PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2019.824.833

Research Article
The Effect of an Ethanol Extract of Purple sweet potato (*Ipomoea batatas* L.) on Exercise-Induced Oxidative Stress in Mice (*Mus musculus*)

[1,4]A.F. Khairani, [1,4]T. Nurhayati, [1,4]P.H.A. Rahman, [2]Nurhasanah, [2]R. Khaerunnisa, [2]S.M.F.R. Jabbar, [1,4]W. Mayasari and [3,4]K. Wahyudi

[1]Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia
[2]Undergraduate Program, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia
[3]Department of Public Health, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia
[4]Research Center of Health System, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia

Abstract

Background and Objective: Strenuous exercise triggers the formation of reactive oxygen species (ROS) that exceed the body's antioxidant defense system. This causes oxidative stress and lipid peroxidation, as indicated by an increase in malondialdehyde (MDA) levels, changes in leukocyte count and function and structural alterations of the skeletal muscle. This study aimed to determine the effect of purple sweet potato (*Ipomoea batatas* L.), which contains the antioxidant anthocyanin, on the prevention of oxidative stress after strenuous exercise. **Materials and Methods:** In this experimental study, 44 male Balb/c mice divided into 3 groups were orally administered different doses of an *Ipomoea batatas* L. ethanol extract (100, 200 and 400 mg kg^{-1}) before swimming as strenuous exercise for 14 days. By the end of the treatment, we histologically tested the gastrocnemius muscle and took blood serum from the mice. Data were analyzed using the Kruskal-Wallis test. **Results:** The *Ipomoea batatas* L. ethanol extract significantly ($p = 0.032$) prevented changes in the leukocyte count and prevented an increase in the MDA level, muscle damage, centralized nuclei in muscle fibers, hypertrophy and hyperplasia to a less significant extent. **Conclusion:** Supplementation with *Ipomoea batatas* L. ethanol extract as an antioxidant after 14 days of strenuous exercise has the significant potential to suppress oxidative stress by preventing decreases in leucocyte counts. Further studies with larger sample sizes and improved procedures are recommended to obtain a complete understanding of the potential antioxidant effect of *Ipomoea batatas* L. on muscle phenotypes after strenuous exercise.

Key words: Strenuous exercise, oxidative stress, leukocyte, malondialdehyde (MDA), purple sweet potato (*Ipomoea batatas* L.), anthocyanin, muscle regeneration

Received: December 23, 2018 Accepted: April 07, 2019 Published: August 15, 2019

Citation: A.F. Khairani, T. Nurhayati, P.H.A. Rahman, Nurhasanah, R. Khaerunnisa, S.M.F.R. Jabbar, W. Mayasari and K. Wahyudi, 2019. The effect of an ethanol extract of purple sweet potato (*Ipomoea batatas* L.) on exercise-induced oxidative stress in mice (*Mus musculus*). Pak. J. Nutr., 18: 824-833.

Corresponding Author: A.F. Khairani, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Jalan Raya Jatinangor KM 21.6, Sumedang, West Java, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Exercise is a type of physical activity consisting of planned, structured and repetitive body movements to improve and maintain physical fitness[1]. The health benefit of exercise is directly proportional to the intensity of the exercise[1,2]. Many people do not know the appropriate duration and frequency of exercise for them, which results in muscle fatigue or aches. Strenuous exercise is physical activity carried out more than 25 min per day[1]. Excessive exercise can cause bruising, muscle injuries, the formation of free radicals, immune system disruption and heart and respiratory dysfunction[3]. Increased oxygen consumption to produce energy during strenuous exercise produces excessive free radicals that can surpass the capacity of the body to clear antioxidants and leads to an increase in reactive oxygen species (ROS), oxidative stress, the apoptosis of leukocytes, muscle membrane damage and the inflammatory response[4-7].

Free radicals can damage the cells of the human body through peroxidation of the lipid components of cell membranes and the cytosol, resulting in a reduction in fatty acids, which can damage cell membranes and organelles. Lipid peroxides subsequently decompose to malondialdehyde (MDA)[8,9]. Therefore, an increase level of MDA can be used as a marker of oxidative stress[10].

Strenuous exercise also triggers apoptosis via two main pathways: (1) A receptor-mediated external pathway through death receptors and (2) An internal pathway through oxidative-mediated apoptosis in mitochondria. Apoptosis is a systemic inflammatory response modulated by ROS, glucocorticoids and cytokines after physical exercise[7]. If the level of free radicals in the body is high and exceeds the limit of the cellular antioxidant system, additional antioxidants from outside of the body may be needed to neutralize the free radicals[4]. The body's ability to produce antioxidants is limited and naturally decreases with age. Purple sweet potato (*Ipomoea batatas* L.) has active antioxidant components, such as anthocyanins, flavonoids, phenolics, carotenoids and polyphenol compounds, that presumably can reduce free radicals[6-8,11-16]. The Sumedang area in West Java Province is a purple sweet potato-producing area in Indonesia. The content of purple sweet potato gives it the potential benefit to be used as a processed product to prevent muscle injury.

Until now, there has been no research discussing the effect of purple sweet potato on muscles after strenuous exercise. The purpose of this study was to identify the effect of an ethanol extract of purple sweet potato on the MDA level; the leukocyte profile; and tissue inflammatory responses, regeneration and hypertrophic responses in muscle after strenuous exercise.

MATERIALS AND METHODS

Ethanol Extract of purple sweet potato (*Ipomoea batatas* L.): Purple sweet potato (*Ipomoea batatas* L.) was obtained from farmers in Tanjungsari, Sumedang for this study. Extraction was performed by soaking the purple sweet potato in 90% liquid ethanol in the Central Laboratory of Universitas Padjadjaran such that 0.68 g of anthocyanin was contained in 100 g of ethanol extract by the differential pH test. The extract solution was administered in three doses: 100, 200 and 300 mg kg^{-1} mouse. To produce the required doses of the extract, it was dissolved in 1 mL of bidistilled water per mouse per day.

Subjects: This study was an experimental laboratory study of 44 male Balb/c mice (*Mus musculus*) 4-6 weeks old with body weights of 25-30 g that were obtained from PT Bio Farma. The study was conducted in the animal laboratory, Faculty of Medicine, Universitas Padjadjaran from July to August 2018. Adaptation was carried out in a cage with a room temperature of 26-34°C and sufficient humidity and lighting. The size of the cage used in this study was 20×32×14 cm. Food in the form of standard food pellets and clean water were provided. The mice were given a seven-day adaptation period prior to the experiment. The research protocol had been reviewed and approved by the Research Ethics Commission of Universitas Padjadjaran Bandung No. 556/UN6.KEP/EC/2018.

Treatment: The experimental animals were divided into a negative control group (the mice underwent exercise without the administration of the *Ipomoea batatas* L. extract), a dose 1 group (the mice underwent exercise with the administration of 100 mg kg^{-1} of the *Ipomoea batatas* L. extract per day), a dose 2 group (the mice underwent exercise with the administration of 200 mg kg^{-1} of the *Ipomoea batatas* L. extract per day), a dose 3 group (the mice underwent exercise with the administration of 400 mg kg^{-1} of the *Ipomoea batatas* L. extract per day) and an untreated group. The experimental animals were randomly distributed into the groups.

After seven days of cage adaptation, swimming adaptation was carried out for six days with increasing swimming time, i.e., 10 min on days 1-2, 20 min on days 3-4 and 30 min on days 5-6. The extract was orally administered to the treatment groups according to the allocated dose with a probe one day before exercise. The exercise was 30 min of swimming per day for 14 days. The size of the swimming pool was 15×15×40 cm and the water temperature was 32°C [17].

Biochemical analysis: At the end of the treatment, 37 mice were anesthetized with diethyl ether. Blood samples were taken into an ethylenediaminetetraacetic acid (EDTA) tube and sent immediately to the Advanced Biomedical Laboratory, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia. The MDA levels in blood samples of 18 mouse were examined with the Thiobarbituric Acid Reactive Substance (TBARS) method and the leukocyte counts in blood samples of 19 mouse were examined according to the protocol.

Analysis of mouse muscle cross-sections: A total of 7 mice were prepared for muscle dissection. The mice were perfused with 4% formaldehyde to fix the muscles. Afterward, the gastrocnemius muscle was dissected from both legs of each mouse and then the muscle tissue was soaked in a 4% formaldehyde solution. The obtained gastrocnemius muscles were blocked in paraffin, cut transversally with a thickness of 5 µm and then stained with hematoxylin and eosin (H and E) in the Cell Biology Laboratory, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung.

The muscle samples were observed under a light microscope using a camera (Optilab Advance Plus) and images were taken using Optilab Viewer Plus 3 software. The histological images of the muscles were processed using ImageJ software version 1.51j8. A total of 1-3 sampling sections were selected from one slide of mouse muscle samples in each group. In each section, images from three cross-sectional areas were taken randomly. The images of the cross-sectional area were taken using 10× objective magnification. Then, from each cross-sectional area, images of 4-5 cross sections were taken using 40× objective magnification.

The fibers were observed in images of the muscle cross-sections using 10×objective magnification and were then counted manually with ImageJ software. The same steps were carried out to measure the diameter of the fibers (Feret's diameter) in images of the cross-sectional area. Using images of the muscle fibers with 40× objective magnification, the Feret's diameters were manually measured with ImageJ software.

The inflammatory area and central nuclei were observed based on the methods of Mancio *et al.*[10]. The area with inflammatory cell infiltration (inflammation area) was measured using ImageJ software. This area is presented as a percentage of the cross-sectional area. The inflamed cells were characterized by staining of the core of the basophils and a small amount of cytoplasm. Meanwhile, the central nuclei were characterized by a round nucleus located in the middle and not in the peripheral muscle cells. Afterward, the percentage of the central nuclear fibers compared to the total fibers was calculated in one cross-sectional area of the muscle tissue sample.

Statistical analysis: The MDA level and leukocyte count are presented as the median difference between each group with the untreated group as a baseline (pretreatment). Therefore, the difference (posttreatment minus pretreatment) was used to indicate the effect of the treatment.

All data describing the inflammatory area, central nuclei, total fiber and the Feret's diameter are presented in the form of median±IQR (interquartile range). The Kruskal Wallis-test was carried out with a significance value of $p<0.05$. The analysis was carried out using IBM SPSS® computer software version 20 for Windows.

RESULTS

MDA level: As shown in Fig. 1, the *Ipomoea batatas* L. ethanol extract had a less significant effect on preventing an increase in MDA. The median MDA value in each group is shown in Table 1. Statistical analysis was used to determine the effect of the *Ipomoea batatas* L. ethanol extract on MDA levels. Analysis was performed by subtracting the average MDA value in the untreated group (2.08 µM) as a baseline value from the MDA level in each group.

Figure 1 shows that the baseline value before treatment was 0 µM (the median MDA level in the untreated group). This means that the negative control, dose 1, dose 2 and dose 3 groups exhibited increased MDA levels after

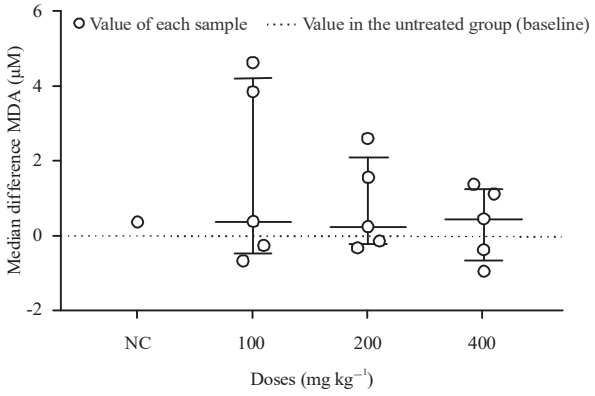

Fig. 1: The effect of an *Ipomoea batatas L.* ethanol extract on the MDA level after strenuous exercise
All values indicate the median difference in MDA values (MDA result-baseline)

Table 1: Median MDA value-baseline after strenuous exercise

Group	MDA value (μM)	MDA-baseline (2.08)	Median (min-max)
negative control	2.46	0.38	0.38
dose 1	1.83	-0.25	0.38 (-0.66 - 4.65)
	5.95	3.87	
	6.73	4.65	
	1.42	-0.66	
	2.46	0.38	
dose 2	3.66	1.58	0.25 (-0.3 - 2.62)
	4.70	2.62	
	1.78	-0.30	
	2.33	0.25	
	1.94	-0.14	
dose 3	2.54	0.46	0.46 (-0.92 - 1.39)
	3.47	1.39	
	1.70	-0.38	
	1.16	-0.92	
	3.21	1.13	

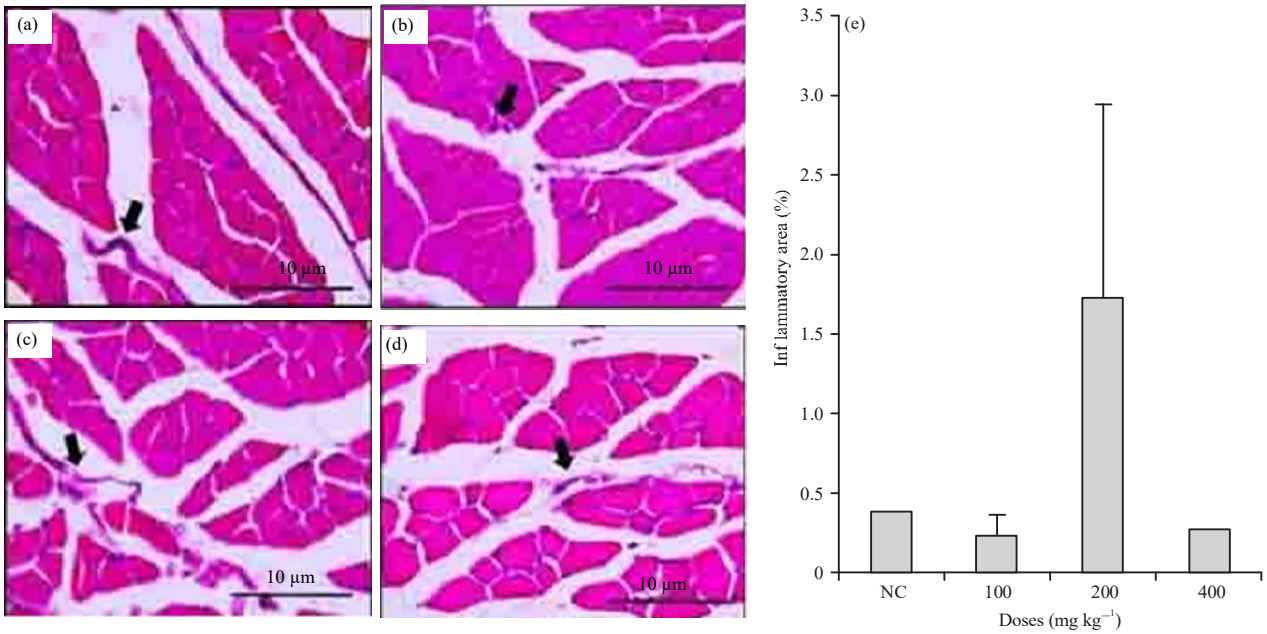

Fig. 2(a-e): Inflammatory area on the gastrocnemius muscles of the mice

The cross-sectional area of the gastrocnemius muscle tissue shows the area with infiltration of an inflamed cell (inflammatory area) pointed by the black arrows in the (a) control group, (b) dose 1 group, (c) dose 2 group, (d) dose 3 group and (e) graph shows the percentage of the inflammatory areas in the control group (KN), dose 1 (D1) dose 2 (D2), and dose 3 (D3). The value of the inflammatory area is presented in median±interquartile range (IQR)

exercise. The dose 1 group that was given 100 mg kg^{-1} of the *Ipomoea batatas* L. ethanol extract per day had the same median MDA level as the negative control group, which was 0.38 μM. Treatment of the dose 2 group with 200 mg kg^{-1} of the *Ipomoea batatas* L. ethanol extract per day suppressed the increase in MDA level and had a median difference of 0.25 μM. Meanwhile, the treatment given to the dose 3 group had a greater effect than that given to the negative control group, with a median difference of 0.46 μM.

Inflammatory area: The inflammation that occurred due to treatment can be identified by calculating the area in the space between adjacent fibers with infiltration of inflammatory cells[10]. The effect of the extract on reducing inflammation is shown in Fig. 2. The groups that were given the ethanol extract of purple sweet potato had smaller inflammatory areas than the negative control group, except for the dose 2 group. The dose 2 group had the largest area of inflammation of 1.75% of the total muscle area, whereas the

Table 2: Median difference in the leukocyte count – baseline after strenuous exercise

Group	Leukocyte count (-10^3 μL^{-1})	Leukocyte-baseline (4.33)	Median (min-max)
negative control	2.6	-1.73	-1.31 (-1.73 - -0.89)
	3.44	-0.89	
dose 1	5.88	1.55	0.75 (-1.53 - 2.67)
	5.08	0.75	
	4.54	0.21	
	2.8	-1.53	
	7	2.67	
dose 2	10.04	5.71	0.61 (-0.71 - 5.71)
	5.66	1.33	
	4.94	0.61	
	3.93	-0.4	
	3.62	-0.71	
dose 3	8.58	4.25	2.79 (1.97 - 4.25)
	8.22	3.89	
	7.12	2.79	
	7.05	2.72	
	6.3	1.97	

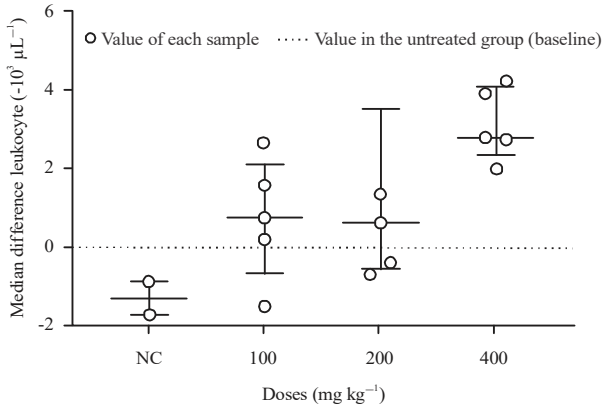

Fig. 3: The effect of an *Ipomoea batatas* L. ethanol extract on leukocyte count after strenuous exercise
All values indicate the median difference in the leukocyte counts (leukocyte count after treatment-baseline)

negative control group had an inflammatory area 0.98% of the total muscle area. This increase in inflammation in the dose 2 group was possibly due to an inflammatory condition caused by a disease in one of the animals in the group. Furthermore, the decrease in the percentage of the inflammatory area between groups was not statistically significant ($p = 0.3$).

Leukocyte count: To identify the effect of the *Ipomoea batatas* L. ethanol extract, leukocytes in each group were counted and the results from each group are shown in Table 2, with the average leukocyte count in the untreated group (4.33-10^3 μL^{-1}) as a baseline. As shown in Fig. 2, the baseline value was 0 μL^{-1} (the median value in the untreated group). Meanwhile, the median leukocyte counts in the negative control group and the dose 1, dose 2 and dose 3 groups after heavy physical exercise were -1.31-10^3 μL^{-1}, 0.75-10^3 μL^{-1}, 0.61-10^3 μL^{-1} and 2.79-10^3 μL^{-1}, respectively, which were all higher than leukocyte count in the negative control group. These results indicate that, after exercise, there was no decrease in the leukocyte count due to apoptosis in the groups given the *Ipomoea batatas* L. ethanol extract. Statistical analysis showed that the differences in leukocyte counts were significant ($p = 0.032$).

Feret's diameter: The cross-sectional area of the skeletal muscle (hypertrophy) in the mice (Feret's diameter) is shown in Fig. 4(a-d). As shown in Fig. 4(e), the Feret's diameter in the negative control group was 250 (10^{-3} mm). Meanwhile, the Feret's diameter in the dose 1, 2 and 3 group, which were 292, 307 and 324.40 (10^{-3} mm), respectively, were larger than that in the negative control group. Statistical analysis showed no significant difference between the Feret's diameters ($p = 0.243$).

Total fiber: The total fiber cross-sectional area (hyperplasia) in the skeletal muscle of the mice is shown in Fig. 5 (a-d). As shown in Fig. 5 (e), in the negative control group, there were 676 fibers in the field of view at 10× objective magnification. Meanwhile, the number of total fibers in the dose 1, 2 and 3 groups, which were 457, 637 and 670, respectively, were lower than that in the negative control group. Statistical analysis showed that the difference in the number of total fibers was not significant ($p = 0.543$).

Central nuclei: The regeneration of striated muscle cells was assessed by examining the percentage of fibers with a central cell nucleus[10]. As shown in Fig. 6, fibers with central nuclei were found in most of the histological features of every group.

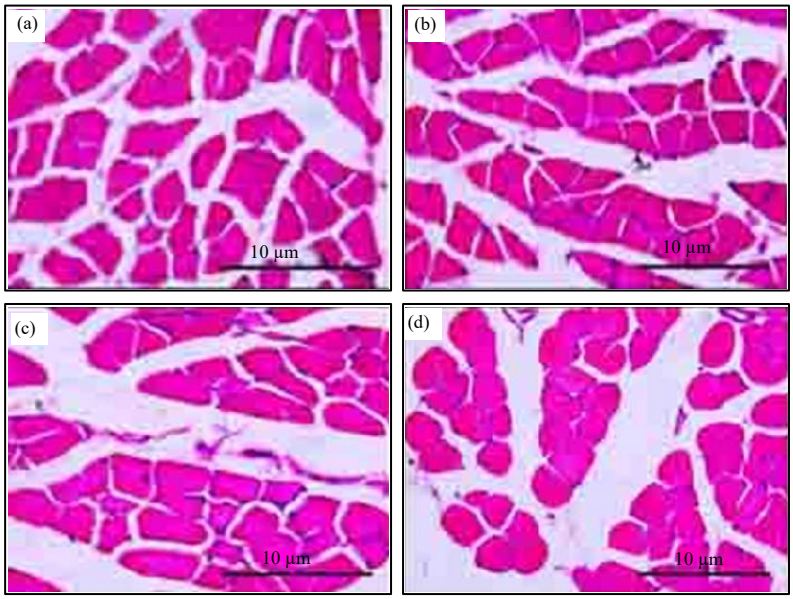

Fig. 4(a-d): Cross-sectional area of the skeletal muscle hypertrophy of the mice (Feret's Diameter)
The cross-sectional area of the gastrocnemius muscle tissue with muscle hypertrophy of the (a) negative control group, (b) dose 1 group, (c) dose 2 group and (d) dose 3 group

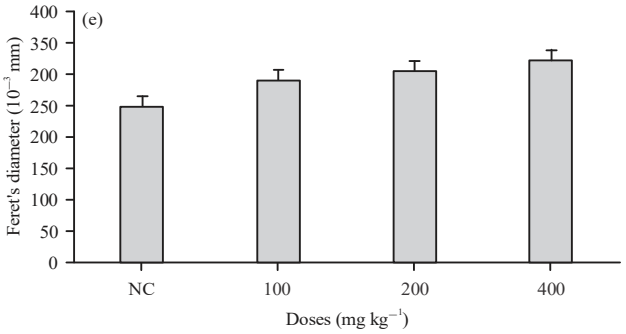

Fig. 4(e): Graph of skeletal muscle hypertrophy of the mice
The value of the skeletal muscle hypertrophy of the mice was calculated as Feret's Diameter and presented in the form of median±IQR

The negative control group had the highest percentage of fibers with a central nucleus of approximately 13%. The other groups treated with the *Ipomoea batatas* L. ethanol extract exhibited fewer nucleated fibers than the negative control group. The lowest percentage was found in the dose 2 group, which was 4.1%. Based on the results shown in Fig. 2, the *Ipomoea batatas* L. ethanol extract reduced the percentage of fibers with central nuclei in each treatment group but this difference was not statistically significant ($p = 0.1$).

DISCUSSION

Strenuous exercise can increase MDA levels[8]. During exercise, free radical formation occurs along with oxidative phosphorylation to form ATP in the mitochondria. Oxygen is needed for this process and reacts with hydrogen to form water. During this process, some oxygen molecules can turn into free radicals. The heavier the exercise is, the more oxygen is needed and the more free radicals that are produced as byproducts[4,8].

Free radicals damage cellular components, such as lipids, lipoproteins, proteins, carbohydrates, RNA and DNA, causing damage to cell-forming macromolecules[10]. In the body, free radicals undergo chain reactions in which one electron is taken from another compound to stabilize the initial molecule while the second become unstable and turns into a new free radical[8]. Antioxidants reduce the negative effects of oxidants by giving electrons to oxidant compounds. The activities of these oxidant compounds can be inhibited directly in the body and indirectly by the formation of new free radicals[18].

Free radicals bind to the lipid membrane through lipid peroxidation to form hydrogen peroxide. Hydrogen peroxide causes the decomposition of aldehyde products with different chain lengths that are toxic to cells. One of the aldehyde compounds produced is MDA. The damage caused by free

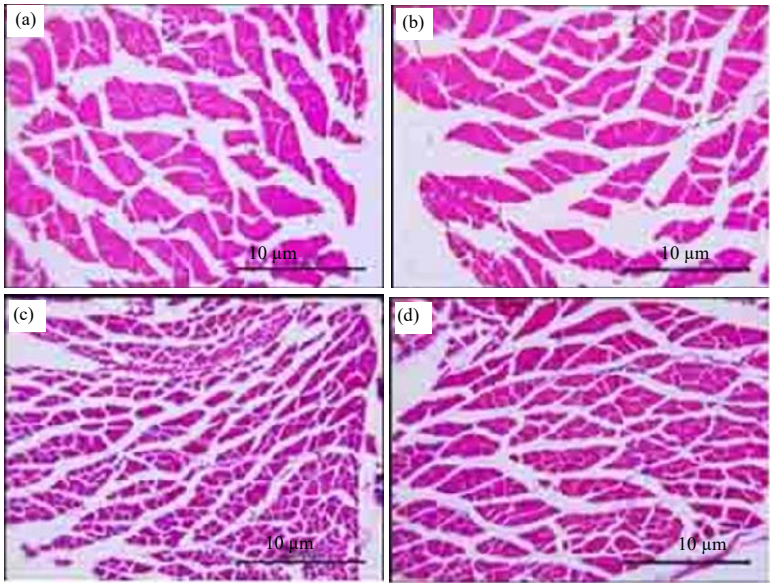

Fig. 5(a-d): Total fiber cross-sectional area (hyperplasia) of the skeletal muscle of the mice
Cross-sectional area of the gastrocnemius muscle tissue with muscle hyperplasia in the negative (a) control group, (b) dose 1 group, (c) dose 2 group and (d) dose 3 group

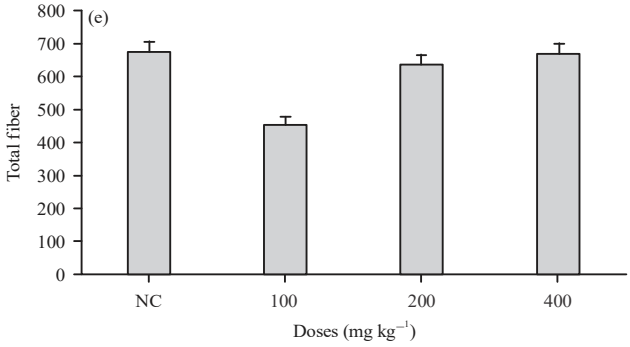

Fig. 5(e): Graph of skeletal muscle hyperplasia of the mice
The total fiber (hyperplasia) value of the skeletal muscle of the mice is presented in the form of median±IQR

radicals to cell membranes causes an inflammatory response. Inflamed cells migrate to the location of the damage to phagocytose the damaged cell.

This study found an increase in MDA levels after exercise in all treatment groups compared to the MDA level in the untreated group. The *Ipomoea batatas* L. extract had no effect ($p = 0.934$) on of the increase in MDA after heavy physical exercise. Figure 1 illustrates the median results in the dose 1 group, which were the same results as those in the control group and indicated that this dose of the extract had no effect. The dose 2 group was able to reduce MDA levels, while the dose 3 group was unable to suppress the increase in MDA. This may be due to a decrease in the enzymatic antioxidant response to physical activity that occurs during and immediately after physical activity but may increase again during recovery. In addition, this response also depends on oxygen consumption and oxidant resistance[9].

The decrease in the MDA level in the dose 2 group indicates a reduced amount of free radicals. This was caused by anthocyanin in the *Ipomoea batatas* L. ethanol extract, which functions as an antioxidant that can inhibit oxidation reactions through radical scavenging, thus decreasing the number of free radicals. Aside from being a scavenger, anthocyanin functions as an antioxidant that inhibits the propagation of oxidation (also called a chain-breaking antioxidant), i.e., breaking the chain of oxidation by donating one hydrogen atom to a peroxyl radical so that the propagation stage is broken and further radical formation is prevented[19]. This is supported by the percentage of inflammatory areas shown in Fig. 2. The percentage of inflammatory areas tended to be lower in the treated groups than in the control group. Therefore, it can be assumed that the antioxidants contained in the ethanol extract of purple sweet potato inhibited the activities of the free radicals produced after strenuous exercise.

In contrast, treatment given to the dose 1 and 3 groups was not able to suppress the increase in MDA. One of the influential factors in this result was the physical endurance of the experimental animals. When the animals carry out heavier

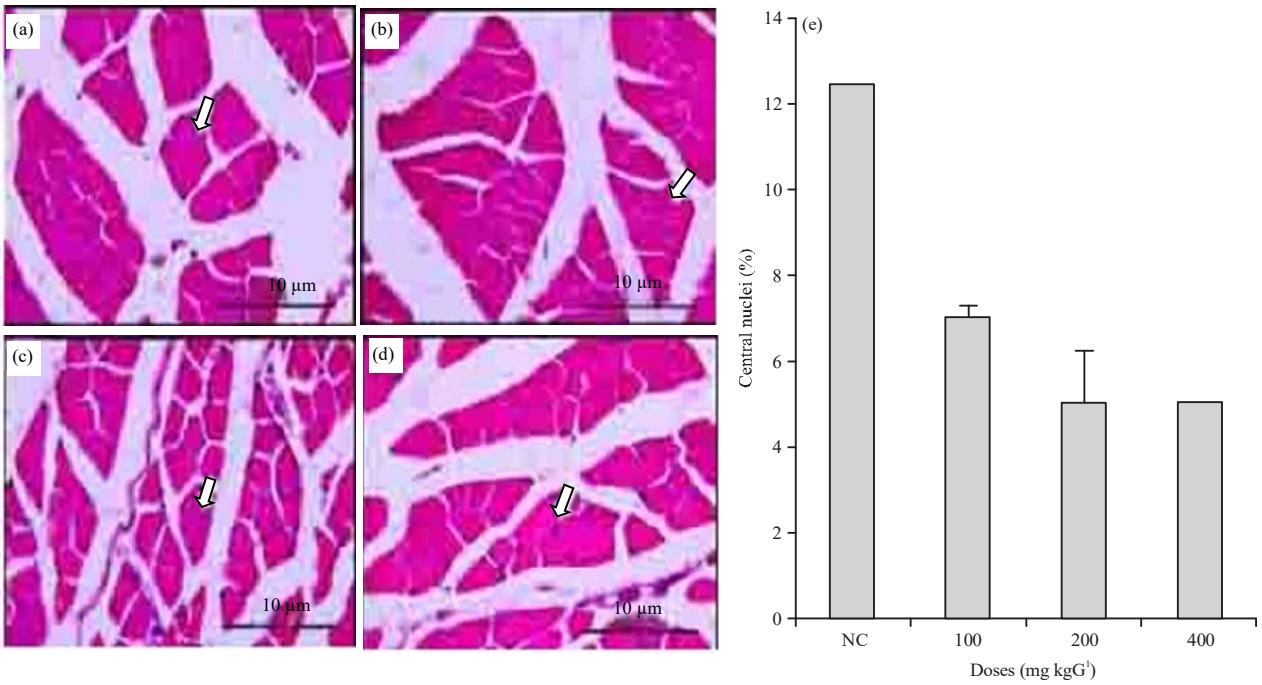

Fig. 6(a-e): Fiber with a central nuclei in the gastrocnemius muscle of the mice

The cross-sectional area of the gastrocnemius muscle tissue shows the muscle cell with central nuclei (inflammatory area) pointed by the white arrows in the (a) control group, (b) dose 1 group, (c) dose 2 group, (d) dose 3 group and (e) graph shows the percentage of the fiber percentage with central nuclei in the control group (KN), dose 1 (D1), dose 2 (D2) and dose 3 (D3). The value of the fiber with central nuclei is presented in median±interquartile range (IQR)

physical activity, there is an increased need for oxygen. Increased oxygen consumption increases the production of ROS in mitochondria. Heavy physical activity also affects the number and function of leukocytes in the blood. In the immune response, the number of leukocytes increases during heavy physical exercise and then decreases after exercise. Leukocyte levels decrease due to their distribution in several tissues and organs or apoptosis. Apoptosis can occur depending on the intensity and duration of the physical activity[7,20].

Figure 3 shows that all treated groups had more leukocytes than the control group due to leukocytosis as an immune reaction. Based on these results, it can be assumed that the *Ipomoea batatas* L. ethanol extract suppressed leukocyte apoptosis. ROS have a role in apoptosis, reducing the level of cellular Bcl-2 and depolarizing the outer layer of the mitochondrial membrane. In addition, ROS affect the intrinsic and extrinsic factors of apoptosis by increasing the expression of Fas[7].

In this study, all doses of the *Ipomoea batatas* L. ethanol extract were able to reduce the effect of ROS on oxidative stress and inhibit apoptosis, as indicated by the higher leukocyte count compared to that of the negative control group. This increased leukocyte count is thought to contribute to the synthesis of cytokines in the skeletal muscle in the form of myokine, which can significantly contribute to the hypertrophic response[21-23]. The myokines that have been identified in the literature are interleukin (IL)-6, IL-7, IL-8, IL-10, IL-13, IL-15, fibroblast growth factor (FGF), leukemia inhibitory factor (LIH) and tumor necrosis factor (TNF)[24]. Strenuous exercise can decrease the leukocyte count through leukocyte apoptosis. In this study, the antioxidants suppressed ROS formed by exercise. The administration of antioxidants from the *Ipomoea batatas* L. ethanol extract suppressed ROS in the body and suppressed leukocyte apoptosis, increasing the leukocyte count. This process is followed by the synthesis of a high level of cytokines and the promotion or acceleration of the hypertrophic response of skeletal muscles. In Fig. 4(a-d), the microscopic Feret's diameter in the negative control group and dose 3 group is a hypertrophic muscle marker.

Muscle cells have the ability to regenerate after being damaged[25]. Inflamed cells produce growth factors and cytokines that activate satellite cells. These satellite cells then migrate to the damaged muscle cells and proliferate into

myoblasts. In this phase, the myoblast, which then fuses into a myotube, has a central cell nucleus like cells in general. During myotube maturation, the cell nucleus approaches the peripheral area of the muscle cell[26].

The regeneration of muscle cells after physical exercise can be observed from the total fiber count and the percentage of fibers with a central nucleus in the muscle tissue. In this study, both the total fiber count and the percentage of fibers with central nuclei in the treated groups were lower than those in the control group. This shows the contribution of antioxidant compounds in the *Ipomoea batatas* L. ethanol extract to the inhibition of free radical activity by radical scavenging, thereby suppressing damage to the muscle membrane. Presumably, the fewer damaged muscle cells there are, the less regeneration occurs.

CONCLUSION

Supplementation with an *Ipomoea batatas* L. ethanol extract as an antioxidant after a strenuous exercise to suppress oxidative stress significantly prevented the decrease in leukocyte count due to apoptosis. In addition, it suppressed the increase in MDA level as a marker of oxidative stress, the number of muscle fibers, central nuclei, hyperplasia and hypertrophy of the mouse skeletal muscle after exercise to a less significant extent.

SIGNIFICANCE STATEMENT

This study discovered novel information about the potential effect of *Ipomoea batatas* L. ethanol extract as an antioxidant to prevent oxidative stress and enhance muscle cell regeneration. Information from this study will help researchers in related fields and provides prospective data regarding natural compounds (anthocyanins) that could compete with oxidative stress and muscle cell regeneration due to strenuous exercise. Thus, further study with a larger number of samples and better procedures is needed to obtain better-quality data on the potential antioxidant effect of anthocyanin as an active compound from *Ipomoea batatas* L. on muscle cell regeneration after strenuous exercise.

ACKNOWLEDGMENTS

This research was supported by the Internal Research Grant Universitas Padjadjaran Riset Fundamental Unpad (RFU) 2018. Many thanks go to Neni Anggraeni for technical assistance.

REFERENCES

1. MacAuley, D., 2013. Oxford Handbook of Sport and Exercise Medicine. 2nd Edn., Oxford University Press, UK., ISBN-13: 978-0-19-966015-5, Pages: 755.
2. Garber, C.E., B. Blissmer, M.R. Deschenes, B.A. Franklin and M.J. Lamonte *et al*., 2011. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal and neuromotor fitness in apparently healthy adults: Guidance for prescribing exercise. Med. Sci. Sports Exerc., 43: 1334-1359.
3. Setiawan, A., 2011. [Factors in the emergence of sports injuries]. J. Media Ilmu Keolahragaan Indones., 1: 94-98.
4. Radak, Z., Z. Zhao, E. Koltai, H. Ohno and M. Atalay, 2013. Oxygen consumption and usage during physical exercise: The balance between oxidative stress and ROS-dependent adaptive signaling. Antioxid. Redox Signal., 18: 1208-1246.
5. Radak, Z., K. Suzuki, M. Higuchi, L. Balogh, I. Boldogh and E. Koltai, 2016. Physical exercise, reactive oxygen species and neuroprotection. Free Radic. Biol. Med., 98: 187-196.
6. Powers, S.K., Z. Radak and L.L. Ji, 2016. Exercise-induced oxidative stress: Past, present and future. J. Physiol., 594: 5081-5092.
7. Kruger, K. and F.C. Mooren, 2015. Exercise-induced leukocyte apoptosis. Exerc. Immunol. Rev., 20: 117-134.
8. Ayala, A., M.F. Munoz and S. Arguelles, 2014. Lipid peroxidation: Production, metabolism and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longevity, Vol. 2014. 10.1155/2014/360438
9. Yavari, A., M. Javadi, P. Mirmiran and Z. Bahadoran, 2015. Exercise-induced oxidative stress and dietary antioxidants. Asian J. Sports Med., Vol. 6. 10.5812/asjsm.24898
10. He, F., J. Li, Z. Liu, C.C. Chuang, W. Yang and L. Zuo, 2016. Redox mechanism of reactive oxygen species in exercise. Front Physiol., Vol. 7. 10.3389/fphys.2016.00486
11. Islam, S., 2006. Sweetpotato (*Ipomoea batatas* L.) Leaf: Its potential effect on human health and nutrition. J. Food Sci., 71: R13-R21.
12. El Husna, N., M. Novita and S. Rohaya, 2013. [Anthocyanins content and antioxidant activity of fresh purple fleshed sweet potato and selected products]. Agritech, 33: 296-302, (In Indonesian).
13. Rumbaoa, R.G.O., D.F. Cornago and I.M. Geronimo, 2009. Phenolic content and antioxidant capacity of Philippine sweet potato (*Ipomoea batatas*) varieties. Food Chem., 113: 1133-1138.
14. Ghasemzadeh, A., V. Omidvar and H.Z.E. Jaafar, 2012. Polyphenolic content and their antioxidant activity in leaf extract of sweet potato (*Ipomoea batatas*). J. Med. Plants Res., 6: 2971-2976.

15. Kang, H., Y.G. Kwak and S. Koppula, 2014. Protective effect of purple sweet potato (*Ipomoea batatas* Linn, Convolvulaceae) on neuroinflammatory responses in lipopolysaccharide-stimulated microglial cells. Trop. J. Pharm. Res., 13: 1257-1263.
16. Teow, C.C., V.D. Truong, R.F. McFeeters, R.L. Thompson, K.V. Pecota and G.C. Yencho, 2007. Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. Food Chem., 103: 829-838.
17. Jones, J.H., 2007. Resource book for the design of animal exercise protocols. Am. J. Vet. Res., 68: 583-583.
18. Naz, S., S.A.R. Naqvi, Z.A. Khan, A. Mansha, M. Ahmad, A.F. Zahoor and Z. Hussain, 2017. Antioxidant, antimicrobial and antiproliferative activities of peel and pulp extracts of red and white varieties of *Ipomoea batatas* (L) Lam. Trop. J. Pharm. Res., 16: 2221-2229.
19. Zhao, J.G., Q.Q. Yan, L.Z. Lu and Y.Q. Zhang, 2013. *In vivo* antioxidant, hypoglycemic and anti-tumor activities of anthocyanin extracts from purple sweet potato. Nutr. Res. Pract., 7: 359-365.
20. Nimmo, M.A., M. Leggate, J.L. Viana and J.A. King, 2013. The effect of physical activity on mediators of inflammation. J. Diabetes Obes. Metab., 15: 51-60.
21. Quinn, L.S., 2008. Interleukin 15: A muscle-derived cytokine regulating fat-to-lean body composition. J. Anim. Sci., 86: E75-E83.
22. Serrano, A.L., B. Baeza-Raja, E. Perdiguero, M. Jardi and P. Munoz-Canoves, 2008. Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. Cell Metab., 7: 33-44.
23. Nielsen, A.R. and B.K. Pedersen, 2007. The biological roles of exercise-induced cytokines: IL-6, IL-8 and IL-15. Applied Physiol. Nutr. Metab., 32: 833-839.
24. Schoenfeld, B.J., 2012. Does exercise-induced muscle damage play a role in skeletal muscle hypertrophy? J. Strength Cond. Res., 26: 1441-1453.
25. Tedesco, F.S., A. Dellavalle, J. Diaz-Manera, G. Messina and G. Cossu, 2010. Repairing skeletal muscle: Regenerative potential of skeletal muscle stem cells. J. Clin. Invest., 120: 11-19.
26. Zanou, N. and P. Gailly, 2013. Skeletal muscle hypertrophy and regeneration: Interplay between the myogenic regulatory factors (MRFs) and insulin-like growth factors (IGFs) pathways. Cell. Mol. Life Sci., 70: 4117-4130.