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Restoration Of Lung Function By Aerobic Vs Anaerobic Exercise: Insights From MDA, TNF-A, And SOD Expression

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ABSTRACT

Introduction: Aerobic and anaerobic exercises play distinct roles in lung function improvement,

each impacting the respiratory system through different mechanisms. Aerobic exercise enhances cardiovascular endurance and respiratory efficiency, while anaerobic exercise focuses on strengthening respiratory muscles and improving short-term oxygen utilization.

Objectives: The aim of this study was to compare the effects and duration required for aerobic and anaerobic exercise to restore lung function, focusing on the expression of malondialdehyde (MDA), tumor necrosis factor alpha (TNF-α), and superoxide dismutase (SOD). These biomarkers were selected due to their roles in oxidative stress, inflammation, and antioxidant defense mechanisms, respectively.

Methods: An experiment was conducted involving four groups: negative control, positive control, aerobic exercise and an anaerobic exercise group, each consisting of five Wistar rats. The experimental protocol included a smoking exposure period of 20 days, followed by aerobic and anaerobic training for five days a week for eight weeks. MDA, TNF-α and SOD expressions were measured by immunohistochemistry techniques

Results: The results demonstrated that both aerobic and anaerobic exercises significantly improved lung function compared to the control groups. Improvements in lung function, indicated by reductions in MDA and TNF-α expressions and increases in SOD expression, were observed as early as day 20 post-treatment in both exercise groups. The study found no significant differences between aerobic and anaerobic exercise in terms of their impact on MDA, TNF-α, and SOD expressions..

Conclusions: In conclusion, this study provides valuable insights into the mechanisms by which aerobic and anaerobic exercises promote lung health, emphasizing the importance of regular physical activity in managing respiratory conditions

Keywords: TNF-α, SOD, MDA, lung function, antioxidant

INTRODUCTION

*C*igarette is a significant source of excessive oxidants, leading to oxidative stress and cellular damages [1, 2]. The complex mix of chemicals in cigarette smoke, including free radicals and reactive oxygen species (ROS), adversely affects health, particularly the respiratory system [3]. Chronic exposure to cigarette smoke is a primary cause of chronic obstructive pulmonary disease (COPD) and it is also associated with the development of other diseases such as cancers and heart diseases [4]. The body has defenses against oxidative stress, including antioxidant enzymes and glutathione; however, persistent cigarette smoke exposure could overwhelm these defenses, leading cellular damages [5].

Cigarette smoking significantly impacts biochemical markers of oxidative stress and inflammation [6]. Malondialdehyde (MDA), an indicator of oxidative stress and lipid peroxidation, significantly increases with cigarette smoke exposure due to the high levels of free radicals and ROS in tobacco smoke [7]. Superoxide dismutase (SOD) neutralizes superoxide radicals; however, chronic cigarette smoke exposure overwhelms this defense, leading to oxidative stress [8]. Tumor necrosis factor-alpha (TNF-α), a cytokine involved in mic inflammation, is elevated in cigarette smokers and contributes to chronic inflammatory diseases such as COPD [9].

Oxidative stress is a physiological condition characterized by an imbalance between ROS

production and the body's detoxification and repair capabilities [10]. Physical exercise, both aerobic and anaerobic, has been shown to affect oxidative stress levels within the body [11]. Aerobic exercises such as running, swimming, and cycling employ aerobic metabolism and oxygen for energy, enhancing antioxidant defenses through hormesis—low-to- moderate oxidative stress stimulating defense mechanisms for improved health and stress resistance [12]. Anaerobic exercises such as weightlifting or sprinting feature short bursts of high-intensity activity independent of oxygen for energy [13]. Despite both exercise types could increase oxidative damage depending on intensity, regular physical activity generally enhances cellular ability to manage ROS accumulation [14].

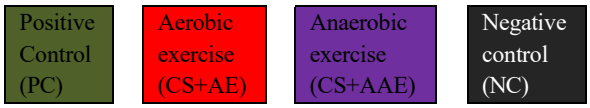
Regular physical exercise enhances exercise capacity, endothelial function, and prevents apoptosis, making it a potential strategy to prevent lung damages [15, 16]. Additionally, physical exercise has been found to increase endogenous antioxidant activities such as SOD and glutathione peroxidase, while decreasing TNF-α levels [17]. To the best of our knowledge, no previous research has investigated the duration needed for aerobic and anaerobic exercise to restore lung function in rats exposed to cigarette smoke, considering SOD, MDA, and TNF-α expression as the markers. Furthermore, it remains unclear which exercise type, aerobic or anaerobic, is more effective for restoring lung function.

OBJECTIVES

The aim of this study was to compare the therapeutic potential of aerobic and anaerobic exercise in repairing rat lungs after cigarette smoke exposure by assessing the lung histopathology and the expressions of SOD, MDA, and TNF-α

METHODS

A posttest-only control group design was used in an experimental investigation. The same lab provided the animals utilized in this investigation. Four groups were used in the experiment: negative control (NC), positive control exposed to cigarette smoke (CS), cigarette smoke plus aerobic exercise (CS+AE group), and Wistar rats (Rattus norvegicus). and cigarette smoke plus anaerobic exercise (CS+AAE group). For each cigarette smoke group, the rats had three subgroups exposed to cigarette smoke for 20, 40, and 60 days. The primary outcomes measured included lung function parameters and biomarkers of oxidative stress at the end of the exposure periods (**Figure 1**). This study was approved by Research Ethics Committee with reference number: 1.184/X/HREC/2020.



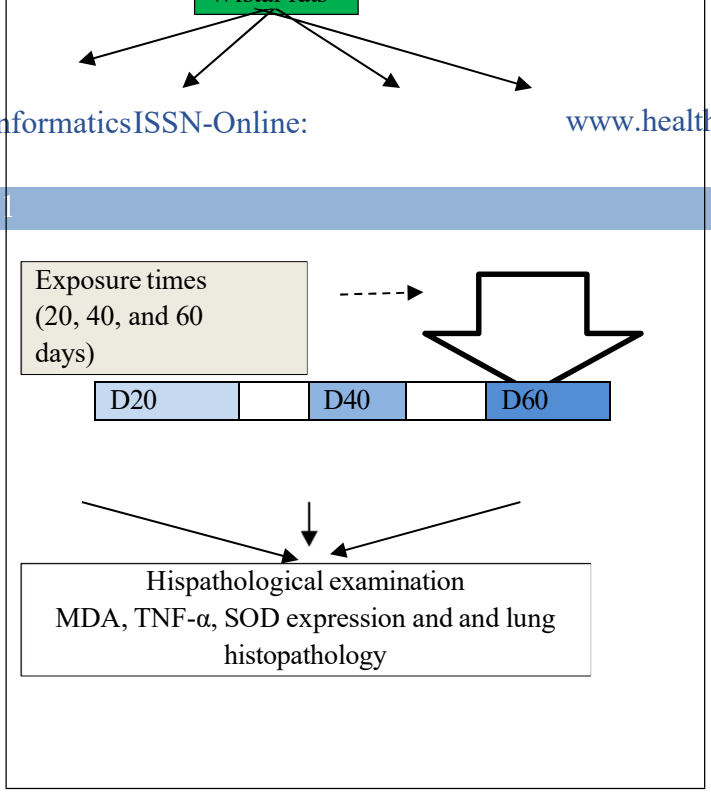


Figure 1. The schematic diagram of the study

Sample size calculation, randomization, and allocation

The sample size was calculated based on Federer's formula of which the minimum sample size for each group was five animals [18]. The animals were divided into four groups randomly using simple random sampling. Included rats were healthy males aged 3–4 months and weighing between 180–220 grams. Females, rats with structural or functional abnormalities, and rats with ongoing infections were excluded. Drop-out criteria included death, infection during the study period, inactivity, or refusal to eat.

Animal preparation

Rats were housed in well-shaded, tranquil rooms with a controlled environment, including temperature and humidity regulation. The animals were provided with uniform food and clean water ad libitum to ensure consistent nutrition. Housing density was maintained in accordance with ethical guidelines, with no more than three rats per cage to prevent overcrowding. Environmental enrichment was provided in the form of nesting materials and objects to encourage natural behaviors, reducing stress. Urine and feces were collected in a tray underneath the cages, which was cleaned every day to ensure hygienic conditions. The temperature and humidity of the ventilated cages were kept at 25–27°C and 50–60%, respectively. Lights were turned on at 5:30 AM and the light-dark cycle was maintained at 12:12. Twice a day, the rats were given formulated pellet meal at a rate of 10% of their body weight.

Experimental procedure

The rats were exposed to ten commercial cigarettes per day, consisting of 3 mg of nicotine and 8 mg of tar, every day for 60 days. The rats were kept in an inhaling chamber, also known as a smoke pump chamber, which had dimensions of 45 cm by 30 cm by 25 cm. Cigarette smoke exposure was conducted using ten cigarettes per group per day for 50 minutes in the smoking pump chamber. The cigarettes were connected to an air pump via a pipe attached to a cigarette holder. The air pump inhaled the smoke and pumped it through the output into the chamber. The different exposure times (20, 40, and 60 days) were selected to evaluate the progression and cumulative effects of cigarette smoke exposure on lung function and oxidative stress markers over time. Rats were maintained in

this smoky air condition (± 3%) for 5 minutes with a 1-minute rest between each cigarette, with a total particle concentration of 300 mg/m³ in the chamber. After 24 hours from the final exposure on days 20, 40, and 60, the rats' lungs were harvested, processed, and stored at -80°C until analysis. Each exposure group consisted of five

animals [19-21]. CS+AE and CS+AAE groups underwent its respective training five days a week for eight weeks.

Histopathological Processing and Microscopic Examination of Rat Lung Tissue

To obtain histological preparations, the rat were sacrificed in accordance with ethical guideline, followed by necropsy for organ sampling, particularly the lungs [22]. Histopathological examination of the lungs was conducted after immersion in 10% Buffered Neutral Formalin for at least five days. The histopathological slides were examined under a light microscope at 40x and 400x magnifications. Data were collected using digital imaging, with specific attention to epithelial cells of the bronchi and bronchioles. Criteria for euthanasia were established to prioritize the welfare of the animals. Rats exhibiting signs of severe distress, pain, or illness that could not be alleviated, as well as those showing signs of unmanageable suffering or significant deviation from normal behavior, were considered for early euthanasia. Monitoring for these symptoms was conducted throughout the experiment. In this study, it was not necessary to euthanize any animals prior to the planned end of the experiment, as all animals remained within acceptable health parameters until the scheduled euthanasia for organ collection. Additionally, the weights of the animals were measured before euthanasia to monitor their health and any potential changes in body condition throughout the experiment [23].

Aerobic and anaerobic training procedures

Aerobic and anaerobic training were conducted using a treadmill, with a customized device used for cigarette smoke exposure and with slight modifications from previous study [24]. The aerobic training regimen involved sessions 5 days per week for 8 weeks, with each session lasting 60 minutes at speeds ranging from 18 to 25 meters per minute. The anaerobic training regimen also spanned 8 weeks with 5 sessions per week. During each anaerobic session, the speed exceeded 25 meters per minute, and the incline was greater than 25%. The anaerobic sessions consisted of 3-minute intervals at 60% and 4-minute intervals at 85% of the maximum speed achieved during the initial test, repeated 7 times, totaling 49 minutes. The remaining time in each session was performed at 60% of the maximum speed. Both training types began with a 5-day adaptation phase, where each session lasted 15 minutes at a speed of 6 meters per minute with an incline ranging from 15% to 25%, including a 5-minute warm- up at 5 meters per minute and the same incline. On days 21, 41, and 61, the rats were euthanized by atlanto- occipital dislocation and necropsied to harvest the lungs. Day 21 provided insights into the immediate effects of cigarette smoke exposure, day 41 revealed the progression of damage, and day 61 allowed for an assessment of long-term and cumulative damage. This design aimed to comprehensively understand how cigarette smoke affects lung health over time.

Histopathological measurement

The histopathological examination was conducted using hematoxylin and eosin staining. Slides were prepared from paraffin blocks, stained and observed under a microscope at 400x magnification. Pulmonary observations covered an area of 1280 x 1024 µm², with five fields of view per slide. The degree of pulmonary damage was assessed based on alveolar edema, alveolar

wall destruction, and inflammatory cell infiltration, as explained previously [25]. The degree of lung damage was assessed based on the presence of alveolar edema, destruction of the alveolar septum, inflammatory cell infiltration, and vascular congestion. Each criterion was scored from 0 to 4, where 0 indicated no histological changes, and 4 indicated the most extensive damage, affecting 76%-100% of the entire field of view. Specifically, alveolar edema was scored as follows: 0 for no changes, 1 for less than 25% involvement, 2 for 26%-50%, 3 for 51%-75%, and 4 for 76%-100%. The same scoring system was applied to the destruction of the alveolar septum, inflammatory cell infiltration, and vascular congestion. The total score, ranging from 0 to 16, indicated the severity of lung damage, with higher scores reflecting greater damage in the lung tissue of the rats [26].

TNF-α, SOD, and MDA expression measurement

The quantification of TNF-α, SOD, and MDA expression was conducted using immunohistochemistry techniques. For MDA expression, we used the PAA590Ge01 Kit from Cloud-Clone Corp., USA. The TNF-α and SOD expressions were quantified using the BZ-087661AF-AM and BZ-0874480F-AP kits, respectively, from BIOANZY, USA. The immunohistochemistry procedure involved the application of these specific antibodies to tissue sections, followed by visualization using a substrate that is chromogenic. In short, samples of lung tissue were embedded in paraffin, sectioned into 4–5 micrometer slices, and preserved in 10% formalin. After being deparaffinized and rehydrated, the sections were heated in a microwave to retrieve the antigen in citrate buffer (pH 6.0). Following chilling and washing in phosphate-buffered saline (PBS), 3% hydrogen peroxide was used to disrupt endogenous peroxidase, and 5% bovine serum albumin in PBS was used to reduce non-specific binding. TNF-α and SOD primary antibodies, and MDA were applied and incubated overnight at 4°C. After washing, a biotinylated secondary antibody and avidin-biotin complex reagent were applied. Expression was visualized with 3,3'-diaminobenzidine, producing a brown precipitate, and sections were counterstained with hematoxylin. TNF- α expression was scored from 1 (minimal) to 4 (very high) based on staining intensity and distribution, allowing for quantifiable comparison across different treatment groups and time points to analyze inflammatory responses.

Statistical analysis

The Shapiro-Wilk test was used to evaluate the normality of the data (Supplementary Table Group 1). To examine differences across groups, the Kruskal-Wallis test was used because the data were not regularly distributed. In the meanwhile, variations within each group according to the length of time spent exposed to cigarette smoke (Day 20, Day 40, and Day 60) were examined using the Friedman test, as these represent paired data with repeated measurements over time [27]. If a significant difference was found using the Friedman test, it was followed by the Wilcoxon Rank Test for further analysis to determine where specific differences occurred between time points. SPSS software v23.0 (IBM, New York, USA) was employed for data analysis, with $p<0.05$ considered statistically significant.

RESULTS

Baseline body weight

Body weight can play a significant role in the context of smoking and its health implications. Research has consistently shown correlations between smoking habits and body weight changes. The subgroup results detailing baseline of body weight for each rat group at various stages of the study in **Table 1**.

Table 1. Baseline body weight data for rat groups before acclimatization, after acclimatization, and after experimental interventions

Group	Before Acclimatization	After Acclimatization	After Experimental Interventions
NC	198 ± 2	197 ± 3	199 ± 1
CS	197 ± 1	199 ± 2	198 ± 1
CS+AE	199 ± 2	200 ± 1	197 ± 2
CS+AAE	200 ± 2	198 ± 1	200 ± 3

Note: Negative control (NC), positive control exposed to cigarette smoke (CS), cigarette smoke plus aerobic exercise (CS+AE group), and cigarette smoke plus anaerobic exercise (CS+AAE group).

Statistical analysis using ANAVA showed no significant differences in body weight between groups at each time point examined ($p > 0.05$ for all comparisons). These results indicate good homogeneity among the rat groups based on body weight, validating the sample selection and interpretation of the study's findings.

TNF-α expression

The expression of TNF-α, a cytokine involved in inflammation, apoptosis, and sepsis in the lungs, showed marked variation among the treatment groups (**Figure 2**). The NC, which did not undergo any experimental intervention, had the lowest TNF-α levels on D20, with a mean of 3.9, representing baseline levels. In contrast, the positive control group (PC) exhibited significantly higher inflammation levels, with the highest TNF-α levels on D20, averaging 13.63. Notably, the group exposed to cigarette smoke combined with aerobic exercise (CS+AE) showed the lowest TNF-α levels on D60, with a mean of 7.5, while the group exposed to cigarette smoke and anaerobic exercise (CS+AAE) demonstrated the lowest TNF-α levels on D60, with a mean of 6.67. These findings highlight the potential efficacy of aerobic and anaerobic exercise interventions in reducing inflammation and apoptosis, possibly counteracting free radicals that trigger inflammatory responses due to cigarette smoke exposure. This is evidenced by the significant reduction in TNF-α levels on H60 compared to other groups.

Kruskal-Wallis analysis of TNF-α expression on D20, D40, and D60 show a consistent upward trend, with the highest mean ranks observed in the group exposed to cigarette smoke (PC) across all time points. Following aerobic (CS+AE) and anaerobic (CS+AAE) interventions, a reduction in TNF-α expression was observed in both treatment groups. Significant differences were noted on D20 and D60, while no significant difference was found on D40. However, on D40, no significant difference was observed, suggesting that TNF-α expression did not vary significantly between control and treatment groups (**Supplementary Table group 2**). Post-hoc Dunn's test was performed for D20 and D60 to further evaluate pairwise differences between control and treatment groups, with the results shown in **Table 3**. Further, Table 3 can be observed that not all treatments show significant relationships.

Moreover, the Friedman test results for TNF-α levels in the negative control group (NC) on day 20, day 40, and day 60 show that the mean values remained consistent at 2.0. In the positive PC, the mean values were also consistent at 2.38 on D20 and D60. For the CS+AE group, there was an increase in the mean TNF-α level from 2 on D20 to 2.5 on D60. Meanwhile, the CS+AAE group showed a decrease in the mean TNF-α level from 2.13 on D20 to 1.75 on D60.

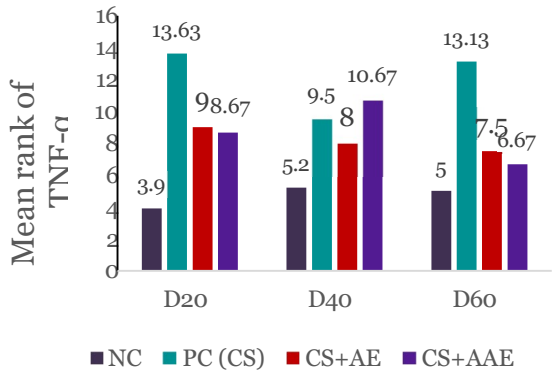


Figure 2. Kruskal-Wallis analysis of TNF-α expression among different treatment groups. NC: negative control, PC (CS): positive control, CS+AE: aerobic treatment, and CS+AAE: anaerobic treatment. The analysis shows significant differences between groups at D20 (p = 0.019) and D60(p = 0.017)

Table 2. The Friedman test for TNFα

Groups	D20 Mean	D40 Mean	D60 Mean	p-Value
NC	2.0	2.0	2.0	1.000
PC	2.38	1.25	2.38	0.050*
CS+AE	2.9	1.5	2.5	0.223
CS+AAE	2.13	2.13	1.75	0.717

**significant <0.05*

Table 3. Post Hoc analysis of TNF-α expression using Dunn's test

Group comparison	p-Value D20	p-Value D60
NC vs. CS+AE	0.592	0.100
NC vs. CS+AAE	0.938	0.100
NC vs. PC	0.010*	0.012*
CS+AE vs. CS+AAE	0.100	0.100
PC vs. CS+AE	0.933	0.186
PC vs. CS+AAE	0.951	0.361

**significant <0.05*

SOD expression

SOD expressions varied among treatment groups (**Figure 3 and Supplementary Table group 2**); however, no significant differences were observed between the groups. At Day 20, baseline SOD expressions in the NC group were 9.1. The PC group exhibited reduced SOD expressions (7.5), indicating oxidative stress from cigarette smoke. Both exercise groups, CS+AE and CS+AAE, showed similar SOD expressions to the PC group (7.5 and 10.17, respectively), suggesting that physical exercise had minimal impact on SOD expressions at this early stage.

By Day 40, SOD expressions showed distinct patterns: NC group decreased to 6.5, PC group

remained at 6.5, indicating persistent oxidative stress from smoke exposure. CS+AE group significantly increased to 11.17, suggesting aerobic exercise enhances antioxidant defenses. CS+AAE group reached 9.33, showing improvement over PC but less than CS+AE. By Day 60, NC group held stable at 9.7, PC dropped to 4, reflecting chronic oxidative damage. CS+AE maintained 9, sustaining aerobic exercises antioxidant benefits. CS+AAE rose to 9.5, showing improved capacity compared to PC, less than CS+AE.

Furthermore, the Friedman test results for SOD levels in the NC showed an increase from 1.7 on D20 to 2.6 on D60. In the PC, the mean SOD levels remained consistent at 2 across D20, D40, and D60. In the CS+AE group, SOD levels increased from 1.67 on D20 to 2.33 on D60, while the CS+AAE group followed a similar trend, rising

from 1.67 on D20 to 2.67 on D60 (**Table 4**).

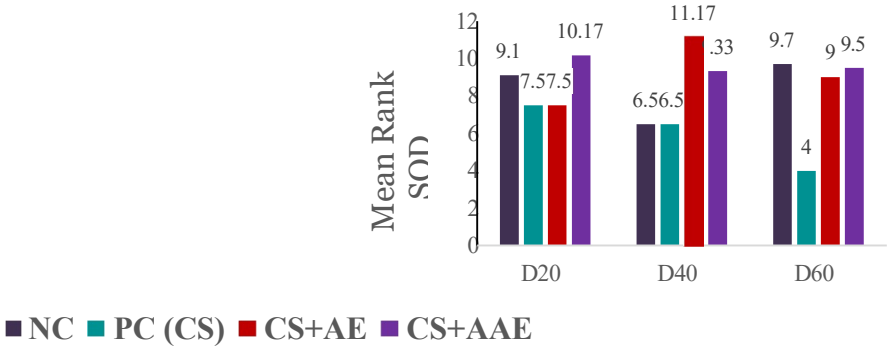


Figure 3. Comparative analysis of SOD expressions across treatment groups. NC: negative control, PC (CS): positive control, CS+AE: aerobic treatment, and CS + AAE: anaerobic treatments.

Table 4. Friedman test for SOD

Groups	D20 Mean	D40 Mean	D60 Mean	p-Value
NC	1.7	1.7	2.6	0.165
PC	2.0	2.0	2.0	1.000
CS+AE	1.7	2.0	2.3	0.670
CS+AAE	1.7	1.7	2.7	0.264

**significant <0.05*

MDA expression

Elevated MDA expressions indicate increased oxidative stress, often associated with various pathological conditions, including exposure to harmful substances like cigarette smoke [28]. **Figure 4** demonstrates significant differences between positive controls and exercise groups. At Day 20, NC had MDA expressions of 6.1, establishing baseline oxidative stress. PC had higher expressions (11.25), indicating smoke-induced stress. CS+AE had 6.75, CS+AAE 11.17, similar to PC, showing initial exercise impact. By Day 40, NC decreased to
4.4. PC increased (10.5), remaining elevated. CS+AE (8.67) showed reduced stress, CS+AAE (10) less than AE. By Day 60, NC remained low (4.6), PC increased (13), chronic damage evident. CS+AE (6.67) sustained reduction, CS+AAE (8.33) showed improvement but less than AE. PC had

significantly higher MDA expressions, indicating prolonged smoke exposure's impact. The Kruskal-Wallis test results showed that while reductions on D20 and D40 were not statistically significant, a significant decrease was observed by D60 (**Supplementary Table group 2 and Table 5)**. These findings demonstrate that both aerobic and anaerobic exercises effectively reduce oxidative stress induced by cigarette smoke exposure, as evidenced by the decreased MDA levels and improved antioxidant status. This is consistent with the findings of increased MDA expressions under oxidative stress conditions, as MDA is a critical marker of lipid peroxidation and oxidative damage [29, 30].

The Friedman test results for MDA levels showed the following patterns: in the NC, MDA levels increased from
1.8 on D20 to 2.2 on D60. The PC followed a similar trend, with an increase from 1.3 on D20 to 3.0 on D60. The Friedman test for MDA showed p-Value more than 0.05 on D20, D40, and D60 for the NC, CS+AE, and CS+AAE groups, indicating no significant differences in MDA expression across these time points for these groups. However, the Friedman test showed significant different for D20, D40, and D60 in the PC group, indicating significant differences in MDA expression for the cigarette smoke exposure group (**Table 6**).

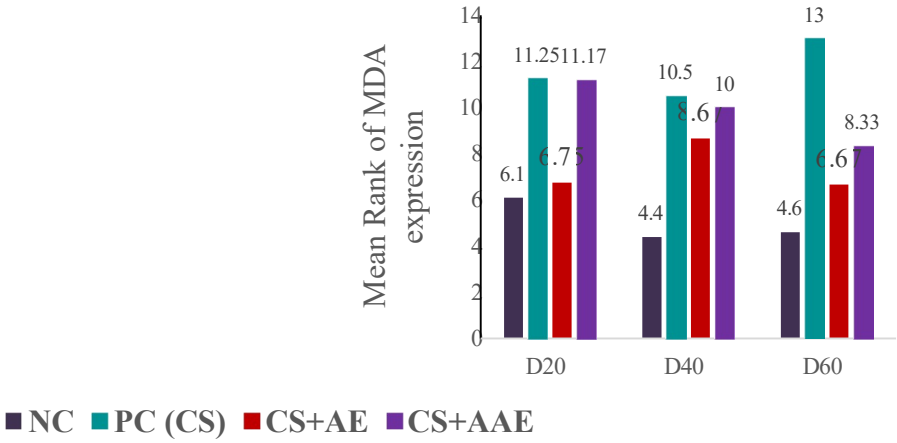


Figure 4. MDA expressions in control and treatment groups across Time points. NC: negative control, PC (CS): positive control, CS+AE: aerobic treatment, and CS + AAE: anaerobic treatments.

Table 5. Post Hoc analysis of MDA expression using Dunn's test

Group comparison	p-Value D60
PC vs. NC	0.021*
PC vs. CS+AE	0.000*
PC vs. CS+AAE	1.000
PC vs. CS+AE	0.000*
CS+AAE vs. NC	0.000*
CS+AE vs. CS+AAE	0.100

**significant <0.05*

Table 6. The Friedman test for MDA

	D20	D40	D60	

Groups	Mean	Mean	Mean	p-Value
NC	1.8	2	2.2	0.670
PC	1.3	1.9	3.0	0.024*
CS+AE	1.3	23	2.3	0.264
CS+AAE	1.8	2.0	2.2	0.867

**significant <0.05*

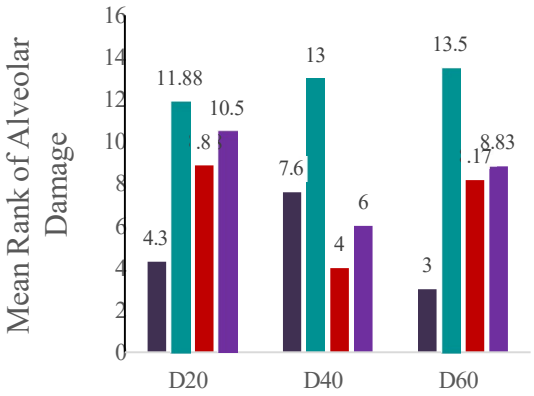
Alveolar damage

Cigarette smoke is a well-documented environmental pollutant known to cause significant damage to lung tissue, particularly the alveoli, which are critical for gas exchange [31, 32]. Prolonged exposure to cigarette smoke leads to oxidative stress, characterized by an imbalance between the production of ROS and the body's antioxidant defenses. This oxidative stress triggers a cascade of inflammatory responses, prominently marked by elevated expressions of pro-inflammatory cytokines such as TNF-α [33].

The histopathological analysis on D20, D40, and D60 showed the highest mean values in the group exposed to cigarette smoke (PC), with the mean values increasing as the duration of exposure lengthened (**Figure 5**). This finding indicates progressive lung damage due to cigarette smoke exposure, with the extent of damage increasing over time. Following aerobic and anaerobic exercise interventions, there was a reduction in lung damage in both the CS+AE and CS +AAE groups on D20 (**Supplementary Table group 2**). These results suggest significant lung repair in rats subjected to aerobic and anaerobic exercise at all time points. Subsequent Post Hoc Dunn Test analysis was conducted for day 20, day 40, and day 60 to evaluate the significant differences between the control and treatment groups, with the results displayed in **Table 7**.

Furthermore, the Friedman test results for histopathological evaluations revealed notable trends: the NC showed a significant increase in damage, with scores rising from 1.3 on D20 to 1.9 on D6. Similarly, the PC experienced

a significant increase in damage, with scores rising from 1 on D20 to 3 on D60. This suggests progressive lung damage due to cigarette smoke exposure. In contrast, the CS+AE and CS+AAE exercises also showed an increase in histopathological scores from D20 to D60. The CS+AE group's scores increased from 1.5 to 3.0, and the CS+AAE group's scores rose from 1.3 to 3.0. However, these changes were not statistically significant, indicating that while both exercise interventions may influence histopathological outcomes, the effect was not sufficient to reach statistical significance within the study's timeframe (**Table 8**).



■NC ■PC (CS) ■CS+AE ■CS+AAE

Figure 5. Alveoli damage score among different control and treatment groups. NC: negative control, PC (CS): positive control, CS+AE: aerobic treatment, and CS + AAE: anaerobic treatments.

Table 7. Post Hoc Analysis of alveolar damage using Dunn's test

Group comparison	p-Value D20	p-Value D40	p-Value D60
NC vs. CS+AE	0.589	1.000	0.661
NC vs. CS+AAE	0.237	1.000	0.427
NC vs. PC	0.037*	0.311	0.002*
CS+AE vs. CS+AAE	1.000	0.027*	1.000
PC vs. CS+AE	1.000	0.161	0.689
PC vs. CS+AAE	1.000	1.000	1.000

Table 8. The Friedman test for lung hispathology

Groups	D20 Mean	D40 Mean	D60 Mean	p-Value
NC	1.3	2.8	1.9	0.042*
PC	1.0	2.0	3.0	0.018*
CS+AE	1.5	1.5	3.0	0.086
CS+AAE	1.3	1.7	3.0	0.061

**significant <0.05*

Figure 6 shows that the positive control group (B, F, J) exhibits severe lung tissue damage with alveolar macrophages covering the alveoli and numerous inflammatory cells. In contrast, the negative control group (A, E, I) shows no alveolar macrophages. The group exposed to cigarette smoke and aerobic exercise (C, G, K) displays reduced alveolar macrophages, indicating less severe lung damage. The group exposed to cigarette smoke and anaerobic exercise (D, H, L) shows inflammation and fibrosis, but to a lesser extent than the positive control group, with fewer alveolar macrophages.

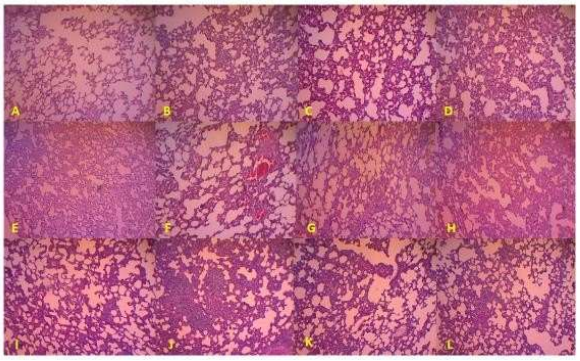


Figure 6. Alveoli damage score median of each different treatment.

DISCUSSION

In distinguishing the aerobic and anaerobic training protocols, the aerobic training involved continuous, steady- state exercises such as running, cycling, or swimming at moderate intensity levels (60-75% of maximum heart rate) for longer durations of 30 to 60 minutes, performed 3-5 times per week. This training primarily utilized the aerobic energy system, relying on oxygen for sustained energy production. The warm-up and adaptation phases included gradually increasing heart rate and muscle temperature to prepare for sustained moderate activity. On the other hand, the anaerobic training protocol consisted of high-intensity, short-duration exercises like sprinting, weight lifting, or high-intensity interval training (HIIT) at 80-95% of maximum heart rate. These sessions lasted between 20 to 30 minutes, with each high-intensity bout ranging from a few seconds to 2 minutes, typically performed 2-4 times per week with adequate rest for recovery. This protocol relied on the anaerobic energy system, characterized by quick bursts of energy, and included a similar warm-up to the aerobic protocol but focused on preparing muscles and joints for explosive, high-intensity movements.

The justification for the aerobic training protocol lies in its well-documented cardiovascular benefits, such as improved heart health, enhanced endurance, and better oxygen utilization, along with metabolic effects like fat reduction, improved metabolic rate, and blood glucose regulation. Long-term adaptations from aerobic exercise include increased mitochondrial density, capillary networks, and aerobic capacity (VO_2 max). Conversely, the anaerobic training protocol is justified by its role in building muscle strength, power, and size, which are essential for overall functional fitness. Anaerobic exercise also boosts metabolic rate and muscle mass, aiding in weight management and fat loss during rest periods. Additionally, it enhances short-term high-intensity performance, speed, and agility, benefiting athletes and individuals engaged in competitive sports.

Furthermore, Tumor Necrosis Factor alpha plays critical cytokine involved in systemic inflammation and is part of the body's immune response. In the context of smoking, research has shown that smokers exhibit higher expressions of TNF-α compared to non-smokers [34]. Significant reduction was observed in TNF-α expressions with aerobic and anaerobic treatments over time in the present study. These findings were aligned with previous studies, showing TNF-α modulation in response to treatments [35-37]. The present study showed no significant difference in TNF-α reduction between aerobic, anaerobic, and positive control groups, indicating a treatment efficacy plateau. TNF-α reduction appears to plateau once a certain treatment threshold is reached. This is particularly relevant in the context of cigarette smoke-induced inflammation, which activates various pathways including oxidative stress and pro-inflammatory cytokines [38, 39]. Therefore, the regulation of TNF-α is critical for managing the inflammatory responses triggered by smoke exposure. Chronic smoke exposure increases TNF- α, contributing to conditions such as COPD [40]. Reducing TNF-α could thus mitigate smoke-induced inflammation. The present study suggested effective TNF-α reduction at Day 20, highlighting potential treatment implications and need for long-term research on optimal dosing and outcomes in chronic inflammation scenarios such as smoking-related diseases.

One of the mechanisms by which cigarette smoke damages the lungs is through oxidative stress, a process in which ROS overwhelm the body's antioxidant defenses [41]. SOD is a critical antioxidant enzyme that helps neutralize ROS. However, the overwhelming presence of ROS in cigarette smoke can impair the function of SOD, leading to cellular damage and inflammation [42]. This imbalance between oxidants and antioxidants, exacerbated by smoking, contributes to the development and progression of various lung diseases [43]. Further, both aerobic and anaerobic exercises enhance antioxidant defenses and reduce oxidative damage [33]. Aerobic exercise boosts

antioxidant enzymes and mitochondrial function, increasing SOD expressions and reducing oxidative damage, while anaerobic exercise enhances antioxidant defenses primarily through muscle strength and resilience against oxidative stress [44]. In the present study, elevated SOD expressions in treatment groups demonstrate physical exercise's effectiveness in combating cigarette smoke-induced oxidative stress, emphasizing its role in improving

lung health and reducing oxidative stress markers even starting from 20 days after initiating physical exercise. By enhancing endogenous antioxidants, both types of exercise support cellular balance and prevent damage, consistent with previous studies [45-47].

The present study found that both aerobic and anaerobic exercises initially provide comparable effects on oxidative stress markers and antioxidant expressions. However, over a longer period, anaerobic exercise appears to offer greater benefits in enhancing antioxidant defenses, as evidenced by significantly higher SOD expressions at Day 60 compared to Day 20. The notable SOD increases at Day 60 in the anaerobic group suggested a delayed enhancement of antioxidant capacity, possibly stems from physiological adaptations such as increased muscle strength and endurance associated with anaerobic training, which contribute to stronger antioxidant defenses over time [48]. Aerobic exercise, observed from the present study, provides immediate and stable improvements in SOD expressions from Day 20 to Day 40, with limited additional gains by Day 60. This may be due to consistent oxidative demands and mitochondrial adaptations associated with aerobic activity, potentially plateauing earlier than anaerobic adaptations [49, 50]. Overall, the findings underscore the differing temporal effects of aerobic and anaerobic exercises on oxidative stress and antioxidant defenses, offering insights for designing effective exercise programs in high oxidative stress conditions such as cigarette smoke exposure.

Exercise interventions have potential to mitigate oxidative damage and improve lung health in prolonged smoke exposure. Both aerobic and anaerobic exercises effectively enhance SOD expressions and contribute to lung function recovery following smoke exposure in the present study, with aerobic exercise showing a more pronounced effect. Lower SOD expressions in the positive control groups underscore severe oxidative stress and compromised antioxidant defenses from prolonged smoke exposure. This is consistent with previous studies showing reduced SOD expressions under oxidative stress conditions, highlighting the critical role of SOD in detoxifying superoxide radicals [51, 52].

Both aerobic and anaerobic exercises in the present study are initially effective in reducing MDA expressions and oxidative stress. However, significant differences in their effects emerge over time, indicating that the impact of each exercise type may vary with prolonged intervention. Regular physical activity effectively reduces MDA expressions, maintain cellular balance and prevent oxidative damage, thus, mitigating cigarette smoke-induced oxidative stress by boosting endogenous antioxidant defenses [53]. Aerobic exercise enhances antioxidants and mitochondrial function, lowering MDA and oxidative damage, while anaerobic exercise also boosts defenses, mainly through muscle strength and resilience against oxidative stress [54].

The present study found that both aerobic and anaerobic exercises are protective against cigarette smoke-induced lung damage, with aerobic exercise being more effective. This suggests aerobic exercise offers more significant therapeutic benefits for improving lung health and mitigating damage caused by cigarette smoke. The higher lung damage in untreated groups highlights the severe impact of prolonged smoke exposure, while the reduced damage in exercise groups underscores the protective effects of regular physical activity. Hence, aerobic and anaerobic

exercises mitigate alveolar damage, enhancing lung tissue resilience and repair. Additionally, the present study showed elevated TNF-α expressions, decreased SOD expressions, and increased MDA expressions collectively contribute to alveolar damage

Conclusions

Both aerobic and anaerobic exercises are equally effective in improving lung function and reducing xidative stress markers as early as day 20 post-treatment by reducing MDA and TNF-α expressions and increasing SOD expressions. Further research is needed to explore the long-term effects and clinical applications of exercise interventions in respiratory diseases

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