

Effect of Mixed Beverage of Red Ginseng and Pomegranate on Motor Ability and Reproductive Function of Mice

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Abstract

Red ginseng and pomegranate compound beverage is developed from the compatibility of traditional edible and medicinal plants, but its function *in vivo* is not clear. In order to explore the effect of red ginseng and pomegranate compound beverage on the exercise ability and reproductive function of mice. Male mice were randomly divided into the following groups: quiet control group, quiet feeding group, exercise control group, exercise feeding group. According to the requirements of the experiment, after 60 days of intragastric treatment, the exhausting swimming time of each group was observed. Afterwards, the mice were euthanized, and their blood, liver, kidneys, muscles and testes were preserved. The contents of superoxide dismutase (SOD), malondialdehyde (MDA), lactate (LA) and testosterone in serum, tissues and organs of mice in each group were measured. Pathological morphology of testis was observed by HE staining. The results showed that compared with the control group, the compound beverage of red ginseng and pomegranate could effectively prolong the loading swimming time. In the high dose group, the activity of SOD in serum and tissues increased significantly, while MDA and LA showed a downward trend. In addition, the content of serum testosterone increased gradually with the feeding dose; the number of spermatogenic cells and Leydig cells increased and arranged more closely in testicular sections. To sum up, the compound beverage of red ginseng and pomegranate can enhance the exercise ability of mice and promote the development and maturation of reproductive organs. Its mechanism may be related to alleviating the oxidative damage of organs and regulating the level of hormones in the body.

Keywords

Red Ginseng Pomegranate; Exercise Ability; Oxidative Damage; Testosterone; Reproductive Function.

1. Introduction

Red ginseng is the dried root and rhizome of *Panax Ginseng* C.A.M., which is a kind of rare Chinese medicine [1]. It is processed by fresh ginseng through infiltration, cleaning, sorting, steaming, drying and drying. After processing, it changes from white to red, producing the unique components of red ginseng. In addition to the saponins common with fresh ginseng (such as Ro, Rb1, Rb2, RC, Rd, Re, Rg1, RF, etc.), it also contains some unique saponins, such as Rh1, Rh2, Rg3, 20(R)-Rg2, Rg5, Rg6, etc. In traditional Chinese medicine, red ginseng is believed to have the effect of invigorating the vital energy, reinforcing and removing the pulse, replenishing qi and absorbing blood. Modern pharmacological studies have also shown that red ginseng has anti-diabetic effect, protective effect on diabetic retinopathy, and certain antioxidant, anti-tumor, anti-aging and other effects[2-4].

Pomegranate (*Punica granatum* L.), also known as pomegranate or sea pomegranate, is a berry plant of the genus Pomegranate in the family Pomegranate. As a kind of medicine and food homologous plant, its efficacy has been paid more and more attention. It has been reported that pomegranate juice is rich in antioxidants, such as soluble polyphenols, tannins, anthocyanins, etc., which has the effect of delaying aging, preventing atherosclerosis and slowing down the carcinogenesis process[5-7]. In addition, some experiments have shown that pomegranate juice can improve sperm concentration, spermatogenic cell density, seminiferous tubule diameter and sperm motility in rats epididymis[8]. Combining the traditional Chinese medicine red with the pomegranate extract to improve the body function through concentration and combination may achieve the effect of complementary and double

the result with half the effort. However, there are few reports on the related medicinal value and mechanism of red ginseng combined with pomegranate.

In this study, different doses of concentrated solution of red ginseng and pomegranate were used to intervene male mice to explore the effects of combination of red ginseng and pomegranate on sexual function and athletic ability of male mice. It provides a theoretical basis for its application in the field of nutrition and medicine and health care.

2. Materials and methods

2.1 Animals

C57bL/6 mice were purchased from Beijing HFK Bioscience. The animals were randomly divided into groups, with 5 animals in each cage and free feeding. The ambient temperature was 22 ± 2 °C and the relative humidity was 40%-50%.

2.2 Grouped animals and feed

A total of 100 male mice were divided into two batches, namely static experiment and exercise experiment, with 50 mice in each batch. After 3 days of adaptive feeding, mice in each batch were randomly divided into 5 groups according to body weight, with 10 mice in each group, namely 1 blank control group (0 times) and 4 red ginseng pomegranate beverage gradient dose groups. In this experiment, the clinical equivalent of adult usage and dosage was 0.5g/kg/d (2.5 times), 1g/kg/d (5 times), 2g/kg/d (10 times) and 4g/kg/d (20 times), respectively. Except for the blank control group mice were given distilled water, each dose group was given the corresponding distilled water diluent of Hongshen pomegranate beverage. Mice were gavaged 0.2ml per 10g body weight, once a day, for 60 days.

3. Results

3.1 Two Effects of red ginseng and pomegranate compound drink on exhaustive swimming time in mice

The loading swimming time of mice is positively correlated with the anti-fatigue effect, which can reflect the exercise ability of mice to a certain extent. As can be seen from Figure 1, exhaustive swimming time of mice in the feeding groups with various concentration multiples was higher than that in the control group. There was a significant difference ($P < 0.01$) between the control group and the feeding group at 10 or 20 times the dose, but the difference was not significant when the feeding group at 2.5 or 2 times the dose. These results indicated that the red ginseng compound beverage could prolong the swimming time and improve the exercise ability of mice in different degrees, and the effect of the 10-fold dose group was the best.

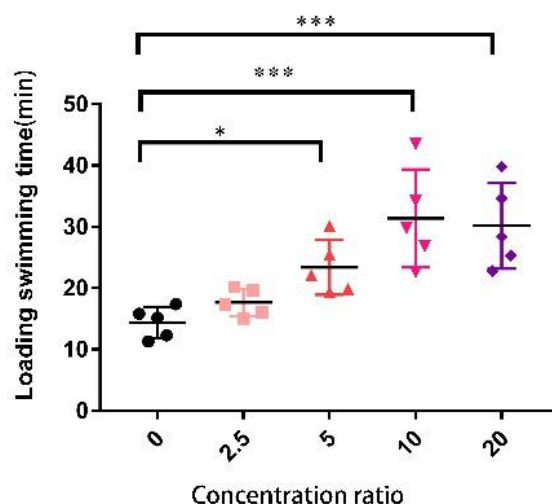


Figure 1. Exhausted swimming time of mice

3.2 Effect of red ginseng and pomegranate compound drink on SOD level in mice

As can be seen from Figure 2, in the static and exercise batches, SOD content in serum, kidney and muscle of mice in the feeding group was increased compared with that in the control group, and the difference was the most obvious when the feeding dose was 10 or 20 times, indicating that high concentration had a better promoting effect on the improvement of antioxidant capacity in mice. In addition, there were more significant differences in serum and kidney tissue between the two groups ($P < 0.05$).

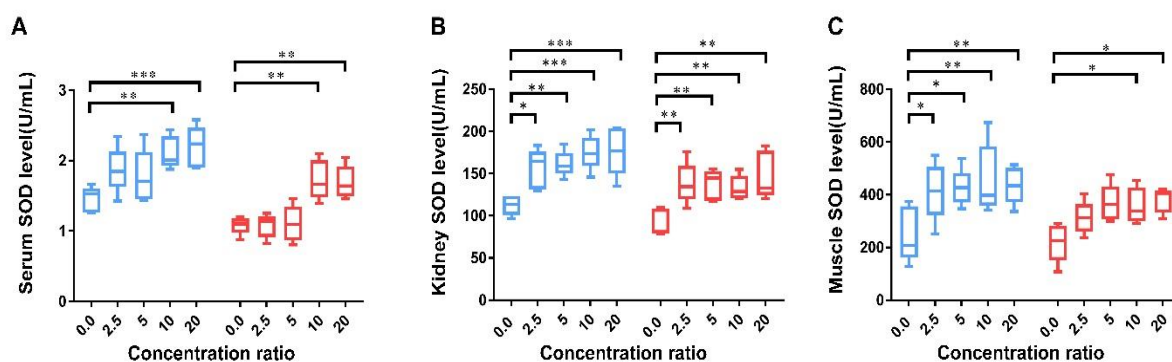


Figure 2. SOD levels in various tissues of mice

3.3 Effect of red ginseng and pomegranate compound drink on MDA level in mice

According to relevant reports, MDA content is negatively correlated with anti-fatigue effect. As can be seen from Fig. 3, in the static and exercise experiments, the MDA contents in serum, kidney and muscle of mice in the feeding group all showed a downward trend compared with the control group, indicating that lipid peroxidation in mice in the feeding group was weakened. In addition, there was a significant difference in serum and muscle between the control group and the 10-fold or 20-fold dose ($P < 0.01$), indicating that the Red Ginseng Pomegranate Compound Drink had a more effective antioxidant effect on blood and muscle tissue.

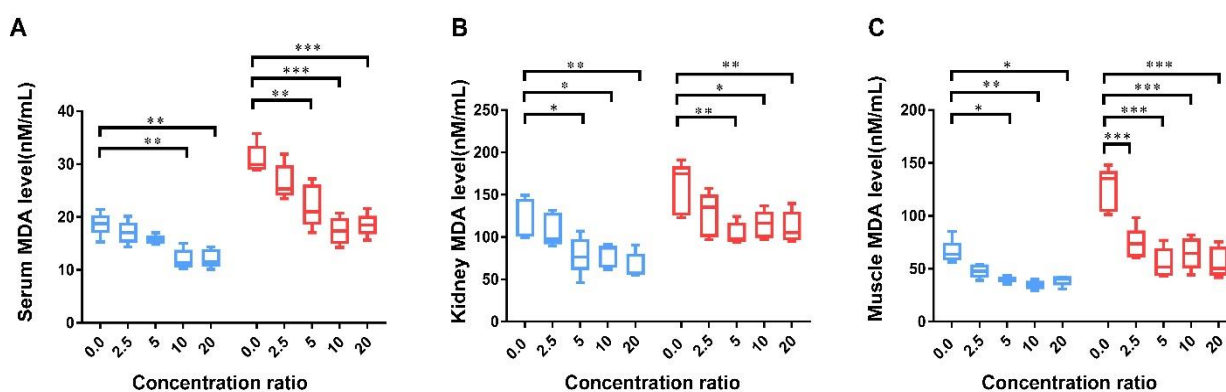


Figure 3. MDA levels in various tissues of mice

3.4 Effect of red ginseng and pomegranate compound drink on LA level in mice

Lactic acid is the body's aerobic or anaerobic exercise which is caused by a metabolic substances, can be seen from the figure 4, the batch experiment, static and movement of mice fed each organization's LA content all have varying degrees of decline, including 10 or 20 times in the serum and muscle tissue dose when compared with the control group with very significant difference ($P < 0.01$), The results indicate that red ginseng and pomegranate compound drink can reduce the accumulation of lactic acid in the body, thus reducing the generation of exercise fatigue.

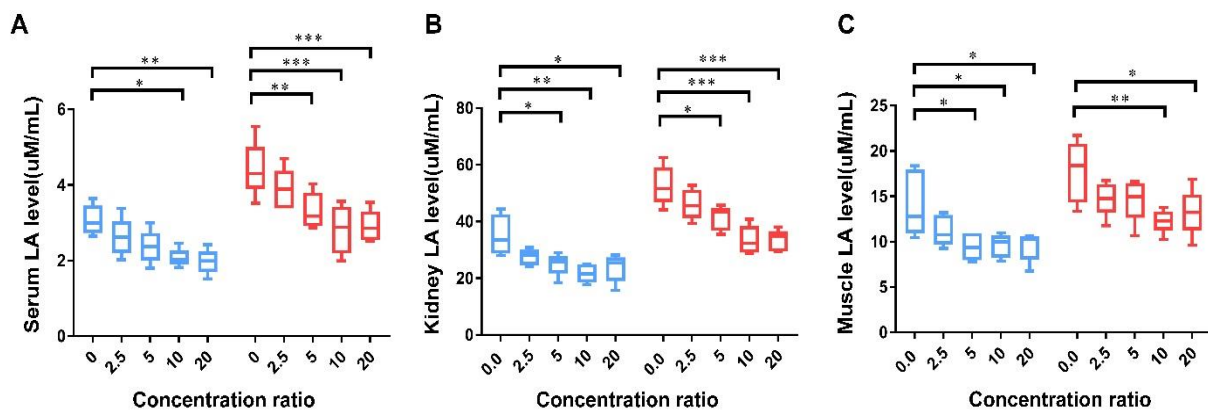


Figure 4. LA levels in various tissues of mice

3.5 Effect of red ginseng and pomegranate compound drink on testosterone level in mice

Testosterone secreted by testicular stromal cells can regulate the expression of genes related to sexual function, which plays an important role in male reproductive ability. As can be seen from Figure 5, with the increase of feeding concentration multiple, the testosterone content of mice gradually increased, and there was a positive correlation between the two. When the feeding dose was 10 or 20 times, there was significant difference in the increase of testosterone level ($P < 0.05$).

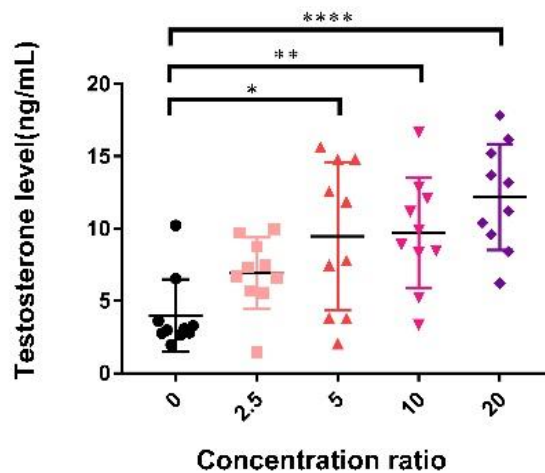


Figure 5. Serum testosterone levels in mice

3.6 Effect of red ginseng and pomegranate compound drink on testis pathological morphology in mice

As can be seen from Figure 6, the testicular tissue of mice in the control group had a complete spermatogenic epithelial structure, complete boundary of spermatogenic tubules, no atrophy or collapse, and the lumen was filled with sperm and developed normally. Compared with the control group, the spermatogenic tubules in the feeding group were more compact, the interstitial tissue was abundant, the spermatogenic cells were closely arranged, the epithelial layer was clear, the supporting cells were clearly visible, and there were a lot of differentiated and mature sperms in the lumen. Compared with each dose, the effect was obvious when the dose was more than 5 times.

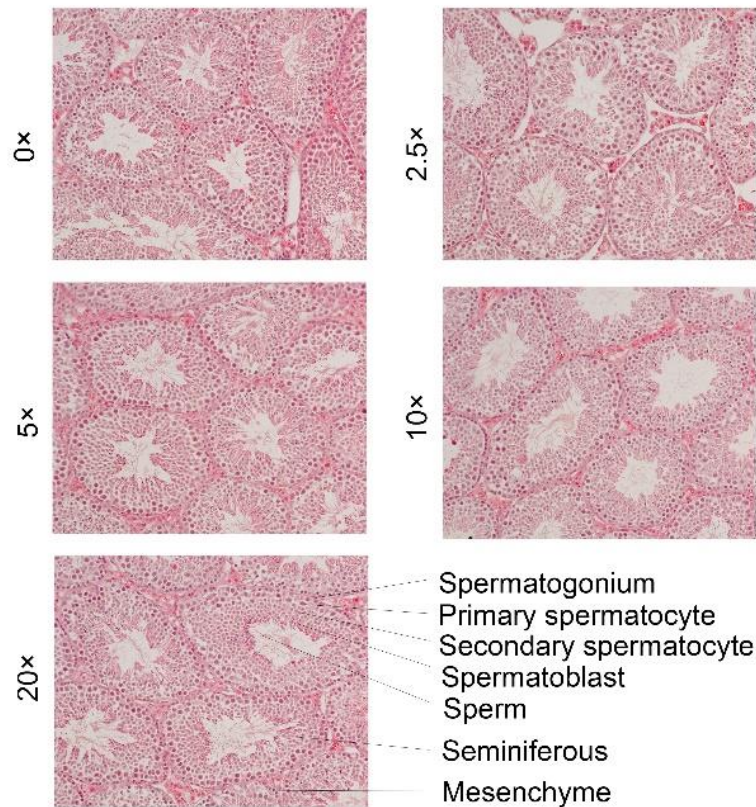


Figure 6. Pathological morphology of mouse testis

4. Conclusion

Exhaustive swimming time is a comprehensive performance of physical ability and a commonly used index to evaluate athletic ability. In the evaluation of health care drugs, the improvement of exhaustive swimming time was identified as a necessary condition for the anti-fatigue effect of the substance. In this study, it was found that red ginseng and pomegranate compound drink could significantly prolong the exhaustible swimming time of mice and enhance their sports ability.

The improvement of exercise ability of mice is closely related to the antioxidant capacity of body tissues. After high-intensity exercise, the physiological and biochemical indexes of mice will change, and a large number of free radicals will be produced, which will cause lipid peroxidation reaction in tissues, thus increasing the end product MDA. It has been shown that MDA can not only inhibit the activity of Ca^{2+} -ATPase and Na^{+} - K^{+} -ATPase in skeletal muscle, but also damage the integrity of biofilms such as muscle cell membrane and mitochondrial membrane[9]. Therefore, the degree of membrane lipid peroxidation and membrane system damage can be understood by measuring the content of MDA[10]. SOD, as one of the components of scavenging free radicals directly in the body, is closely related to aerobic exercise ability. It can convert harmful superoxide free radicals into hydrogen peroxide, thereby reducing the generation of lipid peroxides and protecting cells from oxidative damage[11]. In addition, after intense exercise of muscles, the body's oxygen consumption increases sharply, and the capacity provided by aerobic oxidation alone cannot meet the body's demand, so glycolysis will also become an important energy supply method for exercise [12]. In the process of glycolysis, a large amount of lactic acid is generated, which accumulates in the muscle tissue and leads to an increase in H^{+} concentration and a decrease in pH value, leading to a series of biochemical changes, resulting in muscle fatigue and pain[13]. The results showed that the content of MDA and LA increased after exhaustive swimming in mice. Red ginseng pomegranate complex drink can increase the SOD level of the body, inhibit the lipid peroxidation process in mice, so as to

reduce the degree of free radical damage to the body. In addition, it also plays an anti-fatigue role by accelerating LA clearance.

Male reproductive function is affected by a variety of factors in vivo and in vitro, and the hypothalamic-pituitary-gonad axis is the main regulatory pathway in vivo [14-16]. In this pathway, testosterone secreted by testicular stromal cells is the main hormone regulating male sexual activity. Testosterone can not only promote the synthesis of nitric oxide synthase (NOS) to catalyze the synthesis of nitric oxide (NO) by L-arginine to improve erectile function, but also cooperate with luteinizing hormone (LH) and follicle stimulating hormone (FSH) to promote the development of spermatogenic tubules, spermatogenesis and maturation, thus affecting male reproductive ability and the health level of offspring [17, 18]. The results showed that red ginseng and pomegranate compound drink could significantly increase the serum testosterone content in mice, and showed a dose-dependent effect. The observation of the pathological morphology of testis in mice further verified that it could promote the reproductive development and improve the reproductive function of mice.

In conclusion, Red Ginseng and Pomegranate Compound Drink can prolong exhaustive swimming time of mice, and regulate SOD, MDA, LA and other indexes of each tissue level. It has obvious effect of resisting exercise fatigue and enhancing exercise ability when the dosage is more than 10 times. In addition, red ginseng pomegranate compound drink can significantly increase the testosterone content of mice, promote the development and maturity of their reproductive organs, has a good application prospect.

Acknowledgements

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