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Effects of rambutan (*Nephelium lappaceum*) peel phenolics and Leu-Ser-Gly-Tyr-Gly-Pro on hairless mice skin photoaging induced by ultraviolet irradiation

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ABSTRACT

Rambutan peel phenolics (RPP) have high antioxidant and anti-inflammatory activities. Leu-Ser-Gly-Tyr-Gly-Pro (LSGYGP) possesses good radical scavenging activity and matrix metalloproteinase (MMPs) inhibitory ability. These underlying mechanisms indicated that RPP and LSGYGP may be used for antiphotoaging. Few data on the effects of RPP and LSGYGP on *in vivo* photoaging are available. We evaluated the effects of RPP and/or LSGYGP on ultraviolet (UV)-induced hairless mice skin photoaging. In particular, we analyzed the additive effect of RPP and LSGYGP. The biochemical indices of mice skin, including composition (collagen and hyaluronic acid [HA] contents), oxidant stress (antioxidant enzyme activities and glutathione and malondialdehyde contents), MMPs (MMP-1, MMP-3, and MMP-9 levels), inflammatory cytokines (interleukin (IL)-1α, tumor nuclear factor-α, and IL-6 levels) and the phosphorylation of the mitogen-activated protein kinase pathway, were determined. Results showed a protective effect of RPP and/or LSGYGP on photoaging skin. LSGYGP showed considerable effects on skin collagen and HA contents. RPP showed improved effects on the regulation of the oxidant stress and inflammatory cytokine levels. RPP and LSGYGP exerted an additive effect on the amelioration of the biochemical indices of UV-induced photoaging skin. The histological changes showed that RPP and LSGYGP recovered the changes in skin tissue and endogenous collagen.

1. Introduction

Ultraviolet (UV) irradiation is a key factor leading to skin photoaging (Chen et al., 2016b). UVA (320–400 nm) and UVB (280–320 nm) can cross the atmosphere and exert harmful effects on skin tissues, and chronic exposure to UV can destroy the structure of the extracellular matrix of skin tissues (Ye et al., 2018). On the one hand, UV irradiation produces reactive oxygen species (ROS) to stimulate some cascades, such as mitogen-activated protein kinases (MAPKs) and activated protein 1, which increases matrix metalloproteinase (MMP) secretion (Shah and Rawal Mahajan, 2013). On the other hand, excessive UV irradiation can accelerate the production of proinflammation cytokines, such as tumor nuclear factor-α (TNF-α) and interleukin-1 (IL-1), which stimulate the formation of MMPs (Goettsch et al., 1998). The increase in MMP contents decreases collagen and the formation of collagen fragmentations in skin (Lu et al., 2016).

Phenolics are important secondary metabolites that determine the sensory and nutritional qualities of vegetables, fruits, and other plant products. In recent years, phenolics have received attention because of their high bioactivities. Some phenolic compounds are also proved to have high anti-photoaging activities in previous studies (Koh et al., 2016; Huang et al., 2017; Petruk et al., 2018). Rambutan peel phenolic (RPP) extract is obtained by dynamic separation using macroporous resin from rambutan peel ethanolic extract (Sun et al., 2012; Zhuang et al., 2017a). The total phenolic content of RPP is 877.11 mg gallic acid equivalents/g extract. Thirty-nine phenolic compounds were identified by mass spectrometry, and the geranin content of 122.18 mg/g extract is the highest (Zhuang et al., 2017a). RPP has high Fe^{2+} and Cu^{2+}-chelating activities, effectively inhibits the formation of hydroxyl radical, and efficiently decreases peroxyl radical-induced plasmid DNA strand breakage in an 2,2′-Azobis (2-methylpropionamidine) dihydrochloride (AAPH)-induced DNA damage model (Li et al., 2018). The anti-inflammatory effect of RPP was determined, and RPP clearly inhibits the formation of nitric oxide (NO) and regulates the levels of inducible NO synthase mRNA in LPS-induced RAW 264.7 cells (Li et al., 2018). RPP can also effectively regulate against oxidative stress in H_2O_2-induced HepG2 cells and *D*-galactose-induced aging mice (Zhuang et al., 2017b). The bioactivities of RPP depend mainly on its

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phenolic content and profiles.

Previous studies indicated some gelatin hydrolysates and peptides have good inhibitory photoaging activities (Kim et al., 2018; Lu et al., 2017). Our study also showed that tilapia fish skin gelatin hydrolysates could inhibit skin photoaging *in vivo*. Leu-Ser-Gly-Tyr-Gly-Pro (LSGYGP), a gelatin peptide, was identified from this hydrolysate with a high scavenging hydroxyl radical activity (Sun et al., 2013). The bioactivities of LSGYGP were further determined using UVB-induced mouse embryonic fibroblasts (MEFs). LSGYGP decreases the intercellular ROS production in UVB-induced MEFs and inhibits MMP-1 and MMP-9 activities. Molecular docking simulation showed that LSGYGP inhibits MMP-1 and MMP-9 activities by docking their active sites (Ma et al., 2018). LSGYGP also regulates the UVB-induced intercellular phosphorylation of MAPK pathway (Ma et al., 2018). Thus, LSGYGP can increase the synthesis of collagen in UVB-induced MEFs by regulating oxidative stress and inhibiting MMP activities.

A number of underlying mechanisms, including oxidant stress, inflammatory, radicals, MMPs activity and collagen loss, contribute to the pathogenesis of skin photoaging. According to the relative bioactivities of RPP and LSGYGP evaluated by our previous studies, RPP and LSGYGP could be combined to inhibit skin photoaging. However, study on antiphotoaging by using RPP combined with LSGYGP *in vivo* has not been conducted to date. In this study, we aimed to determine the protective properties of RPP and/or LSGYGP on UV-induced hairless mice photoaging, including skin collagen and hyaluronic acid (HA) formation, antioxidative index, MMP levels, secretion of inflammatory cytokines, and the phosphorylation of the MAPK signal pathway. The effects of RPP and LSGYGP on the skin histological changes were also studied.

2. Materials and methods

2.1. Materials

RPP sample was obtained according to the previous method (Zhuang et al., 2017b). LSGYGP was synthesized by Shanghai Synpeptide Co., Ltd. (Shanghai, China). The commercial kits of protein concentration, CAT activity, SOD activity, GSH-Px activity, HYP content, GSH content, and MDA content were provided by Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). The ELISA kits, including HA, TNF-α, IL-1α, IL-6, MMP-1, MMP-3, MMP-9, *p*-JNK, *p*-ERK, and *p*-p38, were provided by R&D (Systems Inc., Minneapolis, MN, USA).

2.2. Animals

Sixty hairless mice (male, BALB/c nude mice, 20–22 g per body weight [BW]) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). Mice were acclimatized for 1 week to the conditions of the animal room (a 12 h dark/12 h light cycle and ambient temperature ranging from 20 °C to 25 °C). In this process, mice were provided with the standard pellet diets and water freely. All mice experiments were performed according to the animal experimentation guidelines approved by the Animal Care and Use Committee of Kunming University of Science and Technology. Mice were divided into 6 groups, and each group had 10 mice. The six groups are as follows: NC: normal control group, normal saline by oral intake; MC: model control group, normal saline by oral intake; RP: at a dose of 100 mg/kg/day BW RPP by oral intake; PE: at a dose of 100 mg/kg/day BW LSGYGP by oral intake; RPPE-C: at a dose of 50 mg/kg/day BW RPP and a dose of 50 mg/kg/day BW LSGYGP by oral intake; and RPPE-A: at a dose of 100 mg/kg/day BW RPP and a dose of 100 mg/kg/day BW LSGYGP by oral intake.

2.3. UV irradiation

All mice except those in the NC group were irradiated by UVA and UVB tubes (Skin photoaging apparatus HOPE-MED 8140, Tianjin Hepu Industry and Trade Co., Ltd., Tianjin, China), which were positioned 30 cm above the mice. The irradiation intensity was detected by the UVA and UVB radiometers. First, the minimal erythemal dose (MED) of UVA and UVB irradiations on mice skin was determined, and the MED of UVA and UVB were 127.84 and 18.36 mJ/cm^2, respectively. Mice were exposed to UV irradiation 3 times a week for 10 weeks. The UV irradiation intensity was 0.5 MED for 1 week. The intensities of UVA and UVB irradiations were increased by 0.5 MED per week, reaching up to 4 MED. Finally, mice were irradiated at 4 MED for 3 weeks. The total irradiation doses of the UVA and UVB irradiations were 3.32 and 0.48 J/cm^2, respectively.

2.4. Skin hydroxyproline (HYP) and hyaluronic acid (HA) quantitative analyses

Skin HYP content was determined by a hydroxyproline assay kit. The quantification of the skin HA was measured by an ELISA kit. All experiments were performed according to the manufacturer's protocol. The HYP and HA results were expressed by their contents against the skin weight of mice.

2.5. Skin antioxidant indicator analysis

Mice skin was homogenized (1:9 w/v) in ice-cold normal saline. The skin homogenate was centrifuged at 9000 r/min (4 °C, 15 min), and the supernatant was collected. Protein concentration, superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px) activity, catalase (CAT) activity, glutathione (GSH) level, and malondialdehyde (MDA) level in mice skin were performed according to manufacturer's protocols. The SOD, GSH-Px, and CAT results were expressed by their activities against mice skin protein concentrations, and those of GSH and MDA were expressed by their contents against mice skin protein concentrations.

2.6. Skin MMP content analysis

The MMP-1, MMP-3, and MMP-9 contents, which were determined according to the manufacturer's protocols, were analyzed by ELISA kits. The results of different MMPs were expressed by their contents against mice skin protein concentrations.

2.7. Skin proinflammatory cytokine level analysis

The proinflammatory cytokine, including IL-1α, IL-6, and TNF-α levels were determined by the respective kits, which were performed according to the manufacturer's protocols. The proinflammatory cytokine results were expressed by their levels against mice skin protein concentrations.

2.8. MAPK pathway phosphorylation in skin analysis

The phosphorylations of MAPK pathway, including the *p*-extracellular signal-regulated kinases (*p*-ERK), *p*-c-Jun N-terminal kinases (*p*-JNK), and *p*-p38 contents, were determined by the respective kits, which were performed according to the manufacturer's protocols. The *p*-ERK, *p*-JNK, and *p*-p38 results were expressed by their contents against mice skin protein concentrations.

2.9. Skin histological analysis

Mice skin specimens (approximately 1 cm^2) were fixed in the buffered neutral formalin (4%, w/v) for 24 h. Hematoxylin and Eosin (H&

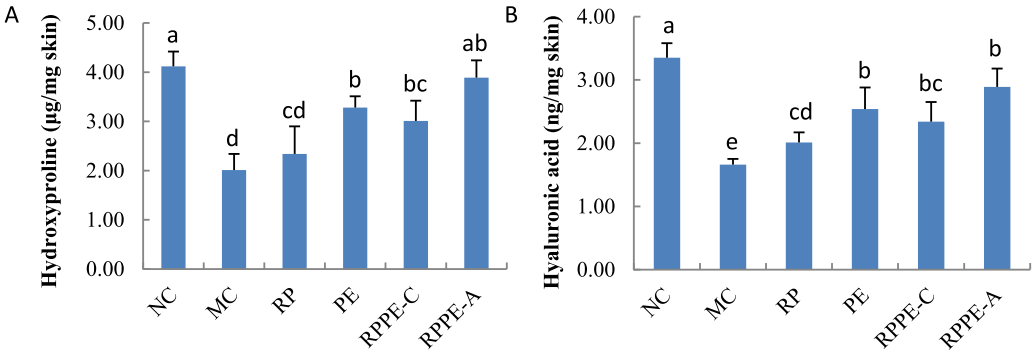

Fig. 1. Effect of RPP and LSGYGP on the hydroxyproline content (A) and hyaluronic acid content (B) of photoaging skin. Data were shown as mean ± standard deviation. Different lowercase letters indicated significant differences ($p < 0.05$).

E) and Van Gieson (VG) staining were performed. The changes in skin histology were determined by an Olympus DP70 Digital Camera System at 200 × magnification for H&E staining and 400 × magnification for VG staining.

2.10. Statistical analysis

The data were presented as mean ± standard deviation. The significant differences between different groups were determined with multiple comparison tests by using SPSS software (SPSS, version 17.0, IBM Inc., USA). A $p < 0.05$ was considered statistically significant, and the significant differences were noted by different lowercase letters for every biochemical index.

3. Results

3.1. HYP and HA contents

As shown in Fig. 1, the HYP and HA contents in the MC group significantly decreased by 51.21% and 50.45% compared with NC group ($p < 0.05$). The RP and PE groups can evidently protect the HYP and HA contents in mice skin. For HYP content, PE group showed higher effect than RP group at a dose of 100 mg/kg/day BW. The effective value of the RPPE-C group was lower than that of the PE group, but both groups had insignificant difference ($p > 0.05$). RPPE-A had insignificant difference with the NC group ($p > 0.05$). For HA content, PE group showed higher effect than the RP group at a dose of 100 mg/kg/day BW. The RPPE-C group had no significant difference with RP and PE groups ($p > 0.05$). RPPE-A group also exhibited insignificant difference with the PE group ($p > 0.05$).

3.2. Antioxidant indices

The results showed that UV irradiation induced a significant difference in the activities of SOD, CAT, and GSH-Px between the NC and MC groups ($p < 0.05$, Fig. 2). For SOD activity, the effect of RP group was significantly higher than PE group ($p < 0.05$). The RPPE-C group had insignificant difference with the RP group ($p > 0.05$), and RPPE-A group exhibited insignificant difference with the NC group ($p > 0.05$). For CAT and GSH-Px activities, the RP, PE, and RPPE-C groups had no significant differences ($p > 0.05$); the RPPE-H group was significantly higher than other groups ($p < 0.05$). The effect of RPPE-A group on CAT activity had insignificant difference with the NC group ($p > 0.05$).

As shown in Fig. 3, compared with the NC group, the GSH content decreased by 42.68%, and that of MDA increased by 141.99% in the MC group. For GSH content, four groups had insignificant difference ($p > 0.05$). However, the value of the RPPE-A group was higher than those of the other groups, and RPPE-A group had insignificant difference with the NC group ($p > 0.05$). For MDA content, the effect of PE group was significantly lower than those of the RP and RPPE-C groups ($p < 0.05$). The RPPE-A groups had insignificant difference with the NC group ($p > 0.05$).

3.3. Inflammatory cytokine levels

As shown in Fig. 4, the changes in inflammatory cytokine levels were evaluated. Compared with NC group, the IL-1α, IL-6, and TNF-α levels were significantly increased via UV irradiation in the MC group ($p < 0.05$). For IL-1α level, RP group had significantly higher effect than the PE group ($p < 0.05$). The RP and RPPE-C groups had insignificant difference ($p > 0.05$), and the RPPE-A group exhibited insignificant difference with the NC group ($p > 0.05$). For IL-6 level, four samples groups showed insignificant difference ($p > 0.05$). For TNF-α level, the sequence of four groups followed the order RPPE-A group > RP group > RPPE-C group > PE group, and RPPE-A group had significant difference with MC group ($p < 0.05$).

3.4. MMP contents

As shown in Fig. 5, UV irradiation induced significant increase in MMP content in mice skin ($p < 0.05$). Compared with the NC group, the rates of the increase in MMP-1, MMP-3, and MMP-9 contents were 87.55%, 65.31%, and 274.57%, respectively. For MMP-1 content, the effects of the RP, PE, and RPPE-C groups had insignificant difference ($p > 0.05$). RPPE-A group showed a higher effect than the RP, PE, and RPPE-C groups. Similar effect was found in the MMP-3 content. For MMP-9 content, the effect of RP group was higher than that of the PE group. The RPPE-A group had insignificant difference with NC group ($p > 0.05$).

3.5. JNK, ERK, and p38 phosphorylation

The effect of the different samples on MAPK pathway is shown in Fig. 6. The results showed that the UV irradiation significantly increased ERK and p38 phosphorylation ($p < 0.05$). The value of JNK phosphorylation in the MC group was higher than that in the NC group, but the difference was insignificant ($p > 0.05$). For the *p*-ERK level, the RP, PE and RPPE-C groups had insignificant differences ($p > 0.05$). The RPPE-A group exhibited insignificant difference with the NC group ($p > 0.05$). For the *p*-p38 level, the PE group had insignificant difference with the MC group ($p > 0.05$). The RPPE-C and RPPE-A groups had significant difference with the MC group ($p < 0.05$).

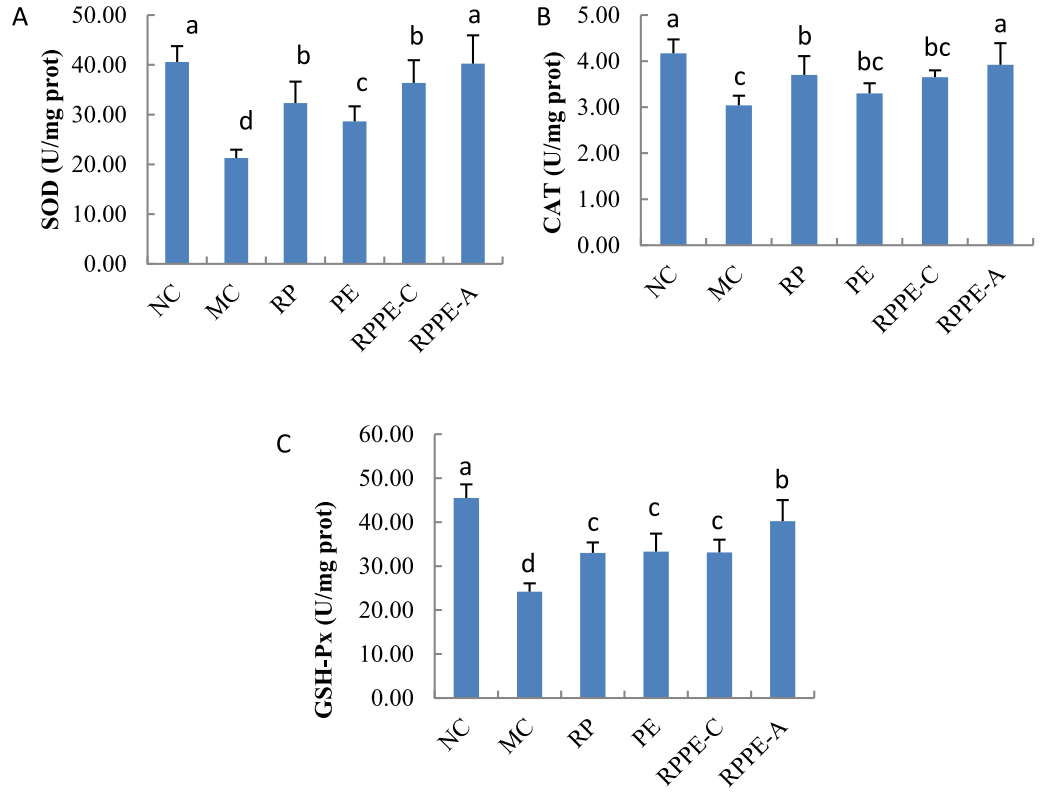

Fig. 2. Effect of RPP and LSGYGP on antioxidant enzymes of photoaging skin. A: SOD activity; B: CAT activity; C: GSH-Px activity. Data were shown as mean ± standard deviation. Different lowercase letters indicated significant differences ($p < 0.05$).

3.6. Histological changes

The H&E staining images of mice skin are shown in Fig. 7A, which displayed the changes in skin structure. UV radiation significantly caused skin tissue alterations. Many abnormal fractured, tangled, degraded, and nonfunctional fibers were expressed in the mice skin of the MC group. The skin in the MC group showed epidermal thickness, sparse dermis, and enlarged sebaceous glands compared with that of the NC group. Minimal damages in UV-induced skin structure were found in the RP and PE groups. This result indicated that RPP and LSGYGP significantly inhibited the UV-induced skin structure alterations. In the RPPE-C group, the dermal collagen fibers were regulated, and the epidermis hyperplasia was recovered. In the RPPE-A group, the structures of mice skin were almost in the same conditions as those of the NC group.

The alterations of skin tissues were further evaluated by staining with VG to study the changes in skin collagen, which appeared as a red deposit under a microscope. As shown in Fig. 7B, compared with the NC group, UV irradiation induced collagen reduction and tortuosity. Additional disordered and looser dermal layer were found in the MC group. The collagen structures in the RP and PE groups were significantly increased, and the effect of PE group was clearly higher than that of the RP group. The improvement of the skin collagen was remarkable in the RPPE-A group.

4. Discussion

Our previous studies showed RPP has good antioxidant activity,

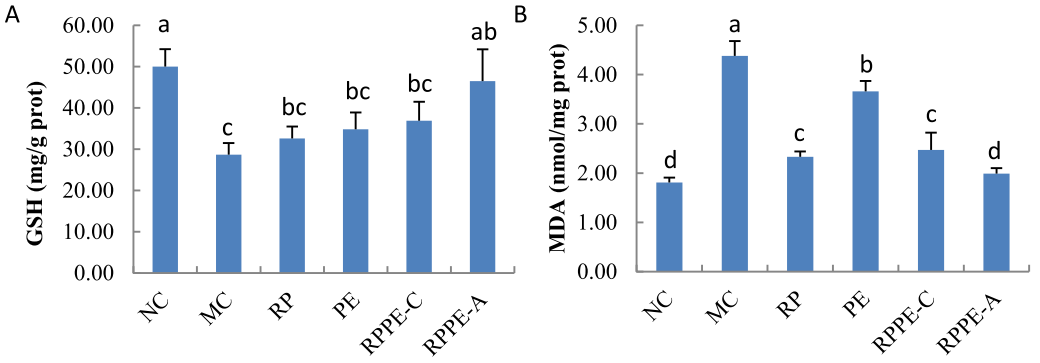

Fig. 3. Effect of RPP and LSGYGP on GSH (A) and MDA (B) contents of photoaging skin. Data were shown as mean ± standard deviation. Different lowercase letters indicated significant differences ($p < 0.05$).

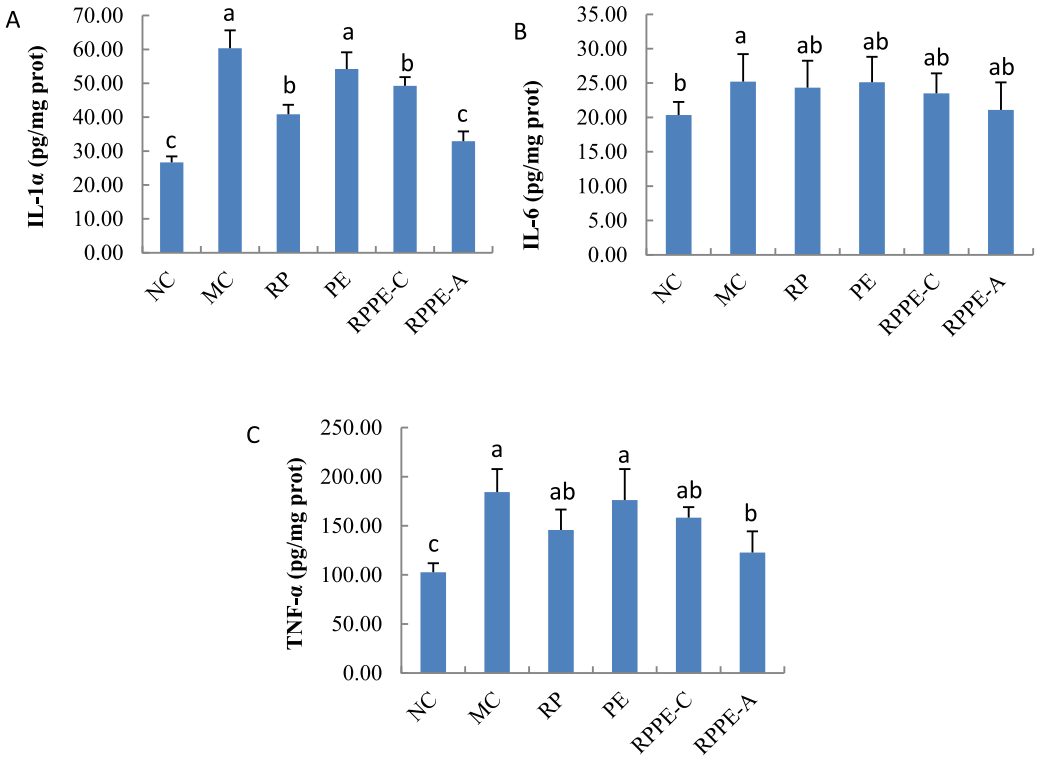

Fig. 4. Effect of RPP and LSGYGP on proinflammatory cytokines of photoaging skin. A: IL-1α content; B: IL-6 content; C: TNF-α content. Data were shown as mean ± standard deviation. Different lowercase letters indicated significant differences ($p < 0.05$).

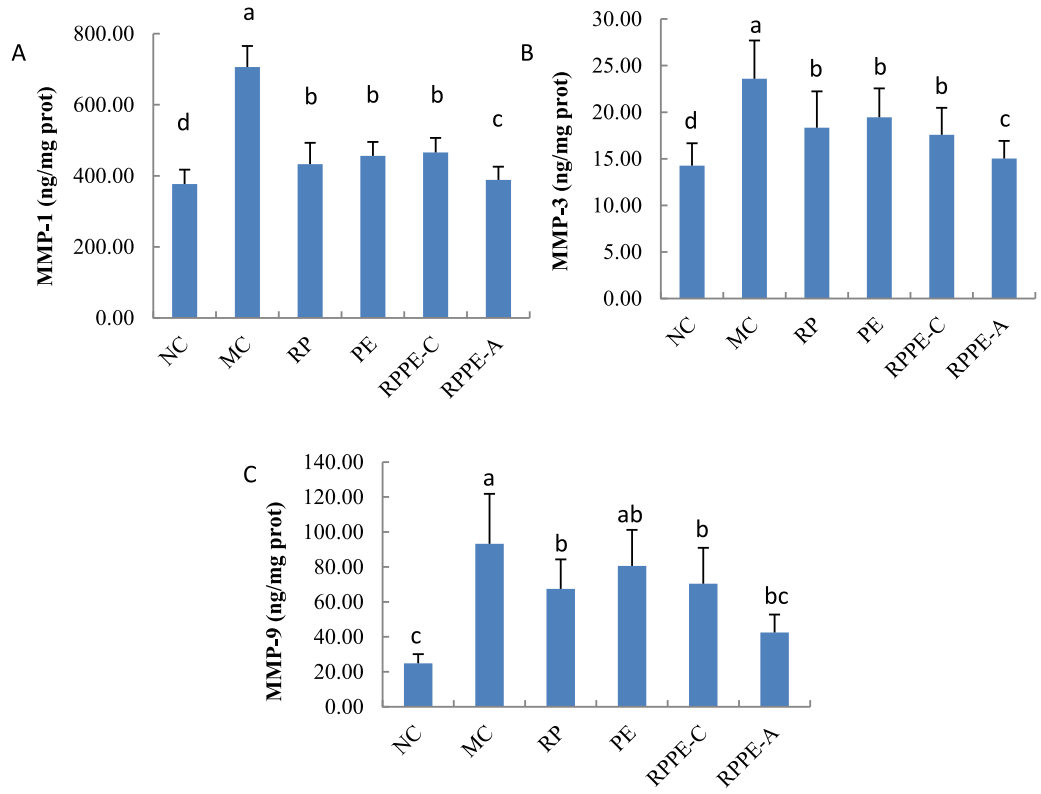

Fig. 5. Effect of RPP and LSGYGP on MMPs contents of photoaging skin. A: MMP-1 content; B: MMP-3 content; C: MMP-9 content. Data were shown as mean ± standard deviation. Different lowercase letters indicated significant differences ($p < 0.05$).

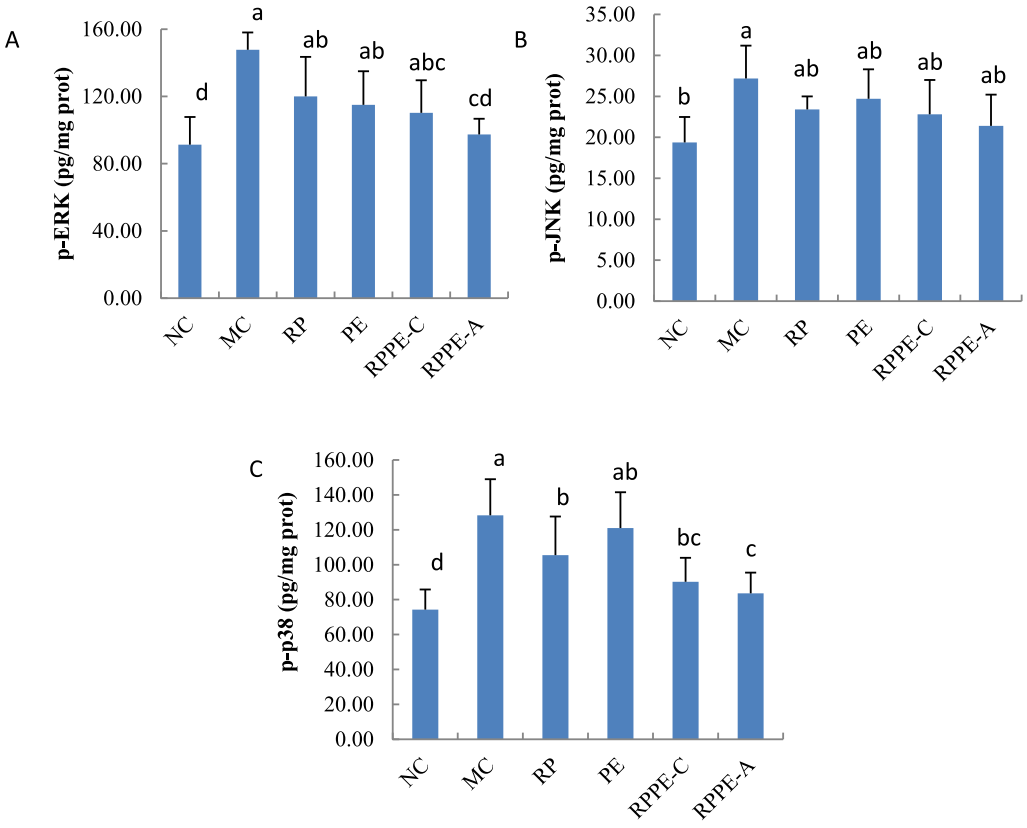

Fig. 6. Effect of RPP and LSGYGP on MAPKs pathway of photoaging skin. A: *p*-ERK content; B: *p*-JNK content; C: *p*-p38 content. Data were shown as mean ± standard deviation. Different lowercase letters indicated significant differences ($p < 0.05$).

anti-inflammatory activity, inhibitory AAPH-induced DNA damage, and regulatory oxidative stress in H_2O_2-induced HepG2 cells *in vitro* (Li et al., 2018; Zhuang et al., 2017b). Some studies indicated that gelatin peptide can induce the increase in fibroblast density and enhance the formation of collagen fibrils in the dermis (Lu et al., 2017; Nguyen et al., 2013). The LSGYGP identified from tilapia fish skin gelatin hydrolysates has high scavenging hydroxyl radical activity and shows protective effect on UVB-induced MEFs *in vitro*. Consistent with these previous reports, we concluded that the RPP and LSGYGP may have an antiphotoaging effect *in vivo* by regulating oxidant stress, decreasing inflammatory reaction, and contributing to the collagen fibers. To confirm the effectivity of RPP and LSGYGP *in vivo* and *in vitro*, we designed the experiment by using UV-induced hairless mice skin photoaging *in vivo*. We also demonstrated and evaluated the additive effect of RPP and LSGYGP on photoaging in UV-induced hairless mice.

Chronic exposure to UV irradiation induces alterations in skin compositions (Liu et al., 2018). Collagen and HA are two key compositions in skin that play important parts in the function of skin (Song et al., 2017). Collagen degradation and damage are major characteristics of photoaging (Xu and Fisher, 2005). HYP is a special amino acid in collagen. Hence, the HYP content is often used as an indicator of the skin collagen content. HA also has an important effect on skin water content because of its good capability to bind water. Meanwhile, HA can support the expansion structure of skin, which contributes to the diffusion of nutrients and metabolites in skin (Xu and Fisher, 2005). In the present study, UV irradiation significantly reduced the HYP and HA contents ($p < 0.05$). RPP and LSGYGP can increase the HYP and HA contents. For the protection of skin collagen and HA contents, the effect of LSGYGP was higher than that of RPP. RPP had minimal effect on the skin collagen in UV-induced mice. This result indicated that the protective effect of LSGYGP on skin collagen secretion was not due to an antioxidative effect, and LSGYGP can increase collagen synthesis and secretion. This result was similar to previous study (Fujii et al., 2013). In addition, many studies indicated that gelatin hydrolysate has been used in the synthesis of the extracellular matrix (Lu et al., 2017; Sun et al., 2018). Zhang et al. (2017) studied that collagen peptides from the skin of silver carp induce a significant increase in skin HA, but tea polyphenols show a minimal effect on HA content despite its high antioxidant activities. These results are similar to those in our study. For HA content, the LSGYGP value was higher than RPP. A previous study proposed that gelatin peptides can be considered as biological messengers, which can stimulate the dermal fibroblasts and increase the HA biosynthesis and content by the underlying cell signaling pathway (Zague, 2008). Pro-HYP from the collagen-derived dipeptide can stimulate the HA synthase gene expression of dermal fibroblasts (Haratake et al., 2015). These results are similar to those of our study.

Oxidative stress is a key factor in the process of UV-induced skin photoaging (Hseu et al., 2012). Approximately half of UV-induced skin damages are from ROS production (Rabe et al., 2006). The antioxidant defense systems, including enzymatic (e.g., SOD, CAT, and GSH-Px) and nonenzymatic molecules (e.g., GSH) are present in the skin (Song et al., 2017). In the present study, the results indicated that the UV irradiation decreased the SOD, CAT, and GSH-Px activities, which decreased the ROS scavenging ability and increased the oxidative damages. Meanwhile, MDA is the typical product of lipid peroxidation process. Hence, MDA is often used to evaluate the degree of lipid peroxidation (Sun et al., 2013). UV irradiation induced the increase in MDA content in the skin (Sun et al., 2013). In this study, RPP and LSGYGP can effectively alleviate UV-induced oxidative stress *in vivo* because of their antioxidative properties. Our results showed that the RPPE-A value was higher than those of other sample groups, which indicated the additive effects of RPP and LSGYGP on the antioxidant indicators in UV-induced photoaging skin.

UV-induced ROS further mediates the proinflammatory cytokine

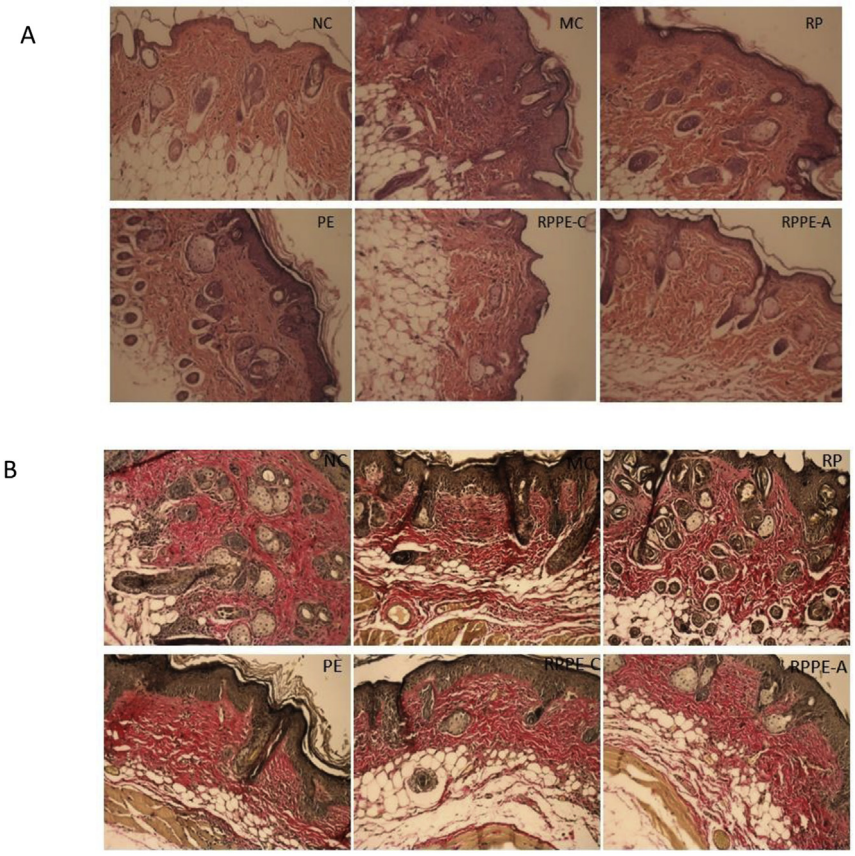

Fig. 7. Effect of RPP and LSGYGP on histological changes of photoaging skin. A: H&E staining images; B: VG staining images.

expression (Gu et al., 2007). Proinflammatory cytokines, including TNF-α, IL-1, and IL-6, play vital actions in UV-induced photoaging mice model (Chen and Hou, 2016). Further UV irradiation destroys the immune system of skin by increasing the proinflammatory cytokine levels (Yang et al., 2019). TNF-α is the primary endogenous mediator of inflammatory reaction. On the one hand, TNF-α causes damage to vascular endothelial cells. On the other hand, TNF-α can trigger alveolar epithelial cells to produce other cytokines, such as IL-6 (Pillai et al., 2005). In this study, TNF-α, IL-1α, and IL-6 levels increased significantly in the MC group. RPP and LSGYGP alleviated UV-induced immune damages and inflammation by decreasing the TNF-α, IL-1, and IL-6 levels. These results proved that the protective effects of RPP and LSGYGP on photoaging skin may be attributed to proinflammatory cytokine inhibition. RPP showed high effect on inflammatory in UV-induced photoaging. According to our previous study, RPP showed good anti-inflammatory activity *in vitro* (Li et al., 2018). These results indicated RPP can produce good activity *in vivo* and *in vitro*. Similar to the antioxidant indicators, the additive effects of RPP and LSGYGP on TNF-α and IL-1α were found, respectively.

MMPs are major collagenolytic enzymes that are responsible for skin collagen degradation (Lu et al., 2016). MMP expression is triggered by UV irradiation in a cytokine-regulated manner. MMPs are classified based on their substrate specificity. MMP-1, -3, and -9 belong to collagenases, stromelysins, and gelatinases, respectively (Kaehaeri and Saarialho-Kere, 1997). In this study, UV irradiation induced an abnormal increase in MMP-1, MMP-3, and MMP-9 secretion. Our results showed that RPP and LSGYGP decreased the up-regulated MMP levels in UV irradiation. The protective effects of RPP and LSGYGP on the MMP contents may be contributed to their antioxidant and anti-inflammatory activities *in vivo*. Our results also indicated that RPP and LSGYGP had good additive effects on MMP secretion in UV-induced photoaging skin because RPPE-A showed higher effect than other experiment groups.

Previous studies showed that oxidative stress and cytokine production in photoaging skin are mainly formed by UV-induced MAPK signaling pathway activation (Chiang et al., 2011; Sun et al., 2016). The activation of MAPKs upregulated MMP expression, and the increased MMP expression induced skin collagen degradation (Chiang et al., 2011). Three distinct MAPK signal pathways, including ERK, JNK, and p38, were reported by previous studies (Kang et al., 2003). Our results indicated that UV irradiation significantly increased ERK, JNK, and p38 phosphorylation. RPP and LSGYGP can exert considerable effects on the UV-induced pathologic alteration. RPP showed higher effect than LSGYGP, and RPP and LSGYGP had an additive effect on ERK and p38 phosphorylation. Chen et al. (2016a) studied the effect of gelatin peptides on skin photoaging by regulating MAPK signaling pathways, and gelatin peptides significantly decrease the UV-induced ERK and p38 phosphorylation. These results are similar to the findings of our study.

The skin has two primary layers in skin, namely, epidermis and dermis. Epidermis which is involved in the creation of a highly effective physical barrier, is the key tissue to protect skin from environmental effect (D'Orazio et al., 2013). Collagen is the major constituent part of the dermis, thereby accounting for 75% of the dry weight (Oikarinen, 1994). Histological analysis indicated that the thickness of epidermis and the changes in connective tissue were the common characteristics in UV-induced photoaging skin. The epidermis thickens to protect the skin from UV irradiation, which can be used as an adaptive response to UV irradiation (Jung et al., 1997). After treatments with RPP and LSGYGP, the thickened epidermis was effectively recovered, and RPPE-A group showed insignificant difference with the NC group ($p > 0.05$). As shown in Fig. 7B, compared with the NC group, UV irradiation induced loose, fractured, and irregular distribution of skin collagen fibers and decreased the collagen content in the MC group. LSGYGP can regulate the structure and distribution of collagen fibers of UV-induced

photoaging skin and inhibit the decrease in collagen content. However, RPP did not recover a visible change in skin dermal collagen. This result was accordance with the change in the HYP contents in the skin (Fig. 1). Fujii et al. (2013) reported that collagen peptides and amla extract exhibit an additive effect on UVB-induced photoaging in hairless mice. However, collagen peptides enhance the production of skin collagen *in vivo*, but amla extract did not. These results were similar to those of our study.

Generally, both RPP and LSGYGP can be used as important anti-photoaging agents, and the mechanisms were partly evaluated. The protective effects of RPPE-C group on skin photoaging had no significant differences with the higher activities between RP group and PE group ($p > 0.05$). The numerical values in RPPE-A group were higher than those of RPPE-C. Many biochemical indices in RPPE-A group had no significant differences with those of NC group ($p > 0.05$), including HYP content, SOD and CAT activities, GSH and MDA contents, IL-1α and IL-6 levels, MMP-9 level and the phosphorylation of ERK and JNK. Therefore, our result showed RPP and LSGYGP had an additive effect on the UV-induced mice skin photoaging. Further studies, which focus mainly on the underlying mechanism of the additive effects of RPP and LSGYGP on the inhibitory photoaging *in vivo*, should be performed.

5. Conclusion

Previous studies showed RPP and LSGYGP have good bioactivities *in vitro*, especially antioxidant activities, anti-inflammatory, and inhibitory MMP levels. Considering these bioactivities, we further evaluated the *in vivo* effects of RPP and LSGYGP on photoaging skin induced by UV irradiation in the study. The additive effects of RPP and LSGYGP were further analyzed. Our results provide a basis for the preventive effects of RPP and LSGYGP on photoaging *in vivo* and suggested that these agents are useful against UV-induced photoaging when taken orally in combination with RPP and LSGYGP.

Conflict of interest

The authors declare no conflicts of interest.

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