Modulation of exercise-induced oxidative stress biomarkers *by* nutraceutical *moringa oleifera* supplementation in football athletes: a randomized double-blind trial

Modulación de los biomarcadores de estrés oxidativo inducido por el ejercicio mediante la suplementación nutracéutica de moringa oleifera en futbolistas: un ensayo aleatorizado doble ciego

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Abstract

Introduction: Acute physical exertion induces oxidative stress through excessive reactive oxygen species (ROS) production and increased lipid peroxidation, disrupting redox homeostasis. Moringa oleifera (MO), rich in polyphenols and flavonoids, has been proposed to attenuate oxidative stress through modulation of antioxidant enzyme activity.
Objective: This study investigated the acute effects of MO supplementation on oxidative stress biomarkers following maximal load exercise in trained football athletes.
Methodology: A randomized, double-blind design, 22 trained male football athletes received either 1000 mg of MO or placebo before performing five sets of 10 squat thrusts at their individualized 10-repetition maximum (10RM) load. Blood samples were collected at baseline, 1 h, and 24 h post-exercise to measure superoxide dismutase (SOD) and malondialdehyde (MDA). Data were analyzed using two-way repeated-measures ANOVA and Pearson correlation ($p < 0.05$).
Results: Compared with placebo, MO supplementation significantly elevated SOD activity and suppressed MDA accumulation at 1 h and 24 h post-exercise ($p < 0.01$), with a strong time × group interaction ($p < 0.001$). A progressive negative correlation between SOD and MDA was observed ($r = –0.50$ at 1 h; $r = –0.71$ at 24 h, $p < 0.05$).
Discussion: Acute MO supplementation can potentially increase enzymatic antioxidant activity and may reduce lipid peroxidation during recovery from maximal exertion.
Conclusions: These findings suggest that acute MO supplementation may support short-term redox responses following maximal exertion and highlight the need for further studies to examine its long-term effects on recovery and performance.

Keywords

Antioxidants response; exercise recovery; nutritional strategy; oxidative damage; resistance training.

Resumen

Introducción: El esfuerzo físico agudo induce estrés oxidativo mediante la producción excesiva de ROS y el aumento de la peroxidación lipídica, alterando la homeostasis redox. Moringa oleifera (MO), rica en polifenoles y flavonoides, se ha propuesto para atenuar el estrés oxidativo mediante la modulación de la actividad de las enzimas antioxidantes.
Objetivo: Este estudio investigó los efectos agudos de la suplementación con MO sobre biomarcadores de estrés oxidativo tras un ejercicio de carga máxima en futbolistas entrenados
Metodología: En un diseño aleatorizado y doble ciego, 22 futbolistas varones entrenados recibieron 1000 mg de MO o un placebo antes de realizar cinco series de 10 squat thrusts con la carga correspondiente a su 10RM individual. Se recolectaron muestras de sangre en el estado basal, a la 1 h y a las 24 h posteriores al ejercicio para medir SOD y MDA. Los datos se analizaron mediante ANOVA de medidas repetidas de dos vías y correlación de Pearson ($p < 0,05$).
Resultados: En comparación con el placebo, la suplementación con MO elevó significativamente la actividad de SOD y redujo la acumulación de MDA a la 1 h y 24 h posteriores al ejercicio ($p < 0,01$), con una fuerte interacción tiempo × grupo ($p < 0,001$). Se observó una correlación negativa progresiva entre SOD y MDA ($r = –0,50$ a la 1 h; $r = –0,71$ a las 24 h, $p < 0,05$).
Discusión: La suplementación aguda con MO puede aumentar potencialmente la actividad antioxidante enzimática y podría reducir la peroxidación lipídica durante la recuperación tras un esfuerzo máximo.
Conclusiones: Estos hallazgos sugieren que la suplementación aguda con MO puede favorecer las respuestas redox a corto plazo después de un esfuerzo máximo y destacan la necesidad de realizar más estudios para examinar sus efectos a largo plazo sobre la recuperación y el rendimiento.

Palabras clave

Respuesta de antioxidantes; recuperación del ejercicio; estrategia nutricional; daño oxidativo; entrenamiento de resistencia.

Introduction

Exercise-induced oxidative stress represents a physiological response triggered by high-intensity physical activity (Powers et al., 2020), characterized by the accumulation of reactive oxygen species (ROS) that exceeds the capacity of endogenous antioxidant systems (Powers et al., 2024; Zulfahmidah & Safei, 2022). In football, repeated sprints, rapid directional changes, and sustained muscle contractions amplify ROS production, leading to oxidative damage in cell membranes, proteins, and DNA, triggering inflammation, impairing energy production, and slowing recovery (Abate et al., 2023; Clemente-Suárez et al., 2023; Wang et al., 2021). Football imposes high physical demands, including repeated high-speed sprints and endurance activities exceeding 19.8 km/h, which substantially increase reactive oxygen species (ROS) production and contribute to exercise-induced muscle damage (EIMD) (Bradley et al., 2016; Lu et al., 2021; Suzuki et al., 2020). This oxidative stress leads to inflammation, impaired muscle regeneration, and reduced performance (Leite et al., 2023; Meng & Su, 2024; Rohnejad & Monazzami, 2023). Nutritional strategies targeting oxidative stress are therefore critical for maintaining performance, promoting recovery and reducing injury risk (Debold, 2015).

Among the potential interventions, Moringa oleifera (MO) has emerged as a nutritionally and pharmacologically relevant candidate, especially in Indonesia, for both dietary and medicinal purposes (Ardiani et al., 2025; Chiş et al., 2023; Pareek et al., 2023; Patil et al., 2021; Trigo et al., 2021). The polyphenols, flavonoids, vitamins, and essential minerals in MO help regulate oxidative and inflammatory processes (Arshad et al., 2025). The bioactive content of MO, especially flavonoids and polyphenols, neutralizes free radicals, reduces lipid peroxidation, inhibits inflammatory pathways, and increases endogenous antioxidant enzymes (SOD, catalase, glutathione peroxidase), thereby strengthening cellular defenses and supporting muscle recovery (Al-Khayri et al., 2022; Chagas et al., 2022; Fatmawati et al., 2021). MO leaves are also contain high levels in vitamins A and C, calcium, iron, and potassium, nutrients that are particularly important for athletes to support endurance, recovery, and muscle and bone health, which are critical for meeting higher metabolic demands (Hodas et al., 2021; Lamou et al., 2016).

Evidence from animal, in vitro, and human studies collectively supports the antioxidant and muscle-protective effects of MO, including reductions in oxidative stress biomarkers (e.g., MDA), enhanced anti-inflammatory responses, leading to improved endurance and recovery. Experimental studies in animals models have shown that high doses of MO leaf extract or MO flavonoids can reduce MDA, TGF-β, and caspase-3, increase muscle mass (Budiningsih et al., 2025), anti-inflammatory cytokines(Ogundare et al., 2025), along with energy and glucose metabolism (Bian et al., 2024; Gao et al., 2022). An in vitro study on C2C12 muscle cells reported that MO protected against oxidative damage by increasing antioxidant enzyme activity and reducing lipid peroxidation (Ceci et al., 2022; Duranti et al., 2021). Furthermore, studies in human subjects have shown that MO supplementation improves antioxidant responses (Arshad et al., 2025; Dong et al., 2024; El Assar et al., 2022), aerobic endurance and performance (Abouzeid et al., 2025), as well as exercise-induced fatigue (Chaudhary et al., 2023; Muscolo et al., 2024; Ray et al., 2023), indicating its potential to modulate physiological stress responses during high-intensity sports such as football. By enhancing SOD activity and reducing MDA, MO may limit oxidative damage during high-intensity intermittent exercise, thereby supporting faster muscle recovery and maintaining performance.

Despite growing evidence of MO's potential effects, research specifically targeting competitive football athletes remains limited. Although previous studies in animals and non-athlete populations have demonstrated the antioxidant and performance-enhancing effects of MO, its acute impact on key oxidative stress biomarkers, such as SOD and MDA, in athletes remains largely unexplored. To our knowledge, no studies have specifically evaluated the acute effects of MO supplementation on SOD and MDA in soccer athletes undergoing high-intensity, intermittent training. This training environment is important to investigate because it has been shown to trigger increased oxidative stress, which can damage muscle cells and slow recovery. Therefore, this study aims to evaluate the acute effects of MO supplementation on oxidative stress biomarkers in a competitive sport context, which is expected to provide a scientific basis for the development of nutraceutical strategies to support athlete recovery and performance.

Method

Study design

This study employed a randomized, double-blind, placebo-controlled trial (RCT) to evaluate the acute effects of Moringa oleifera leaf extract (MOLE) on oxidative stress following high-intensity exercise. Participants were randomly allocated to either the MOLE group or the placebo group. The study was approved by the Institutional Review Board of Stikes Guna Bangsa Approval No. 019/KEPK/VIII/2024 and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all participants prior to enrollment.

Participants

The G*Power 3.1.9.6 software was used to perform an a priori power analysis to determine the required sample size. The calculation was based on a two-way repeated-measures ANOVA design with two groups and three measurement points (baseline, 1 h, and 24 h). Based on (Park et al., 2021), a moderate-to-large effect size (f = 0.29), an alpha level of 0.05, and a desired statistical power of 0.80 were assumed. The analysis indicated that a total of 22 participants (11 per group) would be required, and therefore the sample size used in the present study was considered sufficient to detect meaningful effects.

In the initial phase, a total of 30 male football players competing in League 3 were screened for eligibility. However, 8 players were subsequently excluded: 5 were from higher professional leagues, 1 had a disciplinary sanction, and 2 had a history of prior injuries. Consequently, a total of 22 participants, all male football players aged 19–21 years were included in the study Inclusion criteria were: (1) minimum of three years of competitive football experience, (2) participation in regular structured training ≥5 days per week, and (3) no use of antioxidant supplements in the preceding two months. Exclusion criteria were: (1) history of musculoskeletal injury or surgery within the last two years, (2) diagnosis of cardiovascular, metabolic, or inflammatory disorders, and (3) disciplinary sanctions within the past season.

Randomization and blinding

Participants were randomly assigned (1:1) to the MOLE or placebo group using a computer-generated randomization sequence (block size = 4). Allocation concealment was ensured through sequentially numbered, opaque, sealed envelopes prepared by an independent researcher not involved in data collection. Both MOLE and placebo capsules were identical in appearance, and participants, investigators, and outcome assessors were blinded to group allocation.

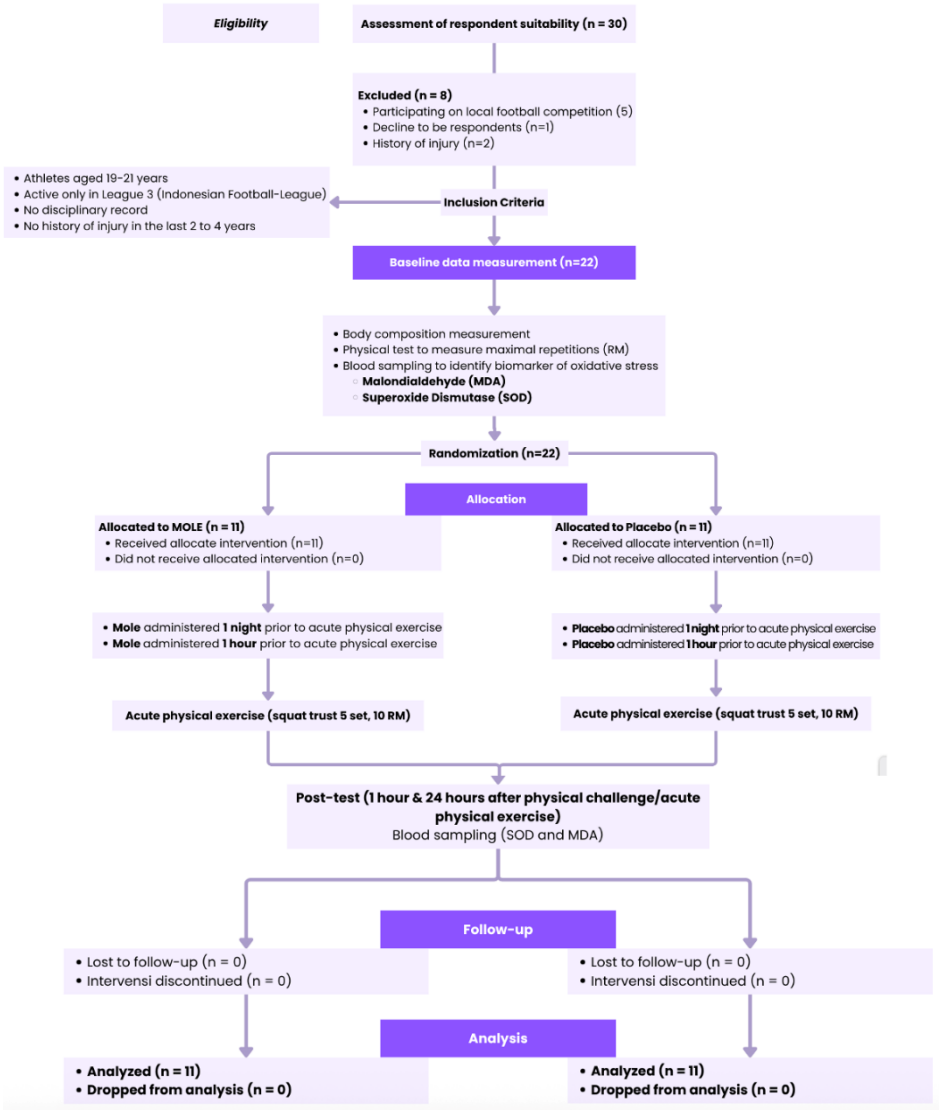
Intervention

The intervention consisted of a commercial MO supplement (Natureline® Moringa, PT. Herba Medica, Indonesia). Each capsule contained 500 mg of dried MO folium extract, processed under Good Manufacturing Practice (CPOB) standards to ensure consistency and safety. According to the manufacturer's certificate of analysis, the extract was standardized to contain ≥20% total polyphenols and ≥10% flavonoids, verified using high-performance liquid chromatography (HPLC). These bioactive compounds, together with essential micronutrients such as vitamin C, vitamin B6, magnesium, and iron, have been recognized for their antioxidant capacity. Participants in the intervention group consumed two capsules (total 1000 mg): two capsule the evening before and two capsule one hour before the exercise protocol. The placebo group received identical capsules filled with inert maltodextrin, matched for weight, size, and color to preserve blinding integrity.

Pre-test controls

Participants abstained from alcohol, caffeine, and strenuous physical activity for 72 hours prior to the study. Meal schedules for the 48 hours prior to the intervention were conducted and supervised by the research team. Participants also adhered to a fixed sleep schedule (9:00 PM–7:00 AM). The detailed procedure is presented in the study's flowchart (Figure 1).

Figure 1. CONSORT Research Flowchart



Exercise protocol

Participants completed a standardized warm-up (10 min jogging, 5 min dynamic stretching) prior to testing. To determine individualized training load, a repetition maximum (RM) test was conducted. This assessment was performed incrementally, whereby athletes lifted progressively heavier loads until they were only able to perform a single successful lift with correct technique, defined as the one-repetition maximum (1RM). From this value, the load for a ten-repetition maximum (10RM) was determined proportionally based on each individual's 1RM capacity. The use of RM testing ensured that each athlete trained at a comparable level of maximal effort, despite individual differences in absolute strength. The exercise protocol consisted of five sets of 10 squat thrust repetitions at the athlete's predetermined 10RM load, with 3-minute rest intervals between sets. Exhaustion was operationally defined as the point at which an athlete failed to complete two consecutive repetitions with correct form, despite standardized verbal encouragement. The quality of execution was monitored by two independent coaches to ensure objectivity, minimize subjectivity in fatigue assessment and ensure that all athletes reached comparable physiological limits relative to their individual strength capacity.

Blood sampling and biochemical assays

Venous blood samples (5 mL) were collected from the antecubital vein at baseline (pre-exercise), immediately post-exercise (blood drawn within 2 minutes of exercise termination, consistently across participants), 1 hour, and 24 hours post-exercise. Plasma was separated by centrifugation (3000 rpm, 10

min, 4°C) and stored at –80°C until analysis. SOD activity was quantified using the Human T-SOD Activity Assay Kit (Hydroxylamine Method, Elabscience, Cat. No. E-BC-K022), with results expressed in U/mL. MDA concentration was measured by the Thiobarbituric Acid Reactive Substances (TBARS) method using the MDA Colorimetric Assay Kit (Elabscience, Cat. No. E-BC-K025), reported in nmol/mL. All assays were performed in duplicate, and intra-assay coefficient of variation (CV) was <5%.

Throughout the duration of the study, there was no missing data, no one dropped out, and no rules were broken. Each participant followed the steps of the procedure systematically, and thus the results represent the true physiological responses without data negligence or error bias.

Data Analysis

Data were analysed using STATA version 14. Descriptive statistics (mean ± SD) were used to summarise the outcomes. Data normality was evaluated using the Shapiro–Wilk test. Between-group differences at each time point were examined using independent t-tests, with Cohen's d reported as the effect size. A two-way repeated-measures ANOVA was conducted to determine the main effects of time, group, and their interaction (group × time) on SOD and MDA. Omega squared (ω^2) was calculated as the effect size for ANOVA outcomes. Post hoc pairwise comparisons (MO vs placebo) across time points were performed with Holm-adjusted p-values, and their effect sizes were expressed using Cohen's d. Correlations between SOD and MDA were examined using Pearson correlation coefficients with 95% confidence intervals (CI). Statistical significance was accepted at $p < 0.05$.

Results

Participants in the placebo group exhibited a significant decline in SOD levels at 1-hour post-exercise, followed by only a slight increase at 24 hours. Concurrently, MDA levels increased at 1 hour and continued to rise at 24 hours, indicating persistent oxidative stress. In contrast, the MO group demonstrated a more preserved SOD response, with a modest decline at 1 hour that stabilized by 24 hours. Although MDA levels initially rose in this group, they showed a slight reduction at 24 hours, reflecting a more favorable oxidative stress profile over time (Figure 2).

Figure 2. The mean changes in SOD and MDA levels were observed at baseline, 1 hour, and 24 hours following acute physical exercise

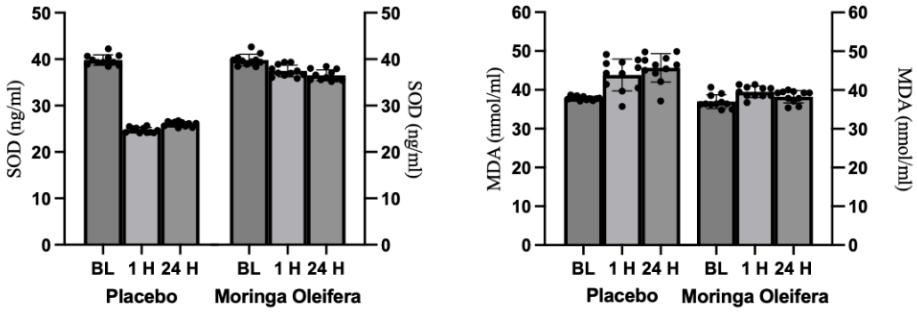
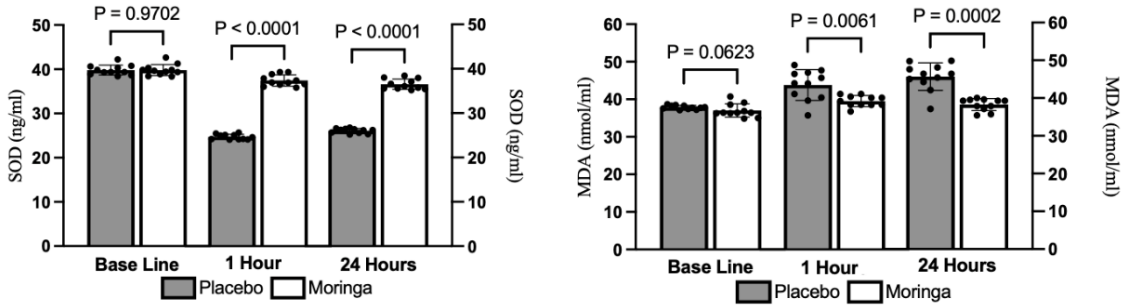


Figure 3 illustrates the differences in SOD and MDA levels between the placebo and MO groups across three time points: baseline, 1 hour, and 24 hours post-exercise. At baseline, there were no significant differences between groups in either biomarker (SOD: $p = 0.9702$, ES = -0.159; MDA: $p = 0.0623$, ES = 0.648). However, at 1 hour post-exercise, the placebo group exhibited a significantly greater reduction in SOD ($p < 0.0001$, ES = -12.922) and a greater increase in MDA ($p = 0.0061$, ES = 1.178) compared to the MO group. These differences persisted at 24 hours, with the placebo group maintaining lower SOD levels ($p < 0.0001$, ES = -11.505) and higher MDA concentrations ($p = 0.0002$, ES = 2.161). These findings suggest that MO supplementation offers superior antioxidant protection, as evidenced by a more stable SOD profile and attenuated MDA elevation following maximal load exercise. The data support the potential role of MO in mitigating exercise-induced oxidative stress and maintaining redox homeostasis.

Figure 3. Group differences in antioxidant and oxidative stress biomarkers over time



Furhtermore, the two-way repeated measures ANOVA showed that both SOD and MDA changed significantly over time, and the patterns of these changes differed between the MO and placebo groups, as indicated by a significant time × group interaction for both biomarkers (Table 1). In terms of SOD, the MO group maintained relatively stable levels with minimal fluctuations, whereas the placebo group exhibited a larger reduction from baseline to 1 hour and 24 hours post-exercise, a pattern that was further confirmed by significant between-group differences in the post hoc analysis. Regarding MDA, the MO group demonstrated a smaller increase, while the placebo group showed elevated values at 1 hour and 24 hours post-exercise, and this pattern was supported by post hoc results indicating significant between-group differences at both time points (Table 2). These findings indicate distinct physiological responses to high-intensity exercise between the two groups, with the MO group displaying a more stable SOD profile and a lower rise in MDA across the observation period.

Table 1. Two-way repeated measures ANOVA results for SOD and MDA biomarkers across time points in MO and placebo groups

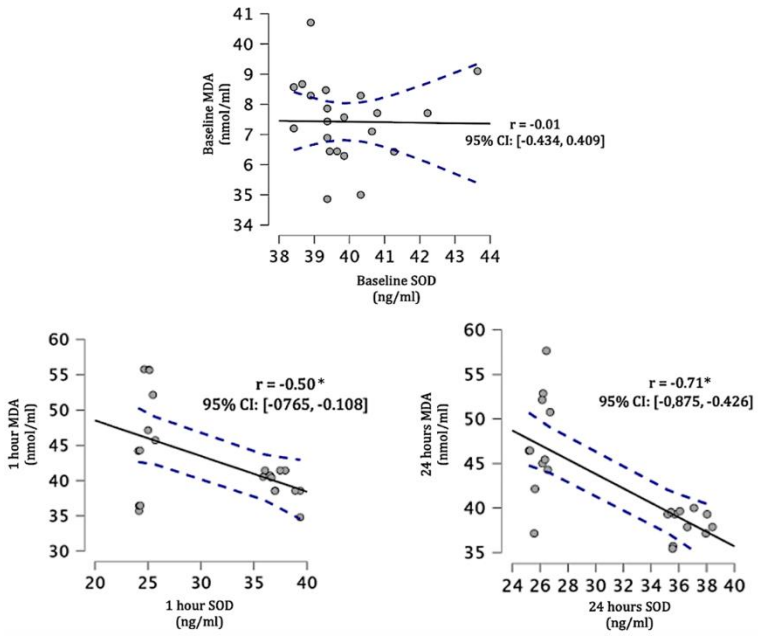
Biomarker	Effect	df	F	p-value	Effect Size (ω^2)
SOD	Time	1.210	949.7	< .001	0.939
	Group × Time	1.210	418.2	< .001	0.872
	Group	1, 20	448.2	< .001	0.914
MDA	Time	1.722	17.391	< .001	0.272
	Group × Time	1.722	7.874	.002	0.136
	Group	1, 20	18.71	< .001	0.297

Table 2. Post hoc comparison of SOD and MDA levels between MO and placebo groups across time points

Biomarker	Comparison (MO vs Placebo)	Mean Diff	SE	t (df=20)	p-holm	Cohen's d
SOD	Baseline	-0.201	0.542	-0.370	.715	-0.188
	1 hour	-12.742	0.420	-30.305	< .001	11.930
	24 hours	-10.492	0.389	-26.981	< .001	9.823
MDA	Baseline	+0.844	0.555	1.519	1.000	0.203
	1 hour	-8.444	1.769	-4.773	.001	-2.030
	24 hours	-9.453	1.263	-7.481	< .001	-2.273

Figure 4 shows the relationship between SOD and MDA levels at three time points: before treatment (baseline), 1 hour, and 24 hours after exercise. In the baseline phase, before MO supplementation and exercise, the correlation between SOD and MDA was weak and not statistically significant ($r = -0.01$, 95% CI: [-0.434, 0.409], $p = 0.95$), reflecting normal physiological conditions with minimal oxidative activity. A significant negative correlation emerged at 1 hour after exercise ($r = -0.50$, 95% CI: [-0.765, -0.108], $p = 0.02$), indicating an acute antioxidant response to exercise-induced oxidative stress. This relationship strengthened at 24 hours ($r = -0.71$, 95% CI: [-0.875, -0.426], $p < 0.001$), suggesting that the inverse association persists and becomes more pronounced during recovery. These progressive change may reflect enhanced activity of the endogenous antioxidant system and could be potentially influenced by MO supplementation.

Figure 4. Correlation between antioxidant SOD and MDA markers post-exercise



Discussion

This study examines the effects of MO supplementation on SOD production in football athlete following maximal resistance training. The research indicated that there was SOD slightly increase in production in the subjects in the MO group even at the 1- and 24-hour marks after maximal resistance training, compared to the placebo group. Therefore, compared to the placebo group, SOD production in the MO group may help attenuate oxidative effects, which are heightened by maximal resistance training and associated with the training. SOD is an enzyme that functions as an antioxidant by preventing oxidative damage from superoxide free radicals through converting them into hydrogen peroxide and oxygen (Azadmanesh & Borgstahl, 2018; Eleutherio et al., 2021). These results align with studies (Ahmed et al., 2020; Aju et al., 2019) which also indicated that the treatment group with MO supplementation displayed enhanced SOD production.

The marginal decrease in SOD production during the one to twenty-four hours post maximal resistance training period potentially related to antioxidant constituents present in MO, namely ascorbate, tocopherol, and polyphenolic compounds, which actively stimulate SOD enzymes (Bhuker et al., 2023; Gómez-Martínez et al., 2020). The body's oxidative defenses in relation to multisession resistance exercises should be supplemented through the antioxidant content in MO. MO was shown to stimulate antioxidant activity and hence SOD and catalase synthesis (Hengpratom et al., 2025; Lim et al., 2024). Several bioactive compounds in MO are thought to modulate inflammatory responses and may enhance the body's defense mechanisms (Dilworth et al., 2020; Saini et al., 2016).

In contrast to what was anticipated, which was highlighted in the previous section, the effect of exercise on MDA concentrations at 1- and 24-hours post-exercise differed. MO supplementation in this study was able to reduce MDA production more than the control group. MDA itself is one of the main biomarkers of lipid peroxides and oxidative damage (Tang et al., 2019; Tsikas, 2017), which in this case was caused by the participants performing maximal resistance training. Though in contradiction to the findings of (Prasetio et al., 2022) who proved that MO supplementation can reduce MDA levels in animals subjected to oxidative stress, the findings of this study demonstrate that MO supplementation, indeed, has the ability to control the increment in MDA levels in the treatment group versus placebo group over 1 hour and 24 hour periods post maximal resistance training. The mechanism for the delayed increment in MDA in the treatment group is attributable to bioactive ingredients present in MA like flavonoids and polyphenols that bolster the endogenous antioxidant system and reduce the oxidative damage of enzymes and lipids to reactive oxidant species (ROS).

Drawing from these results, a more nuanced analysis of the patterns of antioxidant enzyme activity revealed the further protective role of MO. Increased SOD activity coupled with decreased production of MDA suggests regionally optimised redox regulation with increased efficiency, coupled with continuous ensemble enzymatic adaptation. These adjustments may reflect adaptive upregulation of endogenous antioxidant pathways that maintain lipid stability during post-exercise recovery. A similar pattern has been reported by (Javed et al., 2023; Yan & Spaulding, 2020), supports the view that strengthening enzymatic defenses can suppress secondary oxidative products. Collectively, these coordinated responses may support short-term enzymatic and non-enzymatic antioxidant response, as also proposed by (Mohai Ud Din et al., 2025).

This study serves as a preliminary investigation demonstrating that MO supplementation prior to acute physical exertion may enhance redox regulation through increased SOD activity. The observed effects represent an acute adaptive response, reflecting a transient enhancement of endogenous antioxidant capacity that may supports short-term redox stabilization during recovery. This transient enzymatic adjustment may signify a short-term compensatory mechanism to restore redox equilibrium following acute oxidative challenge. Therefore, future long-term studies with larger samples are required to determine whether continued MO supplementation elicits cumulative benefits in oxidative stress modulation, including catalase and GPx activity and antioxidant gene expression, particularly in relation to recovery dynamics and fatigue resistance over extended periods.

Conclusions

Our present study suggests that MO supplementation at a 1000 mg dose, when administered prior to exercise, may help modulate acute oxidative stress by maintaining balanced SOD levels at both 1 hour and 24 hours following high-intensity squat thrust exercise using maximal load. MO may contribute to sustaining antioxidant defence mechanisms during the recovery period after maximal exertion. The findings also indicate that the MO group exhibited lower indicators of oxidative stress than the placebo group, as reflected by lower MDA concentrations, a biomarker of lipid peroxidation. These results underscore the potential of MO as a natural supplement to support redox homeostasis and physiological recovery in response to acute, intensive physical activity.

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