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Anti-fatigue activity on male mice of 70% ethanol and aqueous extract of *Rusa unicolor* antler from East Kalimantan

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Abstract

Energy supply issues, ATP+PC, anaerobic glycolysis, product accumulation, lactic acid, mechanical failure of muscles to contractions and nervous system changes contribute to fatigue. The goal of this study was to determine whether 70% ethanol and aqueous extracts of *Rusa unicolor* antler from East Kalimantan, Indonesia, can reduce fatigue in male mice. A total of sixty-four male mice aged 2-3 months (20-30 g) were randomly assigned to eight groups (negative control, positive control and 3 doses of treatments for each extract). After that, the swimming endurance time was assessed, and blood glucose levels were measured before and after the test. Mice's tiredness is demonstrated by their head remaining submerged for 4-7 s while their tails and legs are stretched. One-way ANOVA was used to statistically analyze the data. The results showed that there were significant differences between all treatments with the negative control group ($p<0.05$). At a dose of 1.3 mg/kg BW of 70% ethanol and aqueous extract of *Rusa unicolor* antlers were able to maintain the stamina of male mice in swimming for 2 hrs 37 mins. Both extracts improve endurance training, delay tiredness, and promote recovery after exercise. Hence, *Rusa unicolor* antler extract can significantly improve athletic ability and facilitate recovery after exercise in male mice by increasing endurance training capacity and delaying tiredness.

1. Introduction

Fatigue is described as the body's inability to maintain the strength it requires (Davis and Bailey, 1997). There are two types of exhaustion: physical or muscular fatigue and mental fatigue (Huang *et al*., 2014). Consuming energy drinks is one way to preserve and regain physical strength. When ingested in large quantities and irregular patterns, it becomes a problem. As a result, there is a need to work toward the development of stimulant medications that are made from natural substances such as plants or animals.

Even though different components that could be employed in the prevention, treatment, or therapy of various diseases have been verified, animal medicine research is still very scarce. Supplements containing deer antler extract are rapidly gaining favor as immune system boosters and stamina boosters. It is utilized in China to restore vital essence, strengthen bones, increase virility, blood nourishment, and male and female sexual activities (Kawtikwar *et al*., 2010). Deer antler is used as a traditional medicine in Indonesia to boost the immune system, increase physical endurance, improve sexual function, and increase bone turnover (Hariyadi *et al*., 2019; Widyowati *et al*., 2020; Widyowati *et al*., 2021). It's commonly made using the decoction method and taken orally.

Through in vivo and in vitro experiments, certain deer antler products demonstrated potential therapeutic benefits for modern disorders such as cardiac function,

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aging (Shao *et al*., 2012), infection (Dai *et al*., 2011), wound healing (Mikler *et al*., 2004), allergic (Kuo *et al*., 2012), osteoarthritis (Zhang *et al*., 2011), immunological dysfunction (Shi *et al*., 2010), and increased stamina (Sleivert *et al*., 2003) However, the underlying mechanisms and bioactive chemicals are mostly unknown (Huang *et al*., 2014). Deer antler products have been proven to be useful in decreasing osteoporosis in mice after ovariectomy in several preclinical investigations (Li *et al*., 2010). The osteoblast proliferation and mineralization process (Lee *et al*., 2011), and the inhibition of osteoclast differentiation are two of the fundamental mechanisms (Choi *et al*., 2013). Deer antler extract has a lipid and protein content from the horn tip to the base, according to a chemical analysis study. Calcium, ash, and collagen content, on the other hand, have increased. Deer antler extract from New Zealand contains minerals such as calcium, phosphorus, sulfur, magnesium, potassium, sodium, manganese, zinc, copper, iron, selenium, cobalt, amino acids, and free fatty acids, according to deer antler analysis (Kawtikwar *et al*., 2010). A study on the supplementing effect of deer antler velvet extract administered to people has been published. According to one study, the gonadotrophic action of velvet deer antler extract improves athletic performance when evaluated over 3000 meters (Yudin and Dobryakov, 1974).

This study was done to evaluate the anti-fatigue activities of 70% ethanol and aqueous extracts of *Rusa unicolor* antler from East Kalimantan, Indonesia on male mice. *Rusa unicolor* is one of the most common types of deer that live in Indonesia.

2. Materials and methods

2.1 Antler materials

The deer antler of *Rusa unicolor* was collected in the middle of February 2019 in UPTD (Technical Implementation Service Unit) of East Kalimantan, Indonesia, and voucher specimens were deposited at the UPTD of East Kalimantan, Indonesia.

2.2 Extraction of Rusa unicolor antlers

The *Rusa unicolor* antler was powdered (991 g) and extracted with 70% ethanol p.a (Merck) (2.0 L x 3) using the maceration method. The 70% ethanol solution was concentrated using a BUCHI rotary evaporator to get 70% ethanol extract (ET, 35.0 g). Besides, the deer antler (430 g) was extracted with 100% distilled water (1.0 L × 3) by applying a continuous percolation method. The aquadest solution was dried by freeze-drying to get an aqueous extract (AT, 6.1 g) (Hariyadi *et al*., 2019; Widyowati *et al*., 2020; Widyowati *et al*., 2021).

2.3 Anti-fatigue test

A tool in the form of a water tank or aquarium with a length of 50 cm, a height of 25 cm, and a width of 30 cm was employed in the swimming endurance method. The water level in the tank or aquarium is important because if the level is too high, test animals will jump out of the aquarium, and if the level is too low, test animals' feet will touch the aquarium's bottom, making it impossible to watch. A post-test control group design was selected for this research. A total of 64 male healthy mice (2-3 months old) with a mean bodyweight of 20-30 g were randomly divided into eight groups, each of which had eight mice: a negative control group, a positive control group (caffeine in pharmaceutical grade), a 70% ethanol extract of *Rusa unicolor* antler group (0.325, 0.65 and 1.3 mg/kg BW/day), and an aqueous extract of *Rusa unicolor* antler group (0.325, 0.65 and 1.3 mg/kg BW/ day). All test animals were given an anti-fatigue treatment before being bathed in a big bath, similar to that used for a tonic test, until fatigue set in, with the mark of the test animal leaving its head underwater surface with vertical head and tail position for more than 7 s. It does not show all four legs moving and the tail stiffens. After the test animals seemed fatigued, they were withdrawn from the bath and the time when they first became tired (T1) was recorded, and male mice were allowed to rest for 30 mins while being dried. After the rest period was ended, the animal test was treated according to each group's test for 30 mins. Then, male mice swam for 30 mins until exhaustion appeared, and the time was recorded as (T2). The difference in swimming endurance time after treatment minus swimming endurance before treatment is the data on the effect of anti-fatigue (Zhang *et al*., 2010). In addition, male mice's blood glucose levels were measured before and after therapy. All experiments were performed at the Animal Research Laboratory of the Faculty of Pharmacy, Universitas Airlangga, Indonesia. Following the Guide for Care and Use of Laboratory Animals issued by the National Institutes of Health revised in 1985. The protocol was approved by the ethical committee of the Faculty of Veterinary, Universitas Airlangga (No.2.KE.176.09.2019).

2.4 Analytical statistic

The results are displayed as mean ± standard deviation (SD). All statistical tests were performed using SPSS 23, and the examination of parametric statistical data was performed using a one-way ANOVA test with a $p<0.05$ significance level, followed by a Post Hoc test to indicate significant differences between group treatments.

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3. Results and discussion

Each test group's swimming endurance time improved as a result of the stamina test effect. When the entire body and part of the head are submerged beneath the surface of the water while the hand, tail, and feet are stretched, the animal is said to be tired (Turner, 1985). Lactate accumulation causes fatigue by interfering with nerve impulses and muscle contractions (Maclaren *et al*., 1989). Chemical constituents contained in deer antlers can help to prevent fatigue by inhibiting the development of lactic acid. Empirically, people believe that *Rusa unicolor* antler extract contributes significantly to human health and performance. Then, according to a number of sources from China, Russia, and Korea, various processed deer antler extract products have a "tonic" impact on people (Bensky *et al*., 1991). According to lymphocyte culture studies and anti-inflammatory characteristics in mice and rats, deer antler velvet has been reported to be utilized as an immunomodulatory drug (Wang *et al*., 1988; Suttie *et al*., 1998). Although this study is still based on rabbit evidence, deer antler products are also referred to as "anti-fatigue" treatments since they are thought to include possible erythropoietic compounds that can improve muscle endurance.

The anti-fatigue or stamina effect of 70% ethanol and aqueous extract of *Rusa unicolor* antler on male mice was researched using the swimming endurance method and blood glucose levels. The first parameter that was observed was the swimming endurance of the test animals. This parameter was used to test the effect of deer antler extract on the resistance of the test animals to physical activity. The swimming endurance test is a pharmacological screening tool used to detect the effect of medicines that affect motion coordination, as well as to test for both a decrease and an increase in central nerve control. The fatigue time acquired from the swimming endurance method on male mice shows the result of the anti-fatigue test (Figure 1).

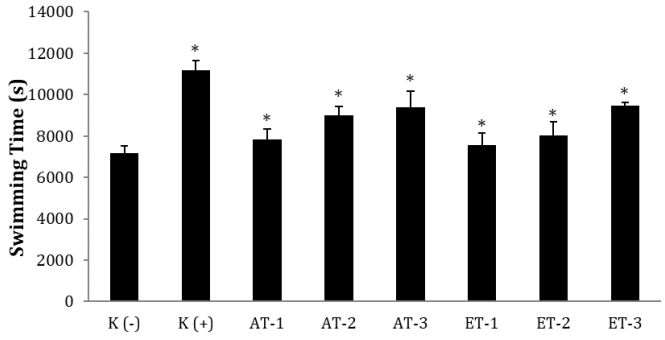

Figure 1. The swimming endurance of male mice with deer antler extracts was compared with the control group. K (-) is the negative control, K (+) is the positive control, AT is the aqueous extract, ET is 70% ethanol extract and *p<0.05.

Figure 1 shows a diagram of the average time difference between fatigue before and after treatment. From the results of the study, it can be seen that there was an increase in swimming endurance time in the positive control and treatment groups. Dosage-dependent administration causes an increase in swimming endurance time which shows that 70% ethanol and aqueous extract of deer antler were able to increase swimming survival time so as to maintain stamina in male mice while swimming for 2 hrs 37 min at a dose of 1.3 mg/kg BW/day male mice. This is due to the activity of the extract which tends to slow down the occurrence of fatigue. Indication of fatigue in mice is determined by the inability of mice to return to the surface of the water for more than 7 seconds with the head and tail vertically below the water surface. This condition is considered a state of fatigue caused by a decrease in the strength of contraction.

To analyze the anti-fatigue effects produced by each test group, an ANOVA test with a 95% confidence interval (p=0.05) was performed. The result has a p=0.004 significance value. The control group and the test group have a significantly different correlation with a significance value of 0.000-0.009, indicating that the test group had an anti-fatigue effect, whereas the caffeine group's relationship with the aqueous extract group (p = 0.083) and the 70% ethanol extract group (p = 0.06) is not significantly different.

Caffeine was employed as a comparative substance in this study. It's a substance that stimulates the ganglion system and the heart muscle, increasing metabolic rate and mental activity while decreasing drowsiness and fatigue (Zhang *et al*., 2010). Caffeine can also inhibit the enzyme phosphodiesterase, preventing ATP from being converted to AMP, and then cAMP can stimulate the enzyme phosphorylase kinase, causing the active phosphorylase to become active, stimulating glycogen conversion to glucose (Anggeluci *et al*., 1999). Glycogen in muscles serves as a glucose reserve that is utilized by muscle cells during contractions, allowing caffeine to provide additional energy. Glycogen is the major source of polysaccharides in human and animal cells, and it serves as a vital source of reserve energy in the body, particularly during activity. Increased glycogen storage in the liver also improves the body's physical endurance (Dohm *et al*., 1983). Caffeine is often used by athletes to prolong exhaustion and boost stamina for this reason.

Low glucose levels cause ATP generation to decrease, resulting in fatigue. Glucose level data after the swimming endurance test is shown in Figure 2. These results indicated that the *Rusa unicolor* antlers extract has a strong ability to maintain blood glucose levels, as indicated by the fact that blood glucose levels in male

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mice treated with the extract are higher than before treatment. This also implies that *Rusa unicolor* antlers extract can maintain ATP synthesis. As a result, resistance to carrying out activities increases. Swimming endurance testing necessitates higher energy production as well as an increase in blood lactic acid levels. Muscle glycogen can alter ATP production when blood glucose levels drop. The glycogen in the liver will then be broken down (Allen *et al*., 2008). The highest glucose levels produced reached 123.25 mg/dL at a dose of 0.65 mg/kg BW/day in male mice. The lowest glucose levels were in the pain group (K-) which causes the inability to survive long swimming in the water and has the effect of being fatigued quickly.

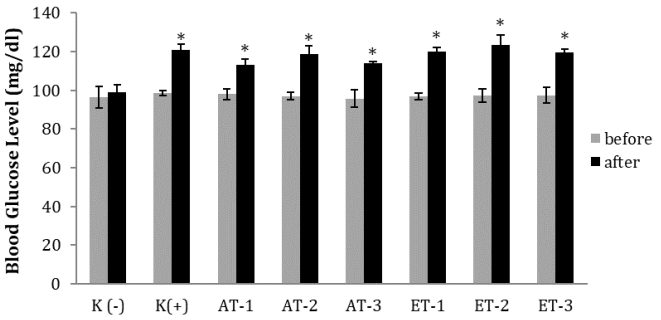

Figure 2. Blood glucose levels of male mice before and after swimming endurance in several test groups. K (-) is the negative control, K (+) is the positive control, AT is the aqueous extract, ET is the 70% ethanol extract and *p<0.05.

The significance value of 0.89 for the glucose levels test before treatment indicates that there is no significant difference between groups. However, the significance value of 0.000 for the glucose levels test after treatment indicates that there is a significant difference between groups. As a result, both the aqueous and ethanol extracts of *Rusa unicolor* antler have the potential to reduce fatigue, despite the fact that caffeine has the most anti-fatigue impact when compared to the other groups with the largest mean difference in swimming endurance time. Caffeine has been shown to have an anti-fatigue or stamina impact in studies (Angelucci *et al*., 2002).

Amino acids found in *Rusa unicolor* antler extract are claimed to have anti-fatigue properties. Amino acids are molecules that combine to form proteins and play a crucial role in a creature's life. Aspartic acid, a non-essential amino acid, can operate as a precursor for gluconic, pyrimidine, and urea production, as well as alleviate weariness and boost energy (Hu *et al*., 2010). According to Suttie and Haines (1998), aqueous deer antler velvet extract supplementation increased rat body weight by 7-10% in a dose-dependent manner after 2 weeks of treatment. Oral supplementation of New Zealand deer antler velvet powder increases isokinetic muscular strength and resistance training response in men receiving supplements. Sleivert *et al*. (2003) reported that Oral supplementation of New Zealand deer antler velvet powder increases isokinetic muscle strength and resistance training response in males getting supplements is larger than in men receiving placebo. Oral administration of 70% ethanol and aqueous extract of *Rusa unicolor* antler to increase stamina did not affect the body weight of the test animals, this was evidenced by the significance value of p≥0.005.

4. Conclusion

Rusa unicolor antler extract can increase endurance training capacity and delay fatigue after exercise in male mice. It indicates that this extract has the potential for development as a stamina product.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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