



Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Experimental research into the influence of *Morchella esculenta* on nitrogen monoxide and NOS system in the rats that have done high strength durability exercises

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ABSTRACT

This paper aims at studying the influence of *Morchella esculenta* on nitrogen monoxide and NOS system in the rats that have done high strength durability exercises. Experiment is conducted for comparison by feeding exopolysaccharide extract to wistar male rats that have done high strength durability exercises to observe the change in the content of NO and NOS in different tissues of wistar male rats that have done high strength durability exercises. Result: the content of NO in the liver tissue of the group that has done exercises and taken drugs is significantly lower than the group that has done exercises ($P < 0.05$); the total activity of NOS enzyme in the cardiac muscle tissue of the group that has done exercises and taken drugs is lower than the group that has done exercises, but the difference is insignificant ($P < 0.05$); the total activity of NOS enzyme in the liver tissue of the group that has done exercises and taken drugs is lower than the group that has done exercises, but the difference is insignificant ($P > 0.05$). Conclusions: the content of NO in the cardiac muscle tissue and the liver tissue in the rats can be changed by supplementing exopolysaccharide extract to improve the constriction of blood vessels in the hearts and the livers of rats having done exercises to stretch blood vessels and increase blood supply.

Keywords: *Morchella esculenta*; High strength durability exercises; NO; NOS

INTRODUCTION

Morchella esculenta, also called "Morchella" and found in 1818, is a rare drug in the world and one of the valuable fungi, as well as a high grade tonic belonging to genus *Morchella* genus, Helvellaceae, Pezizomycetes, Ascomycotina. In shape, it looks like a general pileus, and mainly originates from Shanxi Province, Yunnan Province, Xinjiang, Sichuan Province and other areas[1-3]. According to the researches over recent years, *Morchella esculenta* has the functions of preventing atherosclerosis, cancers, enhancing the immunity and prolonging aging and other biologic functions. At the same time, it also has of relieving fatigue and improving capability of having done exercises, as a sports supplement, it has a broad prospect. This experiment is to further discuss the *Morchella esculenta*'s mechanism to resist oxidation, and the influence on the rats' capability of doing exercises and the change of NO system by supplementing *Morchella esculenta*, and provides a theoretical basis for using *Morchella esculenta* as a kind of nutritious food for sports and health food.

EXPERIMENTAL SECTION

1.1 Materials

Morchella esculenta is picked from Yunnan Province.

1.2 Processing of samples

It is recommended that one person shall take 30 mg/kg.BW/d (the daily recommended mass to be taken is 1.2-2.4g, let us assume 1.8g, and the mass of human body is assumed to be 60kg). The sample is compounded into suspension with distilled water.

1.3 Animal and grouping

In this experiment, wistar male rats are selected provided by the animal experimental center of Norman Bethune College of Medicine of Jilin University. The rats' weight is 260g to 300g, and 24 ones are used in total, they are freely fed and drink freely. The rats are classified into three groups at random: I. Quiet control group; II. High strength durability exercise group; III. High strength durability exercise+taking 300 mg/kg.BW/d of exopolysaccharide extract of *Morchella esculenta*, and filling 0.2mL into their stomachs for continuously 42 days. All of subjects are fed with normal edible feed.

1.4 Extracting exopolysaccharide of *Morchella esculenta*

The *Morchella esculenta* is inoculated into liquid media (glucose 50g, NH_4NO_3 2g, KH_2PO_4 1g, MgSO_4 1g, vitamin B_1 0.1g, distilled water 1L, pH adjusted to 6.5) at 26 °C to cultivate 10d at the speed of 180r/min; the culture medium is collected and condensed; protein is removed by using Sevag method, 95% alcohol precipitate is added, frozen and dried in vacuum to get the exopolysaccharide extract of *Morchella esculenta*(abbreviated as "the extract" below). The main ingredients of the exopolysaccharide extract of *Morchella esculenta* are as follows: total content of sugar 60.12%; the content of protein: 8.91%.

1.5 Experimentation scheme

Benford [8] running bench training method is adopted, and the rats run for six weeks, six days per week, and have a rest on Sunday. One week adaptation training is conducted at first to gradually improve the sports strength above 80% VO_2max until exhausted on the last day. After weighing on the seventh day of the sixth week, the rats' necks are broken to take their brains, cardiac muscles, livers, kidneys, skeletons in the icy physiologic saline to wash away the blood, and dry them with filter paper, and then stored in liquid nitrogen for use in the future. When their physiological indices are measured and they are weighed, the homogenate is centrifugalized at low temperature, and the supernate liquor is taken for use in the near future.

1.6 Testing indices and method

Testing indices: NO and total NOS.

The sample is put in the cold closet for defrosting before the activity of NOS is measured, and then the activity is measured strictly according to the colorimetric methods by using the NOS activity measurement reagent kits provided by Nanjing Jiancheng Bioengineering Institute. The content of NO is measured by using the Nitrate reductase; the protein in the supernate liquor of the homogenate is measured by using the Coomassie Brilliant Blue method.

RESULTS

2.1 The change of NO content in different tissues of the wistar male rats after doing exercises and interfering with the exopolysaccharide extract of *Morchella esculenta*.

Seen from Table 1, the content of NO in the tissues in the wistar male rats after doing exercises rises to different extents, in particular the content of NO in the liver and four-headed thigh that extremely significantly rises ($P < 0.01$), and the content in the kidney and cardiac muscle rises significantly ($P < 0.05$); however, the content of NO in the brain rises insignificantly.

Table 1 The Change of NO Content in Different Tissues of the Wistar Male Rats After Doing Exercises and Interfering in the Exopolysaccharide Extract of *Morchella esculenta*
(Unit: $\mu\text{mol/g prot}$)

Tissue	I Quiet control group	II Benford[8] Running bench	III Benford[8] running bench + exopolysaccharide extract of <i>Morchella esculenta</i>
Brain	3.28±0.23	3.61±0.18	3.31±0.16
Cardiac muscle	1.86±0.55	2.82±0.42*	2.65±0.61
Liver	0.53±0.15	1.45±0.25**	1.02±0.11***
Kidney	1.35±0.36	1.81±0.45*	1.80±0.51
Four-headed thigh	1.96±0.85	3.89±1.26**	3.31±0.89**

Note: *Means significant difference compared with the quiet control group ($P < 0.05$), **Means greatly significant difference compared with the quiet control group ($P < 0.01$); *Means significant difference compared with the exercising control group ($P < 0.05$), ***Means greatly significant difference compared with the exercising control group ($P < 0.01$), the same below.

Compared with the exercising control group, after interfering by adding exopolysaccharide extract of *Morchella esculenta*, the content of NO in the wistar male rats lowers to different extents, in particular that in the liver and four-headed thigh that lowers significantly, in particular those in the liver and the four-headed thigh lower significantly, and those in other tissues only tend to lower; the content of NO in the kidney is changed in significantly, almost equal to that of the exercising control group, but still higher than that of quiet control group; compared with the quiet control group, the content of NO in exercising and drug taking group is still higher, and significantly different in the liver and the four-headed thigh ($P < 0.05$), however, the content of NO in heart and the tissues just tends to lower with no statistical significance.

2.2 The change of NOS content in different tissues of the wistar male rats after doing exercises and interfering with the exopolysaccharide extract of *Morchella esculenta*

Table 2 NOS Content in Different Tissues of the Wistar Male Rats After Doing Exercises and Interfering with the Exopolysaccharide extract of *Morchella esculenta*
(Unit: U/mg prot)

Tissues	I Quiet control group	II Benford Running bench	III Benford running bench + exopolysaccharide extract of <i>Morchella esculenta</i>
Brain	0.51 ± 0.03	0.56 ± 0.08	0.46 ± 0.11 [*]
Cardiac muscle	0.23 ± 0.05	0.37 ± 0.51 ^{**}	0.32 ± 0.09 ^{**}
Liver	0.47 ± 0.01	0.69 ± 0.12 ^{**}	0.60 ± 0.08 ^{**}
Kidney	0.45 ± 0.06	0.98 ± 0.41 [*]	0.87 ± 0.21 [*]
Four-headed muscles	0.16 ± 0.08	0.17 ± 0.07 [*]	0.18 ± 0.06 [*]

Note: ^{*}Means significant difference compared with the quiet control group ($P < 0.05$), ^{**}Means greatly significant difference compared with the quiet control group ($P < 0.01$); ^{*}Means significant difference compared with the exercising control group ($P < 0.05$), ^{**}Means greatly significant difference compared with the exercising control group ($P < 0.01$), the same below.

As shown in Table 1: the activity of NOS in the tissues of the wistar male rats (II) after doing exercises rises. Compared with the quiet control group, the activity of NOS in the cardiac muscles, liver and kidney tissues of wistar male rats in the exercising + exopolysaccharide extract of *Morchella esculenta* group (III) and the exercising control group (II) rise significantly ($P < 0.05$ and $P < 0.01$); however, compared with the group II, that in the group III rises only in the four-headed thigh with insignificant difference; however, that in the cardiac muscles of the group III significantly lowers, and those in the liver, kidney and brain tissues also tend to rise but without significant difference ($P > 0.05$), See table 2.

DISCUSSION

In a whole, the NOS activity in the brains, hearts, livers and kidneys and the four-headed thigh tissues can increase by doing high strength durability exercises, however, the NO content generated also rises, consistent with the rise tendency of NOS. However, that in the livers and the four-headed thigh is greatly significantly different and the rise in other tissues is not so significantly[4].

In the rats in the group that is fed with exopolysaccharide extract of *Morchella esculenta* and has done exercises, the total NOS activity in the tissues tends to lower with significant difference in the content in the cardiac muscle, however, the four-headed muscle is an exception, NOS tends to rise slightly; and the NO content in the tissues also tend to lower, almost consistent with the overall tendency of NOS[5].

The theory may be as follows: the exopolysaccharide extract of *Morchella esculenta* is an efficient free radical scavenger, and can penetrate through the cell membranes, and is not toxic, and also may penetrate blood brain barrier very well; the exopolysaccharide extract of *Morchella esculenta* can scavenge hydroxy radicals, NO, superoxide anions and active oxygen very well[6]. It can reduce the oxygen radicals generated during the exercise, and may improve the scavenging ratio, improve the activity of SOD, and relieve the damage to the endothelial cells by radicals. After the endothelial functions of blood vessels are improved, the concentration of Ca²⁺ in the cytoplasm rises, leading to the rise of cNOS enzymic synthesis, thus leading to the rise of NO synthesis; during the exercise, as the consumption of energy increases, the concentration of ADP rises, and cNOS enzyme may also be activated, and NO synthesis rises.

It is found in the experiment that the total NOS in the brain tissues of the exercising and drug-taking group significantly lowers, maybe because the resistance of exopolysaccharide extract of *Morchella esculenta* to movement causes NO to excessively rise, and damage the brain tissues, and it in fact protects the brain tissues.

CONCLUSION

According to the research results, the content of NO in the cardiac muscles and liver tissues of the rats may be changed by feeding them with exopolysaccharide extract of *Morchella esculenta*, the to improve the constriction of blood vessels in the hearts and the livers of rats having done exercises to stretch blood vessels and increase blood supply. The exopolysaccharide extract of *Morchella esculenta* may scavenge the radicals generated during exercises and regulate NO-NOS system to get to balance, relieve the neurotoxicity of NO medium, further improving the movement capability of the organisms. It means that the ingredients in the exopolysaccharide extract of *Morchella esculenta* may protect the mechanism of the brains, the hearts, livers, kidneys, muscles while the bodies are doing a lot of exercises, and it may relieve the damage to the bodies' functions caused by high strength exercises, improve the bodies' capabilities, and prolong the body fatigue. This research provides a scientific experimentation basis for using the exopolysaccharide extract of *Morchella esculenta* as a supplement for recovering strength.

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