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Experimental research into the influence of morida officinalis how polysaccharide on the rats' capability of having sports

Li Jing Jilin Sports University, Changchun Jilin 130022, (CHINA) E-mail: Jingjing2014@163.com

## ABSTRACT

Purpose of the research: to discuss the influence of Morida Officinalis How Polysaccharide on rats' capability of having sports and metabolism of free radicals of heart muscle. Method: ICR male rats are used as subjects in this research; the swimming training and swimming-until fatigue-of the rats that are supplied with Morida Officinalis How Polysaccharide are increasing, the duration of the rats' swimming until fatigue, serum GOT, CK, Hb, whole blood LD, heart muscle tissue SOD and GSH-Px activity, MDA content are measured to observe the influence of Morida Officinalis How Polysaccharide on the ICR male rats' capability of having sports. Results: The ICR rats from the drug-taking group have their capabilities of having sports significantly improved; immediately after fatigue, the decrement of Hb and the increment of serum GOT and CK, whole blood LD, heart muscle MDA of the drug-taking group are significant; the GSH-Px activity of the drug quiet group is significantly higher than that of the quiet control group. Conclusion: Morida Officinalis How Polysaccharide may significantly improve the ICR rats' capability of having sports and the heart muscles' capabilities of resisting the oxidation by free radicals, and Morida Officinalis How Polysaccharide's pharmacological actions shall be deeply developed and utilized.

# **KEYWORDS**

Morida officinalis how polysaccharide; ICR rats; Heart muscle; Free radicals; Fatigue.

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#### INTRODUCTION

Morinda officinalis How is the dried root of Morinda officinalis How, which is of rubiaceous type, dicotyledons family, tastes sweet with a little bitterness, is a little warm, and is one of the four famous south medicines in China<sup>[1]</sup>. It was originally recorded in Shen Nong's Herbal Classic and was listed as a superior product, and was recorded in the Herbal Classics of every dynasty. Morinda officinalis How's main ingredients include Morida officinalis How polysaccharide, resin, and Morinda officinalis How contains a lot of microelements and Vitamin C. It is found according to related researches over recent years that Morinda officinalis How has such functions of resisting fatigue, free radicals, depression, affecting endocrine and improving immunity.

Free radical is the general name of atoms, ions or molecules containing unpaired electronics on the outermost orbital. Since 1952 when Denham Harman<sup>[2]</sup> proposed the free radical mechanism of aging, people have studied the function of free radicals in the living bodies for more than half a century and more and more studies show that the free radicals in the human bodies, in particular the active oxygen free radicals, play a key role in diseases and aging. As the free radical medicine emerges, people are gradually aware that it is very important to remove excessive active oxygen free radicals in order to prevent and cure diseases. In this paper, the swimming training and swimming-until fatigue-of the rats that are supplied with Morida Officinalis How Polysaccharide are increasing; in this way, the influence of Morida Officinalis How Polysaccharide on the rats' capability of having sports and metabolism of free radicals of heart muscle is studied; the function of Morida Officinalis How Polysaccharide on improving rats' capabilities of having sports is discussed to provide theoretical basis for it to be applied in the field of nutritious sports food better.

#### MATERIALS AND METHOD

#### Extraction of morida officinalis how polysaccharide

Coarse Morida Officinalis How is taken and crushed, dried, and accurately weighed, and then 10 times of water by weight is added to soak Morida Officinalis How for 30 minutes, and then put the same in boiled water for 20 minutes, and then the solution is filtered; three times of alcohol by volume is added to the filtrate, and the mixture is left untouched for a night. After the alcohol is filtered, deposit is collected, and the deposit is dried to get the coarse Morida officinalis How polysaccharide<sup>[3]</sup>. The coarse Morida officinalis How polysaccharide is solved in a right amount of distilled water, the Sevag method is used for removing eggwhite (repeated three times), and alcohol is added until the strength of solution becomes 80.0%; after the solution is left for a night, the deposit is collected. The deposit is washed in anhydrous ethyl alcohol, acetone in turn. The deposit is dissolved in the distilled water and alcohol is added until the strength of solution becomes 70.0%; the solution is left still for a night and then is filtered, washed again and again with alcohol of 70.0%, and the deposit is dried, consequently, pure Morida Officinalis How Polysaccharide is got.

### **Subjects and methods**

# Animals used in the experiment and what to do with them

40 ICR rats are selected, which are eight weeks old, 24-28g by weight, fed for one week in order to be adaptable. In the fourth week, the rats are classified into four groups by their weight: quiet control group, quiet+ Morida Officinalis How Polysaccharide group; and each group contains 10 ones (refer to TABLE 1 for the basic information of the animals and the doses taken). All of the animals are raised in labs at the temperature of  $22\sim27$  0C and relative humidity of 55%-63%; the duration of light varies with the nature, and the rats are fed with basic feed and cool boiled water freely. In the fifth week, the rats are trained to swim under no load for 20 minutes in the water at the temperature ( $30\pm1$ ) 0C and 48cm deep; at 8: 00 in the mornings of the sixth week, all of the animals swim under load until fatigue in groups (TABLE 1); in the seventh week, the rats have a rest. The criteria for fatigue are as follows: the whole head of a rat is submerged in water continuously for eight seconds, and can not float above the water.

### Training and dosing plans for the animals in the experiment

The animals from the training groups begin swimming training under no load from the commencement date of the experiment. In the first week, the rats swim for 30 minutes per day and the duration rises by 10 minutes gradually subsequently per week, and the rats are trained for six weeks in total. When the rats have a rest every Sunday, the rats take 15g of Morida Officinalis How Polysaccharide extract per kg of body weight per day (depending on the dose effectiveness) by using stomach tubes; and the animals from the control groups are fed with equivalent amount of distilled water by using stomach tubes until the completion of the experiment.

# Sampling timing and index testing procedures and methods

On the first day of the seventh week, animals from the groups other than the quiet control group, the quiet+ Morida Officinalis How Polysaccharide group swim under the load which is 9% of their weights until fatigue in groups; the duration of swimming until fatigue is recorded; the criteria for fatigue are the same with those above; blood is sampled from the eye sockets of the animals from the sports control group and the sports+ Morida Officinalis How Polysaccharide group immediately after the animals have sports; and heparin is used for anticoagulation; Hb and LD are tested immediately after blood is sampled; and then the whole blood that has been treated to be anti-coagulated is centrifugalized for 15 minutes; serum GOT (glutamic-oxaloacetic transaminase) and CK are tested; heart muscle tissues are taken out immediately, washed

and cleaned with cooled physiologic (al) saline, and then dried with filter paper and weighed; physiologic (al) saline is added based on W:V=1:9 as homogenate medium and then an electric homogenizer is used for homogenization at low temperature. The mixture is frozen and centrifugalized for 15 minutes at the speed of rotation of 8000r/min; the supernatant is stored in the refrigerator at  $4^{\circ}C$  to test the SOD, GSH-Px and MDA of the heart muscle tissues; and the indices of the two quiet groups are tested as above.

Index testing methods: GOT (Reitman Frankel Method), CK (colorimetric method), Hb (ferricyanic colorimetric method), LD (colorimetric method), total protein (coomassie blue staining method), heart muscle SOD (Xanthinoxidase method), heart muscle MDA (TBA method), heart muscle GSH-Px (DTNB method). All of the index testing reagent boxes are provided by Changchun Far East Biological Engineering Institute.

# Processing of experimental data

The indices before and after the experiment are processed in terms of statistics by using the statistic analysis software of SPSS19.0; and the significance and great significance are set at the levels of P<0.05 and P<0.01; t-testing is used for comparing the averages of two groups.

#### RESULT AND DISSCUSS

# Comparison of rats' weights and duration of swimming under load until fatigue before the experiment

The weights of the rats from all of the groups before being fed with Morida Officinalis How Polysaccharide and swimming are of no significant difference through t-testing (P>0.05). In addition, the durations of the ICR rats from all of the groups under the load equivalent to 5% of the weights until fatigue are of no significant difference through t-testing (P>0.05) (see TABLE 1). It means that before being fed with Morida Officinalis How Polysaccharide, the physiological functions of the rats from all of the groups and their capabilities of having sports are normal, and their basic conditions are of no significant difference and they completely meet the experimentation conditions.

TABLE 1 : Comparison of the weights of the rats from all of the groups and the durations of swimming under load until fatigue ( $\bar{x} \pm sd$ )

Group	Sample sizes	Dose/g·kg-1	Weights before dosage/g	Durations until fatigue /min
Quiet control group	10	_	32.02±1.65	60.88±10.32
Quiet + Morida Officinalis How Polysaccharide	10	15.0	31.65±2.31	61.10±12.68
Sports control group	10	_	$32.41 \pm 1.45$	$59.91 \pm 14.56$
Sports+ Morida Officinalis How Polysaccharide group	10	15.0	32.66±1.29	61.21±11.62

# Influence of morida officinalis how polysaccharide on the durations of icr rats' swimming until fatigue

The sports control group is fed with distilled water; and the sports+ Morida Officinalis How Polysaccharide is fed with Morida Officinalis How Polysaccharide. After six weeks' training under increasing loads, the duration of last swimming until fatigue in the sports+ Morida Officinalis How Polysaccharide group is significantly longer than that of the pure sports group (P<0.01), the duration difference is about 23.16 minutes (see TABLE 2).

 TABLE 2: Influence of Morida Officinalis How Polysaccharide on the Durations of Swimming to Fatigue ( $x \pm sd$ )

Group	Sample size	Durations fatigue/min	Until Duration difference/min	
Sports control group	10	82.91士16.31		
Sports+ Morida Officinalis How Polysaccharide group	10	106.07±20.56**	23.16	

Note:\* means comparison with the quiet control group p<0.05, \*\* means comparison with the quiet control group p<0.01.

#### Influence of morida officinalis how polysaccharide on the biochemical indices of fatigued rats' blood

The serum GOT of the quiet+ Morida Officinalis How Polysaccharide group is significantly lower than that of the quiet control group (p<0.05); immediately after fatigue, Hb decrement of the sports+ Morida Officinalis How Polysaccharide

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group is significantly lower than that of the sports control group (p<0.01); however, the increment of serum GOT and CK, whole blood LD of the sports+ Morida Officinalis How Polysaccharide group are significantly lower than those of the sports control group ((p<0.01, p<0.05), see TABLE 3).

TABLE 3 : Influence of morida officinalis how polysaccharide on the biochemical indices of the blood of fatigued rats  $(\bar{x} \pm sd)$ 

Group	Sample Size	GOT/μ·L <sup>-1</sup>	CK/µ·mL <sup>-1</sup>	Hb/g·L <sup>-1</sup>	LD/mmoL·L
Quiet Control group	10	11.91±1.56	62.10±5.13	130.15 ± 14.60	5.06士0.52
Quiet+ Morida Officinalis How Polysaccharide	10	9.02士1.12*	59.95士5.91	131.27±13.09	5.12±0.46
Sports Control group	10	28.63±2.32*	91.41±5.55*	89.03±13.45*	20.01士2.96*
Sports+ Morida Officinalis How Polysaccharide group	10	15.09±1.96 <sup>▽▽</sup>	79.13±4.12 <sup>▽▽</sup>	$100.21 \pm 14.76^{\triangledown\triangledown}$	$16.87 \pm 2.13^{\triangledown}$

Note:\* means comparison with the quiet control group p<0.05, \*\* means comparison with the quiet control group p<0.01; means comparison with the sports control group p<0.05, means comparison with the sports control group p<0.01.

# Influence of morida officinalis how polysaccharide on the fatigued rats' heart muscle tissue SOD, MDA and GSH-PX

The MDA strength of the heart muscle tissues of the quiet +GSH-Px is significantly lower than that of the quiet control group, and its GSH-Px activity is significantly higher than that of the quiet control group (P<0.05); however, SOD activity of the quiet + Morida Officinalis How Polysaccharide is higher than that of the quiet control group but without significant difference (P>0.05); immediately after the fatigue, the increment of SOD and GSH-Px activity of the liver tissues of the sports + Morida Officinalis How Polysaccharide group is much significantly higher than those of the sports control group (P<0.01); the increment of MDA content in the liver tissues of the sports + Morida Officinalis How Polysaccharide group is significantly lower than that of the sports control group (P<0.05).

TABLE 4: Influence of morida officinalis how polysaccharide on SOD, MDA and GSH-Px of the heart muscle tissues of fatigued rats  $(\bar{x} \pm sd)$ 

Group	Sample size	SOD/U·mg <sup>-1</sup>	MDA /μmol·L <sup>-1</sup>	GSH-Px/U·L <sup>-1</sup>
Quiet control group	10	51.86士2.63	12.10士1.13	52.15士1.85
Quiet + Morida Officinalis How Polysaccharide	10	52.02士1.80	11.95士1.61*	53.27士1.11*
Sports control group	10	63.90士1.92*	18.41士0.63*	65.03±2.05*
Sports + Morida Officinalis How Polysaccharide	10	75.46±1.01 <sup>▽▽</sup>	$14.13 \pm 1.07^{\circ}$	79.21±1.19 <sup>▽▽</sup>

Note:\* means comparison with the quiet control group p<0.05, \*\* means comparison with the quiet control group p<0.01; means comparison with the sports control group p<0.05, means comparison with the sports control group p<0.01.

#### ANALYSIS AND DISCUSSION

Morida Officinalis How is an economic medicinal plant specific to China, contains many chemical ingredients such as fructose, glucose, protein, amino acids, flavonoids, Vr, Ve and inorganic elements and others. At present, Morida Officinalis How and its extracts are playing significant roles in the development and utilization of medicines and healthcare and give out its unique charm.

The research mode of swimming until fatigue has been widely applied by many scholars inside and outside China<sup>[4]</sup>. Duration of having sports until fatigue is the comprehensive embodiment of an organism's anti-stress capability, and antifatigue capability and others, and is an important direct index for measuring the organism's capability of having sports<sup>[5]</sup>. In order to be adaptable to the continuous change of the sports stress, the organism may have the activity of the enzyme removing the free radicals of active oxygen enhanced, and free radical remover or anti-oxidant is supplied to the organism to further prevent lipid peroxidation and protect the cell membranes from being damaged.

According to the test results, the durations of swimming until fatigue of the sports+ Morida Officinalis How Polysaccharide group are significantly longer than those of the sports control group (P<0.01). It means that Morida Officinalis How Polysaccharide may prolong the rats' swimming duration until fatigue and improve the organisms'

capabilities of having sports, and their swimming durations until fatigue and capabilities of having sports depend on the doses. The mechanism is as follows:

①The active ingredients in Morida Officinalis How may effectively promote the synthesis of Hb. Hb, as the carrier for carrying oxygen and carbon dioxide in red cells, has the function of maintaining the balance of pH value of the body fluid, and exerts direct influence on the substance metabolism and the energy metabolism. Increased content of Hb may help to improve the organism's capability of having sports. According to the researches that most of scholars agreed to previously, Morida Officinalis How may effectively inhibit the Hb in the organism from falling down while the organism is doing physical exercises, and promote the synthesis of Hb in the organism while the organism is restoring. Hint: The multiple active ingredients such as amino acids and protein may improve the organism's hematopoietic functions and functions of the circulatory system, promote the regeneration and synthesis of Hb in the organism, thus maintaining the organism's high sports capability.

②The active ingredients in Morida Officinalis How may effectively inhibit the generation of LD in the organism's body while it is doing physical exercises and promote the removal of LD during restoration. According to the research, the blood LD of the two groups immediately after swimming is significant improved, however, the increment of the whole blood LD of the sports+ Morida Officinalis How group is significantly lower. Hint: the active ingredients in Morida Officinalis How, such as amino acids and protein and etc, may effectively improve the skeletal muscles' capability of utilizing oxygen and supplying oxygen to the cardiovascular system so that its capability of oxygen metabolism is improved, the removal of metabolites (LD) inside the bodies is accelerated; the lactic acid accumulated in the body is reduced to maintain the environment inside the bodies relatively stable.

③The main ingredient of Morida Officinalis How is Morida Officinalis How Polysaccharide, which may remove several kinds of active oxygen (ROS) from physical, chemical and biological sources, and may reduce the amount of MDA of the products from the peroxidatic reactions of lipids and improve the activity of SOD and GSH-Px and stabilize the cell membranes, thus improving the rats' capabilities of removing the free radicals while the rats are having sports, helping to resisting fatigue and improving their capabilities of having sports.

Morida Officinalis How contains a lot of vitamin C, Mn and a lot of microelements, which may promote the
metabolism of the energy, resist fatigue and improve the capability of having sports. Besides, Morida Officinalis How may
help to synthesize the glycogen and improve the content of glycogen, accelerate and promote the synthesis of high-energy
phosphate compound of ATP, resist fatigue and improve the capability of having sports.

# RESEARCH CONCLUSIONS

Morida Officinalis How Polysaccharide may significantly improve the duration of ICR rats' swimming until fatigue. It means that Morida Officinalis How Polysaccharide is significant for improving the capability of having sports. Its physiological mechanism is as follows: Morida Officinalis How Polysaccharide may effectively slow down the fall of Hb in the organism's body while the organism is having sports and promote the synthesis of Hb after having sports, reduce LD generated during sports and accelerate the removal of LD; Morida Officinalis How Polysaccharide may significant improve the activity of serum SOD and GSH-Px and reduce the content of MDA in serum. Hint: Morida Officinalis How may remove free radicals, inhibit the damage of lipids arising from peroxidatic reaction and play an active role in prolonging the generation and development of sports-induced fatigue.

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