

ORIGINAL ARTICLE



The Effect of Strawberry (*Fragaria Vesca*) Ethanol Extract to Reduce Plasma MDA Level and Slow the Physical Exhaustion Time of Intermittent Anaerobic Swimming

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Submitted January 16, 2024; Accepted in final form March 22, 2024.

ABSTRACT

Background. Physical exercise can lead to side effects due to the generation of free radicals, specifically during high-intensity exercise such as intermittent anaerobic swimming. **Objectives.** This study investigated the effect of strawberry ethanol extract (SEE) on reducing plasma MDA levels and prolonging the exhaustion time of male Wistar rats induced by intermittent anaerobic swimming. **Methods.** A post-test-only experimental design was adopted, involving 40 male Westar rats divided into 4 groups and induced by intermittent anaerobic swimming for 4 days. Group I and II were given SEE of 250mg/kgBW and 500mg/kgBW per day, respectively. Meanwhile, Group III was given vitamin E of 37.5IU/kgBW daily, and Group IV received a placebo. Plasma MDA level and swimming exhaustion time were measured on the fourth day. **Results.** The result showed that the MDA level of Groups I, II, III, and IV was 0.112 nmol/g (SD 0.098), 0.069 nmol/g (SD 0.038), 0.163 nmol/g (SD 0.101), and 0.434 nmol/g (SD 0.264), respectively. Among these groups, I and II were significantly lower than IV ($p=0.000$) but insignificantly lower than III ($p>0.05$). The exhaustion time of Group I to IV was 30.22 minutes (SD 3.41), 40.9 minutes (SD 6.33), 26.0 minutes (SD 3.6), and 22.5 minutes (SD 1.5), respectively. Groups I and II had significantly slower exhaustion times when compared to Group IV ($p=0.000$). **Conclusion.** The study showed that SEE reduced plasma MDA level and prolonged exhaustion time of male Westar rats induced by intermittent anaerobic swimming, comparable to the effect of vitamin E.

KEYWORDS: *Strawberry Ethanol Extract, Plasma MDA Level, Exhaustion Time, Intermittent Anaerobic Swimming.*

INTRODUCTION

The health benefits of exercise are extensive, including preventing and treating diseases such as diabetes, metabolic syndrome, obesity, and cardiovascular diseases, and also as adjunct therapy in cancer (1-8). Nevertheless, it can also have detrimental side effects by generating free radicals, atoms, or molecules with unpaired electrons (9, 10). These electrons react with other

atoms or molecules, making them unstable and reactive. Free radicals can interact with organic molecules within the body's cells, causing structural and physiological damage, a condition known as oxidative stress, forming the basis of pathogenesis for various diseases. This affects the entire organ system, including cardiovascular, liver, and skin diseases, autoimmune disorders,

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and aging (11–15). Oxidative stress in athletes can accelerate muscle exhaustion by damaging functional proteins within muscle cells, such as calcium channels, leading to a decline in performance (16–18). Furthermore, it can be measured through various methods, such as determining the malondialdehyde (MDA) level resulting from lipid peroxidation by free radicals using the TBARS method (19).

To prevent oxidative stress caused by free radical activity, the body possesses a defense mechanism known as the antioxidant system, which consists of both enzymatic and non-enzymatic components. Enzymatic antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase, play a crucial role in inhibiting the formation of specific free radicals. Meanwhile, non-enzymatic antioxidants comprise reducing agents, such as vitamins E and C, which scavenge unpaired electrons. These antioxidants can be obtained from within the body (endogenous) or external sources (exogenous). When their activities are sufficient to counteract the effect of free radicals, an oxidative-antioxidant balance is achieved, thereby preventing oxidative stress (20–23).

The potential for exercise to induce oxidative stress depends on its intensity and the trained status of the athletes. Properly structured and continuous exercise with mild-moderate intensity can lead to physiological adaptations that increase the presence of antioxidant enzymes such as glutathione peroxidase to counteract the formation of free radicals (24–27). The intensity of the activity was measured by monitoring heart rate and maximal oxygen uptake, subjectively marked by exhaustion level. Higher intensity produces more excellent free radicals, specifically in untrained individuals (16, 17, 26, 28).

The majority of the community remains unaware of proper exercise training patterns. Well-programmed and continuous exercise routines are typically limited to specific groups, such as athletes or fitness club members. For instance, in recreational swimming, many people swim from one end of the pool to another, followed by a brief rest. However, a previous study showed that intermittent anaerobic swimming increases MDA levels (28). High-intensity exercise is often performed among athletes who understand proper training patterns, particularly before and during competitions. Monitoring exercise intensity can be challenging, and feelings of exhaustion are sometimes

disregarded. Based on these factors, the general community and athletes potentially experienced oxidative stress due to increased free radicals during exercise (20).

To avoid oxidative stress from the increased production of free radicals, providing exogenous antioxidant supplements to every individual engaged in exercise activities is necessary. One of the commonly used supplements is vitamin E, known for its ability to scavenge the free radicals present in the body. Furthermore, it prevents oxidative stress by forming complexes with free radicals and donating one of its electrons, thereby terminating the chain reaction of oxidation (22, 23, 29). Another alternative exogenous antioxidant supplement is vegetables and fruits rich in phenolic compounds, which serve as scavengers. Additionally, they may contain other elements, such as vitamin C and minerals, that can enhance the antioxidant capacity (30).

Strawberries (*Fragaria vesca*) are widely loved fruits cultivated in many regions worldwide, including tropical and subtropical areas. They have a red color, a sweet and tangy taste, and are commonly consumed as fresh beverages. Strawberries hold great potential for use as antioxidant supplements. This is because they are rich in various nutritional components and exceptionally high levels of vitamin C. The fruit contains other vitamins and is abundant in polyphenols such as anthocyanins, which act as antioxidants when added to flavanols and ellagitannins. By conducting antioxidant capacity tests using TEAC, ORAC, and FRAP methods, strawberries have been proven to possess more excellent abilities than oranges, tomatoes, broccoli, and other well-known antioxidant-rich fruits and vegetables. The in-vivo tests showed that the fruit can increase antioxidant levels in the body (31–36). Therefore, this study aimed to examine the benefits of strawberries in preventing oxidative stress induced by exercise. This was particulate in the case of untrained individuals engaging in high-intensity intermittent exercise, which theoretically triggers the production of free radicals to a greater extent. We hypothesize that strawberries can provide benefits in exercise-induced oxidative stress, and their efficacy is comparable to the widely used vitamin E supplementation.

MATERIALS AND METHODS

Subjects. The study subjects were 40 male Wistar rats obtained from the Rumah Sakit Hasan

Sadikin Pharmacology Laboratory in Bandung. The rats were given 7-day to acclimatize to the laboratory environment, while an additional two days were provided to adapt to the swimming environment.

The following were the inclusion criteria for the subjects:

- Male Wistar rats
- Aged between 2 to 3 months.
- Weight between 150 to 200 grams

The following were the exclusion criteria:

- Rats showing signs of illness during the adaptation period
- Rats experiencing weight loss of more than 10% during the adaptation period

Materials. The materials used in the study consist of Strawberries (*Fragaria vesca*, Benggala variant) obtained from the local plantation in West Bandung at 08:00 am, Vitamin E tablets (Darya Varia) obtained from Merdeka pharmacy, and materials needed to carry out the Thiobarbituric acid reactive substance (TBARS) assay using high-performance liquid chromatography.

Tools. The tools used in the study consist of tools for Strawberry ethanol extract (SEE) preparation, tools for handling rats, blood collection tools, and tools to carry out Thiobarbituric acid reactive substance (TBARS) assay.

Study Design. This study used a post-test-only randomized experimental design, as shown in Figure 1.

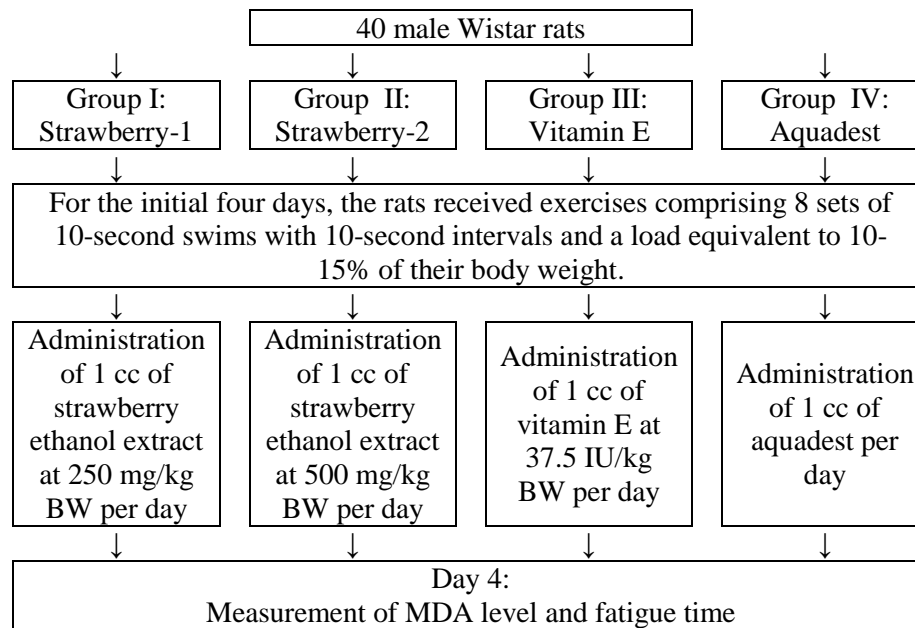


Figure 1. Flowchart of This Study.

Sampling Technique. The "Sample Size Determination" formula by Ralph B. Dell et al. was used in this study:

$$(1) \quad n = 1 + 2C\left(\frac{s}{d}\right)^2$$

n: sample size of each Group

s: estimation of the population's standard deviation for the tested variable

d: difference size in observed results

C: a constant that depends on the desired value of α and β where the amount was calculated using the following formula:

$$(2) \quad C = (Z_{\alpha} + Z_{\beta})^2$$

The observed difference in size (d) was established at 10% of the mean of the tested

variable. This aligned with the agreement in equivalent studies where the slightest clinically significant difference was unknown.

Estimating the population's standard deviation for the tested variable could be obtained from preliminary reports, data from previous studies using similar methods, or most similar literature.

A total of 2 hypothesis tests, namely plasma MDA level and exhaustive swimming test, were conducted, the sample size was calculated, and the largest was selected.

This study's sample size calculation was based on the first hypothesis test, which pertained to the MDA level. The reason for this method was the absence of existing literature with standard

deviation and mean values for the exhaustive swimming test result in Wistar rats receiving SEE.

The standard deviation for MDA level in rats after intermittent anaerobic swimming was 0.18 nmol/ml. Meanwhile, the observed difference size was 10% of the mean MDA value at 0.36 nmol/ml. Based on the above values, the following indicated the required sample size for $\alpha=0.05$ and $\beta=0.05$:

$$\begin{aligned} n &= 1 + 2C\left(\frac{s}{d}\right)^2 \\ (3) \quad n &= 1 + 26\left(\frac{0.18}{0.36}\right)^2 \\ n &= 7.5 \sim 8 \end{aligned}$$

The sample size was 8 for each Group. An additional 20% was included in the calculated sample size to account for potential dropouts. As a result, the minimum total value for the 4 groups was 40.

A convenient sampling technique without specific systematic methods was used for sample selection. The experimental animals were selected based on their ability to meet the inclusion and exclusion criteria until the required sample size was reached.

Preparation of SEE. SEE was obtained from fresh strawberries (*Fragaria vesca* L) of the Benggala variety, acquired from the "All About Strawberry" plantation in West Bandung and handpicked at 08:00 am. One kilogram of the fruit was prepared by removing the leaf sepals, finely ground using a macerator with a cotton pad as a base, and left for 24 hours. Extraction was performed from the outlet beneath the macerator, and the remaining residue was filtered using filter paper, resulting in a solution known as the diluted extract. The new solvent was added to the remaining residue in the macerator, and this process was repeated until the solvent coming out became colorless (usually after 5-6 extractions). Furthermore, the diluted extract was then concentrated using a rotary evaporator until it became thick or no more solvent dripped from the condenser.

Treatment of Rats. The rats were acclimated for 2 days at the Clinical Pharmacology Laboratory of Dr. Hasan Sadikin Hospital in Bandung. Following this, they were allowed to swim freely in a large bucket filled with water to a height of 60 cm and at a temperature of 30°C for 15 minutes to adapt to the swimming environment. Upon the completion of the adaptation phase, the rats were randomly divided into 4 treatment groups:

1. Group I
2. Group II
3. Group III
4. Group IV

All the rats were subjected to heavy exercise, specifically intermittent anaerobic swimming. The activity consisted of 8 sets, each lasting for 10 seconds, and the process was repeated for 4 days. A load of 10% and 13% were attached to the rats' tails on days 1 and 2, as well as 3 and 4, respectively.

The treatments given to the 4 groups were as follows:

1. Group I: SEE 1cc at a dose of 250 mg/kg BW per day
2. Group II: SEE 1cc at a dose of 500 mg/kg BW per day
3. Group III: Vitamin E 1cc at a dose of 37.5 IU/kg BW per day
4. Group IV: Distilled water 1cc per day

MDA Level Examination with the TBARS

Method. Plasma MDA level examination was conducted using the TBARS5 method as follows 5:

Firstly, 3 ml of venous blood was drawn, and the sample was placed into a reaction tube with an anticoagulant. The tube was gently inverted to ensure thorough mixing and left for 30 minutes in a refrigerator. Subsequently, the blood sample was centrifuged at 3000 rpm for 10 minutes. After the centrifugation process, plasma was carefully extracted using a micropipette. The reaction tube was then filled with 700 µl of the analyzed blood plasma, which was mixed with 200 µl, 50 µl, 50 µl, 1500 µl, and 1500 µl of SDS, BHT, EDTA, technical acetic acid, and TBA solutions, respectively. The mixture was heated in a water bath at 100°C for 60 minutes, then removed and cooled in an ice bath.

Furthermore, centrifugation was conducted at 5000 rpm for 10 minutes, and the results were read at an absorbance of 532 nm. The test was performed a minimum of 2 times for each blood sample. The mean of the 2 readings was calculated and converted to µmol/l of MDA level using the following formula:

$$(4) \text{MDA level of sample} = \frac{\text{sample of absorbance}}{\text{standard absorbance} \times \text{standard dilution} \times \text{standard concentration}}$$

Exhaustion Time Test with the Exhaustive Swimming Test. All the rats were subjected to swimming until exhaustion, during which a load of 7% of their body weight was attached to their

tails. The point of exhaustion was indicated when they remained submerged (sank) below the water surface for 10 seconds. Exhaustion time (sank) was recorded for each rat.

Ethics of Using Experimental Animals in the Study. Due to ethical considerations, this study used Wistar rats as experimental animals to replace humans. New substances or tools could not be directly tested on humans initially, and it was essential to ensure their safety through animal testing beforehand. The treatments in this study subjected the experimental animals to various unpleasant experiences, such as inconvenience, discomfort, distress, pain, and, in extreme cases, the possibility of death.

Treatment principles of experimental animals were applied in adherence to the fundamental ethical considerations governing health studies involving animals, commonly referred to as the 3R principles:

1. Replacement: Whenever possible, alternative methods, such as using cells, tissues, in vitro cultures, or invertebrate animals, should be applied to replace experimental animals.

→ In this study, an in vivo model of increasing plasma MDA level induced by intermittent anaerobic swimming was required. Therefore, rats were necessary, and alternative methods were not applicable.

2. Reduction: Efforts were made to minimize the number of experimental animals used by implementing statistical methods, computer programs, biochemical techniques, and alternative models.

→ In this study, the sample size was determined based on the minimum number allowed by statistical literature, namely 8 rats per Group. An additional 10% was included to account for potential dropouts, making 10.

3. Refinement: Steps were taken to minimize or avoid "suffering" from pain or distress.

→ In this study, the rats were adapted to the laboratory environment to reduce stress and ensure their comfort. They were provided with adequate food and water, kept in clean and spacious cages and their living conditions were maintained to appropriate humidity levels. Furthermore, the rats were adapted to a swimming environment using warm and clean water to reduce stress. The load attached to their tails was tied with a comprehensive, half-centimeter rubber band to prevent injuries.

After swimming treatment, the rats were exposed to sunlight to prevent cold. During the blood sampling process, analgesics were used, and the animals were gently wrapped in cloth to keep them calm.

Data Analysis. Data analysis was performed using ANOVA, but when the variance results indicated significant differences, further analysis was conducted by applying Tukey's test with a confidence level of 95% ($p \leq 0.05$). The statistical software SPSS for Windows version 15.0 was employed to perform the analysis.

Study Location and Time. The study was conducted at the Clinical Pharmacology Laboratory of Dr. Hasan Sadikin Hospital in Bandung from June to July 2009.

RESULTS

A total of 40 rats were adapted to the laboratory environment for 7 days, followed by a 2-day acclimation period to the swimming environment. During the study, a rat died and was excluded from data processing. The final number of samples was 39, which still met the minimum criteria of 8 per Group.

Plasma MDA Level Measurement Results. The results of plasma MDA level measurements in rats subjected to a 4-day treatment are presented in [Figure 2](#). Detailed findings can be found in [Appendix 1](#).

Exhaustion Time Measurement Results. Exhaustion time measurement results in rats treated for 4 days are presented in [Figure 3](#).

DISCUSSION

Decreased Plasma MDA Level. After the 4-day treatment, the obtained plasma MDA level data of the 4 groups were statistically analyzed. To determine the appropriate statistical method, the skewness and kurtosis ratios were examined to assess the normality status of the data. The results indicated that the data followed a normal distribution, making it suitable for parametric tests using ANOVA. The parametric test suggested that the results of this study could be considered representative of the same subjects.

ANOVA revealed significant differences among the treatment groups, leading to further investigation with Tukey's test to determine the size of the variations. The results showed that the plasma MDA level of Group I was significantly lower than Group IV by -0.32 nmol/g (95% CI of -0.51 to -0.33) with $p=0.000$. Similarly, Group II

was lower than IV by -0.365 nmol/g (95% CI of -0.54 to -0.18) with $p=0.000$. These outcomes showed a decrease in plasma MDA levels in the

Group of rats receiving SEE compared to those provided with distilled water.

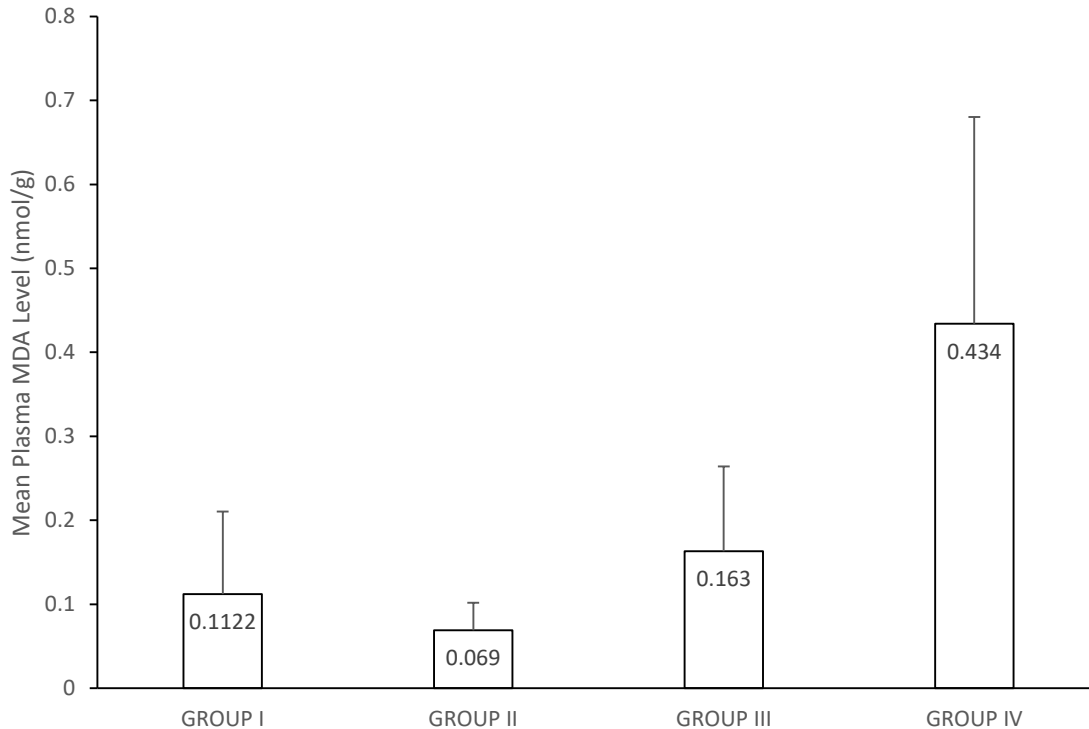


Figure 2. Plasma MDA Level Measurement Result.

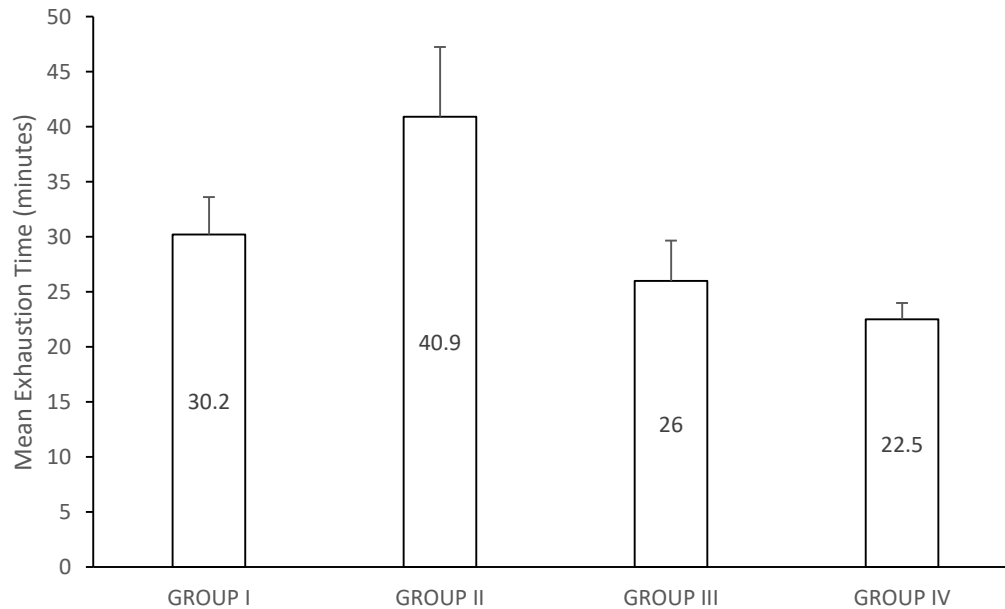


Figure 3. Exhaustion Time Measurement Result.

Plasma MDA levels in rats induced by intermittent anaerobic swimming increased due to

heightened free radical activity. This surge in free radicals was caused by factors such as hypoxia in

tissues, damage to erythrocyte and myocyte membranes, and increased electron leakage during tissue reperfusion. When the number of free radicals exceeds the body's antioxidant defense capacity, it leads to oxidative stress in cell membranes. This, in turn, triggers lipid peroxidation, producing MDA as one of its byproducts.

Strawberry contains high phenolic compounds, particularly anthocyanin pigments and vitamin C. Anthocyanins contribute to the fruit's bright color and exhibit antioxidant properties, while vitamin C also acts as an antioxidant. The administration of SEE increases the body's antioxidant capacity, thereby reducing oxidative stress. This was evident from the significant decrease in plasma MDA levels in the Group receiving SEE.

Plasma MDA level in Group II was insignificantly lower than in Group I, by -0.043 (95% CI of -0.23 to 0.89), with $p=0.925$. This implied that antioxidant activity insignificantly reduced plasma MDA levels at higher strawberry doses. Since only 2 doses were tested, a dose-response graph was not observed to reveal the most effective.

MDA level of Groups I and II, which were -0.0507 nmol/g (95% CI -0.239 to 0.137) at $p=0.886$ and Group II was -0.094 nmol/g (95% CI -0.277 to 0.0893) at $p=0.518$, respectively, was insignificantly lower than Group III. Therefore, SEE and vitamin E showed similar effectiveness in the reduction process.

Slower Exhaustion Time. Based on the results obtained after the 4-day treatment, exhaustion time data of the 4 treatment groups were statistically analyzed. To determine the appropriate statistical method, the skewness and kurtosis ratios were examined to assess the normality status of the data. The results indicated the data followed a normal distribution, making it suitable for parametric testing using ANOVA. The parametric test suggested that the outcomes of this study could be considered representative of the same subjects.

ANOVA showed significant differences among the treatment groups, leading to further investigation with Tukey's test to determine the size of the differences between the groups. The results indicated that the exhaustion time of Group I was significantly longer than IV by 7.72 minutes (95% CI 2.61 to 12.83) with $p=0.001$. Similarly, Group II exhibited a significantly

longer exhaustion time than IV by 18.4 minutes (95% CI 13.42 to 23.37) with $p=0.000$. These outcomes showed a slower time in the Group of rats receiving SEE than in those administered placebo in distilled water.

Exhaustion time in rats induced by intermittent anaerobic swimming was faster due to increased free radical activity. Factors such as hypoxia in tissues, damage to erythrocyte and myocyte membranes, and increased electron leakage during tissue reperfusion contribute to an increase in the formation of free radicals. When free radical activity exceeds the body's antioxidant defense capacity, oxidative stress occurs in muscle cells. This disrupted the sarcoplasmic reticulum calcium channel, resulting in a faster exhaustion time.

Strawberry contains phenolic compounds, particularly anthocyanin pigments and vitamin C. Anthocyanins give this fruit a bright color and exhibit antioxidant abilities, while vitamin C acts as an antioxidant. The body's antioxidant capacity increases by administering SEE, thereby reducing oxidative stress. This was evident from the significantly slower exhaustion time in the Group giving SEE.

The exhaustion time of Group II was significantly longer than Group I by 10.67 minutes (95% CI of 5.56 to 15.78) with $p=0.000$. This implied that a more significant antioxidant activity significantly slowed exhaustion time at higher strawberry doses. Since only 2 doses were tested, a dose-response graph to show the most effective had not been observed.

The exhaustion time of Groups I and II, which were 4.22 minutes (95% CI of -0.887 to 9.33) at $p=0.135$ and 14.90 minutes (95% CI of 9.927 to 19.872), respectively, was slower compared to III, but the difference was not statistically significant for Group II. This indicated that using SEE at 250mg/kgBW was equally effective in slowing exhaustion time as vitamin E, while a dose of 500mg/kgBW was better than vitamin E.

CONCLUSION

Exercise that induces oxidative stress, such as intermittent high-intensity swimming, is commonly conducted by recreational and professional athletes. It can be very detrimental to their health without them knowing the dangers. Meanwhile, the antioxidants to overcome them are available in nature. This study showed that SEE reduced plasma MDA levels and prolonged

exhaustion time, comparable to the effect of vitamin E. The dose of strawberries used in this study, when attached to a portion of a healthy food menu, would be logical and sufficient. As exercise is done regularly, including strawberries in a diet that is done regularly can provide a balance of free radicals-antioxidants and patterns of daily life.

Further research can be continued to determine the minimum and optimal strawberry dose for expected antioxidant results. Measurement techniques and indicators for free radicals in future studies can be adapted to the latest findings and research instruments from other fields.

APPLICABLE REMARKS

- Exercise benefits health but can also lead to exercise-induced oxidative stress, particularly in anaerobic intermittent types.
- Oxidative stress induced by exercise can be mitigated by utilizing antioxidant agents.
- Strawberries are effective antioxidant agents, comparable in efficacy to the well-established vitamin E.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to the staff of the Pharmacology Laboratory at Rumah Sakit Hasan Sadikin Bandung for their assistance during this research.

AUTHORS' CONTRIBUTIONS

Study concept and design: Imas Damayanti, Herri S. Sastramihardja, Trully D. R. Sitorus. Acquisition of data: Imas Damayanti. Analysis and interpretation of data: Imas Damayanti. Drafting the manuscript: Imas Damayanti. Critical revision of the manuscript for important intellectual content: Imas Damayanti, Herri S. Sastramihardja, Trully D. R. Sitorus. Statistical analysis: Imas Damayanti. Administrative, technical, and material support: Imas Damayanti, Agus Rusdiana.

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Study supervision: Herri S. Sastramihardja, Trully D. R. Sitorus.

CONFLICT OF INTEREST

The authors declare that they have no known competing personal or financial interests that could have appeared to influence the work reported in this article.

FINANCIAL DISCLOSURE

The authors declare that they have no known or financial interests related to the material in the manuscript that could have appeared to influence the work reported in this article.

FUNDING/SUPPORT

The authors would like to express their sincere gratitude to the staff of the Pharmacology Laboratory at Rumah Sakit Hasan Sadikin Bandung for their assistance during this research.

ETHICAL CONSIDERATION

The authors declare that all animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences, and the protocol has been approved by Komisi Etik Penelitian Universitas Pendidikan Indonesia (ETHICAL APPROVAL No: 3/UN40.K/PT.01.01/2024).

ROLE OF THE SPONSOR

The Pharmacology Laboratory at Rumah Sakit Hasan Sadikin Bandung provided access to their instruments and assisted with logistical arrangements. However, the laboratory did not offer financial support, and the researchers covered their logistical expenses.

ARTIFICIAL INTELLIGENCE (AI) USE

The authors declare they have not used artificial intelligence (AI) assistance to prepare this manuscript.

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Appendix 1. Data from Measurement of Plasma MDA Level and Exhaustion Time**a) Plasma MDA Level Measurement Results**

Rat Number	Plasma MDA Level (nmol/g)			
	Group 1= SEE 250mg/kgBW	Group 2= SEEd 500mg/kgBW	Group 3= Vit E 37.51U/kgBW	Group 4= Distilled water
1	0.19	0.04	0.11	0.77
2	0.05	0.06	0.28	0.43
3	0.2	0.09	0.08	0.62
4	0.06	0.08	0.3	0.21
5	0.28	0.05	0.27	0.19
6	0.02	0.09	0.06	0.2
7	0.17	0.04	0.08	0.29
8	0.02	0.02	0.06	0.92
9	0.02	0.09	0.26	0.51
10	- dead rat	0.13	0.13	0.2

b) Exhaustion Time Measurement Results

Rat Number	Exhaustion Time (minutes)			
	Group 1= SEE 250mg/kgBW	Group 2= SEE 500mg/kgBW	Group 3= Vit E 37.51U/kgBW	Group 4= Distilled water
1	30	40	25	20
2	32	40	20	23
3	28	45	23	23
4	32	35	30	22
5	26	35	29	24
6	31	30	32	20
7	25	49	25	23
8	32	48	24	24
9	36	40	24	22
10	(dead rat)	47	28	24