Effects of *Dendrobium huoshanense* polysaccharides on antioxidant capacity, mucosal barrier integrity and inflammatory responses in an aging rat ileal model

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**ABSTRACT**

Polysaccharides are important pharmacologically active substances found in the perennial orchid *Dendrobium huoshanense*. In the present study, we sought to determine whether antioxidant and anti-inflammatory mechanisms were involved in *Dendrobium huoshanense* polysaccharides (DHPs)-mediated protection from D-galactose-induced intestine injury in an aging rat model. The effects of DHPs on ileal antioxidant capacity, mucosal barrier integrity and the inflammatory response were investigated in D-galactose-treated rats. The results showed that DHP administration largely attenuated the D-galactose-induced malondialdehyde (MDA), interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-10, Toll-like receptor four and NF-κBp65 content increases and also markedly renewed the total antioxidant capacity and the claudin and occludin contents of ileal tissues from the D-galactose-treated rats. We also found that DHP more markedly affected the IL-6 and TNF-α levels than it did the IL-4 and IL-10 levels in the ileal tissues from the D-galactose-treated rats. These results suggest that DHPs can protect the rat ileum from D-galactose-induced injury by attenuating lipid peroxidation, improving antioxidant capacity and mucosal barrier integrity, correcting imbalanced inflammatory cytokine networks and suppressing the inflammatory response. The novel insights into the mechanism involved in DHPs protection of ileal health in our D-galactose-induced aging model rat advances current understanding of the aging process.

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**Introduction**

Aging is an inevitable life process for any organism including human beings. With increasing age, elderly people’s resistance to disease decreases. The intestinal tract is an important digestive and immune organ of the body, and studies have shown that intestinal senescence precedes the senescence of the body’s other organs, and intestinal senescence can accelerate the senescence of other organs [1]. Thus, protecting intestinal health is very important for delaying the aging process in people.

*Dendrobium huoshanense*, a perennial orchid species, is a well-known medicinal herb in Huoshan County, China. It has been used for centuries in folk medicine and as a health food material in teas and soups when entertaining distinguished guests in China [2]. Recorded in the ‘Compendium of Materia Medica’, an ancient and famous Chinese pharmaceutical work, is that *D. huoshanense* can strengthen Yin and enhance gastrointestinal function, while suppressing stomach Qi, promoting muscle growth and intelligence, eliminating asthma and removing turbidity and unwanted bodily substances and prolonging life [2], suggesting that this plant can improve intestinal health and delay bodily aging. Modern pharmacological studies have found that polysaccharides are the main active ingredients of *D. huoshanense* [3,4]. Many activities have been associated with *D. huoshanense* polysaccharides (DHPs). These include anti-glycation [5], anti-oxidant [6], hepatoprotective [7] and immunostimulating activities [8,9]. However, little research has been done on the anti-aging effect of polysaccharides from *D. huoshanense* or other
Dendrobium species [10–12], and this is especially so for the effects DHPs have on intestine health in aging animals. It is known that maintaining good antioxidant functioning, mucosal barrier integrity and balanced inflammatory cytokine networks in the intestine are necessary for optimal intestinal health. In this study, we investigated the effect of DHPs on antioxidant capacity, mucosal barrier integrity and inflammatory responses using an aging model of rat ilium with the purpose of investigating the experimental basis for developing D. huoshanense as an anti-aging health product.

Materials and methods

Animals

Six-week-old male specific-pathogen-free (SPF) grade Sprague-Dawley rats were purchased from the Laboratory Animal Center of Anhui Medical University (Hefei, China).

Ethics statement

All of the experiments were conducted in accordance with the National Guide for the Care and Use of Laboratory Animals and were also approved by the Bioethics Committee of West Anhui University, China.

Reagents and kits

The malondialdehyde (MDA) assay (thiobarbituric acid method), total antioxidant capacity (T-AOC) assay, and bicinchoninic acid (BCA) protein assay kits were purchased from Nanjing Jiancheng Biotechnology Co., Ltd. (Nanjing, China). Radio immunoprecipitation assay (RIPA) lysis buffer, 30% acrylamide and stripping buffer for western blots were purchased from Beyourtime Biotechnology Corp. (Shanghai, China). D-galactose and tetramethylethylenediamine were purchased from Sigma-Aldrich (TEMED; St. Louis, MO, USA). Sodium dodecyl sulfate (SDS), glycine, 1,1,1-tris(hydroxymethyl)methanamin, ammonium persulfate and Tween 20 were purchased from Solarbio Biotechnology Corp. (Beijing, China). D-galactose and tetramethylethylenediamine were purchased from Sigma-Aldrich (TEMED; St. Louis, MO, USA). Sodium dodecyl sulfate (SDS), glycine, 1,1,1-tris(hydroxymethyl)methanamin, ammonium persulfate and Tween 20 were purchased from Solarbio Biotechnology Corp. (Beijing, China). Interleukin (IL)-4, IL-6, IL-10 and tumor necrosis factor (TNF)-α enzyme-linked immunosorbent assay (ELISA) kits were purchased from Beijing Andy Gene Biotechnology Co., Ltd. (Beijing, China). Enhanced chemiluminescence (ECL) ultra-sensitive luminescence kit and pre-stained protein ladder were purchased from Thermo Scientific (Shanghai, China). Mouse anti-β-actin monoclonal antibody and horseradish peroxidase-labeled goat anti-rabbit IgG were purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd. (Beijing, China). Mouse NF-kBp65 monoclonal antibody was purchased from Santa Cruz Biotechnology (Shanghai) Co., Ltd. (Shanghai, China). Rabbit monoclonal anti-claudin antibody and rabbit monoclonal anti-occludin antibody [EPR8208] were purchased from Abcam PLC (UK) and Toll-like receptor (TLR)4 (S441) polyclonal antibody was purchased from BioWorld Technology Inc. (Louis Park, MN, USA). PVDF (polyvinylidene difluoride) membrane was purchased from Millipore Corporation (Bedford, MA, USA). All other reagents were of analytical grade.

Plant material

The D. huoshanense stems provided by the Anhui Dendrobium huoshan Industrial Development Collaborative Innovation Center were identified as genuine by Professor Chen Nai-fu from the School of Biological and Pharmaceutical Engineering at West Anhui University.

Extraction and isolation

Refined DHP powder was prepared as described by Chen et al. [13]. Briefly, D. huoshanense plant stems were dried at 80 °C to a constant weight, then crushed and sifted over a 60-sized mesh. An appropriate amount of D. huoshanense powder was extracted at 85 °C with 95% ethanol (g: mL = 1: 25) reflux for 1 h, repeating the reflux extraction three times. The extracts obtained were filtered and the filtered residue was re-extracted with water (g: mL, 1:100) for 2 h at 90 °C, repeating the extraction three times. All of the filtrates obtained were mixed and concentrated at low pressure to an appropriate concentration. After that, they were stored at 4 °C for 1 h, and then centrifugated at 4800×g for 15 min. The supernatant was collected to obtain the total polysaccharide extract. Absolute ethanol was added slowly into an appropriate amount of total polysaccharide extract solution until the final ethanol concentration reached 76%, and the resultant product was stored at 4 °C for 24 h, after which it was centrifuged at 4800×g for 15 min. The precipitate was washed in turn with anhydrous ethanol, acetone and ether several times. The precipitate was then dissolved in an appropriate amount of distilled water and deproteinized according to the Sevag method. Finally, the polysaccharide solution obtained was freeze-dried to obtain the refined DHPs. The polysaccharide content in the dried stem from D. huoshanense was 29.4%, as determined by anthrone–sulfuric
It has been reported that DHPs can be further separated into five polysaccharide fractions by diethylaminoethyl cellulose (DEAE-C) column (1.6 × 60 cm) chromatography, and each of these five polysaccharide fractions can stimulate marrow cell proliferation and immunomodulatory activity in intestinal mucosa [14]. Therefore, in the present study, the refined total DHPs preparation was used directly in the animal experiments.

**Experimental design**

Thirty male Sprague-Dawley rats aged at 4 months were randomly divided into the control group, the aging model group and the aging model + DHP group, with 10 rats in each group and raised in 2 cages with five rats per cage. Rats were prepared for the aging group by subcutaneous injection of D-galactose (dose, 125 mg/kg BW once daily) [15]. Rats in the control group were subcutaneously injected with physiological saline once daily, the volume of which was the same as that used for the rats for the aging model group. DHPs was dissolved in distilled water at 4 °C and orally administered to the rats in the aging model group. The homogenate was centrifuged at 10,920 g for 15 min at 4 °C, and the supernatant was collected to determine the levels of IL-6, TNF-α, IL-4 and IL-10 using ELISA kits. The protein concentration in the supernatant was determined by the BCA method. MDA and T-AOC levels were calculated as values per milligram of protein.

**Determination of ileal IL-6, TNF-α, IL-4 and IL-10 levels by ELISA**

About 0.5 g of ileal tissue was placed into a tube containing 5 mL of cold physiological saline and homogenated on ice. The homogenate was centrifuged (15 min, 3000 r/min, 4 °C) and the supernatant was collected to determine the levels of IL-6, TNF-α, IL-4 and IL-10 using ELISA kits. The protein concentration in the supernatant was determined by the BCA method. Cytokine levels were converted to the corresponding levels per milligram of protein.

**Determination of ileal toll-like receptor (TLR)4, NF-κBp65, claudin and occludin levels by Western blotting**

About 0.1 g of ileal tissue was homogenated in 1 mL of RIPA Lysis Buffer (containing 1 mmol/L phenylmethylsulfonyl fluoride) with continuous lysis over 30 min on ice. The lysates were centrifuged at 10,920 g at 4 °C for 15 min. The supernatant was collected and the protein content was measured using a BCA protein assay kit before its analysis. The protein samples were loaded each at a concentration of 10 μg of protein per lane onto a 10% SDS-polyacrylamide gel for electrophoretic separation, and then transferred to PVDF membranes (Immun-Blot PVDF membrane, 0.2 μm).

The membranes were blocked with 5% nonfat powdered milk in 1× Tris-buffered saline (TBS) containing 0.1% Tween 20 (T-TBS) for 2 h at room temperature and then incubated with primary antibodies overnight at 4 °C at the concentrations indicated by their manufacturers; specifically, 1:500 for the TLR4 polyclonal antibody, 1:300 for the mouse NF-κBp65 monoclonal antibody, 1:2000 for the rabbit monoclonal anti-claudin antibody, 1:50000 for the rabbit monoclonal anti-occludin antibody, and 1:1000 for the mouse monoclonal anti-β-actin antibody. The membranes were then treated with horseradish peroxidase-labeled goat antimouse IgG (1:10000) for 2 h at room temperature and washed with T-TBS after each antibody binding reaction. Protein detection was performed using an enhanced chemiluminescence kit (ECL, Millipore Co., USA). The gray values were analyzed using the Beijing Kechuangrui New Biogel Imaging System (Image J software). The gray value of the target protein compared with β-actin was considered to represent the relative expression level of the target protein.
Statistical analysis

All of the data are expressed as the mean values with standard deviation (± S.D.). Single factor analysis of variance (ANOVA) was carried out using Data Processing System (DPS) software (Hangzhou ruifeng information technology co. LTD, Hangzhou, China) and Duncan’s test was used to make multiple comparisons. Values of $p < 0.05$ were considered to indicate a statistically significant difference, and $p < 0.01$ was considered to indicate that the difference was highly significant.

Results and discussion

Appearance of the rats

The D-galactose-induced aging model is a sub-acute aging model. This model is prepared by continuous injections with D-galactose for a certain period of time to increase the concentration of cellular galactose [16]. By the catalysis of aldose reductase, D-galactose is reduced to galactol, which cannot be further metabolized by the cell. As such, it accumulates inside the cells, which affects their normal osmotic pressure, and this is accompanied by the production of a large number of free radicals, resulting in dysfunction and metabolic cellular disorder, thereby causing bodily aging, which is considerably similar to the normal aging process and similar to natural senescence model [16,17]. Furthermore, experimental evidence has shown that 3-month-old rats injected subcutaneously with D-galactose (dose, 125 mg/kg BW once daily) for 40 days can reproduce the aging model similar to 24-month-old rats [18]. Therefore, although older rats may simulate the natural aging process better, the aging model established with rats at the age of 4 months adopted in our experiment can also meet the aging model requirement well. In the present study, compared with the control rats, the rats in the aging group showed lassitude, less activity, dull hair, yellowish parts of hair, and more debilitation than the rats of the control group at the end of the experiment, indicating that the aging model was prepared successfully. The rats in the control group and the aging model + DHP group, unlike the aging-modeled rats, exhibited good spirits, more activity and shinier hair, indicating that DHPs had a positive anti-aging effect.

**MDA and T-AOC**

The causes of aging are so complex that they have not yet been fully elucidated. Increasing numbers of studies now show that oxidative stress plays an important role in aging [19]. Oxidative stress can lead to oxidative damage to DNA, protein degeneration and telomere shortening in the nucleus, resulting in cellular aging [20,21]. MDA levels can reflect the degree of lipid peroxidation injury and indirectly reflect the extent of cellular damage [22], and the level of T-AOC can reflect the ability of the body’s antioxidant defense system to resist oxidative damage. In the present study, the level of MDA in the ileal tissues from the aging-modeled group was significantly higher than that of the control group as well as that of the aging model + DHP group, and the level of T-AOC in the ileal tissues from the aging model group was significantly lower than that of the control group as well as that of the aging model + DHP group ($p < 0.01$) (Table 1), indicating that the aging induced by D-galactose inflicted remarkably high levels of oxidative damage on the ileum. DHPs have good antioxidant activities such that they can significantly alleviate the oxidative damage induced by D-galactose, which might be one of the mechanisms for their anti-aging effects.

**IL-6, TNF-α, IL-4 and IL-10**

Dysregulation of anti-inflammatory and pro-inflammatory cytokine networks plays an important role in the aging process [23]. IL-6 and TNF-α are important pro-inflammatory factors that contribute significantly to inflammation-related aging in healthy elderly individuals [24] as well as in many age-related diseases [25]. Abnormally high levels of IL-6 can lead to immune and neuroendocrine dysfunction [26,27]. IL-6 has long been recognized as important in aging and age-related diseases; hence, it has been called the “gerontologist’s cytokine” [28]. In the present study, IL-6 levels were found to increase significantly in the aging-modeled group ($p < 0.01$) and DHPs were able to decrease IL-6 levels in the aging-modeled rats to a value comparable to that of the control group.

| Group Index | Control | Aging model | Aging model + DHP |
|-------------|---------|-------------|------------------|
| MDA (nmol/mg prot) | 3.73 ± 0.48B | 3.99 ± 0.29B | 5.03 ± 0.44A |
| T-AOC (nmol/mg prot) | 2.88 ± 0.32A | 1.23 ± 0.15C | 2.31 ± 0.20A |
| IL-6 (ng/mg prot) | 226.3 ± 36.20B | 241.9 ± 46.38B | 313.7 ± 20.31A |
| TNF-α (ng/mg prot) | 143.6 ± 27.21B | 169.2 ± 31.20B | 211.8 ± 29.21A |
| IL-4 (ng/mg prot) | 180.60 ± 70.22C | 279.22 ± 58.19B | 371.36 ± 68.80A |
| IL-10 (ng/mg prot) | 158.72 ± 30.82C | 240.78 ± 59.12B | 354.71 ± 71.85A |

Numerical values marked with different uppercase letters in the same row were statistically significantly different ($p < 0.01$).
who carried a TNF-α important role in intestinal inflammation. Aged mice thereby causing bodily aging [26]. TNF-α also plays an important role in intestinal inflammation. Aged mice who carried a TNF-α deletion did not show any intestinal inflammatory response associated with aging [29]. In fact, increasing levels of TNF-α was found to damage macrophage functioning and increase the permeability of the intestinal wall [29]. Many studies have reported on increased TNF-α levels occurring in aging animals [26,30–32]. In the present study, we also found that the TNF-α levels increased in the ileal tissues of the aging-modeled rats and that DHPs administration decreased the TNF-α levels in them to a value comparable to that of the control group (Table 1), further indicating that DHPs have an anti-inflammatory effect.

As age increases and in age-related diseases, a chronic inflammatory state predominates, which – if not properly contained or resolved and if the anti-inflammatory side of the immune system is also similarly dysregulated – will be unable to dampen down the inflammatory episode in a timely effective manner [23]. IL-4 and IL-10 are important anti-inflammatory factors in the body. IL-4 is mainly produced by activated T cell Th2 subsets, and these can antagonize the effect of TNF-α on macrophage activation. Previous studies have shown that the levels of serum IL-4 in D-galactose-induced aging mice [33] and in naturally aging rats [34] decrease significantly. Here, we found that the level of ileal IL-4 increased in the aging-modeled rats. However, Santiago et al. [35] found that IL-4 levels in the duodenum and jejunum tissues from 24-month-old mice increased, whereas the ileal IL-4 level did not. These inconsistencies in the IL-4 level changes in aging animals suggest that IL-4 levels do not undergo any specific changes during aging. IL-10 is a cytokine with anti-inflammatory and anti-oxidative effects [36,37], and is considered to be one of the key anti-inflammatory cytokines in the body [38]. IL-10 can reduce pro-inflammatory factor levels by promoting the degradation of some cytokines encoded by mRNAs, such as IL-1, IL-6 and TNF-α [39]. IL-10 also acts as an antioxidant by blocking the release of reactive oxygen species (ROS) and down-regulating nitric oxide levels [40]. Higher IL-10 serum levels and production by both lymphocytes and monocytes have been reported in elderly people [41]. However, Santiago et al. [35] found that IL-10 levels in the proximal and distal duodenum, and in the distal jejunum and ileum also, decreased in naturally aging mice. In the present study, we found that ileal IL-10 levels in the aging-modeled rats increased, which is contrary to that reported by Santiago et al. [35]. In the present study, we found that DHPs administration decreased the levels of IL-6, TNF-α, IL-4 and IL-10 in the ileal tissues of the aging-modeled rats (Table 1). It should be noted that DHPs administration was able to decrease the levels of IL-6 and TNF-α more dramatically because there were no significant differences in the levels of IL-6 and TNF-α between the control and the aging modeled + DHP group. However, the levels of IL-4 and IL-10 were still much higher in the aging modeled + DHP group than those in the control group. Therefore, these results indicate that DHPs administration had a more obvious effect on pro-inflammatory factors than on anti-inflammatory factors when alleviating the imbalance in the anti-inflammatory factor and pro-inflammatory factor network. However, more research is needed to clarify the changes that occur with IL-4 and IL-10 during aging because of the contradictory results that have been obtained in different studies.

Inflammation is closely related with oxidative stress and aging. The inflammation of aging is characterized by low, asymptomatic, chronic and systemic bodily changes, which is an uncontrollable inflammatory state, also known as inflammatory senescence [42]. Chronic low-grade inflammation can aggravate telomere dysfunction by increasing the DNA damage mediated by ROS, thereby accelerating the accumulation of aging cells, and aging can aggravate chronic inflammation as well as the production of ROS, which causes a vicious circle that hampers tissue regeneration and speeds up senescence [43]. Senescent cells also produce bystander effects on normal peripheral cells by secreting bio-active molecules such as interleukin, chemokines and ROS, thus triggering their senescence [44]. Studies have shown that the in vivo removal of aging cells can significantly delay the development of aging [45]. The intestinal immune system is damaged during aging, making the elderly more likely to develop intestinal infections and inflammation [46]. Therefore, one mechanism for the anti-inflammatory effect of DHPs on the ileal tissues of our aging rats may be ascribed to their antioxidant activities.
**Claudin and occludin**

Intestinal tight junction protein (ZO-1, claudin one and occludin) expression decreases and the mucosal barrier function weakens in naturally aging rats [47,48]. In the present study, we found that claudin and occludin expression in the ileum decreased in the aging-modeled group (Figure 1), indicating that intestinal mucosal permeability increased in this group. This will increase the translocation of endotoxins such as lipopolysaccharide (LPS) from the intestinal bacteria into the blood circulation [49], and increase the release of pro-inflammatory factors such as IL-6 and TNF-α. Increased TNF-α levels can increase the permeability of the intestinal wall [29] and promote the trans-location of LPS, thus forming a vicious circle. Therefore, promoting an increased intestinal mucous barrier function might be an important mechanism for the anti-aging function of DHPs. DHPs might increase the expression of intestinal tight junction proteins by decreasing the damage to the intestinal tight junctions caused by oxidative damage and the inflammation response.

**TLR4 and NF-κBp65**

During aging, the body is in a chronic inflammatory state with increased levels of TLR4 and NF-κB. TLRs are specific pattern recognition receptors that recognize molecules such as LPS and lipoprotein from bacterial pathogens. They activate their downstream signaling pathway, the myeloid differentiation factor MyD88, and further activate the IKK-NF-κB signaling pathway or the JNK-NF-κB signaling pathway to promote the transcription and synthesis of inflammatory mediators such as TNF-α, IL-1 and IL-6 to activate inflammatory responses in the body [50]. There are many TLR types, among which TLR4 plays an important role in LPS signal transduction. Expression of TLR4 in the intestinal tract of aging animals has not been reported previously. However, Shao et al. [51] found that TLR4 expression in the kidneys of 12- and 24-month-old rats was significantly higher than that of 3-month-old rats, and TLR4 expression in 24-month-old rats was significantly higher than that of the 12-month-old rats. Therefore, the increased ileal expression level of TLR4 in the aging-modeled group in the present study might also indicate that the aging model was prepared successfully.

NF-κB is a major transcriptional regulator of inflammation-related genes (including pro-inflammatory interleukin, chemokines, cytokines and adhesion molecules, among others), and itself is also activated by

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**Figure 1.** Levels of claudin, occludin, TLR4 and NF-κBp65 in rat ileum tissue. Note: The data of the same index marked with different uppercase letters indicated that there was significant difference between them \((p < 0.01)\).
pro-inflammatory signals, stress signals and cellular senescence signals [44]. NF-κB regulates the expression of inflammatory genes through its five sub-units, RelA (p65), RelB, c-Related, p50 and its precursor P105, and through p52 and its precursor, P100. These five sub-units can form homo-polymers or hetero-polymers. The classic pro-inflammatory NF-κB form is a P65:p50 hetero-polymer, in which p65 is essential for stimulating target gene transcription [52]. LPS, TNF-α, and IL-1β can all stimulate IL-10 production. IL-10 can block the activation of NF-κB, which is induced by endotoxin in monocytes, thereby inhibiting cytokine production and restricting the acute inflammatory response [53]. It has been found that the level of activated phosphorylated NF-κBp65 in the kidneys of 12- and 24-month-old rats is higher than that of 3-month-old rats, while the level of NF-κBp65 and phosphorylated NF-κBp65 in the kidneys of 24-month-old rats is higher than that of 12-month-old rats [51]. Our study found that the level of ileal NF-κBp65 in the aging-modeled rats was higher than that of the normal control group, while DHPs were seen to decrease NF-κBp65 levels in the ileal tissues of the aging-modeled rats (Figure 1), thereby alleviating the inflammatory reaction in the ileal tissues.

Immunosenescence is not only characterized by reduced levels of peripheral naïve T cells and the loss of immature B lineage cells in the bone marrow, but is also characterized by a decline in the function of macrophages, granulocytes and granulocytes, as evidenced by their diminished phagocytic activities and impaired superoxide generation [54]. Unstimulated neutrophils and lymphocytes isolated from elderly patients accumulate higher amounts of ROS, have decreased superoxidase dismutase (SOD) activities, and are less resistant to cell death compared with the same cells obtained from young individuals [54]. Therefore, high levels of pro-inflammatory cytokines in the elderly might result from continuous stimulation by some inflammatory factors such as increased LPS levels and accumulated oxidative debris, for example. The high level of pro-inflammatory factors in the elderly is unlikely to improve their acquired immune functioning and non-specific innate immunity, but weaken them also. It is interesting how DHPs relieve the inflammatory state in the elderly because DHP has been found to activate macrophages in vitro via its direct binding to TLR4 to trigger TLR4 signaling pathways [55]. It has also been found that DHP-4A (a DHP member) activates RAW264.7 cells to secrete TNF-α, IL-6 and IL-10 by regulating mitogen-activated protein kinase and NF-κB pathways [56]. DHP-4A activates RAW264.7 cells to secrete pro-inflammatory factors such as TNF-α and IL-6 together with anti-inflammatory factors such as IL-10, which might reflect the cell’s self-control of the pro-inflammatory and anti-inflammatory network. However, DHP1A (another DHP member) can decrease the expression of TNF-α, IL-1β, monocyte chemotactic protein-1, macrophage inflammatory protein-2, CD68 and phosphorylated IkBα (p-IkBα) in CCl4-treated mice [3]. The different effects of DHP on inflammatory factors between in vivo and in vitro might be ascribed to the different specific environment. For example, LPS levels increase in the elderly and LPS is a strong inducer of pro-inflammatory responses. LPS can bind directly to TLR4 in vitro rapidly, reaching saturation within 10 s in a concentration-dependent manner [57]. It was also found that 5 μg/mL of LPS can promote the release of nitrous oxide, TNF-α, IL-6 and IL-10 by RAW 264.7 macrophage cells at levels comparable with those induced by 50–200 μg/mL DHP-4A [56]. These studies indicate that DHP may interfere with the interaction between LPS and TLR4 to weaken the inflammation caused by LPS. This might be one reason for the paradox that DHP promoted the release of TNF-α, IL-6 and IL-10 in vitro but reduced TNF-α, IL-6 and IL-10 levels in vivo in the aging-modeled rats; another reason is that DHP has good antioxidant capacity, while oxidative stress is an important accelerator of inflammation. Altogether, although DHPs themselves might still stimulate the release of TNF-α, IL-6 and IL-10 in vivo in the aging rat model through the TLR4 and NF-κB pathways as they do in vitro, they obviously decrease the final levels of these cytokines in vivo by decreasing the absorption of LPS, competing with LPS to combine with TLR4, and relieving oxidative stress.

Conclusions

Our findings showed that the aging induced by D-galactose was characterized by a decrease in antioxidant capacity, decreased expression of intestinal mucosal barrier proteins (claudin and occludin), and increased ileal levels of IL-6, TNF-α, IL-4, IL-10, TLR4 and NF-κBp65 in the aging-modeled rats, which indicates a state of bodily oxidative stress and chronic inflammation and weakened intestinal mucosa barrier functioning. DHPs administration had a good anti-aging effect on the ileum in the D-galactose-induced model of aging, and the underlying mechanism might be related to an enhanced antioxidant capacity, a reduced inflammatory response, and enhanced protection of the mucosa barrier function in the ileum.
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Disclosure statement
The authors declare no conflict of interest.

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