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Oxidative Stress Modulation by Medicago Sativa Extract Slows Axial Elongation in High Myopia: A Randomized Controlled Trial

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ABSTRACT: High myopia is a leading cause of irreversible visual impairment worldwide, driven partly by oxidative stress-mediated axial elongation. Despite low-dose atropine (0.01%) being the current standard, its effect on oxidative imbalance remains limited. This study investigated whether adjuvant Medicago sativa L. extract (EMS) modulates malondialdehyde (MDA) and superoxide dismutase (SOD) levels and consequently slows axial elongation in high myopia. A double-blind randomized controlled trial enrolled 40 adolescents (aged 13-18 years) with high myopia (> -6.00 D) receiving atropine 0.01%. Participants were randomized to EMS 500 mg twice daily (n=20) or placebo (n=20) for six months. Plasma MDA and SOD were measured spectrophotometrically at baseline, month 3, and month 6. Axial length (AL) was measured using A-scan ultrasonography. EMS significantly reduced plasma MDA from 28.81 ± 6.85 to 17.16 ± 3.77 µmol/L ($p<0.001$) while the placebo group showed progressive increase (24.05 ± 6.00 to 29.96 ± 5.03 µmol/L; $p<0.001$). SOD increased markedly in the EMS group (0.99 ± 0.17 to 5.06 ± 1.94 mg/dL; $p<0.001$) versus a decline in the placebo group (0.87 ± 0.20 to 0.65 ± 0.29 mg/dL). The EMS group demonstrated significantly lower axial elongation over 6 months (OD: 0.05 ± 0.03 mm vs 0.45

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± 0.18 mm; OS: 0.06 ± 0.03 mm vs 0.49 ± 0.17 mm; both p<0.001). Changes in MDA and SOD did not directly correlate with axial length changes, suggesting indirect or locally mediated mechanisms. EMS as adjuvant therapy to atropine 0.01% significantly improves oxidative redox balance and attenuates axial elongation in high myopia. These findings support the therapeutic potential of antioxidant supplementation in myopia management.

INTRODUCTION

High myopia, defined as a refractive error exceeding -6.00 diopters, affects approximately 2.7% of the global population and is projected to reach 9.8% by 2050, representing 925 million individuals [1]. Far beyond a refractive inconvenience, high myopia is a recognized cause of irreversible blindness through sight-threatening complications including myopic macular degeneration, retinal detachment, glaucoma, and posterior staphyloma [2][3].

The pathological mechanism central to myopia progression is progressive axial elongation, which stretches and thins the posterior ocular coats. Emerging evidence implicates chronic oxidative stress as a key driver of this process. In the highly oxygenated retinal environment, impaired antioxidant defense leads to accumulation of reactive oxygen species (ROS), which promotes lipid peroxidation, disrupts extracellular matrix homeostasis in the sclera, and ultimately facilitates axial elongation [4][5].

Malondialdehyde (MDA) and superoxide dismutase (SOD) are the most established clinical markers of this oxidative imbalance. MDA, the end product of polyunsaturated fatty acid peroxidation, has been reported to be elevated in the vitreous and plasma of high myopia patients [6]. SOD, the primary enzymatic antioxidant defense that neutralizes superoxide radicals, is consistently reduced in high myopia, and its deficiency has been associated with retinal pigment epithelium (RPE) apoptosis [7]. Together, elevated MDA and reduced SOD reflect an oxidative milieu that may accelerate myopic structural damage.

The current pharmacological standard for myopia control is atropine 0.01% eye drops, which slows axial elongation by approximately 0.36 mm/year compared to 0.41 mm/year in controls [8]. However, atropine does not address the underlying oxidative imbalance; monotherapy leaves substantial residual progression in many patients [9].

Medicago sativa L. (alfalfa) is a polyphenol-rich plant containing flavonoids, saponins, isoflavones, and polyhydroxylated compounds with potent antioxidant activity. Preclinical studies have demonstrated its ability to suppress MDA formation and upregulate SOD through Nrf2-ARE pathway activation [10][11]. However, no clinical trial has yet evaluated EMS as adjuvant therapy specifically targeting the oxidative stress-axial elongation axis in high myopia.

This randomized controlled trial aimed to evaluate whether EMS, combined with atropine 0.01%, could modulate plasma MDA and SOD levels and reduce axial elongation progression in adolescents with high myopia compared to atropine alone. Given the global myopia epidemic and the limited efficacy of current monotherapy, demonstrating the benefit of an antioxidant adjuvant would have significant clinical and public health implications.

METHODS

Study Design and Ethics

A double-blind randomized controlled trial was conducted between January 2022 and December 2024 at the Ophthalmology Outpatient Clinic, Prof. dr. Chairuddin P. Lubis Hospital, Universitas Sumatera Utara, Medan, Indonesia. The study received ethical approval from the Health Research Ethics Committee of Universitas Sumatera Utara (No. 104/KEPK/USU/2024) and was conducted in accordance with the Declaration of Helsinki. All participants and legal guardians provided written informed consent.

Participants

Eligible participants were adolescents aged 13-18 years with high myopia (> -6.00 D) who were already receiving atropine 0.01% eye drops, best-corrected visual acuity (BCVA) of 6/6, and normal complete blood counts. Exclusion criteria included active systemic inflammatory disease, any concurrent ocular pathology other than high myopia, or conditions requiring further

intervention. Dropout criteria included death from unrelated causes, non-attendance at scheduled visits, voluntary withdrawal, or requirement for additional intervention.

Randomization and Blinding

Forty participants meeting inclusion criteria were randomized 1:1 to EMS or placebo using computer-generated permuted block randomization via Research Randomizer (http://www.randomizer.org/), with matching by age and sex. Allocation concealment was maintained by the Faculty of Pharmacy laboratory, which prepared EMS and identical-appearing placebo tablets in coded containers (A or B). Codes were held by an independent pharmacist and not revealed until all data were collected.

Interventions

All participants continued standard therapy with atropine 0.01% eye drops once nightly in both eyes throughout the study. The EMS group additionally received one Medicago sativa L. coated tablet (500 mg; trade name Alfalfa, POM TI.094541131) twice daily for six months. The placebo group received identical-appearing tablets on the same schedule. Assessments were performed at baseline (month 0), month 3, and month 6.

Outcome Measures

Primary outcomes: Change in plasma MDA concentration (µmol/L) and plasma SOD activity (mg/dL) from baseline to month 6.

Secondary outcome: Axial length progression (mm) in both eyes (OD and OS) from baseline to month 6, measured by A-scan ultrasonography (NIDEK US-4000).

Laboratory Measurements

MDA measurement: Plasma MDA was measured using the thiobarbituric acid reactive substances (TBARS) method with MDA OXIS kit (catalogue no. 21044) via spectrophotometry at 586 nm wavelength. EDTA plasma was collected at each time point, centrifuged at 3500 rpm for 15 minutes at 4°C, and stored at -20°C until analysis.

SOD measurement: Plasma SOD activity was determined using the EnzyChrom Superoxide Dismutase Assay Kit (ESOD-100) by UV-Visible spectrophotometry at 440 nm. Results were expressed in mg/dL.

Statistical Analysis

Sample size was determined based on SOD as the most stringent parameter, yielding a minimum of 12 per group; with a 20% dropout adjustment, 15 per group was targeted (final n=20 per group). Data were analyzed using SPSS. Repeated measurements were assessed using repeated-measures ANOVA (or Friedman's test for non-normal data), with post-hoc pairwise comparisons. Between-group comparisons used independent t-test or Mann-Whitney U test as appropriate. Correlations between delta biomarkers and axial length changes used Pearson's or Spearman's coefficient. Statistical significance was set at $p < 0.05$.

RESULTS

Participant Characteristics

Forty participants were randomized (20 per group). Both groups were balanced for sex (10 female, 10 male each; p=1.000) and mean age (EMS 14.0 ± 3.8 vs placebo 14.1 ± 3.6 years; p=0.840). Baseline axial lengths were comparable between groups (OD: 26.19 ± 0.15 vs 26.19 ± 0.12 mm, p=0.936; OS: 26.18 ± 0.16 vs 26.24 ± 0.11 mm, p=0.327). At baseline, the EMS group had significantly higher MDA (28.81 ± 6.85 vs 24.05 ± 6.00 µmol/L; p=0.025) and SOD (0.99 ± 0.17 vs 0.87 ± 0.20 mg/dL; p=0.026). This imbalance is acknowledged as a methodological limitation and is addressed through analysis of within-group change patterns (Table 1).

Table 1. Baseline characteristics of study participants

Characteristic	EMS Group (n=20)	Placebo Group (n=20)	p value
Sex (F/M)	10/10	10/10	1.000
Age (years)	14.0 ± 3.8	14.1 ± 3.6	0.840

MDA baseline (μmol/L)	28.81 ± 6.85	24.05 ± 6.00	0.025*
SOD baseline (mg/dL)	0.99 ± 0.17	0.87 ± 0.20	0.026*
AL OD baseline (mm)	26.19 ± 0.15	26.19 ± 0.12	0.936
AL OS baseline (mm)	26.18 ± 0.16	26.24 ± 0.11	0.327

*p < 0.05. Values expressed as mean ± SD. AL: axial length; OD: right eye; OS: left eye; EMS: Medicago sativa extract.

MDA and SOD Changes Over Time

Over 6 months, plasma MDA progressively decreased in the EMS group (28.81 → 22.73 → 17.16 μmol/L; p<0.001) while progressively increasing in the placebo group (24.05 → 27.33 → 29.96 μmol/L; p<0.001). The between-group difference was already significant at month 3 (22.73 ± 3.98 vs 27.33 ± 4.98; p=0.003) and became more pronounced at month 6 (17.16 ± 3.77 vs 29.96 ± 5.03; p<0.001).

Plasma SOD activity showed a divergent pattern. In the EMS group, SOD increased substantially from 0.99 ± 0.17 to 5.06 ± 1.94 mg/dL over 6 months (p<0.001), with significant gains at each interval. Conversely, the placebo group showed a progressive decline from 0.87 ± 0.20 to 0.65 ± 0.29 mg/dL (p<0.001). By month 6, SOD in the EMS group was nearly eight-fold higher than the placebo group (5.06 vs 0.65 mg/dL; p<0.001) (Table 2).

Table 2. Plasma MDA and SOD levels at baseline, month 3, and month 6 by group

Parameter / Time	EMS (n=20)	Placebo (n=20)	p (between)	p (within EMS)
MDA (μmol/L)				
Baseline	28.81 ± 6.85	24.05 ± 6.00	0.025*	
Month 3	22.73 ± 3.98	27.33 ± 4.98	0.003**	
Month 6	17.16 ± 3.77	29.96 ± 5.03	<0.001**	<0.001
ΔMDA (0→6)	-11.65 ± 5.78	+5.91 ± 3.51	<0.001**	
SOD (mg/dL)				
Baseline	0.99 ± 0.17	0.87 ± 0.20	0.026*	
Month 3	1.70 ± 0.77	0.84 ± 0.31	<0.001**	
Month 6	5.06 ± 1.94	0.65 ± 0.29	<0.001**	<0.001
ΔSOD (0→6)	+4.07 ± 1.95	-0.22 ± 0.23	<0.001**	

*p < 0.05; **p < 0.01. Values expressed as mean ± SD. p (between): independent t-test or Mann-Whitney. p (within EMS): Friedman/repeated-measures ANOVA.

Axial Length Progression

The EMS group demonstrated significantly attenuated axial elongation compared to placebo. Over 6 months, mean AL change in OD was 0.05 ± 0.03 mm (EMS) versus 0.45 ± 0.18 mm (placebo; p<0.001), and in OS was 0.06 ± 0.03 mm versus 0.49 ± 0.17 mm (p<0.001). The placebo elongation rate of ~0.47 mm/6 months translates to approximately 0.94 mm/year, representing rapid progression. The EMS group's rate was ~9-fold lower (Table 3).

Table 3. Axial length at baseline, month 3, and month 6, and delta axial length by group

Parameter	EMS Group	Placebo Group	p value
AL OD Baseline (mm)	26.19 ± 0.15	26.19 ± 0.12	0.936
AL OD Month 3 (mm)	26.21 ± 0.15	26.47 ± 0.18	<0.001*
AL OD Month 6 (mm)	26.24 ± 0.15	26.64 ± 0.19	<0.001*
ΔAL OD (0→6 months, mm)	0.05 ± 0.03	0.45 ± 0.18	<0.001†
AL OS Baseline (mm)	26.18 ± 0.16	26.24 ± 0.11	0.327
AL OS Month 3 (mm)	26.21 ± 0.15	26.52 ± 0.17	<0.001*
AL OS Month 6 (mm)	26.24 ± 0.14	26.73 ± 0.18	<0.001*
ΔAL OS (0→6 months, mm)	0.06 ± 0.03	0.49 ± 0.17	<0.001*

*Independent t-test; †Mann-Whitney U. AL: axial length; OD: right eye; OS: left eye.

Correlation Between Biomarker Changes and Axial Length Progression

Correlation analyses revealed no statistically significant relationships between ΔMDA or ΔSOD and axial length progression in either group. In the EMS group, correlation coefficients were weak ($|r| < 0.20$; all $p > 0.05$). In the placebo group, correlations were similarly non-significant ($|r| = 0.10$-0.28; all $p > 0.05$), including a trend for ΔSOD with ΔAL OS ($r=0.150$, $p=0.528$) and ΔMDA with ΔAL OD ($r=0.183$, $p=0.441$).

DISCUSSION

This is, to our knowledge, the first double-blind randomized controlled trial to demonstrate that adjuvant Medicago sativa L. extract combined with atropine 0.01% produces simultaneous improvements in plasma oxidative stress markers (MDA and SOD) and clinically meaningful attenuation of axial elongation in high myopia. The EMS group achieved a 40.4% reduction in plasma MDA and a 411% increase in SOD activity over six months, with axial elongation reduced by approximately nine-fold compared to the atropine-only placebo group.

The role of oxidative stress in myopia progression is increasingly well-characterized. High myopia creates a state of chronic ocular hypoxia due to choroidal thinning and increased oxygen demand in elongated tissue, which drives ROS overproduction.[4] Francisco et al. demonstrated elevated MDA and reduced SOD in the vitreous of high myopia patients and proposed that this imbalance accelerates retinal and scleral damage.[7] Our results are concordant: the placebo group showed rising MDA and falling SOD over the study period, consistent with progressive oxidative deterioration in actively elongating myopic eyes.

Medicago sativa L. is rich in flavonoids, saponins, and polyhydroxylated polyphenols that scavenge free radicals and activate the Nrf2-ARE transcriptional pathway, upregulating endogenous antioxidant enzymes including SOD, catalase, and glutathione peroxidase.[10,11] Raeeszadeh et al. demonstrated that alfalfa extract reduced MDA by approximately 40% and increased SOD activity in nicotine-induced oxidative stress models.[12] The magnitude of MDA reduction observed in our trial (-40.4%) is consistent with this evidence and was achieved in the context of human high myopia, strengthening translational relevance.

The primary clinical significance of this trial lies in the axial length findings. The placebo group elongated at ~0.94 mm/year — markedly exceeding the 0.41 mm/year reported for atropine 0.01% monotherapy. This may reflect our cohort's high severity (all > -6.00 D with AL > 26 mm). The EMS group's elongation rate of ~0.10 mm/year represents a clinically meaningful reduction, as elongation > 0.20 mm/year is widely regarded as rapid progression. Each additional 1 mm of axial length increases the risk of myopic macular degeneration by approximately 67%.[3] By substantially slowing elongation, EMS adjuvant therapy may therefore reduce the long-term risk of vision-threatening complications.

The lack of significant correlation between ΔMDA/ΔSOD and axial elongation warrants careful interpretation. This finding does not negate the efficacy of EMS, but rather suggests that the relationship between systemic oxidative stress and structural

ocular changes is not a simple linear one. Several explanations are plausible: systemic biomarkers may not accurately reflect local intraocular redox conditions; the primary mechanism of EMS action on axial elongation may involve anti-inflammatory pathways, scleral fibroblast modulation, or other cascades not captured by plasma MDA and SOD; or the six-month follow-up may be insufficient for structural adaptation to emerge from biochemical changes. Notably, some references found that oxidative-hypoxic biomarkers correlated more strongly with structural changes when measured in ocular fluids rather than plasma.[13,14,15]

A 2025 systematic review found limited evidence for nutritional supplementation in myopia control, with most studies examining omega-3 fatty acids and lutein.[15,16] Our data suggest that antioxidant supplementation targeting MDA-SOD balance may represent a complementary approach. Unlike orthokeratology or high-dose atropine, EMS is non-invasive, low-cost, and has a favorable tolerability profile, making it practically applicable across diverse healthcare settings, including resource-limited environments where myopia rates are rising fastest.

LIMITATIONS

This study has several important limitations. The relatively small sample size (n=20 per group), single-center design, and 6-month follow-up limit generalizability and preclude assessment of long-term safety or rebound effects. The imbalance in baseline MDA and SOD between groups, while pre-randomization and not explained by the intervention, may influence interpretation of absolute changes. Systemic biomarkers may not reflect intraocular oxidative status. Compliance was not formally quantified beyond attendance monitoring. A lenient control arm (atropine only, no further antioxidant arm) limits the ability to isolate the independent effect of EMS from atropine.

CONCLUSION

Medicago sativa L. extract as adjuvant therapy to atropine 0.01% significantly reduced plasma MDA, substantially increased SOD activity, and markedly attenuated axial elongation in adolescents with high myopia over six months. These findings position EMS as a promising antioxidant adjuvant for high myopia management, addressing the oxidative component of myopia pathogenesis that is not targeted by atropine alone. Multicenter trials with larger samples, longer follow-up, and intraocular biomarker assessment are warranted to confirm these findings and establish optimal dosing protocols.

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