Supporting Information

The *in Vitro* Protective Effects of Curcumin and Demethoxycurcumin in *Curcuma longa* Extract on Advanced Glycation End Products-Induced Mesangial Cell Apoptosis and Oxidative Stress

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1. Preparation of the AGEs

AGEs were prepared according to following procedure: 0.5 g BSA was added into 10 mM/L phosphate buffered salt of 100 mL (PBS, pH=7.4) and then incubated with 50 mM/L D-glucose in 5% CO₂ at 37 °C for 12 weeks. The free glucose was removed by dialysis overnight in PBS. The final AGEs were stored at -20 °C for further use.

2. Cell treatment

Cells were pre-treated with RAGE-Ab (5 μg/mL), vitamin E (0.1 mM), aminoguanidine (AG, 0.1 mM), curcumin, or demethoxycurcumin (10⁻¹¹-10⁻⁹M) for 30 min, and then 200 μg/mL AGEs was added to co-incubate for 48 h. After being incubated, cell supernatant was taken for determination of SOD and MDA; cells were used for ROS and apoptosis assay.

3. Evaluation on AO/EB apoptosis staining

Acridine orange/ethidium bromide (AO/EB) staining was used to evaluate the attenuation of curcumin and demethoxycurcumin on AGEs-induced apoptosis. Acridine orange can penetrate the integral cell membrane and embed in the nuclear DNA to emit a bright green fluorescence under the fluorescence microscope, while ethidium bromide can only penetrate the damaged cell membrane and then emit jacinth fluorescence. The green fluorescence represented therefore normal cells, while jacinth represented apoptotic ones. After being co-incubated with drugs, cells were treated with AO/EB and then photographed under Olympus IX71 microscope. The green and jacinth areas were calculated by Image-Pro Plus picture analysis software. The relative apoptosis ratio (%) = jacinth area/(jacinth area + green area) ×100%.

4. Flow cytometry analysis for apoptosis

Annexin V-FITC/PI flow cytometry analysis was used to quantify the apoptosis. As shown in Fig. 4, frames are divided into four quadrants: early apoptotic cells (annexin V⁺/P⁻); late
apoptotic cells (annexin V+/P+); viable cells (annexin V−/P−); cells undergoing necrosis (annexin V−/P+). AGEs of 200 μg/mL increased markedly apoptosis to 14.56±0.96%, compared with the BSA group (5.12±0.86%). Curcumin of 10^-11, 10^-10, and 10^-9M reduced significantly the apoptosis ratio to 11.23±0.67%, 8.57±0.73%, and 5.80±0.44%, respectively, while demethoxycurcumin of 10^-11, 10^-10, and 10^-9M decreased significantly apoptosis ratio to 12.43±0.46%, 10.80±0.61%, and 7.67±0.72%, respectively. The results demonstrated that curcumin and demethoxycurcumin had a significant protective activity on AGEs-induced cell apoptosis.
**Fig. 1S** Fluorescence staining for apoptosis. 200 μg/mL BSA (a); 200 μg/mL AGEs (b); RAGE-Ab (5μg/mL)+200 μg/mL AGEs (c); 10^{-9} M C+200 μg/mL AGEs (d); 10^{-10} M C+200 μg/mL AGEs (e); 10^{-11} M C+200 μg/mL AGEs (f); 10^{-9} M DC+200 μg/mL AGEs (h); 10^{-10} M DC+200 μg/mL AGEs (i); 10^{-11} M DC+200 μg/mL AGEs (j). Magnification: 200×.
**Fig. 2S** Fluorescence pictures of ROS. Mesangial cells were incubated with 200 μg/mL BSA (a); 200 μg/mL AGEs (b); 0.1 mM VE+200 μg/mL AGEs (c); 10^{-9} M C+200 μg/mL AGEs (d); 10^{-10} M C+200 μg/mL AGEs (e); 10^{-11} M C+200 μg/mL AGEs (f); 10^{-9} M DC+200 μg/mL AGEs (h); 10^{-10} M DC+200 μg/mL AGEs (i); 10^{-11} M DC+200 μg/mL AGEs (j). Magnification: 200×.
Fig. 3S Annexin V-FITC/PI flow cytometry analysis of curcumin and demethoxycurcumin on AGEs-induced apoptosis. The annexin V-FITC/PI flow cytometry (A) and the apoptosis ratio (B) were obtained. 0.1 mM aminoguanidine (AG, purity≥97%, Sigma) was used as a positive control. The data were taken from three individual experiments and expressed as mean±SD (n=3).