Research Article

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Improvement of skin condition by oral administration of collagen hydrolysates in chronologically aged mice

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Abstract

BACKGROUND: Collagen hydrolysates (CHs) have been demonstrated to have positive effects on skin photoaging by topical application or oral ingestion. However, there has been little research on their influence on skin chronological aging. In this study, 9-month-old female ICR mice were given normal AIN-93M diets containing CHs (2.5, 5 and 10% w/w) from Nile tilapia scale.

RESULTS: After 6 months, the collagen content and antioxidant enzyme (superoxide dismutase and glutathione peroxidase) activities increased significantly ($P < 0.05$), while the survival rate, viscera indices and contents of moisture, fat and non-collagenous protein in skin did not change ($P > 0.05$). The color, luster and quantity of hair were obviously ameliorated. Moreover, the structure of epidermis and dermis, the density and distribution of collagen fibers and the ratio of type I to type III collagen were improved in a dose-dependent manner as shown by histochemical staining.

CONCLUSION: Oral ingestion of CHs increased the collagen content and antioxidant enzyme activities and improved the appearance and structure of skin. These results suggest the potential of CHs as an anti-skin-aging ingredient in nutraceuticals or functional foods.

Keywords: collagen hydrolysates; skin condition; chronological aging; oxidative stress; histological structure; visual appearance

INTRODUCTION

Skin is recognized to protect an organism from deleterious environmental effects and maintain the temperature and balance of electrolyte and fluid. It is also a sensory organ, an endocrine organ producing hormones and vitamins and a biofactory for synthesis, processing and metabolism.[1] However, it suffers progressive morphological, structural and functional deterioration with aging, which impacts not only on cosmetic health but also on psychological and physical health.[1,2] Therefore more and more attention is being paid to improving skin condition in view of the world's increasingly aging population.[3]

Skin condition is known to be influenced by genetic and environmental factors such as hormones, ultraviolet (UV) radiation, nutrition, lifestyle, smoking, etc. Oral nutritional supplementation has long been demonstrated to have beneficial effects on skin by providing vitamins, trace minerals, fatty acids and proteins.[4] Moreover, the protective potential of antioxidant intake has been the subject of a number of studies.[4]

In recent years, the oral supplementation of collagen has received increasing interest owning to its positive influence on skin condition. It could reduce wrinkling, hyperplasia and erythema, increase hydration, elasticity and immunity, repair collagen and elastic fibers and inhibit matrix metalloproteinase activities in a UV-induced photoaging mice model.[5–9] It could also enhance fibroblast and collagen fibril density, improve dryness and pruritus and affect collagen homeostasis shown by other animal models.[2,10–12] Additionally, clinical trials and *in vitro* studies demonstrated that it improved the hydration and elasticity of skin and stimulated the proliferation, migration and hyaluronic acid synthesis of dermal fibroblasts.[2,13–17] However, there has been little research on its effects on chronologically aged skin.

Tilapia are the second most important group of farmed fish after carp and are usually processed as fillets accompanied by large quantities of by-products.[18] As an alternative source to land-based animals, collagen is mainly produced from the by-products and widely used in the food, cosmetic and pharmaceutical industries. In this study, the effect of oral ingestion of collagen from Nile tilapia scale on chronologically aged skin was investigated. The results showed its potential as an anti-skin-aging ingredient in nutraceuticals or functional foods.

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Table 1. Formulation (g kg^{-1}) of AIN-93M purified diet for maintenance of rodents

Ingredient	Content
Cornstarch	465.69
Casein	140.00
Dextrinized cornstarch	155.00
Sucrose	100.00
Soybean oil	40.00
Fiber	50.00
Mineral mix	35.00
Vitamin mix	10.00
L-Cystine	1.80
Choline bitartrate	2.50
tert-Butylhydroquinone (mg kg^{-1})	8.00

MATERIALS AND METHODS

Materials

Collagen was prepared from Nile tilapia (*Oreochromis niloticus*) scale using hot water as in our previous report.[19] After hydrolysis with pancreatin (Sigma, St Louis, MO, USA) at 45 °C for 4 h, the collagen hydrolysates (CHs, average molecular weight 530.64 Da) were spray-dried and then mixed with normal AIN-93M purified diet powder (Table 1) at ratios of 2.5, 5 and 10% (w/w).[20] Finally, the mixtures were granulated and stored at −20 °C until use.

Animals

Healthy female ICR mice (38 ± 4 g, 9 months old) were provided by the Laboratory Animal Research Center, Jiangsu University, China. The mice were housed under controlled conditions of temperature (23 ± 2 °C), humidity (55 ± 10%) and light (12/12 h light/darkness, without any UV radiation).

After acclimatization for 7 days, the mice were randomly divided into five groups of 12 animals each: blank control group (BC, normal diet), whey protein control group (WC, 10% whey), low-dose group (LD, 2.5% CHs), medium-dose group (MD, 5% CHs) and high-dose group (HD, 10% CHs). Meanwhile, newly weaned mice ($n = 12$, 21 days old) were used as young control group (YC, normal diet). The mice were housed in groups and allowed access to water and food *ad libitum*. Body weight and food intake were recorded weekly. After 6 months, the mice were sacrificed by cervical dislocation and their viscera and dorsal skin were collected.

Determination of viscera indices

The spleen, liver and thymus were weighed for determination of viscera indices, which were calculated as follows: index (g kg^{-1}) = visceral weight/body weight.

Proximate analysis

The amino acid profile was determined using an amino acid analyzer after hydrolysis under vacuum with 6 mol L^{-1} HCl at 110 °C for 24 h in the presence of 10 mL L^{-1} phenol. The contents of moisture and fat were determined according to AOAC methods 950.46 and 991.36 respectively. The total protein content was the sum of amino acid residues. The collagen content was calculated from the hydroxyproline (Hyp) content using a conversion factor of 7.46.[21] The non-collagenous protein content was the difference between total protein content and collagen content.

Assay of oxidative stress

The skin was homogenized in normal saline and centrifuged at 2000 × *g* for 10 min. The supernatant was collected for the assays of total antioxidant capacity (T-AOC), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) content using kits from Nanjing Jiancheng Bioengineering Institute, China.

Histochemical staining of skin

The skin was fixed in 100 mL L^{-1} neutral-buffered formalin, embedded in paraffin and sectioned at 5 μm. The slices were then subjected to hematoxylin/eosin (HE), Masson's trichrome or Sirius red staining. All stained samples were observed using an ordinary optical or polarized light microscope.

Statistical analysis

Data are presented as mean value ± standard deviation. Statistical analyses were performed using one-way analysis of variance. Multiple comparisons of means were done by Duncan's multiple range

Table 2. Effect of oral administration of CHs on body weight (g), food intake (g kg^{-1} BW day^{-1}) and mortality rate (%) of chronologically aged mice

Parameter	BC	YC	WC	LD	MD	HD
Body weight	39.54 ± 2.34a	33.67 ± 1.52c	34.73 ± 3.25c	39.21 ± 5.32a	38.65 ± 3.81ab	35.25 ± 2.29bc
Food intake	89.24 ± 3.39bc	109.32 ± 6.36a	91.01 ± 6.05b	84.67 ± 3.50c	84.66 ± 2.62c	92.87 ± 2.45b
Mortality rate	25.00	0.00	50.00	25.00	16.67	33.33

Values are mean ± standard deviation. Means followed by different letters in a row are significantly different at $P < 0.05$.

Table 3. Effect of oral administration of CHs on viscera indices (g kg^{-1}) of chronologically aged mice

Parameter	BC	YC	WC	LD	MD	HD
Liver index	3.60 ± 1.05ab	2.91 ± 0.33b	4.22 ± 1.91a	2.62 ± 0.39b	3.20 ± 0.86ab	3.69 ± 0.61ab
Thymus index	39.33 ± 4.57ab	36.98 ± 2.81abc	40.91 ± 0.87ab	34.42 ± 2.16c	36.49 ± 4.39bc	41.12 ± 3.80a
Spleen index	3.20 ± 0.40b	4.14 ± 0.49a	3.04 ± 0.77b	3.04 ± 0.35b	3.19 ± 0.73b	3.56 ± 0.60ab

Values are mean ± standard deviation. Means followed by different letters in a row are significantly different at $P < 0.05$.

Table 4. Effect of oral administration of CHs on thickness (mm) and main components (g kg^{-1} wet weight) of chronologically aged skin

Parameter	BC	YC	WC	LD	MD	HD
Thickness	0.89 ± 0.08a	0.69 ± 0.12b	0.75 ± 0.05b	0.71 ± 0.06b	0.70 ± 0.06b	0.63 ± 0.07b
Moisture	663.15 ± 27.75a	638.97 ± 35.31a	639.15 ± 37.83a	649.88 ± 18.22a	629.46 ± 49.57a	642.19 ± 43.47a
Fat	183.87 ± 17.62a	182.69 ± 46.19a	192.10 ± 12.05a	199.30 ± 14.82a	209.96 ± 34.63a	191.72 ± 43.31a
Collagen	54.55 ± 6.44c	70.37 ± 12.48ab	61.00 ± 5.36bc	59.20 ± 4.75bc	71.72 ± 8.30ab	82.73 ± 6.64a
Non-collagenous protein	50.41 ± 9.12ab	59.61 ± 10.96a	58.88 ± 3.49a	38.79 ± 6.08b	43.74 ± 6.64b	46.90 ± 10.83ab

Values are mean ± standard deviation. Means followed by different letters in a row are significantly different at $P < 0.05$.

Table 5. Effect of oral administration of CHs on amino acid profile (g kg^{-1} wet weight) of chronologically aged skin

Amino acid	BC	YC	WC	LD	MD	HD
Hyp	7.31 ± 0.86c	9.43 ± 1.67ab	8.18 ± 0.72bc	7.94 ± 0.64bc	9.61 ± 1.11ab	11.09 ± 0.89a
Asp	8.64 ± 1.32b	10.28 ± 0.96a	9.41 ± 0.88ab	8.86 ± 1.25ab	8.76 ± 1.17ab	9.62 ± 1.05ab
Thr	3.26 ± 0.91c	4.98 ± 0.49a	4.49 ± 1.17ab	3.57 ± 0.81bc	3.78 ± 0.78bc	4.32 ± 0.48ab
Ser	7.80 ± 1.00a	8.49 ± 0.89a	8.29 ± 1.40a	8.19 ± 1.53a	7.83 ± 1.00a	7.93 ± 0.85a
Glu	15.90 ± 2.40b	19.32 ± 1.58a	17.70 ± 2.06ab	16.32 ± 1.93b	15.96 ± 2.02b	17.71 ± 1.82ab
Gly	17.66 ± 2.51b	23.13 ± 4.37a	21.13 ± 4.31ab	19.87 ± 3.49ab	21.10 ± 2.46ab	23.19 ± 3.33a
Ala	8.98 ± 1.28b	11.14 ± 1.42a	10.31 ± 1.27ab	9.45 ± 1.22ab	9.77 ± 1.13ab	10.68 ± 1.60ab
Cys	1.62 ± 0.43a	2.19 ± 0.61a	1.71 ± 0.47a	1.70 ± 0.42a	1.56 ± 0.34a	1.62 ± 0.32a
Val	4.35 ± 0.64b	5.20 ± 0.44a	4.89 ± 0.98ab	4.49 ± 0.63ab	4.38 ± 0.57ab	4.71 ± 0.81ab
Met	1.77 ± 0.69a	1.93 ± 0.58a	1.74 ± 0.66a	1.56 ± 0.35a	1.23 ± 0.34a	2.10 ± 0.26a
Ile	3.92 ± 0.73a	4.58 ± 0.49a	4.35 ± 0.20a	4.06 ± 0.69a	3.86 ± 0.60a	4.27 ± 0.71a
Leu	7.14 ± 1.15b	8.38 ± 0.67a	8.00 ± 1.13ab	7.03 ± 0.73b	7.32 ± 0.61ab	7.88 ± 1.10ab
Tyr	3.65 ± 0.70b	4.64 ± 0.41a	4.20 ± 0.69ab	3.72 ± 0.58b	3.69 ± 0.48b	4.08 ± 0.83ab
Phe	4.43 ± 1.15b	5.87 ± 0.81a	5.38 ± 0.86ab	4.42 ± 0.91b	4.68 ± 0.92ab	5.06 ± 1.09ab
His	3.38 ± 0.54a	3.91 ± 0.37a	3.84 ± 0.62a	3.49 ± 0.53a	3.49 ± 0.72a	3.87 ± 0.73a
Lys	7.84 ± 1.02b	9.01 ± 0.95ab	9.22 ± 0.31a	8.08 ± 0.99ab	7.91 ± 0.97ab	8.27 ± 0.89ab
Arg	8.31 ± 1.59a	8.71 ± 0.70a	7.81 ± 1.36a	8.58 ± 1.71a	8.31 ± 1.62a	8.70 ± 1.02a
Pro	9.01 ± 1.10b	11.32 ± 1.72a	10.34 ± 1.34ab	9.96 ± 1.60ab	10.17 ± 1.02ab	11.04 ± 1.58a

Values are mean ± standard deviation. Means followed by different letters in a row are significantly different at $P < 0.05$.

test. Differences at $P < 0.05$ were considered significant. All computations were made with SAS 9.2 (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Body weight, food intake and mortality rate

As shown in Table 2, the body weight of HD (35.25 ± 2.29 g) was significantly lower than that of BC (39.54 ± 2.34 g), which indicated that the CHs had an effect on body weight regulation. This was in accordance with Watanabe-Kamiyama *et al.*[22] but differed from the findings of other authors.[2,9,12] However, the decreased body weight of HD was not accompanied by a decrease in food intake (92.87 ± 2.45 g kg^{-1} body weight (BW) day^{-1}), which was similar to that of BC (89.24 ± 3.39 g kg^{-1} BW day^{-1}) but significantly higher than that of LD (84.67 ± 3.50 g kg^{-1} BW day^{-1}) and MD (84.66 ± 2.62 g kg^{-1} BW day^{-1}). These results were not consistent with the reports of Liang *et al.*[12] and Haratake *et al.*[2] that orally administered CHs did not affect food intake. Moreover, there was no obvious difference in mortality rate between BC (25.00%) and CH-treated groups (16.67–33.33%), suggesting the safety of CHs.

Viscera indices

The liver, thymus and spleen are the primary immune organs and their indices may reflect an organism's immune function.[23] From Table 3, it was seen that the differences in viscera indices among

Table 6. Effect of oral administration of CHs on antioxidant enzyme activities (U mg^{-1} prot) and MDA content (nmol mg^{-1} prot) of chronologically aged skin

Parameter	BC	YC	WC	LD	MD	HD
T-AOC	6.91 ± 0.48a	6.83 ± 0.91a	6.49 ± 0.35a	7.12 ± 0.67a	6.82 ± 0.52a	6.95 ± 0.81a
SOD	112.24 ± 20.34b	158.65 ± 40.76a	138.32 ± 29.91ab	138.85 ± 37.49ab	177.93 ± 34.85a	163.64 ± 22.08a
GSH-Px	46.39 ± 6.36c	72.76 ± 9.94a	55.91 ± 4.30bc	59.03 ± 9.70abc	72.15 ± 5.46a	66.02 ± 8.33ab
MDA	1.40 ± 0.47d	4.19 ± 0.62a	1.90 ± 0.40cd	2.92 ± 0.29b	3.17 ± 0.73b	2.62 ± 0.60bc

Values are mean ± standard deviation. Means followed by different letters in a row are significantly different at $P < 0.05$.

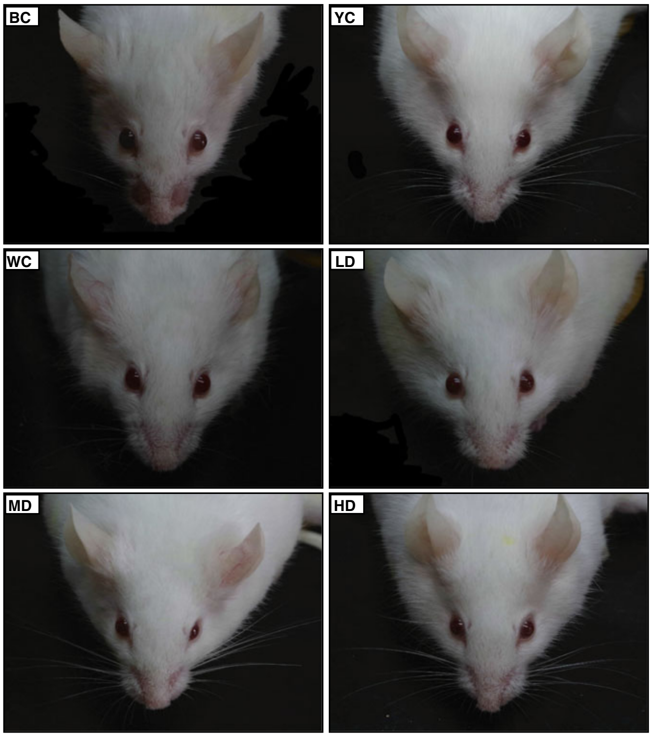

Figure 1. Effect of oral administration of CHs on visual appearance of chronologically aged skin.

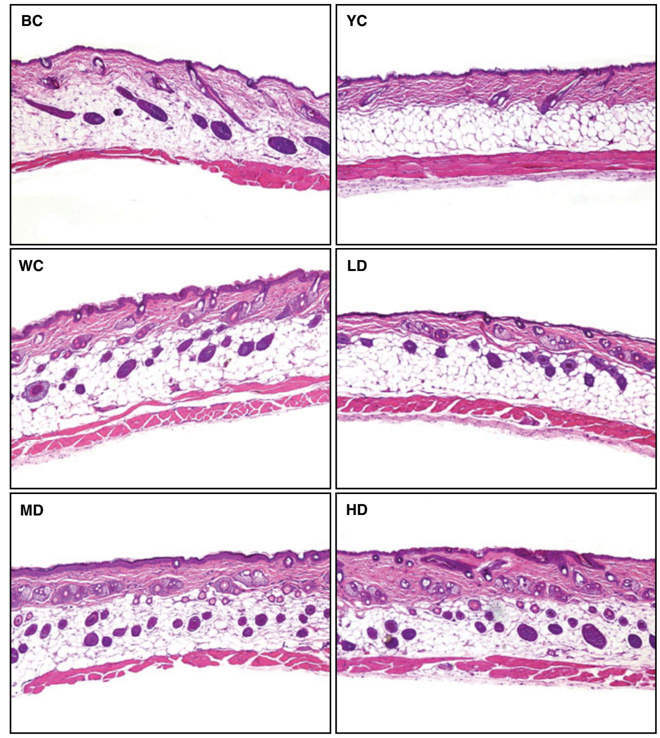

Figure 2. Effect of oral administration of CHs on histological structure of chronologically aged skin (HE staining, 50×).

all groups were not significant except for the thymus index of LD, which indicated that there was no marked atrophy, hyperplasia or swelling in the primary immune organs. This showed that the maximum safe dose of the scale-derived CHs was more than 10%, which was markedly higher than that of the skin-derived CHs (5%). It appeared that the safety of the scale-derived CHs was higher than that of the skin-derived CHs, which could be due to the difference in primary structure (amino acid sequence) of collagens from scale and skin. This might be a reason why the scale-derived CHs have a long history of traditional medicinal use in China rather than the skin-derived CHs.

Thickness, moisture and fat content

It is known that chronologically aged skin is characterized by decreased thickness of the epidermis and dermis.[1] Unexpectedly, the skin thickness of all CH-treated groups (0.63–0.71 mm) was significantly lower than that of BC (0.89 ± 0.08 mm) but similar to that of YC (0.69 ± 0.12 mm) (Table 4).

The moisture content of skin is greatly influenced by ground substances and may be associated with wrinkling and laxity of the skin accompanying aging.[5] It has been reported that the oral ingestion of CHs could increase skin hydration.[5,6,8,9,11,17] However, no significant difference in the moisture content of skin was observed in this experiment, which was in agreement with the clinical trials of Proksch *et al.*[16] Besides, there was also no significant difference in the fat content of skin.

Protein content and amino acid profile

Collagen is responsible for the high strength and resilience of skin and decreases with aging owing to its reduced synthesis and increased degradation.[1] The collagen content of MD (71.72 ± 8.30 g kg^{-1} wet weight) and HD (82.73 ± 6.64 g kg^{-1} wet weight) was significantly higher than that of BC (54.55 ± 6.44 g kg^{-1} wet weight) and similar to that of YC (70.37 ± 12.48 g kg^{-1} wet weight) (Table 4). This indicated the positive effect of CHs on collagen accumulation in skin, which was consistent with previous reports.[2,5–10,12] In addition, the collagen content of WC (61.00 ± 5.36 g kg^{-1} wet weight) was similar to that of BC and significantly lower than that of HD. It thus seemed possible that the CHs increased the collagen quantity of skin in a specific manner.

There was no significant difference in the content of non-collagenous protein between BC (50.41 ± 9.12 g kg^{-1} wet weight) and CH-treated groups (38.79–46.90 g kg^{-1} wet weight), which illustrated that the oral ingestion of CHs did not affect the content of non-collagenous protein. Moreover, the amino acids with relatively higher content in CHs (Gly, Hyp and Pro) increased significantly in HD compared with BC, while the other amino acids were not changed (Table 5).

Oxidative stress

Free radicals are inevitably formed during the metabolism of oxygen in aerobic organisms and are closely related to the occurrence of disease and aging.[24] The cellular antioxidant enzymes can protect tissues from free radical-mediated oxidative injuries. As Table 6 showed, the SOD (112.24 ± 20.34 U mg^{-1} protein (prot)) and GSH-Px (46.39 ± 6.36 U mg^{-1} prot) activities of BC decreased significantly compared with those of YC (158.65 ± 40.76 and 72.76 ± 9.94 U mg^{-1} prot respectively). The CHs did not influence the SOD and GSH-Px activities of LD but significantly increased the SOD (177.93 ± 34.85 U mg^{-1} prot) and GSH-Px (72.15 ± 5.46 U mg^{-1} prot) activities of MD. Moreover, the SOD (163.64 ± 22.08 U mg^{-1} prot) and GSH-Px (66.02 ± 8.33 U mg^{-1} prot) activities of HD were significantly higher than those of BC but similar to those of MD. It appeared that the antioxidant enzyme activities could be enhanced by CHs without dose dependency, which might be due

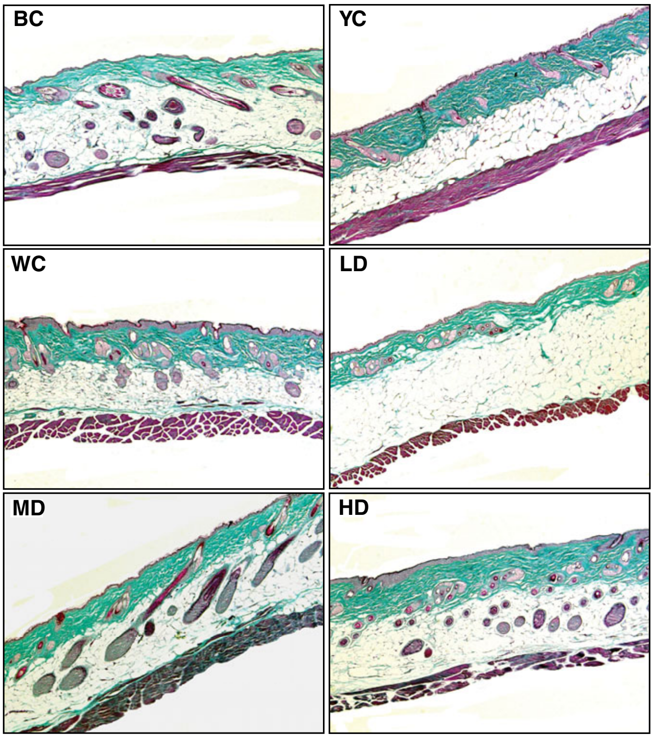

Figure 3. Effect of oral administration of CHs on collagen fibers of chronologically aged skin (Masson's trichrome staining, 50×).

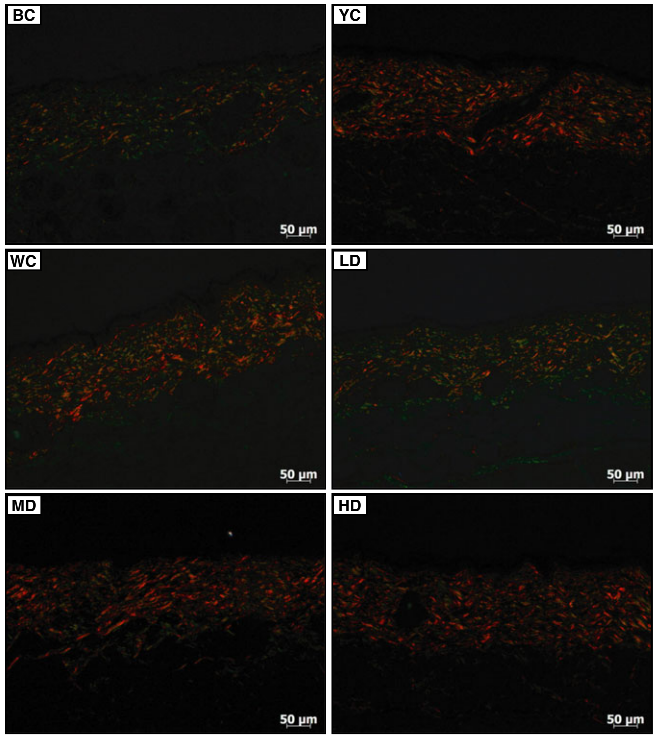

Figure 4. Effect of oral administration of CHs on collagen types of chronologically aged skin (Sirius red staining, 200×).

to the potential toxicity and side effects of high-dose CHs (10%). This indicated the beneficial effect of CHs on the oxidative stress in chronologically aged skin, which was also reported on UV-induced photoaging skin.[6,7,25]

MDA is a product of lipid peroxidation and is usually quantified to estimate the extent of lipid peroxidation.[26] It has been reported that the MDA content of skin increased markedly with aging.[6,7,12,25,26] Surprisingly, the MDA content in BC (1.40 ± 0.47 nmol mg^{-1} prot) was significantly lower than that in YC (4.19 ± 0.62 nmol mg^{-1} prot) and CH-treated groups (2.62–3.17 nmol mg^{-1} prot).

Visual appearance and histological structure

The hair of YC was smooth, glossy and thick, but BC exhibited yellowish, withered and coarse hair, especially severe loss of whiskers (Fig. 1). As expected, the CHs improved the color, luster and quantity of hair and prevented whisker loss. The effect was more marked in MD and HD, which were very similar to YC.

Compared with YC, the epidermis of BC was uneven and coarse, and large quantities of tangled, unordered and degraded fibers were observed in the dermis (Fig. 2). The junction of dermis and hypodermis was also unclear. Nevertheless, the CHs obviously improved the epidermis evenness, dermis thickness and junction clearness. Besides, the fibers in the dermis became abundant, dense and ordered with a dose-dependent tendency. This suggested the beneficial effect of CHs on the visual appearance and histological structure of chronologically aged skin.

Collagen fibers and collagen types

Masson's trichrome staining was employed to determine the distribution of collagen fibers, which were dyed green (Fig. 3). The collagen fibers of BC showed weaker green staining and appeared to be more sparse, fragmented and disorganized. However, they increased dramatically in the CH-treated groups with the dose of CHs, which was compatible with the collagen content shown in Table 4. Moreover, the collagen fibers were obviously denser and more systematic.

Sirius red can distinguish type III (green) from type I (yellow or red) collagen under polarized light.[6] It is known that the ratio of type I to type III collagen decreases gradually with aging. From Fig. 4, it was seen that type I collagen was dominant in YC and type III collagen was hardly seen, but most collagen was type III in BC. The CHs markedly increased the ratio of type I to type III collagen in a dose-dependent manner, which indicated the positive influence of CHs on the collagen matrix homeostasis in chronologically aged skin.

CONCLUSIONS

The effect of oral ingestion of CHs from Nile tilapia scale on chronologically aged skin was investigated with mice in this study. The collagen content and antioxidant enzyme activities of skin were significantly increased. The color, luster and quantity of hair were obviously ameliorated. Moreover, the structure of epidermis and dermis, the density and distribution of collagen fibers and the ratio of type I to type III collagen were markedly improved in a dose-dependent manner. These results suggest the potential of CHs as an anti-skin-aging ingredient in nutraceuticals or functional foods.

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