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LIMITED IMPACT OF ROSELLE FLOWER EXTRACT ON PROTEIN CARBONYL LEVELS AFTER PHYSICAL EXERCISE IN HEALTHY MEN

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Abstract. Limited impact of roselle flower extract on protein carbonyl levels after physical exercise in healthy men. Ayubi N., Rusdiawan A., Hartoto S., Wibawa J.C., Rizki A.Z., Afandi A., Wardani A.P.S., Halip M.F., Jr P.B.D. *Exercise-induced oxidative stress can lead to muscle damage, with protein carbonyls serving as a key biomarker of oxidative modification in proteins. Roselle flower extract contains polyphenols with potential antioxidant properties that may reduce oxidative damage after physical exercise. This study investigated whether post-exercise supplementation of roselle flower extract reduces protein carbonyl levels. A randomized controlled trial with a parallel group pre-test/post-test design was conducted, comparing a placebo group with a treatment group receiving 500 mg of roselle flower extract. The extract did not result in a statistically significant reduction in protein carbonyl levels ($p>0.05$). However, a non-significant decreasing trend was observed, suggesting that future studies should explore larger sample sizes and longer intervention periods to assess potential cumulative effects. Further studies should explore the effects of higher doses of roselle flower extract (e.g., >500 mg/day) and chronic supplementation over several weeks to determine its long-term impact on markers of oxidative stress.*

Реферат. Обмежений вплив екстракту квітів розелли на рівень карбонілів білка після фізичних вправ у здорових чоловіків. Аюбі Н., Русдіаван А., Хартото С., Вібава Дж.К., Різкі А.З., Афанді А., Вардані А.П.С., Халіп М.Ф., Дж Р. П.Б.Д. *Окиснювальний стрес, спричинений фізичними вправами, може призвести до пошкодження м'язів, при цьому білкові карбоніли служать ключовим біомаркером окисної модифікації білків. Екстракт квітів розелли містить поліфеноли з потенційними антиоксидантними властивостями, які можуть зменшити окисне пошкодження після фізичних вправ. У цьому дослідженні було з'ясовано, чи споживання екстракту квітів розелли після тренування знижує рівень карбонілів білка. Було проведено рандомізоване контрольоване дослідження з паралельною групою до/посттестового періоду, у якому порівнювались група плацебо з групою лікування, яка отримувала 500 мг екстракту квітів розелли. Екстракт не призводив до статистично значущого зниження рівня карбонілу білка ($p>0,05$). Проте спостерігалася незначна тенденція до зниження, що свідчить про те, що в майбутніх дослідженнях слід вивчати більші розміри вибірки та триваліші періоди втручання для оцінки потенційних кумулятивних ефектів. У подальших дослідженнях має бути вивчено вплив високих доз екстракту квітів розелли (наприклад, >500 мг/день) і тривалого прийому добавок протягом кількох тижнів, щоб визначити його довгостроковий вплив на маркери окисного стресу.*

Reactive oxygen species (ROS) are continuously generated as by-products of cellular metabolism, especially in mitochondria. When ROS production exceeds the body's antioxidant defense mechanisms, oxidative stress occurs, leading to cellular damage [1]. Prolonged oxidative stress, if unbalanced by antioxidants, contributes to diseases like diabetes, hypertension, cardiovascular disease, cancer, and neurodegenerative disorders [2]. Exercise-induced ROS production is a double-edged sword. While excessive ROS can cause oxidative damage, moderate increases in ROS act as signaling molecules that enhance antioxidant defense mechanisms, mitochondrial biogenesis, and overall cellular adaptation to stress, contributing to long-term health benefits [3]. Depending on the length, level of intensity, and individual's level of physical fitness, exercise can have both beneficial and detrimental effects [4]. However, if the production of ROS is excessive, this will cause oxidative stress to develop and a change in the redox balance toward oxidation, if allowed to continue, will affect physiological mechanisms and disorders in the immune system [1]. Oxidative stress also adversely affects athlete performance and fatigue, which in turn leads to weakened immune function and increased susceptibility to infection [5].

The main endogenous sources of ROS in skeletal muscle include mitochondria, NADPH oxidase (NOX), and xanthine oxidase (XO) [6]. Xanthine oxidase (XO), present in muscle endothelium and cytosol, contributes to the generation of extracellular superoxide (O_2^-) during metabolic stress and ischemia-reperfusion conditions. While XO-derived ROS may play a role in redox signaling, excessive activity may contribute to oxidative damage rather than muscle force generation [7]. In addition, autooxidation of myoglobin or oxidation of hemoglobin to methemoglobin further contributes to oxidative stress in muscle by inducing the formation of peroxide [8]. It can be concluded that the mechanism of increased oxidative stress occurs during the formation of ATP in the mitochondria and then an increase in ROS during the respiration process which triggers muscle damage if antioxidant levels are not able to neutralize ROS [9].

One of the primary by products of free radicals breaking down unsaturated fatty acids is malondialdehyde (MDA), a biomarker of oxidative stress that is created by a class of free radicals known as hydroxyl radicals that induce lipid peroxidation [10]. Another biomarker of oxidative stress is protein carbonyls, which have the advantage of being relatively early in the formation and stability of protein carbonylation [11]. Protein carbonylation is one of the most widely used biomarkers of protein oxidation. Protein carbonylation occurs when ROS modify amino acid side chains, leading to irreversible structural and functional changes in proteins. Proteins with multiple oxidative pathways frequently experience loss of function and alterations in biological activity due to carbonylation, an irreversible deformation brought on by oxidative stress [12].

Protein carbonyl (PC) is considered a reliable biomarker of oxidative stress associated with cell damage, aging, and various age-related disorders [13]. Increased protein carbonyl levels are regarded as an early indicator of ROS exposure since the process is irreversible [14]. During strenuous physical training, data showed that athletes who contributed to the 50 km and 100 km ultramarathon races experienced a significant increase in protein carbonyls [15]. Extreme exertion, such ultra-marathon running, can increase oxygen use by 10-20 times, which will always produce reactive oxygen species (ROS) [1]. Numerous enzymatic and nonenzymatic antioxidants in biological systems can be elevated by exercise-induced ROS, according to recent studies, despite early studies' unfavorable effects [16]. The body has an antioxidant defense system to fight against the oxidative stress that results. Exercise and diet can have an impact on antioxidants body's system consisting of enzymatic and non-enzymatic [17].

Roselle flowers are among the natural materials that possess the capacity to be used as anti-inflammatory and antioxidant substances. Hibiscus Sabdariffa L., commonly referred to as Roselle, is a potent antioxidant due to its polyphenols (anthocyanins, flavonoids, phenolic acids, tannins), polysaccharides, pectin, nonphenolic organic acids, carotenoids, caffeic acid, chlorogenic acid, ascorbic acid, and quercetin are examples of vitamins, minerals, and bioactive components [18]. Anthocyanin is one of the flavonoid polyphenols with potent antioxidant qualities [19]. Anthocyanins in Roselle exhibit strong antioxidant properties by scavenging superoxide anions and other ROS. In addition, they can enhance the activity of endogenous antioxidant enzymes, which contributes to the reduction of oxidative stress and inhibition of protein glycation [20]. Given that Rosella has proven antihyperlipidemic, antihyperglycemic, antioxidant, anti-inflammatory, antihypertensive, and antifibrosis qualities, it may also be investigated further as a drug to lower the risk factor for cardiovascular disease [21].

Roselle is known to have high antioxidant content. Roselle flower extract is believed to be an antioxidant that can reduce free radical levels. The potential of roselle flower extract to reduce exercise-induced oxidative stress by reducing protein carbonyl levels remains unclear, as few studies have directly examined this relationship. Therefore, this study aimed

to evaluate the effects of post-exercise Roselle flower extract supplementation on protein carbonyl biomarkers as indicators of oxidative stress. Therefore, the purpose of this study was to determine the effect of giving roselle flower extract after physical exercise on the oxidative stress response through protein carbonyl biomarkers.

MATERIALS AND METHODS OF RESEARCH

This experimental study used a before and after control group design. Subjects were selected using purposeful sampling based on inclusion and exclusion criteria. After selection, they were randomly assigned to one of two groups: K1 (placebo) or K2 (roselle flower extract, 500 mg). The 500 mg dose of roselle flower extract was chosen based on its safety profile established in human studies. Previous studies have shown that higher doses may provide stronger antioxidant effects, but for ethical considerations and consistency with existing literature, the 500 mg dose was chosen [18]. Supplementation was given in capsule form. Placebo is formulated using only empty capsules that are not given any contents.

Twenty men in good health took part in the study (Table 1 displays subject characteristics). To determine whether volunteers could meet the needs of this study, inclusion and exclusion criteria were established. Students with a normal Body Mass Index (BMI) between the ages of 20 and 25 years were included in the inclusion criteria. Participants were required to be non-regular exercisers, defined as engaging in structured physical activity less than three times per week for at least six months, as assessed via a pre-screening questionnaire. In addition, this study did not involve participants who were under 20 years of age or who had high blood pressure before engaging in physical activity. Additionally, if subjects were taking nonsteroidal anti-inflammatory drugs (NSAIDs), they were excluded. 20 research volunteers were divided into 2 groups, namely the physical exercise + roselle treatment group (n=10), and the control group with physical exercise + placebo (n=10).

Some of the instruments used in this study were blood pressure gauges, height gauges, weight gauges, data collection sheets, stationery, roselle flower extract, and placebo capsules.

The data collection process in this study involved several steps. The research subjects underwent a screening procedure before starting the study. Certain parameters that allowed the data to be included or excluded from the analysis formed the basis of this approach. In addition, they provided consent, agreeing to participate in the study. This research instrument complies with the principles of the Helsinki Declaration and has passed ethical review. The study subjects were randomly divided into two groups: the treatment group, which received roselle flower extract, and the placebo group. The treatment group consumed 500 mg of roselle flower extract, while the placebo group consumed empty capsules. The roselle flower extract was administered in capsule form.

Data collection was carried out for 1 day starting with collecting data on subject characteristics. The research subjects were prohibited from consuming anything before the implementation of the study. One day before the study, the research subjects were given directions to maintain a regular diet and rest pattern. After that, they were asked to warm up. All subjects performed a 2400-meter run at a self-selected moderate-intensity pace, with heart rate monitoring to ensure they remained within 50-70% of their age-predicted maximum heart rate (Hrmax = 220 – age). After the running test, the sample was directed to a shady room not far from the field to rest and wait for the next intervention. Venous blood samples were collected via antecubital venipuncture before and 60 minutes after supplementation. Blood was immediately centrifuged at 3000 rpm for 10 minutes at 4°C, and plasma was stored at -80°C until analysis to prevent oxidative degradation. Blood samples were collected 60 minutes post-intervention, based on previous studies showing that peak absorption of polyphenolic compounds from roselle flower extract occurs within this time frame [22, 23]. However, direct pharmacokinetic measurements were not performed in this study. The analysis used to determine protein carbonyl levels with using the Protein Carbonyl ELISA Kit was carried out at the Faculty of Medicine Laboratory, Brawijaya University Malang, Indonesia. Finally, the researchers analyzed the data and made a written report as a form of accountability (Fig. 1).

Statistical analysis was performed using SPSS 16.0 after data collection. Descriptive statistics was used to summarize the data, including means and standard errors for normally distributed variables and medians for non-normally distributed variables. Normality was assessed using the Shapiro-Wilk test. If data followed a normal distribution ($p>0.05$), a paired t-test was used to compare pre- and post-test values within groups. If the normality assumption was violated ($p<0.05$), the Wilcoxon signed-rank test was applied instead. Statistical significance was defined as $p<0.05$ for all analyses.

This study was conducted in accordance with the principles of the Declaration of Helsinki and has been approved by the Ethics Committee of the Malang Health Polytechnic (Approval Number DP.04.03/F.XXI.31/0486/2024). Written informed consent was obtained from all participants prior to enrollment.

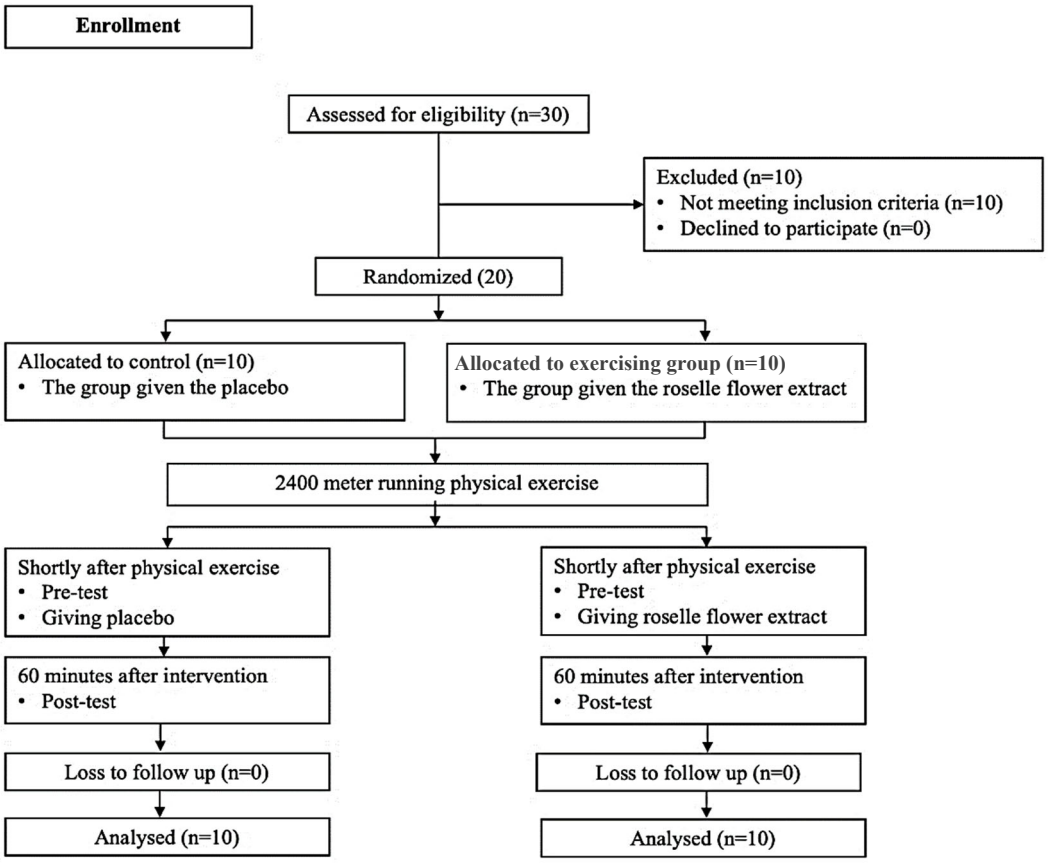

Fig. 1. The CONSORT flowchart

RESULTS AND DISCUSSION

This section presents the data and provides information about the general characteristics of the participants in Table 1. These data allow us to better understand the characteristics of each group. Data are presented as mean ± standard error. Independent t-tests were performed to compare baseline characteristics between Group K1 and Group K2. The results showed no significant differences between the groups for age, height, weight, BMI, or blood pressure ($p \geq 0.05$ for all comparisons).

Table 1

Characteristics of research subjects

Data	Group	N	x±SD	p-value
Age (y)	K1	10	23.70±0.51	0.652
	K2	10	23.30±0.77	
Height (cm)	K1	10	168.70±1.77	0.701
	K2	10	170.20±2.27	
Weight (kg)	K1	10	62.20±2.62	0.722
	K2	10	64.00±3.97	
BMI (kg/m^2)	K1	10	21.79±1.99	0.851
	K2	10	21.99±1.06	
Systolic (mmHg)	K1	10	122.80±3.38	0.962
	K2	10	123.10±4.07	
Diastolic (mmHg)	K1	10	80.00±2.25	0.720
	K2	10	81.50±3.05	

The results of the Shapiro-Wilk test showed that the pre-test and post-test protein carbonyl data did not deviate significantly from the normal distribution ($p>0.05$), thus allowing the use of parametric statistical tests (Table 2).

Table 2

Normality test results

Data	Group	Shapiro–Wilk	
		n	p-value
Protein Carbonyl (pre-test)	K1	10	0.703
	K2	10	0.395
Protein Carbonyl (post-test)	K1	10	0.472
	K2	10	0.183

In group K1 (placebo), protein carbonyl levels decreased significantly after exercise ($p<0.05$), likely due to natural recovery mechanisms. In group K2 (roselle flower extract), there was no statistically significant decrease in protein carbonyl levels ($p>0.05$), indicating that supplementation had no additional effect beyond physiological adaptations. Data are presented as mean ± standard error (Fig. 2, Table 3).

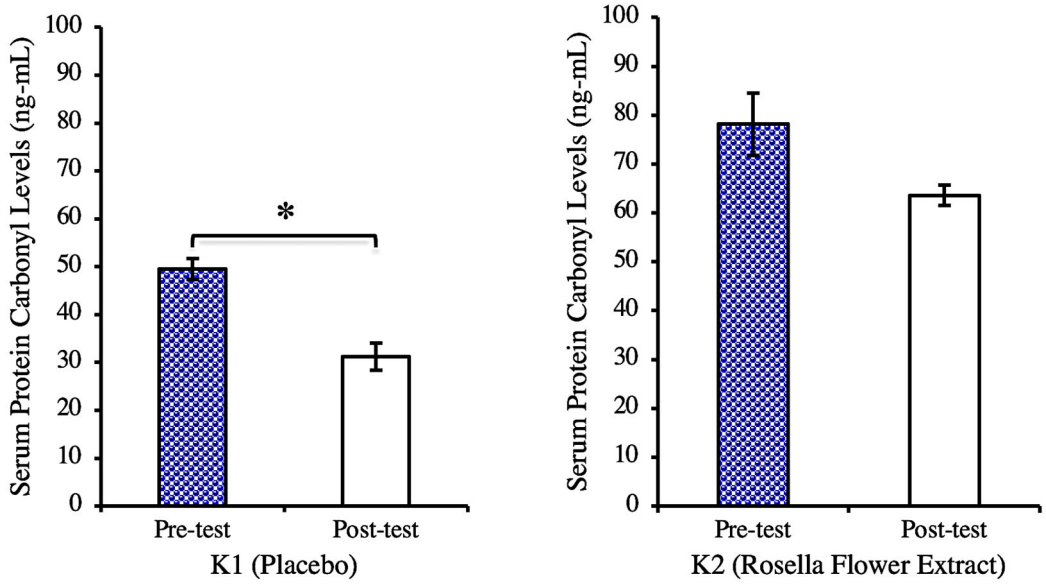

Fig. 2. Research results diagram: group K1 (placebo) and group K2 (roselle flower extract)

Table 3

Results of protein carbonyl levels

Difference Test Method	Group	p
Paired t-test	K1 (pre-test and post-test)	0.001*
	K2 (pre-test and post-test)	0.053

Note. *A significant decrease in protein carbonyl levels was observed in Group K1 (placebo) from pre-test to post-test (p=0.001). However, no significant change was observed in Group K2 (roselle flower extract, p=0.053).

The purpose of this investigation was to ascertain the impact of roselle flower extract supplementation after physical exercise on reducing carbonyl protein levels. The findings of this investigation showed that supplementation of roselle flower extract after physical exercise was not significant in reducing protein carbonyl levels. This was also shown in previous research that administration of 500 mg/day of roselle flower extract for 4 weeks was not able to significantly reduce oxidative stress biomarkers [24]. The cause of the less significant in reducing the levels of carbonyl protein in addition to the samples used being too small, it could be because the dose used was not high enough and the duration of the intervention was not long enough, so this has not had an effect on reducing the levels of carbonyl protein. Although we did not find anti-inflammatory and antioxidant effects of roselle flower extract in our research subjects, several previous studies have shown such effects. In a previous study, polyphenols from roselle flowers were given to patients with metabolic syndrome for 4 weeks. According to the results, the extract showed strong anti-inflammatory activity (decrease in interleukin (IL)-6, IL-1β and IL-8) and increased antioxidant activity (decrease in 8-isoprostane-F2α and increase in serum paraoxonase activity) [25]. In our study, the overall content of roselle flower extract has not been proven to be able to reduce carbonyl protein levels, so it is possible that there are special contents such as flavonoids and polyphenols that can optimally provide a real effect in reducing oxidative stress. Future studies should investigate the chronic effects of roselle supplementation at doses exceeding 500 mg, over durations longer than four weeks, to assess potential cumulative antioxidant benefits.

The findings of earlier research have demonstrated that roselle flower extract supplementation can reduce the expression of NF-kB proteins that play a role in the liver's production of different pro-inflammatory proteins, so roselle is effectively able to suppress inflammatory effects [26]. Further investigation is necessary to ascertain the precise impact of supplementing with roselle flower extract following physical activity on protein carbonyl reduction. In addition to potentially impairing the function of several proteins, protein oxidation is extremely harmful because it alters the redox balance of cells, disrupts the cell cycle, which eventually leads to the death of neurons [27].

Among the oxidative stress processes, protein carbonyls emerge as biomarkers that cause obvious damage to cells [28]. Carbonylated proteins are normally degraded by the proteasome system; however, excessive accumulation can lead to the formation of protein aggregates, which are resistant to degradation and can cause cellular dysfunction [27]. If the cell's carbonylated proteins build up, they will have an impact and cause cytotoxic effects [27]. In addition protein carbonyls make cells more susceptible to apoptotic triggers in addition to causing structural and functional alterations [29]. As a result, protein carbonyls have gained significance as important markers of oxidative stress, due to their sensitivity as well as their structural and functional ramifications [30]. According to the findings of earlier research, giving rats with diabetes mellitus roselle flower extract significantly lowers their protein carbonyl levels [31]. Although this study did not show a significant reduction in protein carbonyl levels, previous studies have shown that roselle flower extract has antioxidant properties that may help reduce oxidative stress [32]. Roselle antioxidant qualities are widely known [33].

According to other study findings, red and white roselle include a variety of phytochemical components that can prevent the negative effects of oxidative stress, such as organic acids, phenolic acids, anthocyanins, and flavonoids [34]. Roselle flower extract has been shown to reduce oxidative stress in obese rats with myocardial infarction, potentially by increasing superoxide dismutase (SOD) activity, increasing glutathione (GSH) levels, and inhibiting NOX2-mediated ROS production [35]. This is because anthocyanins, phenolic acids, and flavonoids can scavenge free radicals such reactive nitrogen species (RNS) and ROS, hence preventing oxidative stress [36]. So although this review did not significantly reduce protein carbonyl levels, the antioxidant effect caused in the previous study can clearly inhibit oxidative stress. The insignificant decrease in protein levels caused in this study could be impacted by a number of variables, including the quantity of samples and the physiological response of the body of each sample in responding to the pharmacological effects of roselle flower extract consumed.

In the control group given placebo in this investigation, it was demonstrated that there was a noteworthy decrease in protein carbonyl levels 60 minutes after physical exercise. This needs to be further explored that the post-test was conducted 60 minutes after exercise and placebo administration. There are indications that this period of time is the body's physiological response in the mechanism of increasing the body's antioxidant levels such as SOD, CAT, GPx. We know that during physical exercise, the body's metabolic rate increases, which can lead to much higher consumption of oxygen, which raises the generation of reactive oxygen species (ROS) [37].

SOD participates in the disproportionation of superoxide free radicals inside and outside cells so that cell membranes and DNA are protected from

damage by oxygen free radicals [38]. SOD enzyme (SOD) plays an important role in balancing the concentration of biological oxidants, which can protect all aerobic organisms from oxidative damage caused by biological oxidants. SOD is one of the most powerful antioxidant enzymes, which can catalyze O_2- to form H_2O_2 to regulate the body's signal transduction [39]. At low concentrations, H_2O_2 acts as a second messenger in redox signaling pathways, modulating inflammation, angiogenesis, and gene expression. However, excessive accumulation of H_2O_2 can contribute to oxidative stress and cell damage [39]. Additionally, H_2O_2 diffuses across the cell membrane through aquaporin aqueous channels, transposing the redox signal from the location where the redox signal is generated to the desired location [40]. So with the duration of the post-test collection in our study there was a range of one hour, at this time the SOD levels increased which will have an impact on reducing oxidative stress triggered by exercise. This is reinforced by previous research that high intensity interval training has been shown to increase SOD levels immediately after the intervention and 3 hours after the intervention also still provides an increase in SOD levels [41].

Research shows that exhausting and prolonged exercise causes inflammation and oxidative stress are harmful to health [42]. The results of previous studies showed that participants who participated in the 50 km ultamaraton running race were shown to provide an increase in protein carbonyl production significantly, the duration and intensity of exercise had an effect on the level of ROS [15]. Another study looked at how running affected the oxidative modification of nucleic acids and discovered that running a marathon instantly caused an acute inflammatory response. This suggests that marathon running also affects endogenous antioxidant activity as an exercise adaptation process of elevated ROS [43].

Another research revealed that 10 weeks of aerobic exercise on a treadmill was shown to reduce protein carbonyl levels [44]. This may be due to an adaptation process that prevents protein carbonyl accumulation during physical exercise. Conversely, it has been demonstrated that a single high-intensity exercise session can raise the lipid peroxidation index [36]. The high intensity of exercise greatly impacts the level of lipid peroxidation that occurs. This is also supported by research from (Ji et al., 2016) that performing a single session of the rate of lipid peroxidation in trained rats' cardiac muscle increased with resistance training [37]. There are inconsistencies in the results of the research that occurred. It should be noted that many factors affect the occurrence of oxidative stress during exercise including the length of intervention, exercise intensity, exercise duration, and data collection period also affect the different results of each study. Acute physical exercise or one session of strenuous exercise is closely associated with an increase in free radical levels, while long-term or chronic exercise of four weeks, eight weeks, or longer is linked to adaptability process that results in an increase in antioxidants in the body [38].

The results of the study prove that exercise can improve mitochondrial function, which is a cell organelle that functions to produce ATP or what is known as an energy warehouse [46]. The reaction mechanism of antioxidant enzymes is very complex and involves several reaction stages and involves various cell organelles. Thus, it can be said that antioxidants in biological systems stop the increase of ROS. Antioxidants prevent the increase in oxidative stress, which reduces oxidative damage to biological components [47]. In fighting free radicals, the enzyme catalase is very important. Since one molecule of catalase can convert millions of H_2O_2 molecules into H_2O and oxygen per second, it has been proven that catalase is one of the most catalytic and efficient enzymes. Due to its outstanding catalytic rate, this enzyme plays an important role in oxidative damage caused by exercise [48]. So in detail, exercise is proven to increase ROS. With the presence of ROS as a trigger for oxidative stress, the body naturally increases the antioxidant defense system in the body such as SOD. Superoxide dismutase (SOD) catalyzes the dismutation of superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2), which reduces oxidative damage. H_2O_2 is then further broken down by catalase (CAT) to water (H_2O) and oxygen (O_2) or by glutathione peroxidase (GPx), which converts H_2O_2 to H_2O using glutathione as a cofactor. So antioxidants are related to each other in an effort to neutralize free radicals triggered by exercise [49].

The findings of earlier research that physical exercise interventions were shown to briefly provide an increase in protein carbonyl levels, but there was a downward trend 60 minutes after the physical exercise intervention and this is a physiological response caused during exercise [50]. It is possible that 60 minutes after exercise, increased SOD activity contributes to the decrease in protein carbonyl levels. However, other antioxidant systems, such as catalase (CAT) and glutathione peroxidase (GPx), may also play a role in reducing oxidative damage. Three isoenzymes make up the antioxidant enzyme SOD. By preserving the body's antioxidant defenses and reducing oxidative stress brought on by an increase in free radicals, SOD is the body's first line of enzymatic defense against free radicals [51]. Regular exercise, on the other hand, can boost the antioxidant system

and lower the body's level of free radicals, enhancing tolerance to oxidative stress and regulating the rate of cell damage [52]. So it can be concluded that the decrease in protein carbonyl levels 60 minutes after physical exercise is a physiological response due to physical exercise by increasing SOD levels which function to reduce free radicals.

CONCLUSION

1. Administration of 500 mg of roselle (Hibiscus sabdariffa) extract after physical exercise did not result in a statistically significant reduction in protein carbonyl levels ($p>0.05$). However, a non-significant downward trend was observed, suggesting that further studies with a larger sample size and longer intervention duration are warranted.

2. The main limitations of this study include the small sample size (n=20) and the single-session intervention rather than a prolonged supplementation period. Future research should investigate higher doses and prolonged administration of roselle flower extract over multiple weeks, considering additional factors such as dietary intake, other oxidative stress biomarkers, and inter-individual variability in exercise response.

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Contributors:

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Halip M.F. – writing – review & editing;

Jr P.B.D. – software, writing – review & editing.

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