Anti-fatigue effect of enzymatic protein hydrolysate from Macadamia

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Abstract. The anti-fatigue effect of macadamia (Macadamia integrifolia) protein and hydrolysate prepared with basic protease were investigated. Random mice grouping was carried out to create 4 groups including the control group and protein hydrolysate groups at low (150 mg/kg·d), medium (500 mg/kg·d) and high (1500 mg/kg·d) doses. All groups were treated by gavage for 28 consecutive days. Weight-loaded swimming test, serum urea nitrogen, serum creatine kinase activity, blood lactic acid and liver glycogen assays were performed to assess its anti-fatigue effect. The results showed that macadamia protein hydrolysate could significantly prolong weight-loaded swimming time, promote synthesis of liver glycogen and decrease blood urea nitrogen and lactic acid. Moreover, it can also significantly reduce serum creatine kinase activity. Therefore, macadamia protein hydrolysate had significant anti-fatigue function.

Keywords: macadamia, protein hydrolysate, anti-fatigue.

1. Introduction
The macadamia (Macadamia integrifolia) is an evergreen native tree indigenous to the coastal rainforests of Australia [1], and is one of the most popular nuts globally. It contains a high content of protein (8-20%) and 17 kinds of amino acids, with a total content of ~25.05% [2]. And it has been found that many bioactive peptides with biological functions can be obtained by hydrolysis of proteins with appropriate proteases, which are easy to digest and absorb, and have the functions of anti-oxidation, lowering blood pressure, lowering blood lipid and immunomodulatory [3-4]. Therefore, development of macadamia peptide products had broad market prospects.

In this study, the anti-fatigue effect of macadamia peptide was studied in order to provide a theoretical basis for the further development of macadamia nut products.

2. Experimental

2.1. Materials and reagents
Macadamia protein is provided by the National Engineering Research and Development Center for Important Tropical Crops. Kunming mice, 6 to 8 weeks of age, half male and half female, weighing 18 to 20 g, were provided by the Experimental Animal Center of Guangdong Medical University.
The content of urea nitrogen (BUN), lactic acid (LA), liver glycogen (LG) and enzyme activity of creatine kinase (CK) in serum were detected by the kit.

2.2. Methods

2.2.1. Pretreatment of macadamia protein hydrolysate. The macadamia protein hydrolysate (MPH) was based on previously reported methods [5].

2.2.2. Experimental animals and feeding. A total of 120 healthy mice (body weight 18-20g) were fed for a period of time (one week) to adapt to the environment (room temperature 20-25 ℃, humidity 40-70%, ventilation 10-20 times/hour, light alternately for 12 hours) before the grouping experiment. Each cage of 5 mice was fed with free diet and standard feed. The drinking bottle was changed at any time. The cage was washed twice a week., after that, the mice were randomly divided into 4 groups: control group (macadamia protein), MPH low dose group (0.15 mg/(g·d]), MPH dose group of 0.5 mg/d (g), MPH high dose group (1.5 mg/d) (g), 20 mice in each group (10 mice used in weight loading swimming experiment, and the other 10 mice for index detection), around the continuous experiments, the continuous sample to 28 d is designed according to the dose.

2.2.3. Assay method[6]. After the last feeding, the mice were put into the water to swim (water depth of 30 cm, water temperature of 25±0.5℃). During the swimming of the mice, the water in the pool should be stirred to ensure that the mice could not stop swimming. The lead block of 5% body weight was loaded in the tail root of the mice, and the time from the beginning of swimming to death was recorded, which was the time of death in weight-loaded swimming (WLS).

Meanwhile, the mice were put into the water to swim (water depth of 30 cm, water temperature of 25±0.5 ℃) and taken out 60 min. Then, venous blood was collected from the intraocular canthus and centrifuged (4 ℃, 3000 r/10min). The content of BUN and enzyme activity of CK in serum were detected by automatic biochemical analyzer. And the content of LA was detected by the kit.

After blood collection, the mice were killed immediately after cervical dislocation and the abdominal cavity was opened. The left lobe of the liver was taken for 80 mg and temporarily stored in liquid nitrogen. After blood collection, the mice were transferred to -80℃ for storage. The glycogen kit was used to detect the content of LG.

2.3. Statistical analysis

All the experiments were repeated in triplicate, and the date were expressed as means ± standard deviations. Statistical analysis was performed on Origin 2020 and SPSS 25.0.

3. Results and discussions

3.1. Effect of MPH on WLS time in mice

As one of the key indexes to evaluate the anti-fatigue effect, the WLS time of mice can more directly reflect the real anti-fatigue effect of the sample. As can be seen from Figure 1, the WLS time of mice with low (150 mg/kg·d), medium (500 mg/kg·d) and high (1500 mg/kg·d) doses of MPH increased by 80.6%, 120.5% and 205.4%, respectively. These values were significantly different from those of the control group (P < 0.05). Also, with the increase of feeding dose of MPH, the WLS time of mice was prolonged, which indicated that MPH had a certain anti-fatigue effect.
3.2. Effect of MPH on LG in mice
As a storage form of glucose, LGs plays a crucial role in maintaining the balance of blood glucose levels in the body. In persistent movement process, LG changes as movement intensity and time, and its excessive consumption and decomposition lag can produce fatigue. As shown in Figure 2, LG content of mice in the low (150 mg/kg·d), medium (500 mg/kg·d) and high (1500 mg/kg·d) dose groups was significantly higher than that in the blank group. Compared with the control group, they increased by 22.37%, 37.5% and 44.4%, respectively, and there was a significant difference (P < 0.05). However, there was no significant difference in LG content between medium and high doses (P > 0.05). It also indicated that MPH had a certain anti-fatigue effect, and the MPH dose was positively correlated with the LG content.

3.3. Effect of MPH on BUN in mice
Under normal circumstances, the production and discharge of urea in the body are relatively balanced, so the content of BUN also remains stable. But the decomposition of nitrogen-containing substances such as proteins and amino acids in sports is accelerated, and the content of BUN will increase. Therefore, BUN is often used as an index to evaluate the anti-fatigue efficacy. Compared with the control group,
the content of BUN in mice in low (150 mg/kg·d), medium (500 mg/kg·d) and high (1500 mg/kg·d) dose groups was decreased by 33.94%, 41.74% and 45.68%, respectively, and there was a significant difference (P < 0.05). The results indicated that MPH could reduce the accumulation of BUN in mice after exercise to a certain extent and had a good anti-fatigue effect.

Figure 3. Effect of MPH on serum urea nitrogen in mice.

3.4. Effect of MPH on CK activities in mice
The activity of CK will directly affect the body's locomotion ability, and the changes in the internal environment of muscle cells during exercise will also directly affect the activity of CK in cells. After exercise training, muscle cells of the body are damaged and CK outflow causes increased CK activity in the blood. Therefore, the activity of CK in serum can used as an important indicator to judge whether muscle or skeletal muscle is damaged or stimulated. Compared with the control group, the content of CK activities in mice in low (150 mg/kg·d), medium (500 mg/kg·d) and high (1500 mg/kg·d) dose groups were decreased by 40.93%, 46.96% and 48.41%, respectively, and there was a significant difference (P < 0.05), but the effect of different doses on the activity of CK was not significant. The result indicated that MPH can effectively prevent muscle damage caused by exercise, reduce the loss of CK in muscle cells, and has a significant anti-fatigue effect.

Figure 4. Effect of MPH on serum creatine kinase activities in mice.
3.5. Effect of MPH on LA content in mice

The level of LA is an important indicator of body fatigue and aerobic metabolic capacity. Compared with the control group, the content of LA in mice in low (150 mg/kg·d), medium (500 mg/kg·d) and high (1500 mg/kg·d) dose groups was decreased by 62.66%, 69.53% and 70.39%, respectively. And there was a significant difference (P < 0.05), but the effect of different doses on the content of LA was not significant. The results indicated that MPH could reduce the accumulation of LA after exercise and had a good anti-fatigue effect.

4. Conclusion

In this study, the anti-fatigue effect of MPH was evaluated through a mice model. The result showed that MPH could prolong the swimming death time of mice (80.6%-205.4%) and delay or reduce the consumption of LG in mice during exercise. The content of LG was 22.37-44.4% higher than that in blank control group. Furthermore, MPH could remove the accumulation of LA and BUN in the blood of mice. The contents of LA and BUN were reduced by 62.66%-70.39% and 33.94%-45.68%, respectively. In addition, the activity of CK in mice was decreased by 40.93%-48.41%. The above results indicated that MPH has a strong anti-fatigue effect and offer an insight into industrial application of MPH.

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