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The leucocytes, neutrophils and superoxydes dismutase (SOD) level after consuming guava juice (*Psidium guajava* L) during aerobic exercise for beginners

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ABSTRACT

Physical exercise is important in preventing and adjunctive therapy certain diseases. However, over physical exercise for beginners may cause oxidative stress and muscle injury leads to generate chemoattractants whic enables to attract neutrophil and monocyte towards the injury and stimulates leucocytes activation. The use of antioxidant-rich fruits to mitigate exercise induced oxidative stress has been applied. This study was conducted to evaluate the effect of guava juice (*Psidium guajava* L) consumption during aerobic exercise on leucocytes, neutrophils and superoxydes dismutase (SOD) level of beginners. Sixteen students of National Land College (*Sekolah Tinggi Pertanahan Nasional*/STPN), Yogyakarta who met the inclusion and exclusion criteria were involved in this study. Subjects were divided into two groups i.e. a group given mineral water as control and another group given 240 mL guava juice 0.67 g/mL daily for 27 days as treatment group. Both groups then underwent aerobic exercise for 30 minutes every day for 27 days. Blood samples were taken before and after exercise on day 1, 7 and 14 for leucocyte and nutrophil count as well as SOD analysis. The leucocyte count and SOD level before and after exercise in both Guava juice and Mineral water groups were not significantly different ($p>0.05$). However, the percentage of neutrophil on day 1 and 7 were significantly higher than that in Mineral water group ($p<0.05$). Moreover, during the exercise, the percentage of neutrophils in Guava juice group significantly increased ($p<0.05$), whereas in Mineral water group was not significantly different ($p>0.05$). It can be concluded that consuming guava juice during exercise for the beginners does not influence leucocyte count and SOD levels. However, it can increase percentage of neutrophil.

ABSTRAK

Latihan fisik penting dalam mencegah dan terapi pendamping untuk penyakit tertentu. Namun demikian, latihan fisik berlebihan bagi pemula kemungkinan bisa menyebabkan stres oksidatif dan luka otot yang dapat menghasilkan *chemoattractants* untuk menarik neutrofil, dan monosit ke daerah luka dan juga mengaktivasi leukosit. Penggunaan buah yang kaya antioksidan untuk mengurangi stres oksidatif akibat latihan fisik telah banyak dilakukan. Penelitian ini dilakukan untuk mengkaji pengaruh pemberian jus jambu merah pada latihan aerobik pemula terhadap leukosit, neutrofil dan kadar superoxydes dismutase (SOD) darah. Enam belas mahasiswa dari Sekolah Tinggi Pertanahan (STPN), Yogyakarta yang memenuhi kriteria inklusi dan eksklusi dilibatkan dalam penelitian. Subjek dibagi menjadi dua kelompok yaitu kelompok kontrol yang diberi air mineral dan kelompok perlakuan yang diberi jus jambu merah 0,67 g/mL sebanyak 240 mL setiap hari selama 27 hari. Kedua kelompok selanjutnya melakukan aerobik selama 30 menit setiap dua hari sekali selama 27 hari. Sampel darah diambil sebelum dan setelah aerobik pada hari ke 1, 7 dan 14

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untuk pemeriksaan leukosit, neutrofil dan kadar SOD darah. Jumlah leukosit dan kadar SOD darah sebelum dan setelah aerobik pada kelompok yang diberi jus jambu merah dan air mineral tidak berbeda nyata ($p > 0.05$). Namun demikian, persentase neutrofil pada hari ke 1 dan 7 aerobik lebih tinggi nyata dibandingkan dengan air mineral ($p < 0.05$). Selin itu, selam aerobik persentase neutrofil pada kelompok yang diberi jus jambu merah naik secara nyata ($p < 0.05$), sedangkan pada kelompok yang diberi air mineral tidak berbeda nyata ($p > 0.05$). Dapat disimpulkan bahwa konsumsi jus buah merah selama latihan fisik bagi pemula tidak mempengaruhi leukosit dan kadar SOD secara nyata, tetapi dapat meningkatkan persentase neutrofil.

Keywords: aerobic exercise - guava juice - leucocytes - neutrophils - SOD level

INTRODUCTION

Numerous studies have revealed a relationship between regular exercise and improvements in physical and mental healths. Physical exercise is important in preventing and adjunctive therapy certain diseases. The beneficial effects of exercise are associated with metabolic improvement and neutralization of some risk factors connected with diseases.[1,2] However, it should be considered that over physical exercise may cause muscle damage called delayed onset muscle soreness (DOMS) due to mechanical trauma followed by oxidative stress.[3]

The DOMS is often experienced by the beginners. Over contraction and unusual use of muscle on the beginners during physical exercise need additional unit motor recruitment. Bone muscle contraction is a mechanical, chemical, and electrical process consisting of six stages called cross bridge cycle. For the beginners or untrained people, power stroke, sliding filament, and disconnecting in cross bridge cycle are mechanical trauma which may cause immune dysfunction, inflammation, oxidative stress, muscle soreness and muscle injury.[4-6]

The muscle injury can generate chemo-attractants which enables to attract neutrophil and monocyte towards the injury site followed by reactive oxygen species (ROS) production.[6,7] Moreover, oxidative stress due to over exercise causes the increase of body temperature and stimulates leucocytes production and activation. In physiological conditions, the ROS production will be neutralized by endogenous antioxidant such as superoxydes dismutase (SOD), glutathione peroxides (GPx), and catalase. Oxidative stress will occur when the endogenous antioxidant is not enough to neutralize ROS production.[8,9]

Recently, there is growing interest in the use of antioxidant-rich fruits or vegetables to mitigate exercise induced oxidative stress. Several fruits or vegetables have been studied for their benefits as dietary supplements and sport nutrition foods in countering oxidative stress during physical exercise.[10] *Psidium guava* L. is a fruit tree commonly known as guava or *jambu biji* in Indonesai, which belongs to the family Myrtaceae. Guava fruit is rich antioxidant such as vitamin C, lycopene and flavonoids. Guava fruit is also a good source of pectin, sugar and minerals.[11-13] Guava fruit has been reported to have antidiarrheal, antibacterial and antioxidant activities.[13,14] Commercially, the fruit is consumed raw or used in making jams, jellies, pastes and juice.[11]

In this study, we reported the effect of guava juice consumption during aerobic exercise on leucocytes, neutrophils and superoxydes dismutase (SOD) level of beginners.

MATERIALS AND METHODS

Subjects

This was a quasi experimental study with nonrandomized pre-post test control group design involving students from National Land College (*Sekolah Tinggi Pertanahan Nasional*/STPN),

Yogyakarta who met inclusion and exclusion criteria. The inclusion criteria were students aged 17-25 years who stay in dormitory, normal nutritional status, normal healthy status based on clinical and hemoglobin examinations, non athletes, no suffered from chronic diseases such as asthma, hipertension, cardiovascular diseases, renal disease, and gartritis, no smoking, and never followed the aerobic exercise program based on frequency, intensity, time, and type (FITT) guidelines minimally in the last 6 years. The exclusive criteria include consuming antioxidant and or vitamin and not willing to be research subject. The study has been approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta.

Procedure of study

On the day that has been agreed, the students were gathered to be selected. An explanation about objectives, benefits and risk of the study was given. Students were then given opportunities to ask eveything related to the study. Sixteen students who fulfilled the inclusion and exclusion criteria were recruited in this study and given an informed consent to be signed. All subjects underwent physical and clinical as well as laboratory examinations. Characteristics of subjects including body weight (BW), body height (BH), sistolic and diastolic blood pressure (SBP and DBP), hear rate (HR), respiratory rate, body temperature, haemoglobin level, leucocyte and neutrophils counts were recorded. Subjects were divided into two groups with 8 students in each group. Group I as control was given mineral water and Group II as treatment group given was given 240 mL guava juice 0.67 g/mL daily for 27 days. The guava juice was given dailay at 05.00 AM. Subjects then underwent aerobic exercise every two days for 27 days consisting 30 minute for exercise, five minutes for warming up and five minutes for recovery. Body weight, blood pressure, HR, temperature of subjects before and after aerobic exercise were monitored and recorded. Food intake of subjects were calculated based on 24-hour food recall for 27 days using nutrisurvey program. Blood samples of subjects after exercise on day 1, 13 and 27 were collected for leucocyte and neutrophil count as well as SOD examination. Leucocyte and neutrophil counts were measured using Hematology Analyzer Sysmex XT-2000i, whereas blood SOD level was measured using cayman kit.

Statistical analysis

Data were presented as mean ± standard deviation (SD). The different of leucocyte and neutrophil counts as well as blood SOD level inter group were analyzed using independent t-test. The different of measurement of each group was analyzed using repeated Anova. A p value $p<0.05$ was considered as significant.

RESULTS

The characteristics of subjects of Guava juice and Mineral water groups before exercise are presented in TABLE 1. No significant difference was observed between Guava juice group and Mineral water group before exercise indicating that both of groups were similar.

TABLE 1. Characteristics of subjects of Guava juice and Mineral water groups

Variable	Guava juice (mean ± SD)	Mineral water (mean ± SD)
Body weight (kg)	58.50 ± 2.75	59.50 ± 3.72
Body height (cm)	171.75 ± 4.17	167.00 ± 4.22
Body Mass Index	19.84 ± 0.86	21.38 ± 1.80
Heart rate (pulse/minute)	82.00 ± 6.05	80.00 ± 6.84
Repiratory rate (x/minute)	19.00 ± 1.51	19.50 ± 2.07
Body temperature (^{o}C)	36.11 ± 0.49	35.99 ± 0.59
Hemoglobin (g/dL)	15.86 ± 0.67	15.16 ± 0.53
Hematocrit (%)	47.45 ± 2.25	45.84 ± 1.84
Leucocyte (μL^{-1})	8217 ± 1862	7478 ± 1716
Neutrophil (%)	54.12 ± 6.13	48.12 ± 7.51

The mean of BW, HR and body temperature of subjects before and after exercise in Guava juice and Mineral water groups are presented in TABLE 2. Change in BW was observed before and after exercise in both groups ($p<0.05$). Heart rate of both groups before exercise was significantly different ($p<0.05$), however it was not significantly differetn after warming up, exercise and recovery ($p>0.05$). The body temperature between Guava juice and Water mineral before exercise was significantly different ($p<0.05$), however after exercise it was not significantly different ($p>0.05$).

TABLE 2. Mean of BW, HR and body temperature of subjects before and after exercise in Guava juice and Mineral water groups

Variable	Guava juice	Mineral water	p
BW before exercise (kg)	58.07 ± 0.21	57.86 ± 0.20	0.000
BW after exercise (kg)	57.86 ± 0.20	59.10 ± 0.35	0.000
HR before exercise (x/minute)	86.14 ± 3 .58	82.53 ± 2 .89	0.007
HR after warming up (x/minute)	125.57 ± 12 .68	123.87 ± 13. 45	0.733
HR after exercise (x/minute)	157.67 ± 0.7 1	158. 01 ± 0. 76	0.236
HR after recovery (x/minute)	121.01 ± 4 .22	117. 92 ± 5 .73	0.116
Temperature before exercise (^{o}C)	35.78 ± 0. 15	35.58 ± 0. 17	0.003
Temperature after exercise (^{o}C)	35.95 ± 0 .21	35.87 ± 0 .27	0.348

The nutrient intake of subjects in Guava juice and Mineral water groups during study are presented in TABLE 3. A significantly higher intake in energy, fat, protein, vitamin C, vitamin E, cuprum, zinc and mangan was observed in Guava juice group compare to Mineral water group ($p<0.05$). However, the carbohydrate intake was not significantly different between two groups ($p>0.05$).

TABLE 3. Nutrient intake of subjects in Guava juice and Mineral water groups

Nutrient intake	Guava juice	Mineral water	p
Energy (kkal)	2807.83 ± 235.85	2405.67 ± 263.81	0.006
Fat (g/day)	99.40 ± 6.71	85.07 ± 11.95	0.033
Protein (g/day)	95.36 ± 5 .98	84.68 ± 11 .26	0.010
Carbohydrate (g/day)	349.50 ± 18 .90	318.99 ± 38. 49	0.064
Vitamin C (mg/day)	225.98 ± 23.70	45.75 ± 13.17	0.000
Vitamine E (mg/day)	7.52 ± 0 .95	5.57 ± 1 .00	0.001
Iron (mg/day)	24.17 ± 10.29	14.97 ± 3 .47	0.031
Cuprum (mg/day)	2.62 ± 0. 36	2.02 ± 0 .29	0.003
Zinc (mg/day)	10.74 ± 1 .01	9.43 ± 1 .07	0.025
Mangan (mg/day)	31.26 + 7.52	24.09 + 5 .42	0.046

The leucocytes count before and after exercise in Guava juice and Mineral water groups are presented in TABLE 4. No significantly difference in the leucocytes count before and after exercise in both groups was observed in this study ($p>0.05$).

TABLE 4. Leucocytes count (μL^{-1}) before and after exercise in Guava juice and Mineral water groups

Exercise	Guava juice	Mineral water	p*	p**	p***
Before exercise	7770 ± 1545	6652 ± 1154	0.123	0.678	0.092
After exercise 1	7687 ± 1939	6686 ± 1035	0.219		
After exercise 7	8076 ± 1440	7564 ± 1153	0.445		
After exercise 14	7340 ± 1735	7108 ± 1734	0.794		

Note : * t-test ; ** Anova on Guava juice group ; *** Anova on Mineral water group

The percentage of neutrophils before and after exercise in Guava juice and Mineral water groups are presented in TABLE 5. No significantly difference in the percentage of neutrophils before exercise and after exercise on day 14 between two groups was observed ($p>0.05$). However, after exercise on day 1 and 7 the percentage of neutrophils in Guava juice group were significantly higher than that in Mineral water group ($p<0.05$). During the exercise, the percentage of neutrophils in Guava juice group significantly increased ($p<0.05$), whereas in Mineral water group was not significantly different ($p>0.05$).

TABLE 5. Percentage of neutrophil (%) before and after exercise in Guava juice and Mineral water groups

Exercise	Guava juice	Mineral water	p*	p**	p***
Before exercise	50.62±5.12	45.75±4.03	0.053		
After exercise 1	62.25±10.09	50.62±8.24	0.024	0.004	0.160
After exercise 7	52.75±5.15	43.87±6.40	0.009		
After exercise 14	51.25±4.30	48.37±8.60	0.412		

Note: * t-test; ** Anova on Guava juice group ; *** Anova on Mineral water group

The blood SOD level before and after exercise in Guava juice and Mineral water groups are presented in TABLE 6. Although the blood SOD level tended to be higher after exercise, however no significantly difference in the blood SOD level before and after exercise in both groups was observed in this study ($p>0.05$).

TABLE 6. Level of blood SOD (U/mL) before and after exercise in Guava juice and Mineral water groups

Exercise	Guava juice	Mineral water	p*	p**	p***
Before exercise	2.31 ± 0.94	2.27 ± 0.98	0.924		
After exercise 7	2.34 ± 1.88	2.49 ± 1.02	0.837	0.247	0.190
After exercise 14	3.06 ± 0.66	3.24 ± 1.46	0.759		

Note: * t-test; ** Anova on Guava juice group ; *** Anova on Mineral water group

DISCUSSION

Regular physical exercise has many health benefits including a reduced risk of certains diseses such as cardiovascular disease, cancer, and diabetes.[14,15] Otherwise, it is also widely accepted that contracting skeletal muscles generate free radicals and that prolonged and intense exercise can result in oxidative stress an muscle injury.[1,6,7,16] Furthermore, the oxidative stress has substantial effect on leucocytes life span. The amounts of several hormones, cytokines and other factors which might influence cellular survival are increased or decreased in organs, tissues and peripheral blood during exercise.[17,18]

This study showed that in Mineral water group the amount of leucocytes and percentage of neutrophil tended to increase after exercise on day 1 and 7, although it was not significantly different. It was indicated that actually aerobic exercise for beginner induce oxidative stress that lead to stimulate leucocytes production due to additional unit motor recruitment and muscle injury. However, ROS production due to the oxidative stress might be neutralized by endogenous antioxidant SOD that found also tended to increase in this study. Although it was also not significantly different. Result of this study is in accordance with study conducted by Shojaei *et al*.[9] that reported that leucocyte increased immediately after 45

minutes cycling with intensity 50% of VO_2 max for untreated human.

Furthermore, in this study also fouund that the amount of leucocytes and percentage of neutrophil tended to decrease after exercise on day 14, although it was not significantly different. These decrease might be caused by leucocytes apoptosis after the prolongation of aerobic exercise. Exercise-induced leucocyte apoptosis has been reported previously. Syu *et al*.[19] demonstrated that acute severe exercise induced an oxidative stress which resulted in acceleration of spontaneous neutrophil apoptosis. Moreover, repeated moderate exercise (30 min a day, 5 days a week at 60% of maximal workload) delayed neutrophil apoptosis.[20] A recent stdy reported that a significant delay of neutrophil apoptosis after marathon run, intensive endurance and downhill running as well as intensive resistance exercise was observed.[21]

Similar results were obtained on Guava juice group for leucocytes which tended increase after exercise on day 1 and 7 and decrease on day 14, whereas SOD level tended to increase on day 7 and 14. Although, these were not significantly different. However, the percentage of neutrophil significantly increased during aerobic exercise. It was indicated that guava juice can protect exercise-induced nutrophil apoptosis.

The effect of guava fruit to protect exercise-induced neutrophil apoptosis might be caused its antioxidant activity. Guava fruit is rich antioxidant such as vitamin C, vitamin E, lycopene and flavonoids. Guava fruit is also a good source of minerals such as copper, iron, zinc, magnesium and selenium.[11-13] Moreover, the antioxidant activity of guava fruit has been proven by some authors.[13,22,23] Antioxidant supplements may benefit exercise performance directly through the reduction of muscle fatigue at the level of contractile function and indirectly through reduction of physiological stressors that negatively impact on training or improvement in the ability to recover from training.[6,24]

CONCLUSION

It can be concluded that consuming guava juice during exercise for the beginners does not signifincantly influence leucocyte count and SOD levels. However, the consuming guava juice can significantly increase percentage of neutrophil.

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