Antifatigue, Antioxidant and Immunoregulatory Effects of Peptides Hydrolyzed from Manchurian Walnut (*Juglans mandshurica* Maxim.) on Mice

FANG Li[†], REN Dayong[†], CUI Lingyu, LIU Chunlei, WANG Ji, LIU Wei, MIN Weihong^{*}, LIU Jingsheng

College of Food Science and Engineering, Jilin Agricultural University; National Engineering Laboratory on Wheat and Corn Further Processing, Changchun 130118, China

Abstract: The Manchurian walnut (*Juglans mandshurica* Maxim.) is rich in proteins, whereas this resource has not been used efficiently. The antifatigue, antioxidative and immunoregulatory effects of Manchurian walnut hydrolysate peptides (MWHPs) were evaluated in this study. MWHPs with a degree of hydrolysis of 32.23% were ultrafiltered and divided into three fractions, namely, high (> 10 kDa), medium (3–10 kDa), and low molecular weight (< 3 kDa), and then fed to mice continuously at doses of 200, 400 or 800 mg/(kg·d). The antifatigue, antioxidative, and immunoregulatory effects of the peptides were tested on the second and fourth weeks of MWHP administration. Results showed that low-molecular-weight MWHPs exerted significant antifatigue (prolonging swimming time, elevating liver glycogen contents, and reducing lactic acid contents), antioxidative (enhancing superoxide dismutase (SOD), GSH-Px, and catalase (CAT) activities and reducing malondialdehyde (MDA) content), and immunoregulatory (raising the immune-organ index and promoting T-lymphocyte proliferation and sIgA secretion in the intestinal tract) effects. This research indicates that MWHPs have potential applications in health care and may be developed as a base for new functional foods.

Keywords: Manchurian walnut; Hydrolysate peptide; Antifatigue property; Antioxidant activity; Immune modulation

1 Introduction

Over the last several years, researchers have shown increased interest in the bioactive peptides of natural foods. Bioactive peptides are peptides with beneficial biological activities that can adjust the physiological function of an organism. As a short amino acid chain, bioactive peptides usually contain 3–20 amino acid residues and positively affect human health^[1]. Several bioactive peptides, including antithrombotic peptides^[2], mineral-binding peptides^[3], antibacterial peptides^[4], angiotensin-I-converting enzyme inhibitory peptides^[5], antioxidant peptides^[6] and immunomodulatory peptides^[7] have been found. They are commonly prepared by protease hydrolysis. Considering their benefits to human health, bioactive peptides may have broad applications in biomedicine, functional foods, and cosmetics.

Juglans mandshurica Maxim., also known as Manchurian walnut, belongs to a group of deciduous broadleaf *Juglans* species in Juglandaceae and is grown on Changbai Mountains, in northeast China^[8]. Walnuts from Changbai Mountains have high nutritional value and are rich in proteins (15%–20%), fats (60%–66%), carbohydrates (1%–15%), vitamins and other micronutrients^[9]. In addition, Manchurian walnut meat can improve the body's lipid profile by decreasing cholesterol levels

and can reduce the risk of coronary heart diseases^[10]. The current utilization of Manchurian walnut mainly is focused on the oil and protein preparation as well as production of other rough-wrought products. Unfortunately, few deep-processed products with high added value are produced from the Manchurian walnut. Nonfat walnut meat, which is a byproduct of walnut oil, contains as much as 45.30% of protein, and 18 kinds of amino acids. However, it is rarely processed and utilized. In fact, it is mostly used in animal feed and other low-value products, meaning a waste of a high-quality protein source. Therefore, utilizing defatted walnut meat for preparation of peptides can realize the high-value use of walnut meat resources. This study investigated the antifatigue, antioxidative, and immunoregulatory effects of Manchurian walnut hydrolysate peptides (MWHPs) of different molecular weights (MWs).

2 Materials and Methods

2.1 Materials and Reagents

Manchurian walnuts were purchased from a market in Changbai Mountains, China; alkaline protease from Novozymes (Copenhagen, Denmark); Hank's solution from Genview; and the RPMI 1640 medium was acquired from HyClone. MTT, concanavalin A (ConA), and dimethyl sulfoxide were purchased from Sigma-Aldrich (USA). Fetal bovine serum were purchased from Zhejiang Hangzhou Bioscience Co., Ltd. (Hangzhou, China), and alkaline phosphatase kits from Nanjing Jiancheng Bioengineering Research Institute (China). Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and secretory immunoglobulin A (sIgA) detection kits were bought from Rendu USA, Inc. (San Diego, CA, USA). All other reagents

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*Corresponding author. E-mail: minwh2000@jlau.edu.cn

[†] FANG Li & REN Dayong contributed equally as first authors.

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in this study were of analytically pure grade.

2.2 MWHP Preparation

Manchurian walnut protein isolates (containing 94.4% of protein) were prepared from Manchurian walnut dregs by the alkali-soluble acid precipitation method^[11]. One hundred milliliters of distilled water were added to 2.6 g of a walnut protein isolate. The solution was mixed at 22540 g for 6 min and then placed in a water bath at 90 °C for 15 min incubation, and its pH was adjusted to 7.2 with 0.6 mol/L NaOH after cooling. Subsequently, 9000 U/g neutral protease was added to the solution to induce enzymolysis for 2 h, after which the mixture was rapidly placed in a 90 °C water bath and cooled for 15 min. After cooling, 7000 U/g alkaline protease was added to induce enzymolysis at 55.5 °C for 3 h (pH 8.4). The final degree of hydrolysis (DH) was 32.23%. The mixture was incubated in a water bath maintained at 90 °C for 15 min to inactivate the enzyme after enzymolysis and then cooled to room temperature; pH of the enzymatic hydrolysate was adjusted to 7.0, and the solution was centrifuged at 3910 g for 10 min. The supernatant was ultrafiltered through filter membranes with MW cutoff of 10 and 3 kDa, and the MWHPs obtained were divided into three fractions according to their MWs, i.e., > 10 kDa (high MW), 3–10 kDa (medium MW) and < 3 kDa (low MW). The components were concentrated in vacuum, freeze-dried, and stored at –20 °C for further use.

2.3 Experimental Animals

Specific pathogen free, 4- to 6-week-old male mice weighing (20 ± 2) g were used. The feeding room was maintained at 22–25 °C and 55%–70% relative humidity. In this experiment, the mice were fed in the feeding room for 5 d for acclimatization and then randomly subdivided into nine experimental groups and a control group. Each group comprised 10 mice. Each of the MWHP MW fractions was gavaged at concentrations of 200, 400, or 800 mg/(kg·d). Deionized water was given to the control group. After the second and fourth weeks of treatment, the mice were weighed, and the antifatigue, antioxidative and immunoregulatory effects of MWHPs were detected. Protocols were carried out in strict accordance with recommendations from the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal experiment protocol was approved by the Committee on the Ethics of Animal Experiments of Jilin Agricultural University. All surgical procedures were performed under ether anesthesia, and every effort was made to minimize suffering.

2.4 Exhaustion Swimming Experiment

Thirty minutes after the last gavage, 5% lead wires were installed in the tails of the mice, and the animals were made to swim in a 40 cm deep basin at constant temperature (30 ± 2) °C. During the experiment, the mice moved with all of their limbs, and the amount of time that passed from when the mice began to swim until their heads got submerged in the water for 10 s and the animals did not surface, i.e., the swimming exhaustion time, was recorded.

2.5 Determination of the Physiological and Biochemical Indexes of Mice

After the last gavage, the mice were allowed to rest for 30 min and then made to swim in a small pool for 30 min. After swimming, the mice were retrieved, and their blood was collected and centrifuged for 15 min at 1410 g to obtain serum, which was kept in a –20 °C freezer. Lactic acid (LA) and MDA contents as well as SOD, GSH-Px, and catalase (CAT) activities in the serum were measured using assay kits according to the manufacturer's protocols^[12].

2.6 Determination of Immune-organ Indexes

The mice were killed by cervical dislocation, and their thymus and spleen were excised, dried with filter paper, weighed, and immediately recorded. The thymus and spleen indexes were calculated by means of the following formulas:

$$\text{Thymus (spleen) index} = \frac{\text{thymus (spleen) weight (mg)}/\text{body weight (g)}}{\times 100\%}$$

2.7 Spleen T Lymphopoiesis Test

The mice were killed by cervical dislocation after the last gavage and then soaked in 70% ethanol for 5 min. Spleen lymphocytes were isolated, and an RPMI 1640-based culture medium was used to adjust the cell population to (2.0 × 10⁶) cells/mL, and trypan blue was used to test cell viability and ensure that it was greater than 90%. Splenic lymphocytes were inoculated into a 96-well microplate 2.0 × 10⁵/well at a volume of 100 µL per well, and the ConA total concentration was found to be 20 g/mL. The culture plate was placed into a 5% CO₂ incubator at 37 °C for 72 h incubation. Ten microliters of CCK-8 (5 mg/mL) was added to each well 4 h before the culture was terminated, and 100 µL of DMSO was added to the plate to dissolve the cells. The absorbance of each well was determined at 570 nm by an ELISA.

2.8 Determination of Phagocytic Activity of Peritoneal Macrophages

The mice were killed by cervical dislocation after the last gavage, and their abdominal skin was disinfected with alcohol. The skin was exfoliated with scissors to expose the abdominal cavity. A syringe with 3 µL of serum-free RPMI 1640 was used to slowly inject this medium into the abdominal cavity, and the intraperitoneal fluid was gently pressed for 5 min. The intraperitoneal fluid was carefully collected with a syringe and centrifuged at 630 g for 20 min. The supernatant was discarded, the cell precipitates were washed thrice with serum-free RPMI 1640, and the concentration of macrophages was adjusted to (2.0 × 10⁵) cells/mL with RPMI 1640 containing 5% of calf serum. Thus, 200 µL of a cell suspension was obtained, added to the wells of a 96-well plate, and cultured at 37 °C and 5% CO₂ for 3 h. The culture medium was discarded, and 100 µL of a neutral red solution (0.1%) was added into each well; the cells were cultured for another 3 h. The neutral red solution was then removed, and cells were washed twice with sterile PBS. One hundred microliters of lysis liquid (glacial acetic acid-ethanol at 1:1) was added to each well and stewing was

performed at 4 °C overnight. The absorbance of the liquid in the wells was tested at 540 nm the next day.

2.9 Determination of the Intestinal Mucosal sIgA

A 10 cm long portion of the small intestine close to the ileocecal junction was excised from the killed mice and split longitudinally. The lumen contents of the intestine were squeezed into a sterile Eppendorf tube using forceps, and 500 mg of intestinal content was diluted with 2.0 mL of PBS, oscillated, mixed, and centrifuged at 2000 *g* and 4 °C for 30 min. The supernatant was obtained, cultured in a sterile EP tube, and then stored at −20 °C for further use. Finally, sIgA content of the intestinal mucosa was measured according to the kit instructions.

2.10 Statistical Methods

One-way ANOVA was performed with the Tukey-Kramer post hoc test. Significance of differences between groups was assessed by Student's *t* test. All the data are expressed as means ± SD. Statistical analyses were performed by means of SPSS 14.0 software for Windows (SPSS Inc., USA). Differences with *P* < 0.05 were considered statistically significant.

3 Results

3.1 Antifatigue Effects of MWHPs

3.1.1 Exhaustion Swimming Time of Mice

Effects of high-, medium- and low-MW MWHPs on the swimming time of mice are shown in Fig. 1a, 1b. MWHPs with low (< 3 kDa) and medium (3–10 kDa) MWs significantly prolonged the swimming time of the mice in the second week. Swimming time values of (91 ± 2.8) min (high-dose group) (*P* < 0.01), (74 ± 4.9) min (medium-dose group) (*P* < 0.05), and (51 ± 4.2) min (control group) were observed. In the fourth week, however, only low-MW MWHPs significantly prolonged the swimming time of the mice.

3.1.2 Hepatic Glycogen Content

As shown in Fig. 1d, MWHPs with low MW significantly reduced losses in hepatic glycogen after 4 week exercise. During the second week, low-MW (< 3 kDa) MWHPs (800 mg/(kg·d)) increased the hepatic glycogen content in mice from (7.7 ± 0.4) mg/g (control group) to (9.4 ± 0.7) mg/g (*P* < 0.01) (Fig. 1c). During the fourth week, the hepatic glycogen content in groups treated with low-MW MWHPs significantly increased (from 14.4 ± 1.2 to 15.1 ± 0.8 mg/g), while that in the control group was (11.8 ± 0.6) mg/g.

3.1.3 LA Content

Fig. 1e and 1f show that low-MW MWHPs could reduce LA content of blood after exercise. During the second week, low-MW MWHPs (800 mg/(kg·d)) induced a decrease in LA content to (3.6 ± 0.4) mmol/L, which was significantly lower than that observed in the control group (6.1 ± 0.3) mmol/L (*P* < 0.001). During the fourth week, LA content in groups treated with high- and medium-MW MWHPs (4.1 ± 0.5) and (4.6 ± 0.4) mmol/L, respectively, was significantly lower than that in the control group (5.6 ± 0.2) mmol/L (*P* < 0.05).

Mice were fed MWHPs at doses of 800 (high dose, H), 400 (medium dose, M) or 200 (low dose, L) mg/kg body weight per day. Deionized water was used as the control. During the second and fourth weeks, antifatigue effects were detected. The values reflect means ± SD of 10 mice. Asterisks represent statistically significant differences: * *P* < 0.05; ** *P* < 0.01, compared with the control group (Con).

3.1.4 Body Weight

During the second and fourth weeks, the average weights of mice in the control group were (32.4 ± 1.9) g and (36.9 ± 2.0) g, respectively, while those in groups treated with MWHPs were (31.6 ± 2.7) g to (35.2 ± 0.7) g and (31.6 ± 1.4) g to (40.8 ± 3.5) g, respectively. Weight differences between the two groups were not significant (*P* > 0.05) (data are not shown).

3.2 Antioxidative Effect of MWHP in Mice

3.2.1 SOD Activity

As shown in Fig. 2a and 2b, during the second week, high-, medium- and low-MW MWHPs did not significantly affect SOD activity in blood after exercise. During the fourth week, SOD activity significantly improved (123.7 ± 1.2) U/mL (*P* < 0.01) only after high-dose treatment (800 mg/(kg·d)) with low-MW MWHPs.

Mice were fed MWHPs at doses of 800 (high dose, H), 400 (medium dose, M) or 200 (low dose, L) mg/kg body weight per day. Deionized water was used as the control. During the second and fourth weeks, the mice were allowed to swim and were retrieved after 30 min. Mouse serum was then collected for SOD detection. The values represent means ± SD of 10 mice. Asterisks represent statistically significant differences: ** *P* < 0.01, compared with the control group (Con).

3.2.2 GSH-Px Activity

Fig. 2c and 2d show that low-MW MWHPs significantly improved GSH-Px activity in blood after exercise (*P* < 0.01). In the second and fourth weeks, GSH-Px activities of (329.6 ± 6.4) U to (342.0 ± 23.8) U and (327.1 ± 11.7) U to (375.8 ± 14.7) U were induced by low-MW MWHPs. By comparison, GSH-Px activities of (274.4 ± 14.0) U and (290.3 ± 12.0) U were seen in the control group. In the fourth week of treatment, the medium-MW MWHP group (400 mg/(kg·d)) also showed improved GSH-Px activity (358.2 ± 12.1) U (*P* < 0.05), although the group with low-MW MWHPs achieved the best effects.

3.2.3 CAT Activity

Fig. 2e and 2f show that only low-MW MWHPs significantly improved CAT activity in blood after exercise. During the second week, CAT activities increased to (13.5 ± 2.0) U/mL (*P* < 0.01) and (12.1 ± 0.6) U/mL (*P* < 0.05), respectively, after high- and medium-dose treatments. During the fourth week, CAT activities of (15.6 ± 1.0) U/mL (*P* < 0.01) and (10.9 ± 1.0) U/mL were detected in the high-dose and control groups, respectively. The changes in other groups were not significant.

Fig. 1 Effects of MWHPs on the exhaustion swimming time (a, b), hepatic glycogen (c, d) and blood lactic acid (LA) contents (e, f) of mice

3.2.4 MDA Content

In the second week, only low-MW MWHPs significantly reduced MDA content of blood after exercise. MDA contents of (10.4 ± 1.3) ($P < 0.001$), (12.3 ± 1.2) ($P < 0.01$) and (15.9 ± 0.9) nmol/mL were detected in the high-dose, medium-dose, and control groups, respectively (Fig. 2g). During the fourth week, low-MW MWHPs (800 mg/(kg·d)) significantly reduced MDA content to (11.2 ± 0.5) nmol/mL, ($P < 0.001$), and medium-MW MWHPs (200 mg/(kg·d)) significantly reduced MDA content to (12.3 ± 0.7) nmol/mL, ($P < 0.01$) (Fig. 2h). By comparison, MDA content in the control group was (15.5 ± 1.0) nmol/mL. The changes seen in other groups were not significant.

3.3 Immunoregulatory Effects of MWHPs on Mice

3.3.1 Spleen and Thymus Indexes of Mice

During the second and fourth weeks, low-MW MWHPs (800 mg/(kg·d)) significantly improved the spleen and thymus indexes of mice ($P < 0.05$) (Fig. 3). In addition, during the fourth week, high-, medium-

and low-MW MWHPs improved the spleen index considerably. Low-MW MWHPs (800 mg/(kg·d)) exerted the most significant effects ($P < 0.01$), whereas other MWHP fractions showed relatively weaker effects.

Mice were fed MWHPs at doses of 800 (high dose, H), 400 (medium dose, M), or 200 (low dose, L) mg/kg body weight per day. Deionized water was used as the control. During the second and fourth weeks, thymus and spleen samples were collected from each group. The thymus and spleen indexes were measured as the ratio of the thymus or spleen weight to the mouse body weight. The values indicate means $\pm$ SD of 10 mice. Asterisks represent statistically significant differences: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs control group (Con).

3.3.2 Proliferation of Spleen T Lymphocytes

During the second week, high-, medium- and low-MW MWHPs showed no significant effect ($P > 0.05$) (Fig. 4) on spleen T-lymphocyte proliferation. During the fourth week, only high-MW MWHPs (800 mg/(kg·d)) promoted spleen T-lymphocyte proliferation significantly ($P < 0.001$).

Fig. 2 Effects of MWHPs on the activities of SOD (a, b), GSH-Px (c, d), and CAT (e, f) and on MDA levels (g, h) in mouse serum

Fig. 3 Effects of MWHPs on the spleen and thymus indexes of mice

Mice were fed MWHPs at doses of 800 (high dose, H), 400 (medium dose, M) or 200 (low dose, L) mg/kg body weight per day. Deionized water was used as the control. During the second a and fourth weeks b, approximately 2×10^5 splenocytes were obtained from the MWHP-treated mice. ConA was used as the positive control. The stimulation index (SI) represents the proliferative capacity of splenocytes from each group after MWHP treatment. The values indicate means $\pm$ SD of 10 mice. Asterisks represent statistically significant differences: *** $P < 0.001$ vs control group (Con).

3.3.3 Phagocytic Activity of Mouse Peritoneal Macrophages

Although high-, medium- and low-MW MWHPs exerted different impacts on peritoneal macrophages phagocytic activity (Fig. 5), their impacts on phagocytic activity were not significant ($P > 0.05$).

Mice were fed MWHPs at doses of 800 (high dose, H), 400 (medium dose, M) or 200 (low dose, L) mg/kg body weight per day. Deionized water was used as the control. In the second a and fourth b weeks, peritoneal macrophages were prepared from the mice treated with MWHPs. The pinocytosis index (PI), which represents the phagocytic activity of peritoneal macrophages from each MWHP-treated group, was measured by determining the accumulation of neutral red in cells via a colorimetric assay. The values represent means $\pm$ SD of 10 mice.

3.3.4 sIgA Content of the Intestinal Mucosa

Low-, medium- and high-MW MWHPs significantly increased sIgA content of the intestinal mucosa (Fig. 6).

Fig. 4 Proliferative analysis of splenocytes from mice treated with MWHPs

Specifically, during the second week of treatment, MWHPs of high (400 mg/(kg·d)), medium (400 and 200 mg/(kg·d)), low (800 and 400 mg/(kg·d)) MWs significantly increased sIgA content of the intestinal mucosa ($18.4 \pm 2.7 \mu\text{g/mL}$ to $19.7 \pm 1.7 \mu\text{g/mL}$). During the fourth week, sIgA contents significantly increased ($14.8 \pm 0.9 \mu\text{g/mL}$ to $18.4 \pm 0.6 \mu\text{g/mL}$) in all other experimental groups except for the groups treated with MWHPs of high (200 mg/(kg·d)) and low (200 mg/(kg·d)) MWs.

Mice were fed MWHPs at doses of 800 (high dose, H), 400 (medium dose, M) or 200 (low dose, L) mg/kg body weight per day. Deionized water was used as the control. In the second a and fourth b weeks, the levels of sIgA in the intestine were determined by an ELISA. The values represent means $\pm$ SD of eight mice. Asterisks represent statistically significant differences: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs control group (Con).

4 Discussion

Antifatigue effects of MWHPs were studied in this paper since fatigue is a common problem in today's society. Over 35% of the world's population experiences fatigue, and middle-aged men account for 60% of this number^[13]. Studies have shown that some bioactive peptides are easily absorbed by the human body, thereby generating energy and eliminating accumulation of fatigue-related metabolites^[14]. Fatigue is a complex physiological and biochemical process. After strenuous exercise, hepatic glycogen decomposes to maintain sugar levels in blood, and glycogen reserves decrease^[15]. When the human body produces a large amount of LA, the H^+ concentration rises and pH of muscular tissues falls, causing a series of physiological and biochemical reactions that promote fatigue.

Exercise tolerance experiments (e.g., the animal swimming test) and biochemical indicator tests (e.g., hepatic glycogen and blood LA) are key methods commonly used to measure the degree of fatigue^[16-17]. Four weeks after MWHP treatment, only low-MW MWHPs ($< 3 \text{ kDa}$) significantly prolonged the swimming time and reduced hepatic glycogen consumption and LA accumulation ($P < 0.01$) in mice. The antifatigue effect of MWHPs was significantly dose dependent, and the optimal antifatigue effect was achieved by a high dose of the treatment (800 mg/(kg·d)).

Numerous studies have shown that many diseases are associated with lipid peroxidation induced by free radicals. MDA, one of the most important products of membrane lipid peroxidation, can intensify membrane damage^[18]. Recent studies have shown that production of large amounts of free radicals is the major cause of fatigue^[19]. Strenuous exercise leads to increased levels of free radicals, oxidative damage to the cell membrane, and fatigue^[20]. A number of antioxidant peptides have been proven to relieve fatigue. A bioactive peptide in soybean has a relatively strong ability to remove hydroxyl and superoxide anion free radicals; thus, it can prolong swimming time and delay the onset of fatigue^[21]. You et al.^[22] also demonstrated that a loach extract peptide can improve antioxidative abilities and eliminate fatigue in mice.

The present study indicates that low-MW MWHPs ($< 3 \text{ kDa}$) significantly increased the activities of SOD, GSH-Px,

and CAT in mice after exercise ($P < 0.01$) as well as reduced MDA content of blood ($P < 0.001$). During the fourth week of treatment, medium-MW MWHPs (3–10 kDa) also increased GSH-Px activities and reduced MDA content of the blood but had no significant effect on SOD and CAT activities. Therefore, low-MW MWHPs showed the best antioxidative effects among the fractions studied.

Fig. 5 Effects of MWHPs on the pinocytosis of peritoneal macrophages

Some bioactive peptides that can promote and regulate immune functions are called immunomodulatory peptides^[23]. Although the mechanism of action of these peptides is unclear, studies have shown that they can enhance lymphocyte proliferation and antibody synthesis. Therefore, they regulate the immune system. In the present research, the immunoregulatory activity of MWHP was studied in immune organs, immune cells, and immune molecules. The thymus and spleen are important immune organs, and their visceral index can reflect the immunological characteristics of an organism to a certain extent^[24]. During the second and fourth weeks of high-dose (800 mg/(kg·d)) MWHP treatment, low-MW MWHPs ($< 3 \text{ kDa}$) significantly raised the immune-organ indices of mice. MWHPs of other MWs increased the spleen index only in the fourth week of treatment, but showed no significant ($P > 0.05$) effect on the thymus index. Low-MW MWHPs also

significantly ($P < 0.01$) promoted spleen lymphocyte proliferation, thus they exhibit a potential antitumor activity.

Fig. 6 Effects of oral administration of MWHPs on secretory immunoglobulin A (sIgA) production in the mouse intestine

Macrophages, as the first line of defense against microbial attacks, can induce and adjust a nonspecific immunoreaction through a variety of cytokines and released active substances^[25]. Conlon et al.^[26] reported that peptides from frog skin secretions can enhance the phagocytic activity of macrophages. The present study suggests that MWHPs exert no significant effect on macrophage activity ($P > 0.05$).

sIgA is the main antibody synthesized by the intestinal mucosal immune system and can defend the intestinal mucosa against the adhesion and engraftment of pathogenic bacteria, thereby reducing intestinal infections^[27]. Masashi et al.^[28] found that food with wheat hydrolysate peptides can dramatically improve sIgA content of the small intestine of mice. Gastrointestinal neuropeptides and bovine casein phosphorylated peptides have similar effects^[29-30]. The results of the present study also confirm that low-, medium- and high-MW MWHPs can increase sIgA levels. According to the immunological experiment, MWHPs (especially those of low MW) promote humoral immunity and cellular immunological responses.

In conclusion, this study showed that low-MW MWHPs exert significant antifatigue (prolonging swimming time

and reducing hepatic glycogen consumption and blood LA accumulation), antioxidative (raising SOD, GSH-Px, and CAT enzymatic activities and reducing MDA content), and immunoregulatory (promoting cellular immunity and humoral immune responses) effects. However, the characteristics and safety of MWHPs need to be further studied.

Conflict of Interest

The authors declare that there is no conflict of interest.

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