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Effect of Mulberry Juice on Blood Lactate and Creatine Kinase in Male College Soccer Players

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Abstract

Soccer is a high-intensity endurance sport that high-intensity moments, where sprints, jumps, acceleration, deceleration, and rapid changes of direction occur repeatedly; actions related to muscle damage promotion. Indeed, these actions during a competitive soccer match promote fatigue and physiological stress in muscle tissue, excessive muscle damage, and delayed-onset muscular soreness (DOMS) in the days after the match (Mohr et al., 2016) Mulberry (Morus alba L.) contained many bioactive components such as alkaloids, anthocyanins, and flavonoids are called polyphenolic compounds (Vijayan et al., 2004; Hasimoto et al., 2008) Mulberry has been reported to protect against cytotoxicity, oxidative stress, and anti-fatigue and pro-inflammatory (Borghei and Ghazalian, 2018; Chen et al., 2017; Jiang et al., 2013) The purpose of this study was to determine the effect of mulberry juice on blood lactate and creatine kinase after high-intensity exercise. Methods: Sixteen males college soccer players of 18-25 years old participated in this study, single-blind, and placebo-controlled trial. The participants received mulberry juice and placebo juice in 2 trials before the treadmill protocol exercise. All the variables were measure before the experiment, post-high intensity treadmill protocol exercise immediately, and after 24 hours. Blood samples were analyzed for lactate and creatine kinase. Results: The results were found that significantly increased blood lactate was seen immediately after high-intensity treadmill exercise and decreased after 24 hours in the placebo and mulberry juice group ($p<.01$). Creatine kinase significantly increased after 24 hours of high-intensity treadmill exercise in the placebo juice group ($p<.01$). Conclusion: This finding suggests that mulberry juice can prevent an increase in creatine kinase after high-intensity treadmill exercise.

Keywords : Mulberry Juice, Blood Lactate, Creatine Kinase, Soccer Players

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Introduction

Soccer is a high-intensity sport with matches lasting approximately 90 minutes. Throughout match play, athletes player cover distances of approximately 10 kilometers and exercise at vigorous intensities of 80-90% of maximal heart rate (HRmax) (Stolen et al., 2005) Athletes player high-intensity moments, such as sprints, jumps, acceleration, deceleration, and rapid changes of direction occur repeatedly; actions related to muscle damage promotion (Mohr et al., 2016). Indeed, these actions during competitive soccer match promote fatigue and physiological stress in muscle tissue, excessive muscle damage, and delayed-onset muscular soreness (DOMS) in the days after the match (Mohr et al., 2016). Furthermore, during a soccer match players perform a substantial number of explosive activities such as jumps, duels, shots, and dribblings (Andersson and Krustrup, 2008) accelerations, decelerations, changes of direction (COD), and sprints (Varley and Aughey, 2013) It is repetitive eccentric contractions that are related to muscle damage (EIMD – exercise-induced muscle damage) (Mohr, et al., 2016; Nédélec et al., 2012) which is clinically presented as muscle soreness and developed two or three days after the match (DOMS – delayed onset of muscle soreness) (Fatourus, et al., 2010; Ispirlidis, et al., 2008) The EIMD is mostly a result of mechanical stress, imbalances in calcium levels, and inflammatory responses with phagocyte infiltration, formation of free radicals, increase of cytokine concentration, and other inflammatory parameters within the muscle (Fatourus, et al., 2010) Players demonstrate a dramatically decline in high-intensity running towards the end of a game (Mohr et al., 2003) causing marked impairment in repeated sprint ability (RSA) (Mohr et al. 2004, 2012; Krustrup et al. 2006) intense intermittent exercise (Krustrup et al. 2010) countermovement jump (Mohr et al. 2010; Mohr and Krustrup 2013) and strength performance (Krustrup et al. 2011; Marshall et al. 2014).

Mulberry (Morus alba L.) belongs to the Morus genus of the Moraceae family (Ercisli and Orhan, 2007) Mulberry is a plant grown in the north and the northeast of Thailand. Mulberry leaves and fruits contained many bioactive components such as alkaloids, anthocyanins, and flavonoids are called polyphenolic compounds (Vijayan et al., 2004; Hasimoto et al., 2008) Mulberry leaves are rich in alkaloids including 1- deoxynojirimycin (DNJ), the most potent glycosidase inhibitor that decreases blood-sugar levels (Kimura et al., 2007; Nakagawa et al., 2010). Anthocyanins are a group of natural polyphenolic compounds responsible for the colouring of fruits, flowers, and leaves.

They are the best source of health benefits as antioxidant and anti-inflammatory compounds (Nitra et al., 2007) Anthocyanin has a high inhibitory ability on lipid oxidation and mulberry anthocyanin extract has antimetastasis activity to inhibit migration of B16-F1 cells (Cui et al., 2008; Ozgen et al., 2009) Flavonoids have commonly occurred in the plant kingdom and mulberry fruit was found to contain at least four flavonoids are rutin, morin, quercetin, and myricetin (Yang et al., 2010) Flavonoids have been recognized to possess anti-inflammatory, antioxidant, antiallergic, antithrombotic, hepatoprotective, antiviral, and carcinogenic activities in human beings (Daimon et al., 2010) Polyphenolic compound content of mulberry fruit was 14.22 mg/g in fresh fruit of Morus nigra L. and 19.43-22.23 mg/g in fresh fruit of Morus alba L. (Ercisli and Orhan, 2007) Polyphenolic compound content in mulberry fruit is much higher than that of other berries including gooseberry (5.2 mg/g), elderberry (19.5 mg/g), chokeberry (20.1 mg/g), red currant (5.4 mg/g), and black currant (13.3 mg/g) (Wu et al., 2004) and even higher than in grape (731.7 mg/kg) (Kallithraka et al., 2005) Mulberry has been reported to protect against cytotoxicity, oxidative stress, and anti-fatigue (Chen et al., 2017; Jiang et al., 2013) And some studies effects of black mulberry supplementation have total phenolic compound content are 18.8 μg/ml on pro-inflammatory markers (plasma interleukin-6 and tumour necrosis factor-α) response in basketball training sport found that black mulberry juice could reduce the rate of plasma interleukin-6 increase immediately and one hour after training and tumour necrosis factor-α trends were downward after supplementation (Borghei and Ghazalian, 2018) By this point, application mulberry juice in sport will be interested. From those above review, the black mulberry supplementation is a preventative effect and can use before in pre-exercise, also use for preventing any damage to athletes. However, the study of mulberry supplementation on muscle fatigue and muscle damage in soccer are limited. Therefore, the researcher aimed to study the effect of mulberry supplementation on blood lactate and creatine kinase after high-intensity exercise in soccer players.

Purpose

The purpose of this study was to determine the effect of mulberry juice on blood lactate and creatine kinase after high-intensity exercise.

Materials and Methods

Participants

Sixteen males college soccer players (mean age, 20.94±1.12 years; mean height, 171.13±3.36 cm; mean mass, 63.24±4.87 kg, Goalkeeper(2), Defender(6), Midfielder(5), Forward(3) volunteered to participate in this study. All participants provided written informed consent before participating in this study, and the investigation was approved by Mahasarakham University ethics committee (009/2020).

Experimental design

The study consisted of a 10-days supplementation in which participants were received 2 juice: placebo juice (PLA) and mulberry juice (MBJ). The participants were consumed PLA and MBJ one bottle per day (300 ml each bottle) for 10 days. The dosage of mulberry juice consists of total phenolic compound content is 1.01 mg/ml. A high-intensity training session was conducted on the eleventh day of the experimental (after 10 days supplementation). Blood samples were collected before the start of the intervention (pre-supplementation), after the high-intensity training session (post-exercise), and 24 hours after the high-intensity training session intervention (post-intervention). Blood samples were collected for analysis of blood lactate, and creatine kinase (CK) concentrations, respectively.

Diet and activity before the experiments

Throughout the study, participants were asked for avoiding eating foods that are high in antioxidants and record their food and drink consumption during the 24 hours before each trial. During the 24 hours before each trial, participants were requested to abstain from vigorous exercise and consumed caffeine-containing beverages and alcohol intake in order to maintain a normal hydration status on the day before the test by drinking at least 6-8 glasses of water and sleeping for 6-8 hours.

Experimental procedures

High-intensity treadmill protocol exercise: The test started at an initial speed of 5 km/h at a gradient of 1% for 3 minutes (Warm-up). The speed at Stage 1 was set at 8 km/h and the gradient was set at 1%. Speed was increased by 2 km/h every 3 minutes until the participant had a heart rate reached 100% maximum heart rate, Rating of Perceived Exertion RPE more than 17 (Borg scale), and tired to be unable to continue the test. The gradient was maintained at 1% throughout the test to maintain biochemical demands similar to flat level running a soccer game (Arent et al.,2010). Heart rate was continuously monitored using a Polar H10 heart rate sensor (Polar Electro Ltd, Kempele, Finland).

Blood sample collection and biomarker assays

Blood samples were collected by medical laboratory technologist, from Surin Hospital and analyzed by medical laboratory Suddhavej Hospital, Faculty of Medicine, Mahasarakham University.

Statistical Analysis

All results are presented as a mean ± standard deviation. To determine the normality of the distribution of all data sets, the Kolmogorov-Smirnov test was used. The effects of mulberry juice and placebo juice on the blood variables were analyzed using an independent sample t-test. A change in blood lactate, and creatine kinase after high-intensity exercise was assessed using one-way repeated-measure ANOVA. When significant results were detected, the Bonferroni post-hoc test was set to detect between-group differences. Statistical analyses were performed using SPSS version 26 (SPSS, IBM Corp., Armonk, NY, USA). Statistical significance was set at p<0.01.

RESULTS

Table 1 Descriptive characteristics of subjects values are mean and S.D.

Variable	Mean $(\bar{x})$	Standard Deviation (S.D.)
Age (years)	20.94	1.12
Weight (kg)	63.24	4.87
Height (cm)	171.13	3.36
BMI	21.59	1.47
Position	**Number**	
Goalkeeper	2	
Defender	6	
Midfielder	5	
Forward	3	

Table 1 presents that the samples were 20.94 years old (± 1.12) weight 63.24 kg (± 4.87) height 171.13 centimeters (± 3.36) and BMI 21.59 (± 1.47)). The sample consists of Goalkeeper(2), Defender (6), Midfielder (5), and Forward (3).

Table 2 Total phenolic compound content of mulberry juice consumed by the participant

Total phenolic (mg GAE /ml)	Mean ($\bar{x}$)	Standard Deviation (S.D.)
mulberry juice concentrated	1.01	0.02

Table 2 presents that the mulberry juice consisted of phenolic compound content were 1.01 ± 0.02 mg GAE /ml.

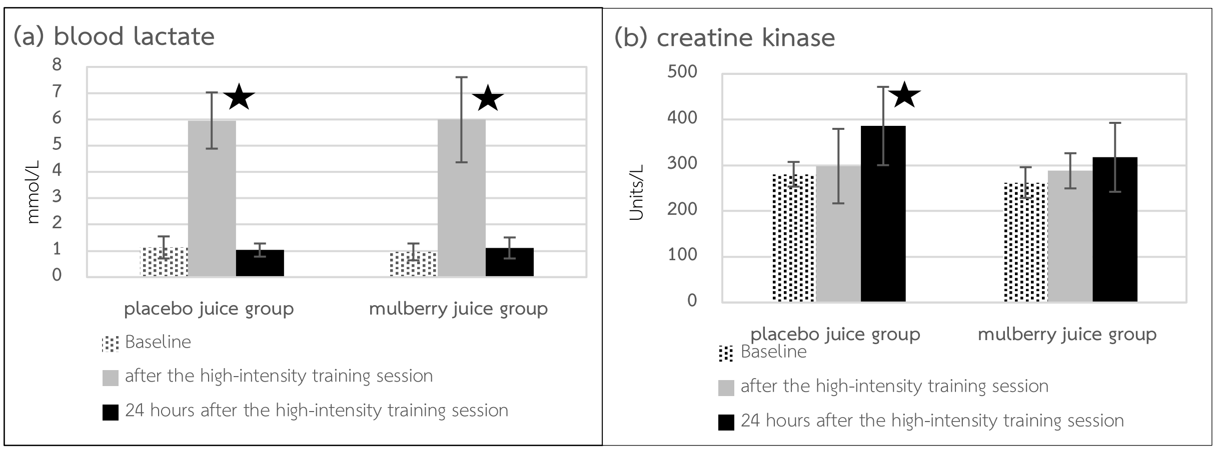

Figure 1 compared mean of (a) blood lactate, and (b) creatine kinase in the placebo juice group and mulberry juice group at the start of the intervention, after the high-intensity training session, and 24 hours after the high-intensity training session

★ = significantly different by one-way ANOVA repeated measure test (p<.01)

Figure 1 (a) shows that blood lactate was increased after the high-intensity training session and decreased 24 hours after the high-intensity training session significantly in the placebo juice group and the mulberry juice group (p<.01). Figure 1 (b) shows that creatine kinase was significantly increased after 24 hours of the high-intensity training session in the placebo juice group (p<.01).

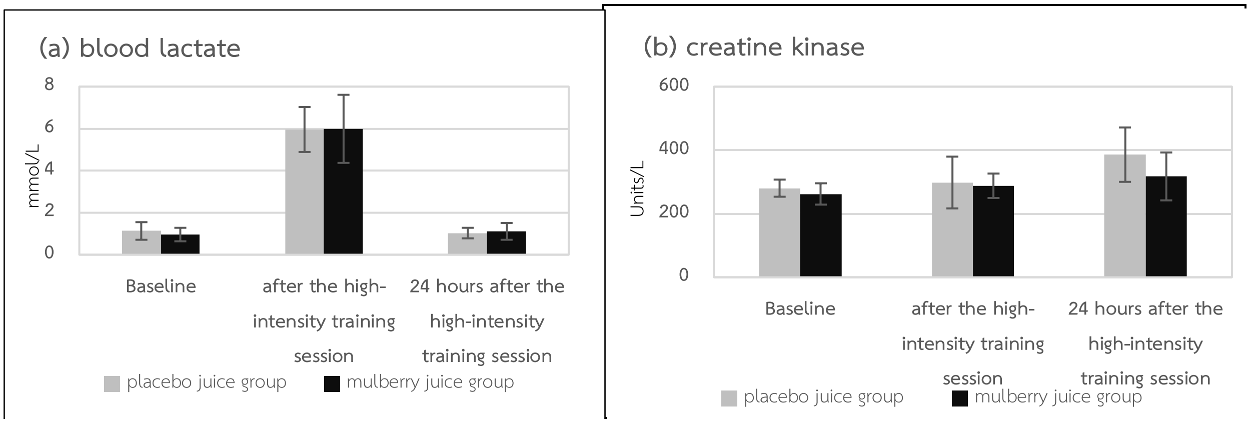

Figure 2 compared mean of (a) blood lactate, and (b) creatine kinase at the start of the intervention, after the high-intensity training session, and 24 hours after the high-intensity training session between the placebo juice group and mulberry juice group.

Figure 2 shows that 24 hours after the high-intensity training session creatine kinase in the placebo juice group was increased more than when compared to the mulberry juice group.

Table 3 compared mean of blood lactate, and creatine kinase at the start of the intervention, after the high-intensity training session, and 24 hours after the high-intensity training session in the placebo juice group and the mulberry juice group

Variables		Mean (S.D.)		Between group
		placebo juice group	mulberry juice group	*p*-value
Lactate				
	Baseline	1.13 (0.42)	0.96 (0.32)	0.21
	after the high-intensity training session	5.96 (1.07)	5.99 (1.62)	0.95
	24 hours after the high-intensity training session	1.03 (0.25)	1.11 (0.40)	0.49
Within Group (time)	*p*-value	0.00*	0.00*	
CPK	Baseline	280.44 (26.97)	262.29 (33.59)	0.10

Variables		Mean (S.D.)		Between group
	after the high-intensity training session	298.27 (81.45)	288.01 (38.44)	0.65
	24 hours after the high-intensity training session	385.94 (85.62)	317.47 (75.37)	0.02
Within Group (time)	*p*-value	0.00*	0.04	

* = significantly different by one-way anova repeated measure test (p<.01)

Table 3 shows that blood lactate was increased after the high-intensity training session and decreased 24 hours after the high-intensity training session significantly in the placebo juice group and the mulberry juice group (p<.01). Creatine kinase was significantly increased after 24 hours of the high-intensity training session in the placebo juice group (p<.01).

Discussion

This study showed that blood lactate was increased after the high-intensity training session in the placebo juice group and mulberry juice group and decreased after 24 hours in both groups. Increased levels of blood lactate in both groups after high-intensity training session compared to before the procedure was due to hypoxia in muscle tissue resulting in anaerobic metabolism, which produced lactic acid (Harahap *et al*., 2021) Blood lactate was decreased after 24 hours due to the mechanism for lactate removal by an increased blood flow to the muscle (Bieuzen et al., 2014) Running or treadmill tests result in an increased metabolic requirement that exceeds the resting level, and lactate production increases above the anaerobic threshold (4 mmol/L). Lactate is the result of pyruvate being reduced by the action of the enzyme lactate dehydrogenase. The accumulated lactic acid can then be re-oxidized in the pyruvate cytosol, which occurs physiologically (Mooren and Klaus, 2005; Guyton and Hall, 2008) The lactate formed will be rapidly eliminated in continuous flow. An individual's ability to remove lactate depends on his or her physical state and fitness. If there is an increment in the level of lactate in the blood circulation, it implies that there is an increment in the entry of lactate into the blood circulation that goes past the liquidation rate of lactate (Ferreira *et al*., 2011) Creatine kinase was increased 24 hours after the high-intensity training session in the placebo juice group. The increase in creatine kinase levels suggests the occurrence of muscle damage after the high-intensity training exercise, which is in agreement with observations already reported (Jówko *et al*., 2012; Oliveira *et al*., 2019) Creatine kinase has been extensively studied as a skeletal muscle damage marker following physical exercise. Studies have demonstrated intensive exercise causes a greater disruption or injury to the muscle tissues which may cause creatine kinase to leak from cells into blood serum (Clarkson and Hubal, 2002; Romagnoli *et al*.,2016)

From the compared within the group showed that blood lactate was increased after the high-intensity training session and decreased 24 hours after the high-intensity training session significantly in both the placebo juice group and mulberry juice group ($p<.01$). Increased levels of blood lactate in both groups after high-intensity training session compared to before the procedure was due to hypoxia in muscle tissue resulting in anaerobic metabolism, which produced lactic acid. Increased levels of lactic acid correspond to Harahap *et al*. (2021) they studied the effect of red dragon fruit juice on reduced levels of lactic acid in soccer players after regular exercise (treadmill exercise). Where the results of their research demonstrate that lactic acid was increased after the procedure compared to before the procedure in both groups (Group RE; regular exercise and no red dragon fruit; Group RE+RD; regular exercise and obtained red dragon fruit juice). Decreased levels of blood lactate in both groups 24 hours after high-intensity training session were due to the mechanism of the body to remove lactate. Creatine kinase was significantly increased after 24 hours of the high-intensity training session in the placebo juice group ($p<.01$). But in the mulberry juice group, creatine kinase was not significantly different. This study corresponds to Jówko *et al*. (2012) they studied the effect of a single dose of green tea polyphenol on blood marker of oxidative stress and muscle damage in soccer players exposed to intense exercise. They found that plasma creatine kinase activity did not change significantly immediately post-exercise, but it increased after 24 hours of recovery ($p < .001$). In the placebo group, after the 24-hours recovery period creatine kinase activity was higher than pre-exercise and post-exercise values, whereas, in the green tea polyphenol group, creatine kinase activity was higher than the pre-exercise value only. This study showed that mulberry juice had a positive trend to inhibited creatine kinase formation after 24 hours of the high-intensity training session.

From the compared between the group showed that blood lactate at the start of the intervention, after the high-intensity training session, and 24 hours after the high-intensity training session was not significantly different when compared between the placebo juice group and mulberry juice group. This study corresponds to Harahap *et al*. (2021) they found that was no substantial deviation in the mean increase in blood lactate levels between the two groups after the intervention (p>0.05). Creatine kinase at the start of the intervention, after the high-intensity training session, and 24 hours after the high-intensity training session was not significantly different when compared between the placebo juice group and mulberry juice group. But 24 hours after the high-intensity training session creatine kinase in the placebo juice group was increased more than when compared to the mulberry juice group. In the study by Jówko, *et al*. (2012) creatine kinase was significant increased 24 hours after the muscle-endurance test in the green tea polyphenols group and placebo group indicates that the exercise-induced oxidative stress disrupted the integrity of muscle cell membranes, but unlike our study creatine kinase was increased 24 hours after the high-intensity training session in the placebo juice group. By this point, there would be chanced in future research.

Recommendations

The results of this study show the effectiveness of mulberry juice to prevent muscle damage. Should be encouraged soccer player application mulberry juice before player or training to protect fatigue and muscle damage. And should be encouraged another sport application mulberry juice before player or training to protect fatigue and muscle damage.

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