JOURNAL LA MEDIHEALTICO

VOL. 05, ISSUE 02 (381-391), 2024
DOI: 10.37899/journallamedihealtico.v5i2.1115

Antioxidant In Reducing MDA Conditions and Increasing SOD Conditions in White Rats (Rattus norvegicus) Wistar Strain Given a High Cholesterol Diet and Induced More Physical Activity

Nova Riana[1], Lenni Dianna Putri[1], Erida Novriani[1]

[1]*University Prima Indonesia, Medan*

**Corresponding Author: Nova Riana*

Article Info

Article history:
Received 31 January 2024
Received in revised form 5 April 2024
Accepted 23 April 2024

Keywords:
Physical Exercise
Antioxidants
Grape Seeds
MDA
Wistar Strain

Abstract

Sedentary lifestyles increase the risk of morbidity and infectious illnesses. Physical exercise can improve immune function but can lead to increased oxidants and reactive oxygen species (ROS), damaging cells and tissues. Crucial in mitigating the cellular damage caused by free radicals are antioxidants, including non-enzymatic antioxidants such as glutathione, vitamins C and E, and others. Moderate to high-intensity physical exercise can result in an increase in ROS and a reduction in SOD. Grape seeds are rich in antioxidants, which have been shown to neutralize free radicals and protect against inflammation, bacteria, ulcers, and cancer. These antioxidants, including phenols and proanthocyanidins, are beneficial to health and have applications in various industries, including energy production, cosmetics, and pharmaceuticals. Research is also being conducted to explore their antioxidant effects on MDA levels. Grape seed extract was tested for its impact on malondialdehyde (MDA) levels in the blood of male Wistar strain white rats. Rats' MDA levels decreased following four weeks of treatment. Rat cages, digital scales, an MDA test kit, and SPSS were some of the equipment used in the study. Grape seed extract effectively reduces MDA levels in rats with hypercholesterolemia, resulting in weight loss and a lee index below 0.3, and increases SOD levels, indicating potential for human use.

Introduction

Nowadays, in the context of globalization, sedentary lifestyles are acknowledged as the fourth main risk factor for an increased risk of morbidity and infectious illnesses. Regular exercise and physical activity can mitigate the link between inactivity and early mortality. Obesity, diabetes, cancer, cardiovascular disease, depression, cancer, and hypertension are just a few of the chronic diseases that physical exercise can help lower risk factors (Hossain et al., 2016).

"Physical exercise" describes scheduled physical activities ranging from weeks to years. Our metabolism varies when we move around, which sets off adaptive mechanisms that bring about a new balance and some helpful adjustments in the body (Magherini et al., 2019). Nonetheless, exercise has both beneficial and harmful effects on one's status. When compared to inactive physical activity, moderate-intensity exercise improves immune function. When you exercise for lengthy periods at a high level, your immune system may suffer (Magherini et al., 2019). According to the research, excessive physical exertion causes muscles to produce more oxidants, creating reactive oxygen species (ROS). Damage to macromolecules, reduced cellular function, apoptosis, necrosis, and exhaustion of enzymatic and non-enzymatic antioxidant systems can result from prolonged exposure to high levels of reactive oxygen

ISSN 2721-1215 (Print), ISSN 2721-1231 (Online)

species (ROS). Consequently, people not used to or trained for intense physical activity should avoid it (Simioni et al., 2018).

Free radicals are produced during exercise by skeletal muscle contractions and increased oxygen intake, making high levels of reactive oxygen species (ROS). Inadequate antioxidant defences cause oxidative damage to cells and tissues (Lu et al., 2021). The presence of one or more unpaired electrons in an atomic or molecular orbital makes an atom, molecule, or molecular fragment a free radical (A & Shah, 2022). The human body uses certain free radicals for good, such as those that combat inflammation, eliminate germs, and regulate the smooth muscles that control the functioning of internal organs and blood arteries (Martemucci et al., 2022). Daily, each cell is attacked by an estimated 10,000 to 20,000 free radicals. Furthermore, when formed in excess or without control, free radicals contribute significantly to the pathophysiology of several illnesses, including cardiovascular disease, diabetes mellitus, Alzheimer's disease, Parkinson's disease, cancer, arthritis, ageing, and so on (Qazi & Molvi, 2018).

The exercise system includes the skeletal muscle, which is a living being. The creation of muscular strength, preservation of muscle content, intracellular signal transduction, gene expression, and other associated functions rely on the physiological level of reactive oxygen species (ROS) to sustain their function (Assi, 2017). Simultaneously, the buildup of reactive oxygen species (ROS) leads to defective contractions and weakened muscles (Thirupathi et al., 2020). It is well-known that exercise-induced skeletal muscle contractions can raise oxidative stress levels and strengthen antioxidant defences by triggering ROS (Taherkhani et al., 2021). While lean muscle force generation does need exercise-induced reactive oxygen species (ROS), excessive ROS levels may lead to contractile failure (Magherini et al., 2019). Muscle contraction is impacted because ROS-sensitive sarcoplasmic reticulum Ca2+ release channels decrease myofibrils' Ca2+ sensitivity (Magherini et al., 2019). Exercise type, intensity, and duration all affect how ROS accumulates in the body.

As a result of the oxygen supply falling short of the body's rapidly increasing demand, numerous tissues and organs release molecules with high levels of activity, including reactive nitrogen species (RNS) and reactive oxygen species (ROS), in response to the common stressor of excessive physical activity (ROS). Oxygen radicals (O2--), hydroxyl radicals (OH—), and hydrogen peroxide (H2O2) are reactive oxygen species (ROS) that play a significant role in the stress response (Powers et al., 2020).

Cell signalling and tissue homeostasis are significantly impacted by reactive oxygen species (ROS), which are generated naturally in the body (Ferreira et al., 2018). However, they contribute to many pathologies, including lipid, protein, and DNA damage, and cause harmful alterations to cell components when created in excess (Lundgren et al., 2018). As a result of their high PUFA content, cell or organelle membranes are especially vulnerable to ROS damage, often known as "lipid peroxidation."

When oxyl, peroxyl, and hydroxyl radicals, among other free radical species, strip electrons from lipids, a process known as lipid peroxidation occurs, producing reactive intermediates capable of undergoing further processes (Bhattacharya, 2015). Lipid peroxidation directly damages phospholipids, which can also trigger programmed cell death. In addition to mediating proinflammatory alterations, oxidized phospholipids can be involved in other inflammatory disorders (Que et al., 2018).

Oxidative stress responses occur when ROS generation surpasses their scavenging capabilities. Under typical physiological circumstances, several cellular processes rely on reactive oxygen species (ROS) (Marreiro et al., 2017). These processes include energy metabolism, signal transmission, and gene expression control. On the other hand, cellular biomacromolecules, including lipids, proteins, and nucleic acids, can be damaged by excessive ROS levels, leading

ISSN 2721-1215 (Print), ISSN 2721-1231 (Online)

to cell damage, ageing, and death (He et al., 2021). According to several studies, low reactive oxygen species (ROS) levels result from frequent or suitable exercise. Additionally, skeletal muscle and other tissues might have their physiological activities compromised if there is an overabundance of endogenous free radicals produced during exercise.

An increase in reactive oxygen species (ROS)—specifically, malondialdehyde (MDA)—and a reduction in superoxide dismutase (SOD), an endogenous antioxidant that suppresses free radical excess—are possible outcomes of moderate to high-intensity physical exercise (Rusip et al., 2021). Antioxidants are the best and most appropriate way to avoid MDA's adverse effects, while other options exist. Research has shown that antioxidants mitigate the harmful effects of reactive oxygen and nitrogen species, which are known to cause cell and tissue damage (Maddu, 2019). An essential function of antioxidants is to neutralize or eliminate free radicals, including reactive oxygen species (ROS). ROS have the potential to mutate DNA, harm cell membranes and proteins, and more. The development of cancer, inflammation, arthritis, atherosclerosis, neurological illnesses (such as Alzheimer's and Parkinson's), diabetes mellitus, and Alzheimer's and Parkinson's if this condition persists (Irawan et al., 2022).

To counteract the damaging effects of free radicals on their cells, organisms have evolved an antioxidant defence mechanism. Vitamins C and E, glutathione, and bilirubin are non-enzymatic antioxidants. In contrast, enzymatic antioxidants like superoxide dismutase (SOD), catalase, and glutathione peroxidase make up the other half of the antioxidant defence system. Antioxidants play a crucial role in halting or postponing the oxidation of biomolecules within and outside cells (Kawamura & Muraoka, 2018). Antioxidants can be found in plants, for example.

Herbs, spices, grains, fruits, and vegetables are just a few plant materials containing natural antioxidants (Vitale et al., 2022). These natural components attract attention because of their ecological and economic significance. Most of these components come from food waste and underutilized plant species (Lourenço et al., 2019). Fruits like grapes are good antioxidants (*Vitis vinifera L.*).

One of the most valuable horticultural products, grapes have been farmed for as long as anybody can remember. Grapes have a low salt, cholesterol, and fat content but a high caffeic acid, ascorbic acid, retinol, and phosphorus content, making them potent cancer fighters. There are several medical uses for resveratrol, a physiologically active and well-characterized component of grapes found in abundance in the pulp. Grapes are very effective antioxidants. Several studies have shown that grapes' phytoalexins and oleanolic acid benefit health (Hussain et al., 2021).

Grape seeds (*Vitis vinifera L.*) include phenols that are beneficial to health, and proanthocyanidins, which are condensed tannins, may also have a nutritional function. Both in vivo and in vitro studies have shown that phenols can serve as antioxidants and neutralize free radicals, with phenols providing more protection than vitamins E, C, and ß-carotene (Lucarini et al., 2018). Additional actions, including those against inflammation, bacteria, ulcers, and cancer, have also been shown (Martin et al., 2020).

The abundant bioactive chemicals in grape seeds may be extracted and used for energy production. The resulting extracts and semi-finished products have applications in agronomy, cosmetics, feed, food, nutraceuticals, and phytochemicals (Lucarini et al., 2018). Because of their high concentration of phytochemicals, which can give hydrogen for scavenging, grape and wine extracts may be able to neutralize free radicals and antioxidants. Grape polyphenols, including quercetin, kaempferol, catechin, and resveratrol, not only function as chelating agents for reactive metal ions (Cu2+), but they also scavenge peroxy and alkoxy lipid radicals (Hussain et al., 2021). Research into the antioxidant effects of grape seed extract in lowering

ISSN 2721-1215 (Print), ISSN 2721-1231 (Online)

MDA levels caused by excessive exercise in rats is of great interest to the scientific community (*Rattus norvegicus*).

Methods

This quantitative experiment uses an actual experiment design (Notoatmodjo, 2022). We used post-test-only control groups. Grape seed extract (Vitis vinifera L.) was tested for its effect on blood malondialdehyde in white rats. Male Wistar strain white rats (Rattus norvegicus) measuring 160-200 gr and 2-3 months old were studied. Rats (Rattus norvegicus) are extensively utilized in biomedical research and exhibit human-like characteristics and physiologies. More significant than mice, this rat adapts better to the lab. These studies employed six Wistar rats per group. This study assessed 24 heads, with one tail per group as a backup if some did not satisfy the criteria. Test animals were randomly assigned to four groups in the field (Wan Mohammad, 2017). This research had independent and dependent factors (Suwarno & Nugroho, 2023). The independent variable in this study is grape seed extract. The amounts of malondialdehyde in white rats are what the experiment measures. The administration of grape seed extract is the independent variable. Rat MDA levels are the dependent variable. Excessive swimming is one of the pre-conditioning factors.

A study on mice and rats showed that grape seed extract, made by macerating grape seeds with ethanol, reduced malondialdehyde levels in their blood serum. After four weeks of therapy, male Wistar strain white rats showed a decrease in MDA levels. The study used an ELISA kit to measure SOD levels in rat serum and involved animal preparation, phytochemical screening, antioxidant activity assessment, treatment administration, serum MDA measurement, and soil quality testing. The study utilized various tools, including rat cages, digital scales, and an MDA test kit for analyzing the concentrations of multiple acids. SPSS was used for data analysis, and Kolmogorov-Smirnov was used for the data normality (Ghozali, 2018). One-way ANOVA assessed the significance between test groups at a 95% confidence level. The LSD Post Hoc Test was used for additional analysis or testing.

Result and Discussion

Mice were fed a high-fat, high-cholesterol diet containing quail egg yolk for 14 days before starting grape seed extract treatment. Rats' body weight was measured using Ohaus scales and index Lee to confirm if high-fat feed induces excess fat or obesity. The diet was given for 14 days before grape seed extract treatment.

Table 1. Body Weight of Rats

Group	Body Weight		Naso-anal Length		Lee index	
	Before	After	Before	After	Before	After
Negative Control	251gr	232gr	204mm	202mm	0.30	0.27
Positive Control	250gr	210gr	203mm	202mm	0.30	0.28
Treatment P1	252gr	205gr	205mm	203mm	0.31	0.28
Treatment P2	252gr	188gr	204mm	202mm	0.30	0.28
Treatment P3	251gr	232gr	204mm	202mm	0.30	0.27
Treatment P4	250gr	210gr	203mm	202mm	0.31	0.28

The study found that rats with high-fat food had a lee index of 0.3, indicating obesity. However, treatment with grape seed extract led to weight loss, as evidenced by the lee index value. The rats treated with grape seed extract did not fall into the obesity category.

Table 2. MDA Descriptive Analysis Results

Group Treatment		N	Mean (nmol/ml)	SD	Max	Min
Control	Before	6	0,15	0,04	0,18	0,10

	After	6	0,14	0,03	0,10	0,17
P-1	Before	6	0,74	0,02	0,78	0,69
	After	6	0,49	0,10	0,38	0,67
P-2	Before	6	0,23	0,03	0,27	0,19
	After	6	0,12	0,02	0,12	0,09
P-3	Before	6	0,14	0,03	0,20	0,14
	After	6	0,09	0,02	0,10	0,05

The study analyzed the mean MDA levels of rats before and after physical exercise and grape seed extract treatment. The control group had a mean MDA level of 0.15 ± 0.04 nmol/ml, while the rats treated with bodily exercise, swimming, and distilled water showed a decrease to 0.14 ± 0.03 nmol/ml. The mean MDA levels in treatment groups 1 and 2 decreased after physical exercise and grape seed extract treatment for 14 days. The mean MDA levels in treatment group 3 decreased after high-fat, high-cholesterol feed and physical exercise. The mean MDA levels in treatment group 3 significantly decreased after physical exercise and grape seed extract treatment. The results suggest that physical exercise and grape seed extract treatment can substantially reduce average MDA levels in white rats fed a high-cholesterol diet and induction.

Table 3. Data On the Average Results of The Study of SOD Levels

Variables	**Control Group Mean ± SD**	**Treatment group 1 Mean ± SD**	**Treatment group 2 Mean ± SD**	**Treatment group 3 Mean ± SD**	**P value**
SOD levels	3.226 ± 0,4368	3,026 ± 0,3112	3,045 ± 0,3636	3.155± 0,3782	
Kolmogorov Smirnov	0,934	0,976	0,897	0,903	0,920
Levene Test	0,890	0,923	0,987	0,945	0,965

The study found that rats treated with grape seed extract and physical exercise for 14 days were able to increase their serum triglyceride (SOD) levels. The rats were given a dose of 5ml/day/head orally using a sonde, indicating that grape seed extract could increase SOD levels despite a high-cholesterol diet and excessive physical activity. The data was tested for normality and homogeneity using the Kolmogorov, Smirnov, and Levene Tests. The results showed no significant differences in all treatments, except for a non-significant difference between the healthy control group, the negative control group, treatment group 1, and treatment group 2.

A straightforward way to measure antioxidant activity is via the DPPH technique. An interaction between a free radical and a hydrogen atom in the test substance causes the DPPH radicals to change colour. A 50 ppm DPPH solution and a 500-ppm black rice extract stock solution are used in the procedure. A spectrophotometer is used to measure the absorbance at 515 nm, which enables a thorough evaluation of the antioxidant activity.

Table 4. Waveform Observation Results

No.	**Sample name**	**IC50 repetition 1**	**IC50 repetition 2**	**IC50 repetition 3**
1	Grape Seed Extract	32,3836	32,5821	32,5884

ISSN 2721-1215 (Print), ISSN 2721-1231 (Online)

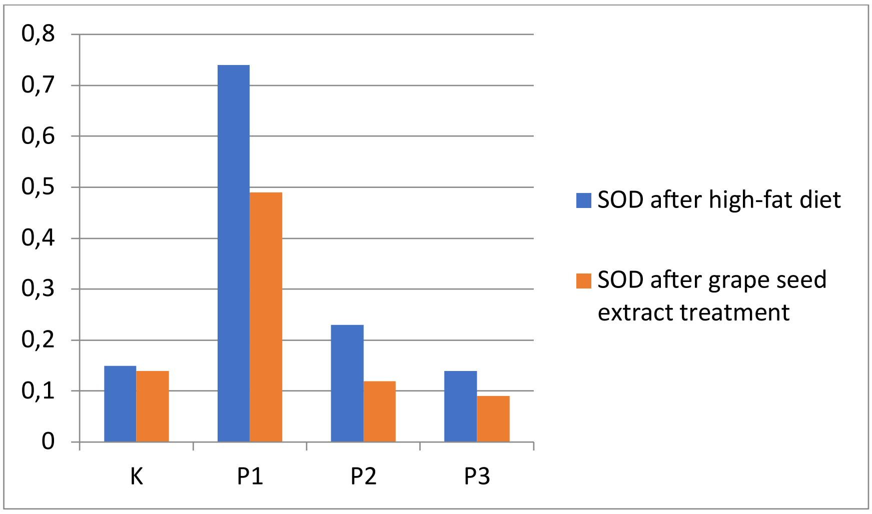

*Figure 1. Graph of SOD levels in all study groups (*p > 0.05 = not significant)*

At a wavelength of 515 nm, the IC50 values at 0 minutes, 30 minutes, and 60 minutes are 32.38, 32.58, and 32.588, respectively. This proves that grape seed extract contains potent antioxidants. A class of chemical substances known as antioxidants can neutralize free radicals by donating an electron or electrons.

Table 5. Data Normality Test Results

Group		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	**Df**	**Sig.**	**Statistic**	**Df**	**Sig.**
MDA Results Before	Control	.172	6	.200*	.957	6	.798
	Treatment 1	.171	6	.200*	.967	6	.869
	Treatment 2	.163	6	.200*	.965	6	.856
	Treatment 3	.207	6	.200*	.918	6	.492
MDA Results After	Control	.205	6	.200*	.908	6	.425
	Treatment 1	.192	6	.200*	.920	6	.502
	Treatment 2	.293	6	.117	.915	6	.473
	Treatment 3	.205	6	.200*	.961	6	.830

*. This is a lower bound of the true significance. a. Lilliefors significance correction

The Kolmogorov-Smirnov test was used to examine the normality of data in each group, revealing a normal distribution of pre- and post-treatment MDA levels in both control and treatment groups.

Table 6. Data Homogeneity Test Results

	Levene Statistic	df1	df2	Sig.
MDA Results Before	.389	3	20	.768
MDA Results After	6.134	3	20	.087

The Levene test determined group homogeneity at a 5% significance level. The results showed that the three treatment groups (control, group 1, and group 2) had common variance or were statistically indistinguishable. The significance values of 0.399 and 6.134 were more than 0.05, indicating that the data was homogeneous.

ISSN 2721-1215 (Print), ISSN 2721-1231 (Online)

Table 7. One-Way ANOVA Test Results

Groups		Sum of Squares	Df	Mean Square	F	Sig.
MDA Results Before	Between	1.236	3	.412	336.387	.000
	Within	.026	20	.001		
	Total	1.263	23			
MDA Results After	Between	.666	3	.222	70.253	.000
	Within	.067	20	.003		
	Total	.729	23			

The researchers conducted a one-way ANOVA test to determine if there was a statistically significant difference in the outcomes of the trial groups. The results showed a considerable difference of 0.000, less than 0.05, indicating that the treatment group significantly differed from the control group. The Post Hoc LSD test was used to compare the mean MDA levels of the different groups, also showing a significant difference of 0.000, less than 0.05, indicating the treatment group's essential differences.

Table 8. LSD Post-hoc Test Results

Dependent variable	(i) Group	(j) Group	Mean Difference (i-j)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
MDA Results Before	Control	Treatment 1	-.59500*	.02020	.000	-.6371	-.5529
		Treatment 2	-.14000*	.02020	.000	-.1821	-.0979
		Treatment 3	-.11667*	.02020	.000	-.1588	-.0745
	Treatment 1	Control	.59500*	.02020	.000	.5529	.6371
		Treatment 2	.45500*	.02020	.000	.4129	.4971
		Treatment 3	.47833*	.02020	.000	.4362	.5205
	Treatment 2	Control	.14000*	.02020	.000	.0979	.1821
		Treatment 1	-.45500*	.02020	.000	-.4971	-.4129
		Treatment 3	.02333	.02020	.000	-.0188	.0655
	Treatment 3	Control	.11667*	.02020	.000	.0745	.1588
		Treatment 1	-.47833*	.02020	.000	-.5205	-.4362
		Treatment 2	-.02333	.02020	.000	-.0655	.0188
MDA Results After	Control	Treatment 1	-.35333*	.03243	.000	-.4210	-.2857
		Treatment 2	.03000	.03243	.366	-.0376	.0976
		Treatment 3	.05500	.03243	.000	-.0126	.1226
	Treatment 1	Control	.35333*	.03243	.000	.2857	.4210
		Treatment 2	.38333*	.03243	.000	.3157	.4510
		Treatment 3	.40833*	.03243	.000	.3407	.4760
	Treatment 2	Control	-.03000	.03243	.366	-.0976	.0376
		Treatment 1	-.38333*	.03243	.000	-.4510	-.3157
		Treatment 3	.02500	.03243	.000	-.0426	.0926
	Treatment 3	Control	-.05500	.03243	.000	-.1226	.0126
		Treatment 1	-.40833*	.03243	.000	-.4760	-.3407
		Treatment 2	-.02500	.03243	.000	-.0926	.0426

*. The mean Difference is significant at the 0.05 level.

The study conducted phytochemical tests on grape seed extract to determine if it contains compounds that can reduce MDA levels and increase SOD in male Wistar rats. The tests included alkaloid, flavonoid, saponin, tannin, steroid/triterpene, and glycoside. The results showed that grape seed extract contains secondary metabolite compounds such as flavonoids, alkaloids, saponins, steroids, and tannins, which can reduce MDA levels and increase SOD levels in rats fed high-fat diets.

Fat and cholesterol-rich diets were consistently fed to rats. Feed was quail egg yolk. This diet raises cholesterol exogenously. Before treatment, the control group had an average MDA level of 0.15 ± 0.04 nmol/ml, ranging from 0.18 to 0.10. After participating in a 20-minute bucket swimming exercise and drinking distilled water daily, the control group's MDA levels decreased to 0.14 ± 0.03 nmol/ml, ranging from 0.10 to 0.17. Before treatment, group 1 had an average MDA level of 0.74 ± 0.02 nmol/ml, ranging from 0.78 to 0.69. Group 1 rats were given orally 2 millilitres of grape seed extract for 14 days after 20 minutes of bucket swimming and reduced to 0.49 ± 0.10 nmol/ml, with peaks of 0.38 and minimums of 0.67.

Before treatment, group 2 had an average MDA level of 0.23 ± 0.12 nmol/ml, ranging from 0.27 to 0.19. Treatment 2 rats' mean MDA levels were measured after 14 days of oral grape seed extract at 3 millilitres daily and 20 minutes of bucket swimming. The value dropped to 0.12 ± 0.02 nmol/ml, from 0.20 to 0.14. The mean MDA level before treatment in treatment group 3 was 0.20 to 0.14 nmol/ml, with a standard deviation of 0.03. After eating a high-fat, cholesterol-rich diet, rats in treatment group 3 exercised by swimming for 20 minutes in a bucket and taking 5 millilitres of grape seed extract orally for 14 days. Treatment group 3 white rats fed a high cholesterol diet, and induction was given grape seed extract orally at 5 millilitres per day and swam in a bucket for 20 minutes. This reduced the average MDA.

The third treatment group had a statistically significant increase in SOD levels. The results showed that GSE increases SOD levels. Compared to the control group, grape seed extract increases SOD levels sequentially in treatments 1 and 2. After receiving 5 millilitres of grape seed extract orally via sonde for 14 days, rats in treatment group 3 were given 20 minutes of bucket swimming. Capable of increasing SOD levels in response to high-cholesterol diet and exercise-induced hypertrophy. Grape seed extract antioxidant test produced a potent category. Antioxidants donate electrons to free radicals to neutralize them. The phytochemical test identified grape seed extract secondary metabolites as flavonoids, alkaloids, saponins, steroids, and tannins. These compounds lower MDA and raise SOD in white rats (*Rattus norvegicus*). Wistar strain fed a high-fat diet with hypercholesterolemia and excessive activity.

Conclusion

Research has shown that grape seed extract can effectively reduce MDA levels and increase SOD levels in white rats (Rattus norvegicus) with hypercholesterolemia and excess activity. The results show that rats treated with grape seed extract experienced weight loss and a Lee index value below 0.3, indicating that the extract is not included in the obesity category. Treatment group 3 rats showed a significant decrease in average MDA and an increase in SOD levels compared to control, treatment 1, and treatment 2. The results of phytochemical tests reveal secondary metabolite compounds in grape seed extract, including flavonoids, alkaloids, saponins, steroids, and tannins, which reduce MDA levels and increase SOD levels in rats. Further research is needed to understand the effect of grape seed extract on reducing MDA and increasing SOD levels in humans.

Acknowledgement

The author would like to express their most profound appreciation to the faculty of medicine, dentistry, and health sciences at Prima Indonesia University and everyone else who had a role in accomplishing this project.

References

A, V. M., S, K. P., & Shah, M. N. A. D. (2022). The Role of Free Radicals and Reactive Oxygen Species in Biological Systems-A Comprehensive Review. *International Journal Of*

Drug Research And Dental Science, *4*(3), 28–41. https://doi.org/10.36437/IJDRD.2022.4.3.E

Assi, M. (2017). The differential role of reactive oxygen species in early and late stages of cancer. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *313*(6), R646–R653. https://doi.org/10.1152/AJPREGU.00247.2017/ASSET/IMAGES/LARGE/ZH60101793480002.JPEG

Bhattacharya, S. (2015). Reactive oxygen species and cellular defense system. *Free Radicals in Human Health and Disease*, 17–29. https://doi.org/10.1007/978-81-322-2035-0_2/COVER

Ferreira, C. A., Ni, D., Rosenkrans, Z. T., & Cai, W. (2018). Scavenging of reactive oxygen and nitrogen species with nanomaterials. *Nano Research*, *11*(10), 4955–4984. https://doi.org/10.1007/S12274-018-2092-Y/METRICS

Ghozali, I. (2018). Aplikasi Analisis Multivariate dengan Program IBM SPSS 25. In *Badan Penerbit Universitas Diponegoro*.

He, Z., Xu, Q., Newland, B., Foley, R., Lara-Sáez, I., Curtin, J. F., & Wang, W. (2021). Reactive oxygen species (ROS): utilizing injectable antioxidative hydrogels and ROS-producing therapies to manage the double-edged sword. *Journal of Materials Chemistry B*, *9*(32), 6326–6346. https://doi.org/10.1039/D1TB00728A

Hossain, M. K., Dayem, A. A., Han, J., Yin, Y., Kim, K., Saha, S. K., Yang, G. M., Choi, H. Y., & Cho, S. G. (2016). Molecular Mechanisms of the Anti-Obesity and Anti-Diabetic Properties of Flavonoids. *International Journal of Molecular Sciences 2016, Vol. 17, Page 569*, *17*(4), 569. https://doi.org/10.3390/IJMS17040569

Hussain, S. Z., Naseer, B., Qadri, T., Fatima, T., & Bhat, T. A. (2021). Grapes (Vitis vinifera)—Morphology, Taxonomy, Composition and Health Benefits. *Fruits Grown in Highland Regions of the Himalayas*, 103–115. https://doi.org/10.1007/978-3-030-75502-7_8

Irawan, C., Elya, B., Hanafi, M., & Saputri, F. C. (2022). Potential of Rhinachanthus nasutus (L.) Kurz Leaves Extract as an Antioxidant and Inhibitor of α-Glucosidase Activity. *Pharmacognosy Journal*, *14*(4), 373–378. https://doi.org/10.5530/pj.2022.14.110

Kawamura, T., & Muraoka, I. (2018). Exercise-Induced Oxidative Stress and the Effects of Antioxidant Intake from a Physiological Viewpoint. *Antioxidants 2018, Vol. 7, Page 119*, *7*(9), 119. https://doi.org/10.3390/ANTIOX7090119

Lourenço, S. C., Moldão-Martins, M., & Alves, V. D. (2019). Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. *Molecules 2019, Vol. 24, Page 4132*, *24*(22), 4132. https://doi.org/10.3390/MOLECULES24224132

Lu, Y., Wiltshire, H. D., Baker, J. S., & Wang, Q. (2021). Effects of High Intensity Exercise on Oxidative Stress and Antioxidant Status in Untrained Humans: A Systematic Review. *Biology 2021, Vol. 10, Page 1272*, *10*(12), 1272. https://doi.org/10.3390/BIOLOGY10121272

Lucarini, M., Durazzo, A., Romani, A., Campo, M., Lombardi-Boccia, G., & Cecchini, F. (2018). Bio-Based Compounds from Grape Seeds: A Biorefinery Approach. *Molecules 2018, Vol. 23, Page 1888*, *23*(8), 1888. https://doi.org/10.3390/MOLECULES23081888

Lundgren, C. A. K., Sjöstrand, D., Biner, O., Bennett, M., Rudling, A., Johansson, A. L., Brzezinski, P., Carlsson, J., Von Ballmoos, C., & Högbom, M. (2018). Scavenging of superoxide by a membrane-bound superoxide oxidase. *Nature Chemical Biology 2018 14:8*, *14*(8), 788–793. https://doi.org/10.1038/s41589-018-0072-x

Maddu, N. (2019). *Diseases related to types of free radicals*. IntechOpen London, UK.

Magherini, F., Fiaschi, T., Marzocchini, R., Mannelli, M., Gamberi, T., Modesti, P. A., & Modesti, A. (2019). Oxidative stress in exercise training: the involvement of inflammation and peripheral signals. *Free Radical Research*, *53*(11–12), 1155–1165. https://doi.org/10.1080/10715762.2019.1697438

Marreiro, D. do N., Cruz, K. J. C., Morais, J. B. S., Beserra, J. B., Severo, J. S., & Soares de Oliveira, A. R. (2017). Zinc and Oxidative Stress: Current Mechanisms. *Antioxidants (Basel, Switzerland)*, *6*(2). https://doi.org/10.3390/ANTIOX6020024

Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., & D'Alessandro, A. G. (2022). Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. *Oxygen 2022, Vol. 2, Pages 48-78*, *2*(2), 48–78. https://doi.org/10.3390/OXYGEN2020006

Martin, M. E., Grao-Cruces, E., Millan-Linares, M. C., & Montserrat-De la Paz, S. (2020). Grape (Vitis vinifera L.) Seed Oil: A Functional Food from the Winemaking Industry. *Foods 2020, Vol. 9, Page 1360*, *9*(10), 1360. https://doi.org/10.3390/FOODS9101360

Notoatmodjo, S. (2022). *Metodologi Penelitian Kesehatan* (3rd ed.). Jakarta: Rineka Cipta.

Powers, S. K., Deminice, R., Ozdemir, M., Yoshihara, T., Bomkamp, M. P., & Hyatt, H. (2020). Exercise-induced oxidative stress: Friend or foe? *Journal of Sport and Health Science*, *9*(5), 415–425. https://doi.org/10.1016/J.JSHS.2020.04.001

Qazi, M. A., & Molvi, K. I. (2018). Free Radicals and their Management. *Am. J. Pharm Health Res*, *6*(04).

Que, X., Hung, M. Y., Yeang, C., Gonen, A., Prohaska, T. A., Sun, X., Diehl, C., Määttä, A., Gaddis, D. E., Bowden, K., Pattison, J., MacDonald, J. G., Ylä-Herttuala, S., Mellon, P. L., Hedrick, C. C., Ley, K., Miller, Y. I., Glass, C. K., Peterson, K. L., … Witztum, J. L. (2018). Oxidized phospholipids are proinflammatory and proatherogenic in hypercholesterolaemic mice. *Nature 2018 558:7709*, *558*(7709), 301–306. https://doi.org/10.1038/s41586-018-0198-8

Rusip, G., Ilyas, S., Lister, I. N. E., Ginting, C. N., & Mukti, I. (2021). The effect of ingestion of red dragon fruit extract on levels of malondialdehyde and superoxide dismutase after strenuous exercise in rats (Rattus norvegicus). *F1000Research*, *10*, 1061. https://doi.org/10.12688/F1000RESEARCH.54254.3

Simioni, C., Zauli, G., Martelli, A. M., Vitale, M., Sacchetti, G., Gonelli, A., & Neri, L. M. (2018). Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. *Oncotarget*, *9*(24), 17181. https://doi.org/10.18632/ONCOTARGET.24729

Suwarno, B., & Nugroho, A. (2023). *Kumpulan Variabel-Variabel Penelitian Manajemen Pemasaran (Definisi & Artikel Publikasi)* (1st ed.). Bogor: Halaman Moeka Publishing.

Taherkhani, S., Valaei, K., Arazi, H., & Suzuki, K. (2021). An Overview of Physical Exercise and Antioxidant Supplementation Influences on Skeletal Muscle Oxidative Stress. *Antioxidants 2021, Vol. 10, Page 1528*, *10*(10), 1528. https://doi.org/10.3390/ANTIOX10101528

Thirupathi, A., Pinho, R. A., & Chang, Y.-Z. (2020). Physical exercise: An inducer of positive oxidative stress in skeletal muscle aging. *Life Sciences*, *252*, 117630.

https://doi.org/10.1016/j.lfs.2020.117630

Vitale, S., Colanero, S., Placidi, M., Di Emidio, G., Tatone, C., Amicarelli, F., & D'Alessandro, A. M. (2022). Phytochemistry and Biological Activity of Medicinal Plants in Wound Healing: An Overview of Current Research. *Molecules*, *27*(11), 3566. https://doi.org/10.3390/molecules27113566

Wan Mohammad, W. M. Z. (2017). Sample Size Calculation in Animal Studies Using Resource Equation Approach. *Malaysian Journal of Medical Sciences*, *24*(5), 101–105. https://doi.org/10.21315/mjms2017.24.5.11

ISSN 2721-1215 (Print), ISSN 2721-1231 (Online)
