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PROTECTIVE EFFECT OF *MOMORDICA GROSVENORI* LEAF EXTRACTIVE ON IMMUNE SYSTEM OF BODY WITH EXERCISE-INDUCED EXHAUSTION AND ITS MECHANISM

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ABSTRACT

This study discussed over the efficacy of *Momordica grosvenori* leaf extractive flavones on the immune system of body through analyzing the effects of flavones on the immune system of body with exercise-induced exhaustion, aiming to provide certain scientific basis and theoretical basis for the development and utilization of economic value and healthcare value of *Momordica grosvenori*. Fifty rats were taken as the research subjects and evenly divided into five groups, 10 in each group. Each rat was gavaged with 200 mg/kg *Momordica grosvenori* leaf extractive flavones. Then the rats were trained for three weeks. The effects of *Momordica grosvenori* leaf extractive flavones on the immune system of rats with exercise-induced exhaustion were observed. It was found that, *Momordica grosvenori* leaf extractive flavones could effectively improve the duration of exhaustive exercise, increase the number of peripheral white blood cells, and increase the content of plasma indexes and it could also protect the immune system of body. *Momordica grosvenori* leaf extractive flavones is effective in improving fatigue resistance capacity and accelerating body recovery and moreover can promote the growth and development of immune organs and strengthen body immune functions.

1. Introduction

Momordica grosvenori is a special plant with important application value and nutritional value in Guangxi and has long been extensively applied in medicines, drinks and seasonings (Chang-Bao et al., 2012). Someone studied the chemical components of Momordica grosvenori leaf recently and found it contained rich flavonoid materials, especially kaempferol and quercetin. In vitro experiment suggested that, Momordica grosvenori extractive flavonoids have strong antioxidant activity and they are effective natural antioxidants which can remove free radicals, improve blood circulation, reduce cholesterol, inhibit the effusion of inflammatory bio-enzyme, promote the healing of wound and relieve pain.

Many people think that physical activity is sure to strengthen immunity (Miyazawa et al., 2015; Murakami et al., 2013). However, the opinion is of divergence because exercise only is not enough to improve the functions of immune system. Many experts and scholars hold that, exercise with different strengths can produce different effects on the immune system of body (Vlachopoulos et al., 2015; Wanner et al., 2014). For example, the high-strength and long-term exercise can inhibit the immune

functions of human body, induce the inhibition of exercise related immune functions, and increase the risks of virus infection. Therefore, it is of great significance to explore which substance can protect immune system. A report suggested that 10-day gavage of Momordica grosvenori leaf extractive flavones to rats significantly increased the proportion of a - Naphthyl Acetate Esterase (ANAE) positive cells and the proportion of rosettes formed in splenocyte, without affecting PPMNP, indicating Momordica grosvenori leaf extractive flavones could improve specific cellular immunity and humoral immunity on the condition of not affecting the non-specific immune function of body, which further revealed the regulatory and protective effects of Momordica grosvenori leaf extractive flavones on body immunity (Di et al., 2011; Dong-Lian et al., 2011; MO et al., 2013). The purpose of this study aimed at excavating and exploring the healthcare function and medical value of Momordica grosvenori leaf extractive flavones, especially the effect on exercise-induced immunity. Flavones extracted from Momordica grosvenori leaves were acted on rats with exercise-induced exhaustion. The experimental analysis proved the regulatory and protective effect of Momordica grosvenori leaf extractive flavones on the immune functions of body, providing a powerful theoretical and factual basis for its value development.

2. Materials and methods

2.1. Research subjects

Fifty healthy and clean male rats, aged 5 weeks and weighed 170 g, were selected. After adaptive feeding, five rats which were not suitable for treadmill exercise were excluded and classified into sedentary group. The remaining 45 rats were randomly and evenly divided into five groups, i.e., 5 for sedentary group, together with the five rats excluded, 10 for repeated exhaustive exercise control group, 10 for once exhaustive exercise control group, 10 for drug intervention combined with repeated exhaustive exercise group and 10 for drug intervention and once exhaustive exercise group. A model of the effect of Momordica grosvenori leaf extractive flavones on immune system of rats after exhaustive exercise was established; experimental treadmill for animals was used as the mode of exercise. All animal experimental operations were made according to the requirements of experimental animal management committee, reviewed and approved by the animal ethics committee and verified by pathologists.

2.2. The establishment of animal exercise model

(1) Except the sedentary group which was treated by conventional feeding but did not take adaptive training, the other four groups experienced three-day adaptive treadmill training;

(2) After three-day adaptive treadmill training, rats in the repeated exhaustive exercise control group and the drug intervention combined with repeated exhaustive exercise group experienced three-week exhaustive treadmill training additionally;

(3) Rats in the repeated exhaustive exercise control group and the drug intervention combined with repeated exhaustive exercise group experienced 6 times of exhaustive treadmill running in one week; at the 7^{th} day, training stopped and blood was collected from the rats.

(4) One day before dissection, once exhaustive treadmill exercise proceeded in the once exhaustive exercise control group and the drug intervention combined with once exhaustive exercise group; but before exercise, all rats were weighed;

(5) The determination criteria for whether rats exhausted included indifferent expression, dull reaction, running in a supine position, and temporary disappearance of righting reflex

2.3. The selection of observation indexes and test indexes

The duration of exhaustive swimming, plasma superoxide dismutase (SOD) activity,

and the content of methane dicarboxylic aldehyde (MDA) were detected and recorded.

2.4. Acquisition and processing of blood specimens

(1) Blood was collected from the exhausted rats using decollation; 4 ml of blood was taken and added into a centrifuge tube loaded with heparin;
(2) Then the blood was added into a large-volume low-speed refrigerated centrifuge and centrifuged at 3250 r/min for 13 min;
(3) The plasma was transferred into tubes respectively and stored in a cryogenic refrigerator as a preparation for the detection of plasma indexes and white blood cells.

2.5. The source of Momordica grosvenori leaf extractive and dose detection

2.5.1. The source of Momordica grosvenori leaf extractive

300 kg of Momordica grosvenori leaves were weighed and put into an extraction pot. After the addition of 3000 L of water, reflux extraction was performed for two hours, followed by cooling and filtration. The above procedures were repeated for four times.

The filter liquor flew through large pore resin absorption column in a proper speed; then it was washed by distilled water till colorless liquid flew out; then it was eluted by 75% ethyl alcohol.

Ethyl alcohol was recycled from the condensed eluent till solid-containing content became 20%; 10 kg of Momordica grosvenori leaf extractive flavones crude products were obtained after spray drying.

The crude products were mixed with 75% ethyl alcohol for reflux extraction. Filtration was repeated for three times. Ethanol solution was merged and condensed. Then ethyl alcohol was recycled till the solid-containing content became 30%. Finally, spray drying was performed. At the end of the experiment, 5 kg of Momordica grosvenori leaf extractive flavones competitive products was obtained.

2.5.2. The determination of the dose of Momordica grosvenori leaf extractive flavones

A certain quantity of Momordica grosvenori leaf extractive flavones was dissolved in a certain amount of water and then gavaged to rats in the drug intervention combined with once exhaustive exercise group and the drug intervention combined with repeated exhaustive exercise group (Genyk et al., 2016; Zehetner et al., 2015). Rats in the sedentary group, once exhaustive exercise control group and repeated exhaustive exercise control group were gavaged with the same dose of normal saline. The gavage was performed at noon 12'o clock. Half an hour after gavage, the rats did exhaustive treadmill training. The gavage was performed once each day, for three weeks.

2.6. Statistical method

Data were statistically processed using SPSS version 18.0. The measured data were expressed as mean ± standard deviation (SD). The comparison between groups was statistically processed using analysis of variance. The difference between groups was processed by t test. $p < 0.05$ meant difference had statistical significance.

3. Results and discussions

3.1. The change of duration of exhaustive exercise of rats under the effect of Momordica grosvenori leaf extractive flavones

Every experimental step proceeded according to the dose of gavage drug and exercise experimental scheme. The comparison between four groups suggested the effects of grosvenori leaf extractive flavones on the duration of exhaustive exercise of rats, as shown in Table 1. It could be seen from table 1 that, under the same training condition and feeding condition, the duration of exhaustive exercise of the drug intervention combined with exhaustive exercise groups was much longer than that of the exhaustive exercise control groups.

Table 1. The change of duration of exhaustive exercise of rats under the effect of *Momordica grosvenori* leaf extractive flavones

Group	Duration of exercise (min)
Once exhaustive exercise control group	31.42±18.13
Drug intervention combined with once exhaustive exercise group	49.25±17.37
Repeated exhaustive exercise control group	43.33±22.15
Drug intervention combined with repeated exhaustive exercise group	61.31±20.27

3.2. The change of plasma indexes of rats under the effect of different doses of Momordica grosvenori leaf extractive flavones

As shown in Table 2, the content of MDA of the once exhaustive exercise control group, drug intervention combined with once exhaustive exercise group, repeated exhaustive exercise control group and drug intervention combined with repeated exhaustive exercise group was significantly/extremely significantly lower than that of the sedentary group, but the differences between those groups were not statistically significant. The SOD activity of the drug intervention combined with once exhaustive exercise group, repeated exhaustive exercise control group, drug intervention combined with repeated exhaustive exercise group was much higher than that of the sedentary group ($p < 0.05$), and the SOD activity of rats taking drugs was not significantly different ($p > 0.05$).

Table 2. The change of rat plasma indexes under the effect of different doses of Momordica grosvenori leaf extractive flavones

Group	Sedentary group	Once exhaustive exercise control group	Drug intervention combined with once exhaustive exercise group	Repeated exhaustive exercise control group	Drug intervention combined with repeated exhaustive exercise group
MDA	3.84±0.94	2.42±0.44	1.87±0.63	1.67±0.43	2.95±1.21
SOD	150.93±8.55	144.49±14.53	171.78±11.19	171.72±8.26	167.23±8.55

3.3. The change of number of peripheral white blood cells of rats in groups under the effect of Momordica grosvenori leaf extractive flavones

The number of peripheral white blood cells was calculated through experiment. The effects of Momordica grosvenori leaf extractive flavones on rats in groups are shown in Table 3. Compared to the drug intervention combined with exhaustive exercise group, the number of peripheral white blood cells of the exhaustive exercise control group was lower, i.e., the data of the drug intervention combined with once exhaustive exercise group and the drug intervention combined with repeated exhaustive exercise group were much higher than the once exhaustive exercise control group and the repeated exhaustive exercise control group ($p < 0.05$); compared to the sedentary group, the number of white blood cells of the peripheral white blood cells was higher, and the difference was statistically significant ($p < 0.05$).

Table 3. The change of white blood cells in peripheral blood of rats in groups under the effect of *Momordica grosvenori* leaf extractive flavones

Group	The number of white blood cells (1×10^9)			
	0th day	8th day	16th day	24th day
Sedentary group	8.45±0.56	8.77±0.27	8.11±0.64	9.19 ±0.75
Once exhaustive exercise control group	8.27±0.53	8.73±0.55	8.85±0.29	9.54±0.37
Drug intervention combined with once exhaustive exercise group	8. 76±0. 43	8. 85±0. 61	9. 26±0. 43	9. 59±0. 29
Repeated exhaustive exercise control group	8.27±0.69	8.87±0.64	9.25±0.44	9.33±0.46
Drug intervention combined with repeated exhaustive exercise group	8. 27±0.93	9. 19±0. 52	9. 38±0. 27	9. 35±0. 26

Momordica grosvenori is one of the special fruits in China and its extractive has special healthcare function and high nutritional value; hence it plays an important role in medicine and healthcare. Why Momordica grosvenori is famous in China and oversea is in a direct correlation to its special healthcare function and high nutritional quality (Mohamed et al., 2012; Yoon et al., 2013; Wu et al., 2013; Allen et al., 2016). For this reason, it is also called east magical fruit in China. We all know that, high-strength or long-term exercise may damage normal physiological balance and even result in the failure of self repair (Quinn et al., 2012; Damasio and Damasio, 2015; Zainol et al., 2012), thereby greatly affecting the health condition of body. Therefore, one hot research topic is the discovery of nutritional substance which can prevent tissue cells from damage, improve physiological tissue of various systems, relieve fatigue during body activity (Segizbaeva et al., 2013; Ferraresi et al., 2015; Leal et al., 2010), protect the health of body, and enhance exercise performance.

As to the change of the number of peripheral white blood cells after exercise, most of experimental results suggested that, the number of white blood cells increased after exercise, which resulted in the isolation of white blood cells from endothelial cells of blood vessel wall (Ronco et al., 2011; Icardo et al., 2012; Jagadeesha et al., 2011; Nugent et al., 2012) and the release of white blood cells from liver and spleen into blood. The results of this study demonstrated the above opinion. The number of peripheral white blood cells of the two exhaustive exercise control groups was higher than that of the sedentary group; the number of peripheral white blood cells of the drug intervention combined with exhaustive exercise group was higher than that of the two corresponding exhaustive exercise group respectively, and the difference was statistically significant. Thus it can be inferred that, long-time administration of Momordica grosvenori leaf extractive flavones or exhaustive exercise can increase the number of peripheral white blood cells, but the long-time administration of certain dose of Momordica grosvenori leaf extractive flavones is more effective in increasing the number of peripheral white blood cells.

4. Conclusions

This study explored the effects of Momordica grosvenori leaf extractive flavones on immune system functions of rats by establishing a rat exhaustive exercise model and detecting relevant immune organ and cell indexes through experiment and found Momordica grosvenori had sound effect in strengthening the immune functions of rats. But due to the limitations of resources and conditions, there were some defects in the experimental and analysis process. We will further supplement and perfect the results in future experiments.

5. References

Allen, K.W., Scott, M.M., Reid, D.R., et al. (2016). An X-band waveguide measurement technique for the accurate characterization of materials with low dielectric loss permittivity. *Review of Scientific Instruments,* 87(5), 33-36.

Chang-Bao, L.I., Li, L.I., Xue-Hui, W.U., et al. (2012). Emulsion formula of Momordica grosvenori seed oil microencapsulation and its property. *Journal of Southern Agriculture,* 43(8), 1203-1207.

Damasio, A., Damasio, H. (2015). Exploring the concept of homeostasis and considering its implications for economics. *Journal of Economic Behavior & Organization,* 126, 125-129.

Di, R., Huang, M.T., Ho, C.T. (2011). Anti-inflammatory Activities of Mogrosides from Momordica grosvenori in Murine Macrophages and a Murine Ear Edema Model. *Journal of Agricultural & Food Chemistry,* 59(13), 7474-81.

Dong-Lian, .C., Run-Jun, H., Shao-Mei, M. et al. (2011). Kinetics of extracting mogroside from grosvenori momordica(C)// Computing, Control and Industrial Engineering (CCIE), 2011 IEEE 2nd International Conference on. IEEE, 2011, 102-104.

Ferraresi, C., Sousa, M.V.P.D., Huang, Y..Y et al. (2015). Time response of increases in ATP and muscle resistance to fatigue after low-level laser (light) therapy (LLLT) in mice. *Lasers in Medical Science,* 30(4), 1259-1267.

Genyk, P., Ehtiati, T., Paudel, K. et al. (2016). 3:00 PMAbstract No. 11 - Quantitative assessment of gastric perfusion following bariatric arterial embolization in porcine model. *Journal of Vascular & Interventional Radiology,* 27(3), S8-S9.

Icardo, J.M., Loong, A.I., Colvee, E. et al. (2012). The Alimentary Canal of the African Lungfish Protopterus annectens, During Aestivation and After Arousal. *Anatomical Record Advances in Integrative Anatomy & Evolutionary Biology,* 295(1), 60–72.

Jagadeesha, K., Dammanahalli, X.W., Zhongjie, S. (2011). Genetic IL-10 deficiency causes vascular remodeling via the upregulation of Nox1. *Journal of Hypertension,* 29(11), 2116-2125.

Leal, J.E.C., Lopes-Martins, R.A., Frigo, L. et al. (2010). Effects of low-level laser therapy (LLLT) in the development of exercise-induced skeletal muscle fatigue and changes in biochemical markers related to postexercise recovery. *Journal of Orthopaedic & Sports Physical Therapy,* 40(8), 524-32.

Miyazawa, K., Kawase, M., Kubota, A. et al. (2015). Heat-killed Lactobacillus gasseri can enhance immunity in the elderly in a double-blind, placebo-controlled clinical study. *Beneficial Microbes*, 6(4), 1-9.

Mohamed, D.A., Al-Okbi, S.Y., El-Hariri, D.M. et al. (2012). Potential health benefits of bread supplemented with defatted flaxseeds under dietary regimen in normal and type 2 diabetic subjects. *Polish Journal of Food & Nutrition Sciences,* 62(2), 103-108.

Mo, W. Gong, M., Liu, T. et al. (2013). Effects of Momordica Grosvenori Flavones on Myocardial Energy Metabolism Enzymes and Expression of PPARα mRNA in Exercise Rats. *Chinese Journal of Experimental Traditional Medical Formulae,* (14), 203-208.

Murakami, H., Kato, S., Ueda, Y. et al. (2013). Reconstruction using a frozen tumor-bearing vertebra in total en bloc spondylectomy can enhance antitumor immunity. *European Spine Journal,* 23(S2), 222-227.

Nugent, H.M., Ng, Y.S., White, D. et al. (2012). Ultrasound-guided percutaneous delivery of tissue-engineered endothelial cells to the adventitia of stented arteries controls the response to vascular injury in a porcine model. *Journal of Vascular Surgery,* 56(4), 1078-88.

Quinn, M.J., Resch, C.T., Sun, J. et al. (2012). NhaP1 is a K+ (Na+)/H+ antiporter required for growth and internal pH homeostasis of Vibrio cholerae at low extracellular pH. *Microbiology*, 158(Pt 4), 1094-105.

Ronco, A.M., Montenegro, M., Castillo, P. et al. (2011). Maternal exposure to cadmium during gestation perturbs the vascular system of the adult rat offspring. *Toxicology & Applied Pharmacology*, 251(2), 137-45.

Segizbaeva, M.O., Donina, Z.A., Timofeev, N.N. et al. (2013). EMG analysis of human inspiratory muscle resistance to fatigue during exercise. *Advances in Experimental Medicine & Biology*, 788(788), 197-205.

Vlachopoulos, D., Barker, A.R., Williams, C.A. et al. (2015). Effect of a program of short bouts of exercise on bone health in adolescents involved in different sports: the PRO-BONE study protocol. Bmc Public Health, 15(361), 1-10.

Wanner, S.P., Costa, K.A, Soares, A.D.N. et al. (2014). Physical exercise-induced changes in the core body temperature of mice depend more on ambient temperature than on exercise protocol or intensity. *International Journal of Biometeorology*, 58(6), 1077-85.

Wu, Y.B., Zheng, L.J., Yi, J. et al. (2013). Quantitative and chemical fingerprint analysis for the quality evaluation of Receptaculum Nelumbinis by RP-HPLC coupled with hierarchical clustering analysis. *International Journal of Molecular Sciences*, 14(1), 1999-2010.

Yoon, J., Lee, J., Kim, S. et al. (2013). Three-dimensional graphene nano-networks with high quality and mass production capability via precursor-assisted chemical vapor deposition. *Scientific Reports*, 3(19), 2491-2491.

Zainol, M., Almeida, G.M., Sherwood, B.T. et al. (2012). Development of an internal standard for the Comet assay: Minimizing intra- and inter-experiment variability in the measures of DNA damage formation and repair. *Atlantis*, 34(2), 167-184.

Zehetner, J., Demeester, S.R., Alicuben, E.T. et al. (2015). Intraoperative assessment of perfusion of the gastric graft and correlation with anastomotic leaks after esophagectomy. *Annals of Surgery*, 262(3), 106-112.