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Modulation of Oxidative Stress Markers and Inflammatory Mediators in Alveolar Damage Induced by Anaerobic Exercise

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Anaerobic exercise, characterized by high-intensity, short-duration physical activity, induces a unique physiological response that includes oxidative stress and inflammation. This study investigates the modulation of oxidative stress markers and inflammatory mediators in alveolar damage induced by anaerobic exercise. Key parameters, including superoxide dismutase (SOD) activity, malondialdehyde (MDA) levels, and tumor necrosis factor-alpha (TNF-α), were analyzed to elucidate their roles in oxidative and inflammatory pathways. Using rats as an experimental model, subjects were exposed to an 8-week anaerobic training regimen, and alveolar tissue samples were evaluated for oxidative stress and inflammatory markers. Histopathological changes and biochemical markers were analyzed across treatment groups and three time points (D-20, D-40, D-60). Results demonstrated a significant group- and time-dependent variations were observed in MDA, SOD, and TNF levels. The MDA levels was high, indicative of lipid peroxidation, while SOD showed elevated oxidative burden across treatment groups. TNF levels were consistently higher in the treatment group, highlighting its role in inflammation. Temporal analyses revealed stabilization at D-40 for certain markers, followed by significant increases at D-60. Correlation analyses indicated strong positive relationships between histopathology and oxidative stress markers, while TNF exhibited a dual role, correlating positively with oxidative stress but negatively with histopathological damage. The findings underscore the multifactorial nature of alveolar damage, driven by the interplay between oxidative stress and inflammation. The interaction network highlights the interconnected roles of MDA, SOD, TNF, and histopathological damage.

Keywords—**Anaerobic exercise; oxidative stress; inflammatory mediators; superoxide dismutase; malondialdehyde; tumor necrosis factor-alpha; alveolar damage.**

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I. INTRODUCTION

Globally, respiratory diseases represent a significant burden on public health, with millions of people suffering from conditions such as chronic obstructive pulmonary disease (COPD), asthma, and acute respiratory distress syndrome [1], [2]. According to the World Health Organization (WHO), respiratory diseases are among the top causes of mortality and morbidity, particularly in low- and middle-income countries [3]. This alarming prevalence underscores the need for innovative approaches to prevent and manage pulmonary health challenges. The consequences of untreated or poorly managed lung diseases include reduced quality of life, decreased work productivity, and increased healthcare costs [4]. Further, the management of pulmonary diseases has traditionally relied on pharmacological interventions, lifestyle modifications, and rehabilitation

strategies [5]. Advances in modern medicine have introduced targeted therapies and biologics that have improved outcomes for many patients. However, these treatments often come with high costs and limited accessibility, particularly in resource-constrained settings [6]. The global focus on preventive measures, including lifestyle changes and physical activity, has emerged as a crucial component of comprehensive pulmonary care.

In recent years, anaerobic exercise has gained traction worldwide, not only as a fitness trend but also for its therapeutic potential. High-intensity interval training and other anaerobic regimens are celebrated for their ability to enhance cardiovascular and muscular fitness [7]. However, the physiological stress induced by anaerobic exercise, especially in individuals with pre-existing conditions, raises questions about its safety and long-term implications [8]. While anaerobic exercise is widely promoted, its impact on oxidative stress and inflammatory pathways in the lungs remains a topic of active investigation. Moreover, the modern world faces increasing exposure to free radicals and reactive oxygen species (ROS) from environmental pollutants, cigarette smoke, and industrial emissions. These free radicals exacerbate oxidative stress, a key driver of cellular damage and chronic inflammation [9]. The interplay between external radical sources and the body's natural antioxidant defenses has profound implications for lung health. Given that anaerobic exercise itself generates ROS, understanding its dual role as both a stressor and a potential therapeutic intervention is critical.

Molecularly, the pathophysiology of oxidative stress involves an imbalance between ROS production and antioxidant capacity. Excessive ROS triggers lipid peroxidation, protein denaturation, and DNA damage, ultimately compromising cellular integrity [10]. In the lungs, these processes can result in alveolar damage, impaired gas exchange, and a heightened inflammatory response [11]. Understanding these molecular mechanisms is essential for developing strategies to mitigate the potential adverse effects of anaerobic exercise on lung health. Moreover, from a real-world perspective, the relevance of studying oxidative stress and inflammation in the context of anaerobic exercise extends to both healthy individuals and those with pre-existing pulmonary conditions. Athletes, fitness enthusiasts, and patients undergoing pulmonary rehabilitation represent distinct populations that may benefit from tailored exercise protocols. Clinicians and researchers must bridge the gap between molecular insights and practical applications to optimize exercise regimens for diverse populations. The clinical implications of this research are significant. Pulmonary rehabilitation programs often incorporate physical activity to improve lung function and reduce inflammation. However, the balance between beneficial and potentially harmful effects of anaerobic exercise must be carefully evaluated. Identifying biomarkers of oxidative stress and inflammation could pave the way for personalized exercise prescriptions, ensuring safety and efficacy.

Despite these advances, several challenges remain. The heterogeneity of exercise responses among individuals, the lack of standardized protocols, and limited data on long-term outcomes pose barriers to the widespread adoption of anaerobic exercise in pulmonary care. Moreover, translating molecular findings into clinical practice requires interdisciplinary collaboration and robust clinical trials. Addressing these challenges will be pivotal in maximizing the therapeutic potential of anaerobic exercise while minimizing risks. In summary, this study aims to investigate the modulation of oxidative stress markers and inflammatory mediators in alveolar improvement induced by anaerobic exercise through different time points.

II. Materials and Method

A. Study Design

This study employed an experimental approach using a post-test-only control group design to evaluate the effects of different interventions on oxidative stress and lung function parameters. The research was conducted using Wistar rats (Rattus norvegicus). The animals were randomly assigned into three groups: a negative control group (Ctr), a positive control group exposed to cigarette smoke (EC), and a treatment group exposed to cigarette smoke combined with anaerobic exercise (TSOD, TMDA, and TTNF, representing superoxide dismutase, malondialdehyde, and tumour necrosis factor, respectively). Each cigarette smoke group was further subdivided based on exposure durations of 20, 40, and 60 days, designated as D-20, D-40, and D-60, respectively. At the conclusion of these exposure periods, key outcomes, including lung function metrics and biomarkers of oxidative stress, were assessed. Ethical approval for the study was obtained from the Research Ethics Committee, with reference number 1.184/X/HREC/2020.

The sample size was determined using Federer's formula, ensuring a minimum of five animals per group [12]. The animals were allocated into three groups using a simple random sampling method. The study included healthy male Wistar rats, aged 3–4 months and weighing 180–220 grams. Exclusion criteria encompassed female rats, those with structural or functional abnormalities, and those exhibiting ongoing infections. Criteria for dropouts included mortality, onset of infections during the study period, inactivity, or refusal to eat.

B. Animal Setting

The rats were housed in well-ventilated, environmentally controlled rooms to maintain optimal conditions. The environment was regulated with a temperature range of 25–27°C and humidity levels of 50–60%. The rats were maintained on a 12:12 light-dark cycle, with lights on at 5:30 AM [13]. To ensure consistent nutrition, formulated pellet feed was provided at 10% of body weight twice daily, and clean water was available ad libitum. The housing density followed ethical standards, with no more than three rats per cage to minimize stress and overcrowding. Environmental enrichment was incorporated to encourage natural behaviors, and trays beneath the cages were cleaned daily to uphold hygiene [14].

C. Experimental Procedure

Rats were exposed to cigarette smoke generated from ten commercial cigarettes per day, each containing 8 mg of tar and 3 mg of nicotine, for seven days a week over 60 days. The exposure was conducted in an inhalation chamber (45 cm x 30 cm x 25 cm) connected to an air pump. Cigarette smoke was introduced into the chamber for 50 minutes daily, with 5 minutes of exposure per cigarette and 1-minute intervals

between each. Exposure durations of 20, 40, and 60 days were employed to investigate the progression and cumulative effects on lung function and oxidative stress markers. At the conclusion of each exposure period, lung tissues were harvested, processed, and stored at -80°C for subsequent analysis. Each experimental group comprised five rats [15].

D. Histopathological Examination

Lung tissues were collected following ethical euthanasia guidelines. The organs were preserved in 10% buffered neutral formalin for at least five days before histological examination. Tissue sections were stained using hematoxylin and eosin (H&E) and analyzed under a light microscope at magnifications of 40x and 400x. Pulmonary assessments focused on alveolar oedema, alveolar wall destruction, inflammatory cell infiltration, and vascular congestion. A scoring system (0–4) quantified the severity of lung damage, where higher scores indicated greater tissue damage. Moreover, histological examination employed paraffin-embedded lung tissues stained with H&E. Observations were conducted at 400x magnification, covering an area of 1280 x 1024 μm^2 with five fields of view per slide. Parameters assessed included alveolar oedema, destruction of the alveolar septum, inflammatory cell infiltration, and vascular congestion. Each criterion was scored on a scale of 0 (no damage) to 4 (extensive damage), providing a total severity score ranging from 0 to 16 [16].

E. Anaerobic Training Procedure

The anaerobic exercise protocol utilized a treadmill and was conducted for 8 weeks, five days per week. Training sessions involved intervals with speeds exceeding 25 meters per minute and inclined greater than 25%. Each session incorporated 3-minute intervals at 60% and 4-minute intervals at 85% of the maximum speed determined during the initial test. These intervals were repeated seven times for a total of 49 minutes, with the remaining time performed at 60% of the maximum speed. A 5-day adaptation phase preceded the training, beginning with 15-minute sessions at 6 meters per minute and inclines ranging from 15% to 25% [17].

F. Measurement of TNF-α, SOD, and MDA Expression

The expression levels of TNF-α, superoxide dismutase (SOD), and malondialdehyde (MDA) were quantified using immunohistochemistry techniques. Lung tissue samples were fixed in 10% formalin, embedded in paraffin, and sectioned to 4–5 µm thickness. After deparaffinization and rehydration, antigen retrieval was performed using citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked, and sections were incubated with primary antibodies specific to TNF-α, SOD, and MDA overnight at 4°C. Visualization was achieved using a chromogenic substrate, followed by hematoxylin counterstaining. TNF-α expression was scored from 1 (minimal) to 4 (very high) based on staining intensity and distribution [18].

G. Statistical Analysis

Data normality was evaluated using the Shapiro-Wilk test. Non-parametric analyses were conducted due to the lack of normal distribution. Differences between groups were assessed using the Kruskal-Wallis test, while the Friedman test was used for repeated measures within groups over time (20, 40, and 60 days). Post hoc analyses employed the Wilcoxon Rank Test to identify specific differences between time points. Statistical significance was set at $p<0.05$. Data analysis was performed using R Version 1.4.1743.

III. Results and Discussion

Anaerobic exercise has long been recognized as a powerful intervention to enhance physical performance, improve metabolic health, and promote resilience against oxidative stress [19]. Unlike aerobic training, anaerobic exercise emphasizes high-intensity, short-duration activities that stimulate glycolytic pathways, leading to adaptations in muscle strength, endurance, and cellular metabolism [20]. This study provides compelling evidence on the significant effects of anaerobic treatments and their temporal dynamics on histopathological damage and associated biomarkers, including MDA, SOD, and TNF. The findings demonstrated clear group-dependent variations, as shown in Fig. 1, Fig. 2, Fig. 3, and Fig. 4, alongside temporal influences, which are critical in understanding the multifaceted nature of alveolar damage.

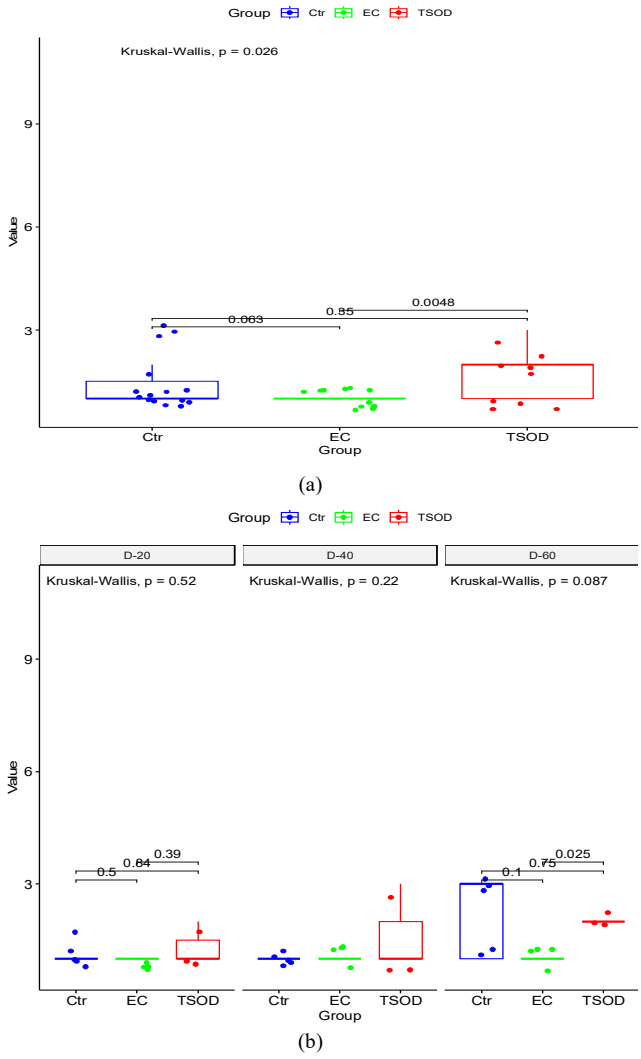

Fig. 1 a) Comparison of measured values among groups (Ctr, EC, TSOD). b) Stratified analysis of measured values across time points (D-20, D-40, D-60) and groups (Ctr, EC, TSOD).

The statistical significance revealed through Kruskal-Wallis tests across all figures emphasizes the robust relationship between treatment groups and the markers studied. Notably, TMDA and TTNF levels were elevated in treated groups, particularly at specific time points, highlighting the biochemical alterations induced by the interventions.

A. Group-Dependent and Temporal Variations in SOD Expression Levels

Based on Fig. 1(a), the results demonstrate a significant variation in the measured values among the different groups (Ctr, EC, and TSOD (SOD)). The test revealed a statistically significant difference between groups, as evidenced by the p-values in the first figure ($p < 0.05$). Specifically, the pairwise comparisons highlighted significant differences between Ctr and TSOD, as well as between EC and TSOD, indicating that the TSOD group exhibited higher median values compared to the other groups. These differences suggest a distinct impact of the respective treatments on the SOD.

Significant differences were observed between groups, with p-values indicating statistical significance for pairwise comparisons, particularly between Ctr and TSOD, as well as EC and TSOD. In Fig. 1(b), where the data is stratified across time points (D-20, D-40, and D-60), the test results varied for each time point. At D-20, significant differences were observed between groups, particularly between Ctr and TSOD, as indicated by the lower p-value (< 0.05). However, at D-40 and D-60, while differences persisted, the level of significance decreased, with p-values approaching the threshold for significance. The TSOD group consistently displayed higher values across all time points compared to the Ctr and EC groups, reinforcing the treatment's potential impact. Overall, these results suggest that the observed differences are both group- and time-dependent, highlighting the influence of specific treatments and temporal effects on the SOD level.

B. Treatment- and Time-Dependent Modulation of MDA Expression Levels

One of the most striking findings was the consistent elevation of TMDA (MDA), an oxidative stress marker, in the TMDA-treated group (Fig. 2). This result aligns with the known role of MDA as a product of lipid peroxidation, a process exacerbated under oxidative stress conditions. The temporal variation observed in TMDA levels suggests a sustained oxidative stress response, especially at D-20 and D-60, with a potential stabilization at D-40. This finding underscores the chronic nature of oxidative stress induced by the treatment, which may serve as a critical factor in alveolar damage progression. The expression levels of TMDA (MDA) exhibited significant differences across groups (Ctr, EC, TMDA), as shown in Fig. 2A and Fig. 2B. The test for Fig. 2(a) revealed a highly significant difference ($p < 0.0001$) among the groups. Pairwise comparisons indicated that TMDA levels in the TMDA group were significantly higher compared to both the Ctr and EC groups, while EC also exhibited a significant increase compared to Ctr. These results suggest that TMDA expression is distinctly modulated by the treatments administered.

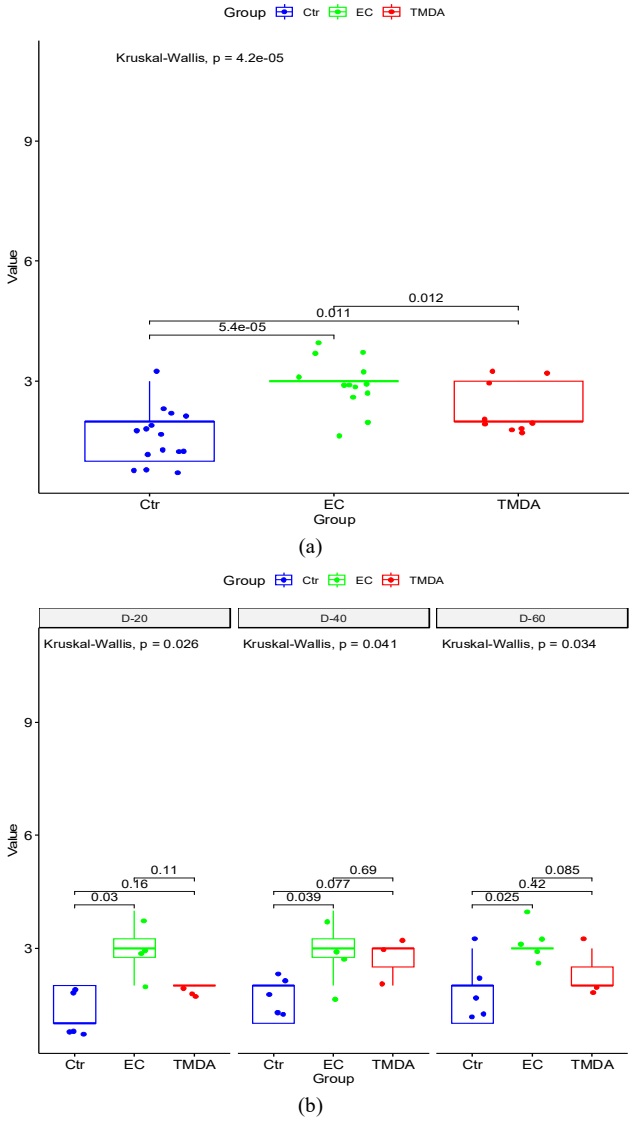

Fig. 2 a) Comparison of TMDA (MDA) expression levels among groups (Ctr, EC, TMDA). b) Analysis of measured values stratified by time points (D-20, D-40, D-60) and groups (Ctr, EC, TMDA).

Significant differences were observed, with the TMDA group showing elevated levels compared to both Ctr and EC ($p < 0.0001$). b) Time-stratified analysis of TMDA (MDA) expression across groups (Ctr, EC, TMDA) at D-20, D-40, and D-60. The test revealed significant differences for each time point, with the TMDA group exhibiting consistently higher levels compared to other groups. In Fig. 2(b), the analysis of TMDA expression stratified by time points (D-20, D-40, and D-60) showed time-dependent variations across the groups. At D-20, significant differences were observed between the TMDA and Ctr groups, as well as between TMDA and EC, with the TMDA group showing consistently elevated levels. At D-40, the Kruskal-Wallis test remained significant ($p = 0.041$), with pairwise comparisons again demonstrating significant increases in TMDA levels in the TMDA group compared to the others. At D-60, similar patterns were observed, with significant differences between TMDA and the other groups ($p = 0.034$). These results underscore the treatment's consistent effect on TMDA expression, which persists across time points.

C. *Group-Dependent and Temporal Dynamics of TNF-□ Expression Levels*

The expression levels of TTNF (TNF) exhibited significant differences across the groups (Ctr, EC, TTNF), as depicted in Fig. 3(a) and Fig. 3(b). In Fig. 3(a), the Kruskal-Wallis test revealed a highly significant difference among the groups ($p < 0.0001$). Pairwise comparisons indicated that the TTNF group had significantly higher expression levels compared to the Ctr group ($p < 0.0001$) and the EC group ($p = 0.0022$). Additionally, EC demonstrated significantly higher TTNF levels compared to the Ctr group ($p = 0.0005$). These findings suggest that the TTNF group exhibited the highest TTNF expression, indicating a marked effect of the treatment on this parameter.

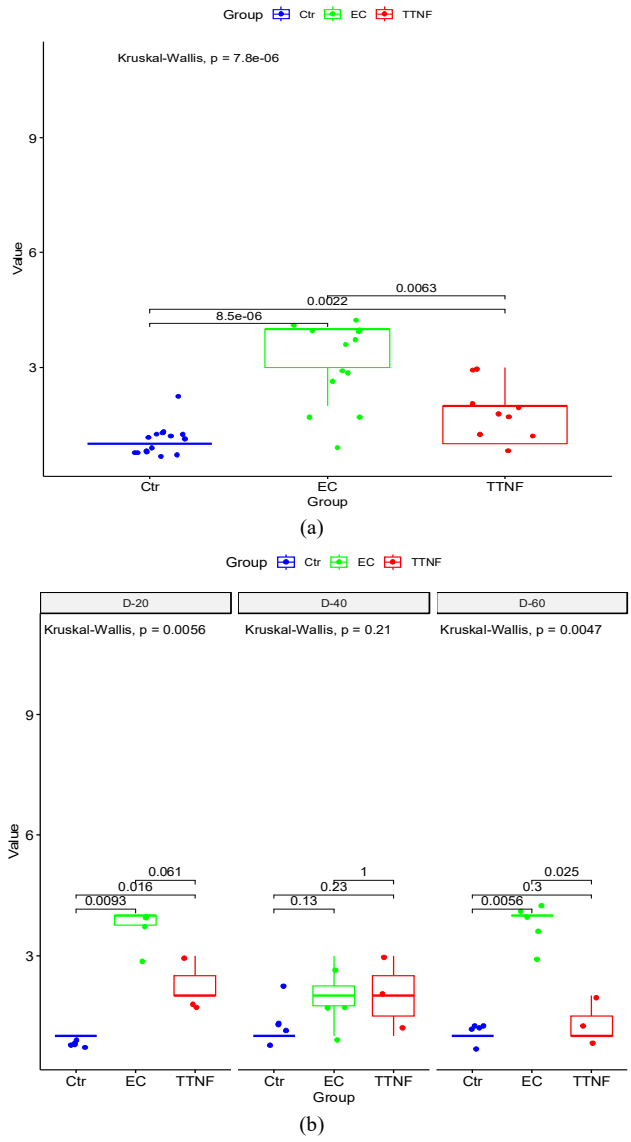

Fig. 3 a) The expression levels of TTNF (TNF) exhibited significant differences across the groups (Ctr, EC, TTNF). b) Breakdown of measured values by time points (D-20, D-40, D-60) and group classifications (Ctr, EC, TTNF).

In Fig. 3b, the time-stratified analysis across D-20, D-40, and D-60 provided further insights into the temporal variations in TTNF expression. At D-20, the Kruskal-Wallis test demonstrated significant differences among groups ($p = 0.0005$), with the TTNF group consistently showing the highest levels compared to both Ctr and EC. At D-40, no significant differences were observed ($p = 0.21$), suggesting a potential stabilization or reduced treatment effect at this time point. At D-60, significant differences re-emerged ($p = 0.0347$), with the TTNF group again showing elevated levels compared to the other groups. These results highlight a dynamic pattern of TTNF expression, with significant group-dependent differences that vary over time.

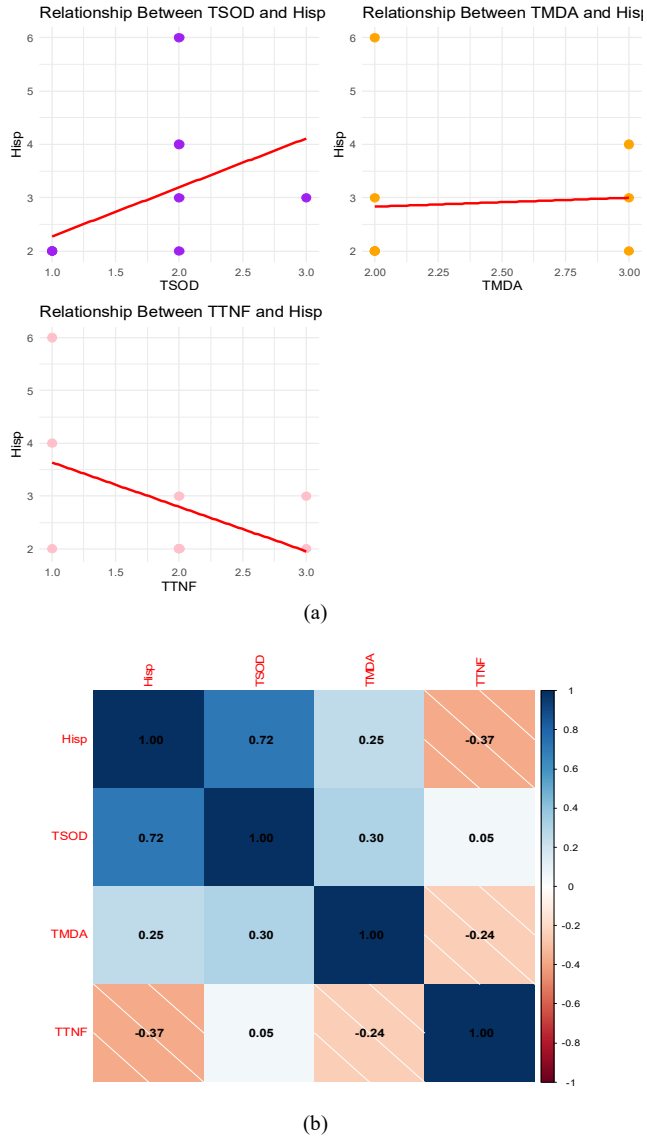

Fig. 4. a) Scatter plots showing relationships between Histopathology (Hisp) and key variables: TSOD, TMDA, and TTNF. Positive correlations were observed for TSOD and TMDA, while TTNF exhibited a negative correlation with Hisp. b) Correlation matrix displaying the relationships among Hisp, TSOD, TMDA, and TTNF.

In addition, the results for TTNF (Fig. 3) reveal its pivotal role as an inflammatory mediator. The significant elevation in TTNF expression in the TTNF-treated group, especially at D-20 and D-60, aligns with previous findings suggesting TNF-α as a major contributor to inflammatory cascades during alveolar injury [21]. Interestingly, the stabilization observed at D-40 suggests a transient adaptation or regulation of the inflammatory response over time. This temporal pattern could reflect a compensatory mechanism within the inflammatory

pathway or an interaction with other cytokines and regulatory molecules. Such findings reinforce the role of TNF-α as both a marker and mediator of inflammation in alveolar damage, consistent with prior investigations into cigarette smoke-induced injury and exercise-induced oxidative stress [22].

D. Exploring Relationships Between Histopathological Damage, Oxidative Stress, and Inflammatory Markers

The scatter plots (Fig. 4(a)) and correlation matrix (Fig. 4(b)) illustrate the relationships among the variables measured in the study, including Histopathology (Hisp), TSOD, TMDA, and TTNF. In Fig. 4(a), the scatter plots provide a visual representation of the linear relationships between Hisp and the selected parameters. The positive correlation between Hisp and TSOD is evident, with the regression line showing an upward trend, indicating that increased TSOD levels are associated with higher histopathological damage scores. In contrast, TMDA displays a weak positive trend with Hisp, while TTNF shows a negative correlation, suggesting that higher TTNF levels are linked to reduced histopathological damage.

Strong positive correlations were observed between Hisp and TSOD, while TTNF exhibited negative correlations with Hisp and TMDA, highlighting complex interdependencies among the parameters. Fig. 4(b) provides a more detailed examination of these relationships through a correlation matrix. The matrix reveals a strong positive correlation ($r = 0.72$) between Hisp and TSOD, confirming the trend observed in the scatter plot. TMDA shows a moderate positive correlation with TSOD ($r = 0.30$) and a weaker relationship with Hisp ($r = 0.25$). Interestingly, TTNF is negatively correlated with both Hisp ($r = -0.37$) and TMDA ($r = -0.24$), while showing a moderate positive correlation with TSOD ($r = 0.35$). These findings suggest that while TSOD is strongly associated with histopathological damage, TTNF may play a protective or modulating role in the observed damage. Overall, these results highlight distinct patterns of association between histopathological damage and oxidative stress markers (TSOD and TMDA), as well as inflammatory responses (TTNF).

Moreover, the results in Fig. 4 further emphasize the interplay between oxidative stress and histopathological damage. The scatter plots reveal a strong positive correlation between TSOD and histopathology (Hisp), suggesting that increased oxidative stress leads to greater tissue damage. TSOD levels, which inversely correlated with antioxidant capacity, were significantly elevated in treated groups, indicating that the oxidative burden may overwhelm endogenous defence mechanisms. These results are consistent with prior studies where oxidative stress was a key driver of structural damage in alveoli under similar experimental conditions [23]. Conversely, the negative correlation between TTNF and histopathological damage suggests a complex role of inflammatory responses, where TNF-α may play both injurious and protective roles depending on its temporal dynamics and interaction with oxidative stress.

E. The interaction network between histopathology, MDA, SOD, and TNF

The interaction network illustrates the intricate relationships between histopathology (Hisp), MDA (TMDA), SOD (TSOD), and TNF (TTNF), highlighting their interconnected roles in the context of alveolar damage (Fig. 5). Histopathology serves as the central parameter, directly influenced by oxidative stress markers (MDA and SOD) and inflammatory mediators (TNF). The positive interaction between MDA and SOD suggests a close relationship between oxidative damage and the body's antioxidant response. This connection underscores the additive nature of oxidative stress, where increased lipid peroxidation (MDA) correlates with heightened oxidative enzyme activity (SOD), reflecting the cellular response to oxidative burden [24]. Subsequently, TNF (TTNF) exhibits a dual role, connecting with both oxidative markers and histopathological outcomes. Its association with MDA and SOD highlights its involvement in the inflammatory response triggered by oxidative stress, while its direct link to histopathology suggests its role as a driver of tissue damage. The negative correlation between TNF and histopathology observed in other analyses suggests a potential modulatory effect, where inflammation may initially play a protective role before contributing to structural damage [25], [26].

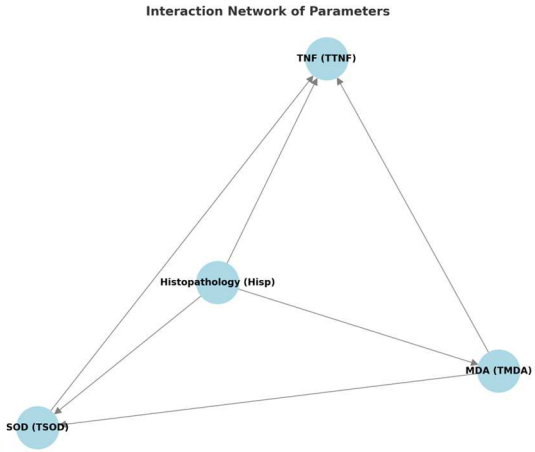

Fig. 5 Interaction network illustrating the relationships between histopathology (Hisp), oxidative stress markers (MDA/ TMDA and SOD/ TSOD), and the inflammatory marker (TNF/ TTNF). The network highlights the central role of histopathology, which is directly influenced by oxidative stress and inflammation

The clinical relevance of these findings cannot be overstated. Alveolar damage, driven by oxidative stress and inflammation, is a hallmark of various respiratory pathologies, including COPD, acute respiratory distress syndrome, and interstitial lung diseases [27]. The results presented here provide insights into the mechanistic underpinnings of such conditions, particularly the interplay between oxidative stress markers (MDA, SOD) and inflammatory mediators (TNF-α). Understanding these relationships is critical for developing targeted therapies aimed at mitigating oxidative and inflammatory damage. For instance, interventions focusing on reducing oxidative stress or modulating TNF-α activity may offer therapeutic benefits in preserving alveolar integrity. When compared to previous studies, this research adds depth by incorporating a time-stratified approach, allowing for a better understanding of temporal changes in biomarker levels [28]–[30]. While previous findings have largely focused on the acute effects of anaerobic exercise or oxidative stress, this study extends the narrative by demonstrating both acute and chronic effects, as evidenced by significant variations at D-20, D-40, and D-60. Moreover, the correlation matrix in Fig. 4(b) provides a

comprehensive view of the interdependencies among biomarkers, a feature often overlooked in earlier research. The strong positive correlation between TSOD and TMDA highlights the additive nature of oxidative damage, while the negative correlation between TTNF and histopathology reflects the complexity of inflammatory regulation.

The observed stabilization of certain markers at D-40 raises intriguing questions about the adaptive capacity of alveoli under prolonged stress. While acute elevations in oxidative and inflammatory markers are expected, the potential for partial recovery or regulation suggests a window of therapeutic opportunity. Targeting this phase with antioxidant or anti-inflammatory treatments could prevent progression to irreversible damage. Such strategies align with clinical efforts to manage chronic respiratory diseases by intervening at early stages of pathogenesis [23]. Moreover, a key limitation of this study is the lack of data on downstream effects of these biomarkers on lung function. While histopathological damage serves as a robust indicator of structural injury, correlating these findings with functional outcomes such as gas exchange or lung compliance would strengthen the translational relevance of these results. Future studies should aim to bridge this gap by integrating functional assessments alongside biochemical and histological analyses.

IV. Conclusion

This study highlights the interplay between oxidative stress, inflammation, and histopathological damage in alveolar tissue under anaerobic conditions. The findings demonstrate that oxidative stress markers (MDA/ TMDA and SOD/ TSOD) and the inflammatory mediator (TNF/ TTNF) significantly contribute to tissue damage, with their effects varying across different treatment groups and time points. Elevated MDA levels indicate heightened lipid peroxidation, while increased SOD levels suggest an overwhelmed antioxidant defence system. TNF, as a key inflammatory marker, exhibits a dual role, contributing to both tissue protection and damage depending on its temporal dynamics and interaction with oxidative stress. Clinically, this study reinforces the importance of early intervention in conditions characterized by alveolar damage. Therapies aimed at reducing oxidative stress and modulating inflammatory responses could offer significant benefits in preserving alveolar integrity and improving patient outcomes. Future research should focus on integrating functional assessments and exploring potential therapeutic agents that can mitigate the harmful effects of these pathways.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research.

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