

The Anti-fatigue Effect of the Extract from Rusa Deer (*Cervus timorensis*) Velvet Antler in Male Wistar rats

Ratsa Sripirom¹ and Rungrudee Srisawat¹⁺

¹ Institute of Science, Suranaree University of Technology, Nakhon Ratchasima Province 30000, Thailand

Abstract. Velvet antler has been used as a traditional animal based medicine to prevent or treat various diseases, promote growth, strengthen body and systematic exhaustion, prevent and repair muscle damage, increase muscular strength and endurance. Thus, the anti-fatigue effects of the rusa deer velvet antler extract (DAV) were investigated in male Wistar rats. Rats received ddd water (1 ml/kg) and DAV (100, 200 or 400 mg/ml/kg) orally once daily for 9 days. After last dose on day 9, rats were tested by forced swimming test. The swimming time to exhaustion was used as the index of the forced swimming capacity. Immediately after exhaustion, blood samples were collected for determination of serum lactate dehydrogenase (LDH), plasma glucose and creatinine using an automatic analyzer. The swimming time to exhaustion was significantly increased in middle and high dose groups (200 and 400 mg/kg DAV) when compared to control group and low dose group (100 mg/kg DAV). Creatinine and glucose levels were significantly increased in middle and high dose groups when compared to control group and low dose group, respectively. No significant difference was found in LDH levels among groups. DAV appears to promote anti-fatigue effects, however, the underlying mechanisms are still not fully understood.

Keywords: anti-fatigue, velvet antler, rusa deer

1. Introduction

Velvet antler has been used as a traditional animal based medicine in the East and in traditional Chinese medicine to prevent or treat various diseases, including cardiovascular disease, gynecological problems, immunological deficiencies and blood cancers, promote tissue repair and also exert health promotion [1]. Velvet antler has been claimed to have a wide range of effects on systematic exhaustion, depression, cold, lower back pain, weak pulse, impotence, spermatorrhea, low white cell counts, regulate the adrenal cortex, regulate energy metabolism, promote sexual function, promote growth and strengthen resistance, anaemia alleviating, anti-aging, anti-cancer, anti-inflammatory, blood pressure control, bone and joint anti-inflammation, growth stimulation, performance enhancement (prevent and repair muscle damage, increase muscular strength and endurance) [2].

Velvet antler composes of many biochemical components including lipids, peptides, carbohydrates and inorganic substances [3]. Many components such as glycosaminoglycans (chondroitin sulphate and glucosamine chondroitin), sphingomyelin, ganglioside, estrone, estradiol, prostaglandins, collagen, amino acid-sugar combinations and growth factors including IGF-1 and epidermal growth factor (EGF) are also found in velvet antler [4]. Deer antler velvet supplementations that contain IGF-1 have been used in runner and weight lifting athletic for many years to enhance muscular strength and improve aerobic performance [5]. Protein and collagens, bioactive compounds found in the deer antler base extract, have been suggested to be the major substances responsible for anti-fatigue effect of the deer antler base extract. The pure compound of deer antler collagens could increase swimming time [6]. It is hope that a bioactive compound found in the

⁺ Corresponding author. Tel.: +66(0)44224631; fax: +66(0)44224185.

E-mail address: srisawat@sut.ac.th.

rusa deer velvet antler extract possesses anti-fatigue and velvet antler could also enhance swimming ability. Therefore, the anti-fatigue effects of the extract from rusa deer velvet antler were investigated in male rats.

2. Materials and Methods

2.1. Preparation of the Rusa Deer Velvet Antler Extract

The rusa deer velvet antlers were purchased from deer farm in Nakhon Ratchasima province in November 2013. The rusa deer velvet antlers were cut off 70 to 75 days after casting from 3 years old rusa stags. The extraction method used in the present study was adapted from the study of Shi *et al.* (2010) [7]. Briefly, the rusa deer velvet antlers were cut into small thin pieces, dried at 60 °C, milled and sieved. The dried milled deer velvet antler (200 g) was extracted four times with 1 L of deionized double distilled (ddd) water in a hot water bath for 5 h and then filtrated through No.1 Whatman filter paper (Whatman Internation Ltd., Maidstone, England). The filtrate was collected, concentrated using a rotary evaporator (Rotavapor model R-205, Bushi, Switzerland) and then converted into crude extract by freeze-drying. The obtained crude extract was stored at -20 °C until further used.

2.2. Animals

Eight weeks old male Wistar rats (weighting 250-300 g) were housed under standard laboratory conditions (12:12 h dark-light cycle, ambient temperature 25±1 °C) with free access to food and water. The experiments were performed following the animal care and use committee guidelines of Suranaree University of Technology. Rats were randomly assigned to four groups of 8 rats each as follows: control group (1 ml/kg ddd water, p.o.) and three treatment groups. The rats in the treatment groups were gavaged with three different doses of the rusa deer velvet antler extract: 100 mg/1 ml/kg for the low-dose group, 200 mg/1 ml/kg for the middle-dose group and 400 mg/1 ml/kg for the high-dose group, respectively. All rats were tested for their exercise ability using forced swimming test.

2.3. Forced Swimming Test

The weight-loaded swimming test was employed in this study to evaluate the effects of the rusa deer velvet antler extract on exercise durability of rats. To avoid circadian variations in physical activity, the test was performed between 10.00 and 17.00 [8]. The procedure for forced swimming test was previously described with some modifications [9]. Briefly, the rusa deer velvet antler extract was administered orally daily (9:00 h) for consecutive 9 days. In order to make the animals to accustom to swim, swimming was carried out on day 1, 3, 5 and 7 for 10 min, distinguished from day 9, in which rats were loaded nothing on their tails. During these periods, those who were unable to learn to swim were excluded from the experiment. Thirty minutes after the last oral administration on day 9, the overnight fasted rats were dropped individually into an plastic pool (90 cm × 45 cm × 45 cm) filled with fresh water maintained at 30±1 °C, approximately 60 cm deep so that rats couldn't support themselves by touching the bottom with their tails. A lead block (5% of body weight) was loaded on the tail root of the rats. The swimming time to exhaustion was used as the index of the forced swimming capacity. The swimming period was considered the time spend floating, struggling and making necessary moments until exhaustion and possible drowning. The rats were assessed to be exhausted when they failed to rise to the surface of water to breath within a 7-s period.

2.4. Determination of Blood Biochemical Variables

Immediately after exhaustion, the rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Whole blood obtained by cardiac puncture was collected into the tube with and without anticoagulant. Blood samples in the tube without anticoagulant were cooled for about 3.5 h at 4 °C, the serum was prepared by centrifugation at a speed of 1000×g at 4 °C for 20 min. Blood samples in the tube with anticoagulant were centrifuged at 2000×g at 4 °C for 5 min. The levels of serum lactate dehydrogenase (LDH), plasma glucose and creatinine were determined by an automatic analyzer [10].

2.5. Statistical Analysis

Results were expressed as mean \pm standard error of mean (SEM). Data were analyzed by one-way ANOVA followed by Tukey and LSD as posthoc tests using the software SigmaStat (version 3.5, Systat Software Inc., USA.). *P*-values less than 0.05 ($P < 0.05$) were considered statistically significant.

2.6. Results

The results of forced swimming capacity test are presented in Fig. 1. The swimming time to exhaustion of middle dose (200 mg/ml/kg DAV) and high dose (400 mg/ml/kg DAV) were significantly higher than control and lower dose (100 mg/ml/kg DAV) groups (Fig. 1). Creatinine levels were significantly increased in middle and high dose groups (200 and 400 mg/ml/kg DAV) when compared to control group (Fig. 2). No significant difference was found in serum LDH levels among groups (Fig. 3). Plasma glucose levels in middle and high dose groups of DAV were significantly higher than low dose group (100 mg/ml/kg DAV) as shown in Fig. 4.

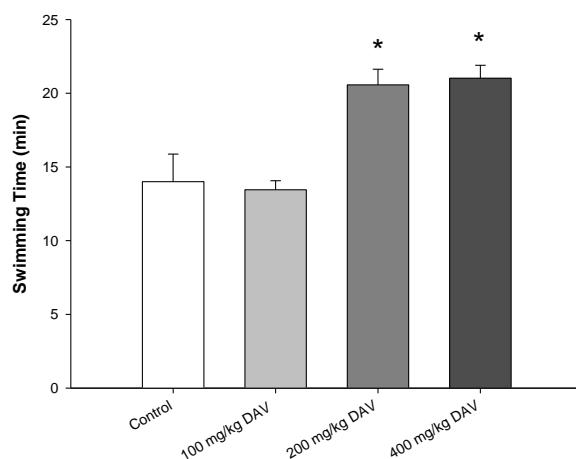


Fig. 1: Effects of 9 days oral administration of the rusa deer velvet antler extract on swimming time to exhaustion in forced swimming test in male rats. Values are expressed as mean \pm SEM; $n=8$ per group. * Indicates a significant difference ($P < 0.05$) when compared to control group and low dose group (100 mg/kg DAV).

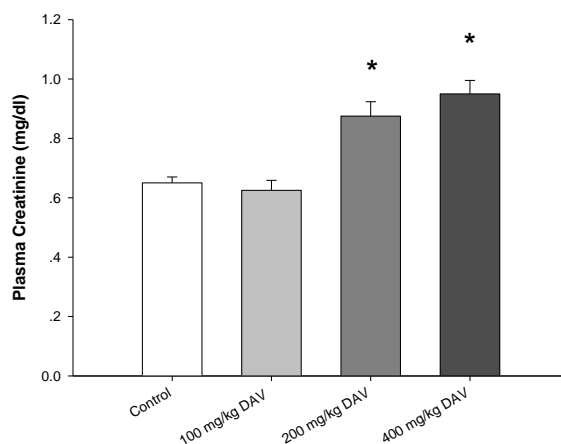


Fig. 2: Effects of 9 days oral administration of the rusa deer velvet antler extract on plasma creatinine levels in male rats. Values are expressed as mean \pm SEM; $n=8$ per group. * Indicates a significant difference ($P < 0.05$) when compared to control group.

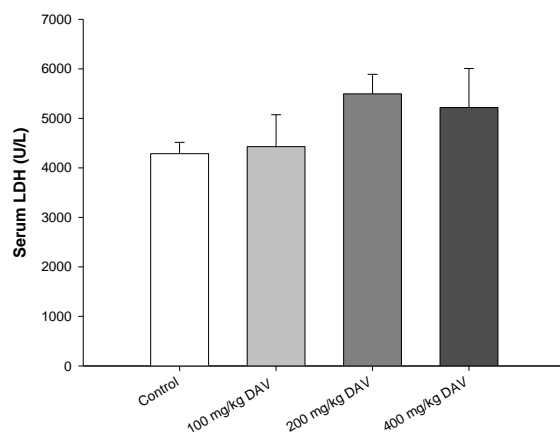


Fig. 3: Effects of 9 days oral administration of the rusa deer velvet antler extract on serum lactate dehydrogenase levels in male rats. Values are expressed as mean \pm SEM; n=8 per group.

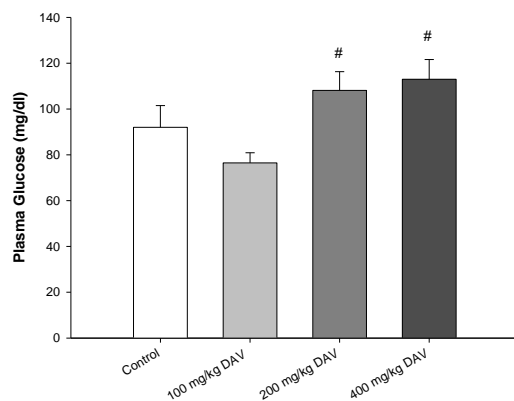


Fig. 4: Effects of 9 days oral administration of the rusa deer velvet antler extract on plasma glucose levels in male rats. Values are expressed as mean \pm SEM; n=8 per group. # Indicates a significant difference ($P<0.05$) when compared to 100 mg/kg DAV treated group.

3. Discussion

In forced swimming capacity test, significant increases in swimming time in rats received rusa deer antler velvet extract at doses of 200 and 400 mg/ml/kg for 9 days were demonstrated (Fig. 1). These findings indicated that the rusa deer antler velvet extract could enhance swimming time durability and may be a potent to anti-fatigue in male rats. Antler velvet has been claimed by traditional Chinese medicine to provide a wide range of effects on systematic exhaustion, regulate energy metabolism and performance enhancement (prevent and repair muscle damage, increase muscular strength and endurance) [2]. Bioactive compounds found in the deer antler base extract such as protein and collagens have been shown to have positive effects on swimming time to exhaustion. The pure compound of deer antler collagens from deer antler base extract have been suggested to have a multitude of beneficial effects on improve the activity of blood lactate dehydrogenase and increase hepatic glycogen [6]. Intra-gastric administration of deer antler base extract for 5 days could prolong the swimming time and increase the adrenal coefficients in a forced swimming test. The findings suggested that anti-fatigue effect of the deer antler base proteins may be associated to the enhancement of adrenal function [7]. Gerrard et al. (1998) [11] showed that 14 days-pretreatment with deer velvet could reduce muscle damage following a simulated downhill run in men who were not trained runners. Moreover, polypeptide-rich deer antler has been used to treat patients with insulin resistance and help to improve their blood sugar profiles [1]. The rusa deer antler velvet extract at doses of 200 and 400 mg/ml/kg for 9 days significantly increased plasma glucose levels compared with control (Fig. 4). These results indicated that the increased demand for glucose by contracting muscle causes an increased glucose uptake to

working skeletal muscle (blood glucose) during prolonged exercise. The rusa deer antler velvet extract at doses of 200 and 400 mg/ml/kg for 9 days significantly increased creatinine levels (Fig. 2). These effects may be due to the increase of creatine kinase levels, responsible for the breaking down of creatine phosphate into creatinine in muscle, in the exercise group than in the sham groups [12]. Serum lactate dehydrogenase levels (shown in Fig. 3) tended to increase, but not significantly difference, indicating that the rusa deer velvet antler extract might induce glycogen degradation *via* lactate dehydrogenase pathway without any muscle tissue damage. According to the study of Stenner, et al. (2005) [13], a significant increase in the levels of serum lactate dehydrogenase released by damaged muscle tissue was observed.

In conclusion, the present findings suggested that the rusa deer extract may be enhancing swimming durability. The rusa deer velvet antler extract appears to promote anti-fatigue effects, however, the underlying mechanisms are still not fully understood.

4. Acknowledgements

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5. References

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