# Effects of *Psoralea corylifolia* L. seed extract on AGEs-induced cell proliferation and fibrotic factor expression in mesangial cells

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**Abstract.** Diabetic nephropathy is a microvascular complication of diabetes that is characterized by mesangial expansion and thickening of the glomerular basement membrane. The production of advanced glycation end products (AGEs) increases in diabetic patients. Activation of the receptor of AGE (RAGE) signaling pathway induces mesangial expansion via the reactive oxygen species (ROS)-mediated production of pro-inflammatory and extracellular matrix molecules. The *Psoralea corylifolia* L. seed (PCS) is a widely used herbal medicine with various biological activities. The current study investigated the effect of PCS extract on mesangial cell proliferation and the RAGE signaling pathway in SV40 MES 13 cells. SV40 MES 13 cells were harvested after treatment with various concentrations of PCS extract at 10 $\mu$g/ml AGEs for 24 h. The results revealed that the PCS extract inhibited AGEs-induced mesangial cell proliferation and cyclin protein expression in a concentration-dependent manner. In addition, the AGEs-induced expression of fibrotic factors, such as transforming growth factor β, fibronectin and collagen, was reduced in mesangial cells after exposure to the PCS extract. The PCS extract also reduced RAGE expression and inhibited the expression of its downstream signaling pathways, such as NADPH oxidase, intracellular ROS and phospho-NF-κB. In conclusion, the data suggested that the PCS extract attenuated AGEs-induced renal mesangial cell proliferation and fibrosis via the suppression of oxidative stress and the downregulation of inflammatory and fibrotic factor expression.

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## Introduction

Diabetic nephropathy (DN) is one of the most important and common complications of diabetes. DN is characterized by glomerular hypertrophy, proteinuria, reduced glomerular filtration and renal fibrosis, resulting in loss of kidney function (1). It is known that mesangial expansion is due to excessive proliferation of mesangial cells and accumulation of extracellular matrix under the pathogenic condition of DN (2,3).

Chronic hyperglycemia is the most common feature in all forms of diabetes mellitus and plays an important role in the development of diabetes-related complications by accelerating the induction of aldose reductase and the irreversible formation of advanced glycation end products (AGEs) (4). Methylglyoxal (MGO), a highly reactive dicarbonyl compound, is the main precursor of AGEs. MGO reacts primarily with arginine residues and, to a lesser extent, with lysine residues, to form MGO-derived AGEs (5,6). It was well known that AGEs play a pivotal role in the development and progression of diabetic complications through various mechanisms (7). Especially, AGEs significantly contribute to the activation of mesangial cell expansion (8). AGEs increase the expression of the receptor for AGEs (RAGE) and interaction of AGEs and RAGE leads to increased oxidative stress by ROS production and NADPH oxidase (NOX) expression, contributing to the development of DN. It is known that NOX4 is mainly expressed in the kidney cortex and is closely related to diabetic nephropathy development (9).

Studies have reported the use of numerous natural products and their active ingredients in the treatment of diabetes and diabetes-related complications (10,11). *Psoralea corylifolia* Linn. seed (PCS) is a widely used herbal medicine with various biological activities, including antitumor, antioxidant, and anti-inflammatory effects (12). It has been extensively used for the treatment of many pathological conditions, such as skin disorders, cancer, inflammatory diseases, neurodegenerative diseases, and kidney disease (12-14). The major active constituents of PCS are coumarins, flavonoids, and meroterpenes. In a previous report, we observed the inhibitory effect of PCS extract on diabetic nephropathy in streptozotocin-induced diabetic mice (15). However, the detailed mechanism has not been studied yet. In this study, we investigated the effects of the PCS extract on the proliferation of mesangial cells, ROS

production, and in the expression of inflammatory and fibrotic factors.

## Materials and methods

*Preparation of PCS extract*. The PCS was artificially propagated and distributed in accordance with the relevant laws and was purchased from an oriental drug store (Kwang Myung Dang Co., Ulsan, Korea). The extraction procedure was performed as described previously (16). Briefly, the dried seeds (300 g) were ground into smaller pieces and extracted twice with 3 L of distilled water, under reflux. The extract was stored in a freezer (-80˚C) for 24 h before it was evaporated in vacuo to produce a dark brownish residue.

*Preparation of AGEs*. The AGEs preparation was performed with a slight modification of a previously reported method (17). AGEs were prepared under sterile conditions by incubating BSA (100 mg/ml, MP Biomedicals) and MGO (1 mol/l, Sigma-Aldrich; Merck KGaA) in phosphate-buffered saline (PBS, pH 7.4) containing 0.02% sodium azide (pH 7.4), at 37˚C for 7 days. Aminoguanidine (AG, 1 mM) was used as a positive control. Control-BSA was prepared using similar incubation conditions but without MGO. After incubation, unreacted carbonyls were removed by extensive dialysis against ammonium bicarbonate buffer (30 mmol/l, pH 7.9, 4˚C). AGEs and control-BSA preparations were further filter-sterilized. AGEs concentrations were determined using the bicinchoninic acid (BCA) protein assay (Pierce; Thermo Fisher Scientific, Inc.) with BSA as a standard before the experimental assay (18). Samples were stored in a freezer at -20˚C until use. The concentration of AGEs and control-BSA was expressed as the concentration of BSA protein added to culture medium.

*Cell culture*. SV40 MES 13 cells were cultured as per the manufacture's guidelines (CRL-1927, ATCC). The base medium for this cell line was 3:1 mixture of DMEM (Welgene) and Ham's F12 medium (Gibco; Thermo Fisher Scientific, Inc.), with 14 mM HEPES. To prepare the complete growth medium, FBS (Gibco; Thermo Fisher Scientific, Inc.) was added to the base medium to a final concentration of 5%. The cells were maintained under standard culture conditions (37˚C in a humidified 5% $CO_2$ atmosphere). The SV40 MES 13 cells were seeded and incubated for attachment overnight. The cells were treated with different concentrations of the PCS extract with 10 $\mu$g/ml AGEs for 24 h, as previously describe (19,20).

*Cell viability assay*. To determine the cytotoxicity of the PCS extract, SV40 MES 13 cells were seeded in 96-well plates ($1x10^4$ cells/well) and incubated overnight for attachment. The cells were then treated with various concentrations of the PCS extract for 24 h. To confirm the inhibitory effect of the PCS extract on AGEs-induced mesangial cell proliferation, the cells were seeded in 96-well plates ($1x10^4$ cells/well) and incubated overnight for attachment. Subsequently, the cells were treated with various concentrations of the PCS extract with 10 $\mu$g/ml AGEs, for 24 h. The D-Plus™ CCK cell viability assay kit (Dongin LS) was used to measure cell viability. The absorbance was measured at 450 nm using microplate reader (Synergy™ 2 Multi-Mode Microplate Reader; BioTek Instruments, Inc.).

*Cell cycle analysis*. The SV40 MES 13 cells were cultured in 6-well plates ($5x10^4$/well). After overnight incubation to promote attachment, the cells were treated with 200 $\mu$g/ml PCS extract with AGEs (10 $\mu$g/ml). After 24 h of treatment, the cells were fixed in 70% ethanol for 30 min at 4˚C. After washing twice with ice-cold PBS, the cells were centrifuged again and stained with 1 $\mu$g/ml propidium iodide (PI) staining solution (Sigma-Aldrich; Merck KGaA). Flow cytometry analysis was performed using a FACSCalibur flow cytometer (Becton Dickinson).

*Measurement of ROS production*. The SV40 MES 13 cells were seeded in a 96-well black plate ($1x10^4$ cells/well). After overnight incubation for attachment, the cells were treated with various concentrations of the PCS extract with AGEs (10 $\mu$g/ml) for 24 h. The cells were stained by 10 $\mu$M CM-$H_2$DCFDA (Invitrogen; Thermo Fisher Scientific, Inc.) for 30 min at 37˚C, after fixed in 10% neutral buffered formalin for 10 min at 25˚C. After wash twice with PBS containing $Ca^{2+}$ and $Mg^{2+}$, fluorescence was detected immediately at an excitation/emission wavelength of 495/527 nm by fluorometer (Synergy™ 2 Multi-Mode Microplate Reader).

*Western blotting*. The SV40 MES 13 cells were seeded in 6-well plates ($5x10^4$/well). After overnight incubation for attachment, the cells were treated with different concentrations of the PCS extract (50, 100 and 200 $\mu$g/ml) with 10 $\mu$g/ml AGEs for 24 h. The cells were harvested, and total protein was extracted using M-PER™ Mammalian Protein Extraction Reagent (Thermo Fisher Scientific, Inc.) containing a protease inhibitor cocktail and a phosphatase inhibitor cocktail (both from Sigma-Aldrich; Merck KGaA). The same amount of total protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a Mini-PROTEAN Tetra Cell (Bio-Rad Laboratories, Inc.) and transferred to a nitrocellulose membrane (GE Healthcare Life Science) using Tetra Blotting Module (Bio-Rad Laboratories, Inc.). The membrane was then blocked with 5% skim milk (BioShop Canada Inc.) or 5% BSA and then incubated with specific primary antibodies and horseradish peroxidase (HRP)-conjugated secondary antibodies (Bethyl Laboratories). Antibodies against β-actin, p-NF-κB p65, and TGF-β1 were purchased from Cell Signaling Technology, Inc. Antibodies against RAGE, fibronectin, and collagen (Col1A1) were purchased from Santa Cruz Biotechnology Inc. Antibodies against NADPH oxidase 4 (NOX4) were purchased from Abcam. Chemiluminescent was developed by Immobilon® Western (EMD Millipore). Detected on the ChemiDoc XRS+ system using the Image Lab software (Bio-Rad Laboratories, Inc.).

*Statistical analyses*. Data are expressed as mean ± standard error. Statistical analysis was done using two-way ANOVA with Tukey's multiple comparisons test in GraphPad Prism 7 software (GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Preparation of AGEs*. To obtain AGEs for the experiment, AGEs formation was induced by co-incubation of MGO

and BSA at 37˚C for 1 week. To confirm the formation of AGEs, fluorescence was measured at excitation and emission wavelengths of 360 and 420 nm, respectively. Aminoguanidine (AG), an inhibitor of AGEs formation, was used as a negative control. When MGO and BSA were incubated together, the observed fluorescence was about 3.4 times higher than that observed with BSA alone, and it was confirmed that this increased fluorescence was inhibited by AG (Fig. 1). This result indicated that the AGEs formation occurred appropriately. The obtained AGEs were quantified after dialysis and then used in the subsequent experiments.

*PCS extract does not show cytotoxicity to SV40 MES 13 cells*. Before examining the effect of the PCS extract on the proliferation of mesangial cells by AGEs, the cytotoxicity of the PCS extract was first examined by cell counting kit-8 (CCK-8) assay. Treatment of SV40 MES 13 cells with various concentrations of the PCS extract (50, 100 and 200 $\mu$g/ml) for 24 h did not show any cytotoxic effect on SV40 MES 13 cells at all the tested concentrations (Fig. 2).

*PCS extract inhibited AGEs-induced proliferation of SV40 MES 13 cells*. To investigate the effect of the PCS extract on AGEs-induced mesangial proliferation, SV40 MES 13 cells were treated with various concentrations of the PCS extract with AGEs for 24 h. AGEs treatment significantly increased the cell proliferation and PCS extract inhibited AGEs-induced mesangial cell proliferation in a concentration-dependent manner (Fig. 3A). The cell cycle is tightly regulated by cyclins such as cyclin A2, cyclin D1, and cyclin E in the kidney (21). We then checked whether PCS extract affects the expression of these cyclin proteins by western blotting. In parallel with the results of SV40 MES 13 cell proliferation, AGEs treatment significantly increased the expression of cyclin A2, cyclin D1, and cyclin E1, and PCS extract treatment inhibited this increase of expression of cyclin proteins in a concentration dependent manner.

Flow cytometry for cell cycle analysis was performed after PI staining. AGEs reduced the Percentages of cells in the G0/G1 phase but increased cells in the G2/M phase, indicating that AGEs could promote cell cycle progression (Fig. 3F). However, co-treatment with the PCS extract increased the proportion of cells in the G1 phase and decreased that in the G2/M phase (Fig. 3F). These results indicate that the PCS extract blocked AGEs-induced cell cycle progression.

*PCS extract inhibited AGEs-induced expression of fibrotic factors in SV40 MES 13 cells*. To investigate the effect of the PCS extract on AGEs-induced fibrotic factor expression, SV40 MES 13 cells were treated with various concentrations of the PCS extract in the presence of AGEs for 24 h. The level of protein expression of fibrotic factor and collagen was examined by western blotting. The expression of TGF-β1 protein, which is a central mediator of fibrogenesis, was increased by AGEs, and PCS extract treatment inhibited this increase. Similarly, the expression of fibronectin and collagen (COL1A1), which are extracellular matrix (ECM) proteins leading to fibrosis, also increased by AGEs, and PCS extract treatment inhibited this increase (Fig. 4). These results indicate that the PCS extract could inhibit AGEs-induced mesangial fibrosis.

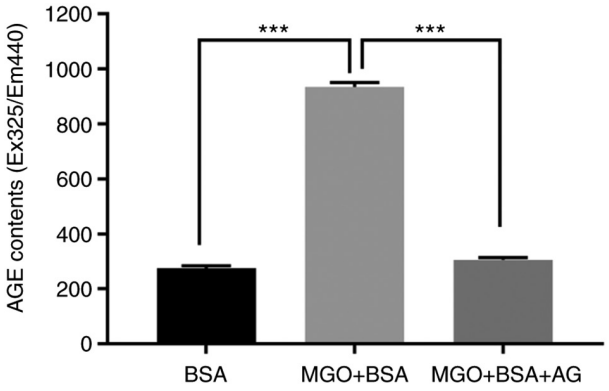



Figure 1. AGEs formation by incubation of BSA with MGO. AGEs formation was induced by co-incubation with 1 mmol/l MGO and 100 mg/ml BSA at 37˚C for 1 week. Fluorescence was measured at excitation and emission wavelengths of 360 and 420 nm, respectively. AG, an AGEs inhibitor, was used as a negative control. Data are presented as the mean ± standard error (n=3; independent experiments). ***P<0.001 as indicated. AGEs, advanced glycation end products; BSA, bovine serum albumin; MGO, methylglyoxal; AG, aminoguanidine.

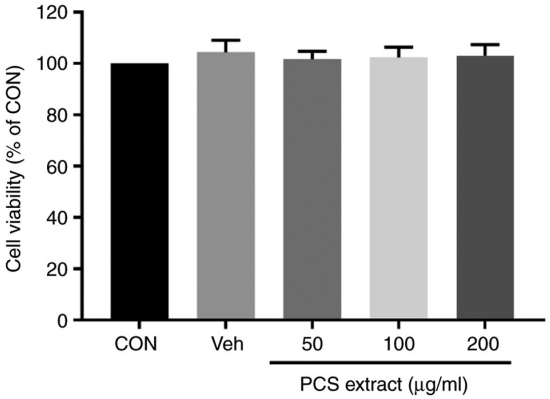
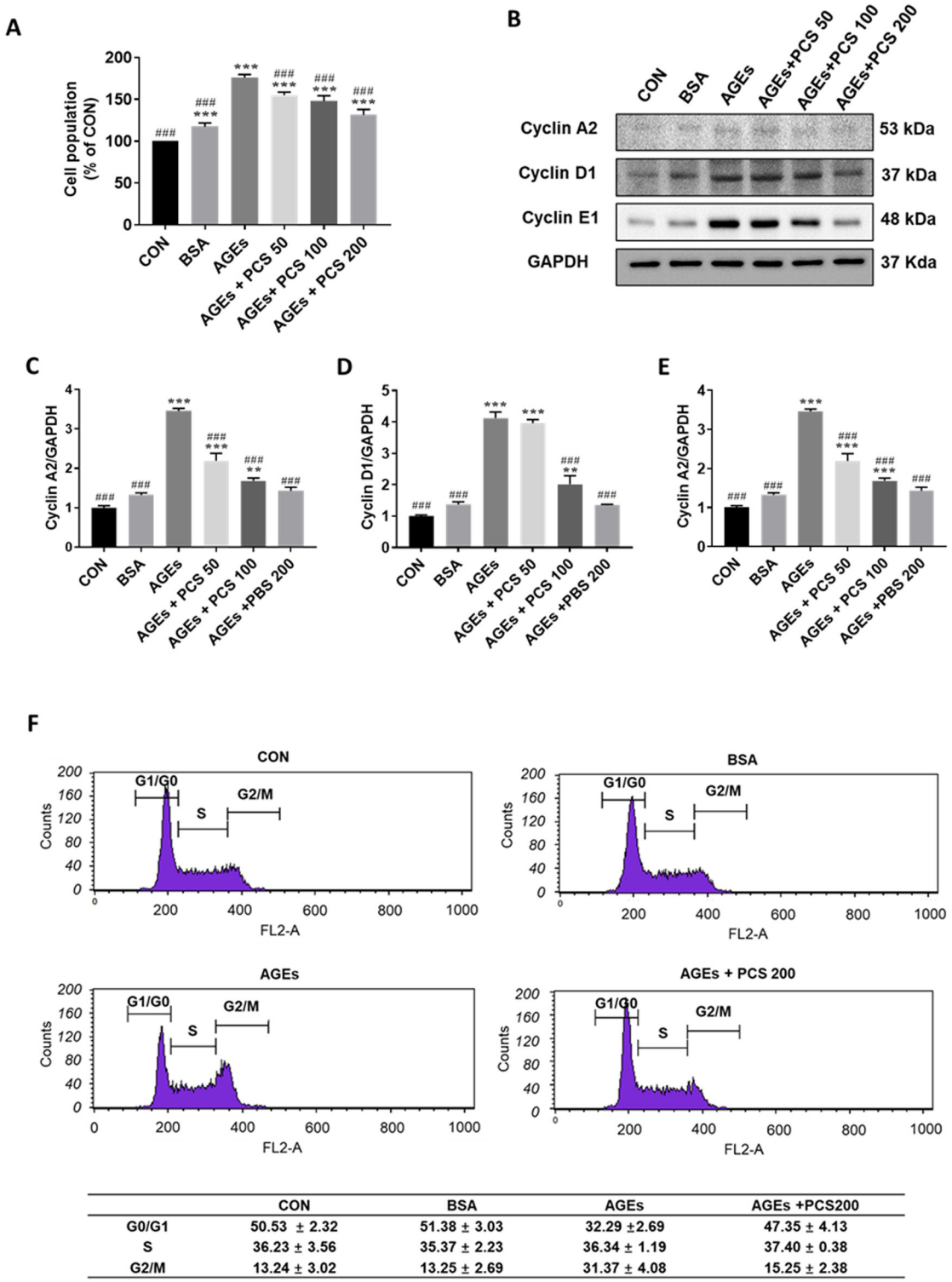



Figure 2. Effect of PCS on the viability of SV40 MES 13 cells. SV40 MES 13 cells were treated with various concentrations of PCS extract (50, 100 and 200 $\mu$g/ml) and incubated for 24 h. Subsequently, a Cell Counting Kit-8 assay was conducted to assess the cell viability. Data are presented as the mean ± standard error (n=3; independent experiments). PCS, *Psoralea corylifolia* L. seed; CON, control; Veh, vehicle.

*PCS extract inhibited AGEs-induced RAGE signaling pathway in SV40 MES 13 cells*. It has been known that AGEs promote ROS production through RAGE, causing oxidative stress (22). AGEs treatment significantly increased the expression of RAGE. However, treatment with the PCS extract led to a concentration-dependent decrease in AGEs-induced RAGE expression (Fig. 5A and B). PCS extract treatment without AGEs also decreased the expression of RAGE protein, which was significantly decreased compared to the control when 200 $\mu$g/ml PCS extract was added (Fig. 5E and F). The expression level of NOX4, which is known to play a major role in ROS production in the kidneys (23), was also significantly increased by AGEs induction, this effect, however, was concentration-dependently decreased upon treatment with the PCS extract (Fig. 5A and C). Therefore, we checked intracellular ROS level. We found that ROS levels were also significantly increased by AGEs treatment, and this increase was inhibited by treatment with PCS extract (Fig. 5F). These results suggest




| | CON | BSA | AGEs | AGEs +PCS200 |
|---|---|---|---|---|
| G0/G1 | 50.53 ± 2.32 | 51.38 ± 3.03 | 32.29 ±2.69 | 47.35 ± 4.13 |
| S | 36.23 ± 3.56 | 35.37 ± 2.23 | 36.34 ± 1.19 | 37.40 ± 0.38 |
| G2/M | 13.24 ± 3.02 | 13.25 ± 2.69 | 31.37 ± 4.08 | 15.25 ± 2.38 |

Figure 3. PCS extract treatment inhibited AGEs-induced mesangial cell proliferation and the expression of cyclin A2, cyclin D1 and Cyclin E1 in SV40 MES 13 cells. SV40 MES 13 cells were treated with various concentrations of PCS extract (50, 100 and 200 $\mu$g/ml) in the presence of AGEs (10 $\mu$g/ml) for 24 h. (A) A Cell Couniting Kit-8 assay was subsequently performed. (B) Cells were harvested and the expression of cyclin A2, cyclin D1 and cyclin E1 were examined vis western blotting. Representative blots are presented. (C-E) Quantification of the results from panel B. (F) The cell cycle was monitored via flow cytometry. The graph represents the percentage of cells at each stage of the cell cycle. Data are presented as the mean ± standard error (n=3; independent experiment). $^{**}$P<0.005 and $^{***}$P<0.001 vs. CON. $^{###}$P<0.001 vs. AGEs. PCS, *Psoralea corylifolia* L. seed; AGEs, advanced glycation end products; CON, control; BSA, bovine serum albumin.

that the PCS extract could inhibit RAGE expression and thereby inhibit NOX4 expression and ROS production. ROS is known to activate a number of transcription factors, which include various inflammation factors such as NF-κB (24). When we checked the expression of phosphorylation of NF-κB in SV40 MES 13 cells, AGEs treatment significantly increased phosphorylation of NF-κB and PCS extract treatment significantly inhibited this increase (Fig. 5A and D). These results

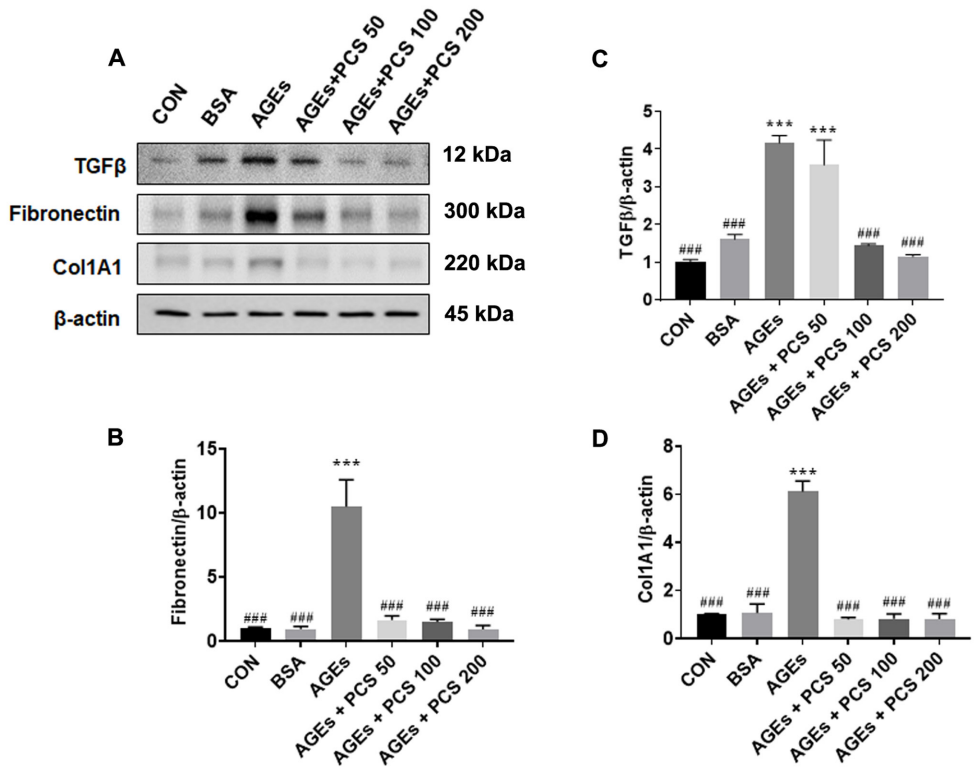



Figure 4. PCS extract treatment inhibited the AGEs-induced expression of fibrotic factors in SV40 MES 13 cells. SV40 MES 13 cells were treated with various concentrations of the PCS extract (50, 100 and 200 $\mu$g/ml) in the presence of AGEs (10 $\mu$g/ml) for 24 h. (A) Cells were harvested and the expression of TGFβ, fibronectin and Col1A1 was examined via western blotting. Representative blots are presented. (B-D) Quantification of the results from Fig. 4A. Data are presented as the mean ± standard error (n=3; independent experiments). $^{***}$P<0.001 vs. CON; $^{###}$P<0.001 vs. AGEs. PCS, *Psoralea corylifolia* L. seed; AGEs, advanced glycation end products; Col1A1, collagen 1A1; CON, control; BSA, bovine serum albumin.

suggest that the PCS extract could inhibit AGEs-induced RAGE-ROS-NF-κB signaling pathways.

## Discussion

The mesangial cells are specialized kidney cells that make up the mesangium of the glomerulus and constitute up to 30-40% of the total glomerular cell population (25). The mesangial cells can synthesize and secrete many protein factors that regulate the structure and function of the glomerulus (26). Alteration in the mesangial cell function is a key factor that results in the progression of glomerular disease in numerous models of chronic renal failure, such as DN. The expansion of the mesangial matrix is one of the hallmarks of DN; it is caused by the proliferation of mesangial cells and the increased deposition of extracellular matrix proteins, such as fibronectin and collagen, into the mesangium (2,3).

In chronic hyperglycemia, AGEs are actively produced and accumulate in the circulating blood and various tissues, resulting in vascular complications in diabetes (27). Several studies have reported that AGEs induce mesangial cell expansion by increasing mesangial cell proliferation and ECM production, and blocking AGEs or AGEs-RAGE signaling can inhibit mesangial cell expansion (28,29). In this study, we demonstrated that PCS extract treatment attenuated AGEs-induced mesangial cell expansion by inhibition of cyclin protein expression and attenuated the expression of fibrotic factors by decrease of NOX4 expression and NF-κB activation.

In order to obtain AGEs, we proceeded with AGEs formation through co-incubation of BSA and MGO (30). MGO, which is a kind of the dicarbonyl intermediates, is known to be a very reactive precursor of AGEs. Co-incubation of BSA and MGO showed significantly increased of AGEs formation and used for this study.

We found that AGEs treatment clearly increased SV40 MES 13 mesangial cell proliferation and PCS extract inhibited this increase of cell proliferation. The major factors that positively regulate the G1 phase are cyclins D1 and E1 (31). Cyclin A2 plays a critical role during the S phase, which is the somatic form of cyclin A (32). It is well known that cyclin A2, E1, and D1 are closely related to the regulation of renal mesangial cell proliferation (33,34). Therefore, the decrease of protein expression of cyclin A2, E1 and D1 by PCS extract might contribute to the inhibition of proliferation of mesangial cells by attenuating the cell cycle.

TGF-β1 is a critical mediator of glomerulosclerosis and fibrosis, leading to end stages renal disease and has a key role in the progression of chronic kidney diseases (35). Increased expression of TGF-β1 mRNA and protein is observed in patients with fibrotic kidney disease, including DN (35). TGF-β1 stimulates mesangial cell proliferation (32). In addition, TGF-β1 induces the production of ECM protein, such as fibronectin and collagen production in various renal cells, including glomerular mesangial cells, renal fibroblasts, and renal tubular epithelial cells (36). PCS extract significantly inhibited AGEs-induced expression of TGF-β1, fibronectin and collagen. Therefore, the decreased

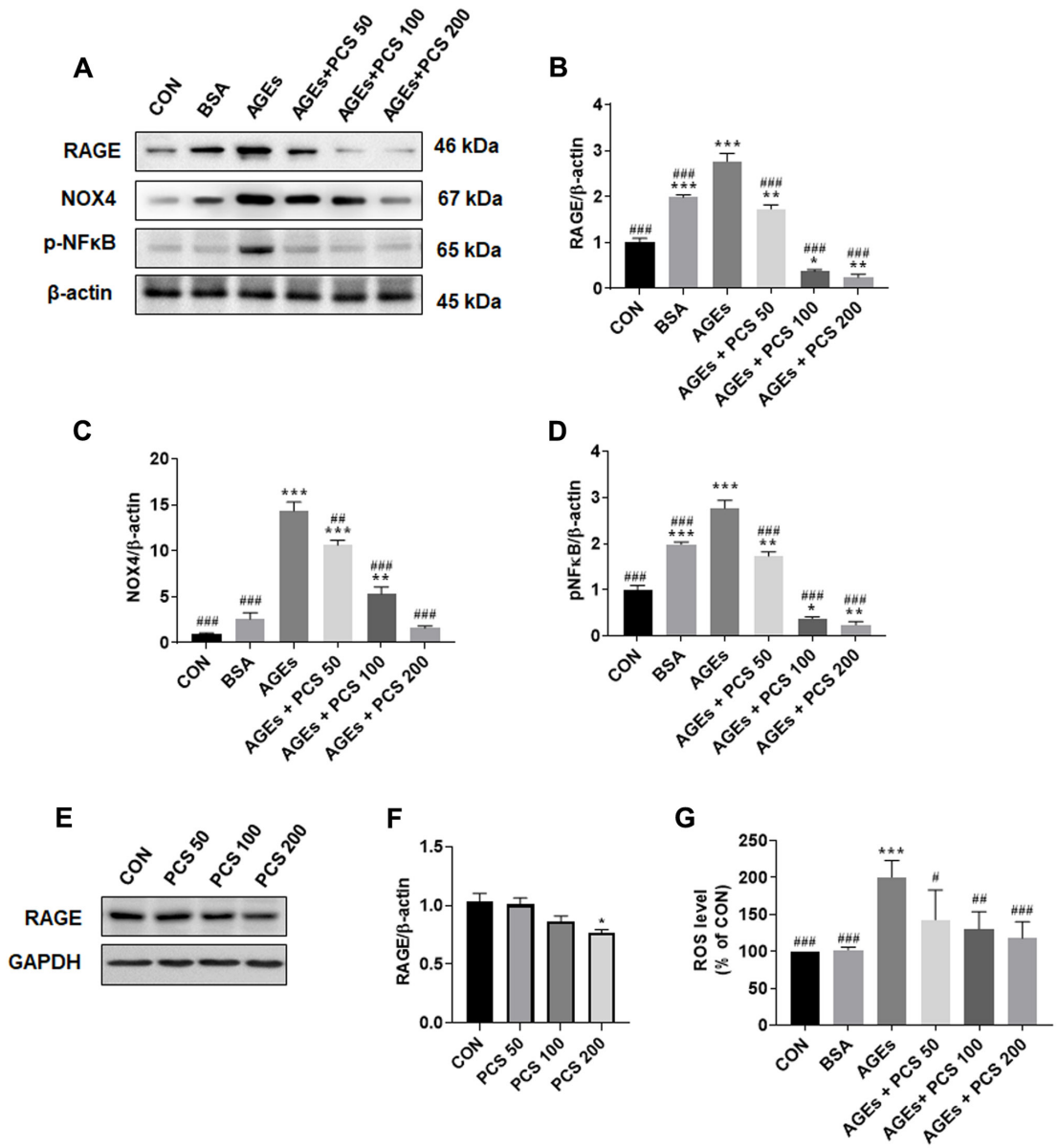



Figure 5. PCS extract treatment inhibited AGEs-induced ROS production and expression of RAGE, NOX4 and p-NFκB in SV40 MES 13 cells. The SV40 MES 13 cells were treated with various concentrations of the PCS extract (50, 100 and 200 $\mu$g/ml) in the presence AGE (10 $\mu$g/ml) for 24 h. (A) After cells were harvested, the expression of RAGE, NOX4 and p-NFκB p65 was examined by western blotting. Representative blots are presented. (B-D) Quantification of the results from Fig. 5A. After treating cells with PCS extract as aforementioned, (E) cells were harvested, and the expression of RAGE was determined by western blotting. The representative blots are presented. (F) Quantification of the results from Fig 5E. (G) ROS levels were measured by staining with 10 $\mu$M CM-$H_2$DCFDA. Fluorescence was detected at an excitation/emission wavelength of 495/527 nm. Data are presented as the mean ± standard error (n=3; independent experiments). $^{*}$P<0.05, $^{**}$P<0.005 and $^{***}$P<0.001 vs. CON; $^{\#}$P<0.05, $^{\#\#}$P<0.005 and $^{\#\#\#}$P<0.001 vs. AGEs. PCS, *Psoralea corylifolia L.* seed; AGEs, advanced glycation end products; ROS, reactive oxygen species; RAGE, receptor of AGEs; NOX4, NADPH oxidase 4; p, phosphorylated; CON, control; BSA, bovine serum albumin.

of TGF-β1 expression by PCS extract contributed to the inhibition of cell proliferation and reduction of ECM protein expression.

It is ideal to conduct experiments with a treatment time optimized for each experiment. However, the experiment was conducted by selecting the treatment conditions for 24 h, as previously describe (19,20), evaluate the change induced by the PCS extract.

AGEs bind to RAGE and activate NAD(P)H oxidase (NOX) pathway, leading to the generation of ROS (22). Diabetic condition elevates NOX4 expression in renal mesangial cells, and NOX4 mediates mesangial cell hypertrophy and extracellular matrix accumulation (37). NOX4 is constitutively active and produces mainly hydrogen peroxide ($H_2O_2$) as the prevalent ROS detected rather superoxide radical anion ($O_2$·-) in kidney (38). Even if superoxide radical anion is produced, it spontaneously undergoes dismutation to form another ROS, hydrogen peroxide (39). It was well known that hydrogen peroxide increases extracellular matrix through TGF-β1 (40). It is ideal to conduct experiments with a treatment time optimized for each experiment. However, the experiment was conducted by selecting the treatment conditions for 24 h, as previously describe (19,20), evaluate the change induced by the PCS extract. Although there

was no experiment of time point to confirm the change of NOX4 expression and ROS level due to the initial reaction of AGEs-RAGE, even after 24-h treatment, PCS extract inhibited the AGEs-induced RAGE expression and subsequently decreased the expression of NOX4 and ROS production. These beneficial effects of PCS extracts might contribute to the prevents of DN.

An increased level of intracellular ROS is known to activate NF-κB and trigger an inflammatory response (41). Our result showed that phosphorylated NF-κB expression, which was induced by AGEs, was also significantly inhibited by PCS extract treatment. Therefore, it is speculated that treatment with the PCS extract suppressed ROS production, thereby downregulating the activation the NF-κB signaling pathway. NF-κB is a transcription factor, which regulates the expression of numerous inflammatory response-related genes during kidney injury (42). Upon activation, the phosphorylated NF-κB translocate into the nucleus and triggers the expression of its target genes, including TGF-β1 and further results in ECM accumulation (43). It was also known that NF-κB signal transduction pathway is related with glomerular mesangial cell proliferation through cyclin D (44). Therefore, treatment with the PCS extract inhibited the AGEs-induced NF-κB activation, resulting in the reduction of the expression of fibrotic factors, such TGF-β, fibronectin, and collagen, and cell proliferation.

We previously reported that PCS extract inhibited AGEs formation *in vitro* and *in vivo* (45). In the present study, we found that PCS extract downregulated the expression of the AGEs receptor, RAGE, and its sub-signaling molecules. In particular, the expression of RAGE protein lower than the CON level by the PCS extraction treatment was observed not only in the AGEs induction but also in the absence of the AGES induction. Further studies are definitely required to elucidate the mechanism underlying the downregulation of RAGE expression by PCS extract. In a previous report, we reported the protective effect of PCS extract and coumarins on diabetic nephropathy in streptozotocin-induced type 1 diabetic mice and psoralen and isopsoralen, which are coumarins and the major components of the PCS extract, improved markers related to mesangial cell damage and fibrosis caused by high glucose (15). In addition, it has been reported that *Hydrangea paniculata*-derived coumarins have beneficial effects on diabetic nephropathy (46,47). Therefore, it is possible that coumarins in the PCS extract may play a role in inhibiting AGEs-induced hyperproliferation of mesangial cells and the downregulation of RAGE and fibrotic factor expression.

In summary, we demonstrated that the PCS extract inhibited the AGEs-induced mesangial cell proliferation by inhibition of the expression of cyclin A2, D1, and E1. In addition, PCS extract inhibited the AGEs-induced ROS production and the expression of RAGE and fibrotic factors. Our findings suggest that treatment with the PCS extract blocks RAGE-ROS-NF-κB-TGF-β1 signaling pathways contributing to the protection against diabetic nephropathy.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

HSJ conceived and designed the current study, and wrote the manuscript. HC and ES contributed to the design of the present study, performed the experiments and wrote the manuscript. All authors read and approved the final manuscript and all authors confirm the authenticity of all the raw data.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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