Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
30 (1): 107-115, 2024
DOI: 10.9775/kvfd.2023.30826

Research Article

Evaluation of the Impact of Long-term Treadmill Exercise on Antioxidant Capacity and Immune Function in Mice

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How to cite this article?
Liu X, Wang X, Wang T: Evaluation of the impact of long-term treadmill exercise on antioxidant capacity and immune function in mice. *Kafkas Univ Vet Fak Derg*, 30 (1): 107-115, 2024.
DOI: 10.9775/kvfd.2023.30826

Article ID: KVFD-2023-30826
Received: 04.10.2023
Accepted: 06.12.2023
Published Online: 11.12.2023

Abstract

Regular exercise is known to confer various health benefits, but the specific impact of exercise intensity on antioxidant capacity and immune function remains complex and incompletely understood. Our study investigates the impact of exercise intensity on antioxidant capacity and immune function in mice. Mice were subjected to various exercise intensities (low, moderate, high) on a treadmill. Antioxidant enzyme activity, oxidative stress markers, ROS production, hemoglobin, hematocrit, leukocyte counts, lymphocyte subsets, and phagocytic activities were assessed. Exercise intensity influenced antioxidant enzyme activity, with low and high intensities showing significant effects. High-intensity exercise led to increased lipid peroxidation but improved the glutathione redox ratio. Exercise, irrespective of intensity, significantly affected immune function, notably increasing leukocyte counts, T-cell and B lymphocyte subsets, and phagocytic activities. Even at lower intensities, exercise profoundly impacts antioxidant capacity and immune function. These findings highlight the relationship between exercise, oxidative stress, and immune modulation.

Keywords: Antioxidant activity, Immune response, Mice, Treadmill exercise

Introduction

Physical exercise contributes to a healthy lifestyle by improving cardiovascular fitness, muscle strength, and weight management [1]. Recent research has increasingly focused on the broader implications of exercise, including its potential impact on the body's ability to combat oxidative stress and bolster immune defenses [2,3]. Adwas et al.[4] suggested that oxidative stress occurs when there is an imbalance between the production of harmful reactive oxygen species (ROS) and the body's ability to neutralize them with antioxidants. Prolonged oxidative stress can lead to cellular damage and contribute to the development of various diseases, including cancer and cardiovascular disorders. Antioxidants play a crucial role in mitigating the harmful effects of oxidative stress [5-9].

The immune system is a complex network of cells and molecules that defend the body against infections and diseases. An efficient immune system is vital for maintaining overall health and well-being [10]. The potential link between exercise, antioxidant capacity, and immune function is an area of growing interest in scientific research.

Long-term treadmill exercise in mice has been the subject of extensive research with significant findings regarding its effects on antioxidant capacity and immune function [11-13]. Research has indicated that prolonged treadmill exercise increases the body's various antioxidants [14]. These antioxidants, including superoxide dismutase (SOD), catalase, and glutathione peroxidase, are crucial in neutralizing harmful reactive oxygen species (ROS), thus mitigating oxidative stress.

Exercise has been associated with a reduction in markers of oxidative stress. The decrease in oxidative stress markers encompasses a lower concentration of ROS and a decrease in lipid peroxidation products [15]. Such a reduction is vital as it helps prevent cellular damage and mitigates the risk of numerous chronic conditions linked to oxidative stress. Moreover, long-term treadmill exercise has positively impacted immune function [16]. Studies have observed that exercised mice exhibit improved immune responses

when subjected to specific antigens or pathogens [17]. This enhancement suggests that exercise may bolster the ability of the immune system to mount effective defenses against infections. Exercise triggers a cascade of physiological responses contributing to improved antioxidant capacity and immune function [14,18]. Regular exercise promotes mitochondrial biogenesis, enhancing cellular energy production. This increase in mitochondrial activity can enhance the body's capacity to neutralize ROS and reduce oxidative stress.

Exercise also promotes the release of anti-inflammatory cytokines, which can modulate the immune response. This modulation is essential for ensuring that the immune system functions optimally, responding effectively to threats while avoiding excessive inflammation, which can be harmful [3]. Furthermore, exercise induces the release of endorphins, which enhance mood and immune regulation. These chemicals positively influence the immune system's activity. In addition to these mechanisms, exercise promotes better circulation, ensuring that antioxidants and immune cells are efficiently transported throughout the body [19]. Improved blood flow facilitates the delivery of these essential components to areas where they are needed most.

Long-term treadmill exercise initiates a series of interconnected events culminating in increased antioxidant capacity and enhanced immune function in mice. Understanding these mechanisms is crucial for advancing our knowledge of exercise physiology and potentially translating these findings into strategies that promote better human health and well-being. Our study focuses on the impact of long-term treadmill exercise on antioxidant capacity and immune function in mice. Previous research has consistently shown that sustained exercise can enhance antioxidant levels, reduce oxidative stress, and improve immune responses in these animals. Complex mechanisms drive these improvements, including increased mitochondrial activity, the release of anti-inflammatory cytokines, and endorphin's influence. These processes collectively underpin the observed benefits of exercise on antioxidant defenses and immune function.

Our primary objective is to investigate the physiological changes caused by long-term treadmill exercise and understand the underlying mechanisms. It may inform strategies to improve health and resilience against oxidative stress and infections in mice.

Material and Methods

Ethics

The Ethics Committee of Hebei Provincial Hospital approved our study and experimental techniques and conducted according to the ethical guidelines on animal experiments in China (Approval no. NK20221008A07)

Animals

Our study acquired 48 three-week-old Kyoto Wistar rats, consisting of 24 males and 24 females, at the start of the experiment. We chose these rats at their current age due to their rapid growth rate, and we estimated that they would be adults at the end of our study, making it suitable for comparative analyses. Moreover, nutritional interventions during this period were associated with sensitive changes in the immune system.

Then, animals were kept in cages (4 per cage) at climate-controlled conditions of 23ºC temperature, 50% humidity, and a day/night cycle of 12 h. Furthermore, they were subjected to a standard diet and supplied with water and food ad libitum. The animals were weighed at the end of every week, and the ponderal homogeneity index was determined and recorded. After the first ten days of acclimatization to these laboratory conditions, the animals were randomly assigned to one of the four experimental groups, with each group consisting of 12 animals; the sedentary group consisted of the non-exercising group that was sacrificed after 28 weeks of training; the low-intensity exercise group was sacrificed at 32 weeks of training; the moderate intensity exercise group consisted were sacrificed at 48 weeks of training and the high-intensity exercise training group were sacrificed at 54 weeks of training.

Exercise Training Program

All animals were subjected to physical exercises at eight weeks of age *(Fig. 1)*. The aerobic treadmill exercise consisted of running on the treadmill for 40 minutes per day, seven days a week. The maximal speed was set to 80% during the 28-, 32-, 48- and 54-week training sessions. The treadmill was inclined at 0º for all experimental groups. At the optimum speed of the treadmill, a speed test was carried out to determine the average running speed of the animals. The exercises consisted of a warm-up on the treadmill for 10 min at 15 m per min, after which the speed was increased gradually to 8 m/min every 60 sec until the animals could not maintain the treadmill belt's pace.

The maximal running speed was estimated as the last speed recorded for at least 60 sec, and the initial weeks of training allowed the animals to adapt to the training exercise. The adaptation was set so every animal could attain the maximum speed before performing subsequent exercises. In the subsequent training exercises, the running speed was slowly increased until the maximal speed was attained every four weeks while maintaining the intensity at 80%. Animals in the sedentary group were placed on a

stationary treadmill to have the same handling effect as those in other exercising groups.

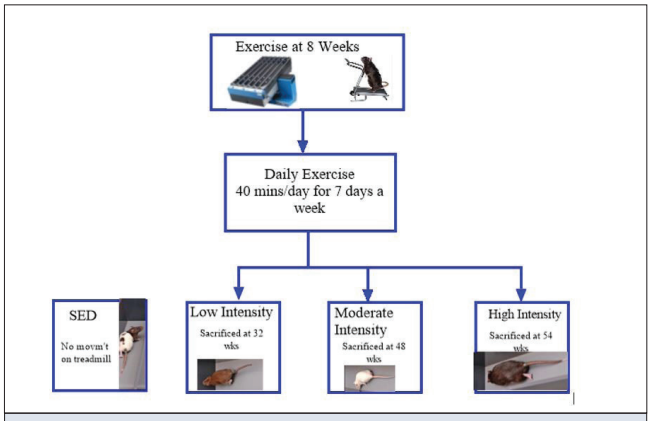

Fig 1. Illustration of the exercise training program

Sample Collection

All animals were anesthetized using intraperitoneal injections of Xylazine and Ketamine at 5 mg/kg of body weight before exsanguination to collect blood samples and tissues. The samples were collected at 28 weeks, 32 weeks, 48 weeks, and 54 weeks. The blood, liver, spleen, and peritoneal macrophages were harvested and processed. The blood was extracted from the heart tissues and ethylenediaminetetraacetic (EDTA) anticoagulated tubes. The EDTA blood samples facilitated the determination of the number of leukocytes, hematocrit, and hemoglobin. Moreover, they were used in characterizing the composition of blood lymphocytes. The liver and peritoneal macrophages were frozen to analyze posterior oxidative stress.

Determining Oxidative Stress

Two grams of frozen liver samples were added to 15% (w/v) of ice-cold phosphate buffer (30 mM, pH = 7.0) before mixing for 5 min to achieve a homogenous mixture. The mixture was applied to different centrifugation cycles at 5ºC: 2000x g for 15 min, 10000x g for 20 min, and 16000x g for 30 min. After that, the supernatant was obtained to estimate the level of antioxidant enzymes and reduce the levels of glutathione and oxidized glutathione ratios. Lipid peroxidation was determined using pellets extracted from the mitochondrial fraction during the initial phase of the centrifugation process. The biuret technique and bovine serum albumin were used to determine the total protein content of the supernatant and the pellets. All antioxidant enzyme assays were performed at room temperature.

Superoxide dismutase (SOD) activity was evaluated spectrophotometrically at 560 nm using the xanthine-xanthine oxidase system. The assays were performed in a modified potassium phosphate buffer (40 mM, pH 7.0) containing EDTA (1.5 mM), hypoxanthine (12 mM), nitroblue tetrazolium chloride (NBT, 12 mM), and approximately 0.3 mg of protein from the enzymatic extract. Catalase (CAT) activity was determined polarographically using a Clark-type oxygen electrode. The reaction occurred in a modified potassium phosphate buffer (45 mM, pH 7.2) with hydrogen peroxide (9.5 M). 0.35 mg of protein from the samples was added to initiate the reaction. Glutathione peroxidase (GPx) activity was assessed spectrophotometrically at 340 nm. The assay involved a modified potassium phosphate buffer (90 mM, pH 7.2), EDTA (1.2 mM), reduced glutathione (GSH, 110 mM), glutathione reductase, NADPH (11 mM), and 0.35 mg of protein from the enzymatic extract. Glutathione reductase (GR) activity was measured spectrophotometrically at 340 nm under magnetic stirring. The reaction system consisted of a modified potassium phosphate buffer (95 mM, pH 7.0), EDTA (0.6 mM), NADPH (11 mM), and 1.4 mg of protein from the enzymatic extract. The addition of oxidized glutathione (GSSG, 120 mM) initiated the kinetic reaction.

Nonspecific lipid peroxidation (LOP) levels were assessed by quantifying lipid peroxides as thiobarbituric acid reactive substances (TBARS). The hepatic lipid extract was combined with 1 mL of thiobarbituric acid (TBA) reagent (0.32% (m/v) TBA, 35% (m/v) trichloroacetic acid (TCA), and 0.012% (m/v) butylated hydroxytoluene (BHT)). This mixture was heated at 95°C for 12 min and then rapidly cooled by immersion in an ice bath. Subsequently, the mixture was centrifugated at 1500× g for 8 min, at 5°C, and the resulting supernatant was collected for further analysis.

Determination of Phagocytic Activity

The process was conducted through flow cytometry analysis, focusing on blood monocytes and granulocytes, using the PhagotestTM kit. Initially, blood was incubated with fluorescein isothiocyanate (FITC)-labeled *Escherichia coli* for 8 minutes at 38°C. Subsequently, the samples were placed on ice, and a quenching solution was added to halt phagocytosis. After a washing step to remove excess reagents, erythrocytes were lysed. Following DNA staining, data acquisition was performed using the Gallios™ Cytometer, and subsequent analysis was conducted utilizing FlowJo v. ten software. Our analysis employed forward-scatter and side-scatter characteristics to select monocyte and granulocyte populations. The quantification of phagocytic monocytes and granulocytes relied on assessing the percentage of FITC+ cells. Additionally, we measured their phagocytic activity using the mean fluorescence intensity (MFI). In each experiment, we compared the proportion of phagocytic cells and their relative phagocytic activity to that of the sedentary (SED) group, with the data from sedentary rats serving as the reference point at 100%.

Lymphocyte Composition in Blood

Blood lymphocyte subsets were identified after erythrocyte osmotic lysis using specific antibodies (mouse anti-rat NKR-P1A, CD45RA, TCRαβ, or TCRγδ) conjugated to FITC. Cells were incubated with saturating antibody concentrations (4°C, 20 min), followed by fixation (0.5% p-formaldehyde) and storage (4°C) until flow cytometry analysis. Each cell sample included a blank control. Data were collected using a Gallios™ Cytometer (Beckman Coulter, Miami, FL, USA). Blood lymphocytes were quantified as subset counts, considering the lymphocyte number from the hematology analyzer and the subset percentages obtained via flow cytometry.

Statistical Analysis

All analyses were carried out in GraphPad Prism version 9.5.1. Descriptive statistics were presented as mean ± SD (standard deviation) for the four experimental groups. We assessed the equality and normality of the results using Levene's and Shapiro-Wilk's tests, respectively. Once these conditions were confirmed, we applied a one-way ANOVA test to evaluate parameters such as phagocytic monocyte and granulocyte proportions, phagocytic activities, and ROS production. In cases where significant differences were observed, we performed Bonferroni's post hoc test to make specific group comparisons.

Variables that did not meet parametric assumptions, such as hemoglobin concentration, hematocrit, number of leucocytes, lymphocytes, monocytes, and granulocytes in the blood, were analyzed using non-parametric tests such as the Kruskal-Walli's test followed by the Mann-Whitney U test to determine significance.

We conducted a repeated measures ANOVA test to analyze time-dependent parameters, including body weight (BW). When comparing two groups, such as males and females, or performance within training, we used unpaired or paired Student's t-tests as needed. We applied Pearson or Spearman correlation tests to assess correlations between variables, considering data normality and equality. We considered differences significant when $P<0.05$.

Results

Baseline Parameters of Body Weight, Food, and Water

We minimized animal stress by measuring animals' body weight, food, and water consumption per week to comprehend their general conditions during the exercise. *Table 1* shows the initial Weight, final Weight, ponderal gain, and average liver weight of all animals in the four experimental groups.

Table 2 displays a standard comparison of the Mean food and water consumption at the experimental protocol's beginning and end.

Performance of Training Groups

Our study evaluated the performance of males and females across the four groups during the training programs. The final exhaustion tests revealed no significant differences between males and females based on the highest distance covered *(Fig. 2)*. A visual analysis of the animals while on the treadmill did not reveal significant differences based

Table 1. *Ponderal homogeneity index (PH), initial and final animal body weights (g), ponderal gain (PG), and mean relative liver weight for all experimental groups*

Groups	PH	Initial Weight (g)	Final Weight (g)	PG	Average Liver Weight (g)
SED	0.53	152.7±12.4	461.7±25.8$^{\dagger,\&}$	0.55±0.03$^{\dagger,\&}$	0.014±0.03
Low Intensity	0.46	156.3±12.2	394.3±12.5$^{*,\&}$	0.50±0.03$^{*,\&,\#}$	0.017±0.01$^{\&,\#}$
Moderate Intensity	0.53	142.9±12.4$^{*,\dagger,\#}$	536.2±11.6$^{*,\dagger,\#}$	0.64±0.02$^{*,\dagger,\#}$	0.010±0.05
High Intensity	0.45	145.6±10.65	429.6±27.7$^{\&}$	0.55±0.02$^{\dagger,\&}$	0.013±0.02

*The values are presented as means ± SD. * P<0.05 compared with SED; † P<0.05 compared with Low Intensity; & P<0.05 compared with moderate intensity; # P<0.05 compared with high intensity; SED (Sedentary Group)*

Table 2. *A standard comparison of the Mean food and water consumption at the experimental protocol's beginning and end*

Groups	Initial Food Consumption (g)	Final Food Consumption (g)	Initial Water Consumption (g)	Final Water Consumption (g)
SED	15.4±0.1	16.2±2.6	20.1±1.4	15.3±2.6
Low Intensity	14.5±5.1	18.4±0.6	19.9±0.5	22.8±1.4$^{\&,*}$
Moderate Intensity	13.9±0.5	18.6±2.6	18.6±0.8	15.1±0.3
High Intensity	14.9±0.8	16.7±0.7	16.3±1.5$^{\&,\dagger,*}$	19.9±1.3

*The values are means ± SD. * P<0.05 compared with SED; † P<0.05 compared with Low Intensity; & P<0.05 compared with moderate intensity, # P<0.05 compared with high intensity; SED (Sedentary Group)*

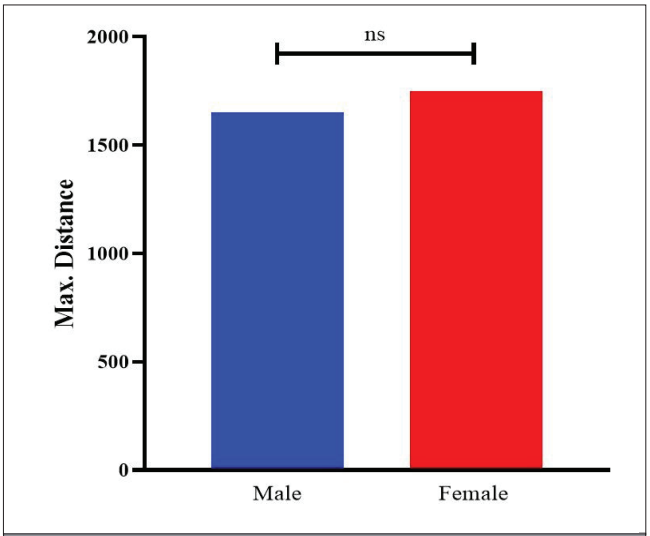

Fig 2. Effect of exercising on the treadmill on the final exhaustion tests

on gender and groups on the level of adaptation to the treadmill.

Antioxidants

Table 3 displays hepatic antioxidant enzyme activity.

Table 4 displays oxidative stress markers: LPO and the GSH/GSSG ratio.

Production of ROS by Macrophages

We analyzed the production of ROS by peritoneal macrophages and showed that the high-intensity exercise group generated the highest levels of ROS compared to other groups *(Table 5)*. Moreover, the ROS was higher in group 3 compared to group 2 ($P<0.05$) and group 2 compared to group 1 ($P<0.05$). These differences in the levels of ROS were statistically significant ($P<0.01$).

Table 5 presents ROS production at 40 and 80 min.

Concentration of Haemoglobin, Haematocrit, and Leukocyte after Exercise

We evaluated the hemograms of experimental groups to identify the effects of exercise intensity on white and red blood cell levels. Our findings showed a significant increase in hemoglobin (HGB) and hematocrit (HCT) in the low-intensity group compared to the sedentary group. Moreover, there were statistically significant differences associated with a decrease in HGB and HCT between groups 3 (moderate intensity) and 4 (high intensity) in comparison to group 1; the levels of HGB and HCT significantly reduced ($P<0.05$) *(Fig. 3)*.

Our analysis of the count of white blood cells *(Fig. 4)* showed that in the high-intensity group, there was two-fold increase in the number of leukocytes, monocytes, and lymphocytes compared to the sedentary group ($P<0.05$).

Table 3. *The activity of hepatic antioxidant enzymes*

Antioxidant Enzymes	SED	Low Intensity	Moderate Intensity	High Intensity
SOD (U.min^{-1}.mg^{-1} protein)	2.19±0.18	2.76±0.11*	2.36±0.21†	2.69±0.04*
CAT (mmol H_2O_2.min^{-1}.mg^{-1} protein)	0.41±0.051	0.48±0.06	0.15±0.010*,†	0.25±0.03*,$^{\dagger,\&}$
GPx (µmol NADPH oxidized.min^{-1}.mg^{-1} protein)	419.4±22.0	353.7±30.6*	371.9±40.9*	332.4±22.6*
GR (µmol NADPH oxidized.min^{-1}.mg^{-1} protein)	30.44±2.33	24.58±0.71*	20.89±2.36*,†	19.73±0.13*,†

*The values are means ± SD (n = 7), with two replicates. * P<0.05 compared with SED; † P<0.05 compared with Low Intensity; & P<0.05 compared with moderate intensity, # P<0.05 compared with high Intensity; SED (Sedentary Group), Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione reductase (GR)*

Table 4. *Oxidative stress markers: lipid peroxidation (LPO) and ratio GSH and GSSG*

Oxidative Stress Markers		SED	Low Intensity	Moderate Intensity	High Intensity
LPO (µM MDA. mg^{-1} protein)	Total Fraction	0.053±0.02	1.16±0.26*	0.61±0.11*,†	0.53±0.11*,†
	Mitochondrial Fraction	0.41±0.14	1.24±0.26*	0.53±0.06†	0.33±0.91†
GSH/GSSG		3.06±0.25	2.68±0.16	3.67±0.91	4.69±1.19*,†

*The values are means ± SD, with two replicates. * P<0.05 compared with SED; † P<0.05 compared with Low Intensity; & P<0.05 compared with moderate intensity, # P<0.05 compared with high intensity; SED (Sedentary Group)*

Table 5. *Production of ROS after 30 and 60 mins*

Time	SED Group	Low Intensity	Moderate Intensity	High Intensity
40 min	5773±934.6	9915±1122.2*,#	10620±986.4#	11087±1001.2*,#
80 min	7026±837.1	10593±1329.1*,#	12587±1255.1#	13004±1342.2*,#

*The values are means ± SD (n = 7), with two replicates. * P<0.05 compared with SED; # P<0.05 compared with high intensity; SED (Sedentary Group)*

Similarly, there was significant increase in groups 3, and 2 compared to group 1 (P<0.05). There were no significant changes in the number of granulocytes across the four groups (P<0.05).

We analyzed the major subsets of lymphocytes in the blood of animals in each group *(Fig. 5)*. There were statistically significant differences in the number of T-cells between the high-intensity and sedentary groups (P<0.05). Moreover, the moderate-intensity and low-intensity groups showed a significant increase in the count of T cells and B lymphocytes (P<0.05).

The effects of exercise training on phagocytic activities showed a statistically significant increase from the sedentary group (baseline) to the high-intensity group *(Fig. 6)*. The baseline percentage of blood phagocytic monocytes and granulocytes was 45% (group 1), which was used as a reference compared to other groups. We noted an increase of 170% within 36 hours of completing the tests in group 4 (P<0.01), 140% after completing tests in group 3 (P<0.01), and 115% after completing tests in group 1 (P<0.01).

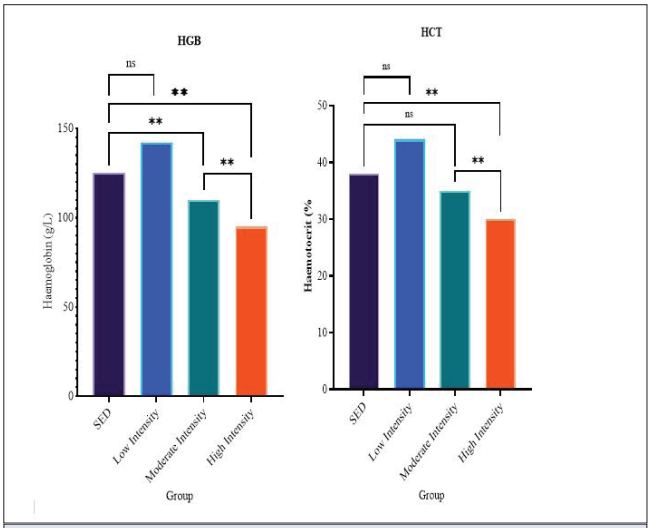

Fig 3. Haemoglobin, Haematocrit, and Leukocyte concentration after exercise, **P<0.05

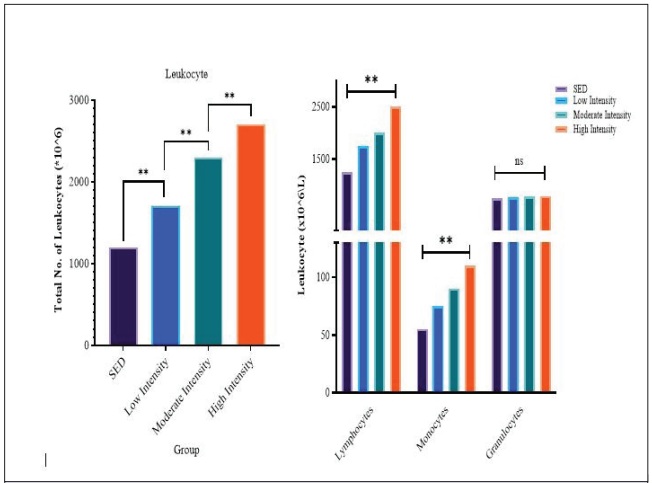

Fig 4. Analysis of the variations in the number of leukocytes per liter, ** P<0.05

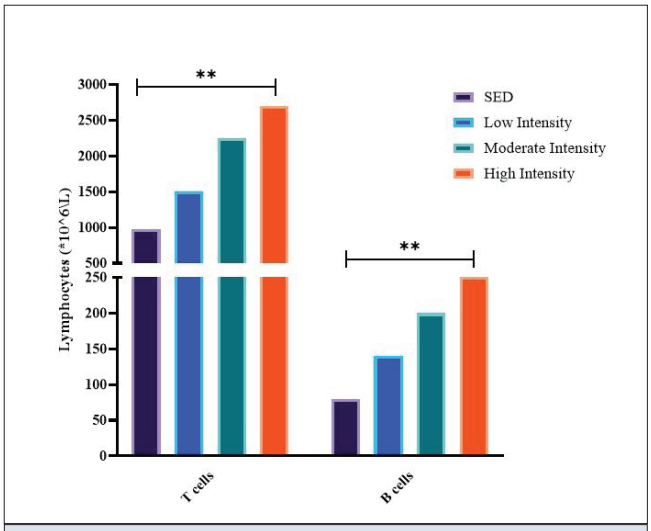

Fig 5. The variations in the levels of lymphocytes across the different groups, ** P<0.05

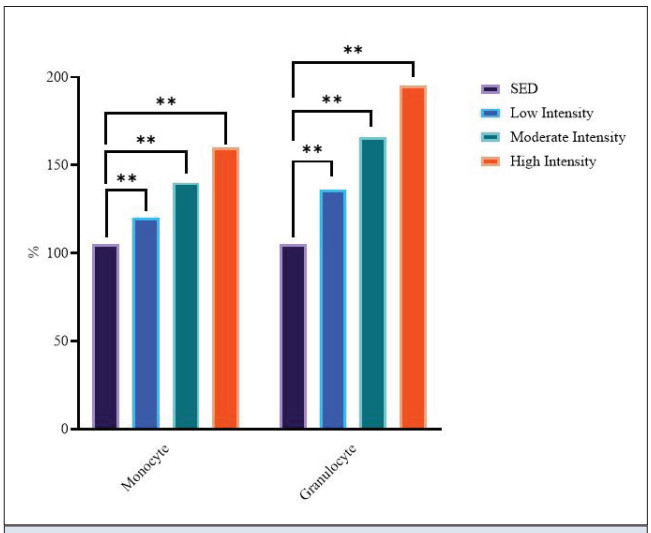

Fig 6. Changes in phagocytic monocytes and Granulocytes across the four groups, ** P<0.05

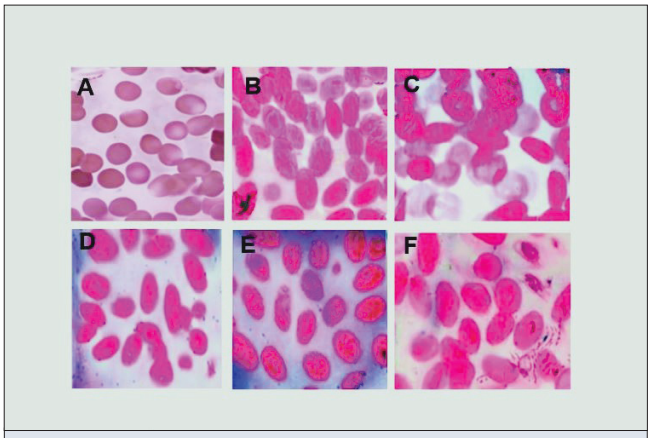

Fig 7. Circulating Lymphocytes across the experimental groups

According to *Fig. 7*, Section A shows the circulating lymphocytes after smearing with Wright's stain, (B) heterophils, (C) Large and small lymphocytes, (D) Erythrocytes and Monocytes, (E) Eosinophils, (F) Basophils. They have quiescent circulation with significantly reduced cytoplasm. The entire cytoplasmic volume is filled with the nucleus whose chromatin is compact and coiled with varying levels of density.

Discussion

Our study investigated the impact of various treadmill exercise intensities on antioxidant enzyme activity, oxidative stress markers, and ROS production in mice. Low and high-intensity exercise increased hepatic antioxidant enzyme activity, specifically in SOD and CAT, while moderate-intensity exercise showed variable effects. Conversely, all exercise intensities resulted in reduced GPx and GR activities. Low and high-intensity exercise groups exhibited elevated lipid peroxidation (LPO), indicating oxidative damage. In contrast, high-intensity exercise positively influenced the GSH/GSSG ratio, suggesting a more reduced cellular environment. ROS production was consistently higher in exercise groups compared to sedentary mice, regardless of intensity.

Our findings were consistent with Ghane et al.[20], who proposed that the increased hepatic antioxidant enzyme activity in SOD and CAT, with low and high-intensity treadmill exercise, can be attributed to the body's response to increased oxidative stress during exercise. Similarly, Thirupathi et al.[15] showed that exercise generates ROS as a natural byproduct of increased metabolic activity. To counteract the potential damage caused by these ROS, the body upregulates the production of antioxidant enzymes like SOD and CAT. SOD converts superoxide radicals into hydrogen peroxide, while CAT neutralizes hydrogen peroxide into water and oxygen. The elevated enzyme activity observed manifests the adaptive response to exercise-induced oxidative stress aimed at preserving cellular integrity.

Valado et al.[21] postulated that the reduction in hepatic glutathione peroxidase (GPx) and glutathione reductase (GR) activities following exercise reflects a more complex interplay of molecular mechanisms. GPx and GR are critical components of the glutathione redox cycle, detoxifying hydrogen peroxide and maintaining cellular redox balance. Exercise-induced changes in GPx and GR may result from adaptations to decreased hydrogen peroxide levels or a shift in alternative pathways utilization during exercise [22]. These alterations in enzyme activity could represent an adaptive response to maintain redox homeostasis in the face of increased exercise-related demands. However, further research is required to elucidate the precise molecular mechanisms at play.

The elevated LPO observed in low and high-intensity exercise groups indicates oxidative damage to cell membranes. Zhou et al.[23] showed that increased metabolic activity generates more ROS during exercise, which can target cellular lipids. ROS can initiate chain reactions, leading to the peroxidation of lipids, ultimately compromising cell membrane integrity. The higher LPO levels suggest that exercise intensity influences the extent of oxidative damage. However, the precise molecular pathways linking exercise intensity to LPO levels and the modulation of lipid peroxidation require further investigation. Our findings aligned with Alizadeh et al.[24], who showed that the high-intensity exercise group's improved glutathione redox ratio (GSH/GSSG) suggests a more reduced cellular environment, this ratio reflects the balance between reduced (GSH) and oxidized (GSSG) forms of glutathione, a vital cellular antioxidant. High-intensity exercise may stimulate the synthesis of GSH or enhance its recycling, reducing GSSG levels and elevating the GSH/GSSG ratio. This molecular response improves cellular redox balance during high-intensity exercise, potentially mitigating oxidative stress.

The higher ROS production in exercise groups at both 40 and 80 min is an expected outcome of increased metabolic activity during physical exertion. Exercise elevates oxygen consumption, leading to more significant ROS generation in mitochondria, the primary site of ROS production [25,26]. Molecular mechanisms behind this phenomenon involve increased electron transport chain activity, which can leak electrons and generate ROS. Furthermore, releasing pro-inflammatory cytokines during exercise can stimulate immune cells to produce ROS. These molecular events collectively contribute to higher ROS levels during exercise, highlighting the complex relationship between physical activity and oxidative stress.

Our study showed that high-intensity exercise significantly increased T-cell counts and phagocytic activities, indicating a robust immune response. Even moderate and low-intensity exercise led to notable increases in T-cells, B lymphocytes, and phagocytic activities, underscoring the immune-boosting potential of physical activity. Exercise can stimulate the release of stress hormones, such as cortisol and catecholamines (e.g., epinephrine), as part of the body's "fight or flight" response. These hormones play a pivotal role in mobilizing immune cells. For instance, cortisol can trigger the release of stored T-cells from the spleen and lymph nodes into the bloodstream [27,28]. T-cells are key players in cell-mediated immunity and recognize and target infected or abnormal cells [29]. The increase in T-cell counts suggests that high-intensity exercise can stimulate the proliferation or mobilization of T-cells, potentially enhancing the body's ability to mount an immune response against pathogens or aberrant cells [30].

Similarly, catecholamines can enhance the trafficking of immune cells, including B lymphocytes, by increasing their adhesion to blood vessel walls and promoting their migration to areas of potential infection [31,32]. This hormonal response contributes to increased T-cell and B lymphocyte counts. The elevated counts of both T-cells and B lymphocytes suggest that exercise, irrespective of intensity, can enhance both arms of the immune system. The molecular mechanisms driving these changes may involve releasing factors like interleukins and growth factors, which support the proliferation and activation of immune cells.

Exercise-induced inflammation is a crucial driver behind the increased phagocytic activity of immune cells. Zhou et al.[33] suggested that physical activity can lead to the release of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). These cytokines act as signaling molecules that alert immune cells to potential threats. They also enhance the phagocytic abilities of monocytes and granulocytes. IL-6, for instance, can stimulate the production of acute-phase proteins, including C-reactive protein (CRP), which can bind to pathogens and facilitate their engulfment by phagocytes. Additionally, TNF-α can activate immune cells, making them more efficient at phagocytosis. Combining these cytokine-driven processes amplifies phagocytic activity, helping the body defend against invading micro-organisms [33].

Exercise-induced changes in hemoglobin levels can be linked to the production of erythropoietin (EPO), a hormone that regulates red blood cell production. Similarly, Tomczyk et al.[34] showed that during exercise, there is an increased demand for oxygen transport to active muscles. This stimulates the kidneys to release EPO, which, in turn, promotes the production of red blood cells in the bone marrow. These newly formed red blood cells are rich in hemoglobin, the oxygen-carrying protein. The increase in hemoglobin levels observed in response to exercise reflects this adaptive response aimed at improving oxygen-carrying capacity to meet the heightened oxygen demands of working muscles.

In conclusion, our study found different responses to exercise, affecting antioxidant capacity and immune function. Exercise intensity influenced antioxidant enzyme activity, with low and high intensities demonstrating significant effects. However, exercise resulted in increased lipid peroxidation, especially at high intensity. The glutathione redox ratio improved at high intensity, indicating better redox balance. Immune function had dynamic responses with exercise, even at lower intensities, significantly affecting leukocyte counts, subsets of lymphocytes, and phagocytic activities. These findings highlight the complex interaction between exercise, oxidative stress, and immune modulation. High-intensity exercise emerged as a robust inducer of immune responses, while even moderate and low-intensity exercise showed substantial effects. These findings provide valuable information for optimizing exercise regimens to promote overall health and immune resilience. Further research into the underlying molecular mechanisms is essential for a deeper understanding of these responses.

Availability of Data and Material

The data presented in this study are available on request from the corresponding author (X. Liu).

Acknowledgment

We are thankful to Ding Yi and Huang Feihong, the heads of the animal department, for their assistance in the research process.

Ethical Approval

The Ethics Committee of Hebei Provincial Hospital approved our study and experimental techniques and conducted according to the ethical guidelines on animal experiments in China (Approval no. NK20221008A07)

Financial Support

None.

Conflict of Interest

The Authors declared that there is no conflict of interest.

Authors Contribution

Conceptualization and Data Collection: X.L.; Methodology, Validation, and Writing-original draft preparation: X.W.; Writing-review and editing with Financial Support: T.W. All authors have read and agreed to the published version of the manuscript.

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