Philippine Journal of Science
152 (6A): 2105-2110, December 2023
ISSN 0031 - 7683
Date Received: 25 May 2023

Consuming *Salak Sidimpuan* (*Salacca sumatrana* Becc.) Juice during Strenuous Physical Activity Reduced Levels of Creatine Kinase and Lactate Dehydrogenase in White Male Rats

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Strenuous physical activity may result in physical harm, as indicated by increased blood parameters like creatine kinase (CK) and lactate dehydrogenase (LDH). However, one effective way to prevent this damage before exercising is by consuming easily digestible foods such as *salak sidimpuan* juice. This study explored the effects of *salak sidimpuan* juice on CK and LDH levels during strenuous physical activity. The study utilized an experimental design with a post-test control design involving 24 white male rats divided into four groups: negative control, treatment group 1 (T1), treatment group 2 (T2), and treatment group 3 (T3). The negative control group underwent strenuous physical activity, whereas the treatment groups underwent the same training with the addition of different amounts of salak juice (1, 2, and 3 mL). The study was conducted for 4 wk in the USU Pharmacy Laboratory, and the results conclusively demonstrated that consuming *salak sidimpuan* juice during strenuous physical activity significantly reduced CK and LDH levels in experimental animals. Moreover, the study found that the more *salak sidimpuan* juice consumed during exercise, the more significantly decreased CK and LDH levels in experimental animals. Therefore, it can be concluded that consuming salak juice during strenuous physical activity can effectively reduce CK and LDH levels in experimental animals.

Keywords: creatine kinase (CK), lactate dehydrogenase (LDH), *salak sidimpuan* juice, strenuous physical activity, white male rats

INTRODUCTION

Extreme physical activity can cause biological damage to the phospholipid membrane by inducing mechanical-metabolic stress. Hypoxia due to exercise can also damage muscle cell membranes (Khajehlandi and Janbozorgi 2018). Creatine kinase (CK) and lactate dehydrogenase (LDH) are indicators of damage to muscle membranes and other tissue structures. These molecules are cytoplasmic and cannot cross the sarcoplasmic membrane barrier. CK molecules are an indirect marker of muscle damage, especially after resistance training or other exercises that require eccentric actions (Callegari *et al.* 2017). LDH is an essential enzyme of the anaerobic metabolic pathway. The LDH enzyme functions to carry out the reverse reaction of converting lactate to pyruvate through the Cori cycle by reducing NAD+ to NADH and *vice versa*. This enzyme becomes more active production during extreme muscle activity when there is an increase in the substrate (Farhana and Lappin 2022).

Serum CK activity will increase for 24 h after strenuous exercise and return gradually to basal levels within 72–96

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h. LDH serum activity will increase 24 h after exercise and be maintained for 48–72 h (Machado *et al.* 2011). CK and LDH enzymes are found in the cellular cytosol of muscle cells, so their appearance in serum or plasma indicates a cellular lesion. CK can also show early detection of fatigue and muscle overload due to exercise. Monitoring of these enzyme changes can serve to monitor recovery strategies after training, reduce muscle damage and the required recovery time, and improve future performance (Barranco *et al.* 2018).

In addition to monitoring recovery strategies after exercise to reduce muscle damage, the role of food consumed during exercise can also reduce CK and LDH levels. Several studies have been conducted on the effect of the consumption of medicinal plants on CK and LDH levels during physical activity. For example, take 500 mg of saffron stigma (ST) capsules daily in two phases – 250 mg after breakfast and 250 mg after exercise; ST consumption can reduce LDH serum levels in the experimental group, but there is no difference in CK serum levels in the control and experimental groups. The mechanism of this change is unclear but is likely related to saffron's antioxidant content. Antioxidants can inhibit the accumulation of free radicals during exercise and ultimately cause a decrease in serum LDH levels in plasma compared to physical activity alone (Niaki *et al.* 2018). Flavonoids in saffron can neutralize free radicals, especially superoxide.

Administration of *Djoscorea bulbifera* (DB) extract for 2 wk in experimental animals during endurance exercise reduced serum CK and increased LDH compared to other experimental animal groups. This decrease in CK serum is associated with DB extract's anti-fatigue activity, thus accelerating lactate clearance from cells and efficient energy utilization with free radical scavenging potential (Narkhede *et al.* 2015).

In another study, giving virgin coconut oil (VCO) 15 mL during sub-maximum physical exercise could significantly reduce CK activity. The antioxidant content of VCO in the form of ferulic acid and p-coumaric acid can restore the body's antioxidant balance and reduce CK activity (Sinaga *et al.* 2020). Administration of *Brassica rapa* L. (BE) extract to experimental animals decreased CK levels and increased LDH levels. The antioxidant content of BE can improve sports performance and reduce physical fatigue (Li *et al.* 2022).

Giving *Pinus korajensis* leaf extract can reduce LDH levels, prevent fatigue, and increase exercise performance potential. The antioxidant activity of street vendors mediates glycogen synthesis and stress control (Lee *et al.* 2022). Antioxidants play an essential role in the human body in reducing oxidative processes and the harmful effects of reactive oxygen species (ROS). They inhibit the development of chronic diseases, as well as lipid peroxidation. Consumption of fruits and vegetables reduces the risk of several dangerous chronic illnesses associated with their antioxidant activity (Gulcin 2020).

However, research on using *salak sidimpuan* fruit in reducing CK and LDH levels after strenuous physical exercise has never been done, so this research is focused on that. *Salak sidimpuan* is a fruit from the City of Padang Sidimpuan, North Sumatra, with antioxidant capacity. This fruit is a monoecious plant with hermaphrodite flowers and seasonal fruit, and the highest amount of fruit production is in May and August each year (Adelina *et al.* 2021). The types of arillus or the color of the flesh of the *salak sidimpuan* fruit vary – ranging from white to reddish-white and dominantly red. *Salak sidimpuan* is rich in flavonoids, glycosides, saponins, tannins, epicatechins, proanthocyanidins, and steroids, which act as antioxidants (Halim *et al.* 2019; Fendiyanto *et al.* 2020). Salak contains phenolic and maleic acid, which play a role in ROS scavenging. Phenolic acids can inhibit cancer cell proliferation and are antimutagenic, anticarcinogenic, and anti-inflammatory. Maleic acid has a bactericidal activity, which can fight pathogenic bacteria (Mazumdar *et al.* 2019). Antioxidants can be essential in reducing muscle damage due to exercise and fatigue. For this reason, the study aims to determine *salak sidimpuan* juice's position in preventing muscle damage to reduce fatigue after strenuous physical exercise.

MATERIALS AND METHODS

Materials

The materials consist of fresh *salak sidimpuan* fruit obtained from snake fruit traders in Medan City, as well as glucose syrup 85^0brix from the Rose Brand Company obtained from the local market in Medan. The tools included knives, bowls, filters, spoons, and slow juicers (Ecohome Brand).

Processing of *Salak Sidimpuan* Juice

First, the fresh *salak sidimpuan* was peeled to separate the fruit from the skin. Second, the peeled fruit flesh was cleaned under running water and drained to dry. Third, the fruit flesh was cleaned, sliced, and separated from the seeds. Fourth, the flesh of the *salak sidimpuan* fruit was put into the juicer without using additional water so that it produced pure *salak sidimpuan* juice. Fifth, *salak sidimpuan* juice was filtered using a two-layer filter to separate the fruit juice extract from the dregs produced from the juice-making process. Sixth, as much as 250 mL of fruit juice was mixed with 10 mL of glucose syrup and

then put in a bottle, which was closed tightly and put in the refrigerator at a temperature of 15 °C.

Chemical Analysis of *Salak Sidimpuan* Juice

Water content analysis was done using the SNI 01-2891-1992 method (gravimetric), carbohydrate using the IKP/K-3 (by difference) method, potassium using the IKP/K-7 (AAS) method, and vitamin C using the KP/K-11 (HPLC).

Experimental Procedure

White male rats (*Rattus norvegicus*) aged 2–3 mo with an average body weight of 150–180 g acclimatized for 1 wk in animal cages at the Pharmacy Laboratory of the Universitas Sumatera Utara (USU). Providing food and drinks *ad libitum* was carried out in a room with a 12-h light/dark cycle, where the lights turn on at 07:00 AM. Humidity and room temperature are regulated at the natural state. This procedure follows the research of Harahap *et al.* (2021). This research is experimental research with a post-test control design. Rats acclimatized for 1 wk in animal cages were given treatment by swimming for 50 min three times a week and administering *salak sidimpuan* juice every day, provided at least 30 min before exercise for 28 d. The rats were divided into four treatment groups – namely, the negative control group (NG), the 1-mL *salak sidimpuan* juice group (T1), the 2-mL *salak sidimpuan* juice group (T2), and the 3-mL *salak sidimpuan* juice group (T3). All rats received food and drink *ad libitum* freely. The negative control group is the group that gets strenuous physical activity and placebo drinking. T1 was the group that received strenuous physical activity and drank 1 mL/ 200 g BW of *salak sidimpuan* juice. T2 was the group that received heavy physical exercise and drank 2 mL/ 200 g BW of *salak sidimpuan* juice. T3 was the group that received heavy physical activity and 3 mL/ 200 g BW of *salak sidimpuan* juice. Ethical approval was obtained from the USU Faculty of Mathematics and Natural Sciences' Animal Research Ethics Committee with number 0488/KEPH-FMIPA/2022.

CK and LDH Tests

Blood sampling was immediately carried out on the last day of the training program. Blood sampling was performed after the rats were anesthetized using ketamine, and blood was obtained from the intracardiac. As much blood as possible was collected, put into a vacutainer tube without EDTA, and stored until the serum and blood plasma were separated, then centrifuged at 5000 rpm for 15 min. Examination of CK and LDH levels was carried out on centrifuged serum. The CK examination used the DGKC/ IFCC method and was optimized by DGKC for LDH, which was carried out at the North Sumatra Health Laboratory.

Statistical Analysis

Data analysis was confidently performed using SPSS version 25. ANOVA (analysis of variance) testing was conducted, and the Bonferroni test was applied for further examination. Significance was determined by comparing the results to a p-value of less than 0.05.

RESULTS

This study aimed to determine the effect of consuming *salak sidimpuan* juice at different doses during strenuous physical exercise on LDH and CK levels in experimental animals. CK and LDH levels are indicators of muscle damage.

Figure 1 shows that strenuous physical exercise significantly increased the average serum CK level with a value of $p = 0.0021$ (p-value < 0.05). Consumption of *salak sidimpuan* juice daily during a strenuous physical exercise program reduced CK levels in experimental animals, but the differences between each treatment group were not significantly different. It can be concluded that strenuous physical exercise can increase LDH and CK serum levels in the experimental group of animals, and administration of *salak sidimpuan* juice during exercise had a beneficial effect in reducing the rate of increase in LDH and CK levels.

Based on the results in Figure 2, it is known that there was a difference in the average LDH value in each

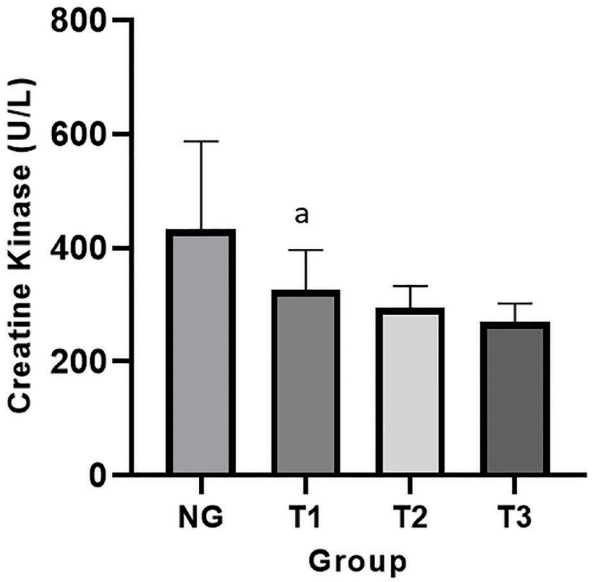

Figure 2. Lactate dehydrogenase Serum levels after four weeks of exercise in the negative control (NG) group, treatment 1 (T1), treatment 2 (T2), and treatment 3 (T3).

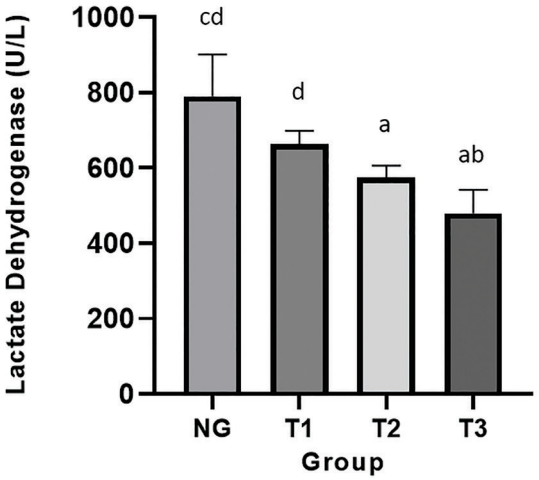

Figure 1. Creatine kinase serum levels after four weeks of exercise in the negative control (NG) group, treatment 1 (T1), treatment 2 (T2), and treatment 3 (T3).

treatment group with a p-value = 0.0001 (p-value < 0.05). Strenuous physical exercise can increase LDH levels in the blood serum of experimental animals compared to the control group. Giving *salak sidimpuan* juice every day during the implementation of the program following heavy physical exercise can significantly reduce blood serum LDH levels in the treatment group with a p-value = 0.0002 (p-value < 0.05). Administration of 1 mL/200 g BW of *salak sidimpuan* juice was sufficient to reduce serum LDH levels.

DISCUSSION

LDH is an enzyme widely distributed in cells of various living systems involved in carbohydrate metabolism to catalyze the interconversion of lactate and pyruvate with the NAD+/NADH coenzyme system. LDH is widely distributed in the body – with high activity in the heart, liver, skeletal muscle, kidney, and erythrocytes and lower activity in the lungs, smooth muscle, and brain (Klein *et al.* 2020). Physiologically, CK provides chemical energy for cells for survival and activities (Gaddi *et al.* 2017).

Even though strenuous physical exercise can increase the risk of inflammation and oxidative stress, which play a role in causing muscle damage due to movement, consuming the right foods can help reduce fatigue after exercise. For this reason, it is necessary to plan an appropriate eating strategy to reduce the inflammatory response and oxidative stress after exercise. Providing optimal nutrition during the post-exercise recovery period can facilitate the repair of muscle damage (Bongiovanni *et al.* 2020).

Consuming 40 g of sesame paste (20 g before starting a workout and 20 g after a workout) can lower markers of muscle damage. The antioxidant activity of sesame seeds can reduce the incidence of oxidative stress, which plays an essential role in causing muscle injury and lipid peroxidation (Barbosa *et al.* 2017).

Administration of an isotonic drink (500-mL water, 32-g carbohydrate, 120-mg calcium, 248-mg chloride, and 230-mg sodium) acutely before the competition has a protective effect and reduces muscle damage. Magnesium and zinc play a role in increasing muscle strength and metabolism. In addition, sports drinks help hydrate the body and prevent dehydration during exercise (Colakoglu *et al.* 2016).

Salak is a fruit with a high antioxidant capacity and vitamin C, which can potentially have beneficial pharmacological effects (Saleh *et al.* 2018). *Salak* also contains phytochemicals such as flavonoids, phenolics, glycosides, and saponins based on laboratory tests conducted at the Pharmacy Laboratory of the University of North Sumatra in 2022. Polyphenols can increase the concentration of ATP and glycogen in muscles and decrease LDH and CK activity. Polyphenols can also induce mitochondrial adaptive redox pathways so that polyphenols can be used as appropriate supplements for sports training. Consumption of vegetables and fruit can meet the need for antioxidants for athletes and people who are physically active (Yavari *et al.* 2015). So, for the next research, we may proceed with additional simple antioxidant experiments such as DPPH radical scavenging effect and total phenolic contents analysis to clarify the major reason for the anti-fatigue effect of *salak sidimpuan* juice. Consumption of *salak sidimpuan* juice as much as 1, 2, and 3 mL 30 min before strenuous physical exercise can reduce LDH and CK levels in experimental animals.

Table 1. Nutritional composition of *salak sidimpuan* juice.

Nutritional composition	Result
Water content	85 g/ 100 g
Carbohydrates	13 g/ 100 g
Potassium	100 mg/ 100 g
Vitamin C	11 mg/ 100 g

Our findings show that consuming fruit drinks, especially *salak sidimpuan* fruit, delayed fatigue after exercise. The nutritional content and antioxidant capacity of *salak sidimpuan* can potentially have a beneficial pharmacological effect on reducing fatigue after exercise. However, this still requires further research to determine the mechanism.

The water content in sports drinks plays a role in carrying oxygen to tissues, hormones, nutrients, and metabolic

waste; regulating blood pH levels; and reducing body heat. Carbohydrates play a role in restoring muscle glycogen reserves (Colakoglu *et al.* 2016). Sodium consumption is effective in lowering CK and LDH levels after athletic activity. Sodium has an ergogenic buffer mechanism as a strong buffer of lactic acid and hydrogen ions, helping to return the environmental pH to its natural state, leading to increased function and delayed fatigue (Samavatisharif *et al.* 2015; Habibineghad *et al.* 2015).

Increased LDH is a sign of the adaptation process of the body's metabolic function during energy metabolism, exercise intensity, muscle stiffness, fatigue recovery, overtraining, and analysis of histopathological damage. Sodium consumption can reduce LDH activity after exercise in non-athlete women, indicating that sodium delays fatigue during exercise (Afolabi and Shittu 2021).

CONCLUSION

After strenuous physical exercise, *salak sidimpuan* juice can reduce plasma LDH and CK levels. Therefore, this study concludes that the consumption of *salak sidimpuan* juice before exercise can reduce the level of muscle damage and has the effect of delaying fatigue after exercise. However, its mechanism for reducing fatigue still requires further research.

ACKNOWLEDGMENTS

The PNBD funded this research from Universitas Negeri Medan.

STATEMENT ON CONFLICT OF INTEREST

The authors declare no conflict of interest that could have appeared to influence the work reported in this paper.

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