Multifunctional Effects of Lactococcus Ferment Lysate for Skin Whitening

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Abstract:

The inflammation produced by exposure to ultraviolet (UV) light has been well documented clinically and histologically. These acute or chronic inflammatory responses cause inflammatory cytokine production from epidermal keratinocytes as well as dermal fibroblasts and other cells, which in turn stimulate melanocytes, often resulting in skin pigmentation. However, not all inflammatory cytokines increase skin pigmentation. Interleukin (IL)-1, IL-6, and tumor necrosis factora (TNFa) are known to suppress skin pigmentation, which suggests the importance of further elucidating the relationship between melanogenesis and inflammation. 【1】 In this study, we screened the cosmetic substances which activate TNFa and evaluated the effect on human skin. In the result, we found Lactococcus Ferment Lysate can inhibit the DNA damage and cell apoptosis caused by UV, activate TNFa and enhance cellular immune function, reduce skin pigmentation after the inflammation. In addition,3D epidermal skin model was irradiated with UVB(50mJ/cm2) for 3 days, the melanin model was photographed, L* was measured with chromatic meter, content of melanin was measured, blackness distribution was detected after section staining. We found the Lactococcus Ferment Lysate can reduce the content and distribution of melanin, lighten skin.

Background: Ultraviolet (UV) radiation activates cell signaling pathways in melanocytes. As a result of altered signaling pathways and UV-induced cellular damage, melanocytes can undergo oncogenesis and develop into melanomas. In this

study, we found Lactococcus Ferment Lysate can increase Human Fibroblast IL-17、NF-κB 、 TNFa signaling pathway related genes were significantly expression. Interleukin (IL)-1, IL-6, and tumor necrosis factor-a (TNFa) are known to suppress skin pigmentation.

Fibroblasts act on melanocytes directly and indirectly through neighboring cells by secreting a large number of cytokines .We guess the Lactococcus Ferment Lysate whitening mechanism is inducing fibroblasts to produce inflammatory factors and inhibiting UV damage.[2]

Methods: Human keratinocytes were irradiated with UVB(300mJ/cm^2), related genes were measured using RNA-seq. Human Fibroblast related genes were measured using RNA-seq. 3D epidermal skin model was irradiated with UVB(50mJ/cm^2) for 3 days, the melanin model was photographed, L* was measured with chromatic meter, content of melanin was measured, blackness distribution was detected after section staining.

Results: DNA replication-dependent nucleosome assembly, DNA replication-dependent nucleosome organization, regulation of neutrophil chemotaxis, nuclear nucleosome, nucleosome, DNA packaging complex, cytokine activity Related genes were significantly up regulated. cellular response to glucose starvation and PERK-mediated unfolded protein response. Related genes were significantly down regulated. IL-17 signaling pathway、Viral protein interaction with cytokine and cytokine receptor、NF-kappa B signaling pathway 、TNF signaling pathway. Related genes were significantly up regulated. Lactococcus Ferment Lysate 5%, It can obviously improve the L* valve, inhibition the Melanin

Conclusion: Lactococcus Ferment Lysate is shown to inhibit the DNA damage of keratinocytes after UVB, by increasing the related genes of DNA

replication-dependent nucleosome assembly and organization, DNA packaging complex and nucleosome significantly, inhibit cell apoptosis by decreasing the related genes of intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress, cellular response to glucose starvation and PERK-mediated unfolded protein response. In addition, it can activation TNFa ， Inhibit inflammation of fibroblast, separately by increasing the related genes of chemotaxis and migration of neutrophils, signal path of IL-17 and decreasing signal path of NF-κB, strengthen skin immune function by increasing related genes of leukocyte chemotaxis and migration, CXCR chemokine receptors binding, GPCRs binding, chemokine receptors binding and cytokine activity.

Keywords: Inflammation, Lactococcus Ferment Lysate, skin whitening

Introduction:

The color of skin is determined by genes(which determine the physiology, structure and functional difference of the skin), external factor(such as UV) and skin inflammation. Key players that regulate human skin pigmentation include melanocytes in the epidermis that synthesize the melanin and neighboring keratinocytes that receive and distribute it in the upper layers of the skin【3】. Other intrinsic factors that help regulate skin pigmentation include fibroblasts in the dermis that affect overlying melanocytes and keratinocytes, endocrine factors from the blood supply, as well as neural factors and inflammation-related factors. Extrinsic factors that directly and/or indirectly affect skin pigmentation include ultraviolet (UV) radiation【4】As known, the pigment melanin including different types, pheomelanin and eumelanin, is produced by melanocytes in a complex process called melanogenesis: melanin synthesis in melanocytes, melanin transport from melanocytes to keratinocytes by melanosome, and melanin distribution in epidermis. 【5】Dermal fibroblasts are traditionally recognized as synthesizing, remodeling and

depositing collagen and extracellular matrix, the structural framework for tissues, helping to bring thickness and firmness to the skin. However, the role of fibroblasts on skin pigmentation arouses concern recently. Fibroblasts act on melanocytes directly and indirectly through neighboring cells by secreting a large number of cytokines (SCF), proteins (DKK1, sFRP, Sema7a, CCN, FAP-α) and growth factors (KGF, HGF, bFGF,NT-3, NRG-1, TGF-β) which bind to receptors and modulate intracellular signaling cascades (MAPK/ERK, cAMP/PKA, Wnt/ -catenin, PI3K/Akt) related to melanocyte functions. These factors influence the growth, the pigmentation of melanocytes via the expression of melanin-producing enzymes and melanosome transfer, as well as their dendricity, mobility and adhesive properties.【2】

Probiotic bacteria are well-established in the food industry, and their benefits for the human body are described in many scientific papers. Interestingly, it is not the whole living probiotic bacterial cell, it is the constituents and metabolites of these bacteria which are essential. A product obtained from a lysate of Lactococcus lactis, which essentially contains the cell debris of this bacterium – such as cell fragments, like DNA, metabolites, cytoplasmic compounds, and cell wall materials. In this study we found Lactococcus Ferment Lysate can induce fibroblasts to produce inflammatory factors IL-17、NF-κB、TNFa. Interleukin (IL)-1, IL-6, and tumor necrosis factor-a (TNFa) are known to suppress skin pigmentation.【1】In evaluate the inhibiting effect of DNA damage: Human keratinocytes were irradiated with UVB(300mJ/cm^2),We found Lactococcus Ferment Lysate can promote inhibiting UV damage.

Materials and Methods

1. Evaluate the inhibiting effect of DNA damage: Human keratinocytes were irradiated with UVB(300mJ/cm^2), related genes of DNA replication-dependent nucleosome assembly and organization, DNA packaging complex and nucleosome were measured using RNA-seq. (figure1)
2. Evaluate the inhibiting effect of cell apoptosis: Human keratinocytes were irradiated with UVB(300mJ/cm^2), related genes of intrinsic apoptotic signaling

pathway in response to endoplasmic reticulum stress, cellular response to glucose starvation and PERK-mediated unfolded protein response were measured using RNA-seq. (figure2)

RNA Quality inspection results						
Experimental group	**Number**	**Concentration**	**Volume**	**Total**	**Integrity value**	**Test conclusion**
Blank control	BC_2	760	45.00	34.20000	9.60	A
positive control	NC_2	568	45.00	25.56000	9.60	A
Lactococcus Ferment Lysate 2%	S2_2	459	45.00	20.65500	9.60	A

A: RNA Quality inspection results. The sample quality meets the quality requirements for database construction and sequencing, and the total amount meets the requirements for database construction for one or more times.

3. Evaluate the inhibiting and activation effect of inflammation: Human Fibroblast related genes of chemotaxis and migration of neutrophils, signal path of IL-17 and NF-κB and TNFa were measured using RNA-seq. (figure3)

RNA Quality inspection results						
Experimental group	**Number**	**Concentration**	**Volume**	**Total**	**Integrity value**	**Test conclusion**
Blank control	BC_1	198	45.00	8.91000	10.00	A
positive control	PC_1	202	45.00	9.09000	10.00	A
Lactococcus Ferment Lysate 2%	S2_2	227	45.00	10.21500	9.80	A

A: RNA Quality inspection results. The sample quality meets the quality requirements for database construction and sequencing, and the total amount meets the requirements for database construction for one or more times.

4. Evaluate the acceleration effect of skin whitening after UVB: 3D epidermal skin model was irradiated with UVB(50mJ/cm^2) for 3 days, the melanin model was photographed, L* was measured with chromatic meter, content of melanin was measured, blackness distribution was detected after section staining. (figure4~7)

Experimental group	Sample name	Dosing concentration	Inducement conditions	model
Blank control	BC	/	UVB （50mJ/cm 2 ）	MelaKutis
negative control	NC	/		
positive control	PC	42.6μg/mL		
Lactococcus Ferment Lysate 5%				

Results

1. 2%Lactococcus Ferment Lysate VS positive control Gene Set Enrichment

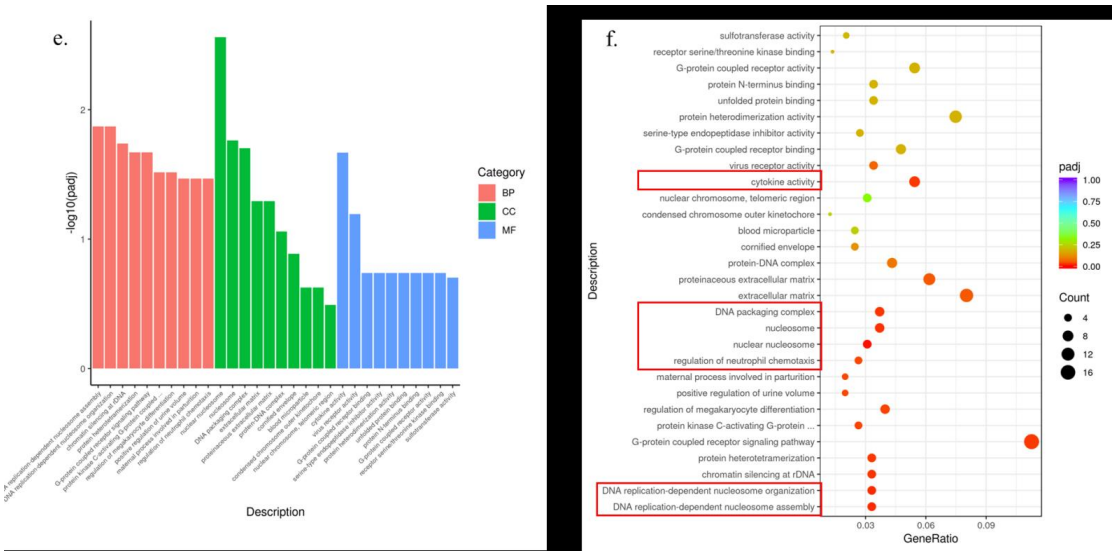

Figure1: Upregulated Gene Set Enrichment

Result: Compared with the positive control, sample Lactococcus Ferment Lysate 2%, DNA replication-dependent nucleosome assembly, DNA replication-dependent

nucleosome organization, regulation of neutrophil chemotaxis, nuclear nucleosome, nucleosome, DNA packaging complex, cytokine activity Related genes were significantly up regulated. Recent evidence suggests that nucleotide excision repair, the physiological repair system that is mostly responsible for the removal of UVR-mediated DNA damage, can be modulated by cytokines, including IL-12, IL-18, and alpha-melanocyte-stimulating hormone.【6】

2. 2%Lactococcus Ferment Lysate VS positive control Gene Set Enrichment

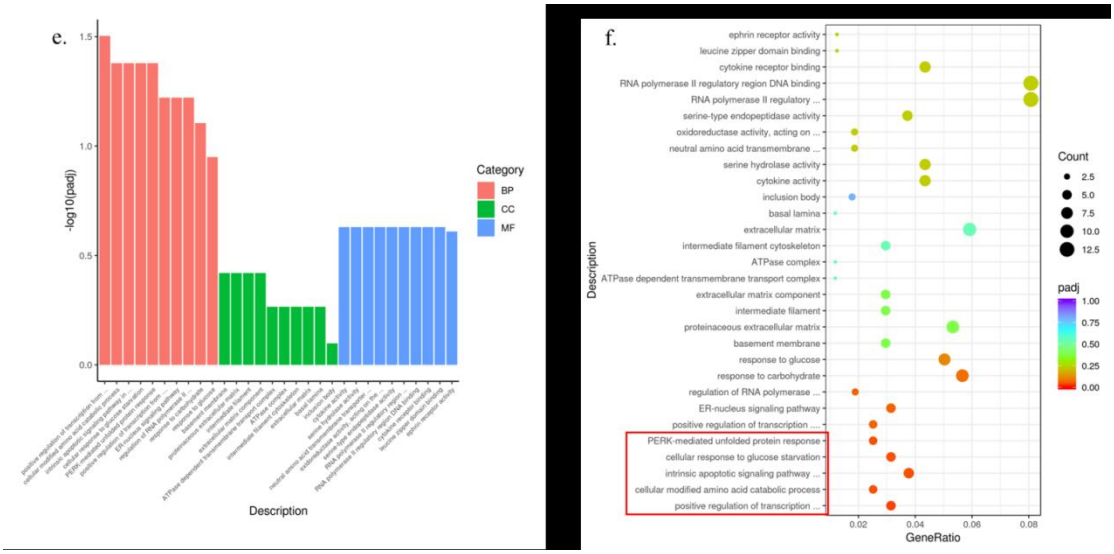

Figure2: Down regulated Gene Set Enrichment

Result: Compared with the positive control, sample Lactococcus Ferment Lysate 2%, cellular response to glucose starvation and PERK-mediated unfolded protein response. Related genes were significantly down regulated. Because repairing radiation-induced DNA damage is an energy-demanding process, glucose starvation impaired DNA double-strand break (DSB) repair.The ER functions in the post-translational processing of protein, including protein folding, maturation, quality control, and trafficking to other cellular compartments. As part of its quality control machinery, when the ER accumulates excess levels of unfolded or misfolded proteins, a distinct series of reactions occurs that slows overall protein synthesis while increasing the production of chaperones and other proteins that increase the fidelity of protein processing. PERK commonly activated to mediate the unfolded protein response

(UPR) and alleviate ER workload. PERK phosphorylates eukaryotic translation initiation factor 2 (eIF2α), resulting in inhibition of most protein synthesis.【7】

3. 2%Lactococcus Ferment Lysate VS blank control Gene Set Enrichment

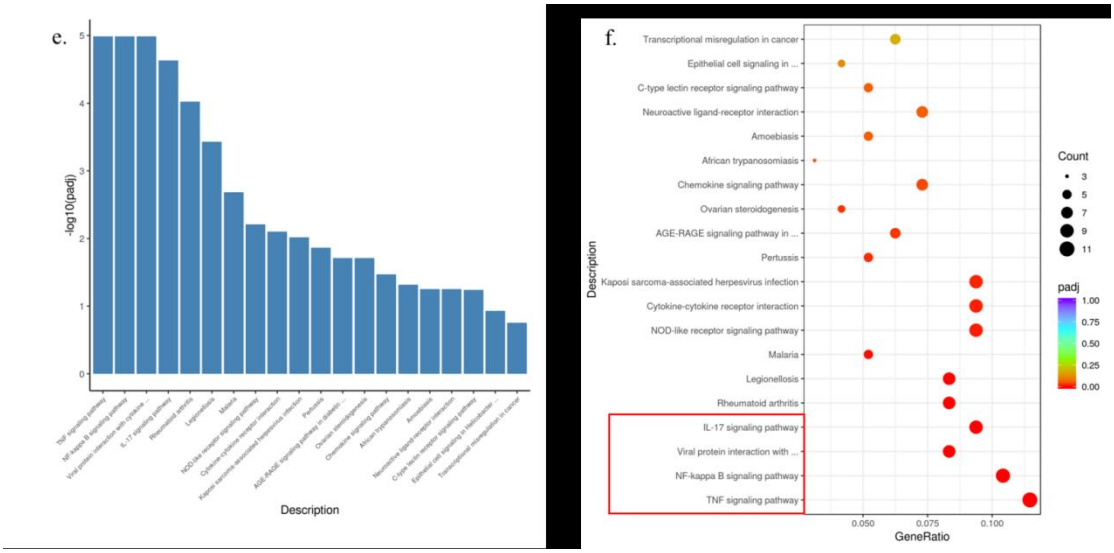

Figure3: Upregulated Gene Set Enrichment

Result: Compared with the blank control group, sample Lactococcus Ferment Lysate 2%, IL-17 signaling pathway、Viral protein interaction with cytokine and cytokine receptor、NF-kappa B signaling pathway 、TNF signaling pathway. Related genes were significantly up regulated.

4. 2%Lactococcus Ferment Lysate VS negative control apparent chromaticity picture

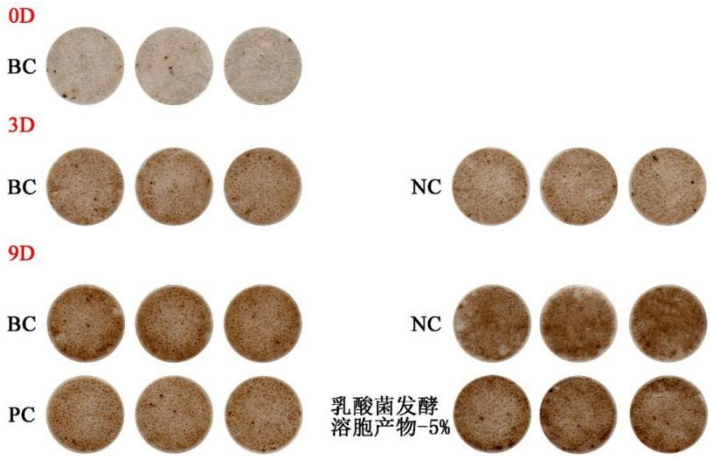

Figure4: 3D epidermal skin model 0D,3D,9D apparent chromaticity picture

Result: Compared with the NC, sample Lactococcus Ferment Lysate 5%, It can obviously improve the melanin model was photographed.

5. 3D epidermal skin model L* value test

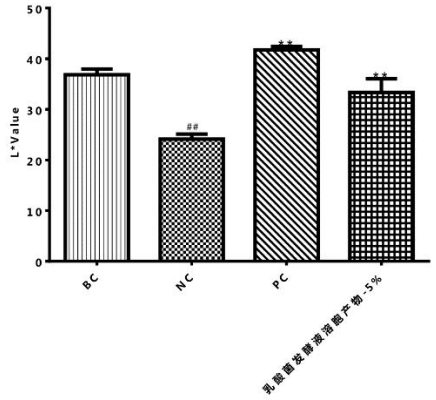

Figure5: Histogram of L* value test results

Result: Compared with the NC, sample Lactococcus Ferment Lysate 5%, It can obviously improve the L* valve

6. 3D epidermal skin model determination of melanin content

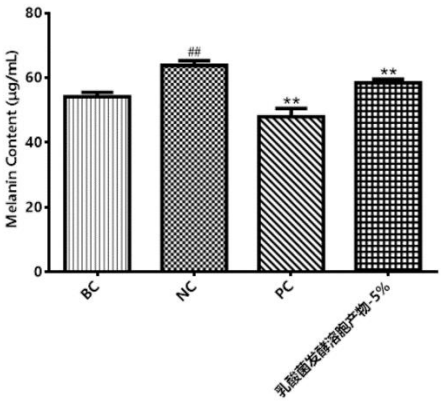

Figure 6: Histogram of melanin content test results

Result: Compared with the NC, sample Lactococcus Ferment Lysate 5%, It can obviously inhibition the Melanin content

7. Take histological photos of the model used for melanin distribution detection in 3D epidermal skin model

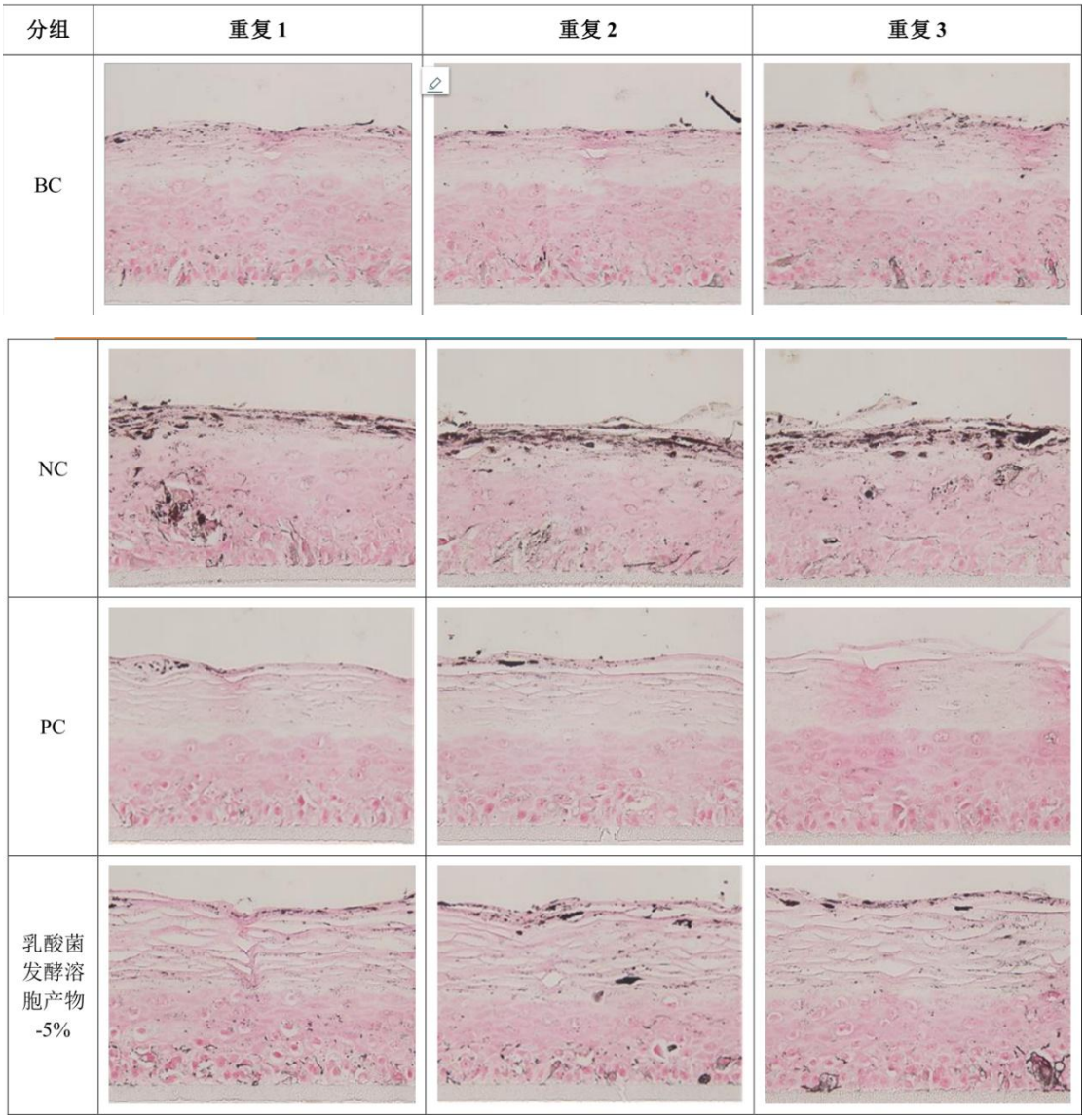

Figure 7: Melanin distribution test results

Result: Compared with the NC, sample Lactococcus Ferment Lysate 5%, Melanin particle distribution decreased significantly

Discussion.

These results demonstrate that Lactococcus Ferment Lysate could be an effective ingredient for cosmetics that exerts a wide variety of biological activities used for skin whitening. It can inhibit the DNA damage and cell apoptosis caused by UV, fight against inflammation and enhance cellular immune function, reduce skin pigmentation after the inflammation, reduce the content and distribution of melanin, lighten skin.

Conclusion.

Lactococcus Ferment Lysate can induce fibroblasts to produce inflammatory factors IL-17、NF-κB、TNFa. In evaluate the inhibiting effect of DNA damage: Human keratinocytes were irradiated with UVB(300mJ/cm2),We found Lactococcus Ferment Lysate can promote cytokine activity, inhibition ellular response to glucose starvation, PERK-mediated

unfolded protein response inhibiting UV damage.3D epidermal skin model was irradiated with UVB(50mJ/cm2) for 3 days， Lactococcus Ferment Lysate can obviously improve the L* valve, inhibition the Melanin, protect DNA from UV damage, lighten skin.

Acknowledgments.

NONE.

Conflict of Interest Statement.

NONE.

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