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Anti-Aging Effect of *Codnopsis Bulleyana* Forrest ex Diels on D-Galactose-Induced Aging Mouse Model

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Abstract:

Codnopsis bulleyana forrest ex Diels is a natural medicinal and edible homologous plant with good antioxidant properties. The purpose of this study was to investigate its anti-aging effect on mice about water extract of *Codnopsis bulleyana forrest ex Diels (cbFeD).* In the animal experiment part, the mice were divided into four groups: control group, D-gal group, vitamin E group and cbFeD group (500 mg/kg/day). The results showed that after the cbFeD gavage treatment, the mice gradually increased their body weight, their daily activities were enhanced, and the atrophy of the organ index was improved. In the two behavioral experiments, the latency time of the novelty inhibition feeding test was significantly shortened, and the preference index of the mice to sugar water was also significantly increased. cbFeD restores D-galactose-induced inflammatory responses and organ damage in mouse liver and kidney tissues. Compared with the mice in the model group, cbFeD significantly improved the anti-oxidative stress capacity of the serum, liver and kidney of the mice. In addition, cbFeD treatment can protect the liver and kidney. The effect of cbFeD treatment is also reflected in the reduction of liver function indexes ALT, AST, and ALP, and the levels of renal function indexes CR and UREA are also significantly reduced. These results indicate that cbFeD has obvious anti-aging activity in the D-gal-induced aging mouse model. Played the role of protecting liver and kidney function and enhancing cognition in mice. The mechanism is related to the enhancement of anti-inflammatory effects and alleviation of oxidative stress in aging model mice to a certain extent.

Keywords*: Codnopsis bulleyana forrest ex Diels, D-galactose, Subacute aging, Anti-aging, Anti-oxidant, Novelty-suppressed feeding test, Sucrose preference test.*

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I. INTRODUCTION

Aging is the degenerative changes in the tissue structure of an organism with the increase of age after development and maturity [1]. The normal function of the body's organs declines, which leads to the weakening of the internal environment's ability to stabilize itself, the reduction of the resistance to the internal and external environmental damage factors, and the tendency to die. It is a natural phenomenon that is continuous, ubiquitous, progressive, inherent, and has destructive effects, cyclic physiological processes [2]. The continuous development of oxidative stress in aging organisms affects the normal maintenance of free radical homeostasis, so that the normal relationship between nutritional status and free radical homeostasis in the body changes to a compensatory continuous degradation relationship [3].

It is well known that oxidative damage caused by excess reactive oxygen species (ROS) is a decisive factor in aging. The formation of excess ROS can cause oxidative stress and affinity reactions, resulting in a redox imbalance, which eventually leads to protein oxidation, lipid peroxidation, mitochondrial and DNA damage, inhibits normal cell function, and even induces apoptosis [4, 5]. Which can be abundantly present in phagocytes, and are also prevalent at low levels in various other tissue cells, and are involved in many membrane receptors. downstream signaling activation [6]. Compared with the extracellular environment, the cytoplasm is usually in a reduced state, which is maintained by the reducing ability of intracellular sulfhydryl compounds, mainly glutathione, vitamin C/E, and thioredoxin (TRX), which can be down-regulated H_2O_2, and lipid peroxide levels, inhibit cellular oxidative stress. Excessive production of reactive oxygen species can cause the depletion of reduced glutathione, vitamin C/E, and thioredoxin, increasing the sensitivity of cells to reactive oxygen species [7]. Due to the complex composition of natural medicines, the side effects are small and it affects the body through multiple targets. Therefore, preliminary exploration of the antioxidant, immunomodulatory and anti-inflammatory effects of natural medicines to prevent and treat aging has become a new hotspot [8].

Comprehensive research shows that cbFeD contains various active chemical components such as flavonoids, polysaccharides, alkaloids, and rich trace elements. At the same time, pharmacological studies have shown that the cbFeD has the pharmacological effects of invigorating qi and blood, moistening the intestines and laxatives, and anti-cancer [9]. The high dose of cbFeD can increase Hb, increase the content of RBC and the concentration of IgG in serum, showing a better immune regulation effect. Has better hydroxyl radical and DPPH scavenging effect [10]. Based on the research on the biological activity of cbFeD, it can be shown that cbFeD has considerable application potential in the field of anti-aging.

II. MATERIALS AND METHODS

2.1. Materials

2.1.1 Plant materials

The fresh *Codnopsis bulleyana* forrest ex diels (cbFeD) were collected in KunmingYunnan Province

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China in August, 2018.

2.1.2 Animals

Healthy male BALB/c mice aged 6-7 weeks were purchased from Hunan Slike Jingda Laboratory Animal Co., Ltd. (license number: SCXK 2016-0002). Mice were raised in separate cages in a clean room with 12 hours of light and a relative humidity of 45%, and they were free to eat and drink.

2.1.3 Experimental chemicals

D-gal and vitamin E were purchased from Tianjin Zhiyuan Chemical Reagent Co., Ltd. D-gal was formulated in normal saline and injected subcutaneously in the neck of mice at a dose of 500 mg/kg body weight, while VE (100 mg/kg) was dissolved in corn oil. Biotechnology and all ELISA kits MDA (MM-0897M1), SOD (MM-0839M1) and GSH-PX (MM-0758M1) were purchased from Enzyme Immunobiology.

2.2. Methods

2.2.1 Extraction of the *Codnopsis bulleyana* forrest ex diels (cbFeD).

After the fresh and tender Codonopis bulleynana Forest ex Diels was washed and cut into pieces, it was placed in distilled water at a ratio of 1:10 of the raw material to liquid, and boiled for three times. Finally, all the collected filtrates were combined. Using a rotary distillation apparatus, the collected sieve liquid was concentrated to an aqueous extract with crude drug content of about 2 g/ml of Codonopis bulleynana Forest ex Diels(cbFeD).

2.2.2 Preparation of subacute aging mice model and treatment

Using D-galactose to establish a natural aging mouse model. D-galactose (500 mg/kg/day) was subcutaneously injected in the D-gal group, VE group and cbFeD group, and the modeling time was 10 weeks. The control group was subcutaneously injected with the corresponding volume of normal saline.

Two mice were randomly selected from each group for observation of brain histopathology sections to determine whether the modeling was completed. After the modeling was completed, the VE group (100mg/kg/d) and cbFeD (500mg/kg/d) group were given continuous gavage for 4 weeks, while the D-gal group and the control group were given corresponding volume of normal saline. During the experiment, the mice were weighed once a week. The mental status and behavioral activities of mice in each group were observed and recorded.

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2.2.3 Behavioral observation

Novelty-suppressed feeding test (NSFT): Before the experiment, all rats were fasted for 24 hours (no drinking), and adapted to the test room environment for 30 minutes. The experimental device is an open box with a length of 50cm, a width of 50cm and a height of 30cm. The bottom of the box is covered with 2cm-thick small sawdust and a white bottom plate with small pieces of food is placed in the center of the bottom of the box. During the experiment, the rat was placed in any corner of the box, and the activity of the rat within 5 minutes was observed through the camera system, and the timing was started from the time when the rat was put in, and the timing ended when the rat picked up the food with its forelimbs to eat, that is, the latency period (latency period), if the animal did not eat for 5 minutes, it was calculated as 5 minutes, and then the rats were put back into the original cage to record the total amount of food consumed within 30 minutes to exclude the influence of individual appetite differences on the incubation period. In addition, the feeding latency in the original cage and the total food intake within 30 minutes of the rats in the new environment were also observed and recorded. Use 70% alcohol to wipe the experimental box between experiments, replace the white paper and food, and eliminate any rat odor and excrement cues [11].

Sugar water preference test (SPT): The experiment is a classic method to detect depressive behavior in mice, and the degree of depression in mice can be assessed according to the sugar water preference index.

Before the experiment, mice were trained to adapt to sugar-sweetened water in a quiet room: two water bottles were placed in each cage at the same time, the first 24 h, both bottles were filled with 1% sucrose water; the second 24 h, a One bottle contains 1% sucrose water and the other bottle contains distilled water. After fasting for 10 h, mice were subjected to sucrose drinking experiments. At the same time, each group of mice was given 1 bottle of 1% sucrose water and 1 bottle of distilled water. After 1 h, 2 bottles of water were taken and weighed, and the consumption of sucrose water, distilled water and total liquid consumption of each group were recorded. On the 7th, 14th, 21st, and 28th day of the experiment, each measurement was performed once. Sucrose water preference (%) = (sucrose water consumption/total liquid consumption) × 100% [12].

2.2.4 Preparation of mice and sample collection

The mouse blood samples were collected by enucleation. After blood collection, the mouse abdominal cavity was dissected to obtain the thymus, heart, liver, kidney and other organs. Liver and kidney tissues were fixed with 10% paraformaldehyde fixative for subsequent slicing experiments, and mouse tissues were frozen in an ultra-low temperature freezer for subsequent tissue protein extraction experiments. In addition, the blood of the mice should be left at 4° C for 1 h after being taken out, and then centrifuged at 3000 r/min at 4° C for 10 min. After centrifugation, the supernatant was taken as mouse serum, which was quickly frozen in liquid nitrogen and then stored in an ultra-low temperature refrigerator.

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2.2.5 Determination of serum indices

The levels of ALT, AST, Cr and urea in the serum of mice in each group were detected by an automatic biochemical analyzer.

2.2.6 Measurement of the anti-oxidation activity in mice serum and organ tissue

The oxidative stress levels in liver and kidney tissues were detected, and the contents of SOD, GSH-Px and MDA in tissue homogenate were detected according to the operation steps of the ELISA kit.

2.2.7 Histological examination of liver and kidney

After the liver and kidney tissues were washed in phosphate buffer at 4°C, the lesions were excised with a sharp blade, immersed in 10% neutral paraformaldehyde solution, and fixed for 48 hours for pathological section production. Rinse the fixed material under running water to remove the residual fixative, and start gradient dehydration after marking. In order to avoid the occurrence of hardening of the material due to the transparency of xylene, we use n-butanol for dehydration.

The whole dehydration process is as follows: the tissue is placed in a mixed solution of several different proportions of ethanol and n-butanol, the concentration gradient of ethanol is from high to low, and the dehydration process is completed by changing a concentration gradient every 2 hours. Then start to soak in wax, soak in 50% n-butanol + 50% paraffin at a constant temperature of 58 ° C for 1 hour; soak in pure paraffin for 1 hour (repeated twice) to complete the wax soaking. Embedding was then carried out as usual. Serial sections at 3 μm were performed on a microtome after embedding numbers. Go through successively: dewaxing of paraffin sections - hematoxylin staining - eosin staining - dehydration and sealing to obtain complete HE sections, and the picture results are collected under the microscope.

2.2.8 Liver histopathological analysis

The liver inflammation analysis scores of mice in each group are shown in TABLE I:

TABLE I. Knodell (HAI) scoring system for the degree of liver inflammation

Portal Inflammation	Score
No portal inflammation	0
Mild (sprinkling of inflammatory cells in ,1/3 of portal tracts)	1
Moderate (increased inflammatory cells in 1/3-2/3 of portal tracts)	3
Marked (dense packing of inflammatory cells in .2/3 of portal tracts)	4

2.2.9 Kindey histopathological analysis

The kindey sections were evaluated for inflammatory cells, glomerular atrophy. Austin scoring system was used to evaluate the degree of renal inflammation:

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Inflammation score: 0, no or little interstitial inflammation; 1, 10%-25% interstitial inflammation; 2, 26%-50% interstitial inflammation; 3, >50% interstitial inflammation.

2.2.10 Statistical analysis

All experimental data were analyzed by software Graph pad9.0, and scientific research was done by AI. (#) $P < 0.05$, significantly different from the control group; (##) $P < 0.01$, significantly different from the control group; (*) $P < 0.05$, significant difference compared with D-gal group; (**) $P < 0.01$, significant difference compared with D-gal group.

III. RESULTS

3.1 cbFed Improved Weight Loss and the General Status of the Aging Mice

Our experiment design was illustrated in Figure 1a. As exhibited in Figure 1b, mice showed a significant weight loss upon D-galactose injection compared to that of the control group. Furthermore, vitamin E or cbFeD treatment significantly reduced weight loss compared with the D-gal group. At the same time, obvious signs of aging were observed in the mice in the D-gal group, including decreased food intake, delayed response, lethargy, and withered and dull fur. Compared with the D-gal group, improved general status of mice in the VE group and cbFeD group were observed in terms of food intake, the amount and gloss of the hair and daily activity.

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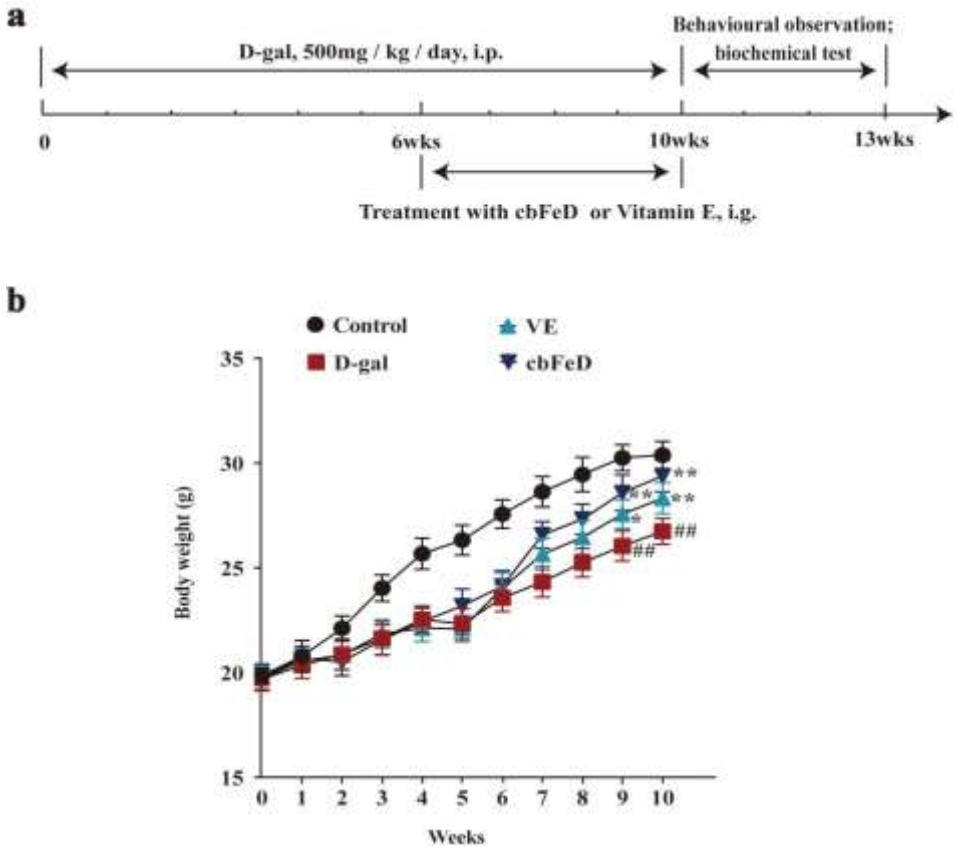

Figure1. Effect of cbFeD on body weight change in aging mice induced by D-galactose.
(a) Graphic illustration of the experiment design.
(b) The body weight of mice during the experiment period.

3.2 cbFeD Alleviated the Depression/Anxiety-Related Behavior of the Aging Mice

Anxiety and depression were commonly observed in aging mice. In order to study the effect of cbFeD on the depression/anxiety-related behavior in aging mice model, novelty-suppressed feeding test (NSF) and sucrose preference test were implemented. As shown in Figure. 2a, latency to feed in mice of the D-gal group was significant longer compared to that of the control group. Treatment with cbFeD and Vitamin E significantly decreased the latency to feed in the NSF test ($P < 0.01$). Furthermore, food consumption of mice in D-gal group was significantly less than the control group while treatment with Vitamin E and cbFeD restored food consumption significantly ($P < 0.05$) as depicted in Figure. 2b. Sucrose preference test also showed there was a significant restoration of the preference index of sucrose in mice of VE group and cbFeD group compared with mice of D-gal group despite a non-significant difference in fluid intake among each experiment groups ($P>0.05$); as depicted in Figure 2c and d. These results indicated that cbFeD alleviates the depression/anxiety-related behavior of the aging mice.

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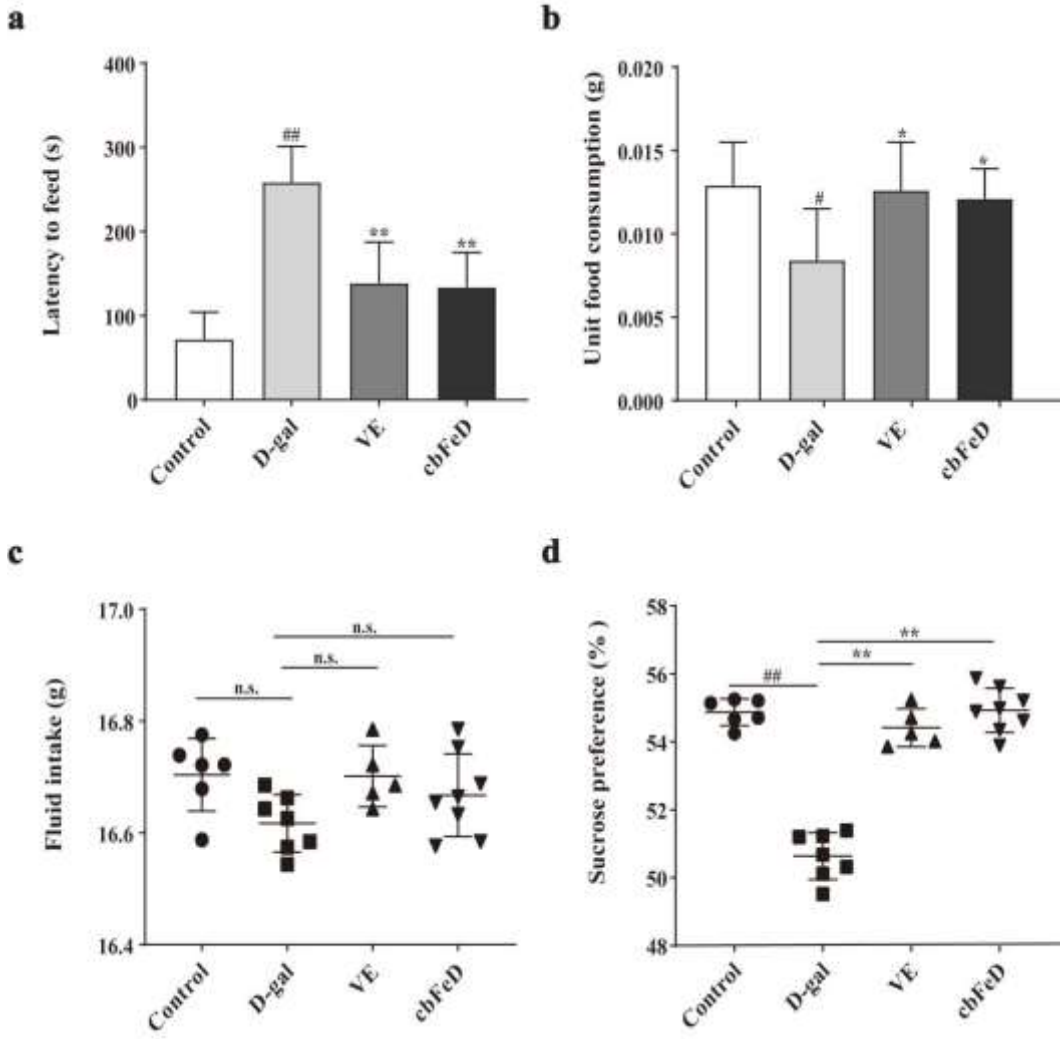

Figure 2. Effect of cbFeD on the emotional and behavioral changes in D-gal-induced aging mice. (a and b) novelty-suppressed feeding test: (a) Latency to feed; (b) Food consumption, (c and d) Sucrose preference test: (c) Fluid intake measurement; (d) Sucrose preference index, each data point represents an individual mouse.

3.3 cbFeD Restored the Organ Indices of the Aging Mice

Changes of the organ index are important parameters for evaluation of aging. As shown in Figure 3, the organ indices of liver, kidney, lung, spleen, and thymus were significantly lower in mice from the D-gal group compared to those of the control group while the heart index didn't show significant difference among these groups. The organ indexes including the liver, kidney, lung, spleen and thymus were significantly restored ($P < 0.05$) after treatment with cbFeD respectively compared to the D-gal group. Besides, treatment with Vitamin E restored these organ indexes with significance in kidney, lung and spleen.

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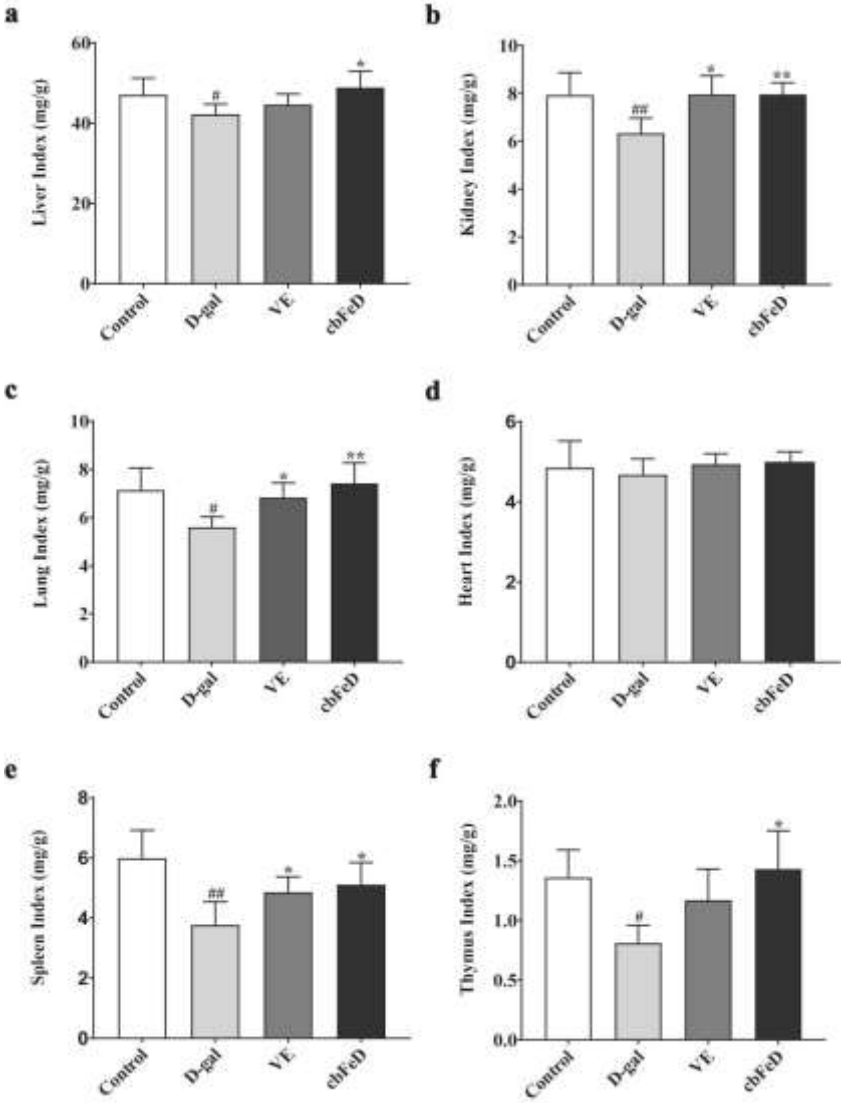

Figure 3. Effect of cbFeD on the organ indices of D-gal-induced aging mice.

3.4 cbFeD Restored the Biochemical Parameters Evaluating the Liver and Kidney Function

As shown in Figure 4a and b, the D-gal group had significantly higher serum ALT and AST levels than the control group due to aging-induced liver damage ($P < 0.05$). After treatment with cbFeD, the levels of ALT and AST in the serum of mice were significantly improved and gradually approached normal levels. At the same time, the effect of cbFeD on the renal function of D-gal-induced aging mice can be seen from the changes in serum creatinine (Cr) and urea levels of mice in each group. As shown in Figures 4c and d, the serum Cr and urea levels of mice in the D-gal group were significantly higher than those in the control group ($P < 0.01$). However, Cr and urea levels recovered significantly after cbFeD or vitamin E treatment, respectively ($P < 0.01$). These results suggest that cbFeD can improve liver and kidney function in D-gal-induced aging mice, and play a protective role in major metabolic organs such as liver and kidney.

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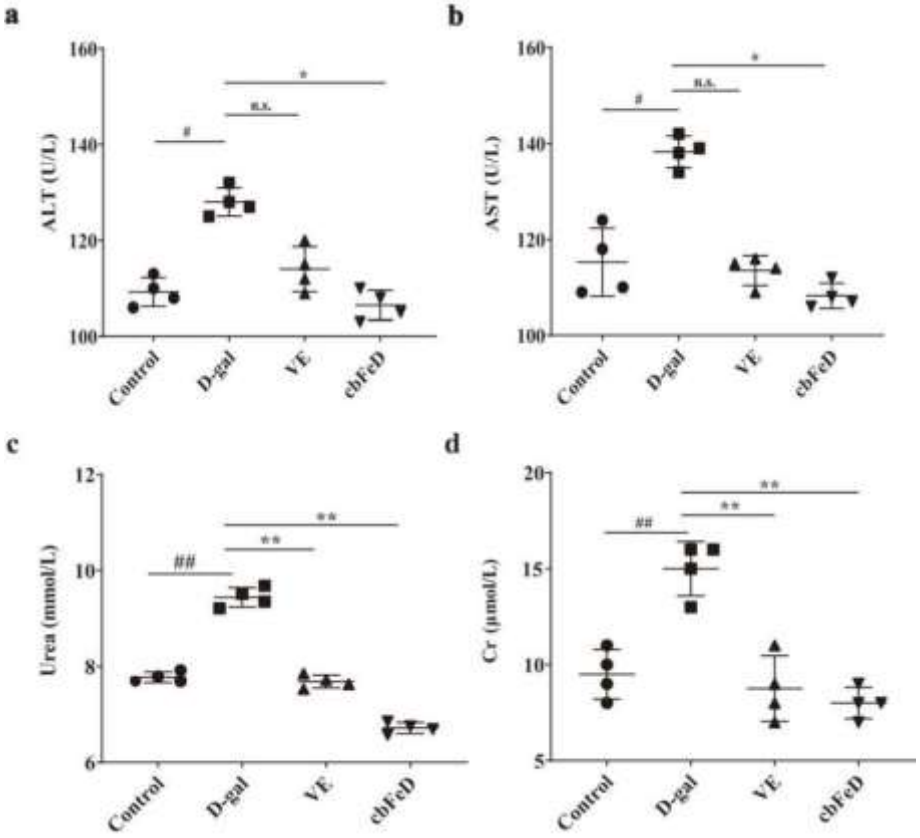

Figure 4. Effects of cbFeD on liver and kidney functions in D-gal-treated mice.
(a) ALT levels in the serum. (b) AST levels in the serum.
(c) Urea levels in the serum. (d) Cr levels in the serum.

3.5 cbFeD Alleviated Histological Changes of the Liver Tissue in D-Gal-Induced Aging Mice

As shown in Figure 5a, while the lobular structure of liver remained intact in mice of the D-gal group, the liver tissue exhibited ballooning degeneration, acidic degeneration and necrosis of hepatocytes with focal infiltration of inflammatory cells. In the liver tissue sections of the mice in the cbFeD group and the VE group, the hepatocytes were neatly arranged radially in the portal vein, and there were fewer inflammatory cell aggregation and hepatocyte ballooning degeneration. We further evaluated the inflammatory infiltration and necrosis of the liver tissue according to Knodell (HAI) score system. According to figure 5b, the inflammation score of liver tissue of mice in D-gal group was 2.83±0.24, which was significantly higher than that of the control group (1.17±0.31). However, treatment with cbFeD or Vitamine E significantly decreased inflammation score of the liver tissue to 1.33±0.24 and 1.5±0.4, respectively (Figure 5b). Necrosis score of the liver tissue in D-gal group (2.42±0.23) was also significantly higher than that of the control group (0.87±0.12) while treatment with cbFeD or Vitamine E significantly decreased necrosis score of the liver tissue to 0.92±0.18 and 0.98±0.14, respectively (Figure 5c).

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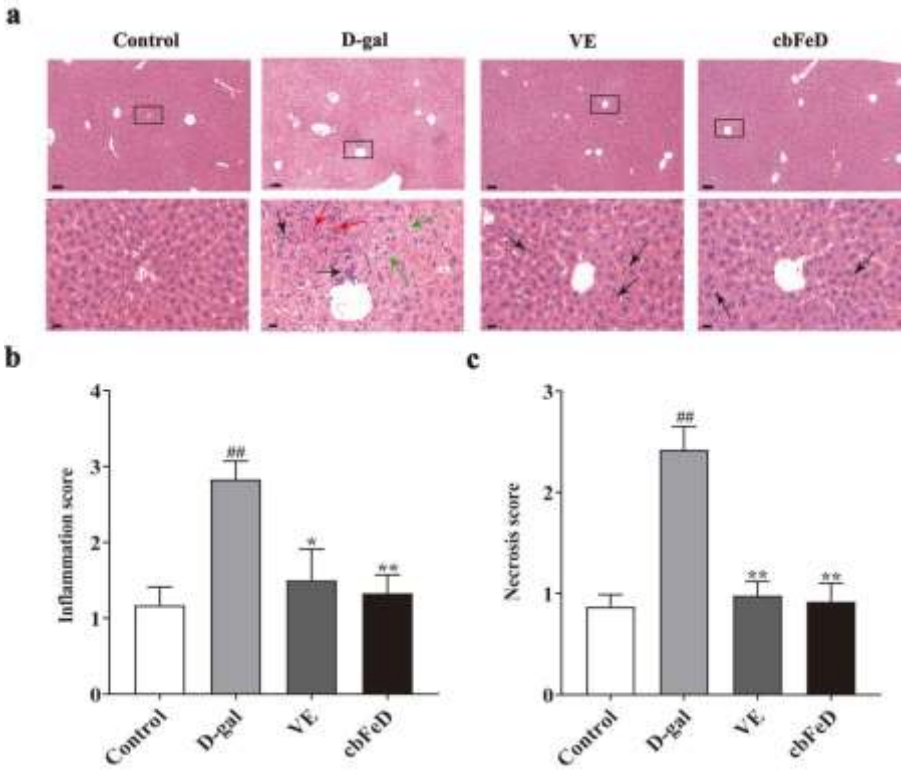

Figure 5. Effect of cbFeD on histopathological changes of liver in D-galactose induced aging mice. (a)HE sections of liver tissue of mice in each group under light microscopy at 100× magnifications (Scale bar = 100 μm) and 400× magnifications (Scale bar = 20 μm).
Green arrow: ballooning of hepatocytes; Red arrow: eosinophilia of hepatocytes;
Black Arrow: inflammatory infiltration.
(b) Inflammation and degeneration scores evaluated. (c) Necrosis score.

3.6 Effect of cbFeD on Histochemical Changes of the Kidney in D-Gal-Induced Aging Mice

Compared with the control group, the renal tissue of the mice in the D-gal group was damaged due to aging, and a large area of glomeruli atrophied or even disappeared in the HE slices, while the glomeruli widened, decreased epithelial cells, and decreased renal function. Tubular epithelial cell edema, etc (As shown in Figure 6). Notably, cbFeD and VE treatment significantly attenuated the unhealthy pathological changes in renal tissue caused by D-gal, with a protective effect on the kidneys, while the phenomenon of renal edema and balloon enlargement in the cbFeD group was largely suppressed. control. These results indicated that cbFeD successfully attenuated D-gal-induced pathological damage in mouse kidney tissue.

We further evaluated the inflammatory infiltration of the kindey tissue according to Austin score system. The inflammatory infiltration in kindey tissue of mice in D-galactose group was widely distributed, and the score was significantly higher than that in control group ($P<0.01$). However, cbFeD or vitamin E treatment can significantly reduce the inflammation score of kindey tissue ($P<0.01$). It is suggested that cbFeD can improve the kindey injury and inflammatory reaction of aging mice induced by D-galactose.

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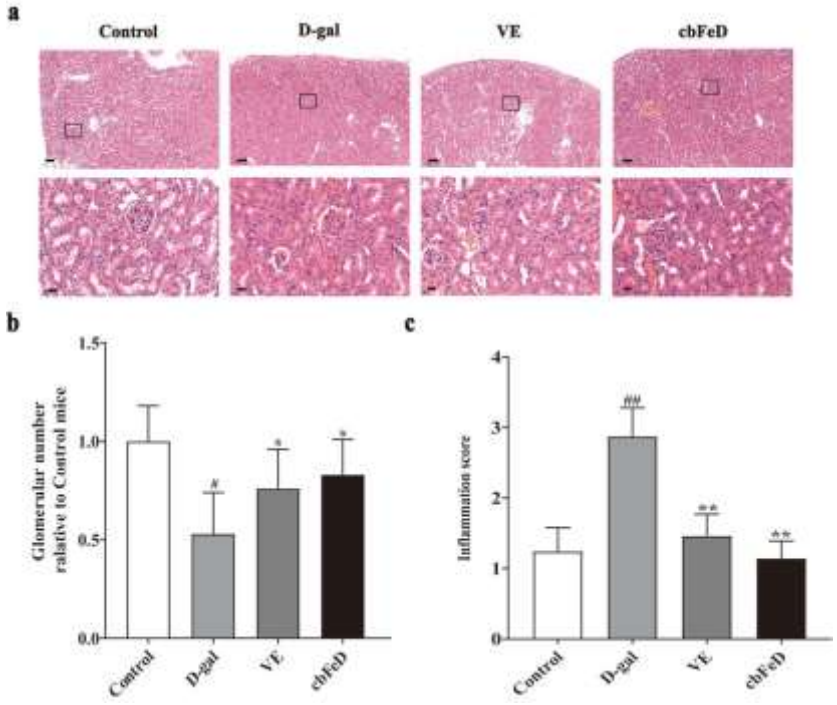

Figure 6. Effect of cbFeD on histopathological appearance changes of kindey in D-galactose induced aging mice. (a) HE sections of kindey tissue of mice in each group under light microscopy at 100× magnifications (Scale bar = 100 μm) and 400× magnifications (Scale bar = 20 μm). (b) Glomerular number relative to Control mice was determined in HE-stained sections. (c) Inflammation and degeneration scores evaluated.

3.7 cbFeD Protected the Liver and Kidney of D-Gal-Induced Aging Mice Against Oxidative Stress

In evaluating the level of oxidative stress in the liver and kidney tissue of D-gal-induced mice, we found that compared with the control group, the content of SOD and GSH-Px in the liver and kidney tissue of the D-gal group mice decreased, while MDA levels were elevated (Fig. 7a, b, c and d). Compared with the D-gal group, the concentrations of SOD and GSH-Px in the liver and kidney were significantly recovered in each treatment group. In addition, MDA decreased significantly after cbFeD and vitamin E treatment. These results suggest that in a mouse model of aging, cbFeD treatment protects the liver and kidneys of mice and largely reduces oxidative stress levels in the organs.

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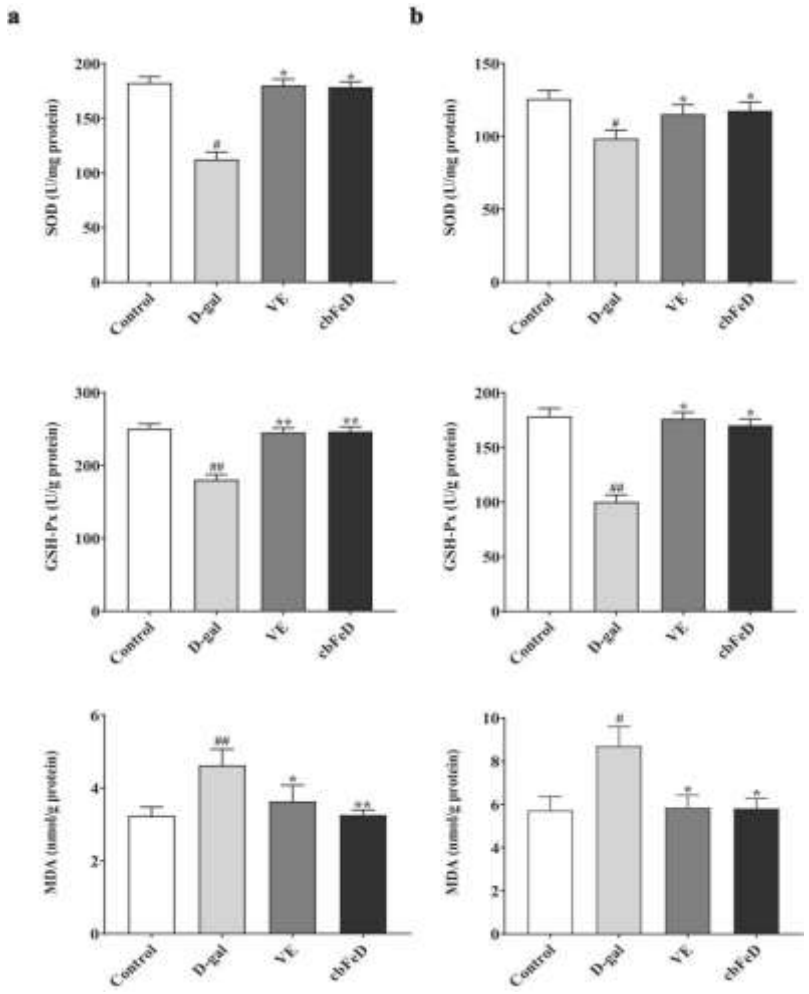

Figure 7. Effects of cbFeD on levels of oxidative stress in mouse liver (a) and kidney (b).

IV. DISCUSSION

D-galactose subacute aging model is a classic model to study anti-aging drugs, and it has been widely accepted [13]. The principle is when continuous high-dose subcutaneous injection of D-galactose will increase the concentration of galactose in the cells of the mouse body, under the catalysis of reductase, it will be reduced to galactitol [14]. Galactitol accumulates in cells and cannot be further metabolized by cells. Normal osmotic pressure will lead to cell swelling and dysfunction. This will lead to reduced immunity, metabolic disorders, decreased activity of antioxidants, accumulation of free radicals and other phenomena, which will eventually lead to aging of the body [15]. In this study, we selected D-gal-induced subacute aging in mice to study the anti-aging effect of cbFeD aqueous extract and explore its underlying mechanism. The results showed that the daily behavior status of the mice in the model group was significantly different from that of the mice in the blank group. At the same time, the spleen, thymus, heart, lung and thymus of the mice in the model group were significantly atrophied, and the organ index was significantly lower than that of the mice in the blank group. In addition, oxidative stress levels such as GSH-PX, SOD viability decreased in serum, liver and kidney of model mice, whereas MDA levels were

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reversed. These results are consistent with previous studies. And under the treatment of cbFeD, the experimental indicators of aging model mice were significantly improved [16, 17].

In the process of aging, the emotional behavior of animals will change significantly. NSF test and Sucrose preference test can detect the emotional changes and other behaviors of animals. After aging, animals will generally have anxiety behaviors, and their feeding latency and preference for sucrose solution in strange environment will be significantly reduced [18, 19]. Therefore, in these two behavioral tests, the feeding latency of the model group mice in the NSF test was prolonged, and the food consumption was significantly reduced, while in the Sucrose preference test, the preference index of the model group mice for sucrose solution was significantly reduced. After intragastric administration, the incubation period of the treatment group was significantly shortened, the food intake was significantly increased, and the preference index for sucrose solution was significantly increased [20].

Modern medical research shows that with the increase of age, Inflammatory cell aggregation caused by immune response in tissues and organs is one of the important causes of aging [21]. Body immune regulation is one of the main methods of anti-aging research. Since the spleen is closely related to humoral immunity and cellular immunity, and plays an important role, spleen atrophy will indirectly reduce the immune function of the animal. The liver and kidney are important metabolic organs. The reduction in their mass will directly affect the animal's metabolic capacity. The liver is also one of the immune organs of animals [22]. The results showed that compared with the control group, the volume of immune organs such as thymus and spleen in the model group was significantly reduced, and the weight of the thymus and spleen was significantly reduced. However, the vitamin E group and the water extract of cbFeD can significantly slow down the atrophy of the thymus, spleen, heart and other organs, and protect the liver and kidneys from oxidative damage.

In addition, the mechanism of aging is closely related to free radical-induced lipid peroxidation. Excessive free radicals can induce lipid peroxidation, and one of the most important ways to cause substantial damage to tissues and organs is to produce lipid peroxidation [23]. Under normal circumstances, the body has an antioxidant system, including SOD (an enzyme that specializes in scavenging superoxide anion free radicals and reducing lipid peroxidation) and GSH-PX (a kind of concentrated catalysis of glutathione into GSSG and Enzymes that stimulate the reduction of toxic peroxides into non-toxic hydroxyl compounds) [24]. Various antioxidant enzymes and various antioxidant nutrients exist in a coordinated and balanced relationship of mutual complementation and interdependence, so there may be a relatively complete defense system. Hydrogen peroxide generated by SOD catalyzed reaction is decomposed by catalase, and ceruloplasmin catalyzes ferrous oxidation, thereby reducing the generation of free radicals by transition metals and promoting free radical damage; there are lipid-soluble antioxidants in cells Vitamin E and PHGPx acting on membrane lipids, and water-soluble vitamin C and SeGPx [25]. The results showed that cbFeD played an antioxidant role in the serum, liver and kidney of mice, enhanced the activities of SOD and GSH-Px to resist the deposition of free radicals in tissues, and reduced the oxidation of unsaturated fatty acids by free radicals. And reduce the content of malondialdehyde to a certain extent, cbFeD can scavenge free radicals in the body and reduce damage to organs, and has a good antioxidant

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effect in the body [26, 27]. In addition, serum ALT, AST and ALP levels and kidney induced by D-gal in mice, and it also significantly reduces the abnormal increase of serum ALT, AST, Urea and Cr, which is harmful to mouse liver. And kidney function to achieve a certain degree of protection [28, 29]. In addition, histopathological examination showed that inflammatory cell infiltration, hepatocyte necrosis, and glomerular atrophy were aggravated in the model group. The treatment of VE and water extract of Codnopsis bulleyana forrest ex Diels can improve the long-term D-galactose to a certain extent, caused liver and kidney structural damage.

Based on the above results, the water extract of Codnopsis bulleyana forrest ex Diels can protect the liver and kidneys of subacutely aging mice induced by D-gal, thereby realizing its anti-aging activity. This may be related to the water extract of Codnopsis bulleyana forrest ex Diels can significantly eliminate free radicals in the body. Improve the body's antioxidant capacity.

V. CONCLUSION

Codnopsis bulleyana is a kind of natural medicinal and food homologous plant. Its protective effect on D-gal-induced subacute aging model mice is mainly through reducing the atrophy of mouse thymus, spleen and other organs, and increasing the key antioxidant enzymes. Activity, reducing the relevant marker enzymes in the liver and kidney of mice, at the same time, the study also shows that its mechanism may be related to its beneficial regulation of oxidation. These results may provide evidence supporting the potential clinical application of *Codnopsis bulleyana* forrest ex Diels as the treatment of age-related diseases.

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