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Improvement effect of enzymic polypeptide from *Paeonia ostii* seed meal on protein-energy malnutrition mouse model

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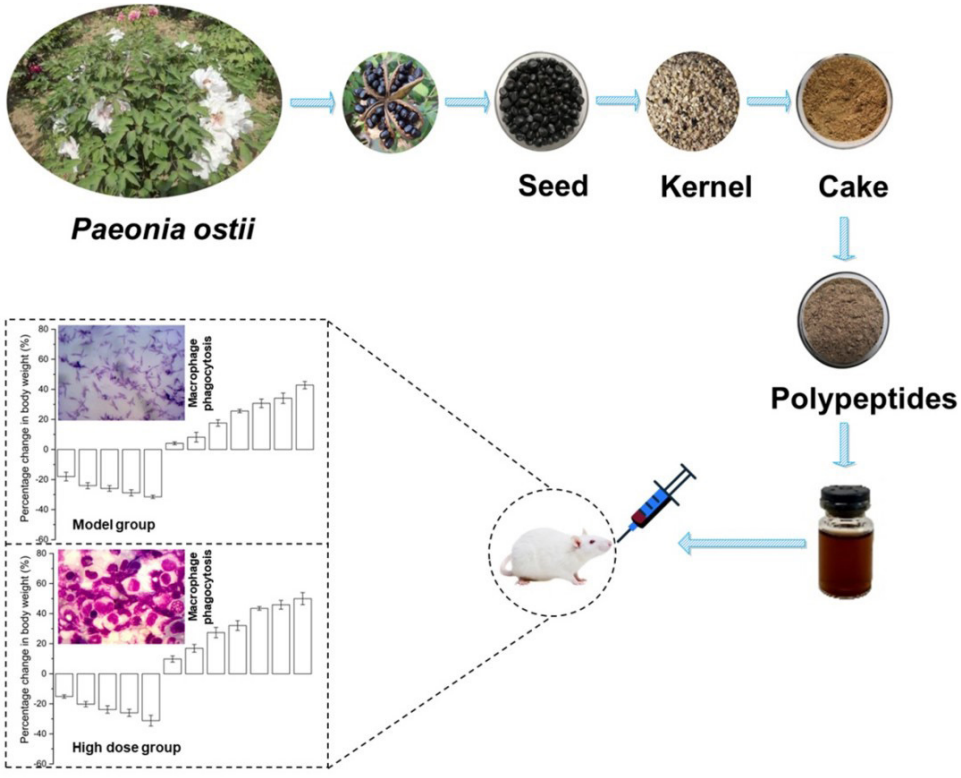
Abstract

The improvement effects of enzymatic polypeptide of *Paeonia ostii* seed meal (APHP) on model mice of protein-energy malnutrition were evaluated by body weight changes, organ indices, levels of SOD, CAT, MDA, total protein and albumin in serum, staining images of major organs and macrophages phagocytosis. The results indicated the mice body weight recovery was dose-dependent with APHP and the body weight change of model mice fed with high-dose APHP (5.0 g/kg) and normal diet showed a same recovery effect. The model mice had higher thymus and spleen indexes than other groups. And the values of SOD, CAT, MDA, total protein and albumin in serum returned to normal level after 30, 60, 45, 30 and 45 days respectively, which was better than those of normal diet. Our research will provide basic data support for new potential applications of APHP and increase the added value of *Paeonia ostii* processing enterprises.

Keywords: enzymatic polypeptide; *Paeonia ostii* seed meal; protein-energy malnutrition; recovery effect.

Practical Application: Practical Application: APHP was obtained by alkaline protease enzymatic digestion from the seed meal of *Paeonia ostii*, a traditional Chinese oil plant. APHP was applied to PEM model mice, which showed excellent protein recovery ability, good ability of anti-oxidation stress, and immune function improvement ability. This APHP can be used as a potential nutritional supplement for PEM patients.

Graphical Abstract



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1 Introduction

Chinese oil peony is a plant of *Paeoniaceae* (Xue et al., 2015), the main varieties of oil peony are *Paeonia ostii* (*Paeonia ostii* T. Hong et J. X. Zhang) and *P. rockii* (*Paeonia suffruticosa* Andr. var.papaveracea (Andr.) Kerner). In 2014, *Paeonia ostii* was listed as the main woody tree species of edible oil by the General Office of the State Council of the People's Republic of China (General Office of the State Council, 2014). According to statistics in 2015, the planting area of oil peony in China was 33.3 $\times 10^3$ ha (Han et al., 2015). The annual output of oil peony seeds was about 99.9 $\times 10^3$ tons (Han et al., 2018). If all the oil peony seeds harvested every year was used to make oil, the yield of this peony seed seed meal would reach a 40.1 $\times 10^3$ tons. These peony seed meals cannot be reasonably developed and utilized, which will cause a lot of resource waste. The utilization and research of peony in China is still in the primary stage (Gao et al., 2018). There are few reports on application of peony seed meal, and its high value-added utilization needs to be further developed.

Nutritional anemia, protein-energy malnutrition (PEM), iodine deficiency disorders and xerophthalmia were the four most common nutritional deficiency diseases in developing countries. Among them, PEM was the most common and difficult to control. Protein-energy malnutrition (PEM) was a state of consistent inadequacy of food and nutrient intake to meet body requirements leading to alterations in body weight, composition, and compromised functioning (Cederholm et al., 2017; Marshall, 2016). The starvation-related deficiency or other with mild to moderate inflammation usually led to PEM (White et al., 2012). PEM was common in hospitalized patients, often undetected and undertreated, with a prevalence of 20% to 50% (Mogensen et al., 2015; Norman et al., 2008). The incidence of adverse consequences in patients with PEM was high (Batool et al., 2015; Martín et al., 2017). The significant effects of PEM on immunocompetence were as follows: i) impaired phagocyte function; ii) antibody secretory decrease of immunoglobulin A; iii) the number decrease of helper T cells; iv) atrophy of thymus and other lymphoid tissues; v) Insufficient complement content; vi) the activity decrease of thymosin (Redmond et al., 1991; Chandra, 1992; Woodward et al., 1992; Ozkan et al., 1993).

The aim of this study is to investigate the improvement effect of enzymic polypeptide from *Paeonia ostii* seed meal on PEM mice. Our research will provide basic data support for new potential applications of enzymatic polypeptide from peony seed meal and increase the added value of *Paeonia ostii* processing enterprises.

2 Materials and methods

2.1 Materials

1-anilino-8-naphthalene-sulfonate (ANS) (Sigma Chemical Co., St. Louis, MO, USA), (5,5'-dithiobis (2-nitrobenzoic acid), DTNB) (Sigma Chemical Co., St. Louis, MO, USA), Sodium dodecyl sulfate (SDS), paraformaldehyde, mercaptoethanol and starch were purchased from Tianxiang Huabo wholesale Station in Harbin, Heilongjiang Province. Total protein test kit, Albumin test kit, Total superoxide dismutase (SOD) test kit, Catalase (CAT) test kit, Malondialdehyde (MDA) test kits, Giemsa stain, Alkaline protease, chicken red blood cells were purchased from Shanghai Yuanye Biotechnology Co., Ltd.

KM mice were provided by Heilongjiang University of traditional Chinese medicine; protein deficient diet (1% casein) and normal diet (20% casein) were purchased from Jiangsu Xietong Pharmaceutical Bioengineering Co., Ltd., all of which were SPF grade.

2.2 Preparation of polypeptide

Paeonia ostii seed meal was provided by Jiangsu Guose Tianxiang oil peony Technology Development Co., Ltd. According to the method in the previous published article (Qiao et al., 2020), the enzymatic polypeptide of alkaline protease was prepared. The crushed *Paeonia ostii* seed meal was extracted by Soxhlet extraction and water extraction method to remove residual oil and polysaccharides. The treated sample was used as raw material and hydrolyzed with alkaline protease. The enzymatic hydrolysis conditions were as followed: solid-liquid ratio of 1:5, pH of 8.0, alkaline protease dose of 3000 U per gram, enzymatic hydrolysis temperature of 50°C, enzymatic hydrolysis time of 4 h. After the end of enzymatic hydrolysis, the hydrolysate was boiled for 10 minutes and centrifuged at 10000 r·min^{-1} for 5 min. The supernatant was dialyzed and vacuum freeze-dried to obtain the enzymatic polypeptide, which was named APHP.

2.3 Determination of polypeptide concentration

The polypeptide concentration was determined using the reported Folin-phenol colorimetric method (Qu et al., 2013). 4 mL of Folin-phenol reagent A and 0.5 mL of sample were mixed and incubated at room temperature for 10 min, then 0.5 mL of Folin-phenol reagent B was added. After incubating for another 30 min at room temperature, the sample absorbance was measured at 500 nm on a spectrophotometer (TU-1810, Puxi General Instrument Co., Ltd., China). The polypeptide concentration of sample was calculated through the standard curve of bovine serum albumin (0-1 mg/mL).

2.4 Other composition analysis

Ash was estimated according to Association of Official Analytical Chemists method (Association of Official Analytical Chemists, 2000). The contents of K, Na, Ca, Mg, Fe, Cu and Zn in the samples were determined according to the wet digestion method proposed (Nascimento et al., 2010). 0.2000 g of sample was added into a polyfluoroethylene tube containing 10 mL of HNO_3 and $HClO_4$ (v/v, 4:1) mixed solvents. The tube was sealed and heated in an aluminum block at 130 °C for 60 min. After cooling to room temperature, 2 mL of H_2O_2 was added and the solution was heated again to 115 °C and kept for 10 min. Then, 0.1 mL of 40% HF was added, heated at 115 °C for 10 min, and 2 mL of H_2O_2 was added a second time until the solution was clear. After digestion and cooling to room temperature, the solution was diluted with 2% dilute nitric acid into a 20 mL volumetric flask. The sample was measured by Vista-MPX Simultaneous ICP-OES (PE, Inc., USA) (Kose et al., 2022).

2.5 Determination of functional properties

Solubility

The APHP (200 mg) was dispersed in 20 mL of distilled water to determine its solubility. The solution was placed in a 25 °C water bath and stirred for 60 min, followed by centrifugation at 4000 r·min^{-1} for 15 min. The protein content in supernatant was determined by biuret method. The total protein content of sample was determined by Kjeldahl method. Polypeptide solubility was calculated as follows (Equation 1):

$$S(\%)=\frac{W_1}{W_0}\times 100 \quad (1)$$

Where, S was the APHP solubility. W_1 was the protein content in supernatant. W_0 was total protein content in the APHP.

Surface hydrophobicity (H_0)

Surface hydrophobicity (H_0) of sample was determined using ANS as a fluorescence probe according to the literature (Alavi et al., 2021). 20 µL of ANS (0.008 M in 0.01 M phosphate buffer, pH 7.0) was added to 4 mL of APHP solution (0.01, 0.05, 0.10, 0.15 and 0.2 mg/mL in 0.01 M phosphate buffer of pH 7.0, respectively). After incubation in the dark for 5 min, the supernatant was obtained by centrifugation at 10000 r·min^{-1} for 5 min. The fluorescence intensity was measured with a fluorescence spectrophotometer (Hitachi F-7000 High-Technologies Corporation, Tokyo, Japan), and the excitation and emission wavelengths were set to 287 nm and 352 nm, respectively. The linear regression equation was established with protein concentration as X-axis and fluorescence intensity as Y-axis, and the slope was regarded as the index of H_0.

Determination of sulfhydryl (SH) and disulfide bond (SS) concentration

The concentration of sulfhydryl (SH) groups of APHP was determined using Ellman's reagent (DTNB), according to the literature (Andlinger et al., 2021). 15 mg of the APHP was dissolved in 3 mL of standard buffer (0.086 M Tris, 0.09 M glycine, and 0.004 M EDTA, pH 8.0) with 8 M urea and 0.05 mL of Ellman's reagent solution (4 mg/mL DTNB in standard buffer). After the solution rapidly mixed and left at 25 °C for 15 min, the absorbance was recorded at 412 nm. The control sample was measured with standard buffer instead of sample solution. The APHP blank was measured with 0.05 mL of the buffer instead of Ellman's reagent solution. The concentration of sulfhydryl groups was calculated according to the following equation (Equations 2-3) (Chandrapala et al., 2011):

$$C_{SH}(\mu\text{mol}\cdot\text{g}^{-1})=\frac{73.53\times\Delta A_{412}\times D}{C} \quad (2)$$

$$\Delta A_{412}=A_{\text{with DTNB}}-A_{\text{without DTNB}} \quad (3)$$

Where, C_{SH} was total free thiol groups. D was the dilution factor. C was the APHP concentration (mg·mL^{-1}). ΔA_{412} was the net absorbance measured at 412 nm by the sulfhydryl groups with/without DTNB.

The concentration of SS bonds was determined according to the following method. In brief, 15 mg of the APHP was dissolved in 3 mL of standard buffer (0.086 M Tris, 0.09 M glycine, and 0.004 M EDTA, pH 8.0) with 8 M urea, 0.02 mL of 0.2% mercaptoethanol was added and incubated at 25 °C for 2 h. 15 mL of standard buffer and 0.05 mL of Ellman's reagent were added in turn. After mixing, the sample absorbance was measured at 412 nm. The concentration of SS groups was calculated according to the following equation (Equation 4):

$$C_{SS}(\mu\text{mol}\cdot\text{g}^{-1})=\frac{C'_{SH}-C_{SH}}{2} \quad (4)$$

Where, C'_{SH} was the concentration of the sulfhydryl groups from reduction of SS groups and total free thiol groups (C_{SH}). Both C'_{SH} and C_{SH} were determined according to Equation 2.

2.6 Animal procedures

60 female KM mice, weighing between 18-22 g, were randomly divided into six groups (Figure 1). The mice in the model group I to V were fed a protein-deficient diet containing only 1% casein, and the mice in the control group were fed a normal diet containing 20% casein. The mice were housed in a rearing cage with a temperature of 20-24 °C, humidity of 35-60%, and a light-dark cycle of 12 h. The weight changes of mice were monitored every 5 days. Mice were considered to have established a model of moderate malnutrition when they were fed a restrictive diet (containing 2% casein) resulting in a weight loss of more than 30% (Garg et al., 2017).

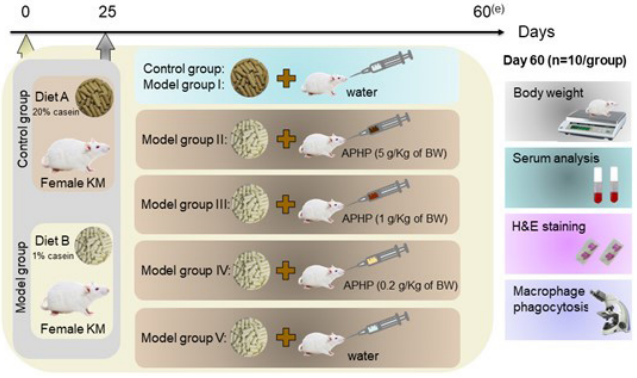

Figure 1. Experimental design. Mice in the control group and model group were fed with Diet A (containing 20% casein) and Diet B (containing 1% casein) respectively for 25 days. During the treatment period (from the 26th day to the 60th day), mice in model II to IV groups were continuously fed with Diet B and gavaged with different concentrations of APHP solution (5,1 and 0.2 g/kg WB, respectively). The mice in model group V were also fed with Diet B, and gavaged with the same amount of water as APHP solution. Mice in the control group and model group I were fed with Diet A and gavaged with the same amount of water as APHP solution. The mice were euthanized on the 60th day. H&E = hematoxylin and eosin; e = euthanasia.

After successful modeling, the model group I was fed with ordinary diet containing 20% casein, and model groups II to IV were fed with different doses of APHP (5.0, 1.0 and 0.2 g/kg, respectively) and were continuously fed with protein-deficient diet to ensure the adequacy of other nutrients. The body weight and nutritional recovery of the mice were recorded and analyzed every 5 days. Mouse blood samples were collected every 15 days to detect and analyze the blood phase index. The study was carried out according to the "Experimental Animal Management Ordinance of Heilongjiang Province".

2.7 Macrophages phagocytosis

Mouse peritoneal macrophages were induced by intraperitoneal injection of 0.5 mL 0.5% starch-saline suspension. After 48 h, 0.5 mL of 5% saline-chicken red blood cell suspension was intraperitoneally injected again. After another 12 h, the mice were dislocated from the cervical spine and 2 mL of saline was injected into the abdominal cavity of mice. The mice were gently pressed on the abdomen for 1 min, the syringe needle was inserted into the abdominal cavity, 1 mL of peritoneal fluid was aspirated, and then dropped into two glass carrier slides, placed in an enamel box with wet gauze, transferred to an incubator at 37 °C for 30 min, rinsed with saline and dried. Cells were fixed with acetone-methanol (V/V = 1:1) solution for 5 min, air-dried, stained with 4% Giemsa phosphate buffer for 30 min, washed with running water, and dried naturally. The morphology of macrophages was observed under an inverted microscope.

2.8 SOD, CAT, MDA, total protein and albumin analysis

The contents of SOD, CAT, MDA, total protein and albumin in serum of mice collected in the above experiments were detected by kits.

2.9 Organ morphological analysis

At the end of the experiment, the mice were sacrificed by cervical dislocation and completely autopsied after the last weighing. The organs of thymus, spleen, liver, kidney, brain and heart were weighed and stored in 4% paraformaldehyde. The tissue samples were routinely embedded in paraffin, sectioned and stained with haematoxylin and eosin, and then observed and photographed under the microscope.

2.10 Statistical analysis

All the experiments were carried out in triplicate and all data were expressed as mean ± standard deviation (SD).

3 Results and discussion

3.1 APHP composition and properties

In Table 1, the protein content of APHP (85.79%) was higher compared to *Paeonia ostii* seed meal (41.60%). In addition, the ash content of APHP was 5.49%, of which the Na content was 0.984%, which was caused by the use of NaOH to adjust the pH of the solution during the enzymatic hydrolysis extraction of APHP. Solubility was one of the most important functional properties of enzymatic hydrolysis polypeptide, which was related to hydrophilic amino acids (Trevino et al., 2007). APHP with low H_0 (81.56) had excellent solubility and 98.84% solubility in aqueous solution of pH 7.0 at 25 °C. Low H_0 indicated that more hydrophilic groups and regions were formed on the surface of APHP during the enzymatic hydrolysis process. Disulfide bonds were usually formed by the interaction of intra- and inter-proteins (Zhang et al., 2022). High free sulfhydryl content contributed to increase protein activity, including antioxidant activity (Hu et al., 2022) and stability (Zou et al., 2022). Compared with *Paeonia ostii* seed meal, APHP had lower contents of disulfide bonds (11.64 μmol/g) and higher contents of free sulfhydryl groups (15.29 μmol/g), which indicated that APHP underwent the unfolding reaction and reduction reaction of disulfide bonds. So, the APHP had greater activity than *Paeonia ostii* seed meal.

3.2 Changes of body weight

Figure 2 showed the feeding regimen and body weight changes of mice during the modeling and treatment phases (60 days

Table 1. The composition and properties of APHP.

Item		*Paeonia ostii* cake	APHP
Composition	Protein (%)	41.60 ± 2.08	85.79 ± 3.28
	Ash (%)	5.20 ± 0.46	5.49 ± 0.75
	Polypeptide concentration (mg/g)	27.98 ± 4.11	465.41 ± 30.27
Mineral contents	K (g/100 g)	0.269 ± 0.037	0.485 ± 0.044
	Ca (g/100 g)	0.622 ± 0.032	0.202 ± 0.019
	Na (g/100 g)	0.014 ± 0.001	0.984 ± 0.049
	Mg (g/100 g)	0.266 ± 0.013	0.303 ± 0.011
	Cu (g/100 g)	0.006 ± 0.0004	0.003 ± 0.0005
	Fe (g/100 g)	0.134 ± 0.017	0.018 ± 0.002
	Zn (g/100 g)	0.052 ± 0.006	0.006 ± 0.0004
Functional properties	Solubility (%)	--	98.84 ± 3.94
	Surface hydrophobicity (H_0)	--	81.56 ± 4.08
	Total free Sulfhydryl groups (C_{SH}) (μmol/g)	5.79 ± 0.48	15.29 ± 0.76
	Total Sulfhydryl groups (C'_{SH}) (μmol/g)	36.75 ± 2.83	38.56 ± 1.92
	Disulfide bond (C_{SS}) (μmol/g)	15.48 ± 1.77	11.64 ± 1.58

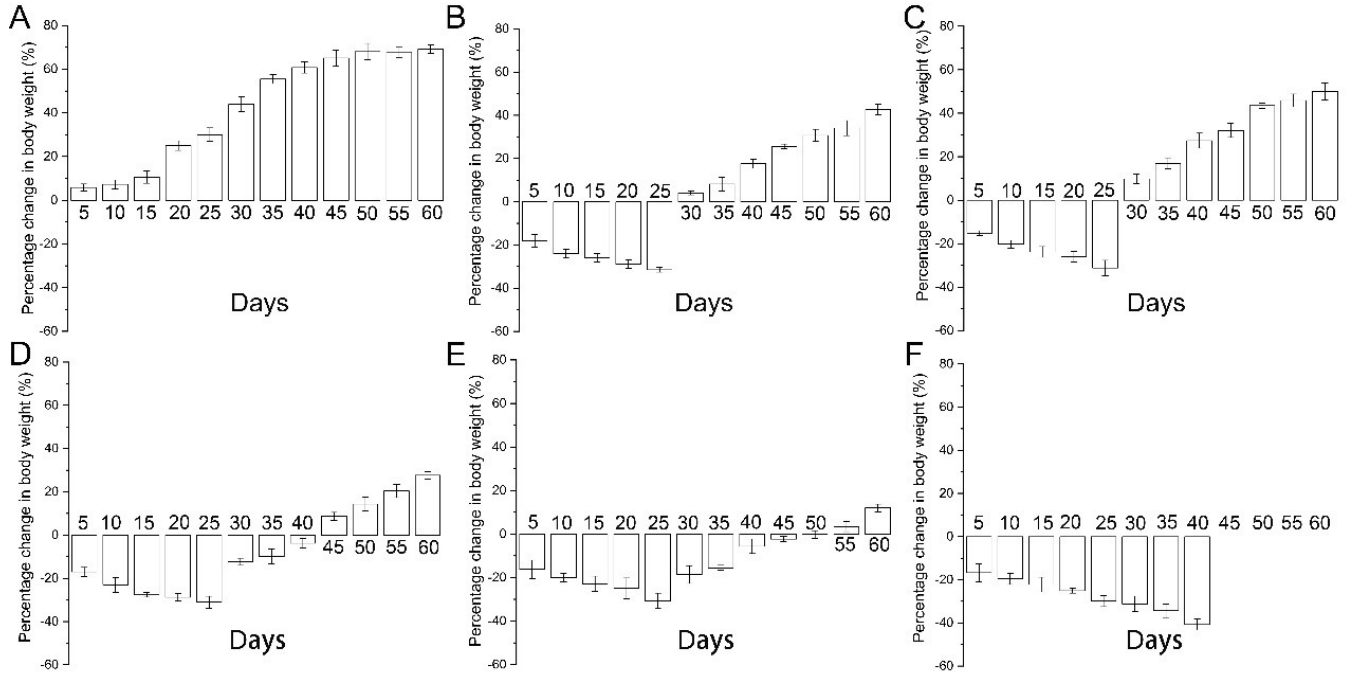

Figure 2. Effect of different doses of APHP on percentage change in body weight (%) of protein-energy malnutrition mouse model. (A) Control group: Control group mice fed with normal diet. (B) Model group I: Protein-energy malnutrition mice fed with normal diet. (C) Model group II, (D) Model group III and (E) Model group IV: Protein-energy malnutrition mice fed with protein-deficient diet and different dose of APHP (5.0, 1.0, and 0.2 g/kg, respectively). (F) Model group V: Protein-energy malnutrition mice only fed with protein-deficient diet.

in total). On the 25th day of the experiment, the mice in each group lost more than 30% of their body weight, which marked the successful establishment of moderate malnotrition mouse model. At the same time, a new feeding regimen was started, the mice in the control group continued to be fed with normal diet; mice in model group I were only fed with normal diet; mice in model groups II, III and IV were fed with protein-deficient diet in addition to different doses of APHP by gavage (5.0, 1.0 and 0.2 g/kg, respectively); mice in model group V were only fed with protein-deficient diet. On the 30th day, the body weight of mice in model group I to IV began to recover, especially the body weight of mice in model groups I and II had returned to the pre-model level. This indicated that feeding high-dose of APHP and normal diet had the same recovery effect on mouse body weight. On the 40th day, only the mice in model group V continued to lose body weight. For the ethical consideration, the experiment of mice in model group V was stopped. On the 45th day, the body weight of mice in model group III also returned to the pre-model level. On the 55th day, the body weight of mice in model group IV also returned to the pre-model level. On the 60th day, the experiment was terminated. At this time, the body weight of mice in the control group, in model group I and in model group II increased by 69.16 ± 1.88%, 42.81 ± 2.43% and 49.98 ± 3.99% respectively. It was seen that APHP significantly better than the mice in model group I. This also confirmed that APHP had better improvement effect on protein-energy malnutrition mouse model than normal diet due to its high absorption rate and good biological activity.

On the whole, the body weight of mice (the control group) fed with normal diet showed a continuous increase trend (Figure 2A), while the body weight of mice (model group V) fed with protein-deficient diet showed a continous decrease trend (Figure 2F). The body weight of mice (model group I) fed with normal diet (20 g/kg casein) and mice (model group II) fed with high-dose of APHP (5.0 g/kg) showed similar recovery trends and similar inflection point time (Figure 2B-2C). Figure 2D-2E showed the effects of medium-dose (1.0 g/kg) and low-dose (0.2 g/kg) of APHP on the body weight of mice (model groups III and IV), respectively. From Figure 2C-2E, it was found that there were significantly differences in the inflection point time of body weight change in mice, which indicated that the body weight recovery of mice was dose-dependent with APHP.

3.3 Organ index

As shown in Figure 3, the thymus and spleen indexes of model group II fed with high-dose of APHP were higher than those of other groups, including the control group fed with normal diet, which indicated that the APHP could effectively improve the immune function of mice. The model group I and II fed with normal diet and high-dose of APHP had similar liver, kidney, brain and heart indexes. The results showed that high-dose of APHP had more comprehensive recovery effect than intact protein. Because the normal diet was rich in oil (7% oil content), it caused the fat accumulation and some inflammation in the liver. Therefore, the control group fed with normal diet had higher liver index than other groups. The results were also consistent with the results of liver pathological section of the control group. The liver index of model group V fed with protein-deficient diet was higher than that of other groups except the

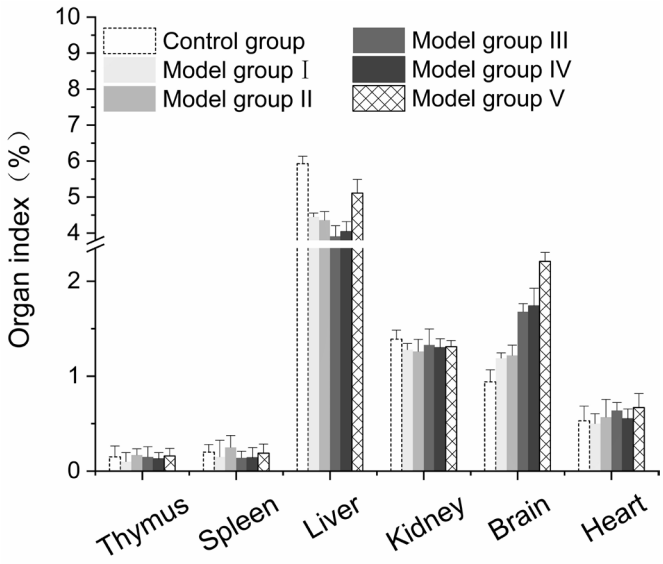

Figure 3. Effect of different doses of APHP on organ indexes of protein-energy malnutrition mouse model, including thymus, spleen, liver, kidney, brain and heart. Control group: Control group mice fed with normal diet. Model group I: Protein-energy malnutrition mice fed with normal diet. Model group II, Model group III and Model group IV: Protein-energy malnutrition mice fed with protein-deficient diet and different dose of APHP (5.0, 1.0, and 0.2 g/kg, respectively). Model group V: Protein-energy malnutrition mice only fed with protein-deficient diet.

control group and its brain index was higher than that of other groups. The results were related to low body weight, brain tissue abnormal swelling and inflammation in model group V, which was also confirmed by the results of brain pathological section.

3.4 Determination of SOD, CAT, MDA, total protein and albumin in serum

The physiological activities of the human body are closely related to oxygen. Due to incomplete reduction, a large number of toxic reactive oxygen species (ROS) are generated during the process (Omoregie & Osagie, 2011; Powers et al., 2011), including hydrogen peroxide, hydroxyl radical and superoxide radical, which results in a series of oxidative stress reaction (Fu et al., 2022). As a marker, MDA is often used to detect the oxidative stress (Reiter et al., 2014; Ho et al., 2013). There are some antioxidant mechanisms against ROS production and oxidative damage in cells (Li et al., 2022). Cellular antioxidant enzyme, including SOD and CAT, can effectively remove hydrogen peroxide and lipid peroxide, and efficiently detoxify superoxide anion (Mruk et al., 2002). The results of SOD, MDA and CAT in mouse serum showed that the values of CAT and SOD increased and value of MDA decreased with the increase of APHP dose (Figure 4A-4C). From Figure 4, it was seen that values of SOD, MDA and CAT in model group II fed with high-dose of APHP returned to the normal level after 30, 60 and 45 days. The mean values of SOD, MDA and CAT in control group were 87.2 U/mL, 5.76 nmol/

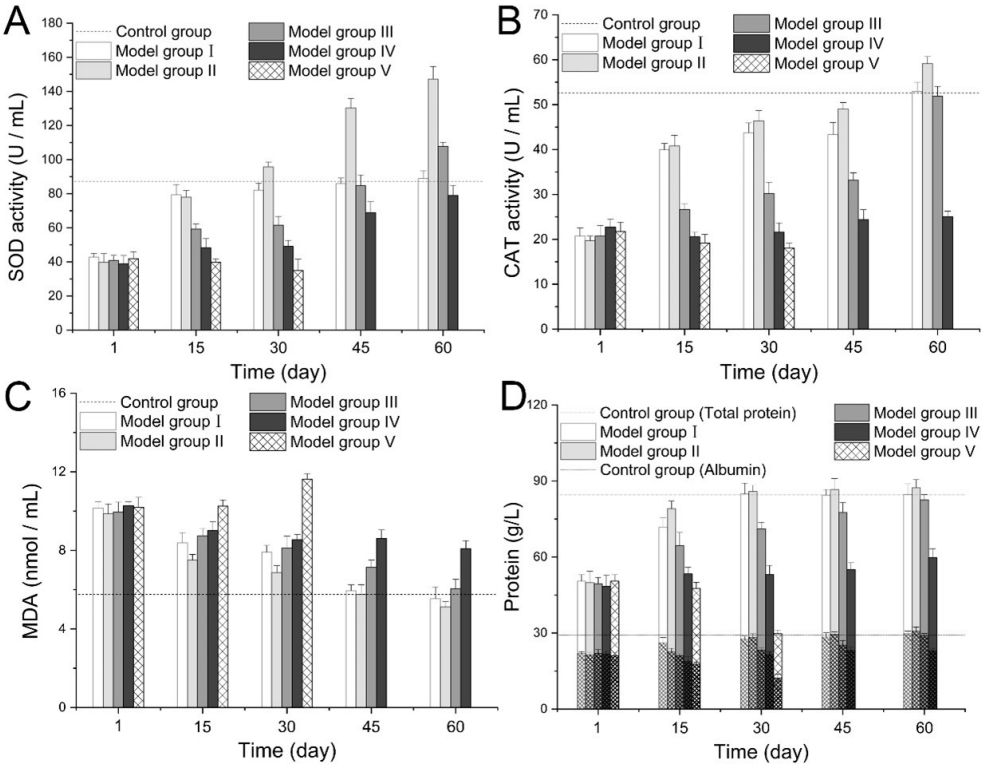

Figure 4. Effect of different doses of APHP on the superoxide dismutase (A), activities of catalase (B), methane dicarboxylic aldehyde (C) and the contents of total protein and albumin (D) in mouse serum of protein-energy malnutrition. Control group: Control group mice fed with normal diet. Model group I: Protein-energy malnutrition mice fed with normal diet. Model group II, Model group III and Model group IV: Protein-energy malnutrition mice fed with protein-deficient diet and different dose of APHP (5.0, 1.0, and 0.2 g/kg, respectively). Model group V: Protein-energy malnutrition mice only fed with protein-deficient diet.

mL and 52.7 U/mL, respectively. APHP was superior to the intact protein of normal diet, which could effectively improve SOD and CAT activities and inhibit MDA production. It had a good ability of anti-oxidation stress.

The total protein content in human tissue fluid plays an important role in health (Murakami et al., 2015). Human immune function is closely related to total serum protein, which can not only maintain plasma pH, vascular osmotic pressure and transport various metabolites, but also regulate the physiological function of transport substances. Total protein in serum is also used to reflect the nutritional status of the body (Koyama et al., 2016). Albumin not only affects the integrity of microvessels, antioxidant and inflammatory pathway regulation, but also improves the recruitment and function of immune cells (O'Brien et al., 2014; Vincent et al., 2016). By detecting the contents of total protein and albumin in mouse serum, it was found that the values of total protein and albumin were also dose-dependent with APHP, and increased with the increase of APHP dose (Figure 4D). The values of total protein and albumin in serum of model group II fed with high-dose of APHP had better recovery ability than that in serum of model group I fed with normal diet, especially albumin, which also implied that the high-dose of APHP restored the body's immune function very well. In this study, the values of total protein and albumin in serum of model group II returned to normal after 30 and 45 days, respectively. However, the values of total protein and albumin in model group V fed with protein-deficient diet showed a downward trend during the experiment.

3.5 Analysis of H&E staining and macrophage phagocytosis

The morphology and structure of cerebral cortex in control group fed with normal diet were normal without swelling. The cytoplasm and nucleus staining were clearly visible, and no obvious inflammatory cells were found (Figure 5A1). However,

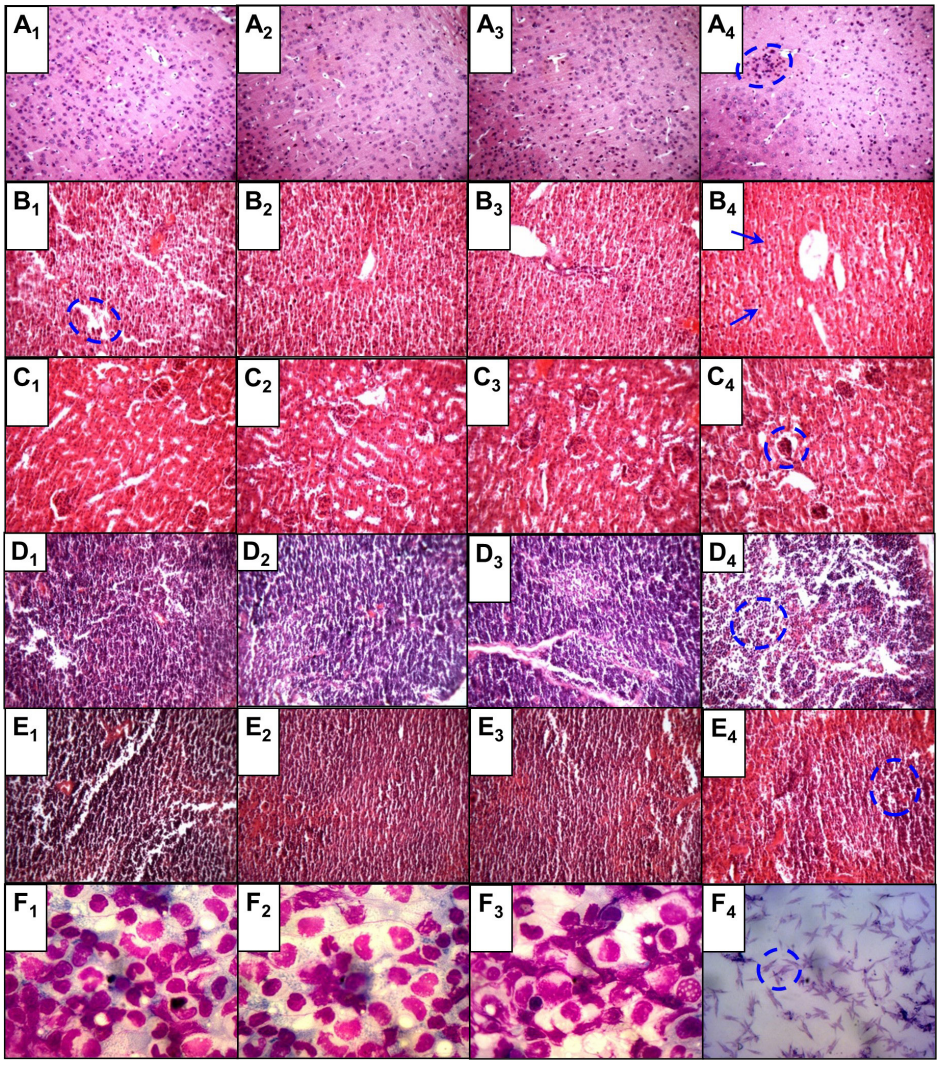

Figure 5. Effect of APHP on H&E staining images of the major organs, immune organs and macrophages phagocytosis, including (A) brain, (B) liver, (C) kidney, (D) thymus, (E) spleen and (F) the macrophages' phagocytosis of chicken red blood cell (CRBC). (A1-F1) Control group: Control group mice fed with normal diet. (A2-F2) Model group I: Protein-energy malnutrition mice fed with normal diet. (A3-F3) Model group IV: Protein-energy malnutrition mice fed with protein-deficient diet and the dose of APHP (5.0 g/kg). (A4-F4) Model group V: Protein-energy malnutrition mice only fed with protein-deficient diet.

the morphology of cerebral cortex tissue in model group V fed with protein-deficient diet was relatively complete, the tissue structure was loose and there was abnormal swelling. The cytoplasm of some nerve cells was deeply stained and the nucleus was obviously pyknotic. The inflammatory cells were scattered and occasionally gathered in piles, as shown by the circle in Figure 5A4. The morphology and structure of cerebral cortex were similar in model group I fed with normal diet (Figure 5A2) and model group II fed with high-dose APHP (Figure 5A3), and close to the control group fed with normal diet. The morphology and structure of liver tissue in control group fed with normal diet was loose and adipose tissue was obviously visible, as shown by the circle in Figure 5B1. In model group V fed with protein-deficient diet, the arrangement of hepatocytes was irregular and the gap between hepatocytes became narrower, as shown by the arrows in Figure 5B4. Some hepatocytes were fused, nuclei were pyknosis and the number of hepatocytes decreased. In model group I fed with normal diet (Figure 5B2) and model group II fed with high-dose APHP (Figure 5B3), the morphology and structure of liver tissue were similar and superior to that in control group. The glomeruli and tubules in the kidney tissue were significantly atrophied in model group V fed with protein-deficient diet, as shown by the circle in Figure 5C4. The morphology and structure of kidney tissue were similar in control group fed with normal diet (Figure 5C1), model group I fed with normal diet (Figure 5C2) and model group II fed with high-dose APHP (Figure 5C3). In model group V fed with protein-deficient diet, the normal structure of thymus tissue was destroyed and loose, as shown by the circle in Figure 5D4, and the connective tissue proliferated and the number of lymphocytes decreased significantly. The morphology and structure of thymus were similar in model group I fed with normal diet (Figure 5D2) and model group II fed with high-dose APHP (Figure 5D3), which was better than that in model group V fed with protein-deficient diet, but weaker than that in. control group fed with normal diet. It was seen that the spleen tissue structure was loose and the lymphocyte was significantly reduced in model group V fed with protein-deficient diet, as shown by the circle in Figure 5E4. The morphology and structure of spleen tissue were similar in model group I fed with normal diet (Figure 5E2) and model group II fed with high-dose APHP (Figure 5E3), superior to model group V fed with protein-deficient diet.

The microscopic image of the macrophages' phagocytosis of chicken red blood cell (CRBC) was shown in Figure 5F. The microscopic image of macrophages' phagocytosis of CRBC in model group V fed with protein-deficient diet was significantly different from that in the other three groups, and there was no obvious macrophages' phagocytosis of CRBC, as shown by the circle in Figure 5F4. The macrophages' phagocytosis of CRBC in model group II fed with high-dose APHP (Figure 5F3) was slightly stronger than that in model group I fed with normal diet (Figure 5F2), but lower than that in control group fed with normal diet (Figure 5F1). Due to the extreme lack of protein for a long time, the average body weight and immunity of the model mice were lower. This situation was significantly improved by feeding APHP.

4 Conclusion

In this study, the improvement effect of enzymatic polypeptide of *Paeonia ostii* seed meal was evaluated on model mice of protein-energy malnutrition by the body weight changes, organ indexes, levels of SOD, CAT and MDA in serum, total protein and albumin in serum, tissue section images of major organs. The results indicated the body weight recovery of model mice was dose-dependent with APHP. The model mice fed with high-dose APHP had higher thymus and spleen indexes and similar liver, kidney, brain and heart indexes with the model mice fed with normal diet. In addition, the recovery abilities of SOD, CAT, MDA, total protein and albumin in model group fed with high-dose APHP were stronger than that in model group fed with normal diet, and the recovery abilities were dose-dependent with APHP. In general, APHP showed excellent protein recovery ability, good ability of anti-oxidation stress, and immune function improvement ability. Our research will provide basic data support for new potential applications of APHP and increase the added value of *Paeonia ostii* processing enterprises.

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