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"Adaptogen plants in delaying skin-aging: A holistic bio-chemical/mechanical coupling observation system construction"

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Abstract

Adaptogens can nonspecifically enhance human resistance. However, there are still few studies on their specific effects and synergistic mechanisms in local skin applications. Using the MGO-induced senescent damaged fibroblast model we established, we successively studied three adaptogenic plants (*Withania somnifera, Gentiana scabra*, and *Physalis alkekengi*). Experimental verification showed that adaptogenic plants can significantly inhibit the synthesis of CML, effectively improve cell viability, and enhance cell proliferation, migration, and adhesion abilities. At the same time, by upregulating the expression of integrin β1 and TGF-β1 and downregulating the expression of MMP-2/MMP-9, they maintain the dynamic balance of key ECMs, increase the Young's modulus and adhesion force of cell mechanical properties, maintain the biochemical/mechanical coupling of ITG-ECM, and achieve a balance between intracellular ECM events and extracellular functions, thus protecting aging skin. This study provides a theoretical basis for the research and development of new plant-based ingredients for retarding skin aging.

Keywords: Aging signs evaluation, Anti-aging, Bioavailability, Omics

1. Introduction

With the improvement of people's living standards, there is an increasing focus on skin health and anti-aging. Skin aging is affected by both endogenous and exogenous factors. It not only affects appearance but also reflects complex changes at the cellular and molecular levels. In numerous studies on retarding skin aging, plant-based ingredients have become the focus due to their natural, gentle nature, and wide availability. Among them, adaptogenic plants have attracted significant attention.

Adaptogenic plants can nonspecifically enhance human resistance and help the body maintain homeostasis under stress. However, currently, our understanding of their specific effects and

synergistic mechanisms in local skin applications is limited. The high-sugar diet of modern people leads to the massive accumulation of AGEs in the dermis of the skin, accelerating skin aging. The skin aging process is closely related to the structural and functional changes of ECM components and their receptors. AGEs can disrupt the balance between ECM synthesis and degradation, damaging the skin's dynamic equilibrium. Although some studies have revealed part of the effects of AGEs on the skin, the complete mechanism of skin aging caused by a high-sugar diet remains unclear, lacking a sufficient evidence chain and experimental imaging data.

This study focuses on the role of adaptogenic plants in retarding skin aging. From the perspective of cell mechanics, we examined the effect of adaptogenic plants on the biochemical/mechanical coupling of ITG-ECM, aiming to provide a theoretical basis for the development of new plant-based ingredients for retarding skin aging, promote innovation in the field of cosmetic ingredients, and meet consumers' demands for efficient and safe skin care products.

2. Development of adaptogen-based cosmetic botanical ingredients based on the *In silico* platform

Adaptogenic plants are a type of medicinal plants that can non-specifically enhance human resistance[1]. The concept was introduced into China by Academician Xiao Peigen in the 1960s. They can help the body maintain internal homeostasis under stress, thereby resisting external pressures. Adaptogens have similar efficacy and applications to tonifying traditional Chinese medicine and "ginseng-like" drugs in various countries. However, there are few studies on their specific effects and synergistic mechanisms in local skin applications.

Therefore, through the *In silico* development platform for plant-based cosmetic ingredients we constructed, we conducted a bibliometric analysis of 1,683 articles and 1,364 patents, revealing the potential value of 109 adaptogenic plants in dermatology. For the first time, we proposed that the mechanism of adaptogenic plants acting on the skin may be to regulate the balance of ECM protein components as Figure1, so as to protect the normal functions and health of the skin[2]. Subsequently, our research on adaptogenic plants aims to develop new plant-based ingredients for retarding skin aging, providing innovative ingredient options and development ideas for the cosmetics industry.

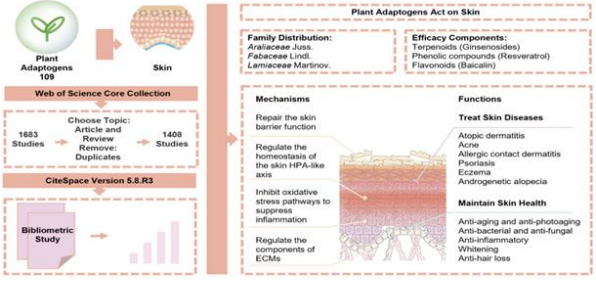

Figure 1. Mechanism of adaptogenic plants acting on the skin

3. Skin glycosylation mechanism and scientific hypothesis

Under the high-sugar diet of modern people, AGEs accumulate in large amounts in the dermis of the skin, accelerating skin aging. Numerous studies have shown that an increased level of AGEs in the skin is more likely to cause skin problems, such as sallow complexion and deepening wrinkles. However, few studies have delved into the mechanism by which a high-sugar diet leads to skin aging.

Therefore, our research team established a mouse aging model damaged by a high-sugar diet. Through comprehensive research in multiple aspects, we found that a high-sugar diet can cause senescent damage to the skin as Figure2[3]. Its damage to the skin is reflected in promoting the accumulation of AGEs, causing damage to the dermal structure, inhibiting the expression of dermal ECM proteins, disrupting the balance of ECM protein components, sup-pressing the expression of their receptors, and affecting the normal functions of ECM receptors, resulting in impaired cell behaviors such as proliferation, migration, and adhesion. This result provides an effective evidence chain for the senescent damage caused by a high-sugar diet to the skin.

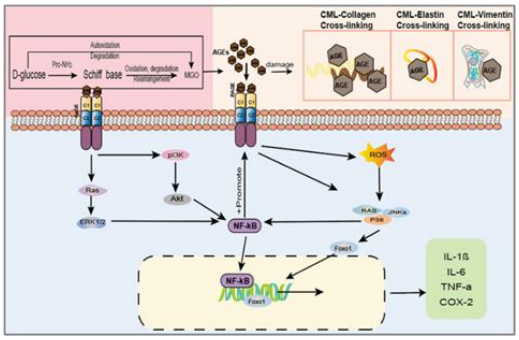

Figure 2. Molecular mechanisms of AGEs damage to skin dermis cells

Through our previous research on adaptogenic plants and the skin glycation mechanism, we hypothesized that adaptogenic plants can integrate the biochemical coupling between proteins and the extracellular matrix to combat skin aging.

4. Development of anti-aging plant materials based on ITG-ECM biochemic-al/mechanical coupling

To verify the crucial role of the "ITG-ECM" biochemical/mechanical coupling in the an-ti-aging effect of adaptogenic plants, our research team selected three adaptogenic plants (*Withania somnifera, Gentiana scabra*, and *Physalis alkekengi*) for research through the *In silico* platform. First, we used network pharmacology to predict the pharmacological relevance and potential molecular mechanisms of Gentiana scabra extract in skin anti-glycation. Then, we established an MGO-induced glycated damaged skin fibroblast model. Through biochemical and cell experiments, we explored and verified the inhibitory effect of gentiopicroside on the glycation reaction[4].

On the basis of this research, we further explored the mechanism of the effect of *Withania somnifera* root extract at the cellular level. We found that it can restore the expression level of the integrin β1 receptor, upregulate the TGF-β1 signal, down-regulate the MMP-2/MMP-9 signal, and bidirectionally regulate the balance of four ECM proteins, including COL1, FN1, LM5, and TNC[5].Based on the MGO-induced senescent damaged fibroblast model, we selected the extract of *Physalis alkekengi* (PAE), a representative adaptogenic plant, to continue the research on the integrin-mediated biochemical/mechanical coupling mechanism.

4.1 Effect of MGO on cellular mechanical properties

Research has shown that glycation directly leads to disorders in the dynamic equilibrium of skin cells and repair disorders by disrupting cell proliferation, migration, and adhesion functions[6]. In this study, skin fibroblasts were stimulated with MGO to establish a senescent fibroblast model with high expression of AGEs, simulating the accumulation of AGEs in the skin. Detection by a Pavone microscope revealed that the stiffness of cells after MGO stimulation was significantly reduced, indicating that MGO would damage the mechanical properties of cells as Figure3.

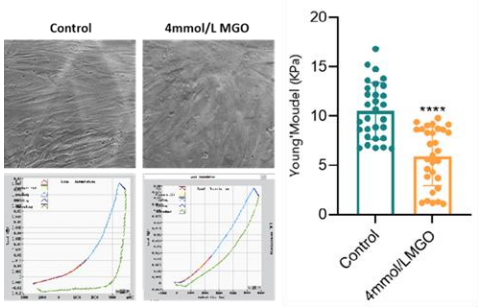

Figure 3. Changes in cell stiffness after MGO stimulation

4.2 Effect of PAE on cellular mechanical properties

The high accumulation of AGEs in the body can change the cell microenvironment, resulting in a decline in the skin's ability to resist exogenous damage, structural disorders, in-creased susceptibility to infection, and aggravated aging. At the cellular level, it also manifests as a decline in migration, adhesion, and other behavioral functions. Our research team used a Pavone nanoscope to measure cell stiffness, a scratch assay to detect cell migration ability, and a fluorescence assay to detect cell adhesion ability to evaluate the improvement effect of PAE on the imbalance of behavioral functions caused by cell senescent damage at the cellular level.

4.2.1 PAE modulates cellular stiffness

Detected by a Pavone nanoscope, compared with the MGO model group, the Young's modulus of cells in the 62.5 μg/mL PAE treatment group was significantly increased, indicating that

PAE has an obvious regulatory effect on cell stiffness as Figure 4. It is speculated that PAE may improve cell mechanical properties by enhancing the stability of the cytoskeleton.

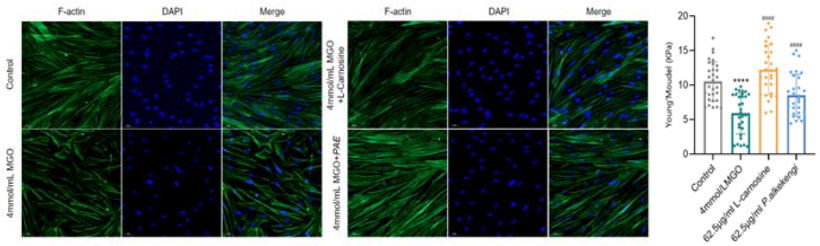

Figure 4. Regulation of cell stiffness by PAE

4.2.2 PAE regulates cell migration force

Since cell senescent damage is related to impaired migration ability, this experiment used a scratch assay to explore the improvement effect of PAE on the migration ability of MGO-induced senescent damaged fibroblasts. The results showed that within 48 hours, high, medium, and low concentrations of PAE could significantly enhance the migration ability of MGO-damaged cells, with no significant difference compared with the L-carnosine positive control group as Figure 5.

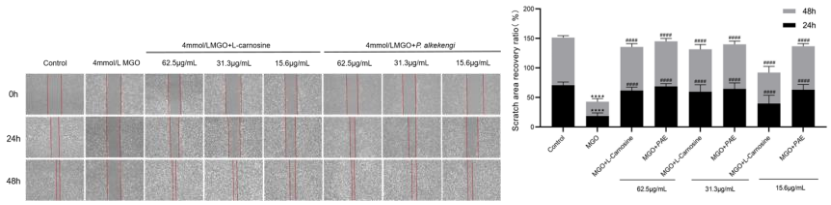

Figure 5. Effect of PAE on MGO-induced cell migration ability

4.2.3 PAE regulates cell adhesion

Since cell senescent damage is related to impaired migration ability, this experiment used a scratch assay to explore the improvement effect of PAE on the migration ability of MGO-induced senescent damaged fibroblasts. The results showed that within 48 hours, high, medium, and low concentrations of PAE could significantly enhance the migration ability of MGO-damaged cells, with no significant difference compared with the L-carnosine positive control group as Figure 6.

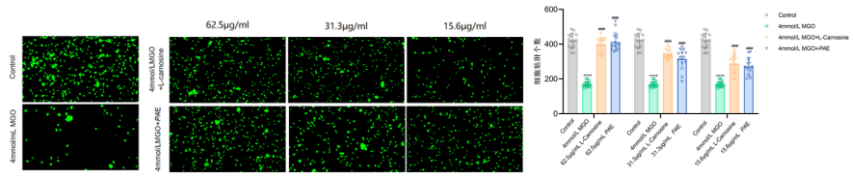

Figure 6. Effect of PAE on MGO-induced cell adhesion ability

In summary, PAE has a significant effect on restoring the migration and adhesion abilities of skin fibroblasts, effectively improving MGO-induced cell damage, and maintaining the normal levels of cell migration and adhesion functions.

4.3 Biomechanical regulation of ITG-ECM interactions by adapted protoplasts

Integrins are a class of key anchoring proteins on the cell membrane that connect the extracellular ECM to the intracellular cytoskeletal proteins and act as two-way hubs for signal transmission between cells and their extracellular microenvironment[7]. They can connect the cytoskeleton to the microenvironment composed of ECMs and act as biochemical/mechanical signal sensors for cells[8]. That is, integrin ITG is equivalent to an anchor cable, mechanically pulling the "water cube" structure composed of cells and ECMs outside the cell, and triggering a series of events inside the cell under the regulation of the biochemical/mechanical changes of ITG-ECM[9].

We used different detection methods such as ELISA, WB, and immunofluorescence staining to study the effect of PAE on regulating MGO-induced senescent damaged cells at the cellular level. The results showed that MGO treatment significantly inhibited the expression of related proteins such as COL1 in cells, while PAE could significantly enhance the ability of MGO-damaged cells to secrete COL1 protein. PAE could upregulate the expression of COL1 and the ITGB1 receptor in cells, with no significant difference compared with the positive control group as Figure7-9.

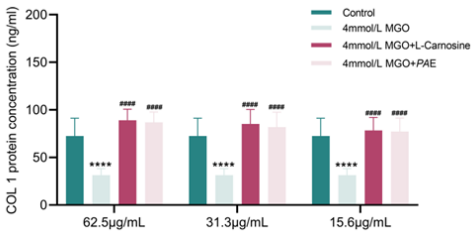

Figure 7. Detection of COL and ITGB1 protein expression by Elisa method and analysis of the results

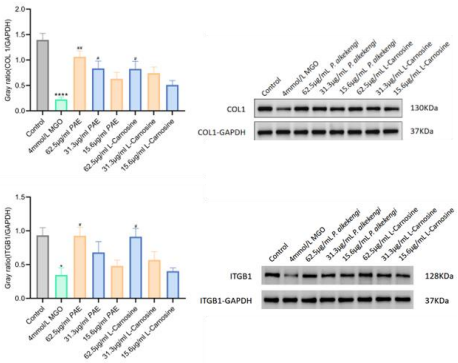

Figure 8. Detection of COL and ITGB1 protein expression by WB method and analysis of results

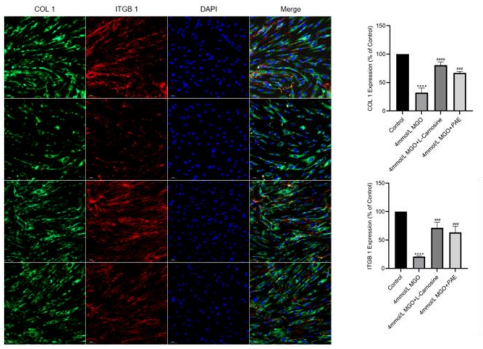

Figure 9. Detection of COL and ITGB1 protein expression by IF method and analysis of results

In summary, PAE can regulate the ITG-ECM interaction of MGO-induced damaged skin fibroblasts, protect damaged skin fibroblasts, promote the normal expression of the integrin receptor ITGB1, and maintain the normalization of the ITG-ECM interaction.

4.4 PAE regulates ECM protein homeostasis reconstitution

Dermal fibroblasts mainly regulate ECM synthesis and degradation through the TGF-β signaling pathway. This signaling pathway can promote ECM gene expression and downregulate MMPs to promote ECM protein synthesis[10-11].

Therefore, our research team used ELISA, WB, and IF methods to multi-dimensionally measure the changes and expression of extracellular ECM proteins. We used qRT-PCR and WB methods for double verification to detect the changes in the expression levels of TGF-β1 closely related to ECM production and MMP-2 and MMP-9 closely related to ECM degradation, exploring the mechanism of PAE's protective effect on the imbalance of ECM functions.

4.4.1 PAE regulates protein expression of ECMs

Using methods such as ELISA, WB, and immunofluorescence staining, the effects of PAE on the protein components (ELN, FN1, LM5) of ECMs in cells with senescence damage induced by MGO were investigated. As we found, compared with the MGO model group, high, medium, and low concentrations of PAE could significantly restore the ability of MGO-damaged cells to secrete ECMs proteins, and there was no significant difference in the effect compared with the positive control group of L-carnosine as Figure10-12.

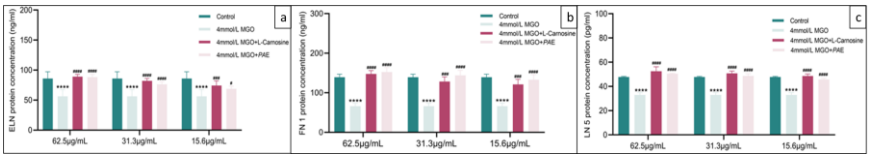

Figure 10. Protein expression of ECMs by ELISA: (a) ELN; (b) FN1; (c) LM5

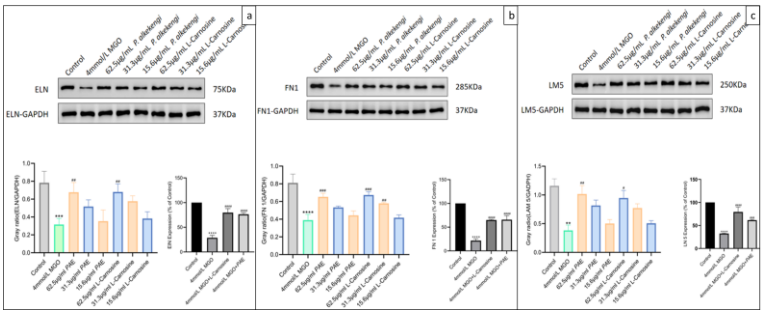

Figure 11. Detection of ECMs protein expression by WB method: (a) ELN; (b) FN1; (c) LM5

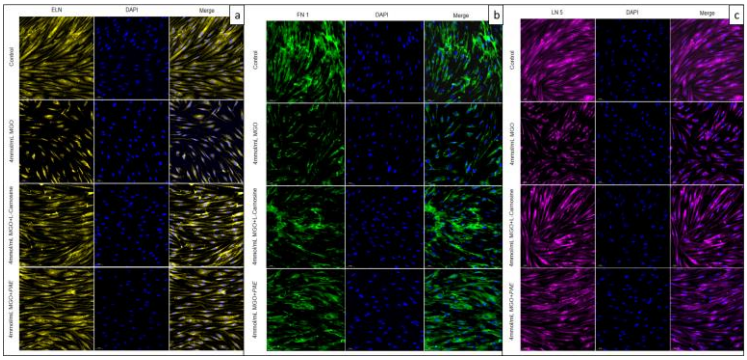

Figure 12. Immunofluorescence method to detect the protein expression of ECMs: (a) ELN; (b) FN1; (c) LM5

4.4.2 PAE regulates TGF-β1 expression, which is closely related to the generation of ECMs

The TGF-β1 signaling pathway plays a central role in the biosynthesis of ECMs. The qRT-PCR method and WB method were used to investigate the ability of PAE to regulate TGF-β1 as Figure 13. Compared with the MGO model group, both 62.5 μg/mL and 31.3 μg/mL of PAE could significantly up-regulate the relative expression level of TGF-β1 in MGO-damaged cells.

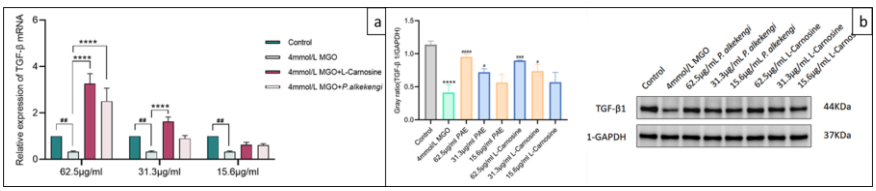

Figure 13. Detection of the relative expression of TGF-β1 gene: (a) qRT-PCR; (b) WB

4.4.3 PAE regulates the expression of MMP-2 and MMP-9, which are closely related to the degradation of ECMs

The increased expression of MMP-2 and MMP-9 is the main cause of the breakdown of ECM protein components. We used qRT-PCR and WB methods to investigate the ability of PAE to regulate the expression of MMP-2 and MMP-9 in MGO-induced senescent damaged cells. Compared with the MGO model group, 62.5 μg/mL and 31.3 μg/mL of PAE could significantly inhibit the ability of MGO-damaged cells to secrete MMP-2 and MMP-9 as Figure 14-15.

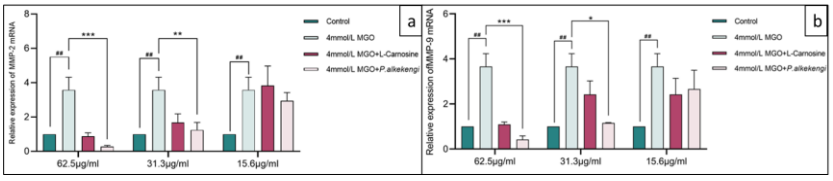

Figure 14. Relative gene expression detected by qRT-PCR: (a) MMP-2; (b) MMP-9

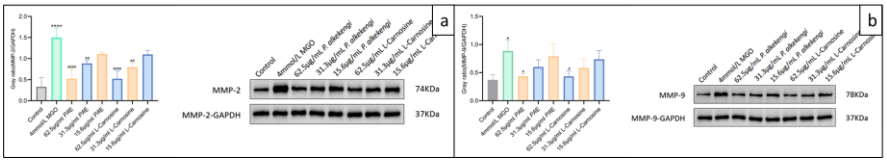

Figure 15. Relative gene expression detected by WB: (a) MMP-2; (b) MMP-9

In summary, PAE can regulate the content of ECM protein components (ELN, FN1, LM5) secreted by MGO-induced damaged skin fibroblasts to approach normal levels. It can also bidirectionally regulate the stability and balance of extracellular matrix production and degradation by upregulating the TGF-β1 pathway and downregulating the MMP-2 and MMP-9 pathways, protecting the normal function of the ITG-ECM interaction in fibroblasts.

5. Summary and Prospect

This study focuses on the delay of skin aging by adaptogenic plants. A senescence model of mice damaged by a high-sugar diet was established, confirming that a high-sugar diet can promote the accumulation of AGEs, disrupt the balance of the protein components of the ECM, and lead to impaired cellular behavior and function. Taking *Withania somnifera, Gentiana scabra*, and *Physalis alkekengi* as the research objects, it was found in the cellular senescence damage model induced by MGO that adaptogenic plants can inhibit the synthesis of CML, enhance cell viability, as well as proliferation, migration, and adhesion abilities. By regulating the expression of integrin β1, TGF-β1, MMP-2/MMP-9, the dynamic balance of the ECM and the functional balance inside and outside the cells can be maintained, thereby protecting the aging skin. However, the current research is mainly

based on a two-dimensional monolayer cell model. In the future, it is necessary to construct a three-dimensional skin organoid containing multiple types of cells to simulate the ECM hardening and cellular mechanical responses related to aging, so as to obtain more realistic experimental data.

6. References

[1] YI FAN. A preliminary review of studies on adaptogens: comparison of their bioactivity in TCM with that of ginseng-like herbs used worldwide [J]. Chinese Medicine, 2018, 13.

[2] LIU Xiaoxing, YI F*.Bibliometric Study of Adaptogens in Dermatology: Phar-macophylogeny, Phytochemistry, and Pharmacological Mechanisms [J].DDDT, 2023:17 341–361.

[3] Li Z W, Liu X X, Shi J Y, et al.Unveiling the mechanism of high sugar diet induced ad-vanced glycosylation end products damage skin structure via extracellular matrix-receptor interaction pathway[J]. Journal of cosmetic dermatology,2024,23(7):2496-2508.

[4] Chunyu C, Xiaoxing L, Li L, et al. Study of the mechanism by gentiopicro-side protects against skin fibroblast glycation damage via the RAGE pathway[J]. Scientific Reports,2024,14(1):4685-4685.

[5] Liu X, Chen C, Lin Y, et al.Withania somnifera root extract inhibits MGO-induced skin fibroblast cells dysfunction via ECM-integrin interaction[J]. Journal of Ethnopharmacology,2024,323117699-.

[6] Liao H, Pastar I, Chen W. Rosiglitazone modulates the behaviors of diabetic host-derived fibroblasts in a carboxymethyllysine-modified collagen model[J]. Wound Repair and Re-generation, 2012, 20(3): 435-443.

[7] Kim S, Kim S, Hwang A-R, et al. Apelin-13 inhibits methylglyoxal-induced unfolded protein responses and endothelial dysfunction via regulating AMPK pathway[J]. International Journal of Molecular Sciences, 2020, 21(11): 4069.

[8] Sejersen H, Rattan S I S. Dicarbonyl-induced accelerated aging in vitro in human skin fibroblasts[J]. Biogerontology, 2009, 10(2): 203-211.

[9] Alikhani M, Maclellan C M, Raptis M, et al. Advanced glycation end products induce apoptosis in fibroblasts through activation of ROS, MAP kinases, and the FOXO1 tran-scription factor[J]. American Journal of Physiology, 2007, 292(2): C850-C856.

[10] Lazarev N V. General and specific effects of drugs[J]. Farmakologiia I Toksikologiia, 1958, 21(3): 81-86.

[11] Brekhman I I, Dardymov I V. New substances of plant origin which increase nonspecific resistance[J]. Annual Review of Pharmacology, 1969, 9: 419-430.