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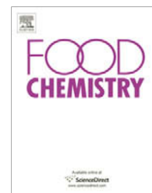

The effect of pacific cod (*Gadus macrocephalus*) skin gelatin polypeptides on UV radiation-induced skin photoaging in ICR mice

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ABSTRACT

Gelatin was extracted from Pacific cod (*Gadus macrocephalus*) skin and hydrolysed sequentially with pepsin and alkaline protease. The hydrolysates were fractionated into two ranges of molecular weight (PEP1: 2000 Da < Mr < 6000 Da; PEP2: Mr < 2000 Da) using ultrafiltration membranes. In this present study, we investigate the protective effects of both polypeptides against ultraviolet radiation-induced skin photoaging by the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and the contents of glutathione (GSH), malondialdehyde (MDA), hydroxyproline (HYP) in photoaging skin tissue. The arrangement of collagen fibres in skin tissue was examined by Van Gienson stain. UV radiation-induced decrease in the antioxidase activity and depletion of reduced glutathione (20.4%) in the skin of hairless mice in a model group. Compared with the model, both polypeptides can enhance the activities of SOD, GSH-Px, CAT and the contents of GSH and HYP, and reduce the content of MDA, which minimised the skin photo damage. Moreover, the results of histology study confirmed that both polypeptides could protect collagen fibres in skin.

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1. Introduction

The development speed of the fisheries industry has been very fast in recent years. Fish offal, a non-edible part produced in large quantities by the fisheries industry, represents a significant source of waste or pollution. Cod is one of the important fish-catches of the world and its meat is used entirely for cod fillet production. But the cod skin is largely underutilised and discarded as waste, which leads to environmental pollution. Recent study shows that fish skin provides the best source of gelatin because of its high availability, reducing pollution, no risk of disease transmission, no religious barriers and possibility of higher yields of collagen (Senaratne, Park, & Kim, 2006).

Solar UVB (280–320 nm) and UVA (320–400 nm) radiations are known to elicit cutaneous damage (Deeba, Farrukh, & Hasan, 2007). Globally, the incidence of all types of skin disease induced by solar ultraviolet radiation has increased over the past several decades. Reactive oxygen species (ROS), such as H_2O_2, superoxide anion and singlet oxygen, which are induced by UV radiation, are thought to be involved in cancer, aging and various inflammatory disorders (Steenvoorden & Beijersbergen van Henegouwen, 1997).

The human antioxidant defence system is equipped with enzymatic scavengers, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) hydrophilic scavengers, e.g. glutathione and lipophilic radical-scavengers, such as tocopherols (Ratnam, Ankola, Bhardwaj, Sahana, & Ravi Kumar, 2006). It was found that the activities of SOD, CAT and GSH-Px were damaged after exposure to UVA, UVB or combinations, which result in superoxide anion-scavenging activity decrease and oxidative damage to the cell (Steenvoorden & Beijersbergen van Henegouwen, 1997; Iizawa, Kato, Tagami, Akamatsu, & Niwa, 1994). It would therefore be beneficial if antioxidase activity could be maintained or increased.

Antioxidant activity exists in gelatin hydrolysates from cobia skin (Yang, Ho, Chu, & Chow, 2008), Alaska Pollock skin (Kim et al., 2001), Jumbo flying squid skin (Lin & Li, 2006), hoki fish skin (Mendis, Rajapakse, & Kim, 2005) and Jumbo squid skin (Mendis, Rajapakse, Byun, & Kim, 2005). It was also reported that collagen polypeptides from *Apostichopus japonicus* (Wang et al., 2008) and peptides from *Chlamys farreri* (Yu, Li, Liu, & Wang, 2004) showed protective effects against ultraviolet radiation-induced skin photoaging.

However, there were no previous investigations of the protective effects of peptides from Pacific cod skin gelatin against ultraviolet radiation-induced skin photodamage. The skin of humans exposed to sun damage resembled that of hairless mice subjected to UV irradiation, including hypertrophy of the epidermis, an

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increase in the number of elastic fibres, and disordered collagen fibres in the dermis (Isoda, Ueda, Imayama, & Tsukahara, 2001). Thus, it is feasible to estimate the effects of our samples using ultraviolet radiation-induced skin photoaging. In this work, we investigate the changes of the antioxidase activity in skin tissue and the arrangement of collagen fibres.

2. Materials and methods

2.1. Materials

Pacific codfish skins were provided by Qingdao Fusheng Food Co. Ltd. (Shandong, China). These skins were taken to the laboratory and stored at −20 °C until used.

ICR male mice were obtained from Vital River Lab Animal Technology Co. Ltd. (Beijing, China). After acclimatisation for one week, they were subjected to the following experiments. All experimental procedures were carried out in accordance with standard guidelines for the care of animals and were approved by the Welfare Committee of the Centre of Experimental Animal, Qingdao, China.

2.2. Preparation of polypeptide

The frozen codfish skins were thawed to 10 °C in a cool room, cut into pieces (10 × 2 cm) and then washed under running tap water. The skin pieces were treated with 0.05 N NaOH to remove non-collagenous proteins for 40 min at a solid to solution ratio of 1:6 (w/v) and washed with distilled water until the pH became neutral. The cleaned skins were further soaked in 0.2% (w/w) H_2SO_4 for 40 min at a solid to solution ratio of 1:6 (w/v) and rinsed with distilled water until neutral pH was reached. After the above pretreatment, the swollen fish skins were homogenised in a high-speed tissue homogeniser (Cany Precision Instrument Co. Ltd., Shanghai, China) and extracted with distilled water (1:8, w/v) for 24 h at 45 °C with continuous stirring. Then the mixture was centrifuged at 4000*g* for 30 min to remove insoluble material (Lin & Li, 2006; Grossman & Bergman, 1992). The supernatants were concentrated at 37 °C using a vacuum evaporator and lyophilised. The freeze-dried product, gelatin, was stored at −20 °C.

To gain polypeptides, pepsin and alkaline protease were used. One gramme of freeze-dried gelatin was added to pepsin and alkaline protease (hydrolysate: enzyme = 100:1,w/w) in 100 ml of 0.1 M sodium phosphate buffer (pH 7.0). The hydrolysis was performed at 45 °C for 2 h in a 50 rpm shaking water-bath incubator, and it was ended by heating the mixtures at 100 °C for 5 min to inactivate the protease activity. The solution was centrifuged at 8000g for 10 min at 4 °C. Then the supernatants were subjected to ultrafiltration using 6 and 2 kDa molecular weight cut-off membranes (Shanghai Institute of Applied Physics, Chinese Academy of Sciences, China) to obtain PEP1 (2 kDa < Mr < 6 kDa) and PEP2 (Mr < 2 kDa). Both of them were freeze-dried and used for experiments.

2.3. Determination of the molecular weight distribution

Molecular weight distributions of PEP1 and PEP2 were determined by gel permeation chromatography on a TSK gel 3000 PWXL column (Tosoh, Tokyo, Japan), using a high-performance liquid chromatography system (Agilent 1100, USA). The mobile phase used was 50% acetonitrile (v/v) in the presence of 0.1% trifluoroacetic acid. The samples were eluted at a flow rate of 0.5 ml/min and monitored at 225 nm at 25 °C. A molecular weight calibration curve was prepared from the average retention times of the following standards: cytochrome *C* (12,500 Da), insulin (5734 Da), vitamin B12 (1355 Da), hippuryl-histydyl-leucine (429.5 Da), and glutathione (307.33 Da) (Sigma, USA).

2.4. Amino acid composition

Polypeptide samples were hydrolysed with 6 M HCl at 110 °C for 24 h, and the hydrolysates were analysed on a Hitachi 835-50 amino acid analyser (Hitachi, Tokyo, Japan).

2.5. Experimental groups

Male ICR mice were randomly divided into the following six groups (10 mice in each group): group a, normal group; group b, model group; group c, at dose of 50 mg kg^{-1} d^{-1} bw PEP1 group; group d, 200 mg kg^{-1} d^{-1} bw PEP1 group; group e, at dose of 50 mg kg^{-1} d^{-1} bw PEP2 group; group f, 200 mg kg^{-1} d^{-1} bw PEP2 group. Animals in the normal group and model group were given normal saline at the same volume. All animals were maintained on a 12 h light/dark cycle and fed a standard rodent chow diet. All mice, except the normal group, were irradiated with the same UV source.

2.6. UV irradiation

Two 40 W UVA tubes (wavelength range: 320–400 nm, peak wavelength: 365 nm) and one 40 W UVB tube (wavelength range: 290–320 nm, peak wavelength: 297 nm) constituted the UV source. The distance from the lamps to the animals' backs was 30 cm. The light intensity was measured with a UVA-radiometer and a UVB-radiometer (Photoeletric Instrument Factory of Beijing Normal University, China).

Before UV irradiation, normal saline and polypeptides were preventively irrigated into the mice's stomachs for two weeks. One day before the UV radiation, the mouse back was depilated with 8% sodium sulphide over the depilation area of 9 cm^2. Initially, we measured the minimal erythema dose (MED) on mouse dorsal skin. Mice were irradiated three times weekly (Monday, Wednesday and Friday). Then, intensities of UV were increased by 1 MED per week until week 6, and then maintained at 4 MED up to the 10th week.

2.7. Van Gieson stain

Skin specimens were then taken for histochemical investigation 24 h after the final irradiation. Mouse skin samples were fixed in 4% buffered neutral formalin solution for 24 h, and embedded in paraffin. Serial sections (7 μm) were mounted onto silane-coated slides and visualised with Van Gieson stain (Montes, 1996). Examination was done by light microscopy at 200× magnifications.

2.8. Antioxidant activity of polypeptide in vivo

For antioxidant activity assays, the skin samples were homogenised (1:9 w/v) in ice-cold normal saline. Homogenates were centrifuged at 8000*g* for 15 min (4 °C) and the supernatants were collected and stored at −20 °C.

The activities of SOD, CAT and GPH-Px, and the contents of GSH and MDA in the back skin tissue homogenate were examined by reagent kit (NanJing JianCheng Bio Inst, Nanjing, China). The principles of these kits are briefly as follows.

SOD activity was determined using the xanthine oxidase method, based on its ability to inhibit the oxidation of hydroxylamine by the xanthine–xanthine oxidase system. CAT activity was measured according to the ammonium molybdate spectrophotometric method, based on the fact that ammonium molybdate could rapidly terminate the H_2O_2 degradation reaction catalysed by CAT and react with the residual H_2O_2 to generate a yellow complex which could be monitored by the absorbance at 405 nm. GSH-Px activity was measured by quantifying the rate of H_2O_2-induced oxidation of

GSH to oxidised glutathione (GSSG), catalysed by GPH-Px. MDA was determined by the thiobarbituric acid (TBA) method, based on its reaction with TBA to form thiobarbituric acid-reactive substances (TBARS). The GSH content in the supernatant was measured by reaction with dithionitrobenzoic acid (DTNB) and monitored by absorbance at 412 nm.

The total protein content in the supernatant of homogenate extracts was determined according to the Bradford method, using bovine serum albumin as a standard (Bradford, 1976).

2.9. Hydroxyproline

The hydroxyproline content was determined after hydrolysis of the sample in 6 M HCl for 4 h at 130 °C, using the colourimetric method recommended by ISO 3496(E).

2.10. Statistical analysis

The spss 11.5 statistical software programme was used for analyses of variance. The hypothesis testing method included one-way analysis of variance (ANOVA), followed by least significant difference (LSD) test. $P < 0.05$ and $P < 0.01$ indicated statistical significance. All the results were expressed as means ± standard deviation for 10 mice in each group.

3. Results

3.1. Determination of the molecular weight distribution

The gel permeation chromatography technique was applied to analyse molecular weight distribution of the sample. There was a linear relationship between the retention time and the logarithm of the molecular weight in the range of 100,000–100 Da ($r^2 = 0.9945$) and the formula for calculation was as follows: $\lg M = -0.2721t + 7.7887$. The molecular weight distributions of PEP1 and PEP2 are presented in Fig. 1. The results showed that there were two main fractions in PEP1 with molecular weights of 6700 Da (54.2% of total protein) and 3000 Da (44.5% of total protein), respectively. The molecular weight of the main peak of PEP2 was 1450 Da and its relative proportion was more than 90%.

3.2. Amino acid composition

The main amino acids in collagen are glycine, proline, hydroxyproline and alanine, with a small number of residues of phenylalanine and tyrosine. The data in Table 1 showed that the amino acid compositions of PEP1 and PEP2 were similar to that of cod skin gelatin. PEP1 and PEP2 had high contents of glycine, glutamic acid, arginine, hydroxyproline and proline residues and small amounts

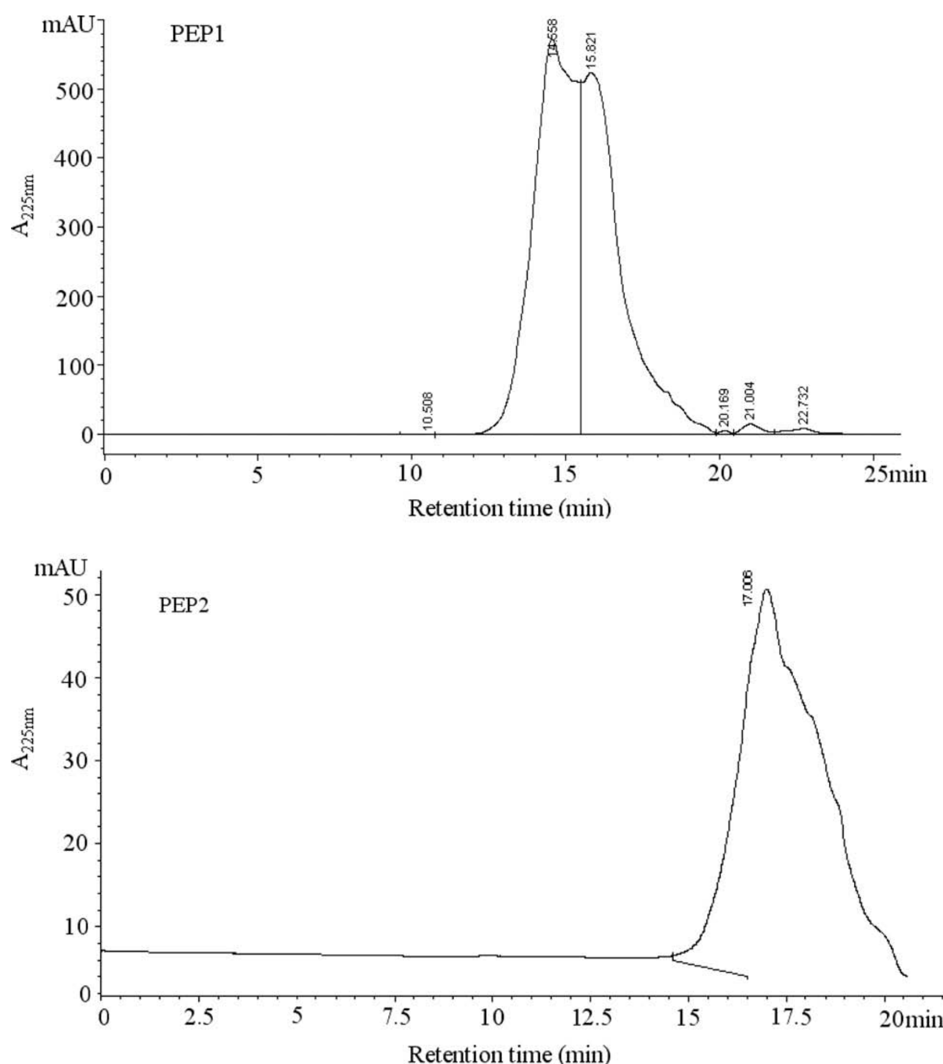

Fig. 1. Molecular weight distribution profiles of PEP1 (2000 < Mr < 6000 Da) and PEP2 (Mr < 2000 Da) using a high-performance liquid chromatography system analysis. (Column: TSK gel 3000 PWXL; flow rate: 0.5 ml/min; elution buffer: 50% acetonitrile (v/v) in the presence of 0.1% trifluoroacetic acid.)

Table 1
Amino acid composition of gelatin and polypeptides (g/100 g).

Amino acid	Gelatin	PEP1	PEP2
Aspartic acid	5.37	6.85	8.00
Threonine	2.09	2.64	2.59
Serine	4.47	5.55	5.26
Glutamic acid	8.72	10.4	11.6
Glycine	31.0	21.3	20.4
Alanine	7.75	9.37	8.22
Cystine	0.83	0.79	1.13
Valine	2.14	2.49	2.50
Methionine	2.58	2.50	2.57
Isoleucine	1.06	1.13	1.11
Leucine	1.96	2.17	1.92
Tyrosine	0.48	0.47	0.72
Phenylalanine	1.56	1.77	1.63
Lysine	2.58	3.21	3.06
Histidine	0.90	1.06	0.72
Arginine	6.88	7.77	6.09
Proline	12.7	15.4	14.7
tryptophan	0	0	0
Hydroxyproline	6.92	5.08	7.75
Total	100	100	100

of tyrosine, cysteine, histidine and methionine residues. Tryptophan residues were absent in all samples. Amino acids are the building blocks of proteins and they can promote collagen synthesis.

3.3. In vivo antioxidant activity

As shown in Table 2, chronic exposure of hairless mice to UVA and UVB irradiation for a period of several weeks, led to a significant decrease of SOD, GSH-Px and CAT activities. Compared with the normal group, the activities of SOD, GSH-Px and CAT in the model group, decreased by 15.5%, 31.5%, 31.4%, respectively.

Superoxide dismutase (SOD), an intracellular antioxidant enzyme which exists to combat oxidative stress, catalyses the conversion of superoxide to H_2O_2. It would therefore be beneficial if SOD activity could be maintained or increased. Our results show that there was a significant increase in the SOD activity in skin tissues-administered with PEP1 or PEP2.

The Se-dependent enzyme, glutathione peroxidase (GSH-Px), is active against H_2O_2 and lipid peroxides by catalysing the reactions of GSH. Catalase (CAT) is another enzyme that scavenges H_2O_2 in the skin, which dismutates H_2O_2 into water and O_2, and is a constituent of peroxisomes.

As shown in Table 2, PEP1 or PEP2 administration greatly elevated CAT and GSH-Px activities in the skin ($P < 0.05$, $P < 0.01$, respectively). Both treatments, at a dose of 200 mg/kg, had the maximal increase in total antioxidant capacity in the skin. The maximal activity of all antioxidant enzymes in the skin was achieved by the administration of PEP1 at a dose of 200 mg/kg. PEP1 treatment, at a dose of 200 mg/kg, maximally increased the SOD, GSH-Px, and CAT activities in the skin and, compared with model group, the activities increased by 21.0%, 41.6% and 57.9%, respectively.

3.4. Content of malondialdehyde and glutathione in skin tissue

The most widely used index of lipid peroxidation is malondialdehyde (MDA) formation, often assayed with the thiobarbituric acid (TBA) assay. The results clearly demonstrate that UVA and UVB radiation resulted in an increase of the MDA formation of lipid peroxidation products (Table 3). Glutathione is considered as a free radical-scavenger or as a cofactor for protective enzymes. UVA- and UVB-induced peroxidation is associated with the loss of intracellular GSH. As shown in Table 3, UV irradiation caused a significant ($P < 0.01$) decrease (20.4%) in the levels of GSH when compared with the normal group. PEP1 or PEP2 treatment at low dose (50 mg/kg) decreased the MDA levels in the skin ($P<0.05$), and had no influence on the GSH level in the skin ($P > 0.05$). PEP1 or PEP2 treatment, at doses of 200 mg/kg, significantly inhibited the formation of MDA and increased GSH level in the skin tissue ($P < 0.01$). Therefore, peptide-pretreated groups showed significant increases in levels of GSH and decreases in levels of MDA when compared with corresponding UV-exposed groups (model group).

3.5. Hydroxyproline content

The content of collagen was estimated by hydroxyproline (the hydroxylated form of the collagen-specific amino acid proline). Our results demonstrated that the content of hydroxyproline in the mice skin was significantly reduced ($P < 0.01$) after exposure to UVA and UVB. This may be the reason for wrinkle formation in photoaged skin. As shown in Table 4, the administration of PEP1, at 50 and 200 mg/kg, and PEP2, at 200 mg/kg, resulted in hydroxyproline in the skin tissue being significantly increased ($P < 0.05$ and $P < 0.01$), while there was no significant difference in the content of hydroxyproline between the PEP2 (50 mg/kg) treatment group and the model group ($P > 0.05$).

This increase may be due to the antioxidant effect and amino acid compositions of PEP1 and PEP2. In view of the present results, it can be concluded that PEP1 and PEP2 possess protective effects against ultraviolet damage in mice skin tissues and the effect of PEP1 is better.

Table 2
Effect of polypeptides on antioxidant enzymes in the skin tissue homogenate of the mice's backs.

Group	Dosage (mg/kg)	CAT (U[a]/mg protein)	SOD (U[b]/mg protein)	GSH-Px (U[c]/mg protein)
Normal	–	4.23 ± 0.71	23.2 ± 1.99	34.7 ± 7.13
Model	–	2.90 ± 0.87***	19.6 ± 3.51***	23.8 ± 3.74***
PEP1	50	3.92 ± 0.47**	23.0 ± 1.41*	29.2 ± 6.27
	200	4.58 ± 0.46**	23.7 ± 1.01**	33.6 ± 4.07**
PEP2	50	3.84 ± 0.47**	22.4 ± 2.01*	28.4 ± 4.16
	200	4.07 ± 0.57**	23.1 ± 3.44**	30.4 ± 3.64*

All the results were expressed as means ± standard deviation for 10 mice in each group.
[a] One unit of catalase activity was defined as the amount of enzyme that reduces 1 μmol of H_2O_2 per second under defined conditions.
[b] One unit of SOD activity was defined as the amount of the enzyme inhibiting the oxidation by 50%.
[c] One unit of glutathione peroxidase is defined as the amount of the enzyme leading 1 μmol GSH oxidised per min.
* $P < 0.05$ vs. model group.
** $P < 0.01$ vs. model group
*** $P < 0.01$ vs. normal group.

Table 3
Effect of polypeptides on glutathione (GSH) and malondialdehyde (MDA) in the skin tissue homogenate of the mice's backs.

Group	Dosage (mg/kg)	GSH[a] (mg/g protein)	MDA[b] (nmol/mg protein)
Normal	–	74.8 ± 4.07	5.12 ± 0.59
Model	–	59.5 ± 3.12***	6.96 ± 1.44***
PEP1	50	62.4 ± 2.71	5.43 ± 1.45*
	200	65.7 ± 4.34**	5.31 ± 0.36**
PEP2	50	62.8 ± 5.21	5.94 ± 0.89*
	200	65.0 ± 3.94**	5.14 ± 1.20**

All the results were expressed as means ± standard deviation for 10 mice in each group.
[a] GSH content was expressed as mg per g protein.
[b] MDA values were expressed as nmol per mg protein.
* $P < 0.05$ vs. model group.
** $P < 0.01$ vs. model group.
*** $P < 0.01$ vs. normal group.

Table 4
Effect of polypeptides on hydroxyproline (HYP) in the skin tissue homogenate of the mice's backs.

Group	Dosage (mg/kg)	HYP[a] (mg/g skin)
Normal	–	1.07 ± 0.14
Model	–	0.74 ± 0.063***
PEP1	50	0.87 ± 0.10*
	200	0.94 ± 0.13**
PEP2	50	0.85 ± 0.13
	200	0.91 ± 0.071*

All the results were expressed as means ± standard deviation for 10 mice in each group.
[a] HYP content was expressed as mg per g skin.
* $P < 0.05$ vs. model group.
** $P < 0.01$ vs. model group.
*** $P < 0.01$ vs. normal group.

3.6. Histological changes of collagen

The Van Gieson stain was obtained to determine the distribution of collagen, which appeared as a red deposit. In the normal group, the collagen fibre bundle architecture has a thick parallel arrays beneath an epidermal layer (Fig. 2a), as described previously (Iocono, Ehrlich, Keefer, & Krummel, 1998). UV irradiation resulted in collagen reduction when compared to the normal group (Fig. 2a and b). PEP1 or PEP2 treatment groups displayed an increased collagen deposition which was evident from increase in the hydroxyproline of the skin tissue (Table 4).

In the present study, PEP1 or PEP2, at the doses of 50 or 200 mg/kg, which were useful for enhancing the activity of antioxidant enzymes, led to a protective effect of the collagen fibres below the epidermis. There was no significant difference between the PEP1 (200 mg/kg) treatment group and the normal group.

4. Discussion

The detrimental effects of UV radiation on the skin were associated with the generation of reactive oxygen species (ROS), such as superoxide anion radical ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$), and singlet oxygen ($^1O_2$) (Yasui & Sakurai, 2000). Reactive oxygen species (ROS), induced by UVA and UVB irradiation, played a substantial role in collagen oxidation and degradation (Wlaschek et al., 2001). To protect against oxidative damage, skin cells have evolved a complex antioxidant defence system which includes nonenzymatic antioxidants such as glutathione (Yapar, Kart, Karapehlivan, & Citil, 2007) and several enzymatic antioxidants, such as superoxide dismutase (SOD), catalase(CAT) and glutathione peroxidase (GPH-Px) (Hou, Guo, Li, & Wang, 1996; Zhang et al., 2008). However, enhanced oxidative stress, induced by UV radiation, is accompanied by decreases in activities of SOD, CAT and GPH-Px (Steenvoorden & Beijersbergen van Henegouwen, 1997; Shindo, Witt, Han, & Packer, 1994). The present investigation clearly shows that there was a significant decrease in the SOD, CAT and GSH-Px activities in skin tissues exposed to UVA and UVB irradiation.

Glutathione is considered to be free radical-scavenger or a cofactor for protective enzymes, which plays a pivotal role in the cellular defence against oxidative damage (Moysan et al., 1993; Demir et al., 2003). Repetitive UVA irradiation, with short intervals,

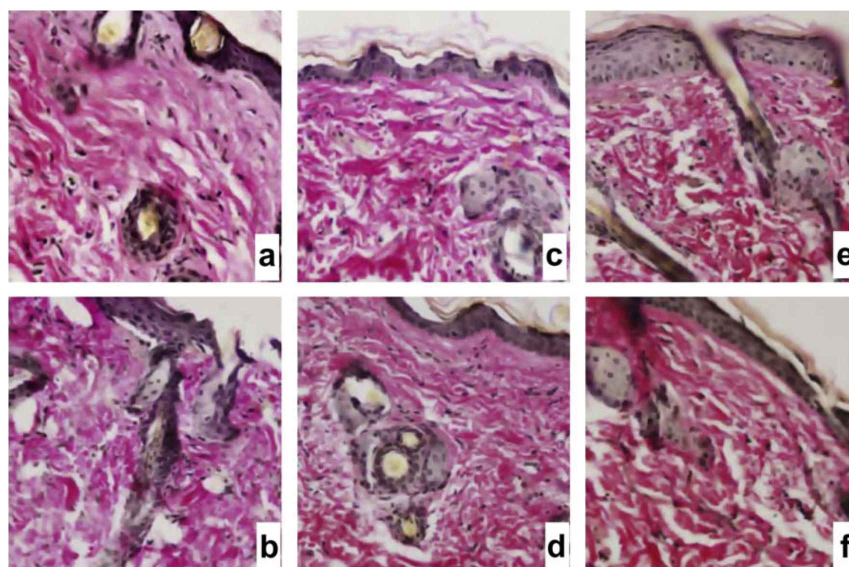

Fig. 2. Mouse skin samples fixed in 4% buffered neutral formalin solution for 24 h, and embedded in paraffin. A representative cross section of the skin from all groups visualised with Van Gieson stain as viewed with light microscopy (original magnification ×200). (a) Normal group; (b) model group; (c) PEP1treatment group (50 mg/kg); (d) PEP1 treatment group (200 mg/kg); (e) PEP1 treatment group (50 mg/kg) and (f) PEP1 treatment group (200 mg/kg); all mice, except the normal group were irradiated with the same UV source. Collagen fibres are identified by the red colour. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

leads to a lower GSH level due to leakage and oxidation of GSH (Merwald et al., 2005). GSH depletion of cultured human skin cells makes them sensitive to UV-induced mutations and cell death (Prasad, Ramachandran, Pugalendi, & Menon, 2007).

The most widely used index of lipid peroxidation is malondialdehyde (MDA) formation. The increase of MDA production indicates that peroxidative damage increases with the photoaging process. Our results clearly demonstrate that both peptides give a decrease of the MDA formation of lipid peroxidation products by UV irradiation.

A topical polypeptide (PCF), isolated from *C. farreri*, provided a photoprotective effect against chronic skin damage induced by ultraviolet radiation (He, Kutala, Kuppusamy, & Zweier, 2004; Wang et al., 2002). Our results indicated that both polypeptides (PEP1 and PEP2) could greatly enhance the level of GSH and markedly promote SOD, GSH-Px and CAT, while the amounts of MDA and ROS were decreased. In view of the present results, it can be concluded that PEP1 and PEP2 showed reducing effects against oxidative damage in skin tissues and protected the skin against UV-induced damage.

On the other hand, the protective effect on the skin tissue may be related to the amino composition of polypeptide. Five (or four) specific amino acids, namely, L-arginine and/or L-glutamine, as well as L-valine, L-isoleucine and L-leucine, at a specific ratio, are useful for inhibiting skin aging and healing wounds, and are associated with collagen synthesis (Murakami, Hitoshi, Kobayashi & Hisamine, 2006; Williams, Abumrad, & Barbul, 2002). The amino compositions of PEP1 and PEP2 are similar to what Murakami had reported. This may be the reason why the PEP1 or PEP2 could enhance the collagen in skin tissue and obviously reduce the wrinkles of skins. Therefore it is useful for protecting mice skin against ultraviolet radiation-induced skin photoaging.

5. Conclusions

In the present study, PEP1 or PEP2, at doses of 50 or 200 mg/kg, which were useful for enhancing the activities of antioxidant enzymes, led to a protective effect of the collagen fibres below the epidermis. Although ultimate outcome of this cannot yet be completely assessed, the evidence indicates that the effects of PEP1 or PEP2 against the UV-induced skin damage were reasonably good during the study, and the effect of PEP1 was better.

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