Naringin: Bridging Correlation and Causation in Methylglyoxal Metabolism and Skin Lightening

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1. Introduction

Advanced glycation end-products (AGEs) are formed through non-enzymatic glycation reactions between reducing sugars and proteins, known as the Maillard reaction[1]. This reaction occurs spontaneously under physiological conditions and plays a critical role in the pathogenesis of various diseases. Accumulated AGEs in skin lead to stiffening and loss of elasticity. Additionally, AGEs production contributing to skin discoloration has attracted widespread attention. AGEs are known to affect skin color through two mechanisms. First, AGEs bind to the receptor for AGEs (RAGE) on melanocytes, triggering intracellular signaling pathways that activate melanogenesis. This leads to increased melanin production, which darkens the skin[2]. Second, AGEs themselves, with their characteristic yellowish-brown color, accumulate in the skin, causing a sallow and dull appearance[3]. This accumulation is further exacerbated by AGEs cross-linking with extracellular matrix proteins like collagen and elastin, disrupting their structure and function and altering the skin's optical properties[4].

Methylglyoxal (MGO), a key dicarbonyl intermediate in the formation of advanced glycation end products (AGEs), plays a central role in initiating glycation reactions[5]. Reducing MGO levels effectively curbs the generation of MGO-derived AGEs, emerging as a promising strategy for anti-glycation defense[5, 6]. In the skin, multiple enzymes are involved in metabolizing glucose and dicarbonyl compounds. Among these, the glyoxalase system, comprising glyoxalase 1 (GLO-1) and glyoxalase 2 (GLO-2), critically attenuates MGO toxicity [7]. Under physiological conditions, this system detoxifies MGO through a glutathione (GSH)-dependent catalytic reaction, converting it into the less toxic metabolite D-lactate. However, during aging or metabolic stress, the rate of MGO formation surpasses the capacity of GLO-1-mediated metabolism, leading to glyoxalase system dysfunction[8]. This imbalance accelerates AGEs accumulation and exacerbates skin discoloration.

Against this backdrop, natural bioactive compounds capable of enhancing GLO-1 activity have emerged as promising therapeutic candidates for anti-glycation and skin-lightening interventions[9]. Naringin, a flavonoid glycoside abundant in citrus fruits, has garnered increasing attention due to its diverse biological activities in antioxidant and anti-inflammatory properties[10]. Here, we investigate the anti-glycation effects of naringin on the skin, focusing on its

dual mechanisms of action: inhibiting RAGE-mediated signaling pathways and upregulating GLO-1 activity, offering a novel approach for combating AGEs-induced skin discoloration.

2. Materials and Methods
2.1 Chemicals and reagents
Naringin was obtained from Zhejiang Moda Biotechnology Co., LTD. AGEs used throughout this study were prepared by incubating bovine serum albumin (BSA, Aladdin, China) and D-glucose (Aladdin, China) at 37℃ for 7 days according to previous report[11].

2.2 Cell culture
B16-F10 mouse melanoma cells and A375 human melanoma cells were obtained from the Shanghai Institutes for Biological Sciences (Shanghai, China). Cells were cultured in DMEM (Gibco, USA) complete medium, supplemented with 10% FBS (Procell, China), and 1% peni-cillin-streptomycin (Biosharp, China).

2.3 AGEs production
To assess AGEs production, 30 µg/mL BSA was glycated by incubating at 37℃ for 7 days in the presence of 300 µg/mL D-glucose with or without naringin (50, 100, 200 µM). A blank control was prepared by incubating BSA in PBS without D-glucose and naringin. Following incubation, AGEs production was quantified by measuring fluorescence at 335/385 nm (excitation/emission) using a microplate Reader (Tecan, Switzerland).

2.4 Tyrosinase activity assay
To measure tyrosinase activity, B16 cells were pretreated with or without 50 µg/mL naringin for 24 h, followed by incubation with 100 µg/mL AGEs for another 24 h. Cells were then lysed using RIPA lysis buffer (Beyotime, China). A total of 150µL of cell lysate was transferred to a 96-well plate, and 20 µL of 0.1% L-DOPA (Aladdin, China) was added to each well as the substrate. Plates were incubated at 37°C for 1 h to allow tyrosinase-mediated oxidation of L-DOPA to dopaquinone. The reaction was quantified by measuring the absorbance of dopaqui-none at 490 nm using a microplate reader.

2.5 Melanin production
To quantify intracellular melanin production, B16 cells were pretreated with or without 50 µg/mL naringin for 24 h, followed by incubation with 100 µg/mL AGEs for another 24 h. Cells were then lysed using RIPA lysis buffer (Beyotime, China), and cellular debris was removed by centrifugation. The melanin content in the supernatant was determined by measuring absorbance at 490 nm using a microplate reader.

2.6 Real-time PCR
A375 human melanocytes were treated with 100 µg/mL AGEs with or without 50 µg/mL nar-ingin for 24 h. Total RNA was extracted using the total RNA isolation kit (Generay, China) according to the manufacturer's instructions. Subsequently, cDNA synthesis was performed

using the HiScript II Q RT SuperMix for qPCR (Vazyme, China), following the provided protocol. Quantitative real-time PCR was conducted with the ChamQ SYBR Color qPCR Master Mix (Vazyme, China) on TThe CFX Connect Real-Time PCR Detection System (BIORAD, USA), using the following primer sequences: RAGE: Forward: 5′-ACGGCTGGTGTTCCCAATAA-3′, Reverse: 5′-TGTTCCCTTCACAGATACTCCCTTC-3′; GAPDH: Forward: 5′-GGAGCGAGATCCCTCCAAAAT-3′, Reverse: 5′-GGCTGTTGTCATACTTCTCATGG-3′. Results were normalized to those for GAPDH.

2.7 GLO-1 activity

Skinovo-Epi reconstructed human epidermal models (RHE, Regenovo, China) were used to evaluate glyoxalase 1 (GLO-1) activity. The control group received no treatment, while the MGO group was exposed to 5 mmol/L MGO (Sigma,USA) for 24 h to induce glycation stress, followed by replacement with normal culture medium and continued cultivation for 5 days. The naringin group was topically applied with 50 µg/mL naringin for 5 days after MGO treatment. 1 mmol/L aminoguanidine (AG, a known GLO-1 inducer) was selected as positive control[12]. At the end of the culture period, epidermal samples were lysed and GLO-1 activity was evaluated by Glyoxalase I Activity Assay Kit (Sigma,USA) according to the manufacturer's instructions.

2.8 Immunofluorescence staining

RHE models were used for immunofluorescence detection of GLO-1 and Nε-carboxymethyl-lysine (CML) expression. After indicated treatment, the models were fixed in formalin, dehydrated, embedded in paraffin, and sectioned into 6 µm slices. Sections were permeabilized with 0.1% Triton X-100, blocked with 5% BSA, and incubated overnight at 4°C with anti-GLO-1 (Abcam, USA) and anti-CML (Abcam, USA). Then, fluorescent secondary antibodies (Abcam, USA) were applied for 1 h in the dark. Nuclei were counterstained with DAPI (Invitrogen, Sigma). Samples were mounted and imaged by fluorescence microscope (ECLIPSE Ni-U, Nikon, Japan), with fluorescence intensity quantified by ImageJ.

2.9 Molecular docking

The crystal structure of BSA (PDB ID: 3V03) and RAGE (PDB ID: 6XQ9) were obtained from RCSB Protein Data Bank. The molecule of naringin (CID: 442428) was obtained from PubChem. Docking was performed with AutoDock 4.2, defining a 100 Å×100 Å×100 Å grid (0.375 Å spacing) and the Lamarckian Genetic Algorithm. The conformation with the lowest binding energy was selected to analyze key amino acid residues and interactions between naringin and target proteins sing PyMOL 2.5.1.

2.10 Statistical analysis

Statistical analyses were conducted using GraphPad Prism 8.0 (GraphPad Software, USA). Data from three independent experiments are presented as mean ± S.D. For comparisons across multiple groups, one-way analysis of variance (ANOVA) was performed followed by Tukey's post-hoc test to correct for multiple comparisons. Statistical significance was defined as $p < 0.05$.

3. Results

3.1 Inhibition effects of Naringin on AGEs formation

During AGEs formation, active dicarbonyl compounds react with lysine and arginine residues in proteins. AGEs inhibitors competitively bind to and block these protein glycosylation sites[6]. To evaluate naringin's binding ability to protein glycosylation sites, molecular docking simulations were conducted (Figure 1a). The results showed that naringin formed stable hydrogen bonds with Lys113, Arg143, and Arg434 of BSA, with a binding energy of -10 kcal/mol. Naringin was embedded within the negatively charged core of BSA, suggesting that it inhibits non-enzymatic glycosylation by competitively binding to protein glycosylation sites.

Utilizing the fluorescent properties of AGEs, a quantitative analysis of AGEs generation was performed in a BSA-glucose glycosylation model (Figure 1b). The results revealed that naringin inhibited AGEs formation in a dose-dependent manner. Compared with the blank control group, the inhibition rates of naringin on the relative production of AGEs at 50, 100, and 200 µM were 10.22%, 17.43%, and 31.59%, respectively. These findings clearly demonstrate that naringin can effectively suppress AGEs formation, highlighting its potent anti-glycation activity.
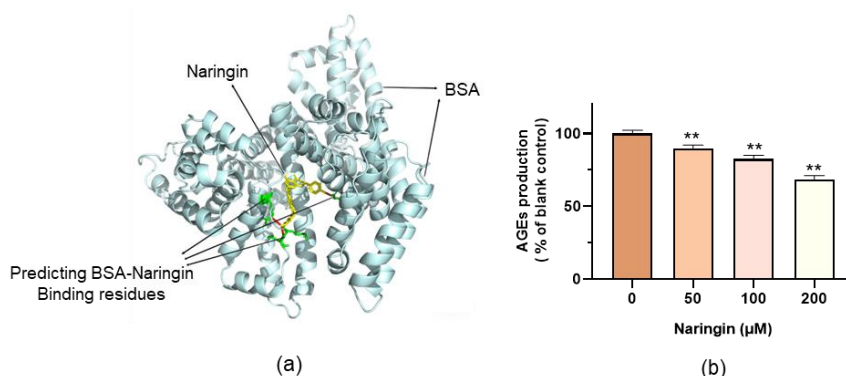

(a)   (b)

Figure 1. Naringin suppresses AGEs formation by competitively binding to protein glycosylation sites. (a) Predicted binding mode of naringin with BSA from molecular docking analysis. (b) Inhibitory effect of naringin on AGEs formation in the BSA-glucose glycation system. All data represent mean ± S.D. from at least three independent experiments; **$p < 0.01$ vs. blank control group.

3.2 Naringin inhibits AGEs-induced melanogenesis by inhibiting tyrosinase activity

AGEs are known to promote melanogenesis by enhancing tyrosinase activity, the key enzyme in melanin synthesis[2]. To determine naringin's effect on AGEs-induced pigmentation, we evaluated tyrosinase activity and melanin production in AGEs treated B16 melanocytes. AGEs alone increased tyrosinase activity by 32.9% compared to blank control ($p < 0.01$), whereas naringin pretreatment significantly limited this increase to 1.6% ($p < 0.01$, indicating effective inhibition of tyrosinase activity (Figure 2a). Consistently, AGEs elevated intracellular melanin content by 23.4% compared to the blank control ($p < 0.01$), but this accumulation was suppressed to 2.4% in naringin-pretreated cells ($p < 0.01$, Figure 2b). These findings demonstrate that naringin inhibits AGEs-induced melanogenesis in B16 cells by suppressing tyrosinase activity.
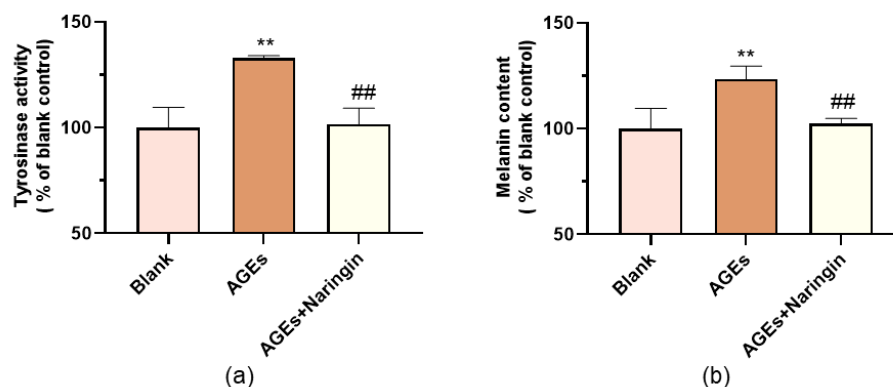
(a)　　　　　(b)

Figure 2. Naringin inhibits AGEs-induced melanogenesis by suppressing tyrosinase activity. (a) Tyrosinase activity and (b) Intracellular melanin content was measured after cells were pretreated with 50 μg/mL naringin for 24 h, followed by 100 μg/mL AGEs treatment for 24 h. All data represent mean ± S.D. from at least three independent experiments; **p < 0.01 vs. blank control group；## p < 0.01 vs. AGEs group.

3.3 Naringin disrupts AGEs - RAGE interaction and represses RAGE expression

To explore the molecular mechanism by which naringin antagonizes AGEs signaling, molecular docking was performed to assess interactions between naringin and the RAGE[13]. Docking results revealed that naringin binds to the ligand-binding pocket of RAGE with a minimum binding energy of -7.8 kcal/mol (Figure 3a). Key interactions included hydrogen bonds with critical amino acid residues Glu24, Asn25, Thr27, Arg29, and Glu32, all essential for AGEs recognition, suggesting competitive inhibition of AGEs-RAGE binding. qPCR analysis further confirmed that naringin treatment reduced AGEs-induced RAGE mRNA levels from 64.3% to 19.6% in A375 melanocytes (Figure 3b).These results indicate that naringin directly disrupts AGEs-RAGE interaction and repress RAGE gene expression in melanocytes.
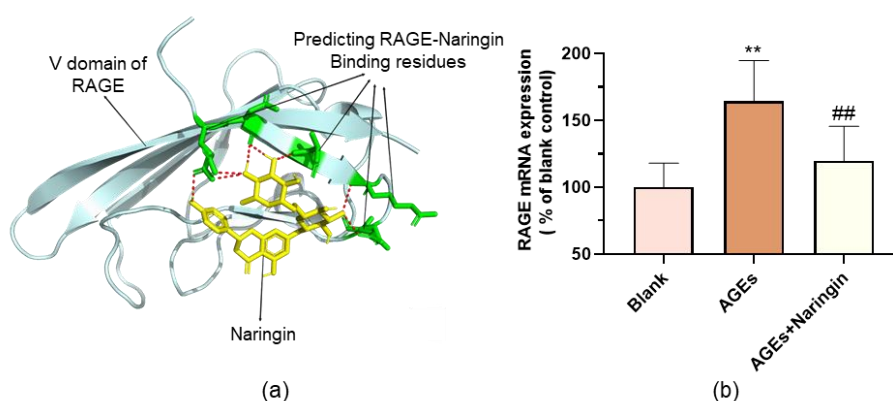

(a)　　　　　(b)

Figure 3. Naringin disrupts AGEs-RAGE interaction and represses RAGE gene expression. (a) Predicted binding mode of naringin with RAGE's V domain from molecular docking analysis. (b) mRNA level of RAGE was measured after AGEs treatment for 24 h. All data represent mean ± S.D. from at least three independent experiments; **p < 0.01 vs. blank control group；## p < 0.01 vs. AGEs group.

3.4 Naringin reduces the generation of AGEs by restoring GLO-1 function

GLO-1 is a key enzyme in detoxifying methylglyoxal (MGO), a major precursor of advanced glycation end products (AGEs), by converting it into non-toxic metabolites[5]. During aging or metabolic stress, excessive MGO accumulation overwhelms the glyoxalase system, leading to dysfunction in AGEs clearance and subsequent skin pigmentation. Here, we evaluated the protective effect of naringin against MGO-induced glycation stress (Figure 4a).

In blank control group, baseline GLO-1 activity was 190.7 ± 9.2 units/L (Figure 4b). Exposure to MGO for 24 h significantly drastically impaired GLO-1 function in RHE models, reducing activity by 78.7% to 40.7 ± 3.6 units/L ($p < 0.01$). Remarkably, topical treatment with 50 μg/mL naringin for 5 days following MGO exposure restored GLO-1 activity to 217.4 ± 6.4 units/L- 172.5% higher than the MGO group ($p < 0.01$) and reaching the blank control levels. The positive control, 1 mmol/L AG, increased GLO-1 activity to 110.9 ± 6.1 units/L ($p < 0.01$ *vs.* MGO), validating naringin's potent role in restoring GLO-1 activity.

Immunofluorescence analysis of CML (Figure 4c), a representative AGEs, revealed a 130.3% increase in MGO-treated models ($p < 0.01$ vs. blank control), concurrent with a 67.0% reduction in GLO-1 protein expression (Figure 4d). Naringin treatment significantly attenuated CML accumulation to baseline ($p < 0.001$ vs. MGO) and restored GLO-1 expression to control levels, demonstrating a strong correlation between GLO-1 recovery and AGEs reduction

These results demonstrate that naringin mitigates MGO-induced glycation damage by restoring GLO-1 activity and expression, thereby reducing AGEs formation in human epidermal models.
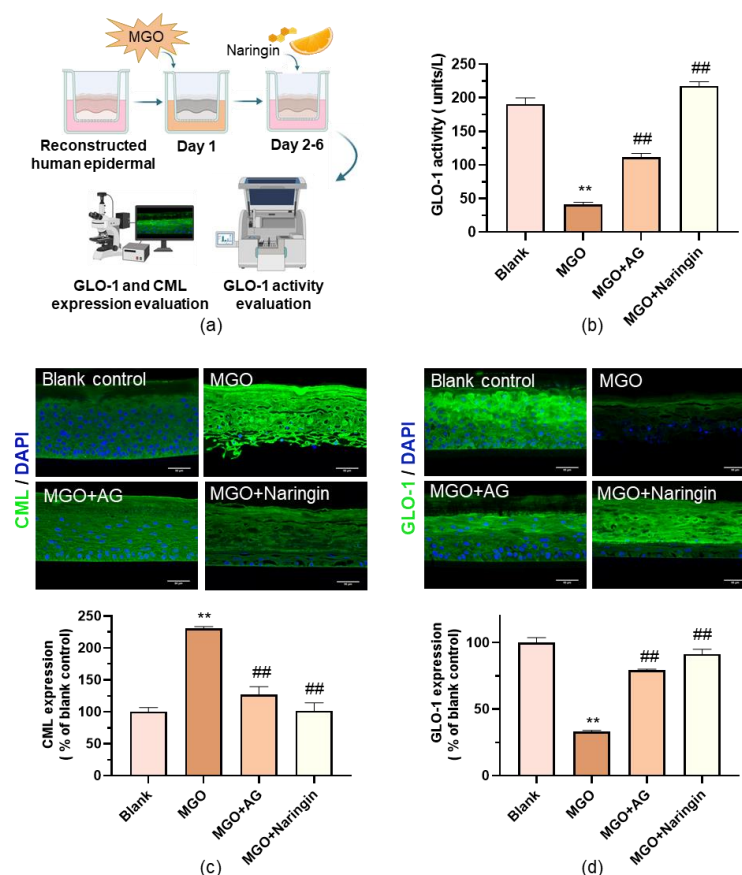

Figure 4. Naringin inhibits AGEs formation by restoring GLO-1 function. (a) Schematic of an experiment to evaluate the effect of naringin on GLO-1 and AGEs formation. (b) GLO-1

activity, (c) GLO-1 expression, and (d) CML expression was measured after naringin treatment for 5 days. Scale bar = 50 μm. All data represent mean ± S.D. from at least three independent experiments; **p < 0.01 *vs.* blank control group；## p < 0.01 *vs.* AGEs group.

4. Discussion

Our study demonstrates that naringin, a citrus-derived flavonoid, exerts multifaceted effects against glycation-induced skin discoloration by targeting both the formation of AGEs and the AGE-RAGE pathway driving melanogenesis, while concurrently restoring the metabolic function of key detoxifying enzymes GLO-1.

Naringin was found to inhibit AGEs formation by competitively binding to protein glycosylation sites, as evidenced by molecular docking studies showing stable interactions with protein glycosylation amino acid residues. In melanocyte models, naringin attenuated AGEs-induced melanogenesis by suppressing tyrosinase activity and intracellular melanin accumulation. Mechanistically, naringin bound to the ligand-binding domain of RAGE, reducing RAGE expression at both the mRNA and functional levels, thereby blocking RAGE-mediated pro-melanogenic signaling pathways.

Crucially, naringin restored the activity of GLO-1, a rate-limiting enzyme in the detoxification of reactive dicarbonyl precursor of AGEs. By enhancing GLO-1 function, naringin promoted the conversion of MGO to non-toxic metabolites, reducing the accumulation of downstream AGEs such as CML. This dual action, interrupting AGEs formation/signaling and reinforcing metabolic detoxification, addresses both the pigmentary and structural consequences of skin glycation stress.

As a natural bioactive compound with established safety profiles, naringin presents promising applications in cosmetic fields[14]. Topical formulations containing naringin may mitigate skin dullness and hyperpigmentation by targeting both the yellowish AGEs deposition and melanin overproduction. Additionally, its role in restoring GLO-1 activity suggests potential for preventing glycation-related extracellular matrix damage, such as collagen cross-linking, which contributes to skin stiffening and loss of elasticity. Our findings provide novelty insights in combating AGEs-induced skin discoloration, suggesting the potential applications of naringin in skin lightening products.

5. Conclusion

Our study highlights a novel dual mechanism for naringin: (1) direct inhibition of AGEs-RAGE interaction and RAGE-dependent melanogenesis, and (2) activation of the glyoxalase system to enhance MGO metabolism. This distinguishes naringin from single-target anti-glycation agents, offering a comprehensive strategy to combat the complex pathogenesis of glycation-induced skin discoloration.

6. References

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