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Effect of polysaccharides from *Tremella fuciformis* on UV-induced photoaging

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ABSTRACT

Tremella fuciformis Berk, a popular nutritious mushroom in China, has attracted increasing attention due to its various bioactivities. In the present work, *T. fuciformis* polysaccharides (TP) were extracted with hot water. It mainly consisted of mannose, as well as 10.77% (w/w) uronic acid. In additional, the anti-photoaging effects of TP were evaluated using a 30-day UV-irradiated animal assay. TP could efficiently reduce the water and collagen losses of the skin, and inhibit the increase of glycosaminoglycans. Moreover, a histopathological study showed that, UV-induced skin structural alterations were alleviated as well as repairing endogenous collagen breakdown and maintaining the ratio of type I/III collagen, after oral treatment of TP. The activities of SOD, GSH-Px and CAT were increased compared to the irradiated control group without treatment. Accordingly, TP can be used as a potential functional food supplement for skin function protection.

1. Introduction

Human skin with its three distinct skin layers (epidermis, dermis, and hypodermis), is an effective physical and chemical barrier against the detrimental effects of harmful components, including ultraviolet (UV) radiation (Hou et al., 2012). Recent studies indicated that overexposure to UV radiation, particularly to UVB (290–320 nm), is the main cause of skin photo-damage, which is characterised by wrinkling, scaling, dryness, irregular pigmentation, poor elasticity and glossiness (Fisher et al., 1997; Ichihashi et al., 2003). Chronic exposure to UV radiation affects cellular chromatophores and photosensitisers, which could induce the generation of reactive oxygen species (ROS) (Fan, Zhuang, & Li, 2013; Hou et al., 2012). Overproduction of ROS in the epidermis destroys the steady-state balance of antioxidant defence systems causing oxidative stress leading to DNA damage, activation of signalling pathways related to cell (or tissue) growth, differentiation, senescence, and connective tissue alterations (Lim et al., 2014). These reports indicated that bioactive compounds with antioxidant activity might prevent skin photoaging through reduction of ROS induced by UV irradiation.

It's worth noting that the use of skin care products for maintenance of healthy skin is on an increase in recent years (Lim

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et al., 2014). Moreover, research has focused on the development of antioxidant materials for the prevention of skin photoaging and for the maintenance of healthy skin. Various pharmacological studies have shown that some dietary supplements have potential chemoprophylactic activities, such as green tea (Katiyar, Elmets, & Katiyar, 2007), ferulic acid (Prasad, Ramachandran, Pugalendi, & Menon, 2007), collagen polypeptide (Fan et al., 2013; Hou et al., 2012; Zhuang, Hou, Zhao, Zhang, & Li, 2009), soy phosphatidylserine (Choi et al., 2013), and so on. Antioxidants have been shown to be effective against UV irradiation, thus maintaining skin heath. Polysaccharides in plants, epiphytes and animals are a potential source of bioactive compounds. Researches have focused on those material which show strong biological activities, including antioxidant, anticancer, anti-inflammatory, and immunostimulating activities (Du et al., 2014; Wen et al., 2011; You et al., 2013; Zhu et al., 2013). Previous reports indicated that polysaccharides could attenuate the risk of skin damage induced by UV radiation using cell models (Ku, Lee, Moon, & Lee, 2008; Moon et al., 2008, 2009).

The white jelly mushroom *Tremella fuciformis*, belonging to the *Tremellaceae* family of the class Heterobasidiomycetes, also called 'snow fungus' or 'silver ear', is mainly distributed in subtropical regions, especially in China (Chang, 1998). The fruiting body of *T. fuciformis Berk* is a popular nutritious food and a traditional Chinese medicine used as a tonic for weakness and aging (Xu et al., 2012). As a type of mushroom, polysaccharides are one of the main bioactive components of *T. fuciformis*. The literature indicated that *T. fuciformis* polysaccharides (TP) had an antioxidant activity by scavenging superoxide anion and hydroxyl radicals (Zhang, Wang, Zhao, & Qi, 2014). Other pharmacological activities observed include antitumor, antidiabetic, anti-inflammatory, and immunomodulating activities (Bin, 2010; Cho et al., 2007; Jiang et al., 2012; Kiho, Morimoto, Sakushima, Usui, & Ukai, 1995; Shi et al., 2014; Wasser, 2002). Lai, He, Zhao, and Dong (2010) found that TP has an excellent moisturising effect as well as anti-wrinkle effect in topical applications. These effects were mediated by improving skin texture, lowering skin harshness and increasing skin flexibility. Additionally, a type of water-soluble homogeneous polysaccharide from *T. fuciformis* (WTF-B) protected the haematopoiesis system, stimulated stem cell proliferation, and prevented radiation induced genotoxicity. It could be used as a potential radioprotector (Xu et al., 2012).

It's clear that skin photoaging is currently perceived as a major problem for consumers based on their expenditures for such products (Katiyar et al., 2007; Pandel, Poljsak, Godic, & Dahmane, 2013). Thus, the development of effective preventive agents and strategies on the reduction or control of UV-induced skin damage is desired by consumers. Additionally, strategies that include dietary sources of antioxidants have been shown to help prevent skin photoaging (Yoon et al., 2014). However, in the previous studies, TP were applied by painting on the surface of the skin or were used in some skin protection products. Does TP have a skin protective effect after oral consumption as a potential source of antioxidants? How does consumption affect the skin photoaging system? The effect of oral consumption of TP on skin photoaging induced by UV radiation has never been studied until now. Therefore the purpose of the present work was to study oral treatment with TP in Sprague-Dawley (SD) rats and begin to determine the possible mechanism(s) for any positive effect of the oral treatment.

2. Materials and methods

2.1. Chemicals and reagents

Standard samples of xylose (Xyl), arabinose (Ara), glucose (Glc), galactose (Gal), fructose (Fru), mannose (Man), fucose (Fuc), and glucuronic acid (GlcA), and dextrans (Mw 5200~6,100,000) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Commercial kits used for determination of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and hydroxyproline were purchased from Jiancheng Institute of Biotechnology (Nanjing, Jiangsu, China), and a rat glycosaminoglycan (GAG) assay kit was obtained from Shanghai Yueyan Biological Technology Co., Ltd (Shanghai, China). Absolute alcohol, hydrochloric acid, potassium bromide (KBr), xylene, phenol and sulphuric acid were obtained from Guangzhou Reagent Co. (Guangzhou, Guangdong, China). All other chemicals used were of analytical grade (Guangzhou Reagent Co.).

The dry fruiting body of *T fuciformis Berk* was obtained from Infinitus Co., Ltd. in Guangzhou (China), and ground into fine powder using a laboratory mill (FW100, Taisite Instrument Co., Ltd., Tianjin, China). The materials were stored at room temperature in a desiccator until use, which was a maximum of 10 weeks.

Sprague-Dawley (SD) female rats (180–220 g), aged 6~7 weeks, were obtained from the Guangdong Medical Laboratory Animal Center (Guangdong, China). The rats were housed in a temperature-controlled animal room (21–26 °C, and relative humidity of 40-70%), with free access to water and food, which was prepared by the Guangdong Medical Laboratory Animal Center, containing 60% corn and bran mixture (1:1), 20% protein, 5% fat, and 4% coarse fibre. All experimental procedures were carried out in accordance with standard guidelines for the care of animals that were approved by Welfare Committee of the Centre of Experimental Animal, Guangdong, China.

2.2. Preparation of polysaccharides

Dried *T. fuciformis* fruiting body powder was refluxed with 95% ethanol at 70 °C in a water bath for 2 h to remove ethanol-soluble substances. Then the mixture was filtered through Whatman No. 1 filter paper (Hangzhou, China), and the residue was dried at 45 °C for 12 h. These dried residues were extracted three times with 50 volumes (v/w) of boiling water for 4 h each time. The extract was centrifuged at 3000 *g* for 15 min at 4 °C (Allegra X-15R, Beckman Coulter Co., Ltd., Brea, California, USA), then filtered through Whatman No. 1 filter paper. The supernatants were pooled and concentrated in a rotary evaporator (Hei-VAP, Heidolph, Germany) at 60 °C under reduced pressure using a vacuum pump (SHZ-D, Yuhua Instrument Co., Ltd., Gongyi, China). Four volumes of absolute alcohol were added. The mixture was kept overnight at 4 °C, and the precipitate was obtained after centrifugation (3000 *g*, 20 min, 4 °C). The polysaccharides TP were got after drying by freeze drying

(ALPHA, 1-2 LD plus, Marin Christ Co., Osterode, Germany) and stored at room temperature in a desiccator until use, which was a maximum of 2 months. The total polysaccharide content of TP used for further analysis was 89.62%, calculating as glucose equivalent, on dry weight basis. And its protein content was 1.32%.

2.3. Chemical composition of TP

Total sugar content was determined using phenolic-sulfuric acid method using D-glucose as the standard (Dubois, Gillis, Hamilton, Rebers, & Smith, 1956). Briefly, 1.0 mL polysaccharides solution was mixed with 1.0 mL of phenol solution (5%), then 5.0 mL of concentrated H_2SO_4 was added and mixed thoroughly, and determined absorbance at 490 nm after 30 min. Uronic acid was measured according to the previously described method using D-glucuronic acid as the standard (Filisetti-Cozzi & Carpita, 1991). Firstly, 40 μL of 4 M sulfamic acid-potassium sulfamate (pH 1.6) was added to 0.4 mL of sample solution, mixed thoroughly, followed by adding 2.4 mL of concentrated H_2SO_4, which contained 75 mM sodium tetraborate. Then the mixture was heated for 20 min in a boiling water bath with the tubes capped after vortex mixing. After cooling in an ice bath, 80 μL of 0.15% (w/v) m-hydroxydiphenyl in 0.5% (w/v) NaOH was added and then stirred in vigorously by vortex mixing. The absorbance was measured at 525 nm after the pink colour developed to completion in about 5 min.

Other compositional properties of TP, including neutral monosaccharide composition and molecular weight were determined as described previously with some modification (Wen et al., 2011). Briefly, 10 mg of polysaccharides sample was hydrolysed using 10 mL of 2 M trifluoroacetic acid at 100 °C for 2 h. After removing the regent at 60 °C under reduced pressure, derivatisation of the released monosaccharides was then carried out using the trimethylsilylation reagents as follows: the hydrolysed TP mixture was dissolved in 2 mL of pyridine, and then 0.4 mL of hexamethyldisilazane, and 0.2 mL of trimethylchlorosilane were added and kept at room temperature for 5 min. The supernatant (4000 *g*, 10 min, 4 °C) of trimethylsilylated derivatives (1 μL) was loaded onto a GC-2010 gas chromatography system (Shimadzu, Tokyo, Japan), which was equipped with a RTX-5 capillary column (30 m, 0.32 mm ID, 0.25 um, Shimadzu, Kyoto, Japan) and a flame ionisation detector. The analysis was done as follows: the initial column temperature was kept at 130 °C for 1 min, and programmed from 130 to 180 at 2 °C /min, holding for 3 min at 180 °C, then increasing to 220 at 10 °C/min, and finally holding for 3 min at 220 °C; and the injection temperature as well as the detector temperature were 230 °C. The model of splitless injection was adopted. Based on the comparison of the retention time of sample with standard substance, the monosaccharide composition of TP was identified. Additionally, inositol was used as the internal standard to quantify the monosaccharide content with an internal standard method.

High-performance gel permeation chromatography (HP-GPC) was used for the determination of the TP molecular weight distribution. A Waters HPLC apparatus (Waters 1525, Waters Co. Ltd., Milford, MA, USA), a TSK-GEL Guard Column (PWXL 6.0 × 40 mm), TSKGEL4000K gel column (PWXL 7.8 × 300 mm) and TSK-GEL5000K gel column (PWXL 7.8 × 300 mm) (TOSOH Co. Ltd., Yamaguchi, Japan), and a Waters 2414 Refractive Index Detector were used. The molecular weight of TP is based on the assumption that the elution volume is proportional to the molecular weight.

The infrared spectrum of TP was done using a Fourier transform infrared (FT-IR) spectrophotometer (Bruker, Ettlingen, Germany) equipped with OPU3.1 software. A sample (2 mg) was ground with KBr power (200 mg) and pressed into pellets for 16 scans transformation infrared spectra determination over a frequency range of 4000–400 cm^{-1}.

2.4. Animal treatment and UV irradiation

Female SD rats were fed *ad libitum*, before the study, standard diets (regular food and water *ad libitum*) for one week for adaptation prior to any active ultraviolet treatment. They were then randomly divided into the following 5 groups (12 rats in each group): NC (control group: no irradiation, negative control); PC (irradiated control group with no treatment, positive control); TP-1 (low dose TP: at a dose of 100 mg/kg/day by oral intake using lavage needle); TP-2 (medium dose TP: 200 mg/kg/day); TP-3 (high dose TP: 300 mg/kg/day). Animals in the NC and PC groups received the same volume of distilled water. All rats were allowed free access to the regular diet and water during the experimental period.

To generate UV radiation, a skin phototoxicity assay detector (HOPE-MED 8130B, Tianjin Development Zone Hepu Industry & Trade Co., Ltd., Tianjin, China) was used as a UV source with UVA (peak wavelength: 365 nm) and UVB (peak wavelength: 297 nm). Animals, except those assigned to the NC group were exposed to UV light 3 times weekly (Monday, Wednesday, and Friday) on their back for 4 weeks while in their regular cages at 2016 mJ/cm^2 UVA and 82 mJ/cm^2 UVB as the minimal erythemal dose (MED) (Zhuang et al., 2009) during the 1st week, 2 MED (4032 mJ/cm^2 UVA and 164 mJ/cm^2 UVB) during the 2nd week, 3 MED during the 3rd week, and 4 MED during the 4th week, yielding a total dose of 60.5 J/cm^2 and 2.46 J/cm^2 of UVA and UVB, respectively. Before each UV radiation session, each rat's back (including the NC) was denuded using sulfureted sodium (8%, Jinshan Co.Ltd., Chengdu, China) over the depilation area of 5 cm × 5 cm, and the distance from the lamps to the rat's back was approximately 30 cm.

2.5. Observation of the clinical signs and body weight

Animals were observed once a day throughout the stabilisation and experimental periods to note any visible clinical signs or death. The body weight of animals was measured once before the treatment began and once a week during the study period to determine any major changes in health, as well as to observe the radiated skin area to measure any visible skin damage.

2.6. Antioxidant activity in serum

At the end of the experimental period, animals were deprived of food for 12 h at staggered times but allowed free access to water. Blood was collected from the ventral abdominal artery and put into centrifuge tubes after the animal was anaesthetic with 50 mg/kg body weight of sodium phenobarbital through intraperitoneal injection. After centrifugation at 8000 *g*

for 15 min at 4 °C (Avanti J-E, Beckman Coulter Co., Ltd.), the serum was frozen and stored at –80 °C until analysis within 2 months. The activities of SOD, GSH-Px, CAT in the serum were determined using the respective diagnostic kits. A microplate reader (Spectra Max 190, Molecular Devices, USA) was used to measure the respective absorbance of samples and standards as provided in the kits and following the instructions provided by the manufacturer of the kits. No independent tests were done to validate the results using the test kits.

2.7. Measurement of water content

The water content of the skin was determined as previously described by Hou et al. (2012). Briefly, the dorsal skin (1 cm^2) was collected immediately after the animal was sacrificed at the end of the experiment, and the wet weight was measured, followed by drying in an oven at 105 °C for 4 h before determining its dry weight. The water content of the skin was evaluated as the percentage of weight loss of dry sample, and calculated as:

$$\text{water content}\ (\%) = 100 \times (W_0 - W_4)/W_4$$

where W_0 was the weight of skin before drying and W_4 was the weight of skin after drying for 4 h.

2.8. Measurement of total glycosaminoglycan content

The total glycosaminoglycan (tGAG) content was measured by Azure I analysis as previously described (Hou et al., 2012; Oh et al., 2011). Briefly, the dorsal skin samples were cut into small pieces with a scissor, and were degreased with chloroform:methanol (2:1) to remove subcutaneous fat. Dry skin tissue (30 mg) was powdered thoroughly with a bullet blender (Blue5, Next Advance Co., Averill Park, NY, USA) for 2 min, treated overnight with 0.1 M NaOH, and then neutralised with HCl using a pH meter (FE20, FiveEasy, Mettler-Toledo International Inc., Columbus, Ohio, USA). Before adjusting the pH to 7.4, the mixtures were heated at 100 °C for 5 min to inactivate microorganisms. After subjecting the skin sequentially to alkaline proteinase (2%, w/w) and trypsinase (3%, w/w) digestions at 50 °C for 12 h, respectively, an equal volume of 0.2 M of sodium phosphate buffer (pH 7.4), containing 6.4 mg papain, 18 mg EDTA and 8 mg cysteine was added and incubated at 50 °C for another 24 h. The reaction was terminated by heating the mixtures to 100 °C for 5 min. Finally, TCA (15%, w/v) was added and the mixtures were centrifuged at 3000 *g* for 15 min. The supernatants were used for determining GAG: tGAG content in the samples was measured according to the manufacturer's protocol using the rat glycosaminoglycan (GAG) assay kit with a microplate reader at 450 nm. And the amounts of tGAG was expressed in terms of the dermatan sulphate-B (by dry powder weight) used as a standard.

2.9. Measurement of total collagen content

Previous studies have indicated that hydroxyproline is almost exclusive to collagen and accounts for about 13.4% of collagen in dermis (Kong et al., 2013; Lin et al., 2014). Therefore, hydroxyproline content can be used to obtain an estimate of the total collagen by multiplication using the reciprocal factor 7.46.

The extraction process of hydroxyproline was conducted as described previously (Zhuang et al., 2009). Briefly, the dorsal skins (30 mg) were hydrolysed with 6 M HCl at 110 °C for 24 h. After centrifugation at 3000 *g* for 15 min, the supernatants were immediately collected for later determination of the hydroxyproline content according to the manufacturer's protocol using a commercial hydroxyproline kit and the microplate reader at 550 nm. The amounts of hydroxyproline were expressed in terms of the hydroxyproline standard provided by the manufacturer.

2.10. Histological analysis

A dorsal skin sample (about 1 × 1 cm) from each sacrificed animal at week 4 was fixed in 4% neutral formalin solution for 24 h, followed by embedding in paraffin. Serial sections (4 μm) were mounted on silane-coated slides, and the tissues were observed after Haematoxylin & Eosin (H&E) and Masson staining (Hou et al., 2012). Images were obtained and recorded using an Olympus BX41 microscope and Image-Pro Express 5.1.1.14 Pathology Image Analysis System (Olympus Corporation, Tokyo, Japan) at 100 × magnifications.

The thickness of the epidermis and dermis, and the counts of karyocyte were measured at 10 randomly selected locations per slide while under the microscope. Histological alterations were evaluated and quantified using the image analysis program.

Immunohistochemistry for type I and III collagen was done using strept avidin-biotin complex (SABC) with the specimens removed. Antibodies for collagen I and III were purchased from Abcam Chemical Company (Cambridge, MA, USA). Kits and other reagents were obtained from Boster Biological Technology Co., Ltd. (Wuhan, China). Briefly, serial sections were firstly dewaxed and hydrated with xylene, anhydrous alcohol, 95% of alcohol, and PBS sequentially. Then 3% H_2O_2 was used to block the endogenous peroxidase, and the tissue was washed with PBS 3 times for 3 min each time. After that, the tissue was immersed in 0.01 M of sodium citrate buffer solution (pH 6.0) and boiled for 15 min for antigen retrieval, followed by washing with PBS for 3 times. After adding 5% bovine serum albumin blocking solution at room temperature for 15 min, an appropriate volume of diluted primary antibody was added, and the sections were kept overnight at 4 °C before washing with 0.01M PBS (pH 7.2-7.4). After incubation with biotinated secondary antibody at 37 °C for 20 min, a PBS wash was done as described above, followed by incubating with straptived-HRP complex (SABC), including A solution (avidin) and B solution (biotin-HRP) at 37 °C for 20 min, and a PBS wash. Then the tissue section was stained with DAB chromogenic reagent kit, and washed using distilled water. After that, haematoxylin and saturated disodium hydrogen phosphate solution were used for counterstaining and differentiation, respectively. Finally, the tissue section was mounted on silane-coated slide after dehydration with alcohol and xylene, respectively. These were examined under the microscope and images captured at 400× magnifications.

2.11. Statistical analysis

Statistical analyses were performed using SPSS software 19.0 (SPSS Inc., Chicago, IL, USA). Results were subjected to ANOVA, and differences between means were located using Tukey's multiple comparison test and $p < 0.05$ were regarded as significant.

3. Results and discussion

3.1. Structure characteristic of TP

The recovery of polysaccharides from the fruiting body of *T. fuciformis* was about 28.9%, of the starting dry weight. As shown in Table 1, the content of total sugar and uronic acid were 89.6 and 10.8%, respectively, of the crude polysaccharides.

The neutral monosaccharide composition of TP showed five neutral monosaccharides, including fucose, xylose, mannose, galactose, and glucose, whose molar ratio was 0.85:1.00:1.59:0.01:0.30 (Table 1). Additionally, the molecular weight of TP was 3430 KDa in the present study.

The FT-IR spectrum of TP is seen Fig. 1a. It showed the typical peaks of polysaccharides. The peaks at 3440, 2930, and 1050 cm^{-1} correspond to stretching vibrations of O-H, C-H, and C-O, respectively. The signal at 1720 cm^{-1} corresponds to the C-O bending vibration of uronic acid. Moreover, signals at 920 and 800 cm^{-1} correspond to the β-linked glycosyl and α-linked glycosyl residues, respectively (You et al., 2013; Zhang, Wang, Han, Zhao, & Yin, 2012).

The results indicated that mannose was the predominant monosaccharide and constructed the backbone for TP, as similar to previous report, which indicated that polysaccharides of tremella compose of a mannan backbone and side chains containing a small percentage of xylosyl, arabinosyl, fucosyl, glucosyl, and glucuronic acid residues. They also pointed out that the backbone of TP consisted of (1→3)-linked β-D-mannopyranoside (Jiang et al., 2012). However, the proposed chemical repeating units of the fruiting body polysaccharides (Fig. 1b) in *T. fuciformis* was observed to be D-glucurono-D-xylo-D-mannan with a backbone composing of (1→3)-α-Man*p*, and β-Xyl*p*-, and β-GlcA*p*- containing side chains (De Baets & Vandamme, 2001). The most important species in *Tremellaceae* family are *T. mesenterica*, *T. fuciformis*, and *T. aurantia*, and their sugar compositions are the same but found in different ratios with relatively high uronic acid in *T. fuciformis* (Khondkar, Aidoo, & Tester, 2002). A large amount of uronic acid may be responsible for the health function of this polysaccharides, owing to polysaccharides with a high content of uronic acid exhibiting relatively stronger biological activities (Chen, Zhang, & Xie, 2004; Wang, Dong, Ma, Cui, & Tong, 2014). The results also showed that glucuronic acid was the major uronic acid in TP.

Table 1 – Yield and chemical composition of TP (% (w/w) of dry weight).

Yield (%)	Total sugar (%)	Uronic acids (%)	MW ($\times 10^6$ Da)	Neutral sugar (mole ratio)				
				Fuc	Xyl	Man	Gal	Glc
28.92	89.62	10.77	3.43	0.85	1	1.59	0.01	0.3

3.2. General observations

No deaths were noted in all five group animals, and their body weights increased during the 4-week experimental period without any significant difference between treatments. While the dorsal skin for the PC showed skin damage, including erythema, drying, and escharosis after UV radiation exposure particularly later in the study; this damage was alleviated with all TP treatments. No skin damage was seen in the NC group. Thus, TP showed a macroscopic skin protective effect against UV radiation.

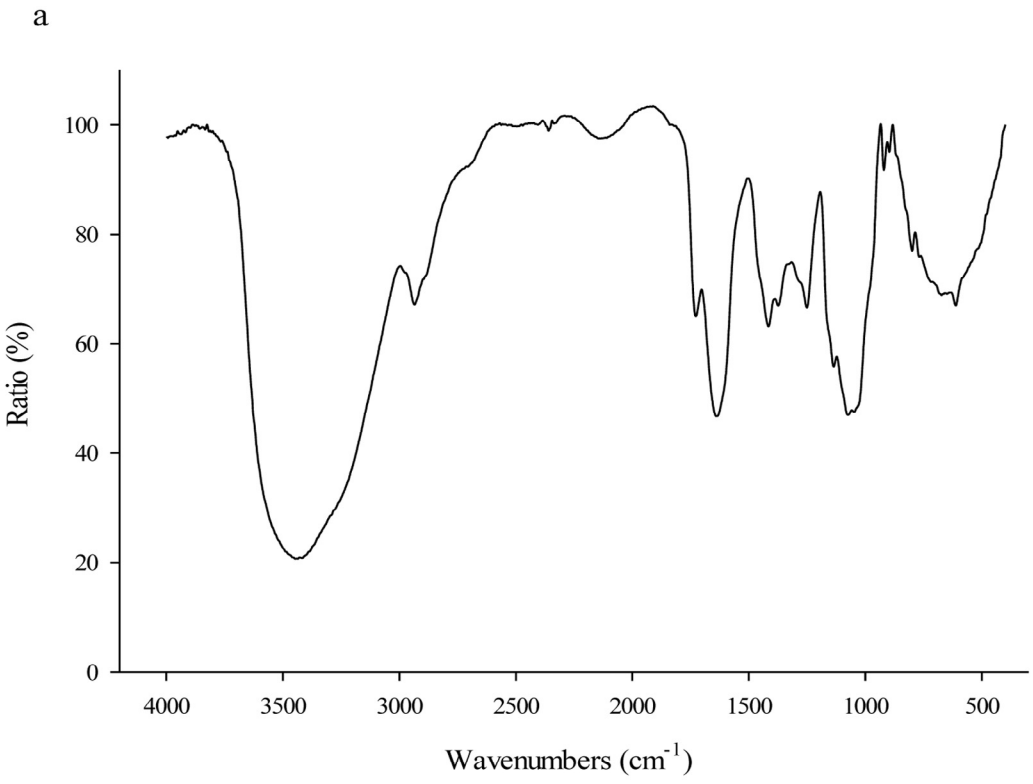
3.3. Effects of TP on histological alterations

Generally, repeat UV irradiation resulted in the skin structure alterations. According to the histological analysis, the NC group showed relatively complete structure without disruptive changes (Fig. 2a). However, the histopathological features of overexposure to UV light was observed in the PC group (Fig. 2b), including changes in the epidermis: like hyperkeratosis and parakeratosis in epidermal tissue, increases of prickle cells, increases in the thickness of the epidermis, and overproduction of kerationocyte; and changes in the dermis: such as disappearance of dermal-epidermal junctions, twisted and disorganised collagen fibres, congestion, haemorrhages and the inflammatory infiltration phenomenon, masses tangled and degraded elastic fibres, and sebaceous hyperplasia, as published previously (Choi et al., 2013; Lin et al., 2014).

The results of H&E staining showed that the skin of animals treated with different doses of TP (TP-1~TP-3) showed sebaceous hyperplasia, but the thickness of the epidermis was significantly reduced, along with haemorrhages and diffuse inflammatory sites were ameliorated. There were also fewer disorganised fibres in the dermis. The histology results suggested that TP may have protective effect against both UV induced epidermal and dermal changes.

The thickness of epidermis and dermis of the PC group increased significantly compared to the NC group ($p < 0.05$, Table 2). In the case of the TP-supplemented groups, the epidermis thickness decreased significantly compared to the PC group ($p < 0.05$). These results showed that TP at all tested doses significantly reduced UV-induced epidermal hyperplasia in a dose-dependent manner. However, no significant difference was found in the dermis thickness between the PC group and the TP-treated groups ($p > 0.05$). In addition, the karyocyte counts of the PC group had about a 4-fold increase compared with the NC group. However, oral administration of TP (TP-2 and TP-3) significantly decreased the numbers of karyocytes compared to the PC group ($p < 0.05$).

The epidermal serves as a barrier to protect the body by synthesis of the protein keratin (Rittie & Fisher, 2002). Its thickness is used as a parameter to directly reflect the protective effect of TP on UV-induced epidermal hyperplasia, which led to skin aging due to skin damage (Gail, 2002). Moreover, a previous report pointed out that an increase in epidermal thickness is responsible for the protection of dermis from damage, which if damaged would lead to formation of wrinkles and loss of elasticity in skin (Yaar & Gilchrest, 2001). Macroscopic alterations, which include increase of skin wrinkles, epidermal hyperplasia, decrease of fragmented collagen fibres and elastic



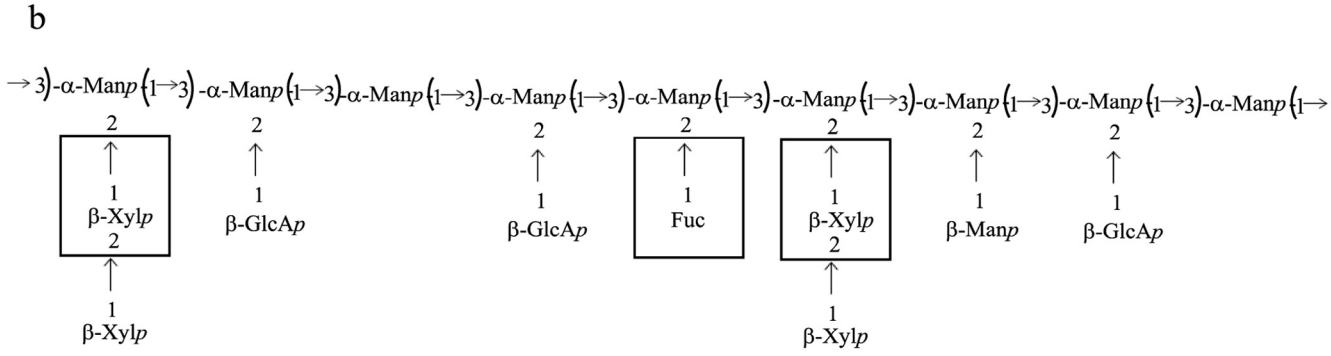


Fig. 1 – Structure characteristics of TP: IR spectra of TP (a), and proposed chemical repeating units of D-glucurono-D-xylo-D-mannan of fruit body polysaccharide from *Tremella fuciformis* (b, De Baets & Vandamme, 2001).

fibres, and tangled and degraded elastic fibres, were ameliorated with supplementation of food nutrients essential for skin metabolism, as described in recent studies (Puglia, Offerta, Saija, Trombetta, & Venera, 2014; Yoon et al., 2014). Moreover, the representative signs of damaged skin recovery involve improvement in transepidermal water loss and skin thickness (Davis, Chen, & Beno, 1996), which were observed in TP-supplemented groups in a dose-dependent manner. This suggests TP have the ability to improve photoaging skin damaged recovery.

3.4. *Effects of TP on skin water content*

The water content of dorsal skin of SD rats was measured at the end of treatment. The results (Table 3) suggested that UV radiation exposure caused water loss, as the skin water content in NC group was significantly high than that of PC group ($p < 0.05$). Additionally, the water content in TP treated groups increased with increased TP. As mentioned before, the water content of the skin is strongly influenced by ground substances, which may be responsible for wrinkle formation in photoaged skin (Jung, Cha, Lee, Chun, & Kim, 1997). It seems that TP showed a protective effect against water loss caused by UV radiation in a dose-dependent manner.

3.5. *Effects of TP total glycosaminoglycan (tGAG) content*

The effect of TP on total glycosaminoglycan (tGAG) content of the tested animal after UV irradiation exposure was studied. As shown in Table 3, a significant increase of tGAG was

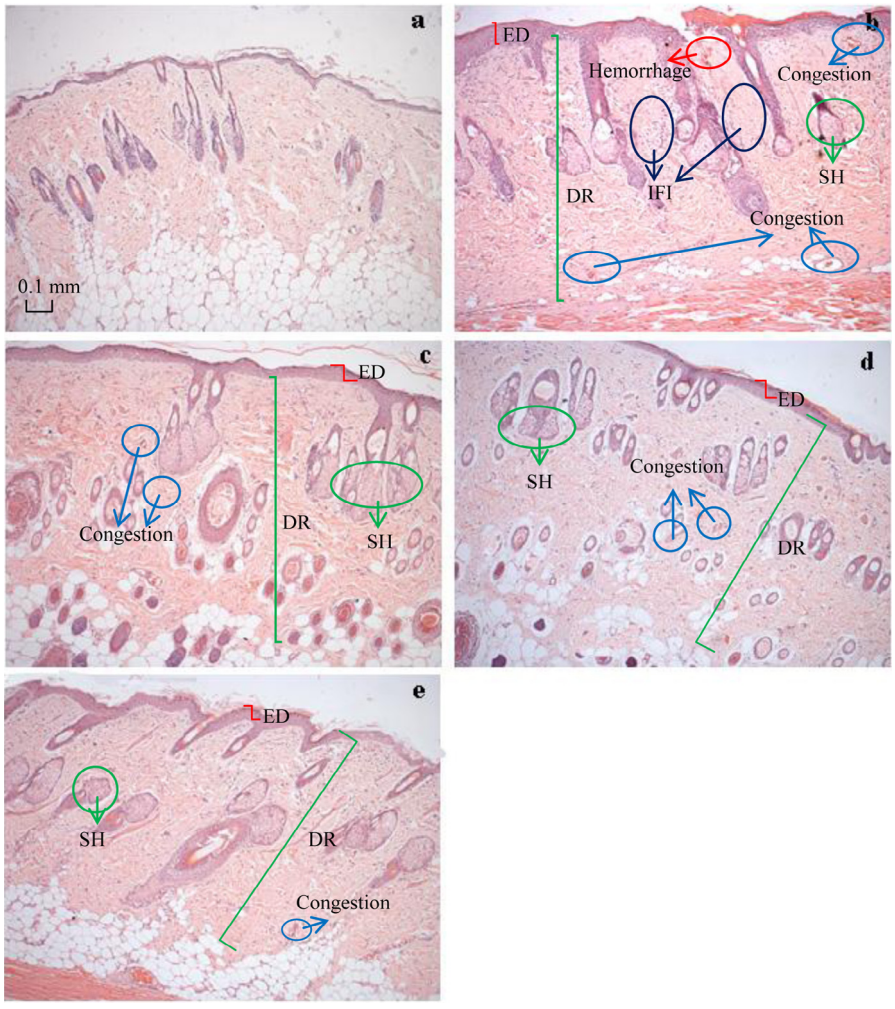


Fig. 2 – Effect of TP on the morphology of photoaging skin (original magnification 100×). (a) NC; (b) PC; (c) TP-1; (d) TP-2; and (e) TP-3. ED, epidermis; DR, dermis; SH, sebaceous hyperplasia; IFI, inflammation infiltration.

observed in rat skins after chronic UV irradiation exposure, whereas there was a significant decrease of tGAG levels in the TP treated groups ($p < 0.05$). GAG, the main component of connective tissue in skin, significantly decreased with aging (Jung et al., 1997). However, chronic UV irradiation induced increased tGAG, according to previous reports (Hou et al., 2012; Zhuang et al., 2009), similar to what was found in this study. Moreover, skin GAG partly affected the characteristic changes in skin during photoaging. The significant increase in GAG induced by UV light may be related to the structure alterations of dermal connective tissue, including damage of collagen, elastic fibres, and the amorphous ground substance of skin (Takahashi, Ishikawa, Okada, Ohnishi, & Miyachi, 1995). The effect of inhibiting the tGAG increase with TP may be associated with protection of collagen synthesis in skin.

Table 2 – Effect of TP on the skin thickness and karyocyte counts of SD rats exposed to UV irradiation at the end of the administration period.

Groups	Epidermis thickness (mm)	Dermis thickness (mm)	Karyocyte counts (unit/0.04 mm^2)
NC	0.020 ± 0.005^a	0.761 ± 0.076^a	34 ± 4^a
PC	0.103 ± 0.018^b	1.093 ± 0.257^b	122 ± 27^b
TP-1	0.073 ± 0.016^c	0.997 ± 0.134^b	91 ± 18^b
TP-2	0.068 ± 0.020^c	0.946 ± 0.131^b	71 ± 13^c
TP-3	0.063 ± 0.019^c	0.935 ± 0.148^b	70 ± 10^c

Values with no letter in common are significantly different ($p < 0.05$).

3.6. *Effects of TP on collagen content*

Collagen was a major component of skin dermis, constituting 75% of the dry weight of the dermis (Hou et al., 2012). There was a significant decrease in the total collagen content between the NC and PC groups ($p < 0.05$), which demonstrated that a reduction of total collagen content was induced by UV irradiation, as seen in most previous studies (Table 3). After treatment with TP (middle and high dose), the collagen content in the UV-induced animal skin was enhanced by 22 and 26%, respectively as compared to the PC group although the low dose did not show any significant increase. The results suggest that TP-supplementation could prevent collagen losses induced by UV irradiation.

Table 3 – Effect of TP on biochemical indices after UV irradiation exposure at the end of the administration period.

Group	Water content (%)	tGAG (μg/mg FW[1])	Total collagen (μg/mg FW)	SOD (U/mL)	GSH-Px (μmol/mL)	CAT (U/mL)
NC	70.26 ± 2.18[a]	18.15 ± 0.98[a]	38.63 ± 5.87[a]	208.1 ± 12.4[a]	1.16 ± 0.08[a]	4.01 ± 0.74[a]
PC	64.65 ± 2.32[b]	27.68 ± 1.24[b]	24.69 ± 3.62[b]	166.4 ± 4.6[b]	0.41 ± 0.09[b]	2.93 ± 0.64[b]
TP-1	66.12 ± 1.60[bc]	24.98 ± 1.22[c]	26.50 ± 5.00[b]	169.4 ± 10.3[b]	0.62 ± 0.09[c]	2.98 ± 0.81[b]
TP-2	67.51 ± 0.94[c]	23.02 ± 1.64[c]	30.18 ± 5.00[c]	178.9 ± 13.8[b]	0.65 ± 0.08[c]	3.18 ± 0.77[ab]
TP-3	68.95 ± 1.98[ac]	20.68 ± 0.67[d]	32.72 ± 5.43[c]	190.9 ± 15.6[c]	0.81 ± 0.08[d]	3.36 ± 0.59[ab]

Values with letter in common are significantly different ($p < 0.05$).
[1] Fresh weight of animal skin.

Skin collagen mostly consisted of type I and III collagen, among which type I collagen was the most abundant extracellular matrix protein in skin (more than 70%), acting as the basis for tissue structure and cellular functions (Wulf, Sandby-Moller, Kobayasi, & Gniadecki, 2004). Previous studies indicated that UV radiation induced a significant decrease of type I collagen but an increase of type III collagen, thereby leading to a significant increase in the ratio of type III collagen to type I collagen (Fan et al., 2013; Hou et al., 2012). Immunohistochemistry assay was used to measure the expression of type I and type III collagen in dermis, and the collagen fibres were stained brown yellow and the intensity of colour reflected the amount of collagen. As shown in Fig. 3a, type I collagen in the NC group was thick and distributed throughout a large part of the slices while a small amount of type III collagen was found. On the contrary, the content of type I collagen obviously decreased, along with increasing type III collagen in the PC group (Fig. 3b). Additionally, the type I collagen was observed to be tangled and cracked after UV irradiation. After treating with TP, especially the higher dose (TP-2 and TP-3), an increase in type I collagen was observed along with a reduction of type III collagen. Thus, TP showed a protective effect for type I collagen, which may be part of the mechanism by which TP protects against photodamaged skin.

Collagen, one of the main components of the extracellular matrix of dermal connective tissue, acts as a structural support system. Preventing the loss of collagenous extracellular matrix helps keep the skin elastic and strong. The degradation of collagen and the accumulation of abnormal elastin in the superficial dermis were considered as the prominent histological features of photoaging skin (Hou et al., 2012). Matrix metalloproteinases (MMPs), which were responsible for the inhibition of synthesis and degradation of the collagenous extracellular matrix in skin, could be induced by activating cellular signalling transduction pathways after UV exposure, consequently causing skin photoaging (Moon et al., 2008). Moreover, collagen synthesis was decreased along with reducing expression of type I collagen. Therefore, UV-induced expression of MMPs, especially MMP-1, MMP-3, and MMP-9, which are the main enzymes responsible for procollagen breakdown in skin, results in activation of pathways that lead to the damage to skin structure, such as increase of type III/type I collagen. Previous reports demonstrated that fucoidan, a functional polysaccharide, showed potential protective effect on photoaging damage by preventing UVB-induced MMP-1 expression and inhibiting down-regulation of type I procollagen synthesis (Moon et al., 2009). This may be one of the possible mechanisms that would explain the current results, and it's worthy to be performed in the future work.

3.7. *Effects of TP on oxidative stress in serum*

One factor underlying the pathology of skin photoaging, including water loss, skin wrinkling, and pigmentation was an increase of ROS caused by oxidative stress (Briganti & Picardo, 2003; Pandel et al., 2013). This suggests that the protective effect of TP against UV may be related to its antioxidant activity. Activities of antioxidant enzymes, including SOD, GSH-Px, and CAT, the common enzymatic antioxidants in antioxidative defence system, were generally considered as parameters to evaluate the antioxidant levels of organism (Lin et al., 2014). As shown in Table 3, the activities of SOD, GSH-Px, and CAT in the PC group were significantly decreased compared with that of NC group ($p < 0.05$). However, TP-3 administration greatly elevated SOD activity in the skin ($p < 0.05$) compared to PC group, although no significant enhancements were found using the low and medium doses (TP-1 and TP-2). Meanwhile TP (at all doses tested) significantly increased GSH-Px activity by 51~98% ($p < 0.05$), along with slight improvements of CAT activity (2~15%). These results suggested that TP could protect the antioxidant enzymes.

It has been reported that approximately 50% of UV-irradiated skin damages, such as erythema, oedema, inflammation, and subsequent pigmentation, were due to overproduction of ROS, which included the superoxide anion radical ($\cdot O_2^-$), the hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2) (Rabe, Mamelak, McElgunn, Morison, & Sauder, 2006; Zhuang et al., 2009). These radicals in excess might cause irreversible damage to biomacromolecules, including DNA, nucleic acid, lipids, and protein, which could further result in skin diseases (Ichihashi et al., 2003; Katiyar et al., 2007). In the present work, the activities of SOD, CAT and GSH-Px, which are considered as important indexes for oxidative stress evaluation, were measured. And the results indicated that increasing ROS induced by UV irradiation were involved in connective tissue alterations, as previously described (Hou et al., 2009; Lin et al., 2014; Pandel et al., 2013). Moreover, TP administration could protect these enzymes. This suggests that TP might be able to scavenge free radicals. *T. fuciformis* polysaccharides were found to scavenge superoxide anion and hydroxyl radicals in a previous report (Bin, 2010). Furthermore, it has been known that UV-induced ROS could aggravate lipid peroxidation in membranes and cellular components, degenerate fibroblasts in dermis, and consequently impair the skin structure leading to formation of wrinkles (Hou et al., 2009; Masaki, 2010). According to the results as mentioned above, the anti-photoaging properties of TP, including reduction of water loss, amelioration of skin structure alternation, and increase of collagen content, may be due to its protective effects on antioxidant enzymes.

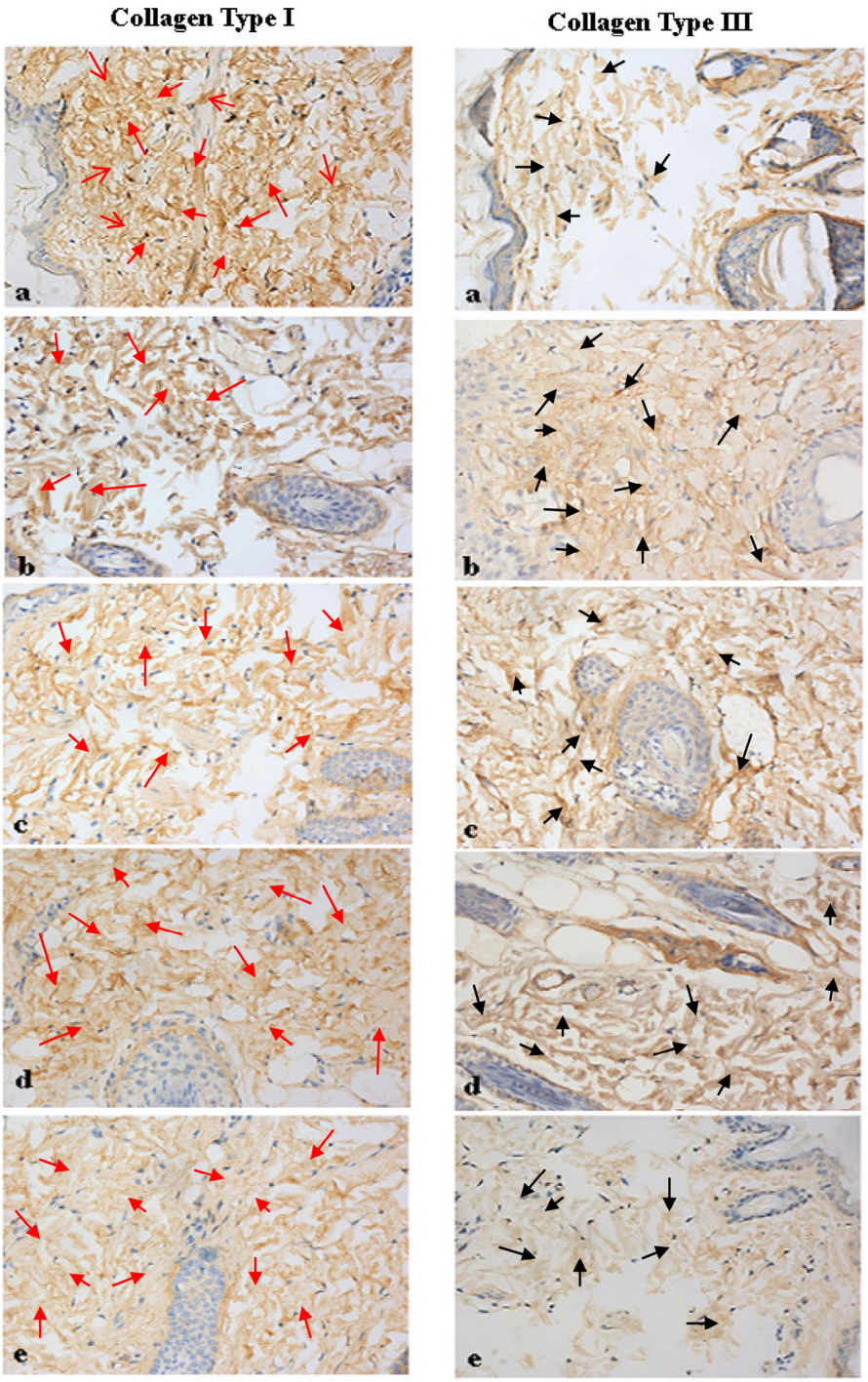


Fig. 3 – Effect of TP on the expression of type I and III collagen after UV irradiation exposure at the end of the administration period (original magnification 400×). (a) NC; (b) PC; (c) TP-1; (d) TP-2; and (e) TP-3. → type I collagen; → type III collagen. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Conclusion

The anti-photoaging properties of TP including reduction of skin water loss, amelioration of skin structure alterations, and an increase of collagen type I content, are presumably due to TP's protective effects on antioxidant enzymes which inhibit lipid peroxidation. Accordingly, TP is a potential therapeutic agent to prevent skin photoaging, and can be utilised as a potential functional food supplement for skin function protection in the future.

Conflict of interest

The authors declared no conflicts interest.

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