Antiaging activity of low molecular weight peptide from *Paphia undulate*

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Abstract  Low molecular weight peptide (LMWP) was prepared from clam *Paphia undulate* and its antiaging effect on D-galactose-induced acute aging in rats, aged Kunming mice, ultraviolet-exposed rats, and thermally injured rats was investigated. *P. undulate* flesh was homogenized and digested using papain under optimal conditions, then subjected to Sephadex G-25 chromatography to isolate the LMWP. Administration of LMWP significantly reversed D-galactose-induced oxidative stress by increasing the activities of glutathione peroxidase (GPx) and catalase (CAT), and by decreasing the level of malondialdehyde (MDA). This process was accompanied by increased collagen synthesis. The LMWP prevented photoaging and promoted dermis recovery and remission of elastic fiber hyperplasia. Furthermore, treatment with the LMWP helped to regenerate elastic fibers and the collagen network, increased superoxide dismutase (SOD) in the serum and significantly decreased MDA. Thermal scald-induced inflammation and edema were also relieved by the LWMP, while wound healing in skin was promoted. These results suggest that the LMWP from *P. undulate* could serve as a new antiaging substance in cosmetics.

Keyword: *Paphia undulate*; low molecular weight peptide; antiaging; antiultraviolet radiation; skin wound healing in scald rats

1 INTRODUCTION

Marine organisms comprise approximately half of the total global biodiversity and are a good source of bioactive peptides with various biological activities, including antioxidant and antimicrobial action. Therefore, they are potential sources of active components for functional cosmetics and foods or in nutraceutical and pharmaceutical products (Kim and Wijesekara, 2010; Harnedy and FitzGerald, 2012). It was reported that peptides play an important role in dermal wound healing, antiaging and photoaging skin care products (Bauza et al., 2004; Perrin et al., 2004; Singer et al., 2007), however, studies on bioactive peptides from marine species are rare. *Paphia undulate*, a marine mollusk, is one of the most important marine mariculture shellfishes in Southern China. It is traditional seafood in China and serves as a good source of protein (Fang et al., 2009). Remarkable antifatigue and antioxidant activities were reported for *P. undulate*, revealing its medicinal value and provides a new utilization pathway (Lin et al., 2010; Fang et al., 2011).

Skin aging is a degenerative process that can be macroscopically recognized by wrinkles and destruction and thinning of the elastic fiber network (Giacomoni and Rein, 2001; Lee et al., 2008). The rate of aging may be related to the nutrient supply

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The weight of evidence indicates that the general loss of molecular fidelity drives the aging process (Hayflick, 2007). Alternatively, alterations with the passage of time are superimposed with the effects produced by environmental factors, including ultraviolet radiation, traumatisms, cigarette smoke and infections. There are different theories (e.g., protein error theory, free radical theory) to explain the aging phenomenon (Rattan, 2006). Among them, the free radical theory of aging appears to be the most popular and widely proposed theory in the literature. The reaction of active free radicals, produced naturally in organisms, with cellular constituents initiates the changes associated with aging (Harman, 2009). Together with increasing age, the human organism has to face an increasing imbalance between antioxidant defense systems and prooxidant formation. The antioxidant defense system, which consists of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), prevents the excessive formation of free radicals and plays a role in the removal of reactive oxygen species (ROS) (Nohl, 1993; Wei et al., 2001). A decrease in the biomarker malondialdehyde (MDA) is an indicator of the protection of the skin against oxidative stress (Sanchez et al., 2011).

In this paper, low molecular weight peptides (LMWPs) were isolated from *P. undulate*. The antiaging effects of LMWP on normal aged mice, D-galactose-aged rats and ultraviolet damaged rats and its protective effects on thermal scald wound in rat skin were investigated.

2 MATERIAL AND METHOD

2.1 Pretreatment of *P. undulate*

Live *P. undulate*, purchased from the Shanzi Market in Foshan, China, was decontaminated in clean, artificial sea water for 48 h, followed by elimination of the clam and internal organs. The meat was rinsed well with distilled water, homogenized, divided into small samples (10 g/sample) and refrigerated (-20°C).

2.2 Animals and reagents

SD mice (Grade SPF, Certificate No. SCXK (Yue) 2008-0002), half male and half female, weighing 20±2 g, were obtained from the animal center of Guangzhou University of Traditional Chinese Medicine. All animals were cared for and treated in accordance with the Guiding Principles in the Care and Use of Animals. Papain, with a nominal activity of 2×10^6 U/g, was obtained from Pangbo Enzyme Co., Ltd. (Guangxi, China). Sephadex G-25 was procured from Yubo Biotechnology Co., Ltd. (Shanghai, China). Ovalbumin, cytochrome C, insulin, glucagon and blue dextran 2000 were purchased from Weijia Technology Co., Ltd. (Guangzhou, China). Commercial CAT, GPx, MDA and SOD kits were from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Vitamin E (Vit E) was obtained from Xingquin Pharmaceutical Co., Ltd. (Guangzhou, China). SOD Milk (100 mL), used as positive control substance, was obtained from Beijing Dabao Cosmetics Co., Ltd. All other chemicals and solvents used in this study were analytical grade.

2.3 Preparation of enzymatic hydrolysates

Enzymatic hydrolysis was carried out by incubation with papain for 4 h under optimal conditions (pH 6.3, 55°C). The enzyme to substrate and substrate to deionized water ratios were 1:100 (w/w) and 1:3 (w/v), respectively. The resulting hydrolysate was immediately heated in a boiling water bath for 10 min to inactivate the enzyme, and then centrifuged for 10 min at 2450×g (Chen et al., 2010).

2.4 Molecular weight determination

The enzymatic hydrolysate was separated on a Sephadex G-25 column using deionized water as the eluent and the fractions collected were measured at 280 nm. The molecular weight was calibrated using ovalbumin, cytochrome C, insulin and glucagon, and the apparent elution volume (Ve) was recorded. Blue dextran 2000 was used to determine the void volume of the column. The molecular weight was calculated from the equation below (Wang et al., 2009).

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\log \text{MW} = -0.0063 V_e + 5.3368 \quad (R^2 = 99.95\%)
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The fractions isolated by Sephadex G-25 were designated as high, medium, and low molecular weight peptide (HMWP, MMWP, and LMWP; Table 1), and the LMWP fraction with the lowest molecular weight of 655.84 Da was studied for antiaging activity.