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Basic Study

Effect of moxibustion at Shenque (CV 8) on immune system in rats with different degrees of exhaustive exercise

艾灸神阙穴对不同程度力竭运动大鼠免疫系统的影响

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Abstract
Objective: To investigate the effect of moxibustion at Shenque (CV 8) on the immune system in rats with different levels of exhaustive exercise.
Methods: Fifty-six male Sprague-Dawley (SD) rats were randomly divided into a blank group (*n*=8), an exhaustive group (*n*=24), and a moxibustion group (*n*=24). The exhaustive group was randomly divided into a 1-time exhaustive group, a 4-time exhaustive group and a 7-time exhaustive group, with 8 rats in each group. According to the treatment time, the moxibustion group was randomly divided into a 1-time moxibustion group, a 4-time moxibustion group and a 7-time moxibustion group, with 8 rats in each group. Rats in the exhaustive groups and the moxibustion groups were subjected to replicating the exhaustive swimming models. Rats in each moxibustion group received mild moxibustion for 15 min immediately after the exhaustive modeling, once every other day. Twenty-four hours after the corresponding exhaustive exercise, the rats in each group were tested for the levels of serum immunoglobulin (Ig) G, IgA, IgM and acid phosphatase (ACP), and the morphological changes of spleen tissues were observed. The level of IgA was detected by immunoturbidimetric assay, and the levels of IgG, IgM and ACP were detected by enzyme-linked immunosorbent assay (ELISA).
Results: Compared with the 1-time exhaustive group, swimming time of rats in the 4-time exhaustive group was significantly prolonged (P<0.01), and swimming time of rats in the 7-time exhaustive group was significantly shortened (P<0.01). Compared with the 7-time exhaustive group, exhaustive swimming time of rats in the 7-time moxibustion group was significantly prolonged (P<0.01). Compared with the blank group, the IgG level in the 1-time exhaustive group was significantly decreased (P<0.01), and the levels of IgG, IgA and IgM in the 4-time exhaustive group and the 7-time exhaustive group were all significantly decreased (P<0.05 or P<0.01), while the ACP level was increased significantly (both P<0.01). Microscopically, the number of splenic corpuscles in the 1-time exhaustive group was reduced; the center of some splenic corpuscles in the 4-time exhaustive group was damaged; the number of splenic corpuscles in the 7-time exhaustive group was reduced, and there was no obvious germinal center. Compared with the 4-time exhaustive group, the IgA level in the 4-time moxibustion group was significantly increased (P<0.01), and the ACP level was significantly decreased (P<0.01). Compared with the 7-time exhaustive group, the levels of IgG, IgA and IgM in the 7-time moxibustion group were significantly increased (all P<0.01), and the ACP level was significantly decreased (P<0.01). Microscopically, the number of splenic corpuscles in the 1-time moxibustion group was reduced; the center of some splenic corpuscles in the 4-time moxibustion group was damaged together with hyperplasia of some splenic corpuscles; blast cells were proliferated in the center of some splenic corpuscles in the 7-time moxibustion group.
Conclusion: Moxibustion at Shenque (CV 8) can improve the levels of IgG, IgA and IgM, reduce the ACP level, repair damaged spleen tissues, and enhance the immunity of the body to some extent in the long-term fatigue rats.
Keywords: Moxibustion Therapy; Moxa Stick Moxibustion; Point, Shenque (CV 8); Exhaustive Exercise; Fatigue; Immunoglobulins; Acid Phosphatase; Rats

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【摘要】目的：探讨艾灸神阙穴对不同程度力竭运动大鼠免疫系统的影响。方法：将 56 只雄性 Sprague-Dawley (SD)大鼠随机分为空白组(*n*=8)、力竭组(*n*=24)和艾灸组(*n*=24)，根据造模时间，将力竭组随机分为力竭 1 次组、力竭 4 次组和力竭 7 次组，每组 8 只。根据治疗时间，将艾灸组随机分为艾灸 1 次组、艾灸 4 次组和艾灸 7 次组，每组 8 只。力竭组及艾灸组大鼠复制游泳力竭模型。艾灸各组大鼠力竭造模后即刻温和灸神阙穴 15 min，隔日 1 次。各组大鼠完成相应力竭运动 24 h 后，检测血清免疫球蛋白(Ig) G、IgA、IgM 和酸性磷酸酶(ACP)含量，并观察脾组织形态变化。采用免疫比浊法检测 IgA 含量，采用酶联免疫吸附(ELISA)法检测 IgG、IgM 和 ACP 含量。结果：与力竭 1 次组比较，力竭 4 次组大鼠力竭游泳时间明显延长(P＜0.01)，力竭 7 次组大鼠力竭游泳时间明显缩短(P＜0.01)。与力竭 7 次组比较，艾灸 7 次组大鼠力竭游泳时间明显延长(P＜0.01)。与空白组比较，力竭 1 次组 IgG 含量明显降低(P＜0.01)，力竭 4 次组和力竭 7 次组 IgG、IgA 及 IgM 含量均明显降低 (P＜0.05 或 P＜0.01), ACP 含量明显升高(均 P＜0.01)。镜下可见，力竭 1 次组脾小体数量减少，力竭 4 次组部分脾小体中心坏死，力竭 7 次组脾小体数量明显减少，无明显的生发中心。与力竭 4 次组比较，艾灸 4 次组 IgA 含量明显升高(P＜0.01), ACP 含量明显降低(P＜0.01)；与力竭 7 次组比较，艾灸 7 次组 IgG、IgA 和 IgM 含量均明显升高(均 P＜0.01)，ACP 含量明显降低(P＜0.01)。镜下可见，艾灸 1 次组脾小体数量减少，艾灸 4 次组部分脾小体中心坏死的同时发现部分脾小体增生，艾灸 7 次组部分脾小体中心母细胞增生。结论：艾灸神阙穴可提高长期疲劳大鼠机体的 IgG、IgA 和 IgM 含量，减低 ACP 含量，修复被破坏的脾组织，在一定程度上增强了机体的免疫能力。

【关键词】灸法；艾条灸；穴，神阙；力竭运动；疲劳；免疫球蛋白；酸性磷酸酶；大鼠

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With the increase of life pace and work intensity, the load on people has been gradually increasing. When the fatigue level exceeds the limits or the duration of exertion is too long, the body will experience decreased immunity, premature aging, etc., and lead to various chronic diseases such as chronic fatigue syndrome[1]. How to speed up the recovery of the fatigue and enhance the immunity are the key to preventing aggravation of fatigue. Previous studies have found that the change of exhaustive time in long-term exhaustive swimming rats is parabolic, and moxibustion at Shenque (CV 8) can effectively improve the immunity and prolong the exhaustive swimming time[2-4]. To further explore the effect of moxibustion at Shenque (CV 8) on the immune system of rats with different degrees of exhaustion, this experiment was designed. According to the characteristics of the change of exhaustive time, 1-time, 4-time and 7-time exhaustive exercises corresponding to the ascending, peak and descending segments on the parabola were selected respectively in this study. They were taken as the representatives of different exhaustive models to evaluate the effects of moxibustion at Shenque (CV 8) on serum immunoglobulin (Ig) G, IgA, IgM and acid phosphatase (ACP) and morphological changes of spleen in rats with different degrees exhaustive exercise.

1 Materials and Methods

1.1 Laboratory animals and grouping

Fifty-six clean male Sprague-Dawley (SD) rats, weighing (200±10) g, were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. [license number: SCXK (Beijing) 2012-0001]. The rats were housed in different cages, with 4 rats per cage. The temperature of the breeding room was kept at 20-24 ℃, with humidity of about 50% and 12 h shift of light and dark (light time was 07:00-19:00). All animals free access to food and water. The rats were adaptively fed for 7 d and adaptively swam once on the 3rd day and the 6th day respectively, 3 min/time. All animal experiments followed the relevant regulations of experimental animal management in Hebei University of Chinese Medicine.

This study had tried to reduce the sufferings and the number of animals being used. The rats were randomly divided into a blank group (*n*=8), an exhaustive group (*n*=24), and a moxibustion group (*n*=24) using the random number table. According to the duration of modeling and treatment, the exhaustive group and the moxibustion group were further randomly divided into a 1-time exhaustive group, a 4-time exhaustive group and a 7-time exhaustive group, a 1-time moxibustion group, a 4-time moxibustion group and a 7-time moxibustion group, respectively, with 8 rats in each group.

1.2 Main reagents and instruments

IgA, IgG and IgM kits (Shanghai Senxiong Technology Industrial Co., Ltd., China); ACP kit (Nanjing Jiancheng Bioengineering Institute, China); self-made constant temperature water tank (200 cm×80 cm); moxa stick (specification: 7 mm×117 mm, Henan Nanyang Han Medicine Moxa Co., Ltd., China); homemade rat box for moxibustion (national patent of utility model, patent number: ZL201120193244.8)[5]; TD10001 electronic balance (Tianjin electronic balance Instrument Co., Ltd., China); TDL-5-A centrifuge (Shanghai Anting Scientific Instrument Factory, China); FJ-2021γ-radioimmunication counter (Xi'an 262 Factory, China); AU400 automatic biochemical analyzer [Kelgewani (Shanghai) Analytical Instrument Co., Ltd., China]; DP73 digital microscope

(Olympus, Japan); HMIAS-2000 microscopic image analysis system (Wuhan Tongji Medical University, China).

1.3 Model preparation

After 7-day adaptive feeding, an exhaustive exercise model was prepared with reference to the literature[6]. The rats in the exhaustive group and the moxibustion group were placed in a constant temperature water tank [water depth: 50 cm, water temperature: (30±2) ℃], with a piece of lead equal to 5% of the body weight tied at the root of the tail (1-5 cm from the tail end) for exhaustive exercise (exhaustive standard: rat swimming movement was obviously out of balance and could not continue; the nose tip was under water for 5 s and could not return to the surface).

The rats in the exhaustive and the moxibustion groups performed the corresponding numbers of exhaustive exercise, and the exhaustive exercise was performed every other day in the multiple exhaustive exercise groups. The rats in the moxibustion groups received moxibustion once after each exhaustive exercise.

1.4 Intervention methods

Blank group: Rats were placed in a special rat moxibustion box for 15 min, once every other day for 7-time without any interventions.

One-time moxibustion group: Immediately after exhaustive swimming, the rats were placed in a special rat moxibustion box, mild moxibustion at Shenque (CV 8) to receive for 15 min[7], only once.

Four-time moxibustion group: Immediately after exhaustive swimming, the rats were placed in a special rat moxibustion box, and the mild moxibustion at Shenque (CV 8) to receive for 15 min[7], once every other day for a total of 4 treatments.

Seven-time moxibustion group: Immediately after exhaustive swimming, the rats were placed in a special rat moxibustion box, and the mild moxibustion at Shenque (CV 8) to receive for 15 min[7], once every other day for a total of 7 treatments.

One-time exhaustive group: Immediately after exhaustive swimming, the rats were placed in a special rat moxibustion box for 15 min without moxibustion, only once.

Four-time exhaustive group: Immediately after exhaustive swimming, the rats were placed in a special rat moxibustion box for 15 min without moxibustion, once every other day for 4 times.

Seven-time exhaustive group: Immediately after exhaustive swimming, the rats were placed in a special rat moxibustion box for 15 min without moxibustion, once every other day for 7 times.

1.5 Investigation items

1.5.1 Exhaustive time

The last exhaustive swimming time of the rats in each exhaustive and moxibustion group was recorded by one same person by second and correct to the nearest 0.1 s.

1.5.2 Serum biochemical indicators

Twenty-four hours after rats in each group completed the corresponding exhaustive exercise (rats in the blank group and the 7-time exhaustive group at the same time), 3 mL blood was collected by dislocation and centrifuged at 3 000 r/min for 10 min. The supernatant was collected and stored by cryopreservation for later examination. IgA was detected by immunoturbidimetric assay; IgG, IgM and ACP were detected by double-antibody enzyme-linked immunosorbent assay (ELISA).

1.5.3 Spleen histomorphology

After the rats were sacrificed by dislocation, spleen was quickly removed on ice and placed in 10% formalin. After fixation, dehydration, waxing, embedding, sectioning and hematoxylin-eosin (HE) staining, the general morphology of spleen was observed under low power microscope.

All tests were completed by the Research Center of Hebei University of Chinese Medicine.

1.6 Statistical methods

Statistical analysis was performed using SPSS version 13.0 software. The measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Multiple group comparison was analyzed by ANOVA and multiple comparison *q*-test. The comparison between two groups was conducted by least significant difference (LSD). The *t*′-test was used for data with heterogeneity of variance. $P<0.05$ indicated that the difference was statistically significant.

2 Results

2.1 Comparison of exhaustive swimming time

Compared with the 1-time exhaustive group, the exhaustive swimming time of rats in the 4-time exhaustive group was significantly prolonged ($P<0.01$), and that in the 7-time exhaustive group was significantly shortened ($P<0.01$). Compared with the 4-time exhaustive group, the exhaustive swimming time of rats in the 7-time exhaustive group was significantly shortened ($P<0.01$). Compared with the same time exhaustive group, there was no statistically significant difference in exhaustive swimming time in the 1-time moxibustion group and the 4-time moxibustion group (both $P>0.05$), the exhaustive swimming time of rats was significantly longer in the 7-time moxibustion group ($P<0.01$). The detail is shown in Table 1.

2.2 Comparisons of serum IgG, IgA, IgM and ACP levels

Compared with the blank group, the IgG level in the 1-time exhaustive group was significantly decreased ($P<0.01$), while the IgA, IgM and ACP levels were not statistically different ($P>0.05$); the levels of IgG, IgA and IgM were significantly decreased ($P<0.05$ or $P<0.01$), and the ACP levels were significantly increased in the 4-time exhaustive group and 7-time exhaustive group

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(all *P*<0.01). The detail is shown in Table 2.

Compared with the blank group, the IgG level was significantly lower (*P*<0.01), and the levels of IgA, IgM and ACP were not statistically different in the 1-time moxibustion group (all *P*>0.05); the IgG level was significantly decreased (*P*<0.01), and there were no statistically significant differences in IgA and IgM levels (both *P*>0.05), and ACP level was significantly increased in the 4-time moxibustion group (*P*<0.01); there were no statistically significant differences in IgG, IgA and IgM levels (all *P*>0.05), but ACP level was increased in the 7-time moxibustion group (*P*<0.05), (Table 2).

Compared with the 1-time exhaustive group, there was no statistically significant difference in the levels of IgG, IgA and IgM (all *P*>0.05) and ACP level was increased (*P*<0.01) in the 4-time exhaustive group; the levels of IgA and IgM were significantly decreased (*P*<0.01, *P*<0.05), and the ACP level was significantly increased (*P*<0.01) in the 7-time exhaustive group. Compared with the 4-time exhaustive group, there were no statistically significant differences in the levels of IgG, IgA and IgM (all *P*>0.05), but the ACP level was significantly increased in the 7-time exhaustive group (*P*<0.01). Compared with the 1-time moxibustion group, the levels of IgG and ACP were increased in the 4-time moxibustion group (both *P*<0.05); the IgG level was increased in the 7-time moxibustion group (*P*<0.01). Compared with the 4-time moxibustion group, there were no statistically significant differences in the levels of IgA, IgG, IgM and ACP in the 7-time moxibustion group (all *P*>0.05). The detail is shown in Table 2.

Compared with the same time exhaustive group, there were no statistically significant differences in IgA, IgG, IgM and ACP levels in the 1-time moxibustion group (all *P*>0.05); the IgA level was significantly increased (*P*<0.01), and the ACP level was significantly decreased in the 4-time moxibustion group (*P*<0.01); the levels of IgG, IgA and IgM were significantly increased (all *P*<0.01), and ACP level was significantly decreased in the 7-time moxibustion group (*P*<0.01). The detail is shown in Table 2.

Table 1. Comparison of the exhaustive swimming time of rats ($\bar{x}\pm s$, s)

Group	*n*	Exhaustive swimming time
1-time exhaustive	8	422.33±33.71
4-time exhaustive	8	463.06±42.24 1)
7-time exhaustive	8	390.53±60.10 1)2)
1-time moxibustion	8	419.04±33.01
4-time moxibustion	8	484.26±47.29 3)
7-time moxibustion	8	495.50±45.79 3)4)

Note: Compared with the 1-time exhaustive group, 1) *P*<0.01; compared with the 4-time exhaustive group, 2) *P*<0.01; compared with the 1-time moxibustion group, 3) *P*<0.01; compared with the 7-time exhaustive group, 4) *P*<0.01

Table 2. Comparison of serum IgG, IgA, IgM and ACP levels ($\bar{x}\pm s$)

Group	*n*	IgG (mg/mL)	IgA (mg/mL)	IgM (mg/mL)	ACP (U/L)
Blank	8	13.97±0.55	2.85±0.07	1.71±0.08	55.07±2.60
1-time exhaustive	8	11.66±0.31 1)	2.78±0.05	1.60±0.03	58.28±2.56
4-time exhaustive	8	12.45±0.66 1)	2.61±0.39 2)	1.53±0.10 1)	67.38±3.34 1)3)
7-time exhaustive	8	12.04±0.73 1)	2.45±0.41 1)3)	1.46±0.15 1)4)	76.83±3.72 1)3)5)
1-time moxibustion	8	11.76±0.54 1)	2.94±0.14	1.65±0.15	55.54±1.53
4-time moxibustion	8	12.64±0.51 1)7)	2.95±0.09 5)	1.62±0.08	60.22±2.34 1)5)7)
7-time moxibustion	8	13.22±0.93 6)	2.90±0.20	1.62±0.08	58.72±1.65 2)

Note: Compared with the blank group, 1) *P*<0.01, 2) *P*<0.05; compared with the 1-time exhaustive group, 3) *P*<0.01, 4) *P*<0.05; compared with the 4-time exhaustive group, 5) *P*<0.01; compared with the 1-time moxibustion group, 6) *P*<0.01, 7) *P*<0.05

2.3 Comparison of splenic morphology

Observation under light microscope: In the blank group, the splenic structure was intact, and there were no obvious abnormalities in the marginal zone of the white pulp and red pulp, the splenic sinus and the splenic cord; the splenic corpuscle was clear, and the central blast was visible in the splenic center where a lot of mature lymphocytes were around; the arterial lumen was visible in the center of the periarterial lymphatic sheath, and lymphocytes were scattered around. The 1-time exhaustive group showed a decrease in the number of splenic corpuscles with a large number of lymphocytes around; the 4-time exhaustive group showed partial necrosis of the splenic corpuscle center; and the 7-time exhaustive group showed a significant reduction in the number of splenic corpuscles, with no obvious germinal center; the 1-time moxibustion group also showed a decrease in the number of spleen corpuscles with a large number of lymphocytes around; the 4-time moxibustion group showed partial necrosis of the splenic corpuscle center along with hyperplasia of some corpuscles; the 7-time moxibustion group showed blast proliferation at the center of some splenic corpuscles. The detail is shown in Figure 1.

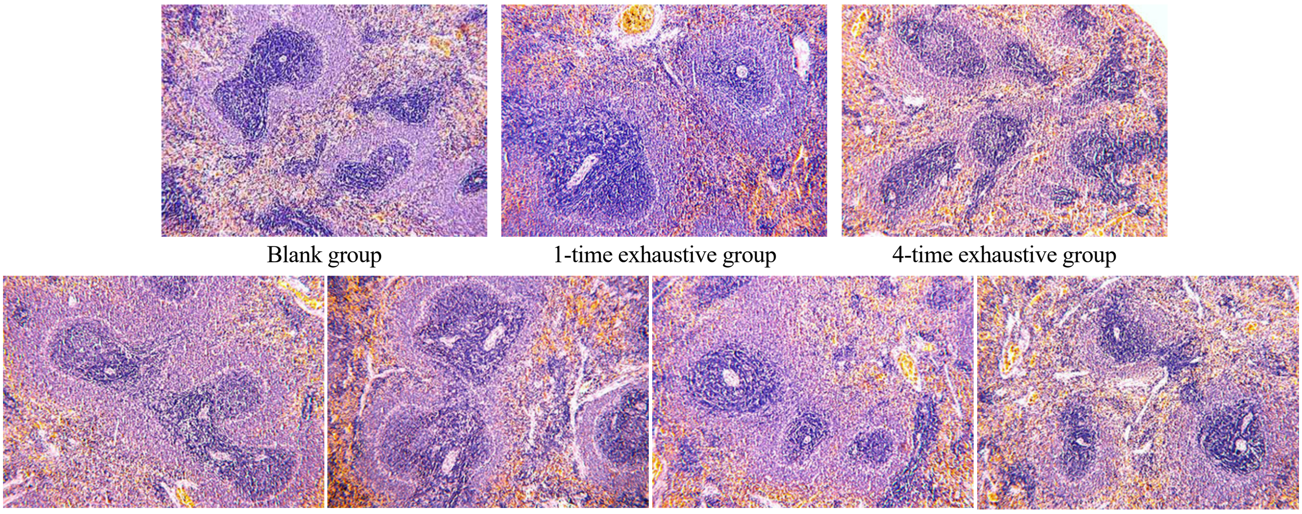

Figure 1. Morphological manifestations of rat spleen in each group (HE, ×40)

3 Discussion

Exercise fatigue refers to bodily function unable to maintain at a particular level and/or the overall inability to maintain the predetermined exercise intensity. Studies have shown that regular moderate training can increase the IgG, IgA and IgM levels and enhance the body's immunity *in vivo*. However, long-term fatigue exercise can lead to a decrease in the body's IgG, IgA and IgM levels, and inhibit the body's immunity[8]. Immunoglobulin refers to a group of globulins with antibody activity or similar chemical structure. It is generally divided into five categories: IgG, IgA, IgM, IgD and IgE. Among them, IgG, IgA and IgM are often used as indicators to evaluate the body's immune function and anti-infective ability[9]. At the same time, the spleen is the largest peripheral lymphoid organ, and it is also the main base for the production of antibodies, especially IgM[10]. The splenic corpuscle, also known as the lymphoid nodule, consists of a large number of B cells. The larger lymph nodes show germinal centers. When stimulated by antigen, the lymphoid nodules and spleen volume are increased to enhance cellular immunity and humoral immunity[11-12]; when immunity is inhibited, spleen tissue will shrink[13]. ACP is a non-specific lysosomal enzyme in the body[14], and plays an important role in the body's inflammation repair and immune regulation, participating in the repair of immune and cell damage[15].

In Chinese medicine, qi and blood are the material foundation for the body strength. They are closely associated with the spleen and kidney. The spleen is the acquired foundation and source of qi and blood production. Tonifying spleen qi helps to alleviate exercise fatigue and, at the same time, activate and facilitate the Yuan-primordial qi to defend the body against exogenous pathogens[16]. The kidney is the congenital foundation and acts as the driving force of the entire body. Tonifying kidney qi can therefore increase the secretion of androgen and improve the bodily function[17]. Shenque (CV 8) is located where the Yuan-primordial qi originates from. Applying moxibustion to this point can supplement yang, regulate qi and benefit both the congenital and acquired foundation. Studies have suggested that applying moxibustion to Shenque (CV 8) helps to regulate nervous and endocrine system and boost the immunity[18-19].

The results of this study showed that there were no significant differences in IgG, IgA, IgM, and ACP levels and spleen morphology between the 1-time exhaustive group and the blank group. It may be because that the transient exercise fatigue caused by 1-time exhaustive exercise can be restored by the autoregulation of the body. The immune indicators were decreased to various degrees, and the ACP repair system was activated in the 4-time and 7-time exhaustive groups. The exhaustive swimming time in the 7-time exhaustive group was significantly shorter than that in the 1-time and 4-time exhaustive groups, indicating that the body function was decreased after the 4th exhaustive swimming, and the body gradually became weak. Various indexes in the 4-time and 7-time moxibustion groups were all better than the same time exhaustive groups, especially in the 7-time moxibustion group which had more obvious advantages, suggesting that the weaker the body, the more obvious the moxibustion effect. The results further confirmed the two-way benefit regulation of acupoints.

This study showed that long-term exhaustive exercise would lead to a decrease in serum immunoglobulins, impaired spleen tissues, and decreased immune defense; moxibustion at Shenque (CV 8) could improve the immune system of the body by increasing the immunoglobulin levels of the long-term fatigue body, reducing the ACP level, and repairing the damaged spleen tissues. In the future, we should further study the effects of moxibustion at Shenque (CV 8) on the immune system by focusing on the thymus indicators and immune cells, thus to provide a more scientific and comprehensive experimental basis for improving the immunity of the body by moxibustion.

Conflict of Interest

The authors declared that there was no potential conflict of interest in this article.

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Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria.

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