB transactivation in HUVECs transfected with luciferase reporter plasmids. NF-κB is a transcription factor that regulates production of VCAM-1 and MCP-1[15]. Luciferase assay results showed gomisin N inhibited TNF-α-induced transactivation of NF-κB in HUVECs (Fig. 5A). Western blot analysis results showed gomisin N drastically suppressed TNF-α-induced phosphorylation of IκBα that is an upstream protein of NF-κB signaling pathway (Fig. 5B) and the degradation of basal IκBα (Fig. 5C). In addition, gomisin N inhibited phosphorylation of IKK that is an upstream protein of IκBα in a concentration-dependent manner (Fig. 5D).
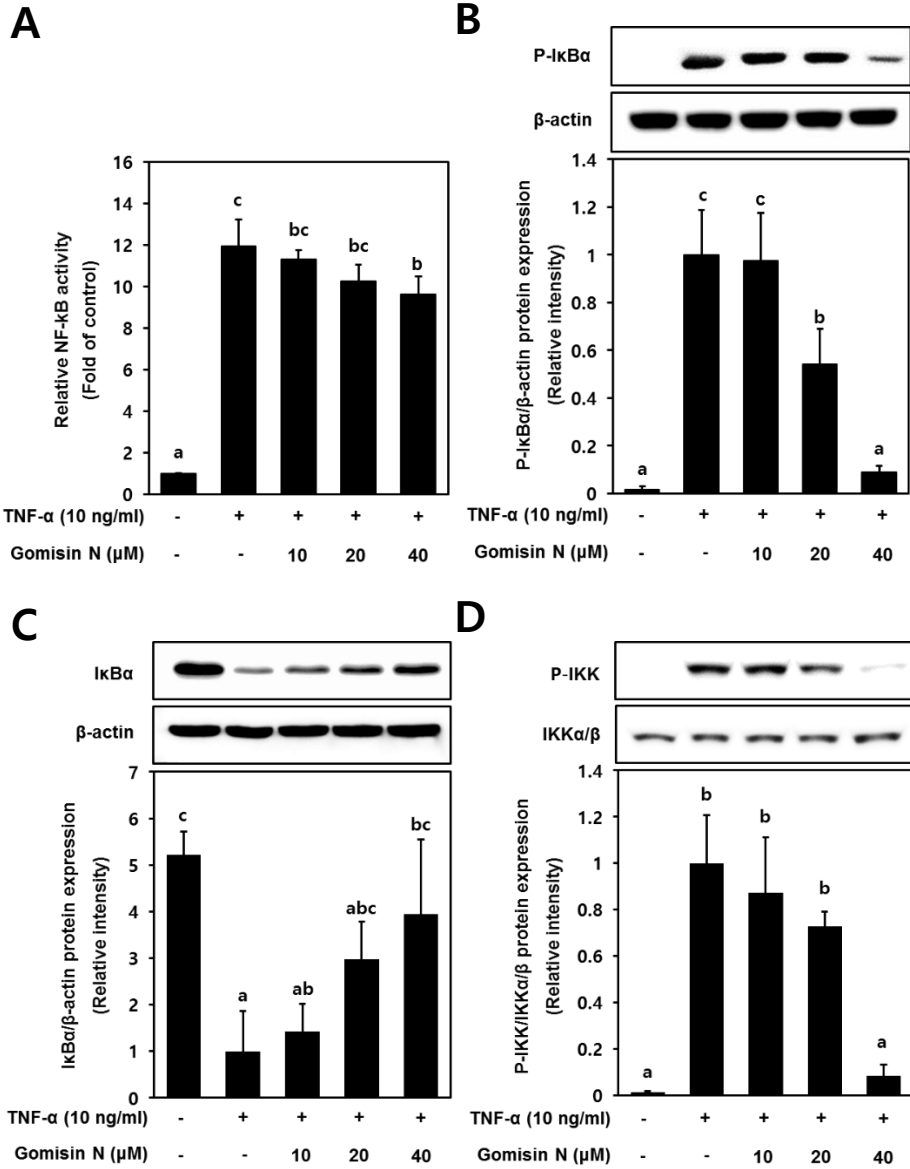
**Figure 5**

**Figure 5. Effect of gomisin N on TNF-α-stimulated NF-κB signaling pathway in HUVECs**

**A.** A luciferase reporter gene assay shows effect of gomisin N on TNF-α-stimulated NF-κB transcriptional activity. Western blot analyses show the effect of gomisin N on phosphorylated and total protein expressions related to NF-κB signaling pathway. **B.** β-actin protein expression levels was used as a control of phosphorylated IκBα and **C.** total IκBα. **D.** Total protein expression levels of IKK was used as a control of phosphorylated IKK. Data (n = 3) represent the means ± SD. Means with different letters are significantly different from each other at $P < 0.05$.

## 6. Gomisin N reduces MST3/STK24 activity

To find a direct target protein of gomisin N in suppressing TNF-α-induced signaling pathway, a kinase profiling analysis was conducted by kinase assay service (Reaction Biology Corporation). Activities of 369 human wild type kinases were analyzed with 20 μM gomisin N. From the results, I found 6 kinases which have less than 60% of enzyme activities (Table 1). In order to select the target, I excluded 4 kinases which are not expressed in HUVECs and 1 kinase which is less associated with monocyte adhesion. Finally, I selected MST3/STK24 as the target of gomisin N. Gomisin N also significantly attenuated the activity of MST3/STK24 with 8.1 μM of the half maximal inhibitory concentration (IC50) (Fig. 6A).

# Table 1

**Table 1. Kinase profiling of gomisin N (20 μM)**

| Kinase | % Enzyme activity (relative to DMSO controls) |
|---|---|
| MST3/STK24 | 41.16 |
| DAPK1 | 45.30 |
| ROS/ROS1 | 46.32 |
| STK33 | 55.59 |
| CAMK4 | 55.80 |
| RIPK4 | 59.45 |

Kinase profiling analysis of gomisin N was performed in duplicate mode at a concentration of 20 μM. 369 whole human type kinases were tested to find the target of gomisin N by kinase assay service (Reaction Biology Corporation). We selected 6 kinases whose activity was reduced by more than 40% by gomisin N.
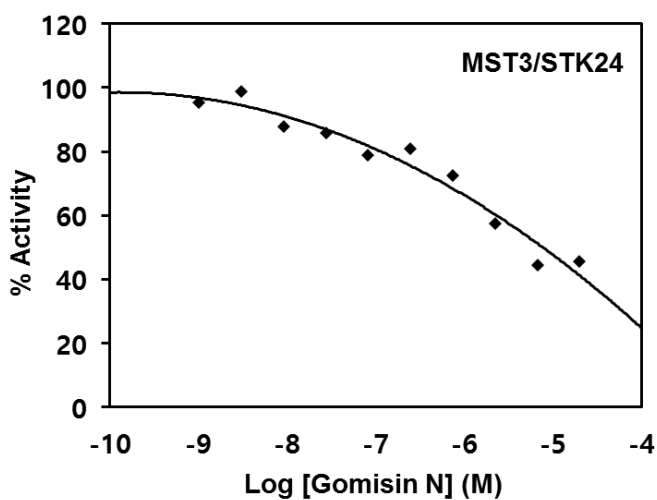
**Figure 6**

**Figure 6. Effect of gomisin N on MST3/STK24 activity**

Gomisin N was tested for MST3/STK24 inhibitory activity from a 10-dose $IC_{50}$ assay with 3-fold serial dilution starting at 20 μM.

# 7. Gomisin N suppresses TNF-α-induced phosphorylation of ERM in HUVECs

Next, I investigated the effect of gomisin N on phosphorylation of ERM family protein, a substrate of MST3/STK24, which is known as making docking system by binding VCAM-1 and up-regulating NF-κB signaling pathway indirectly[16, 17]. Western blot analysis results showed gomisin N markedly inhibited TNF-α-induced phosphorylation of ERM in HUVECs (Fig. 7A).

# Figure 7



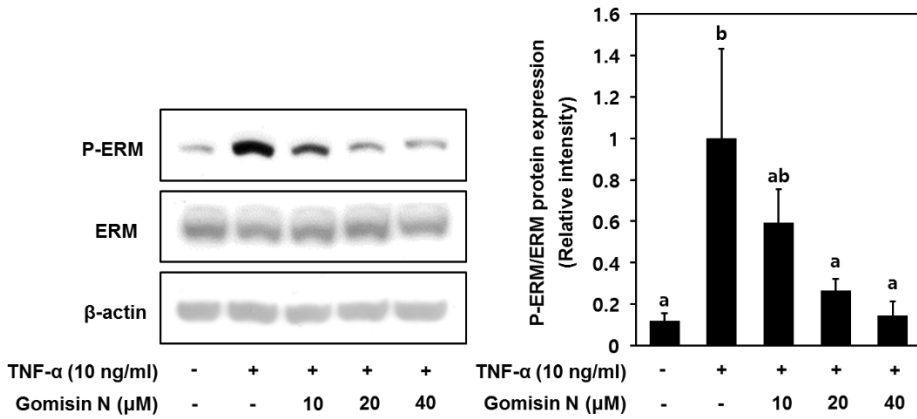**Figure 7. Effect of gomisin N on TNF-α-stimulated ERM phosphorylation in HUVECs**

Western blot analysis shows inhibitory effect of gomisin N on TNF-α-stimulated phosphorylation of ERM. Data (n = 3) represent the means ± SD. Means with different letters are significantly different from each other at $P < 0.05$.
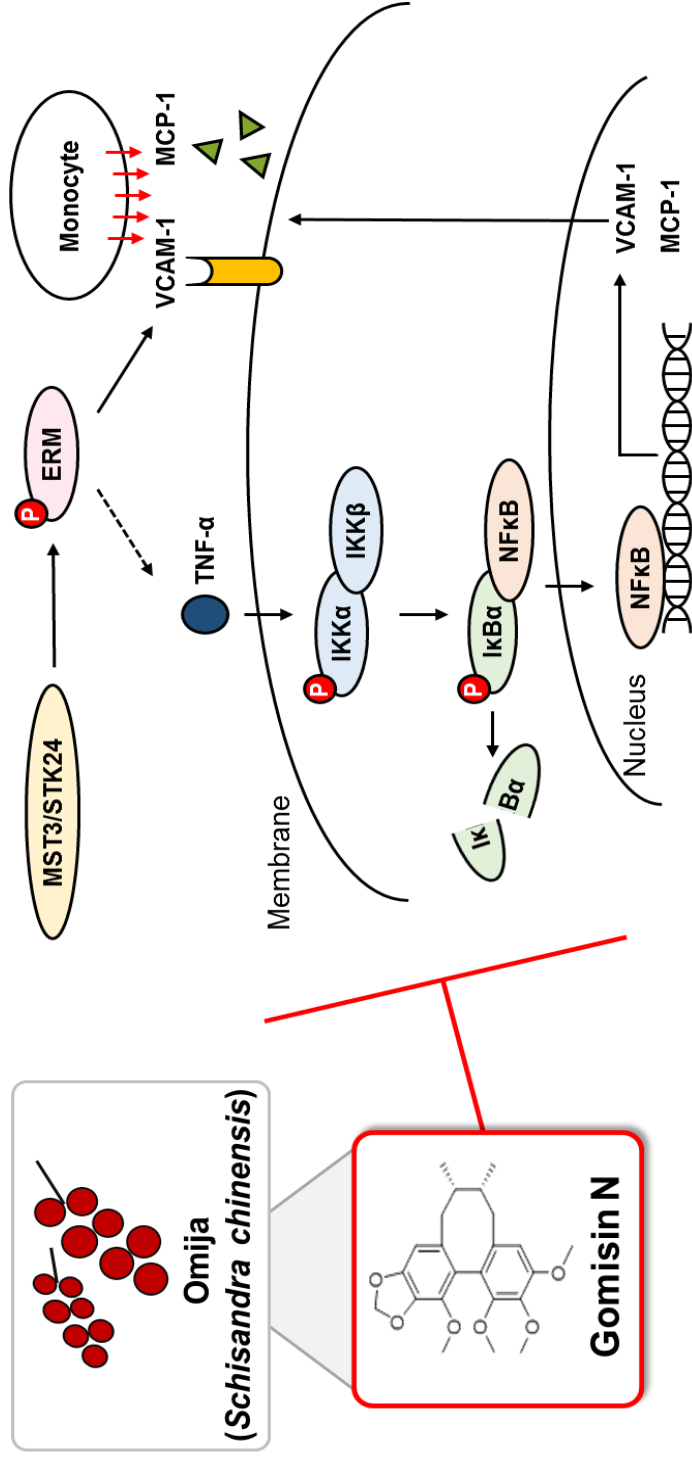
# Figure 8



Figure 8. Proposed mechanism of gomisin N

# Ⅳ. DISCUSSION

Dibenzocyclooctadiene lignans have been isolated to more than 300 species so far, and new compounds and their bioactivities are still being discovered and studied. Most of them are known to be abundant in *Schisandra[18]* and *Kadsura[19]* genera belonging to Schisandraceae family. Omija (*Schisandra chinensis*), a Korean medicinal plant, has also various kinds of lignans. Recent studies show that Omija contains about 5-9 mg/g of schisandrin, 2-3 mg/g of gomisin N and 1-2 mg/g of gomisin A[20-22]. Because these three lignans are highly concentrated in Omija, we asked if one of these is a bioactive compound of Omija which can exert the preventive effects of early stage of atherosclerosis.

In the present study, the preventive effect of gomisin N on atherosclerosis was determined by monocyte-endothelial adhesion assay. This is the first time to compare the three major components of Omija on monocyte-endothelial adhesion. Among the three lignans contained in Omija, we found that gomisin N is the most effective. Previous studies reported that gomisin N has the effects of improving sleep-behavior[23],

anti-obesity[24], anti-melanogenic[25] and so on. Especially, gomisin N exhibits inhibitory effects on nitric oxide production in lipopolysaccharide-stimulated RAW 264.7 macrophages[26]. Because of its anti-inflammatory properties, it is thought to have effectively worked against atherosclerosis which is aggravated by chronic inflammatory responses.

The monocytes that move around the blood vessels usually do not stick strongly to vascular endothelial cells[27]. However, when the endothelial cells are stimulated by a key pro-inflammatory cytokine such as TNF-α or oxidized LDL, they increase the secretion of MCP-1 and expression of VCAM-1. MCP-1 attracts monocytes into vascular intima and VCAM-1 causes the monocytes to adhere firmly to endothelial cells[28, 29]. We identified that gomisin N, which inhibited monocyte adhesion most effectively, also suppressed TNF-α-induced protein and mRNA expression levels of VCAM-1 and MCP-1 in a concentration-dependent manner at 10 to 40 μM. NF-κB signaling pathway is known to promote inflammatory reactions in atherosclerosis via regulating the

expression of VCAM-1 and MCP-1[30]. IκB proteins inhibit NF-κB signaling by tightly binding to NF-κB dimers until N-terminal phosphorylation by stimulated IKKs. Upon stimulation, IKKs phosphorylate IκBs leading to subsequent degradation of these proteins[31]. We demonstrated that gomisin N significantly inhibited the TNF-α-stimulated activity of the NF-κB transcription factor and also inhibited TNF-α-induced phosphorylation IκBα and IKK at 20 and 40 μM respectively. Taken together, these results indicate that gomisin N inhibits monocyte-endothelial adhesion, at least in part, via the inhibition of NF-κB signaling. However, these results these results did not show how gomisin N inhibits the phosphorylation of IKKs.

In this study, we also found that MST3/STK24 is a molecular target of gomisin N. MST3/STK24 plays a role in regulating cytoskeleton and mitogen-activated protein kinase (MAPK) signaling[32]. In particular, it is expressed in HUVECs and known to phosphorylate ERM protein involved in monocyte adhesion[33]. The ERM family which is known as substrate of MST3/STK24 is a key organizer of specialized membrane

domains. As ERM is activated by phosphorylation in the threonine residue, its folded structure expands and it can bind to other proteins[34]. Recent study reported that ERM indirectly promotes the NF-κB pathway by binding to ERM binding protein 50 (EBP50) with positive feedback[17]. Furthermore, it builds an endothelial docking structure to facilitate monocytes adherence by making a complex with VCAM-1[16]. We showed that gomisin N significantly inhibited the activity of MST3/STK24 and also suppressed TNF-α-stimulated phosphorylation of ERM in HUVECs. However, it remains to be determined how gomisin N structurally binds MST3/STK24 and regulates its downstream signaling.

In conclusion, the present study demonstrates that gomisin N significantly inhibited THP-1 monocyte-endothelial adhesion *in vitro*. We have shown that its efficacy is based on a mechanism that down-regulates NF-κB signaling pathway involved in VCAM-1 and MCP-1 expression by targeting MST3/STK24. Therefore, gomisin N is expected an active compound of Omija for preventing early stage of

atherosclerosis. However, it is necessary to develop processing

techniques of Omija to increase contents of gomisin N and validate its

efficacy *in vivo* and clinical studies.

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# 국문 초록

오미자는 오래 전부터 독특한 색과 맛 식품으로 사용되었지만, 풍부한 생리 활성 물질을 가지고 있어 약초로도 사용되었다. 한국 전통 의학서인 동의보감에는 오미자의 혈액순환 촉진 효능에 대하여 기록되어 있으며, 최근 연구들은 오미자의 심혈관 질환 보호 효과에 대해 보고하고 있다. 그러나 오미자의 유효 성분과 그 작용 기전에 대해서는 아직 밝혀져 있지 않다.

따라서 본 연구에서는 사람 혈관 내피 세포인 HUVEC과 단핵구인 THP-1 세포주를 이용하여 오미자의 유효성분을 밝히고, 그 성분이 염증으로 유도된 혈관 내피 세포에서 단핵구의 부착에 미치는 영향과 그 메커니즘을 규명하고자 하였다.

오미자에 다량 함유된 3가지 리그난들의 효능을 비교한 결과, 고미신 N이 가장 효과적으로 TNF-$\alpha$로 유도된 단핵구의 혈관 내피 세포에 대한 부착을 가장 효과적으로 억제 시켰다. 고미신 N은 단핵구의 부착에 관여하는 VCAM-1과 MCP-1의 발현을 단백질과 mRNA 수준에서 농도 의존적으로 저해하

였으며, NF-κB 합성 경로의 IKK와 IκBα 단백질의 인산화 또한 하향 조절하는 것으로 나타났다. 타겟 스터디를 위해 키나아제 프로파일링 및 $IC_{50}$ 분석을 진행하였고, 고미신 N이 MST3/STK24의 활성을 유의하게 저해하였으며, 혈관 내피 세포에서 그 기질인 ERM의 TNF-α 유도된 인산화를 억제하는 것을 밝혔다.

결론적으로, 고미신 N은 MST3/STK24를 타겟으로 하여, NF-κB 합성 경로를 하향 조절하였고 염증으로 유도된 혈관 내피 세포에 단핵구 부착을 현저히 저해하였다. 이러한 결과는 고미신 N이 심혈관 질환의 근본적인 병태인 동맥경화의 초기 단계를 예방할 수 있는 기능성 물질임을 시사한다.