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Antioxidant effect of chromium picolinate on chronic exercise-induced oxidative stress in male rats

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Chromium picolinate influences antioxidant mechanisms, potentially affecting oxidative stress levels during prolonged aerobic exercise. This study investigates the effects of chromium supplementation on Catalase (CAT) activity and Malondialdehyde (MDA), advanced oxidation protein products (AOPP), and reduced glutathione (GSH) levels in chronic aerobic exercise in male rats. Twenty-eight male Wistar albino rats were divided into 4 groups containing 7 mice each (control, exercise, chromium, and chromium + exercise). Supplemented rats received chromium picolinate (8 µg/kg/day) daily for eight weeks. Exercise was performed on a rat treadmill at an average speed of 15 cm/s for 20 min, five days a week for eight weeks. At the end of the 8th week of the experimental period, blood samples were taken. CAT, MDA, AOPP, and GSH analyses were performed. It was observed that the chromium + exercise group induced a significant reduction in CAT activity compared to the other three groups (− 8.6 to − 12%, $p < 0.05$). MDA values meaningfully increased (18.2–25.7%, $p < 0.001$) in all groups, except the controls after the 8-week intervention. All groups demonstrated an increase in AOPP (8.1–12.3%, $p < 0.001$), but not the controls. In GSH, all experimental groups showed a significant elevation (30.3–45.8%, $p < 0.001$) compared to the control group ($p < 0.001$) following an 8-week intervention period. The present findings indicate that supplementation with chromium picolinate, whether administered alone or in conjunction with aerobic exercise, led to modulations in oxidative stress and redox status indices in male rats following an 8-week aerobic exercise regimen. The observed reduction in CAT activity may suggest a lowered oxidative challenge; however, this finding should be interpreted with caution, as decreased antioxidant enzyme activity can also reflect a potential limitation in defense capacity.

Keywords Redox balance, Free radicals, Endurance training, Skeletal muscle, Oxidative damage

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During exercise, the metabolic rate and oxygen consumption increase, releasing reactive oxygen species (ROS) in the mitochondria[1]. In addition, the activation of phospholipase A2 leads to an increase in reactive species through the reaction of various enzymes[2]. ROS produced by exercise has beneficial effects, such as stimulating glycogen synthesis, reducing the risk of infection, and facilitating adaptation to exercise[2–4]. However, excessive ROS can cause muscle damage, immune disorder, and fatigue by changing cell structure and function[5].

In general, the body has sufficient reserves of antioxidants to cope with ROS production under physiological conditions. This system contains antioxidant vitamins, glutathione, thiols, and antioxidant enzymes[6]. Each of these antioxidants plays a unique role in the cell and functionally complements the others. In addition, interorgan transport of certain antioxidants is likely to occur. These antioxidant defense systems maintain hemostasis for normal cell function both at rest and during light exercise[7]. Disruption of the balance between oxidants and antioxidants, especially in favor of oxidants, leads to the compromise of vital cellular structures such as membrane lipids, proteins, and DNA, resulting in the development of pathological events in living organisms[8]. However, when ROS production is excessive, such as during prolonged aerobic exercise, or when the antioxidant defense is severely hampered by nutritional deficiencies and pharmacological intervention, the inadequate defense can be suppressed by ROS, leading to extensive cell and tissue damage[8].

There are many reasons to examine the effects of antioxidant supplements on exercise performance and post-exercise recovery. Mitochondrial adenosine triphosphate production is not 100% efficient and therefore superoxide radicals are formed in large quantities during exercise[9]. As more oxygen is consumed during exercise, more superoxide anions are released and can cause oxidative stress. Muscle damage causes free radical production[10], which inhibits healing. Finally, the endogenous defense mechanism is insufficient to scavenge the increased types of free radicals, and antioxidant supplements can help prevent oxidative stress at this point[11]. Many antioxidant compounds such as chromium are widely used by athletes to reduce muscle fatigue and increase their performance[12].

Antioxidant supplements may have a direct or indirect effect on exercise performance. Its direct effect is that it can reduce muscle fatigue during the contraction process[13]. The indirect effect can be counted as reducing physiological stressors that negatively affect exercise or improving post-exercise recovery[14]. Chromium picolinate differs from classical antioxidants such as vitamins C and E by influencing both glucose metabolism and insulin sensitivity, which may indirectly affect oxidative stress pathways. Unlike vitamins that mainly act as radical scavengers, chromium contributes to cellular redox regulation via trace element roles in enzymatic reactions[12–14]. This unique dual role makes it a promising candidate for exercise-related oxidative stress research. In this study, it is thought that chromium supplementation in addition to the exercise applied in rats will provide an excellent model to examine the dynamic balance between oxidative challenge and antioxidant defense in the biological system. In this direction, the study aims to examine the effects of chronic aerobic exercise together with chromium supplementation on CAT activity, MDA, AOPP, and GSH levels in male rats.

Materials and methods

Registration

The study protocol was registered on the Open Science Framework (https://osf.io/vfrd5/). No deviations from the registered protocol occurred during the implementation of the study.

Experimental design and animals

Male Wistar rats (8 weeks old, 220–350 g body weight) were obtained and used in this study from Van Yüzüncü Yıl University Experimental Animals Laboratory. The care and procurement of the experimental animals were carried out at the Van Yüzüncü Yıl University Experimental Animals Laboratory. Rats were maintained in a standard laboratory environment (temperature: 22 ± 2 °C, relative humidity: 55 ± 5% and 12/12-hour light/dark cycle). The animals were given a standard diet that was regularly renewed daily, and their hygiene was maintained. This study was approved by Van Yüzüncü Yıl University Animal Experiments and Local Ethics Committee (2023/07–33) and conducted in accordance with internationally recognized ethical standards for laboratory animal use and care, including the ARRIVE guidelines and the European Community Directive (86/609/EEC). To prevent pain and suffering in the experimental animals and to respect animal rights, anesthetic agents such as xylazine and ketamine were used. The dose of ketamine was set at 100 mg/kg, and the dose of xylazine was set at 15 mg/kg. The rats were subjected to the procedures following these administrations. This practice is necessary to avoid unnecessary pain in the animals and to conduct the study in accordance with ethical standards.

Euthanasia criteria for experimental animals typically include several conditions. These include situations where the animal is experiencing uncontrollable, severe pain or suffering, as well as conditions involving untreatable diseases or injuries. Euthanasia is also considered when the animal's overall quality of life is significantly compromised. Once the experimental objectives have been met and the animal will not provide additional valuable data, euthanasia may be performed. Clinically significant health problems or infections, along with abnormal, aggressive, or otherwise atypical behavior, are also criteria for euthanasia. Additionally, if the animal does not respond to treatment and has no chance of recovery, euthanasia is considered. At the end of the experimental period, all 28 rats were humanely euthanized under anesthesia for the purpose of sample collection. At the end of the experimental period, all rats were deeply anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (15 mg/kg, i.p.), followed by euthanasia via decapitation. Blood samples were collected by cardiac puncture immediately after decapitation.

A preliminary power analysis was performed to determine an adequate sample size and ensure the generalizability of the results when assigning the experimental groups. To determine the sample size to be included in this study, a power analysis was performed with reference to the study conducted by Pancar et al. 12 (5% margin of error, 95% power), and the sample size resulted in at least 28 rats with groups of 7 members each. Twenty-eight male Wistar albino rats (8 weeks old, 220–350 g body weight) were included in the study, with 7

rats housed in each of the 4 separate cages. The cages are Type II Eurostandard, with dimensions of 265 × 205 mm x 140 mm (± 10 mm). The base living area is 350 cm^2 (± 5 cm). The cages are made of transparent polycarbonate material and are autoclavable up to 121 °C. The rats were obtained from Van Yüzüncü Yıl University Experimental Animals Laboratory and were cared for and fed in the same center. The rats were randomly divided into the following four groups: control group ($n = 7$), animals in this group did not receive any treatment and were given standard feed; exercise group ($n = 7$), animals in this group were fed a daily standard diet and performed 20 min of daily treadmill exercise, five days a week for eight weeks; chromium group ($n = 7$), in addition to the daily standard diet, animals in this group were given chromium (Ocean brand chromium picolinate component) supplementation by gavage method (8 µg/kg/day) once a day for eight weeks; chromium + exercise group ($n = 7$), in addition to the daily standard diet, animals in this group were given a single dose (8 µg/kg/day) of chromium picolinate (Ocean brand chromium picolinate component) supplementation by gavage method for 8 weeks, and 20 min of daily treadmill exercise, five days a week for eight weeks.

The present study employed a posttest-only control group design, in which a single measurement was taken at the end of eight weeks to assess the final effects of the interventions. The experimental groups were homogeneously established, ensuring that subjects shared the same age, gender, genetic background, and environmental conditions, thereby eliminating significant differences between groups at the start of the experiment and making pre-tests unnecessary[15,16]. This design, widely used in animal studies, offers ethical and methodological advantages by minimizing stress on the animals, which can negatively impact results, especially when invasive pre-test procedures are involved. In our case, obtaining blood and tissue samples for a pre-test would have required decapitating the rats, an irreversible procedure that raises ethical concerns and could disrupt the study's progression. To address these concerns, we only collected blood and tissue samples at the conclusion of the 8-week period to evaluate the long-term effects of the interventions. This approach not only prioritizes animal welfare but also enhances the reliability of the results, making it the most appropriate and ethically sound methodology for our study[16].

Treadmill workout programs

The treadmill exercise program in this study was performed using a treadmill (MAY-TME 0804, Commat Ltd., Ankara, Turkey) designed for rats. The animals assigned for exercise were given treadmill training for two weeks. They were trained for 5–10 min a day, at 5 m/min speed and 0% incline, on a treadmill consisting of six separate lanes designed for rats. Then, the rats did jogging exercise for 20 min every day, five days a week for eight consecutive weeks, at an average speed of 15 cm/s, in accordance with the treadmill protocol developed by Rico et al.[4].

Biochemical analysis

Following the conclusion of the experimental applications (week 8), a dosage of 90 mg/kg ketamine was administered intraperitoneally to all rats. After the animal's thorax was shaved, cleaned with alcohol, and opened with a vertical incision from the midline, the heart was directly cannulated, and a blood sample was taken. And centrifuged at 4,000 rpm for 5 min to separate the serum fraction. The serums were transferred into pre-assigned and numbered Eppendorf tubes. It was stored at -80 °C until analysis. CAT, MDA, AOPP, and GSH analyses were performed using Andy Gene ELISA kits (AndyGene Biotechnology Co., Ltd., Fengtai District, Beijing, CHINA) according to the manufacturer's protocol. All biochemical parameters were tested following the recommended procedures provided in the assay kits as previously reported[17].

Statistical analysis

The SPSS-21 package program was used in the analysis of the data. The distribution of the data was examined with the normality-homogeneity test, and it was determined that the variances showed normal distribution. Descriptive statistics for the featured features were expressed as mean and standard deviation. A one-way ANOVA analysis was conducted to statistically analyze all parameters. A Tukey test was employed to compare the various groups. Eta squared was reported as the effect size for the ANOVA model, while Cohen's d was used to indicate the effect size for between-group comparisons. The statistical significance level was set at 5% in the calculations.

Results

All results are presented in detail in Table 1; Figs. 1, 2, 3 and 4. The chromium + exercise group showed significant reductions in CAT ($p < 0.001$) compared to the control (− 12%), exercise (-8.6%), and chromium (-8.7%) groups. The exercise and chromium groups reduced CAT by 3.7% and 3.6%, respectively, compared to the control group ($p < 0.05$) (Fig. 1). The Cohen's d values indicated moderate to very large effects, with 0.77 between the control and exercise groups, 1.24 between the control and chromium groups, and 3.31 between the control and the combined chromium + exercise groups. MDA values meaningfully increased in the exercise (+ 18.2%), chromium (+ 18.2%), and exercise and chromium (+ 25.7%) groups compared to controls ($p < 0.001$) (Fig. 2). No differences were found among the exercise, chromium, and chromium + exercise groups after the 8-week intervention. For MDA, the Cohen's d values were 1.69 between the control and exercise and chromium groups, and 2.22 between the control and the exercise + chromium group. The exercise, chromium, and chromium + exercise groups significantly raised the AOPP values by 8.1%, 11.4%, and 12.3%, respectively, compared to controls ($p < 0.001$) (Fig. 3). No changes were observed in AOPP among the exercise, chromium, and chromium + exercise groups following the conclusion of the 8-week intervention period. For AOPP, Cohen's d was 1.12 for the comparison between the control and exercise groups, 1.39 for the control and chromium groups, and 1.50 for the control and the exercise + chromium groups. In GSH, the exercise (+ 30.3%), chromium (+ 45.8%), and chromium + exercise (+ 40.6%) groups displayed substantial improvements compared to the control group ($p < 0.001$) (Fig. 4). No

Parameters	Control	Exercise	Chromium	Chromium + exercise	p	η_p^2
CAT (U/L)	370.3 ± 9.7	356.7 ± 23.0[a]	357.0 ± 11.6[a]	326.0 ± 16.2[a, b,c]	0.000	0.639
MDA (mmol/L)	0.66 ± 0.06	0.78 ± 0.08[a]	0.78 ± 0.08[a]	0.83 ± 0.09[a]	0.004	0.512
AOPP (mmol/L)	40.79 ± 4.04	44.10 ± 1.00[a]	45.42 ± 2.36[a]	45.82 ± 2.44[a]	0.012	0.446
GSH (mmol/L)	1.55 ± 0.23	2.02 ± 0.21[a]	2.26 ± 0.24[a]	2.18 ± 0.37[a]	0.000	0.638

Table 1. Changes in CAT, MDA, AOPP, and GSH levels in all groups after an 8-week intervention. CAT, catalase; MDA, malondialdehyde; AOPP, advanced oxidation protein products; GSH, reduced glutathione; [a]significantly different from control, $p < 0.001$; [b]significantly different from exercise, $p < 0.05$.; [c]significantly different from chromium, $p < 0.05$. Values are expressed as mean ± SD.

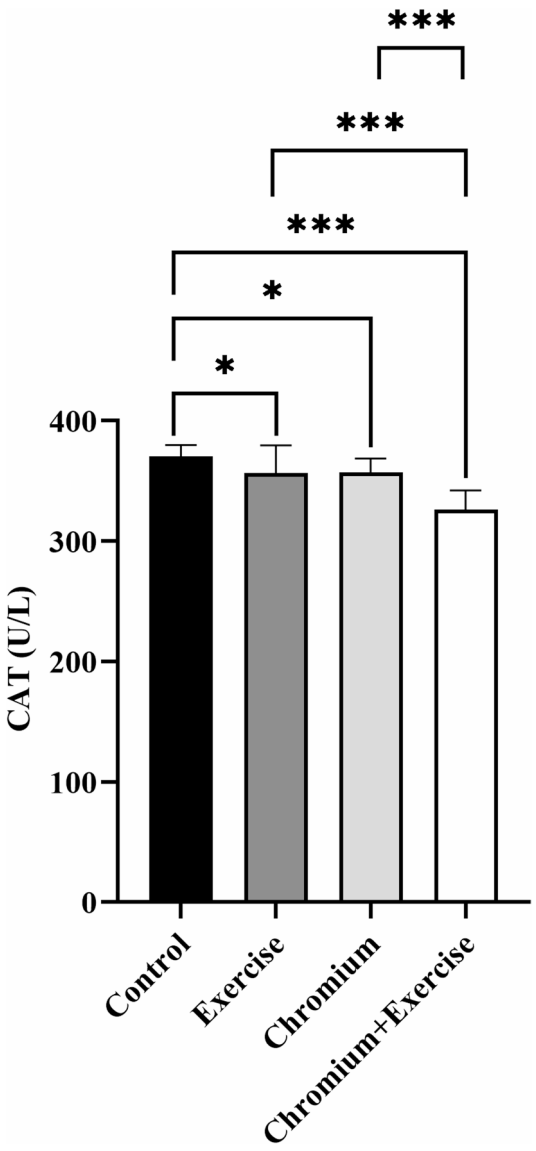

Fig. 1. Catalase (CAT) activity (U/L) across experimental groups. Vertical bars represent mean ± SEM. Asterisks and connecting lines indicate significant pairwise differences between groups (*$p < 0.05$, ***$p < 0.001$).

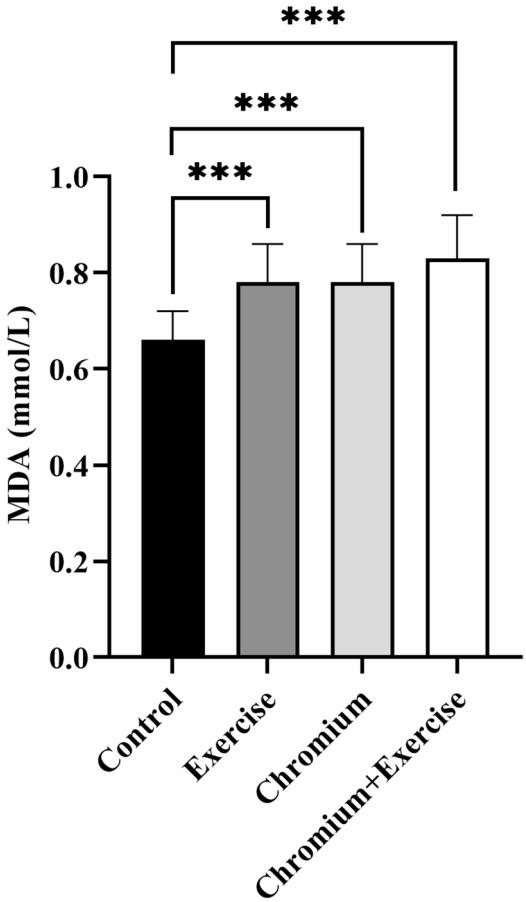

Fig. 2. Malondialdehyde (MDA) levels (mmol/L) in experimental groups. Data are presented as mean ± SEM. Asterisks and connecting lines indicate significant pairwise differences between groups (***$p < 0.001$).

significant differences were detected among the exercise, chromium, and chromium + exercise groups in GSH levels. For GSH, Cohen's d was 2.13 for the comparison between the control and exercise groups, 3.02 for the control and chromium groups, and 2.04 for the control and the exercise + chromium groups.

Discussion

In this study, the effects of chromium picolinate supplementation and treadmill exercise on CAT activity, MDA, AOPP and GSH levels were investigated. The objective of this study was to ascertain the extent of oxidant damage in rats subjected to prolonged strenuous exercise and to evaluate the efficacy of chromium picolinate, which contains high levels of antioxidant compounds, in preventing this damage. To this end, MDA and AOPP levels were measured, which serve as indicators of oxidative stress. In addition, CAT enzyme activity and GSH levels were also examined, which demonstrate the defense response against oxidative stress. In our study, a comparison of the interventional groups with the control group after chronic exercise revealed significant between-group differences in CAT, MDA, AOPP, and GSH levels in the chromium, exercise, and chromium + exercise groups among male rats. Additionally, supplementation with chromium picolinate in conjunction with aerobic exercise resulted in distinct changes in CAT enzyme activity compared to chromium or exercise alone. Unlike classical antioxidants such as vitamins C and E, chromium picolinate may exert dual effects by both modulating redox balance and influencing glucose-insulin homeostasis, thereby indirectly attenuating oxidative stress during prolonged exercise. This dual mechanism highlights its novelty as an intervention in exercise-induced oxidative stress[14]. These findings suggest a potential role of chromium picolinate in modulating exercise-induced oxidative stress and redox status in male rats; however, further studies exploring upstream regulators (e.g., Nrf2, NF-κB) and downstream tissue-level mechanisms (e.g., histology, mitochondrial function) are needed to clarify the physiological significance of these changes.

Numerous studies are in line with findings from our study reporting that MDA increases after exercise. For example, Güllü[11] reported that MDA values increased significantly after maximal acute exercise in 10 endurance athletes and 10 sedentary athletes[18]. Kanter et al.[19] showed an increase in plasma MDA following extreme endurance training applied to elite athletes[20]. Marzatico et al.[21] found high plasma MDA in sprinters who performed high-level sprint training and marathoners who trained at a high level[22,23]. The similarity in the applied exercise duration and type is thought to be effective in obtaining such results. On the other hand, conflicting results have been reported regarding MDA. Alessio et al.[24] showed that MDA did not change in

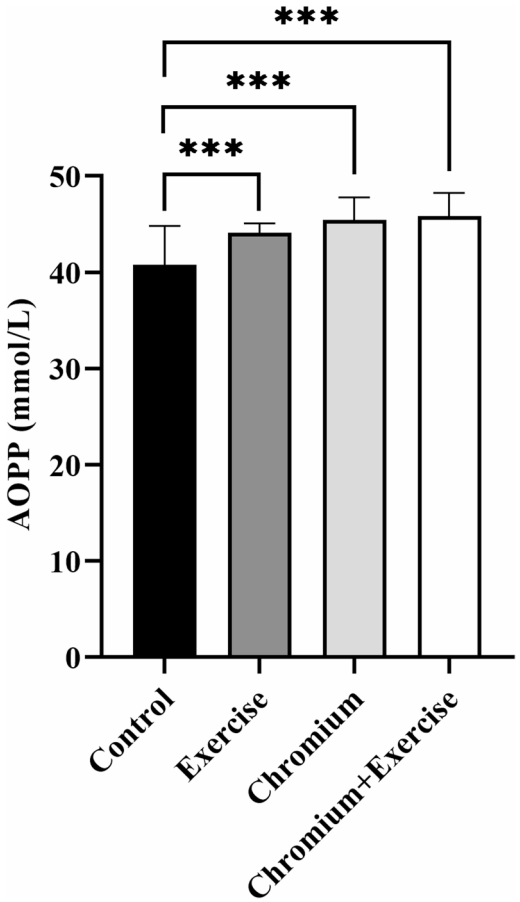

Fig. 3. Advanced Oxidation Protein Products (AOPP) levels in experimental groups. Data are presented as mean ± SEM. Asterisks and connecting lines indicate significant pairwise differences between groups (*$p < 0.05$, ***$p < 0.001$).

continuous aerobic exercise in untrained adults[25]. Selamoğlu[20] found a statistically significant decrease in MDA in long-distance runners[26]. Çelik et al.[22] reported that MDA levels decreased after acute exercise performed by football players[27]. Aksu et al.[28] reported that acute exercise did not cause oxidative stress in the rat brain (prefrontal cortex, striatum, and hippocampus)[29]. These studies do not show parallelism with the present study. Such discrepancies likely reflect differences in species, training status, exercise modality/intensity/duration, and the specific oxidative markers assessed (e.g., TBARS vs. MDA). The different results in exercise-induced serum MDA levels are thought to be due to the varying exercise durations applied to the different subjects. For example, in our study the subjects were rats. In study by Alessio et al.[24], the subjects were untrained adults, in the study by Selamoğlu[20], the subjects were long-distance runners, in the study by Çelik et al.[22], the subjects were football players. In the study by Aksu et al.[28], the subjects were rats, however the antioxidant measured was TBARS and not MDA.

When serum CAT, AOPP, and GSH levels were compared with the control group, significant differences were observed between the groups in the chromium, exercise, and chromium + exercise groups ($p < 0.05$). In a study conducted by Thirumalai et al.[30], rats were subjected to an intense and comprehensive swimming exercise program for five days. The results indicated that oxidative stress occurred, and GPx and GSH values were significantly decreased in the rats that exercised. subjected rats to an intense and comprehensive swimming exercise program for five days and reported that oxidative stress occurred, and GPx and GSH values were significantly decreased in the rats that exercised. This difference is thought to be due to the difference in the exercise stimulus and the duration of the exercise program[31,32]. In the study by Thirumalai et al.[30], the exercise stimulus was swimming, and the duration of the exercise program was 5 days. On the other hand, in our study, the exercise stimulus was running on a treadmill, and the duration of the exercise program was 8 weeks. Similarly to our study, Sahlin et al.[33] found that MDA and total GSH increased in acute exercise[23].

In a study that assessed plasma antioxidant levels in elite runners before and after exercise, it was reported that while MDA levels increased after exercise, there was no significant difference in GPx levels[34]. Chronic endurance training strengthens antioxidant defense systems[24,28]. Burneiko et al.[31] reported that SOD values increased, CAT values decreased, and serum TOS levels increased significantly in the liver tissues of rats exercising two and five days a week in a study conducted for eight weeks[35]. Although decreased CAT activity might reflect lower oxidative burden and reduced need for enzymatic defense, it could alternatively suggest

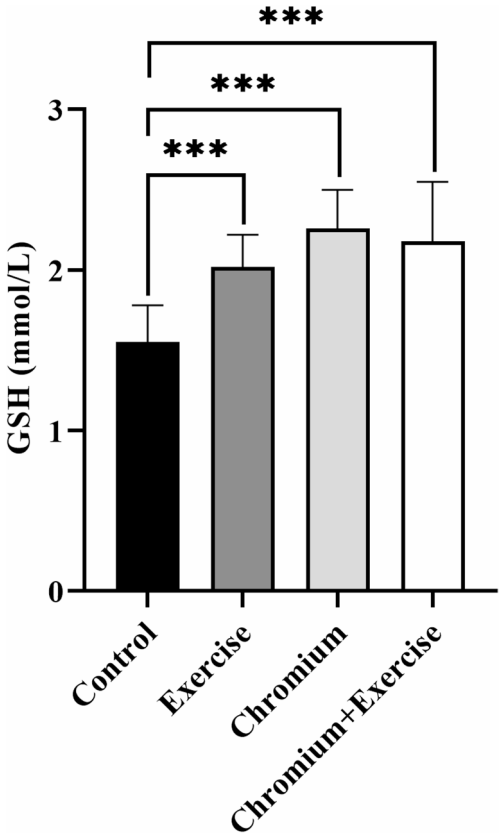

Fig. 4. Glutathione (GSH) levels in experimental groups. Data are presented as mean ± SEM. Asterisks and connecting lines indicate significant pairwise differences between groups (*$p < 0.05$, ***$p < 0.001$).

compromised antioxidant protection. The use of antioxidant agents can protect against the harmful effects of radicals by partially preventing the formation of free radicals caused by exercise and may also reduce the side effects that may occur. Interestingly, it has been reported that the use of antioxidants such as vitamins C and E provides partial protection against exercise-mediated oxidative damage in both humans and rats[36]. Venditti et al.[37] investigated the effect of vitamin E on oxidative stress caused by a swimming program for rats, conducted five days a week for 10 weeks, with gradually increasing duration. They examined the rat liver and compared groups, including those exercising and taking vitamin E. While the GSH value in liver homogenates was at the lowest level in this group, it was reported to be at the highest level in the exercise group without taking vitamin E[37].

The findings of these studies lend further support to the existing body of research in this field[17,19,38]. It is postulated that the similarity in the applied exercise duration and type is responsible for the observed results. In the study of Marmett et al.[39], rats were supplemented with chromium picolinate. The study found that trained groups demonstrated increased exercise tolerance, compared to sedentary groups. In the gastrocnemius, SOD activity was higher in the groups that underwent aerobic training (residual oil fly ash exposure and training, trained and supplemented and trained groups) compared to all sedentary groups (residual oil fly ash exposure and sedentary, residual oil fly ash exposure and supplemented, sedentary, and sedentary and supplement groups). Activity levels of SOD, CAT, and TBARS in lung tissues did not differ among groups. Similarly, in heart tissue, no differences were observed among groups in relation to SOD, CAT, and TBARS activity levels. Collectively, these studies suggest that chromium supplementation may confer tissue-specific and context-dependent antioxidant effects, warranting further detailed evaluation.

The limitations of the study include the use of only male rats. Future studies should confirm these findings separately in both male and female rats, as exercise-induced oxidative stress may potentially affect hormone fluctuations between the sexes. Additionally, the present findings were limited to a single exercise protocol in active young rats. Further research should be conducted to confirm these findings with subsequent exercise practices over a longer period in more diverse populations with different exercise protocols. Moreover, it would be beneficial for future studies to investigate additional markers in order to provide data related to a wider spectrum of oxidative stress and redox status.

Conclusion

Aerobic exercise, chromium supplementation, and combined chromium supplementation plus aerobic exercise appear to influence oxidative stress and antioxidant capacity in male rats. The combination of chromium picolinate supplementation and aerobic exercise was associated with more pronounced alterations in catalase

enzyme activity compared to chromium supplementation or exercise alone. These findings highlight a potential antioxidant role of chromium picolinate in exercise-induced oxidative stress; however, additional mechanistic investigations are required to clarify the underlying pathways and physiological relevance.

Strengths, limitations, and recommendations

This study has notable strengths, including a controlled four-group design enabling independent and combined evaluations of exercise and chromium supplementation, the use of a validated chronic treadmill protocol, and the simultaneous assessment of oxidative stress (MDA, AOPP) and antioxidant markers (CAT, GSH), providing a comprehensive redox profile.

However, limitations include the use of only male rats, restricting generalizability across sexes, reliance on a single exercise protocol, endpoint-only measurements without time-series analysis, and the absence of mechanistic investigations such as signaling pathways or histological analyses.

Future research should incorporate both sexes, evaluate different chromium forms and doses, extend follow-up to recovery phases, and include molecular and mitochondrial assessments. Comparative exercise models and combination trials with other antioxidants (e.g., vitamins C and E) are also recommended to broaden applicability and mechanistic insights.

Data availability

The research data supporting the findings of this study are available on the Open Science Framework (OSF) at: https://osf.io/vfrd5/. No deviations from the pre-registered protocol occurred during the conduct of the study.

Received: 17 May 2025; Accepted: 21 October 2025
Published online: 12 November 2025

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Acknowledgements
This study acknowledges the fund support from Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R422), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Author contributions
M.S., B.Ö., N.H.A., M.S.U. and N.M.E. designed and supervised the study; M.S., B.Ö., N.M.E., M.S., V.Ç., S.Ö., Y.G.G., M.A., M.S.U., Y.E.Y. and K.K. collected the data; M.S., B.Ö. and S.B.A. carried out the statistical analyses; B.Ö., N.M.E. and A.B. drafted the manuscript; N.H., B.B.S., G.V.G. and A.B. reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

Funding
Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R422), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki, and the animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of Van Yüzüncü Yıl University (2023/07–33,) Animal Experiments and Local Ethics Committee (2023/07–33). All experiments were performed in accordance with internationally recognized standard ethical guidelines for laboratory animal use and care (EEC Directive 86/609/EEC of 24 November 1986) as described in European community guidelines.

Additional information
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