



## Experimental research into the influence of supplemented spirulina on the form of erythrocytes and the content of hemoglobin of anaemic rats that have done exercises

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### ABSTRACT

The paper aims at verifying the influence of supplemented spirulina on the form of erythrocytes and the content of hemoglobin of anaemic ICR male rats that have done exercises. The method adopted: scanning electronic microscope and full automated hematology analyzer are used to studying the influence of supplementing spirulina on the form of erythrocytes and the content of hemoglobin of rats. Results: The malform ratio of the erythrocytes of ICR male rats in the group doing exercises is significantly higher than the control group, and the group supplemented with spirulina ( $P < 0.01$ ); the content of hemoglobin of ICR male the rats from the exercising group is significantly lower than that from the normal control group and the group supplemented with spirulina ( $P < 0.01$ ); the content of hemoglobin of ICR male rats from the exercising group is significantly lower than that from the normal control group and the spirulina group ( $P < 0.01$ ); the content of hemoglobin of ICR male rats from the spirulina group is lower than the normal control group ( $P < 0.01$ ). Conclusion: Spirulina can significantly reduce the malform ratio of erythrocytes of ICR male rats after doing exercises, and improve the content of hemoglobin of ICR male rats after doing exercises; this paper provides an experimentation basis for application and development of spirulina in the field of exercises.

**Keywords:** Spirulina, Form of Erythrocytes; Content of Hemoglobin; scanning electronic microscope

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### INTRODUCTION

Spirulina generally means the two types of spirulina belonging to arthrospira edible to human beings and animals, namely, Arthrospira maxima and Arthrospira platensis. The spirulina is rich in proteins, contains up to 60-70%, and has more nutriment than general food, and is rich in  $\beta$ carotene that is 15 times of that in the carrots; the spirulina is also rich in vitamin; it contains several kinds of trace elements necessary for human bodies; it also contains chlorophyll A which contains porphyrin with its similar to hemoglobin of human bodies and animals, and is the direct materials for human beings' and animals' hemoglobin.

Sports anaemia is a phenomenon that often occurs during athletic sports and influences the sportsmen's functions. According to the related research, long-term and high strength exercises may cause the form of organisms' erythrocytes to be abnormal, the level of hemoglobin to lower; however, the abnormal form of erythrocytes not only causes the ability to do exercises to lower, but also is an important reason for sports anaemia and fatigue arising from exercises[1-3]; the content of hemoglobin is closely related to the adaptability of sportsmen, their status to do exercises and exercises results. In this study, the influence of supplementing spirulina on the form of erythrocytes and the content of hemoglobin of anaemic rats that have done high strength exercises is demonstrated according to the characteristics of spirulina through experiments, and the experiment basis is provided for research and development of spirulina in sports nutrition.

## EXPERIMENTAL SECTION

### 1.1 Subjects

45 two-month  $30 \pm 2.53$ g ICR male rats provided by the animal experimental center of Norman Bethune College of Medicine of Jilin University.

### 1.2 Compounding of spirulina

The spirulina is compounded into spirulina solution containing 0.125g of crude drug per ml and then is put in 4e refrigerator for future use.

### 1.3 Process flow of the experiment

The duration of the experiment is 6 weeks. After the subjects are fed for two days for their adaptability, they are classified into three groups at random: normal control group, exercising group, exercising+spirulina group (abbreviated as the "spirulina group"); each group consists of 15 ones; the spirulina group is fed with spirulina (1.5g/per kg of weight); the normal control group and the exercising group are fed with distilled water of equal volume; refer to the R&D Design and Statistic Method of Chinese Traditional Medicine (Edited by He Shilin) for the calculation of the dose of drugs. Except the normal control group, rats from other groups swim without load 3h after being fed with drugs, for 30 min every day in the 1<sup>st</sup> and 2<sup>nd</sup> weeks, 60 min every day in the 3<sup>rd</sup> and 4<sup>th</sup> weeks, and 90 min every day in the 5<sup>th</sup> (water temperature  $28 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$  and water about 35cm deep). On the 41<sup>st</sup> day, rats from each group will swim to its maximum extent and the swimming duration is recorded; the maximum extent is measured by no exposure above the water after the animal sinks. After the rats that have swum have a rest for 30 min, they are fixed with wood plates and blood is sampled from their heart.

### 1.4 Measurement of the content of hemoglobin

French ABX MICROS full automated hematology analyzer is used for measuring the content of whole blood HGB.

### 1.5 Making the specimen of the erythrocyte scanning electronic microscope

Heparin is centrifugalized at the speed of 1500r/min for 10 min, the supernatant liquor is discarded, 0.86% NaCl is used for washing it, and the mixture is centrifugalized, and the supernatant liquor is discarded, three times in total. It is fixed with 2.5% glutaraldehyde, and gradiently eluted by using ethanol, then dried at the critical point by using CO<sub>2</sub>, coated with E-1010 carbon coater, the form of erythrocyte is observed by using the Philips XL30 scanning electronic microscope, 1000 ones shall be observed per specimen. The form of the erythrocyte is observed and the number of abnormal erythrocytes is calculated by referring to the classification methods of the forms of erythrocytes reported by Pan Li and others.

### 1.6 Processing of data

X<sup>2</sup> testing is adopted for comparing the percentages of abnormal erythrocytes (ratio of abnormal erythrocytes); variance analysis is used for comparing the HGB contents, and SPSS 19.0 statistic software is used for processing the data.

## RESULTS

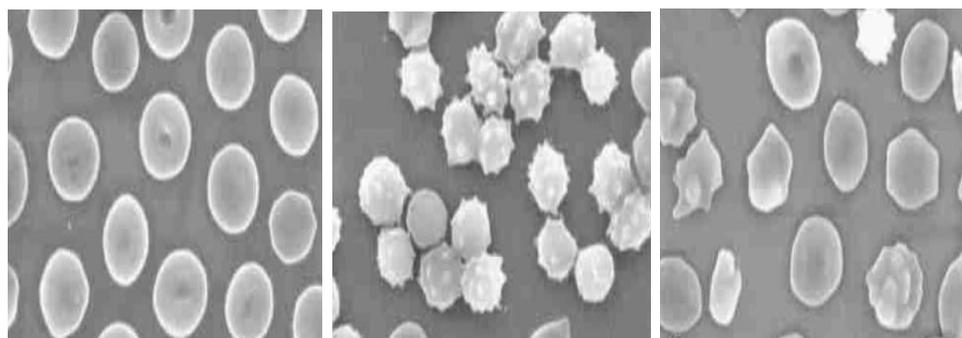
### 2.1 The influence of spirulina on the form of erythrocytes of ICR male rats having done exercises

#### 2.1.1 Observing the forms of erythrocytes of the three groups of ICR male rats with scanning electronic microscope

The erythrocytes of the ICR male rats in the normal control group are in the shape of double concave disc with regular smooth surfaces with fewer abnormal ones (Picture 1). The abnormal erythrocytes of the rats in the exercising group significantly rise and most are thorn shaped (Picture 2). The abnormal erythrocytes of the ICR male rats in the exercising+spirulina group significantly lower (Picture 3).

#### 2.1.2 Comparison of the abnormal erythrocyte ratio of the three groups of ICR male rats

The abnormal erythrocyte ratio of ICR male rats in the exercising group is significantly higher than that of the other two groups ( $P < 0.01$ ); although that of the ICR male rats in the exercising+spirulina group is higher than that in the normal control group ( $P > 0.05$ ), it is significantly lower than that of the exercising group ( $P < 0.01$ ). It means that the spirulina may significantly reduce the abnormal erythrocyte ratio of ICR male rats that have done high strength exercises (See Table 1).



Picture 1

Picture2

Picture 3

Table 1 Comparison of the Abnormal Erythrocyte Ratio of the Three Groups of ICR Male Rats (X±SD)

	Abnormal erythrocyte ratio (%)
Control group	25.83±9.51
Exercising group	89.23±15.96**
Single drug group	50.12±11.32*

Note: \*Means significant difference ( $P < 0.05$ ), \*\*Means extremely significant difference ( $P < 0.01$ ).

## 2.2 Influence of spirulina on the Content of HGB of Exercising Rats

The content of HGB of male rats from the exercising group is significantly lower than that of the other two groups ( $P < 0.01$ ); the content of HGB of ICR male rats from the exercising+spirulina group is lower than that of the normal control group ( $P > 0.05$ ); the content of HGB of ICR male rats from the exercising+spirulina group is extremely significantly higher than that of the exercising group ( $P < 0.01$ ). It means that the content of HGB of the exercising rats can be significantly increased by supplementing spirulina (See Table 2).

Table 2 Comparison of HGB Contents of All Groups of Rats (X±SD)

	Content of hemoglobin (g/L)
Control group	112.36±17.61**
Exercising group	67.58±10.32
Single drug group	86.91±15.39*

Note: \*Means significant difference ( $P < 0.05$ ), \*\*Means extremely significant difference ( $P < 0.01$ ).

## DISCUSSION

Normal erythrocytes are necessary for the rats to have normal functions. According to the research results, the percentage of abnormal erythrocytes of the ICR male rats from the exercising group is significantly higher than that of the normal control group ( $P < 0.01$ ), it means that long-term high strength exercise may significantly increase the abnormal erythrocytes. However, abnormal erythrocytes may affect the oxygen carrying function, and cause the fall of their ability to do exercises on one hand, and may also affect their ability to deform, hinder the micro-circulation, and it is one of the important reasons for fatigue arising from exercises[4]. Besides, it may also affect the rheological property, and may be retained in the reticuloendothelial system and vulnerable, it is one important factor for causing sports anaemia. According to the research results, the abnormal erythrocyte percentage of ICR male rats from the exercising+spirulina group is significantly lower than that from the exercising group ( $P < 0.05$ ), it means that spirulina may significantly lower the abnormal erythrocyte ratio of rats that have done high strength exercises.

The content of HGB is an index that may reflect the function and the comprehensive nutrition of bodies, for a lot of aspects, in particular durability, HGB is an important factor that may affect the ability to do exercises[5]. According to the research results, the HGB content of ICR male rats from the exercising group is significantly lower than that from the normal control group; the HGB content of the ICR male rats from the exercising+spirulina group is significantly higher than that of the exercising group. According to the experiment, long-term high strength exercises may lower the HGB content, however, the spirulina may significantly improve the HGB content of ICR male rats that have done high strength exercises[6]. Although we don't know the mechanism that causes HGB content to fall down, according to the research and the results over recent years, we believe that long-term high strength exercises cause the abnormal erythrocytes to significantly fall, and the erythrocytes to be vulnerable, it is one of the main reasons to cause HGB content to fall down. The spirulina may significantly reduce the abnormal erythrocyte ratio of rats that have done exercises, and thus may significantly increase the HGB content of ICR male exercising rats.

### CONCLUSION

The spirulina may significantly reduce the abnormal erythrocyte percentage of rats that have done high strength exercises for a long term, improve HGB content, and may relieve the damage to the bodies arising from high strength exercises, as well as improve the ability to do exercises and prolong the fatigue of bodies. It provides scientific testing basis for using spirulina as a nutrient supplement for recovering strength after high strength durability exercises.

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