Progress in Nutrition 2019; Vol. 21, N. 4: 882-888 DOI: 10.23751/pn.v21i4.8596

Effect of *Lepidium meyenii Walp.* on the immune function of boxers

Hao Ren

Sanquan College of Xinxiang Medical University, Henan 453003, China; Pukyong National University, Busan 608-737, Korea - Email: haor_rh@yeah.net

Summary. *Objective:* To explore the effect of *Lepidium meyenii Walp.* on immune function of boxers. *Methods:* Thirty athletes who received boxing training in Pukyong National University were randomly divided into three groups and then divided into three intervention stages to ensure that each group underwent different doses of *Lepidium meyenii Walp*. The levels of red blood cells, white blood cells and immunoglobulin (Ig) in the blood of the athletes were detected before and after each intervention stage. *Results:* After taking different doses of *Lepidium meyenii Walp.*, there was no significant change in the level of red blood cells ($P > 0.05$); the level of white blood cells in the low and high dose groups increased significantly ($P < 0.05$; $P < 0.01$); the levels of IgA, IgM and IgG in the high and low dose groups increased significantly ($P < 0.05$), but the difference of increase amplitude was not large. *Conclusion: Lepidium meyenii Walp.* can improve the immune function by raising the level of white blood cells and Ig in athletes.

Key words: *Lepidium meyenii Walp.*, immune function, white blood cells, immunoglobulin

1. Introduction

Boxing is a sport of unarmed fighting with gloves. Its victory condition is to get more points or knock down opponents while avoiding attack as much as possible in the course of attack and defense. Boxers need to fight with their bare hands on a square platform. This process is a mental, physical and technical confrontation. In order to gain advantages in the process of confrontation, boxers need a lot of training, including pace and various punching skills. Therefore, for boxers, a large amount of daily exercise is essential. In order to maintain the healthy development of the body, besides a balanced diet, it is essential to improve their anti-fatigue ability and recovery ability (1). Especially when the boxing match enters the see-saw fight, the endurance and resilience of the athletes become the key to victory or defeat. *Lepidium meyenii Walp.* has anti-fatigue and endocrine regulation functions, so that it can assist boxers in daily exercise (2). In addition, when boxers exercise or compete, they will inevitably have wounds, and skin, especially, will have varying degrees of damage. Once the wounds are improperly handled, bacteria or viruses will invade the body, causing varying degrees of illness, as well as internal injuries. Diseases caused by bacterial viruses will greatly affect the daily performance of athletes, so how to improve the immune function of boxers has become an important part of the sports field (3). In addition to the above functions, *Lepidium meyenii Walp.* can also improve the immunity of the human body. Therefore, *Lepidium meyenii Walp.*-related products have been applied in the field of sports. Li et al. (4) detected the molecular structure of *Lepidium meyenii Walp.* using fiber chromatography and found that it had reductive ability through antioxidant experiments and that it could promote the proliferation and phagocytosis of immune cells. Lee et al. (5) fed Andean trout with

different doses of *Lepidium meyenii Walp.*. The results showed that the trout groups which were fed with *Lepidium meyenii Walp.* not only grew fast, but also had a high survival rate; *Lepidium meyenii Walp.* increased the number of white blood cells in trout, so as to improve the survival rate of trout and larvae. Through virtual screening of estrogen receptors and verification of osteoblast pharmacological activity, Hao et al. (6) revealed that *Lepidium meyenii Walp.*'s effective anti-osteoporosis component, N-benzyl-palmitamide, could promote osteoblast proliferation, differentiation and mineralization and promote bone formation by enhancing the expression of osteogenesis-related genes. This paper briefly introduced the origin, shape and nutrient composition of *Lepidium meyenii Walp.*, and then took 30 athletes who were trained for boxing in Pukyong National University as an example.

2. *Lepidium meyenii Walp.*

Lepidium meyenii Walp. (7), a herb growing at high altitudes in the Andes, is divided into annual and biennial varieties, but there are no significant differences in functional pharmacodynamics and cell chromosomes. *Lepidium meyenii Walp.* is regarded as food locally, but also as a natural herb. *Lepidium meyenii Walp.* belongs to Lepidium (8) in the perspective of botanical classification. It is like white radish in overall appearance. The main edible and medicinal parts of *Lepidium meyenii Walp.* are the tuberous rhizomes buried in the soil. The leaves exposed to the ground during growth are basically linear drill-shaped. The edges of the leaves are serrated and can grow up to 23 cm. They droop to the ground in the process of growth. The underground tuber parts are usually 10-14 cm long and the maximum diameter can be about 3-5 cm. The natural rhizome has two colors, yellow and purple, and the longest root fibrous is up to 15 cm at the bottom. *Lepidium meyenii Walp.* with purple rhizome contains much iodine; the darker the color, the more the content. It tastes sweet and bitter. In the growth process of *Lepidium meyenii Walp.*, the tuberous rhizomes can be obtained only 7 to 9 months after sowing, and after flowering for about one month, the fruit can be obtained. *Lepidium meyenii Walp.*'s growth conditions are very strict. In addition to planting in high altitude areas, it is necessary to ensure that the temperature difference between day and night exceeds 30°C, and there must be sufficient water in the growing area (9). Because of the harsh growth conditions, the cultivation of *Lepidium meyenii Walp.* is difficult to expand and the yield is difficult to increase. Another key reason is the predatory absorption of soil fertility. After harvest, it is necessary to make the cultivated land rest for more than seven years. Therefore, the yield of *Lepidium meyenii Walp.* can not be improved in any case.

Lepidium meyenii Walp. is rich in nutrients. 100 g of dried *Lepidium meyenii Walp.* contains 10.2 g of protein, 2.2 g of fat, 8.5 g of dietary fiber and 59 g of carbohydrate. Compared with common tuber crops such as sweet potato and radish, it has higher protein and dietary fiber (10). Alkaloids that regulate human endocrine system can also be extracted from *Lepidium meyenii Walp.*, which is the secondary metabolite of *Lepidium meyenii Walp.*

As *Lepidium meyenii Walp.* is rich in nutrients and secondary metabolites such as alkaloids, it also has a considerable degree of health care functions besides being a food (11), including improving fertility, regulating endocrine, improving anti-fatigue ability and antioxidant capacity, and strengthening immune function. Moreover, *Lepidium meyenii Walp.*, as the main food of local people for many years, is not found to be toxic to human beings. Various toxicity studies have proved that *Lepidium meyenii Walp.* is a non-toxic crop. Although *Lepidium meyenii Walp.* has been proved to be a non-toxic crop, due to its fertility and endocrine regulation function, excessive consumption may cause physical damage, so it is necessary to regulate the dosage.

3. Example analysis

3.1 Research subjects

As shown in Table 1, 30 athletes who received boxing training in Pukyong National University were selected. All of them have been trained for more than three years. Their average age was about 20 years old, their average height was about 175 cm, and their average weight was about 70 kg. They had been informed. They have no family history and have not been injured

Table 1. Basic information of research subjects

Various indicators	Group 1	Group 2	Group 3
Number/n	10	10	10
Average age/year	20.1±0.1	20.2±0.1	20.0±0.2
Boxing training time/year	3.5±0.2	3.6±0.1	3.4±0.3
Height/cm	175.1±2.3	175.2±1.2	175.3±0.3
Weight/kg	70.2±0.3	71.1±0.2	70.1±0.1
Family history	None	None	None
Being injured in recent 3 months?	None	None	None

in the past three months. Among them, no family history was required (12) to prevent some genetic-related immune diseases from affecting the experiment, and no injury in the last three months was required to prevent the immune system from working after injury, resulting in immune indicators beyond the normal range. Thirty athletes were randomly divided into three groups and numbered **1**, **2** and **3**.

3.2 Main instruments and reagents

Main instruments included automatic biochemical analyzer, automatic blood cell analyzer, high-speed centrifuge, refrigerator, disposable transfusion tube, aseptic test tube, and pipette.

Main reagents included sterile water, saline, anticoagulant, biochemical analyzer kit reagent, and blood cell analyzer kit reagent.

The preparation of *Lepidium meyenii Walp.* tonic: *Lepidium meyenii Walp.* dried tablets produced from Lijiang, Yunnan province, were processed into fine powder and made into capsules in a size of 250 mg each capsule. Moreover, the capsules which was made of edible starch and had the same specification were used as placebo. There was no difference in appearance between the two tonics. All the above preparation works were carried out in sterile environment.

3.3 Experimental process

As shown in Table 2, the experiment was divided into three stages of tonic intervention to ensure that each group of athletes were given three different doses of tonic intervention. Four *Lepidium meyenii Walp.* capsules were given to the high dose group, two *Lepidium meyenii Walp.* capsules was given to the low dose group, and the placebo group was given four starch capsules. During the whole experiment, the athletes were not told what kind of capsules they were taking, and the prescribed dosage was not allowed to talked between groups.

Table 2. The experimental scheme of supplement intervention stage

	The first stage	The second stage	The third stage
Group 1	High dose	Placebo	Low dose
Group 2	Low dose	High dose	Placebo
Group 3	Placebo	Low dose	High dose

Firstly, each stage lasted for three weeks, during which each group took daily supplements as shown in Table 2, and there was an interval of 2 weeks between every two stages to eliminate the effects of supplements on the body. During the whole experimental process (including three intervention stages and two elution stages), all three groups of boxers had the same daily training and eating. Daily training included: **1** jogging for 2 km in the morning and having breakfast after 30 minutes' rest; **2** doing warm-up activities for 15 minutes, including rope skipping and sandbags beating, after resting for one hour; **3** group antagonistic boxing training after warming-up; **4** resting until lunch time after group boxing training; **5** free activity time for 2 h after lunch; **6** warming up for 15 minutes after the free exercise and doing boxing exercises and confrontation

training; **7** the time after dinner and before 22:00 was free time, and the time after 22:00 was sleep time.

3.4 Index test

At the beginning and end of each stage, the indicators were detected, including blood physiological indicators (red blood cell and white blood cell count) and specific immune detection indicators (immunoglobulin (Ig) A, IgM and IgG) (13).

The procedure of each test is the same. First, the athletes kept fasting for 12 hours the night before, and then 10 mL of venous blood was collected with a disposable transfusion tube in the morning at the fasting state, 5 mL for the detection of blood physiological indicators and 5 mL for the detection of specific immune indicators.

Detection of blood physiological indicators: 15 μL of venous blood was transferred into a sampling bottle with a pipette. The red blood cell and white blood cell counts were detected by putting the sampling bottle into the automatic blood cell analyzer. The blood sample of each person was detected three times, and the average value was taken.

Specific immunoassay indicators: Samples containing venous blood were centrifuged with a high-speed centrifuge at a centrifugal speed of 3500 r/s, for 15 minutes, to obtain the separated serum; the separated serum was loaded into a sterile test tube and stored in refrigerator at -20°C; through automatic biochemical analyzer and associated reagents, the content of IgM, IgG and IgA in the serum was detected using immune turbidimetry (14). The detection of each subject repeated three times, and the average value was taken as the final result.

3.5 Statistical analysis

The collected data were input into EXCEL and analyzed by SPSS software (15). The calculation results are expressed as $\bar{x} \pm s$ and processed by t test. Difference was thought having statistical significance if the value of P was smaller than 0.05.

3.6 Experimental results

As shown in Table 3, the number of red blood cells in the normal human blood was 3.5~*5.5*x10^{12}/L, the number of white blood cells was 4.0~*10.10*x10^{9}/L, and the red blood count and white blood count of the boxers before and after taking different doses of *Lepidium meyenii Walp.* were within the normal range. Different doses of *Lepidium meyenii Walp.* had no effect on the red blood count in the athletes' blood. Although there was slight change before and after taking *Lepidium meyenii Walp.*, there was no significant difference, and the value of P was larger than 0.05. There was no significant change in the white blood count in athletes' blood before and after taking placebo, and the value of P was higher than 0.05. The white blood count in the blood increased significantly before and after taking low dose of *Lepidium meyenii Walp.*, and the value of P was smaller than 0.05. The white blood count in the blood before and after taking high dose of *Lepidium meyenii Walp.* significantly increased, and the value of P was smaller than 0.01. As shown in Figure 1, the white blood cells in the blood of athletes after taking *Lepidium meyenii Walp.* increased with the dosage, but did not exceed the normal range.

As shown in Table 4, the serum IgA content in the normal subjects ranged from 0.76 to 3.90 mg/mL, the IgM content ranged from 0.40 to 3.45 mg/mL,

Table 3. Changes of red blood cells and white blood cells before and after taking different doses of *Lepidium meyenii Walp*

		High dose	**Low dose**	**Placebo**
Erythrocyte count (10^{12}/L)	Before taking	4.95±0.28	4.93±0.31	4.95±0.25
	After taking	4.93±0.29	4.92±0.29	4.94±0.26
Leukocyte count (10^{9}/L)	Before taking	5.33±0.75	5.32±0.74	5.34±0.78
	After taking	5.67±0.66**	5.53±0.67*	5.38±0.76

*Note: * indicated that there was a significant difference compared to before taking, P < 0.05; ** indicated that there was a significant difference compared to before taking, P < 0.01.*

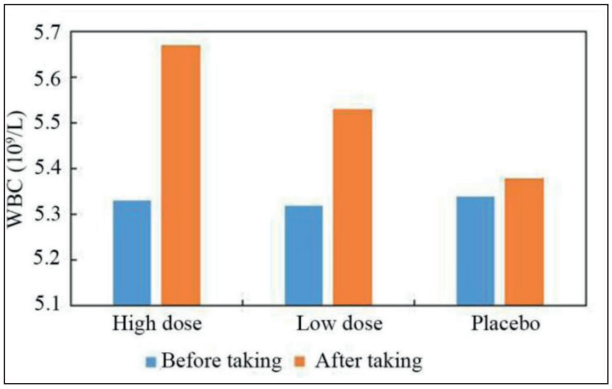

Figure 1. Changes of number of white blood cells before and after taking different doses of *Lepidium meyenii Walp*

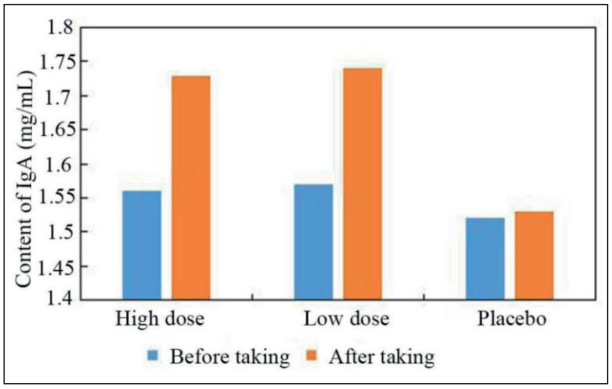

Figure 2. Changes of serum IgA level before and after taking different doses of *Lepidium meyenii Walp*

and the IgG content ranged from 6.00 to 16.00 mg/mL; the serum IgG content of athletes in three groups before and after taking different doses of *Lepidium meyenii Walp.* was within the normal range; the serum IgG content was the highest, followed by the content of IgA and IgM. The three kinds of Ig increased significantly after taking low and high doses of *Lepidium meyenii Walp.*, and the value of P was smaller than 0.05; the three kinds of Ig had no significant changes after taking placebo, and the value of P was larger than 0.05.

The change of the serum IgA content before and after taking different doses of *Lepidium meyenii Walp.* is shown in Figure 2. The specific values are shown in Table 4. It was found from Figure 2 that the serum IgA content of athletes after taking *Lepidium meyenii Walp.* did not change significantly with the increase of dosage, and the final value does not exceed the normal range of IgA.

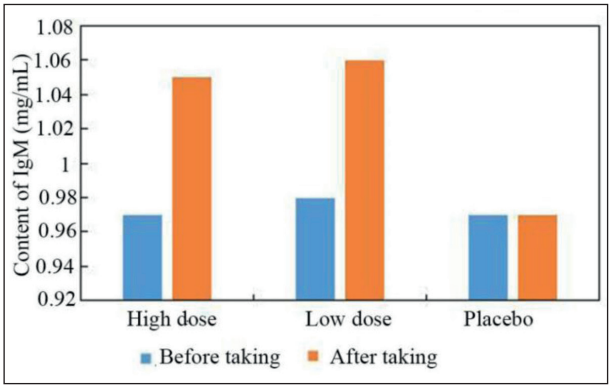

Figure 3. Changes of serum IgM level before and after taking different doses of *Lepidium meyenii Walp*

The change of serum IgM content before and after taking different doses of *Lepidium meyenii Walp.* is shown in Figure 3. The specific values are shown in Table 4. It was found from Figure 3 that the serum

Table 4. Changes of serum Ig levels before and after taking different doses of *Lepidium meyenii Walp*

		High dose	**Low dose**	**Placebo**
IgA (mg/mL)	Before taking	1.56±0.53	1.57±0.56	1.52±0.51
	After taking	1.730.58*	1.74±0.59*	1.53±0.52
IgM (mg/mL)	Before taking	0.97±0.38	0.98±0.38	0.97±0.46
	After taking	1.05±0.41*	1.06±0.43*	0.97±0.39
IgG (mg/mL)	Before taking	9.25±1.65	9.26±1.59	9.23±1.98
	After taking	10.06±2.09*	10.02±1.55*	9.25±1.91

** indicated that there was a significant difference compared to before taking, P < 0.05.*

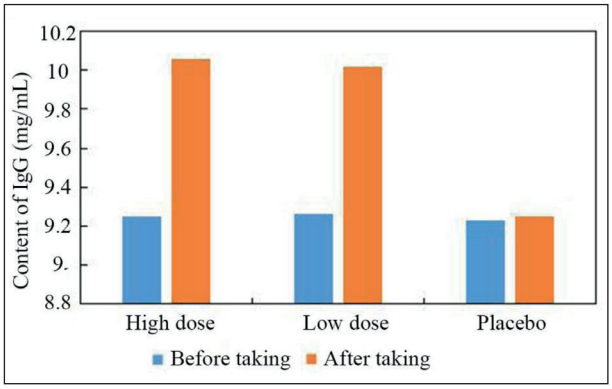

Figure 4. Changes of serum IgG level before and after taking different doses of *Lepidium meyenii Walp.*

IgM content after taking *Lepidium meyenii Walp.* did not change significantly with the increase of dosage, and the final value did not exceed the normal range of IgM.

The change of serum IgG content before and after taking different doses of *Lepidium meyenii Walp.* is shown in Figure 4. The specific value is shown in Table 4. It was found from Figure 4 that the increase of serum IgG content of athletes after taking *Lepidium meyenii Walp.* did not change significantly with the increase of dosage, and the final value did not exceed the normal range of IgG.

4. Discussion and analysis

Boxers need to consume a lot of energy in competition or training, at which time the oxygen demand is greatly increased. As hemoglobin in red blood cells can combine with oxygen and carbon dioxide to achieve intracellular and extracellular material exchange and promote energy conversion, the content of red blood cells in the body can affect the endurance of athletes: more red blood cells can transport more oxygen, promote cell respiration, and produce more energy, and more oxygen can also inhibit cellular anaerobic breathing, reduce the production of lactic acid in muscle, and improve exercise endurance. The results of red blood cell test of boxers showed that the change was not obvious after the athletes took different doses of *Lepidium meyenii Walp.*, and moreover its content was within the normal range. Therefore, it was speculated that the fluctuation of blood cell content was caused by training and normal metabolism, and *Lepidium meyenii Walp.* had little effect on blood cells.

The number of white blood cells will reduce after boxers experience a large number of high-intensity exercises, and sometimes leukopenia may occur. The former can recover to the normal level after conditioning, while the latter may develop into a long-term low immunity, which will not only affect the daily life of boxers, but also force them to end their boxing career earlier. After all, boxing is a fierce antagonistic sport, and injury is common in competition. White blood cells is a part of non-specific immunity in human immune system. It is differentiated from hematopoietic stem cells in bone marrow. It is generally divided into granulocyte, monocyte and lymphocyte by morphological function. The results of this study showed that the number of white blood cells increased after taking different doses of *Lepidium meyenii Walp.*, and the larger the dosage, the greater the increase, but not beyond the normal range. The reason is that *Lepidium meyenii Walp.* can promote the production of testosterone, and testosterone can promote the proliferation and differentiation of hematopoietic stem cells. In addition, secondary metabolites in *Lepidium meyenii Walp.* can also promote the proliferation and differentiation of white blood cells, neutralize the hypochlorous acid in white blood cells, and prolong the life of white blood cells.

Ig in serum is a component of specific immunity in human immune system. Its principle is to combine with pathogens *in vivo* to label pathogens and activate other immune cells. Ig is classified into five types: A, M, G, E and D. The content of IgE is too low, and the function of IgD is not clear. Therefore, only three Igs, i.e., IgA, IgM and IgG, were in this study. The results showed that the content of IgG was the highest, followed by the content of IgA and IgM. Three kinds of Ig increased after the athletes took different doses of *Lepidium meyenii Walp.*, but the dose had no significant influence on the increased content of Ig.

In conclusion, *Lepidium meyenii Walp.* can improve the content of non-specific immune white cells and specific immune globulin, thereby enhancing the immune function of athletes.

5. Conclusion

This paper briefly introduced the origin, shape and nutrient composition of *Lepidium meyenii Walp.* and then took 30 athletes who received boxing training in Pukyong National University as an example. The athletes were randomly divided into three groups and then divided into three stages of supplement intervention to ensure that each group receive different doses of *Lepidium meyenii Walp.* intervention. Finally, the content of red blood cells, white blood cells and Ig in athletes' blood were detected before and after each intervention stage. The results are as follows. *Lepidium meyenii Walp.* had no effect on the content of red blood cells in the blood of boxers, but it can effectively improve the level of white blood cells; the larger the dosage of *Lepidium meyenii Walp.*, the better the effect. *Lepidium meyenii Walp.* could effectively raise the level of IgA, IgM and IgG in athletes' serum, but the effect had no significant relationship with the dosage of *Lepidium meyenii Walp*. In conclusion, *Lepidium meyenii Walp.* can effectively improve the immune function of boxers by improving the level of immune cells and immune substances.

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Correspondence:
Hao Ren
China Guangfa Bank, No. 218,
Shengli Road, Xinxiang, Henan 453000, China
E-mail: haor_rh@yeah.net