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Oral Red Pomegranate (*Punica granatum*) Extract Administration to Elevate Blood Glutathione Peroxidase Levels in Female Wistar Rats (*Rattus Norvegicus*) Following Maximum Physical Activity

Liena[1], Rahma Sari[2], Fioni[3*]
[1,2,3]Master Study Program in Biomedical Sciences, Faculty of Medicine, Dentistry and Health Sciences, Prima Indonesia University, Medan

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ABSTRACT

There is a significant danger to human health from oxidative stress. This disorder does not have any apparent symptoms. However, it is linked to the start and progression of many diseases. There are also no established medical procedures for detecting oxidative stress. Finding out how oral administration of red pomegranate (Punica granatum) extract increases blood glutathione peroxidase levels in female rats (Rattus norvegicus) strains is the goal of this experimental quantitative study, which employs a pre-test-post-test control group design. Maximum physical activity was induced in Wistar rats. Twenty-four animals were used in the experiments, with six in each group. Three, four, and five milliliters of red pomegranate extract were used in this experiment. With an antioxidant value of 8.33 ppm, pomegranate extract (Punica granatum) was determined to have a very robust action, according to the research. In terms of composition, the 5-milliliter dose of red pomegranate (Punica granatum) extract outperforms the others when compared to average levels of glutathione peroxidase. Researchers discovered that supplementing with red pomegranate extract (Punica granatum) considerably raised glutathione peroxidase levels, suggesting that this fruit may aid in the body's antioxidant defenses against free radicals generated by strenuous exercise.

Email :
dr.fioni@unprimdn.co.id

1. INTRODUCTION

Physical activity can improve brain health, weight management, disease prevention, bone and muscle strength, and daily function (Angulo et al., 2020; Oppert et al., 2021). Physical activity is any body movement produced by skeletal muscles that consumes energy, including unstructured activities of daily living, pre-arranged, deliberate, or repetitive exercise, grassroots sports, and competitive and regular physical activity. Meditative activities, including walking, cycling, and sports, are healthy (Simioni et al., 2018). However, excessive use can induce harmful adverse effects. Maximum physical activity increases oxidative stress and decreases antioxidants (Powers et al., 2020).

Oxidative stress occurs when the body's antioxidant system is out of balance with oxidation (Bhatti et al., 2022; Silina et al., 2022; Verhaegen et al., 2022). Oxidative stress is dangerous. This condition has no symptoms but is linked to different diseases (Akbari et al., 2022; El Assar et al., 2022). Oxidative stress is not routinely tested in medicine. The detrimental effects of oxidative stress can develop without notice. Therefore, damage is often done unnoticed. According to Galano & Alvarez (2019), oxidative stress is a silently dangerous substance (Galano & Alvarez-Idaboy, 2019).

When the body's detoxification mechanisms are overwhelmed by the formation and buildup of reactive oxygen species (ROS) in cells and tissues, a condition known as oxidative stress results (Pizzino et al., 2017). Activity, intensity, and duration determine the accumulation of ROS in the body. Research has revealed that cells can use low levels of reactive oxygen species (ROS) generated by moderate-intensity activity to transmit signals from growth factors. Because free radicals are an

inevitable byproduct of fast-growing oxygen consumption, an imbalance in cellular oxidation-antioxidant equilibrium can result from engaging in maximal physical activity, which promotes excessive ROS formation (Wang et al., 2021).

Forming free radicals, highly reactive chemical entities containing unpaired electrons requires much energy (Galano & Alvarez-Idaboy, 2019; Lim et al., 2022; Polidori & Mecocci, 2022; Silina et al., 2022). Biological systems typically have a half-life of less than 10−6 seconds for free radicals despite their tremendous reactivity. Although reactive oxygen species (ROS) and other oxygen species usually are non-reactive, they can produce free radicals when exposed to certain conditions (Engwa, 2018).

The biological consequences of reactive oxygen species (ROS), the most prevalent free radicals in the body, have been the subject of much research both in vivo and in vitro (Checa & Aran, 2020; Nakamura & Takada, 2021; Tauffenberger & Magistretti, 2021). When conditions are right, this free radical type can do wonders against infections, inflammation, and cancer. Lipid peroxidation damage to biological membranes and macromolecular materials can occur when the body's antioxidant system becomes dysregulated, which can happen after exposure to diseases or certain exogenous drugs and toxins. This leads to disorders in free radical metabolism. Many reduced oxygen species, including hydroxyl free radicals, superoxide anion, and hydrogen peroxide, are examples of reactive oxygen species (Jomova et al., 2023).

An umbrella term for oxygen-derived free radicals, reactive compounds, and ions is "Reactive Oxygen Species" (ROS) (Ribeiro et al., 2018; Sessa et al., 2020). ROS are organisms that can be useful or harmful depending on their concentration. Avoiding oxidative stress requires regulation of ROS generation and release (Checa & Aran, 2020; Nakai & Tsuruta, 2021). Cells are said to be in a state of "oxidative stress" when these levels surpass defense mechanisms. Biomolecules, including lipids, proteins, and DNA, are vulnerable to damage from elevated ROS levels. Cell death can occur due to changes in intrinsic membrane features such as fluidity, enzyme activity, ion transport, protein cross-linking, DNA damage, and suppression of protein synthesis (Jomova et al., 2023; Nakamura & Takada, 2021; Tauffenberger & Magistretti, 2021).

Exogenous and endogenous reactive oxygen species exist. Some organic molecules in the environment react non-enzymatically with oxygen to form free radicals (Nakamura & Takada, 2021). Free radicals can come from environmental pollutants, cigarette smoke, alcohol, radiation, ozone, UV light, pesticides, anesthetics, pharmaceuticals, industrial solvents, etc (Nakai & Tsuruta, 2021; Sessa et al., 2020). Endogenous sources include enzymatic activities in living organisms that create free radicals. These include the respiratory chain, cytochrome P450, phagocytosis, and prostaglandin production processes. Free radicals are generated via mitochondrial, phagocyte, inflammatory, and arachidonic pathways. Also, iron and transition metal processes, peroxisomes, xanthine oxidase, etc. Producer of endogenous free radicals (Jomova et al., 2023; Tauffenberger & Magistretti, 2021).

The body develops defense systems against foreign and endogenous free radicals to prevent cellular damage. Direct and indirect bodily mechanisms may be involved. First, indirect processes eliminate or transform free radicals without directly acting on them. The natural defense mechanism uses antioxidants to bind or convert free radicals into less reactive forms. This defensive system includes enzymatic and non-enzymatic antioxidants (Engwa, 2018; Galano & Alvarez-Idaboy, 2019; Nakai & Tsuruta, 2021; Qiu et al., 2022).

Glutathione peroxidase is an antioxidant enzyme. The kidneys synthesize glutathione peroxidase, found in practically all organs but mainly in the liver. Cytosol and mitochondria are typical subcellular sites. Eliminating selenium, an enzyme cofactor at the active site, drastically reduces enzyme activity. Glutathione peroxidase oxidizes reduced glutathione (GSH) to break down hydrogen peroxide or lipid hydroperoxides (Jomova et al., 2023; Mironczuk-Chodakowska et al., 2018). Compounds with a low molecular weight antioxidant (LMWA) capacity to inhibit oxidative damage via direct or indirect interactions with reactive oxygen species (ROS) are part of the non-enzymatic system's antioxidant arsenal. Plant extracts are one such source (Engwa, 2018).

For a long time, people have relied on plant-based diets as a source of essential nutrients. Traditional medicine depends on a wide range of plant remedies for various ailments. Many medical systems advocate using plant-based compounds for illness prevention and treatment, a practice rooted in the long-established value of natural goods (Moga et al., 2021). Among these is its role as an antioxidant. An imbalance in the number of free radicals is thought to be the primary cause of most human diseases, which brings the idea of employing plants as antioxidants into sharper focus due to oxidative stress conditions (Engwa, 2018).

Several diseases, including diabetes, cancer, heart disease, atherosclerosis, and aging, have been accelerated by oxidative stress caused by high amounts of free radicals. Because of their involvement in illness prevention, phytochemical substances found in plants and several vitamins can be utilized to avoid this condition. The ability of plants to bind free radicals and protect cells from damage is related to their antioxidant capabilities. Therefore, phytochemicals found in plants are seen as potential substitutes for conventional antioxidants that people can take. The red pomegranate, or Punica granatum, is an example of a plant that has antioxidants.

Traditional red pomegranate orchards can be found in the United States, Afghanistan, China, Iran, India, and Pakistan. Pomegranates play a significant part in people's well-being. Pomegranate's antioxidants and phenolic bioactive components are essential for good health. These chemicals can be discovered in pomegranate juice, peel, and fruit (Qahir et al., 2021). A study by Leesombun et al. found that pomegranate extract contains various beneficial phenolic compounds. These compounds include flavonoids (anthocyanins and catechins), tannins (ellagitannins and ellagic acid derivatives: punicalagin, punicalin, and pedunculagin), and phenolic acids (hydroxycinnamic and hydroxybenzoic acids) (Leesombun et al., 2022).

An alternative treatment option for regulating reactive oxygen species (ROS) during wound healing and increasing the activity of the glutathione peroxidase (GPx) enzyme, which suppresses ROS by neutralizing it—specifically, by changing hydrogen peroxide (H_2O_2) to water—is red pomegranate extract, which contains flavonoid compounds with antioxidant potential. An enzyme known as glutathione peroxidase (GPx) relies on selenocysteine. The most crucial enzyme in cells that transforms H_2O_2 to water is GPx (Strycharz-Dudziak et al., 2019).

The objective of this research was to find out whether female Wistar rats (*Rattus norvegicus*) who had been induced to exercise to their maximal capacity might increase their blood glutathione peroxidase levels by taking an extract from red pomegranates (*Punica granatum*).

2. METHOD

This study employs a methodology standard in experimental quantitative research: a controlled experiment in a controlled environment (Notoatmodjo, 2022). Serious experimental study involves controlling all external variables that can impact experimental activities; this is what we mean when we say an experiment is true. In this work, the effects of oral administration of red pomegranate (*Punica granatum*) extract on boosting blood glutathione peroxidase levels in female Wistar rats (*Rattus norvegicus*) were investigated using a pre-test-post-test control group approach. The rats were subjected to maximal physical exercise.

The female Wistar rats (*Rattus norvegicus*) used in this research were 2-3 months old and weighed 160-200 gr. The number of study samples was determined by Kendall using the 3R Principle (Replacement, Reduction, and Refinement) (Kendall et al., 2018). We shall divide the 24 male Wistar rats into four groups for this experiment. There were six mice in each group. The dependent variable is one of two types of study variables; the other is the independent variable. A variable that is dependent on other factors is called a dependent variable, whereas a variable that is independent of other variables is called an independent variable (Suwarno & Nugroho, 2023). The course of treatment with red pomegranate (*Punica granatum*) extract served as the study's independent variable. The dependent variable is a female Wistar rat's (*Rattus norvegicus*) blood glutathione peroxidase level.

All the necessary equipment was utilized in this investigation, including rat drums, gloves, cannulas, vials, porcelain cups, digital scales, sondes, markers, blenders, pipettes, EDTA tubes, feed

containers, rotary evaporators, ovens, micropipettes, filter paper—two things: a mask and an Eppendorf tube. The list of components includes red pomegranate (*Punica granatum*) extract, high-fat meal, distilled water, 96% ethanol, and female rats of the Wistar strain.

Acclimatizing test animals for seven days at the Animal House, Faculty of Mathematics and Natural Sciences, Medan State University, was the initial study step. Then, we will produce red pomegranate (*Punica granatum*) extract and a phytochemical test (tannin, flavonoid, alkaloid, and steroids/terpenoid).

Then, 4 mL of simplicia stock solution with concentrations of 0, 1.25, 2.5, 5, 10, and 20 ppm was added to the tube to test antioxidant activity. Each tube was incubated for 30 minutes with 1 mL DPPH solution. Each tube was placed in a cuvette and read on a 517 nm UV-Vis spectrophotometer to determine antioxidant activity.

Next, test animal preparation: 28 white mice, 160-200 grams and 2-3 months old, were separated into four groups of six. We believed white mice's stomach volumes were 3-5 ml. Thus, this trial gave red pomegranate extract in 3, 4, and 5 doses. To determine the best dose, this variation is done. During the 14-day treatment, mice swam in a bucket for an hour every morning. After maximum physical activity, mice were cleaned with a towel and rested for 1 hour before receiving red pomegranate extract with a blunt or stomach probe according to dose and group. Sonde is carefully placed in the stomach. Red pomegranate extract is pumped in after verifying that the sonde entered the stomach.

Glutathione peroxidase enzyme activity was measured in plasma on days 0, 7, and 14. The research mice were fasted for 10-12 hours and given distilled water before blood was drawn. Rat orbital vein blood samples were obtained with a one cc hematocrit capillary pipette. Plasma is produced by centrifuging blood at 3,000 rpm for 10 minutes. Pipetting the plasma into another Eppendorf tube prepares it for testing. Then, a reaction mixture containing one mM glutathione, 0.2 mM NADPH, and 0.24 units of glutathione reductase in 0.1 M Tris-HCl buffer (pH 7.2) was used to assess G-Px levels in blood samples spectrophotometrically. Add 0.25 mM H2O2 and measure absorbance at 340 nm for 5 min to start the reaction. GPx is measured in U/mg.

The research data was analyzed using SPSS 25.0 for Windows. The Kolmogorov-Smirnov test ($p > 0.05$) assessed data normality. The significance between groups was tested using a one-way analysis of variance (One-way ANOVA) with a 95% confidence level ($p < 0.05$). The Post Hoc Test with LSD was used for further analysis.

3. RESULTS AND DISCUSSION

Research Result

Table 1. Characteristics of Test Animals

Component	Group P0	Group P1	Group P2	Group P3
Types of Rats	*Rattus norvegicus*			
Gender	Female			
General condition	White fur, healthy and active			
AVG Initial B/W (gr)	198	195	197	194
AVG Final B/W (gr)	195	194	195	191

The DPPH (2,2-diphenyl-1-picrylhydrazyl) technique was used to test red pomegranate (Punica granatum) extract antioxidant activity. The IC50 values for compounds are categorized as strong (50-100), moderate (100-150), weak (151-200), or powerful antioxidants. A higher level of antioxidant activity is associated with a decreased IC50 value (Molyneux, 2004).

The red pomegranate (Punica granatum) extract's IC50 value was calculated from the regression equation $y = ax + b$. The y coefficient in this equation is IC50, while the x coefficient is the extract concentration needed to lower 50% of DPPH radical activity. The computation showed that red pomegranate (*Punica granatum*) extract has an IC_{50} of 8.33ppm.

Table 2. Antioxidant Test

No	Concentration	% Inhibition	IC_{50}
1	1 ppm	4.54	8.33 ppm
2	2 ppm	9.81	
3	4 ppm	23.69	
4	8 ppm	48.21	
Linear Equations = y= 11.974x + 4.061			

Table 3. Phytochemical Test

Secondary Metabolites	Reactor	Color	Result
Flavonoid	Mg + concentrated HCl	Yellow	+
Saponin	Air + HCl	Yellow and foamy	+
Tannin	$FeCl_3$	Blackish green in color	+
Steroid / Triterpenoid	Liebermann-Burchard	Reddish in color	+
Alkaloid	Wagner	Orange in color	+

Phytochemical analyses revealed the presence of secondary metabolite chemicals in red pomegranate (*Punica granatum*) extract. The substances included in this were alkaloids, triterpenoids, tannins, saponins, and flavonoids. These chemicals will subsequently lower malondialdehyde levels in the serum blood of highly active Wistar white rats (*Rattus norvegicus*).

Table 4. GPx levels

No	Group	Repetition	GPx levels	
			Pre-test	Post-test
1	Control Group (P-0) (Aquades)	1st	33.15	47.65
2		2nd	33.67	46.33
3		3rd	34.21	45.13
4		4th	32.65	41.78
5		5th	35.21	47.12
6		6th	32.09	42.34
		Mean	**33.49**	**44.61**
7	Treatment Group P-I (Red pomegranate extract 3 ml)	1st	34.15	60.34
8		2nd	36.33	61.56
9		3rd	38.78	62.33
10		4th	33.58	59.56
11		5th	31.41	62.33
12		6th	33.13	61.09
		Mean	**34.56**	**61.20**
13	Treatment Group P-II (Red pomegranate extract 4 ml)	1st	39.54	68.21
14		2nd	32.71	66.45
15		3rd	40.02	71.23
16		4th	32.26	69.89
17		5th	31.51	70.23
18		6th	33.43	69.88
		Mean	**34.91**	**68.62**
19	Treatment Group P-III (Red pomegranate extract 5 ml)	1st	36.32	78.44
20		2nd	33.12	79.56
21		3rd	31.81	76.23
22		4th	30.76	75.18
23		5th	32.56	77.45
24		6th	36.21	72.25
		Mean	**33.46**	**76.51**

Table 4 shows pre- and post-GPx exam outcomes. GPx (U/mg) is the enzyme needed to oxidize one nmol NADPH per minute in 1 mg of protein. According to study group observations, the control and treatment groups had different blood serum GPx levels. See table for treatment group III's 5 ml dose, which had a lower post-test average of 76.51U/mg. The control group, which exercised vigorously and drank only distilled water, had an average post-test GPx of 44.61U/mg. It outperforms the others. An average post-test result for treatment group I at 3 ml was 61.20U/mg, while treatment group II had 68.62U/mg.

Table 5. Normality Test Results

Group	Extract Dose	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
GPx levels	Control	.176	6	.200*	.957	6	.793
	Treatment P1	.179	6	.200*	.926	6	.548
	Treatment P2	.198	6	.200*	.957	6	.797
	Treatment P3	.140	6	.200*	.966	6	.864

*. This is a lower bound of the true significance

a. Lilliefors Significance Correction

According to Table 5, the Kolmogorov-Smirnov Test determined normalcy. The significance was 0.200. A p-value> 0.05 indicates regularly distributed data (Ghozali, 2018). This implies that the data is regularly distributed. After confirming the data is normally distributed, the Levene test assesses if each variety of the study population group is homogeneous.

Table 6. Test of Homogeneity of Variances

		Levene Statistical	df1	df2	Sig.
GPx levels	Base on Mean	1.281	3	20	.308
	Base on Median	1.190	3	20	.339
	Based on Median and with the adjusted df	1.190	3	15.957	.345
	Based on trimmed mean	1.279	3	20	.309

*. This is a lower bound of the true significance

a. Lilliefors Significance Correction

Table 6 shows the Levene homogeneity test results. The significant column probability is 0.308. Data homogeneity is indicated by a significance probability more prominent than 0.05 (Ghozali, 2018). Thus, the control group, treatment group-1, treatment group-2, and treatment group 3 are homogeneous or have the same variance.

Table 7. Results of the ANOVA Test

		Sum of Squares	df	Mean Square	F	Sig.
GPx levels	Between Groups	3332.745	3	1110.915	234.195	.000
	Within Groups	94.871	20	4.744		
	Total	3427.615	23			

Table 7 shows the One-Way ANOVA test's significant value as 0.000 or less than 0.05 (Ghozali, 2018). These statistics show a substantial difference between the control and treatment groups.

Table 8. Post Hoc Bonferroni Test Results

Dependent Variable: GPx levels

LSD

Test	Experimental Group (I)	Experimental Group (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bonferroni	Control (P0)	(P1)	-16.58833*	1.25745	.000	-19.2113	-13.9653
		(P2)	-24.01500*	1.25745	.000	-26.6380	-21.3920
		(P3)	-31.90500*	1.25745	.000	-34.5280	-29.2820
	Extract	(P0)	16.58833*	1.25745	.000	13.9653	19.2113

Dependent Variable: GPx levels
LSD

Test	Experimental Group (I)	Experimental Group (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
	Dosage 3 ml	(P2)	-7.42667*	1.25745	.000	-10.0497	-4.8037
	(P1)	(P3)	-15.31667*	1.25745	.000	-17.9397	-12.6937
	Extract	(P0)	24.01500*	1.25745	.000	21.3920	26.6380
	Dosage 4 ml	(P1)	7.42667*	1.25745	.000	4.8037	10.0497
	(P2)	(P3)	-7.89000*	1.25745	.000	-10.5130	-5.2670
	Extract	(P0)	31.90500*	1.25745	.000	29.2820	34.5280
	Dosage 5 ml	(P1)	15.31667*	1.25745	.000	12.6937	17.9397
	(P3)	(P2)	7.89000*	1.25745	.000	5.2670	10.5130

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

Table 8, LSD Post Hoc Test is used to determine whether groups have significant differences from other groups. The results of the Post Hoc LSD test analysis in this study showed a significance value smaller than 0.05, which means that all groups had significant differences from other groups (Ghozali, 2018).

Discussion

Research on the effects of red pomegranate (Punica granatum) extract on GPx levels in female Wistar white rats (Rattus norvegicus) given maximum swimming activity for 1 hour. Female Wistar strain white rats (Rattus norvegicus) were divided into four groups: one received distilled water, the other three received red pomegranate extract (Punica granatum) in three doses. The first treatment group received 3 ml of red pomegranate (Punica granatum) extract. Treatment group two received 4 ml of red pomegranate (Punica granatum) extract. The final group received 5 ml of red pomegranate (Punica granatum) extract. Researchers next tested different therapies on each group. The goal was to determine which red pomegranate (Punica granatum) extract dose increased GPx levels in white Wistar rats (Rattus norvegicus) following maximum physical exercise.

Regular physical activity has positive effects on health. But it has nasty side effects that could hurt your health if you do it too much. According to research, oxidative stress increases, and antioxidant levels decrease during maximal physical exercise (Powers et al., 2020). One type of free radical that the body encounters frequently is reactive oxygen species (ROS). When conditions are right, this free radical type can do wonders against infections, inflammation, and cancer. However, the body's antioxidant system can get dysregulated and lead to extreme metabolic imbalance diseases under specific circumstances, like after being exposed to illness or certain exogenous medications and poisons. The body has safeguards to prevent cellular damage in reaction to free radical levels, which can originate from external or internal sources. The antioxidants used by this defense system either bind free radicals or transform them into less reactive forms, exerting direct action against free radicals. Antioxidants, both enzymatic and non-enzymatic, comprise this protective mechanism (Engwa, 2018).

Enzymes like glutathione peroxidase (GPx) are beneficial antioxidants. Decomposing hydrogen peroxide and other species, such as lipid hydroperoxides and glutathione peroxidase, facilitates the oxidation of reduced glutathione (GSH) (Engwa, 2018). There are several ways in which plant phytochemicals and some vitamins might protect cells against free radical damage and associated illnesses. As a result, phytochemicals found in plants are now thought to be the best long-term option for supplementing the human body's natural defenses against oxidative stress. The red pomegranate, or Punica granatum, is an example of a plant that has antioxidants. The health benefits of pomegranate cannot be overstated. There is strong evidence that the pomegranate's bioactive phenolic content and antioxidants benefit human health. One possible antioxidant role for the flavonoid chemicals found in red pomegranate extract is to improve antioxidant defenses and neutralize reactive oxygen species (ROS). To corroborate this theory, scientists started investigating whether or not red pomegranate

(Punica granatum) extract might raise GPx levels in Wistar white rats (Rattus norvegicus) during intense swimming exercise.

This study's trial phase lasted 14 days of observation and generated data that required processing and testing, necessitating many data analyses. It all starts with a normal test. We use SPSS's Kolmogorov-Smirnov test to check if the collected data is normally distributed. The outcome is that all test groups' data follows a normal distribution, with a significance level of 0.000. Therefore, the data can be said to follow a normal distribution or represent the population as a whole.

The homogeneity test comes up next. This test must be administered to determine if the study participants are representative of a normally distributed population. The obtained results indicate a significance level of 0.308. It may be inferred that the control and treatment groups 1, 2, and 3 represent the same population, as the resulting significant probability value is greater than 0.05. The One-Way ANOVA test was used to assess the effectiveness and significance of this homogeneous and normally distributed data.

The significance level that emerged from this test is 0.000, which is higher than the threshold of 0.05. The results show that there are statistically significant differences between the three groups (control, treatment 1, and treatment 2); hence, post hoc LSD is required as a follow-up test. If there were significant variations in the mean GPx levels between the groups, we ran additional tests using post hoc LSD. All groups differed significantly from one another, according to the study's Post Hoc LSD test results, which had a significance value of 0.000, which is less than 0.05.

The results indicate that after performing maximal physical activity and receiving therapy with red pomegranate (Punica granatum) extract and distilled water, the GPx levels of the mice in each group increased. The variation in average post-test values reveals the variation in the growth of GPx levels. On average, the GPx level was 44.61 U/mg in the control group that received only distilled water. The average value for treatment group 1, which got 3 milliliters of red pomegranate (Punica granatum) extract, was 61.20 U/mg. The average result for treatment group 3 was 76.51 U/mg, while treatment group 2 received an average of 68.62 U/mg from a 4 ml dose. After reaching their maximal aerobic capacity, white Wistar rats (Rattus norvegicus) were shown to have higher glutathione peroxidase levels when given 5 ml of red pomegranate (Punica granatum) extract, according to this comparison.

According to results from the study group that received red pomegranate extract (Punica granatum). They enhanced GPx levels compared to the group who received distilled water alone. This is because components, including tannins, alkaloids, steroids, triterpenoids, and flavonoids, are present in the red pomegranate (Punica granatum) extract, which contains antioxidants. The antioxidant properties of these bioactive substances have been demonstrated by Adeeyo et al. (2021). Because of their antioxidant properties, antioxidants can aid the body in obtaining more antioxidants to combat free radicals generated by intense physical exertion (Adeeyo et al., 2021). This evidence suggests that red pomegranate (*Punica granatum*) extract increases GPx levels in Wistar white rats (*Rattus norvegicus*) after intense exercise.

4. CONCLUSION

This study's findings about secondary metabolite components in red pomegranate (*Punica granatum*) extract were as follows: flavonoids, triterpenoids, saponins, alkaloids, and tannins. At the end of a vigorous workout, these chemicals raise the amount of glutathione peroxidase in the blood serum of Wistar white rats (*Rattus norvegicus*). According to the findings, pomegranate extract (*Punica granatum*) possesses antioxidant activity that falls within the powerful category, with a concentration of 8.33 ppm. The study found that 5 ml of red pomegranate (*Punica granatum*) extract was more effective than other doses when comparing average glutathione peroxidase levels. Therefore, it is clear that after engaging in vigorous swimming for an hour, female Wistar white rats (*Rattus norvegicus*) can have their glutathione peroxidase levels increased by red pomegranate (*Punica granatum*) extract. Experiments and observations suggest that when taken orally, red pomegranate (Punica granatum) extract can considerably raise glutathione peroxidase levels. A

comprehensive analysis of the compounds found in red pomegranate (*Punica granatum*) extract is required for future studies, as is the development of novel functional food components that may be transformed into a marketable product and evaluated in human trials. Further testing for malondialdehyde (MDA) levels, a biomarker for free radical presence, is also required.

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