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Exercise attenuates oxidative stress and cartilage degeneration in a rat model of knee osteoarthritis

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ABSTRACT

Background: Oxidative stress contributes to cartilage degeneration and disease progression in knee osteoarthritis (OA). Although exercise is widely recommended for OA management, the comparative effects of aerobic, resistance, and combined exercise on oxidative stress and cartilage preservation remain unclear. This study aimed to evaluate the effects of different exercise modalities on oxidative stress biomarkers and cartilage histopathology in a rat model of knee OA.

Methods: Thirty-five male Wistar rats were randomly allocated into five groups (n = 7 per group): non-OA, OA non-exercise (OA + NE), OA + aerobic exercise (OA + AE), OA + resistance exercise (OA + RE), and OA + combined exercise (OA + CE). Knee OA was induced using intra-articular monosodium iodoacetate (MIA), followed by 8 weeks of exercise intervention. Body weight ratio, knee diameter, synovial fluid malondialdehyde (MDA), superoxide dismutase (SOD), SOD/MDA ratio, and cartilage histopathology based on Osteoarthritis Research Society International scoring were evaluated.

Results: Significant differences were observed in knee diameter changes among groups ($p < 0.001$), with all exercise-treated groups demonstrating reduced joint swelling compared with the OA + NE group. The exercise-treated group also showed lower MDA levels and higher SOD/MDA ratios than the OA + NE group, although these differences did not reach statistical significance (MDA: $p = 0.333$; SOD/MDA ratio: $p = 0.074$). Histopathological analysis revealed lower OARSI grades in all exercise groups. The OA + CE group demonstrated the mildest cartilage degeneration and the greatest preservation of cartilage structure.

Conclusion: Exercise intervention was associated with reduced joint swelling and improved cartilage preservation in experimental knee osteoarthritis. Combined exercise demonstrated the most favorable histopathological profile, indicating potential complementary benefits of aerobic and resistance training. Further studies are required to confirm these findings and clarify their clinical relevance in knee OA management.

Keywords: exercise, knee osteoarthritis, Osteoarthritis Research Society International, oxidative stress.
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INTRODUCTION

Osteoarthritis (OA) is one of the most widespread musculoskeletal diseases in the world and a major cause of disability and pain. Furthermore, OA is a chronic degenerative joint disease that affects the structural and functional integrity of joint tissue, with articular cartilage degeneration as the major symptom.[1–3] According to the World Health Organization (WHO), in 2019, the disease affected approximately 528 million people worldwide, of whom 73% were aged 55 or older.[4] Several studies have shown that knee OA is the most prevalent, with an estimated 365 million people affected globally.[5]

Pathogenesis of OA includes a stepwise loss of cartilage, structural changes in the whole joint organ, which is predominantly propagated by chronic low-grade inflammation.[1–3] The process of cartilage degeneration is promoted by impaired joint homeostasis, where catabolic activities predominate. These catabolic pathways are mediated by lipid mediators, pro-inflammatory cytokines, and reactive oxygen species (ROS) derived from synoviocytes, osteoblasts, and chondrocytes. Moreover, long-lasting inflammation and deterioration of the joint surface favor oxidative stress, which is defined as an imbalance between the excessive production of ROS and insufficient antioxidant protection.[6,7]

One of the major oxidative products of lipid peroxidation is malondialdehyde (MDA), commonly considered a marker of oxidative stress resulting from ROS-mediated membrane damage.[8] Meanwhile, superoxide dismutase (SOD) is an important endogenous antioxidant enzyme that catalyzes the dismutation of superoxide radicals to less harmful species, protecting joint tissues against oxidative damage.[7] The state of increased MDA levels and decreased SOD activity is an imbalance that leads to redox dysregulation and can be a strong contributor to OA progression. These processes enhance the structural joint damage and cartilage degeneration.[6,7,9]

As oxidative stress has a central role in the pathophysiology of OA, therapeutic strategies to restore redox homeostasis are increasingly gaining significant attention. The modern treatment of

Table 1. Comparison of body weight ratio, knee diameter changes, and oxidative stress parameters among experimental groups

Variables	Group					p-value
	Non-OA	OA + NE	OA + AE	OA + RE	OA + CE	
Body weight ratio	1.87 ± 0.32	1.74 ± 0.38	1.52 ± 0.22	1.88 ± 0.44	1.49 ± 0.19	0.066a
ΔKnee diameter, mm	0.06 (-0.20 – 0.43)	1.58 (0.23 – 2.57)	0.08 (-1.96 – 0.59)	0.62 (-0.90 – 0.98)	0.08 (-0.64 – 0.66)	<0.001b
MDA, µmol/mg protein	2.12 ± 0.90	4.85 ± 3.19	1.44 ± 2.12	1.92 ± 0.99	1.32 ± 0.65	0.333c
SOD, unit/mg protein	4367 ± 919	5578 ± 2211	2933 ± 1560	4152 ± 1210	5052 ± 2505	0.055c
SOD/MDA ratio, unit/µmol	2170.51 (1275.88 –5767.69)	996.87 (547.15 –7103.81)	3708.25 (534.22 –6777.72)	2407.56 (1308.83 –5825.35)	3651.08 (2205.43 –20111.31)	0.074b

Values are presented as mean ± standard deviation (SD), except for Δ knee diameter and SOD/MDA ratio, which are presented as median (minimum – maximum). Δ, delta or change; µmol, micromole; AE, aerobic exercise; CE, combined exercise; MDA, malondialdehyde; mg, milligram; mm, millimeter; NE, non-exercise; OA, osteoarthritis; RE, resistance exercise; SOD, superoxide dismutase.
aWelch ANOVA
bKruskal-Wallis test
cOne-way ANOVA

OA is mostly devoted to the analgesia of pain and inflammation using non-steroidal anti-inflammatory drugs (NSAIDs).[10] However, pharmacological interventions do not target the oxidative and inflammatory processes underlying structural joint damage. This shows that non-pharmacological methods altering oxidative stress pathways can provide further disease-modifying prospects.

In this context, physical exercise has been supported as a first-line management intervention for chronic pain in knee OA, with analgesic properties similar to simple analgesics and NSAIDs.[11,12] Different exercise forms, such as aerobic, muscle strengthening, neuromuscular training, balance training, proprioceptive, and aquatic therapy, have been introduced into clinical practice. In addition to symptomatic improvement, the intervention has been suggested to increase antioxidant protection systems and to improve cellular redox homeostasis.[13–16] However, there is still limited information on the mechanistic connection between particular exercise modalities and the control of oxidative stress in OA.

Although previous studies have shown positive outcomes of aerobic exercise or resistance training on outcomes associated with OA, there have been limited direct comparisons between these parameters. Therefore, there is uncertainty whether these exercise modalities have different effects on the oxidative stress biomarkers, including MDA and SOD, or whether the benefits of oxidative status can be translated into structural preservation of articular cartilage. The majority of studies have also been on functional outcomes or single molecular markers, as only a few measure both oxidative stress biomarkers and histopathology of cartilage. This shows a knowledge gap on the comparative impacts of aerobic, resistance, and combined exercise on redox equilibrium and cartilage degeneration.

The mechanistic pathways of OA progression within controlled experimental conditions can be successfully examined using preclinical animal models. Compared to human studies, rat models are directly evaluated regarding joint tissues, biomarkers of oxidative stress, and a detailed histopathological analysis of cartilage destruction, which is commonly challenging to acquire because of ethical and practical constraints. Therefore, this study aims to explore the impact of resistance, aerobic, and combined exercise on the oxidative stress indicators (MDA and SOD) as well as histological cartilage integrity in a rat model of knee OA. A combination of biomechanical measurements of redox balance and Osteoarthritis Research Society International (OARSI)-based histopathological evaluation enables the study to provide a mechanistic understanding of the effects of various exercise modalities on OA progression. The method provides a new understanding of the potential of exercise to be a disease-modifying tool in addition to its well-known application in symptom management.

METHODS

Experimental design

This study used 35 male Wistar rats aged 7-8 weeks, weighing 150-200 g, obtained from Bio Farma, Indonesia. The animals were kept in 2-3 animals/cage under controlled environmental conditions (23.0 oC ± 1.0 oC, 12-h light/dark cycle), where food and water were provided ad libitum. After a 1-week acclimatization period, the body weight of all the animals was recorded as the baseline. Rats were weight-stratified according to their baseline body weight and randomly allocated into five experimental groups (n = 7 in each group) using a random number generator in Microsoft Excel to ensure comparable baseline body weight across groups. The experimental groups consisted of non-OA; OA non-exercise (OA + NE); OA with aerobic exercise (OA + AE); OA with resistance exercise (OA + RE); and OA with combined exercise (OA + CE).

All experimental procedures involving animals were reviewed and approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia (Approval No. 1758/UN2.F1/ETIK/PPM. 00.02/2024). Animal care, housing, experimental procedures, and euthanasia were conducted in accordance with institutional guidelines for the care and

use of laboratory animals and complied with internationally accepted principles for animal welfare.

Induction of knee osteoarthritis

The knee OA induction protocol was performed in all groups except the non-OA group. Before OA induction, all rats were weighed, followed by anesthesia utilizing ketamine (73 mg/kg bw) and xylazine (8.8 mg/kg bw). The rats were positioned supine with the knee maintained in 90° flexion, and the hair around the right hind knee joints was shaved with an electric razor. The right hind knee joint received a single intra-articular injection of 50 µl of saline comprising 2 mg Monoiodoacetate (Sigma-Aldrich, Saint Louis, United States) through the infrapatellar ligament utilizing a 27-gauge, 0.5-inch needle.[17,18] Exercise interventions were initiated 2 weeks after OA induction.

Adaptation exercise protocol

Before the exercise intervention, rats underwent a 1-week adaptation period to familiarize them with the exercise equipment and reduce stress. Rats within the aerobic exercise groups were habituated to treadmill running (10 m/min, 10 min/day) using a motor-driven treadmill (ZH-PT, Zhongshidichuang Science & Technology Development, Co., Ltd., Beijing, China).[14,19] Rats in the resistance exercise groups were trained to climb a 1-meter ladder inclined at 80° without additional load, 3 times per day.[20] The OA + CE, the adaptation phase consisted of 3 weekly aerobic sessions and 2 weekly resistance sessions, implemented in accordance with the respective protocols outlined above.

Determination of maximum carrying capacity

The maximum carrying capacity was determined before the initiation of resistance training. Rats performed repeated ladder climbs under gradually increasing loads attached to the tail, starting 50% of body weight and increasing by 30 g after each successful climb.[20] The maximum load successfully carried before failure was recorded and used to prescribe training intensity.

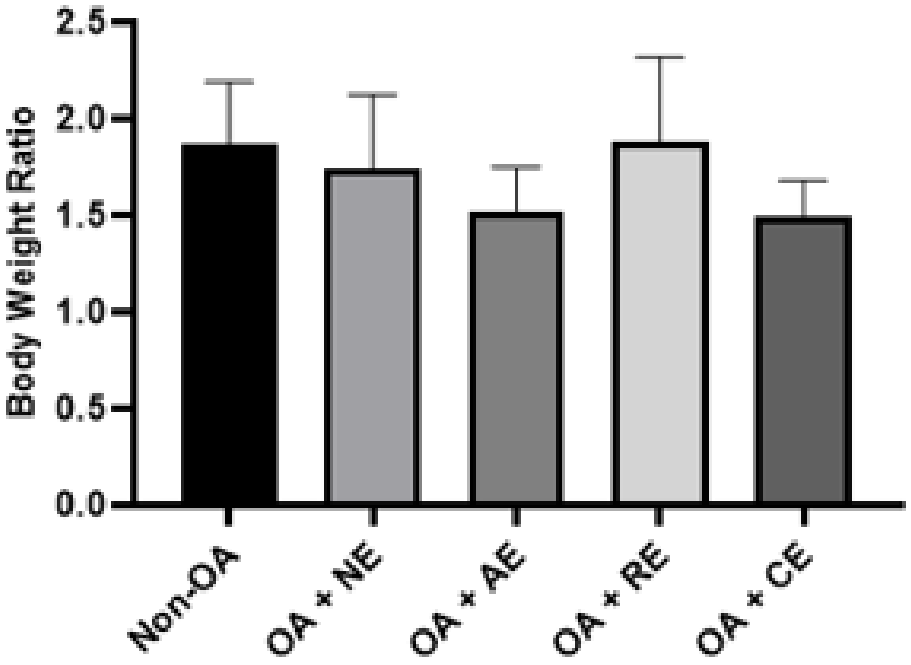

Figure 1. Body weight change, expressed as the post- to pre-induction ratio, across experimental groups. Data are presented as mean ± standard deviation (SD) (n = 7 per group). Non-OA, non-osteoarthritis control group; OA + NE, osteoarthritis non-exercise group; OA + AE, osteoarthritis + aerobic exercise group; OA + RE, osteoarthritis + resistance exercise group; OA + CE, osteoarthritis + combined exercise group.

Resistance exercise sessions

Rats in the RE group performed ladder climbing on a 1-meter ladder inclined at 80°, completing 8 sets per day with 2-minute inter-set rest periods, 3 times per week for 8 weeks. Resistance training was progressively increased according to the following scheme 20: 1. Weeks 1-2: 50% of maximum load; 2. Weeks 3-4: 75% of maximum load; 3. Weeks 5-8: 90% of maximum load.

Determination of maximal exercise test (MaxET) and aerobic training protocol

Before aerobic training, rats in the OA + AE and OA + CE groups underwent a maximal exercise test (MaxET) on a graded treadmill at incremental speeds of 5 m/min until exhaustion. The highest running speed achieved was used to prescribe aerobic training intensity. Aerobic exercise consisted of treadmill running for 30 min/session at progressive intensities of 60% MaxET (weeks 1-2), 70% MaxET (weeks 3-4), and 80% MaxET (weeks 5-8), performed 5 times weekly for 8 weeks.[14,19]

Combined training protocol

Rats in the OA + CE group underwent aerobic training 3 times weekly and resistance training twice weekly on alternating days for 8 weeks, following the respective protocols described above.

Measurement of knee diameter

Measurements were performed at baseline during OA induction and upon completion of the experiment. During the measurement procedure, rats were anesthetized and positioned supine with the hind limbs flexed at 90 °. A digital caliper was used to measure the diameter of the right hind limb across the mediolateral part of the joint. All measurements were performed under standardized positioning conditions by the same examiner, and the average of 3 repeated measurements was analyzed.

Sample collection

Following 8 weeks of intervention, all rats were euthanized by high-dose ketamine. All experimental animals had their right hind knee joints and synovial fluid collected. Knee joint tissues were removed, washed with sterile NaCl solution, and

preserved in phosphate-buffered saline (PBS) to be analyzed further.

Synovial lavage fluid was produced from the right knee with intra-articular injection of 0.5 mL sterile saline, followed by flexion-extension and aspiration-reinjection. Centrifugation (3000 rpm, 15 min, 24 °C) was performed on the recovered fluid, followed by MDA, SOD, and total protein analyses of the supernatant.

Histological analysis

Right hind knee joints were fixed in 10% neutral-buffered formalin (72 h), decalcified in 10% formic acid (2 weeks), embedded in paraffin, and sagittally sectioned at 5 uM thickness. Histological assessment was performed by staining sections with hematoxylin and eosin (H&E) and Safranin-O/Fast-green. The histological effects have been observed under the binocular light microscope (Leica DM500, Leica Microsystems, Germany) at 100x magnification, and the images captured with an inbuilt digital camera (Leica ICC50 HD). HE staining was used to analyze cartilage morphology and structural abnormalities, and Safranin-O/Fast green staining was used to analyze the amount of proteoglycan in the cartilage matrix. Histological severity was graded using the OARSI (Osteoarthritis Society International) scoring system (grade 0-6).[7,8]

Measurement of oxidative stress parameters

Protein quantification

Total protein levels were measured using the Warburg-Christian method with spectrophotometric detection at 280 nm. The value for each sample was calculated by comparing the absorbance of the test solution to a linear bovine serum albumin (BSA) standard curve and quantified in milligrams per milliliter (mg/mL).

Malondialdehyde (MDA) assay

MDA levels were measured using the thiobarbituric acid reactive substances (TBARS) assay, as described by Wills. The absorbance of the MDA-TBARS complex was measured at 530 nm using a UV spectrophotometer. MDA concentration in both the standard and the samples was determined by measuring the

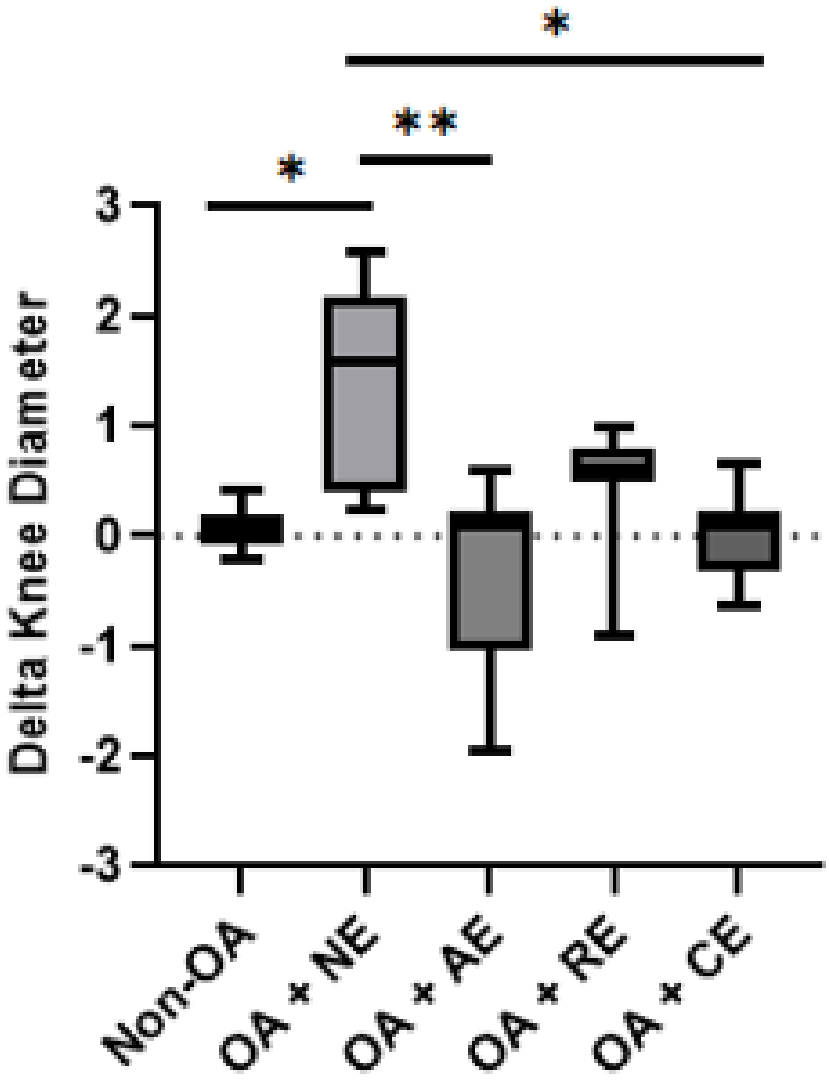

Figure 2. Changes in knee expressed as Δ knee diameter (post-intervention minus pre-induction) across experimental groups. Data are presented as medians, with whiskers indicating the minimum and maximum values (n = 7 per group). Positive values indicate an increase in knee diameter, while negative values indicate a reduction. Statistical significance is indicated as *p<0.05, **p<0.01, and ***p<0.001. Non-OA, non-osteoarthritis control group; OA + NE, osteoarthritis non-exercise group; OA + AE, osteoarthritis + aerobic exercise group; OA + RE, osteoarthritis + resistance.

intensity of the pink chromogen formed and calculated using a linear standard curve, with tetraethoxypropane as the standard. Trichloroacetic acid (TCA) and thiobarbituric acid (TBA) reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Results were expressed in micromolar (μM) and normalized to total protein concentration (μmol/mg protein).

Superoxide dismutase (SOD) activity assay

Total SOD activity was measured using a SOD activity assay kit (Elabscience, USA), according to the manufacturer's instructions. The assay was based on the generation of superoxide anion radicals (O2-) through the xanthine-xanthine oxidase reaction system. These radicals react with hydroxylamine to produce nitrite, which subsequently forms a colored compound upon reaction with a chromogenic developer. In addition, SOD activity was quantified by measuring the degree of inhibition of nitrite formation, according to the manufacturer's calculation formula.

To account for variations in sample concentration, MDA levels, and SOD activity were normalized to the total protein concentration of each sample. The

final values were expressed as µmol/mg protein for MDA and U/mg protein for SOD activity.

Statistical Analysis

Data were analyzed applying IBM Statistical Package for the Social Sciences (SPSS, version 29; IBM Corp., Armonk, New York, United States), and graphs were generated using Prism 8 (GraphPad Software, La Jolla, California, United States). Data normality was assessed before analysis using the Shapiro-Wilk test. For normally distributed data, one-way analysis of variance (ANOVA) was used to compare differences among groups, followed by Tukey's post hoc test for multiple comparisons. For data that did not meet normality assumptions, the Kruskal-Wallis test was applied, followed by Dunn's post hoc test.

RESULTS

Each animal was subjected to the study protocol and incorporated in the ultimate analyses to obtain a total of seven animals per group. **Table 1** summarizes the outcome of body weight ratio, Δ knee diameter, and oxidative stress parameters.

The body weight ratio was not significantly different across groups ($p = 0.066$). Although there was a tendency towards lower body weight ratios in the OA + AE and OA + CE groups compared to the OA + RE, no statistically significant differences were found. This showed that the exercise interventions had no negative effects on the overall growth and body weight gain during the experiment (**Figure 1**).

Knee diameter changes were measured as Δ knee diameter (post-pre). A large difference was found between groups ($p < 0.001$). OA + NE showed the largest knee diameter change, which indicated consistent joint swelling after inducing OA. However, all the exercise-treated groups had significantly lower Δ knee diameter values. These results indicate that aerobic, resistance, and combined exercise programs mitigated the OA-related joint inflammation and swelling (**Figure 2**).

Figure 3 shows differences in synovial fluid MDA levels. The OA + NE group had the highest MDA level compared to all other groups, while aerobic, resistance, and

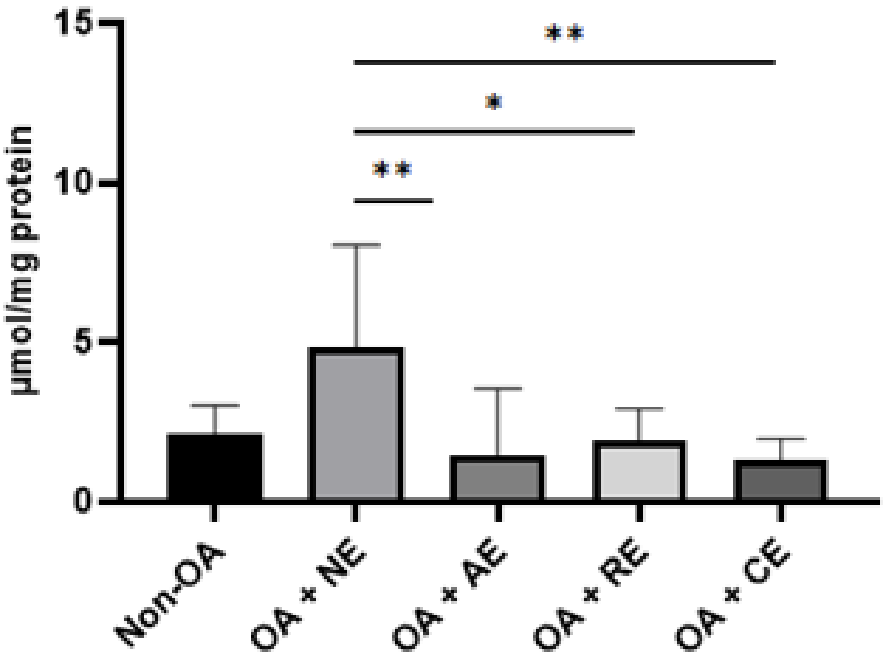

Figure 3. Malondialdehyde (MDA) levels in knee synovial fluid across experimental groups. Data are presented as mean ± standard deviation (SD) (n = 7 per group). Statistical significance is indicated as *p<0.05 and **p<0.01. µmol/mg, micromole per milligram; Non-OA, non-osteoarthritis control group; OA + NE, osteoarthritis non-exercise group; OA + AE, osteoarthritis + aerobic exercise group; OA + RE, osteoarthritis + resistance exercise group; OA + CE, osteoarthritis + combined exercise group.

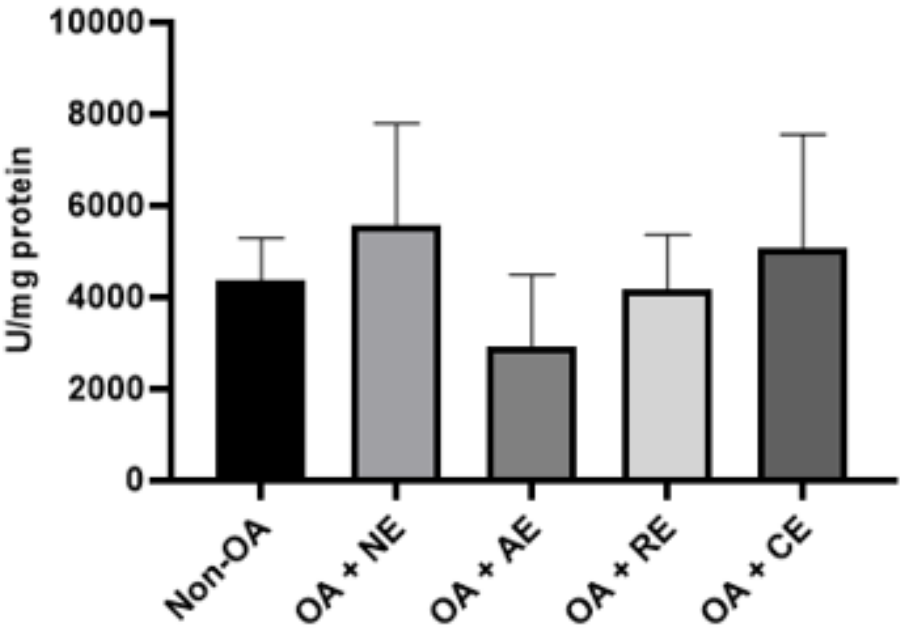

Figure 4. Total superoxide dismutase (SOD) activity in the knee synovial fluid across experimental groups. Data are presented as mean ± standard deviation (SD) (n = 7 per group). Non-OA, non-osteoarthritis control group; OA + NE, osteoarthritis non-exercise group; OA + AE, osteoarthritis + aerobic exercise group; OA + RE, osteoarthritis + resistance exercise group; OA + CE, osteoarthritis + combined exercise group; U/mg, unit per milligram

combined exercise had significantly lower MDA levels. Although the overall one-way ANOVA did not demonstrate a significant difference among groups (p = 0.333), post hoc comparisons showed consistently lower MDA levels in all exercise-treated groups compared with the OA + NE group. Collectively, these findings suggest that exercise intervention was associated with reduced lipid peroxidation.

Total SOD activity between groups did not significantly differ (p = 0.055) in terms of antioxidant response, as shown in **Figure 4**. However, the OA + CE group showed numerically better SOD activity when compared to the OA + AE and OA + RE groups. Although the value obtained did not reach statistical significance, these results indicated a propensity toward an improvement of antioxidant defense in response to combined exercise training.

The ratio of total SOD activity to MDA levels (SOD/MDA ratio) was calculated to further assess oxidative stress status. Based on the results, there were no statistically significant differences between groups (p = 0.074). However, the exercise-treated groups indicated a high SOD/MDA ratio as compared to the OA + NE group. These results suggest a shift toward a better redox balance after exercise intervention, particularly in the OA + AE and OA + CE groups (**Figure 5**).

The knee OA severity was categorized by OARSI scoring system. Samples that had OARSI scores of 0, 1-2, and 3-6 were classified as non-OA, low-grade knee OA, and high-grade knee OA. As presented in **Figure 6**, all the animals in the non-OA group had normal cartilage morphology (OARSI grade 0), proving the absence of osteoarthritic lesions. However, animals in the OA + NE group showed OA lesions in most cases in the grades 3-6, which was evidence of severe cartilage degeneration.

The exercise intervention was correlated with a change to lower OARSI grades. The OA + AE and OA + RE groups were primarily grade 2 (6/7 samples), and the OA + CE group was primarily grade 1 (6/7 samples). These results suggest that exercise intervention was linked to better cartilage structure maintenance, with combined exercise having the highest propensity to preserve cartilage.

The results were further supported by the histopathological observations.

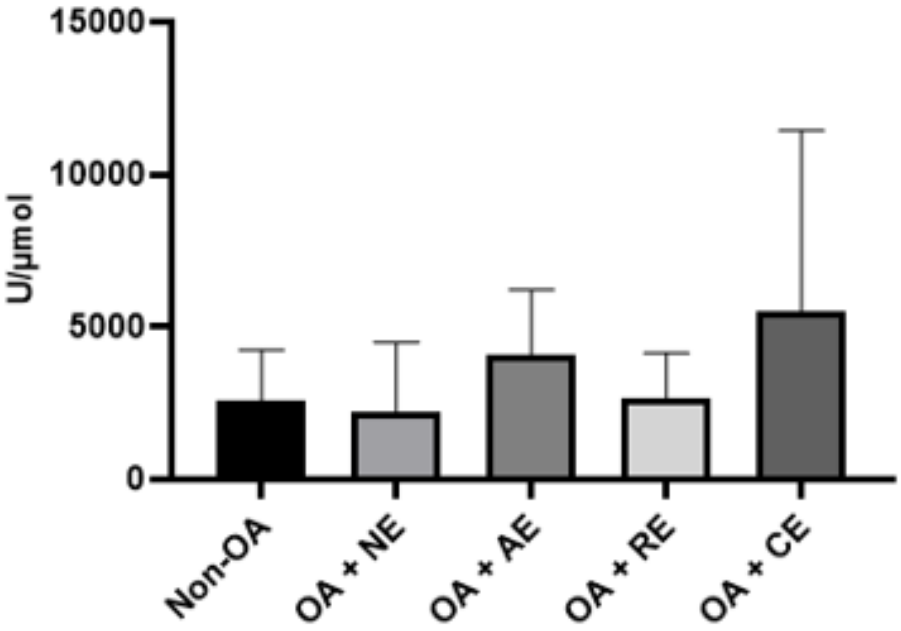

Figure 5. Ratio of total superoxide dismutase (SOD) activity to malondialdehyde (MDA) levels (SOD/MDA) in knee synovial fluid across experimental groups. Data are presented as mean ± standard deviation (SD) (n = 7 per group). Non-OA, non-osteoarthritis control group; OA + NE, osteoarthritis non-exercise group; OA + AE, osteoarthritis + aerobic exercise group; OA + RE, osteoarthritis + resistance exercise group; OA + CE, osteoarthritis + combined exercise group; U/µmol, unit per micromole.

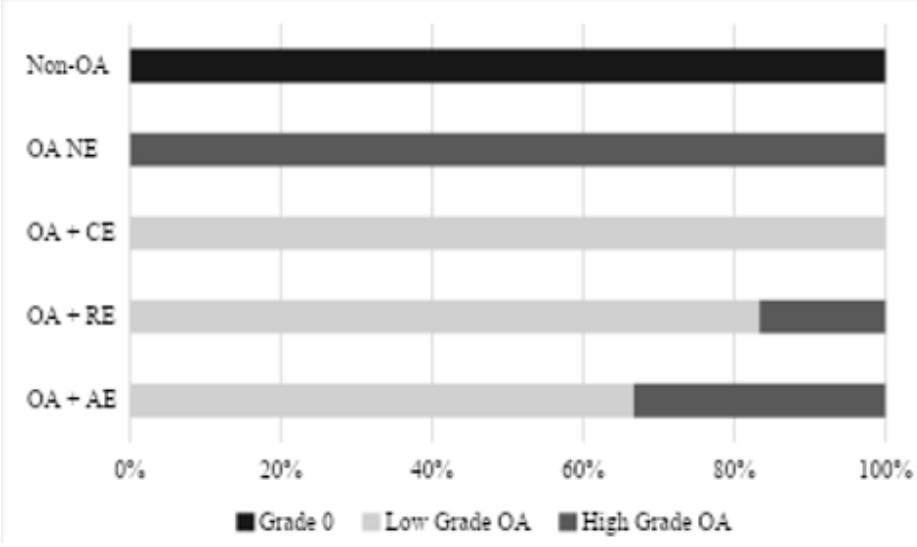

Figure 6. Distribution of cartilage histological severity across experimental groups based on the Osteoarthritis Research Society International (OARSI) scoring system. Samples were categorized as non-OA (grade 0), low-grade OA (grades 1-2), and high-grade OA (grades 3-6). Data are expressed as percentages of samples within each category. Non-OA, non-osteoarthritis control group; OA + NE, osteoarthritis non-exercise group; OA + AE, osteoarthritis + aerobic exercise group; OA + RE, osteoarthritis + resistance exercise group; OA + CE, osteoarthritis + combined exercise group.

In the OA + NE group, representative hematoxylin and eosin (H&E) and Safranin O/Fast Green stains showed widespread cartilage surface irregularity, matrix loss, and structural disruption. Within architecture, there was better preservation of proteoglycan staining and surface integrity. The mildest changes in

histopathology were observed in the OA + CE group, which correlated with the domination of lower OARSI (**Figure 7**).

DISCUSSION

This study examined the impact of aerobic, resistance, and combined exercises on oxidative stress biomarkers and cartilage integrity in a rat knee OA model. The key results were that the OA induction was effective in causing joint swelling, oxidative stress imbalance, and cartilage degeneration. Furthermore, exercise intervention was shown to lower MDA levels and reduce knee swelling in comparison to the OA non-exercise. Although no significant differences were observed in SOD activity or SOD/MDA ratio, exercise-treated animals demonstrated a tendency toward improved redox balance. Furthermore, exercise intervention was associated with lower OARSI grades and improved cartilage preservation, with the combined exercise group demonstrating the most favorable histopathological profile.

In this study, knee OA was induced by intra-articular injection of monosodium iodoacetate (MIA). Increased knee swelling, altered oxidative stress parameters, and histopathological evidence of cartilage degeneration in the OA non-exercise group indicated successful OA induction. OA induction also resulted in a lower body weight gain ratio compared with the non-OA group, although the difference was not statistically significant. This tendency may reflect reduced mobility and altered metabolic status associated with OA progression. These results were consistent with previous studies that described joint swelling as a hallmark of inflammatory and degenerative changes in experimental OA models.[21]

The OA non-exercise group also demonstrated higher synovial fluid MDA levels than the non-OA group, indicating increased lipid peroxidation and oxidative stress following OA induction. Excessive reactive oxygen species (ROS) production contributes to cartilage matrix degradation and chondrocyte dysfunction through oxidation of lipids, proteins, and extracellular matrix components.[6] Clinical studies have reported increased lipid peroxidation in OA patients, reflected by elevated levels of MDA and 4-hydroxynonenal (4-HNE) in synovial tissue compared with controls.[22] In addition, chondrocyte lipid peroxidation contributed to collagen oxidation and

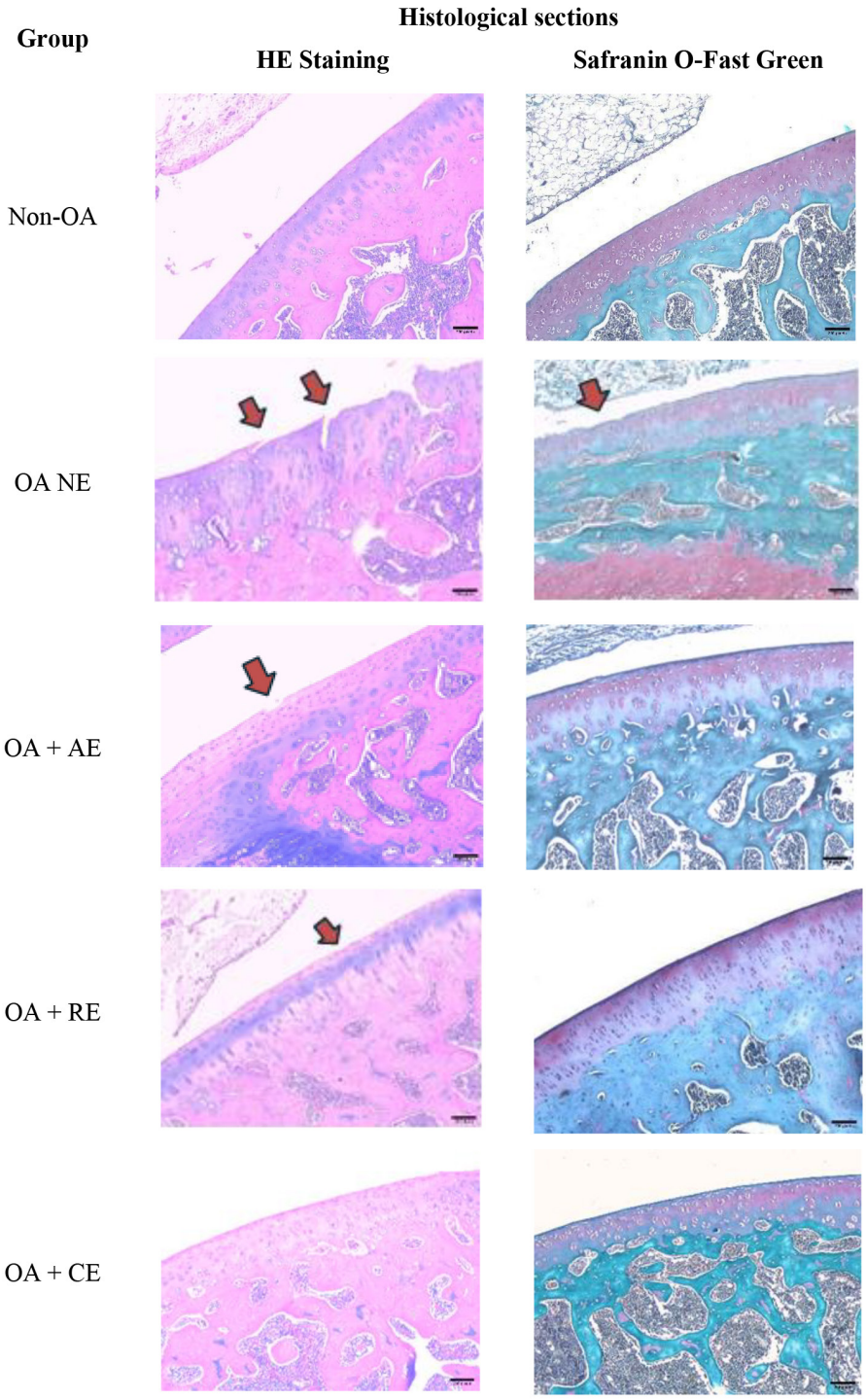

Figure 7. Representative histological sections of knee articular cartilage stained with HE and Safranin O/Fast Green across experimental groups (n = 7 per group). Arrows indicate areas of cartilage surface disruption and matrix degradation. Images were obtained at 100x magnification. Non-OA, non-osteoarthritis control group; OA + NE, osteoarthritis non-exercise group; OA + AE, osteoarthritis + aerobic exercise group; OA + RE, osteoarthritis + resistance exercise group; OA + CE, osteoarthritis + combined exercise group.

degradation, thereby increasing cartilage susceptibility to mechanical fatigue and accelerating OA progression.[22,23]

Although total SOD activity tended to be higher in the OA non-exercise group than in the non-OA group, the difference was not statistically significant. This increase may represent an early compensatory response to elevated oxidative stress. However, the apparent increase in antioxidant activity was insufficient to fully counterbalance lipid peroxidation, as reflected by the higher MDA levels observed in the OA non-exercise group. The absence of significant differences in SOD activity may also reflect the dynamic and adaptive nature of endogenous antioxidant responses, which vary with disease stage and the timing of sample collection.[23]

Similarly, the SOD/MDA ratio tended to be lower in the OA non-exercise group than in the non-OA group, suggesting impaired oxidative balance. Previous studies have shown that reduced antioxidant capacity contributes to oxidative damage, chondrocyte degeneration, and cartilage destruction during OA progression.[6,24] Xiao et al. also reported that osteochondral defects were associated with progressive increases in MDA, accompanied by reductions in antioxidant defense mechanisms over time.[23] Collectively, these findings support the role of oxidative stress in OA pathogenesis and further confirm the successful induction of OA in the present study.

Histopathological evaluation further confirmed successful OA induction. OA lesions were evident in all animals in the OA non-exercise group and were mostly located in OARSI grades 3 to 6, showing advanced cartilage degeneration. The results were shown by structural disorganization, cartilage surface irregularity, and reduced Safranin O staining intensity, indicating progressive matrix degradation and proteoglycan loss. Other histopathological alterations described by Takahashi et al. showed progressive cartilage degeneration following induction of MIA. These included weak staining of chondrocytes in the early stages, and characteristic OA changes, such as fibrillation, fissuring, subchondral bone changes, and osteophyte formation at a later stage.[25] The histopathological changes in MIA-induced OA models were similar to human OA.[25,26] These results suggested that intra-articular MIA injection effectively replicated major pathological changes associated with knee OA, such as inflammation, oxidative stress changes, and cartilage degeneration.

Exercise interventions proved to have positive impacts on various parameters related to OA development. The body weight ratios were significantly different across groups, although aerobic and combined exercise groups were more likely to show lower weight gain compared to the resistance exercise group. This trend was also physiologically significant, since better weight management would decrease mechanical load on the knee joint and maintain cartilage.[27,28] Moreover, previous studies have established that mechanical loading can increase the rate of cartilage degeneration, while physiological loading facilitates cartilage metabolism and joint homeostasis.[27,29] This tendency is physiologically relevant since better weight regulation reduces the mechanical stress on the knee joint and preserves cartilage.

Exercise-treated animals also demonstrated lower MDA concentrations than the OA non-exercise group. Although these differences did not reach statistical significance, the findings suggest a tendency toward reduced lipid peroxidation following exercise intervention. The lowest MDA levels were observed in the combined exercise group, followed by the aerobic and resistance exercise groups. Previous studies have shown that exercise can alleviate oxidative stress by increasing mitochondrial efficiency, enhancing antioxidant homeostasis, and decreasing ROS formation.[14,30,31]

Based on the results, there were no statistically significant differences in total SOD activity and SOD/MDA ratio between groups. However, the exercise-treated groups tended to have higher SOD activity and SOD/MDA ratio than the OA non-exercise group. This trend showed the partial recovery of the balance between antioxidant defense and lipid peroxidation after exercise intervention. The lack of statistically significant differences in SOD-related parameters showed the dynamic and adaptive character of endogenous antioxidant reactions that might change depending on the stage of OA development and the time when the samples were collected. Additional studies with more oxidative stress indicators, such as glutathione (GSH), 4-HNE, or 8-OHdG (8-hydroxy-2-deoxyguanosine), provided a more detailed insight into oxidative changes during OA progression and exercise adaptation.

Further evidence of the positive effects of exercise intervention was presented by the histopathological findings. All the exercise-treated patients compared to the OA non-exercise group had lower OARSI grades and improved cartilage structure preservation. The proportion of low-grade OA lesions and the least significant histopathological changes were observed in the combined exercise group, showing that a combination of aerobic and resistance exercise could be complementary in its effects on metabolic and biomechanical processes.

Oxidative metabolism and cellular redox regulation have been reported to be enhanced by aerobic exercise. However, resistance exercise was found to improve muscle strength and joint stabilization, causing a decrease in abnormal mechanical loading of the articular cartilage.[27,29,32,33] The positive histological picture presented in the combined exercise group could be due to the combination of these complementary adaptations. Despite the descriptive differences between the exercise modalities, the general direction toward better cartilage preservation in the combined exercise group shows the possible benefits of multimodal exercise in OA management.

From a translational standpoint, the results indicated that exercise interventions could be used to alleviate oxidative damage and cartilage integrity in OA. Although all models showed similar effects on oxidative stress biomarkers, the combined exercise group had the best histopathological profile. These results show that aerobic and resistance exercises should be used complementarily because of their combined effects based on both metabolic and biomechanical processes. Exercise intervention was realized when OA induction had caused mild-to-

moderate cartilage degeneration that corresponded to OARSI grades 23. This has clinical implications in relation to individual patients with early-stage knee OA, since exercise interventions can delay structural cartilage degeneration and enhance joint preservation.

This study offered new evidence about the possible effects of various exercise modalities on oxidative stress regulation and histopathological maintenance of cartilage in an experimental OA model. However, there are several limitations associated with the experimental process. First, only male Wistar rats were used, which restricted the extrapolation of the results to females, where hormonal effects might influence OA progression and exercise responses. Second, histopathological evaluation was semi-quantitative based on OARSI scoring and could be vulnerable to observer bias despite the standardized evaluation criteria. Third, the intervention time was restricted to 8 weeks and might not completely represent long-term exercise adaptations or the chronic course of OA. Fourth, the sample size could be small and might have limited statistical power to identify the differences between exercise modalities in terms of oxidative stress biomarkers. Fifth, the analysis did not examine the oxidative and inflammatory pathways of cartilage degeneration in general, but concentrated specifically on MDA and SOD. Therefore, future studies with bigger samples, prolonged interventions, both sexes, and more molecular biomarkers are recommended to help better elucidate the mechanisms and clinical value of exercise interventions in OA management.

CONCLUSION

In conclusion, this study shows that exercise intervention can decrease joint swelling and enhance cartilage preservation in experimental knee osteoarthritis. Although no statistically significant differences were observed in oxidative stress biomarkers, exercise-treated groups consistently demonstrated lower MDA levels, higher SOD/MDA ratios, and milder cartilage degeneration than the OA non-exercise group. The best cartilage histopathological profile is shown in combined exercise. These results suggest the potential of combining aerobic and resistance exercise as a non-pharmacological approach to managing knee OA. Moreover, further studies are required to validate the results and establish their clinical relevance.

ETHICAL CONSIDERATION

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia, registration number 1758/UN2.F1/ETIK/PPM. 00.02/2024.

CONFLICT OF INTEREST

This study was conducted without any conflict of interest.

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AUTHOR CONTRIBUTIONS

CFH prepared study designs, collected and analyzed data, and wrote manuscripts. DISS, HW, and PEW directed data collection and revised the manuscript. All authors contributed to drafting and approved the final manuscript.

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