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# Aqueous Extract of *Chrysanthemum morifolium* Enhances the Antimelanogenic and Antioxidative Activities of the Mixture of Soy Peptide and Collagen Peptide

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## ABSTRACT

The possible synergistic effect between the aqueous extract of *Chrysanthemum morifolium* (菊花 Jú Huā) (AECM) and the peptide mixture (PM) containing soy peptide and collagen peptide was investigated in an ultraviolet (UV) irradiation–induced skin damage mouse model. The irradiated mice were treated with the PM or PM + AECM (containing PM and AECM), respectively. Both PM and PM + AECM groups displayed an apparent photoprotective effect on the UV-irradiated skin damage of mice. Histological evaluation demonstrated that the epidermal hyperplasia and melanocytes in the basal epidermal layer of the UV-irradiated skin in mice decreased when treated with either PM or PM + AECM. Further study showed that soy peptide, collagen peptide, and AECM also inhibited the activities of mushroom tyrosinase with $IC_{50}$ values of 82.3, 28.2, and 1.6 μg/ml, respectively. Additionally, PM + AECM reduced melanogenesis by 46.2% at the concentration of 10 mg/ml in B16 mouse melanoma cells. Meanwhile, the UV-induced increase of antioxidative indicators, including glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and malondialdehyde (MDA), was reduced significantly after treatment with 1.83 g/kg/dbw of PM + AECM. This evidence supported the synergistic antioxidative effect of AECM with PM. These results demonstrated that oral intake of PM and AECM had synergistic antimelanogenic and antioxidative effects in UV-irradiated mice.

**Key words:** Antimelanogenic, Antioxidant, *Chrysanthemum morifolium*, Collagen peptide, Soy peptide

## INTRODUCTION

Ultraviolet (UV) radiation can cause serious skin damage that is generally characterized by wrinkling, roughness, laxity, and pigmentation. UV radiation generates reactive oxygen species (ROS) and, thus, leads to oxidative stress. Oxidative stress induces pro-inflammatory cytokines, which in turn increase the intracellular levels of ROS.[1] The ROS induced by UV radiation assist melanin biosynthesis and DNA damage, which results in up-regulation of the gene for tyrosinase, the rate-limiting enzyme in melanin biosynthesis, and subsequently in epidermal hyperpigmentation.[2-4] Therefore, the inhibitors of tyrosinase or antioxidants may suppress melanogenesis in the epidermal layer of the skin. ROS are also found to activate the cytoplasmic signal transduction pathways in the resident fibroblasts. This activation relates to growth, differentiation, senescence, and connective tissue degradation and also causes permanent genetic changes.[5] Considering that UV-induced oxidative stress mediates adverse effects in

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the skin and body, regular intake of antioxidants in combination with an antioxidant topical treatment is a useful way to reduce the pigmentation and harmfulness of UV radiation.[6]

The flowers of *Chrysanthemum morifolium* (菊花 Jú Huā) (Compositae) are commonly used in tea and as an herbal drug in China. They have been reported to possess antibacterial, antifungal, anti-spirochetal, anti-inflammatory, and antioxidant activities.[7] The flavonoids, alkaloids, and sesquiterpene lactones are thought to contribute to the pharmacological activities of *C. morifolium*.[7-9] A recent report indicated that the flavonoids in the extracts of *C. morifolium* protected the brain, liver, and kidney against lead-induced oxidative damage in mice. Moreover, the extracts provided significant protection against cerebral ischemia and reperfusion injury in rats through their antioxidant effect.[10,11]

Soy peptide (SP) and collagen peptide (CP) have been used as important active components in medicinal and food industries because of their excellent bioactivity, biocompatibility, good penetrability, and lack of irritation.[12] Our previous study confirmed that SP had synergistic antioxidant activities with CP. The mixture of SP and CP showed good antioxidant activity in the senescent mouse model.[13] However, neither the additive nor the synergistic effect of the aqueous extract of *C. morifolium* (AECM) and peptides has been reported yet. By examining the *in vivo* effects of the peptide mixture (PM) in the presence or absence of AECM, we set out to investigate whether AECM has a synergistic effect in a UV-irradiated mouse model and to uncover the possible mechanisms for the antimelanogenesis and antioxidant activities.

## MATERIALS AND METHODS

### Materials

The SP was obtained from Fuji Oil (Osaka, Japan). The CP was obtained from Haishi (Zhoushan, China). The air-dried and powdered aerial parts of *C. morifolium* were extracted with water. Nuclear fast red, tyrosinase, l-tyrosine, alpha-melanocyte stimulating hormone (α-MSH), and all other chemicals were obtained from the Sigma-Aldrich company (Shanghai, China) and were of analytical grade.

### Mice treatment and UV radiation

Four-week-old female Kuming (KM) mice [weighing 25 ± 3 g, SCXK (Xiang) 2009-0004] were obtained from Hunan SJA Lab Animal Ltd. Mice were housed under controlled conditions [SYXK (Yue) 2008-0003] with 55 ± 5% relative humidity at a temperature of 25 ± 1°C (12 h light/dark cycle). The experiments were carried out according to the Chinese legislation on the use and care of laboratory animals and were permitted by the Institutional Animal Care and Use Committee of Guangzhou Institute of Pharmaceutical Industry. Mice were given free access to pellets and drinking water during the experiment. After 5 days acclimation, the back of each mouse was denuded using sulfureted sodium over the depilated area of 15 $cm^2$, and each mouse was randomly assigned to one of five groups, with 12 mice in each group. The groupings are as follows:

Negative control (NC) group: Normal group, normal saline by oral intake

Vehicle/UV group: Model group, normal saline by oral intake

PM (SP: CP = 1:1) group: A dose of 1.67 g/kg/dbw of the PM by oral intake

PM + AECM (SP: CP: AECM = 1:1:0.2) group: A dose of 1.83 g/kg/dbw of the PM containing AECM by oral intake

Vitamin C (Vit C) group: A dose of 200 mg/kg/dbw by oral intake

All mice were given the substances in the same volume of 0.2 ml. The samples were freshly prepared and given to the mice every day using a stomach sonde needle. All the mice, except the ones in normal group, were irradiated with the same UV source.

The back areas of the mice were irradiated for 30 min every day with 235 mJ/$cm^2$ of UV each time for 30 consecutive days. The source of light was a UVB-313EL lamp (Q- Panel Lab Products, Westlare, USA). The distance from the lamps to the animals' back was 25 cm. During the period of exposure, the mice were group housed in a stainless steel irradiation chamber and the animals could freely move around in the chamber. A non-irradiated group of animals was included as a control. The animals were anesthetized, sampled, and sacrificed after the final irradiation.

### Histological evaluation of mouse skin

Hematoxylin and eosin (H and E) staining was conducted for a routine examination of the tissue and to measure the epidermal thickness, fibrous tissue, and adipose tissue. The pathological status of the skin was evaluated by taking 10 representative sample measurements per tissue section under the microscope.

### Measurement of DOPA-positive melanocytes in mice

The mice were sacrificed after the final samples were administered. Biopsies were obtained from the UV-irradiated central dorsal skin, and 3,4-dihydroxyphenylalanine (DOPA) staining of the epidermal sheet was performed.[14] Briefly, the biopsies were fixed in 10% buffered formalin and then incubated with 0.0056 mol/l of DOPA solution. After being fixed in Bouin's fixative, dehydrated with a sequence of ethanol solution, and embedded in paraffin, 5-μm-thick serial sections were cut. The sections were stained with 0.1% nuclear fast red and then observed for melanocytes by a photomicroscope.

### Sample collection and preparation

The whole blood collected was diluted prior to measurement. The dorsal skin and liver were removed and immediately placed in ice-cold 50 mM potassium phosphate buffer (pH 7.4). Tissues were cleaned by rinsing carefully in buffer and then homogenized at 20,000 rpm in 50 mM potassium phosphate buffer (pH 7.4) for 15-25 s in a tissue homogenizer (model T25-S1; IKA Labortechnik, Staufen, West Germany). Tissue sample specimens ranging in size from 25 to 100 mg were further homogenized in 1.0 ml of ice-cold buffer. The homogenates were centrifuged (Beckman High-Speed Refrigerated Centrifuge, model J2-HS) for 10 min at 10,000 g and 4°C. The supernatants were used for immediate enzyme activity assays or stored at - 70°C until analysis. All enzymes were assayed within 3 weeks.

### Assay for GSH-Px and SOD activity

Glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities were assayed by using the GSH-Px