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EXPERIMENTAL RESEARCH ON THE ANTI-FATIGUE EFFECT OF TRIBULUS TERRESTRIS IN SPORTS FOOD

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ABSTRACT

Objective: This paper aims to study the anti-fatigue effect of *Tribulus terrestris* in sports food, which provides theoretical and practical basis for the prevention of exercise fatigue. Methods: 24 healthy SD rats were divided into control group, exercise fatigue group and *Tribulus terrestris* + exercise fatigue group. After seven-day adaptive feeding, the two experimental groups took five-week increasing load treadmill training. The rats in *Tribulus terrestris* + exercise fatigue group took intragastric administration of *Tribulus terrestris* extract once a day before exercise. By the fourth week, 24 hours after the training, the blood was obtained from the orbital venous plexus of each narcotized rat; the liver and spleen of each rat were frozen for later use; their abdominal venous blood was collected; the numbers of natural killer cells and natural killer T cells of the rats were detected by a flow cytometer; the content of hemoglobin and muscle glycogen in the rats was detected by Sysmex automatic blood cell analyzer.

Results: In comparison with control group, there was an evident decrease in the weights and NK cell numbers of the rats in exercise fatigue group, indicating that in the exercise fatigue group, the exercise-induced immune of the rats with exercise fatigue was suppressed. In comparison with exercise fatigue group, numbers of NK and NKT cells of the rats in *Tribulus terrestris* + exercise fatigue group increased significantly (even greater than those of the control group), which showed that *Tribulus terrestris* extract could enhance the immunity of the rats which took excessive exercise and improve their fatigue resistance. In comparison with the control group, hemoglobin and muscle glycogen content of the rats in exercise fatigue group decreased more obviously, while a significant increase of hemoglobin and muscle glycogen level was found in *Tribulus terrestris* + exercise fatigue group (with no significant difference from the control group). Conclusion: *Tribulus terrestris* extract can improve the exercise-induced immune-suppression of the rats with exercise fatigue and enhance their anti-fatigue ability.

1.Introduction

Due to its distinct clinical effect and extensive pharmacological effects, *Tribulus terrestris* has been applied as a kind of medicine for a long time in China (Talasaz et al., 2010). Since the 1960 s, a lot of foreign scholars have studied the chemical composition of *Tribulus terrestris*; in China, the relevant studies were not carried out until the 1980 s (Hu et al., 2009; Berkman et al., 2009). Gradually, the researches on the composition of *Tribulus terrestris* have become more refined and penetrated. According to a large number of studies, it is generally recognized that *Tribulus terrestris* mainly contains compounds (saponin, flavonoid, biology class and polysaccharide) as well as amino acids, terpenes, fatty acid, inorganic salt (Hamed et al., 2009; Ranjithkumar et al., 2013). Saponin compounds, whose content is the highest in *Tribulus terrestris*, are endowed with the advantages of depressurization, antitumor, strengthening sexual function, anti-aging and protecting myocardium (Chan et al., 2014).

Tribulus terrestris is widely applied in sports owing to the fact that it contains no prohibited ingredients while it can improve the blood testosterone level of the body and promote the synthesis of protein, thereby increasing the athletes' muscle strength and improving their athletic ability (Koncic and Tomczyk, 2013). A lot of studies have revealed that the saponin in *Tribulus terrestris* could increase the content of sex hormones in human body (Bourke, 2012); especially, it could increase the content of male hormones which contributed to enhancing anabolism, promoting the recovery of athletes and preventing exercise-induced fatigue. Wang et al. (Wang *et al.*, 2013) found that the nourishment containing *Tribulus terrestris* could improve the athletic ability of long-distance runners by improving synthesis and oxygen carrying capacity of their bodies. Esfandiari et al. (Esfandiari et al., 2011) learned that Tri*bulus terrestris* extract could inhibit the decomposition of protein and amino acid, promote protein synthesis and increase hemoglobin content. Borrione (Borrione et al., 2012) et al. revealed that *Tribulus terrestris* extract was able to increase hemoglobin content and glycogen reserve; in addition, it could accelerate protein synthesis as well as inhibit the decomposition of protein and amino acids, which further demonstrated the anti-fatigue effect of *Tribulus terrestris.* In view of the research situation, we selected 24 Sprague-Dawley (SD) rats as the subjects, aiming to study the anti-fatigue effect of *Tribulus terrestris* extract as a kind of sports food.

2. Materials and methods

2.1. Subjects

We selected 30 healthy male SD rats (180~220 g) from the Experimental Animal Center of Liaoning Province and purchased standard rodent feed. The rats were kept in a clean animal room (relative humidity: 45-65%; room temperature: 22-27 °C). After being fed adaptively for 7 days, all the rats were screened through exercise (15 m/min, 10 min/day), and 24 of them were selected for the experiment on account of their good adaptability. They were randomly divided into control group, exercise fatigue group and *Tribulus terrestris* + exercise fatigue group, each group including eight rats. No exercise load was exerted on the control group; 30 min before each training, each rat in *Tribulus terrestris* + exercise fatigue group was given intragastric administration of the solution (25 mg/ml) of *Tribulus terrestris* extract (total saponins > 60%), while the control group and the exercise fatigue group took the same volume of normal saline by gavage.

2.2. Experiment method

The rats in the control group were given conventional feeding without exercise; the rats in the other two groups took treadmill training bearing increasing load from Monday to Saturday every week and took rest every Sunday. The specific training plan is shown in Table 1.

Table 1. The training plan for the exercise fatigue group and *Tribulus terrestris* + exercise fatigue group

Weeks	Intensity (m/min)	Gradient (°)	Exercise duration (min)
The first week	15	5	25
The second week	20	5	45
The third week	25	10	65
The fourth week	30	10	85
The fifth week	30	15	85

Every Sunday, the rats in each group were weighed. By the fifth week, 24 hours after the final training, all the rats were treated with moderate anesthesia, and their orbital venous plexus blood was collected and preserved in a cryogenic refrigerator at -50 °C for later detection. Then, all the rats were put to death; their abdominal cavity vein blood, livers and spleens were all collected, labeled and preserved in the refrigerator at -50 °C for later use. The numbers of natural killer (NK) cells and natural killer T (NKT) cells of the rats in the three groups were detected: 100 μl of rat venous blood was added to a flow tube; with antibody labeling added, the flow tube was kept away from light for 30 min; 500 μl of Optilyze C hemolysis reagent was added; after shaking, the tube was kept away from light for 30 min; 500 μl of ISOTON III was added; then, the sample was detected by a flow cytometry.

In this study, Sysmex automatic blood cell analyzer was used to detect the hemoglobin (Hb) and muscle glycogen in rats. For the statistical analysis on the experimental data, SPSS 17.0 was used; the inter-group differences in mean values were compared using the method of single factor analysis of variance; the results were presented as mean ± standard deviation (x ± SD); $p < 0.05$ means the differences were significant.

3. Results and discussions

3.1. Changes of apparent conditions and weights of the rats in the three groups

During the five-week exercise with increasing load, as the exercise time and intensity were under control, there was no death in the three groups. In the fourth and fifth weeks, all the rats in exercise fatigue group showed depression, slow reaction and hair loss; in *Tribulus terrestris* + exercise fatigue group, such phenomena were rare. Before the experiment, there was no difference in the weights of the rats in the three groups; since the third week of the experiment, weights of the rats in exercise fatigue group were obviously lower than those in the control group, the difference was significant ($p < 0.05$); the difference was the maximum at the fifth week. By supplementing *Tribulus terrestris* extract, weights of the rats in *Tribulus terrestris* + exercise fatigue group were markedly lower than those of control group ($p < 0.05$). In the fourth and fifth weeks, weights of the rats in *Tribulus terrestris* + exercise fatigue group were higher than those of exercise fatigue group; the difference was significant ($p < 0.05$). The results are shown in Table 2.

Table 2 Weights of the rats in three groups with increasing load training and supplement of *Tribulus terrestris* extract (g)

Week	Control group (n=8)	Exercise fatigue group (n=8)	*Tribulus terrestris* + exercise fatigue group (n=8)
One week before exercise	197.82±7.15	199.21±9.36	200.84±4.58
The first week	262.26±19.78	257.18±12.21	260.27±11.42
The second week	305.26±10.77	289.54±17.25	291.04±10.48
The third week	368.12±8.95	312.83±15.62	325.91±15.20
The fourth week	379.47±21.54	315.95±16.51	336.49±20.54
The fifth week	398.43±23.72	324.59±21.82	355.19±24.51

3.2. Changes in the numbers of NK and NKT cells of the rats in three groups

After the increasing load exercise, numbers of NKT and NK cells of the rats in three groups are shown in Figure 1. It can be seen that the numbers of NKT and NK cells of the rats in *Tribulus terrestris* + exercise fatigue group were still lower than those in control group ($p < 0.05$); however, they were in the tendency to increase in comparison with the exercise fatigue group. In comparison with exercise fatigue group, numbers of NKT ($p < 0.05$) and NK ($p < 0.05$) cells, which were higher than those of the control group, increased significantly in *Tribulus terrestris* + exercise fatigue group.

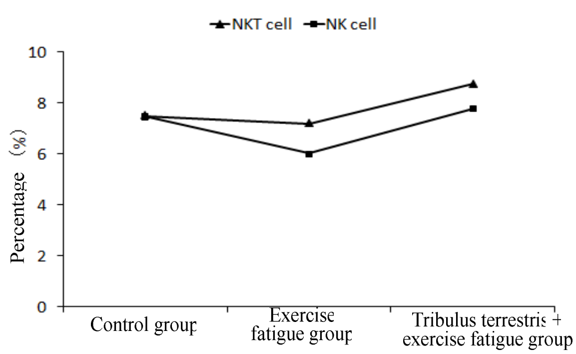

Figure 1. Numbers of NKT and NK cells of rats in three groups after increasing load training and supplement of *Tribulus terrestris* extract

3.3. Changes in hemoglobin and muscle glycogen of the rats in three groups

Table 3 shows the changes in hemoglobin and muscle glycogen of the rats in three groups. In comparison with control group, hemoglobin content of the rats in exercise fatigue group declined significantly ($p < 0.05$). Apparently, the tendency could be inhibited by taking in *Tribulus terrestris* extract, which was reflected in the fact that the hemoglobin content of the rats in *Tribulus terrestris* + exercise fatigue group increased ($p < 0.05$) in comparison with exercise fatigue group. In comparison with the control group, the muscle glycogen content of the rats in *Tribulus terrestris* + exercise fatigue group increased significantly ($p < 0.05$); due to exercise fatigue, muscle glycogen content of the rats decreased obviously ($p < 0.05$); nevertheless, *Tribulus terrestris* extract could obviously inhibit the decrease of muscle glycogen caused by exercise fatigue.

Health conditions can be reflected in weight changes. In motion experiments, body weights can be used to analyze the influence of training on the body and the adaptability of the body (Buijsse *et al.,* 2009). If there is a progressive decrease in body weight, it is assumed that the training might be arranged improperly or there are diseases in the body. Under normal conditions, body weights might fluctuate by 10%. If the fluctuating value is greater than 10%, the weight is abnormal (Schafer *et al.*, 2011). In this study, in the later period of increasing load training, the weights of the rats were in a declining trend in exercise fatigue group and *Tribulus terrestris* + exercise fatigue group. By the end of the training, in contrast with the control group, the weights of the rats in the two experiment groups decreased by 18.5% and 10.9% (both beyond the normal range of weight fluctuation). In this study, the weights of the rats in *Tribulus terrestris* + exercise fatigue group increased significantly compared with those in exercise fatigue group, which indicated that *Tribulus terrestris* extract could restrain the decreasing trend of weights. The data regarding the change of weight indirectly indicated that *Tribulus terrestris* extract might be able to benefit muscle growth and a well-built body.

As the lymphocytes with natural kill ability, NK cells are important for the immune defense responses of the body. NKT cells are T cell subgroup of special type; on the surface of NKT cells, there are both T cell receptors and NK cell receptors. Movement can lead to the change of the number and function of immune cells among which NK cells are the most sensitive to such changes; accordingly, NK cells can be used as a monitoring index for the early diagnosis of exercise fatigue (Paust et al., 2010; Vonarbourg et al., 2010).

Table 3. Content of hemoglobin and muscle glycogen of the rats in three groups

Week	Control group (n=8)		Exercise fatigue group (n=8)		*Tribulus terrestris* + exercise fatigue group (n=8)	
	Hemoglobin (g/dl)	Muscle glycogen (mg/g tissue)	Hemoglobin (g/dl)	Muscle glycogen (mg/g tissue)	Hemoglobin (g/dl)	Muscle glycogen (mg/g tissue)
One week before exercise	14.56±0.89	1.32±0.52	14.51±0.98	1.35±0.21	14.71±1.20	1.36±0.19
1st week	14.62±1.23	1.35±0.65	14.22±1.21	1.30±0.09	14.65±1.65	1.30±0.29
2nd week	13.92±2.56	1.34±0.54	13.85±2.55	1.21±0.15	14.03±2.06	1.26±0.13
3rd week	14.78±1.75	1.36±0.71	13.24±3.01	1.11±0.29	13.86±1.87	1.21±0.22
4th week	14.59±2.02	1.33±0.46	12.63±2.98	1.08±0.32	14.12±1.34	1.17±0.34
5th week	13.66±2.71	1.35±0.39	12.51±3.54	1.05±0.34	14.02±1.46	1.14±0.51

NKT cell is a sensitive index that reflects the change of immune function after exercise, under the influence of intensity and amount of exercise. In the study, we found that with the supplement of *Tribulus terrestris* extract, there was a noticeable increase in the amounts of NK and NKT cells which were even greater than those of the control group, indicating that *Tribulus terrestris* extract could improve the immune function of over-exercise rats by increasing the numbers of NK and NKT cells. The increasing NKT cells not only benefits the differentiation of CD_8^+ killer T cells, but also greatly enhances the killing activity of NK cells (Witte et al., 2010) and improves the immune function of the body.

Hemoglobin, a kind of oligomeric protein (containing iron) in red blood cells, mainly plays its physiological function by transporting oxygen and carbon dioxide; it has a buffer effect on acidoid and can regulate the intracorporal potential of hydrogen (Parshina et al., 2013). Some researches (Garvican et al., 2010; Holden, 2013) found that hemoglobin content was influenced by the nutrition intake, exercise load and rest time of athletes during training and competition. Therefore, it is necessary to determine the hemoglobin content in order to grasp the nutritional status and physical function of athletes (Joseph et al., 2013). The data in Table 2 showed that after intense exercise, intracorporal hemoglobin content of the rats in exercise fatigue group decreased more obviously than that of *Tribulus terrestris* + exercise fatigue group, which indicated that *Tribulus terrestris* extract was beneficial to the synthesis of hemoglobin or was able to inhibit the injury of hemoglobin caused by free radicals, thus hemoglobin content was relatively high. Muscle glycogen reserves can directly influence the athletic ability of the body. Previous studies (Maga *et al.,* 2013) considered that the increase of muscle glycogen content might be one of the reasons why *Tribulus terrestris* extract prolonged the exercise duration of rats and improved their exercise capacity. In this study, muscle glycogen content was higher in *Tribulus terrestris* + exercise fatigue group than in exercise fatigue group ($p < 0.05$), which shares some similarity with the opinion of the previous studies.

4. Conclusions

Excessive exercise can result in exercise fatigue which reduces immunity of the body; consequently, there is exercise-induced immune-suppression which leads to the decrease of athletes' resistance as well as infectious diseases, which is harmful to their training or competition. Therefore, for the sake of athletes' exercise capability, it is significant to find a solution to avoid the negative effect of exercise fatigue. Considering that *Tribulus terrestris* extract is a kind of sports food, we studied its effect of resisting exercise fatigue based on increasing load training on 24 SD rats. The results showed that *Tribulus terrestris* extract could enhance the inhibited immune function of the rats with exercise-induced fatigue by increasing the numbers of NK and NKT cells; it could effectively restrain the decrease of hemoglobin content caused by excessive exercise; in addition, it could increase muscle glycogen content, thus to improve energy reserves and aerobic exercise ability of the body, and eventually to improve the fatigue resistance of the body.

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