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Research article

Moringa oleifera hydroalcoholic leaf extracts mitigate valproate-induced oxidative status in the extraorbital lacrimal gland in a rat model

Burcin Alev-Tuzuner [a,b], Sehkar Oktay [c], Eda Cergel [d], Gulsum Elik [e], Umar Faruk Magaji [f,g], Ozlem Sacan [f], Refiye Yanardag [f], Aysen Yarat [c,*]

[a] Istanbul Gelisim University, Faculty of Dentistry, Basic Medical Sciences, Biochemistry, Avcilar, Istanbul, Turkiye
[b] Istanbul Gelisim University, Life Sciences and Biomedical Engineering Application and Research Centre, Istanbul, Turkiye
[c] Marmara University, Faculty of Dentistry, Basic Medical Sciences, Biochemistry, Maltepe, Istanbul, Turkiye
[d] Haliç University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Eyupsultan, Istanbul, Turkiye
[e] Egil District State Hospital, Department of Nutrition and Dietetics, Egil, Diyarbakir, Turkiye
[f] Istanbul University-Cerrahpaşa, Faculty of Engineering, Department of Chemistry, Avcilar, Istanbul, Turkiye
[g] Federal University Birnin Kebbi, Department of Biochemistry and Molecular Biology, Birnin Kebbi, Kebbi, Nigeria

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ABSTRACT

Dysfunction of the extraorbital lacrimal gland (ELG) can lead to loss of vision due to damage to the epithelium of cornea. The broad-spectrum anti-epileptic drug sodium valproate (SV) has numerous side effects. *Moringa oleifera (M.oleifera)* is widely used as a food and in folk medicine. The effects of orally administered SV and *M. oleifera* hydroalcoholic leaf extract on rat ELG were investigated in this study by analysing both antioxidant and oxidant parameters. Additionally, boron level and tissue factor (TF) activity were determined. Protein changes were detected by sodium dodecyl sulfate gel electrophoresis (SDS-PAGE). Significantly lower values of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and total antioxidant status (TAS) were observed in the SV group compared to the control group. Treatment with Moringa extract significantly increased SOD, CAT and TAS values in the Moringa given SV group (SVM). While no significant differences were observed between the sialic acid values of the groups, lipid peroxidation (LPO), nitric oxide (NO) and total oxidant status (TOS) values were significantly elevated in the SV group compared to the control group. Due to the effect of Moringa extract, LPO, NO and TOS levels were significantly decreased in the SVM group compared to the SV group. TF activity was not meaningfully altered between groups. Compared to control rats, oxidative stress index (OSI) level significantly increased, whereas the boron level decreased in the SV group. Moringa extract treatment noticeably reduced OSI in the SVM group. According to SDS-PAGE, decreases in the density of protein bands with molecular weights of 51, 83, and 90 kDa were observed in SV given rats compared to the other groups. These decreases were reversed by the administration of Moringa extract. Moringa extract has shown protective properties arising from anti-oxidant potential, especially with its very low OSI value. Individuals undergoing SV treatment and having ELG complications might consider using Moringa extract to mitigate valproate induced damage.

1. Introduction

The tear film, which is made up of electrolytes, water and proteins, is produced by an exocrine gland called the extraorbital lacrimal gland (ELG). It protects the eye from environmental factors and helps maintain the integrity of the ocular surface. Dysfunction of the lacrimal gland is associated with ocular surface damage (especially the cornea), visual impairment, ocular distress and dry eye illnesses (Garg and Zhang, 2017). Oxidative stress caused by reactive radicals is implicated in lacrimal gland dysfunction due to cellular injury and contributes to the development of dry eye, among other alterations (de Souza et al., 2021).

Sodium valproate (SV), derived from valeric acid, is widely used as an anticonvulsant for epilepsy and other seizure disorders (Ghodke-Puranik et., 2013). It also has applications in the treatment of migraine, manic-depressive disorders, anxiety and related psychiatric

* Corresponding author. Marmara University, Faculty of Dentistry, Department of Basic Medical Sciences, Biochemistry, Basibuyuk, Maltepe, 34854, Istanbul, Turkiye.
E-mail addresses: btuzuner@gelisim.edu.tr (B. Alev-Tuzuner), nsehkar@yahoo.com (S. Oktay), eda.cergel.95@gmail.com (E. Cergel), gulsumelik.9@gmail.com (G. Elik), umar.faruk@fubk.edu.ng (U.F. Magaji), osacan@iuc.edu.tr (O. Sacan), yanardag@iuc.edu.tr (R. Yanardag), ayarat@marmara.edu.tr (A. Yarat).

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Table of abbreviations

AU	Arbitrary unit
C	Control group
CM	Moringa extract given control group
ELG	Extraorbital lacrimal gland
SV	Sodium valproate
SVM	Moringa extract and sodium valproate given group
T	Tissue

conditions. Although it is generally well tolerated, it can cause a number of side effects, including indigestion, weight gain, dysphoria, fatigue, dizziness, drowsiness, hair loss, headache, nausea, vomiting, sedation, and tremors (Kostrouchova et al., 2007).

Impaired ocular motility and retinal vascular autoregulation are common side effects of antiepileptic drugs. Others include retinopathy, maculopathy, glaucoma, myopia and optic neuropathy (Hamed, 2019). SV has been shown to suppress antioxidant protection, leading to lens oxidation and the pathogenesis of cataracts (Gogri et al., 2021). However, the effect of SV on the ELG is unknown.

Moringa oleifera (*M. oleifera*), commonly called the miracle tree, is from the Moringaceae family, it is used as a medicine and food source (Ghimire et al., 2021). *M. oleifera* owes its therapeutic potential to phytochemicals such as alkaloids, flavonoids, carotenoids, tannins, anthraquinones, anthocyanins, and proanthocyanidins (Abdul Razis et al., 2014). The plant's leaves are rich in protein, provitamin A, vitamins C and E, polyphenols and minerals (such as calcium, copper, iron, magnesium, potassium, zinc etc.) (Gopalakrishnan et al., 2016; Leone et al., 2016; Hukkeri et al., 2006). The plant exhibits various pharmacological effects ranging from anticancer (Parvathy and Umamaheshwari, 2007), antibacterial (van den Berg and Kuipers, 2022), antioxidant (Peñalver et al., 2022), anti-inflammatory (Cheenpracha et al., 2010), immunomodulatory (Sudha et al., 2010), antifungal (Chuang et al., 2007), antidiabetic (Jaiswal et al., 2009; Magaji et al., 2020) and hepatoprotective (Buraimoh et al., 2011) activities. There are no reported studies on its effect on ELG. Therefore, this study aims to determine the potential protective impacts of *M. oleifera* extract on ELG in rats exposed to SV.

2. Materials and methods

2.1. Chemicals

Analytical grade chemicals and reagents used in this study were purchased from Merck, Sigma-Aldrich and Fluka.

2.2. Plant materials

Fresh leaf samples of *M. oleifera* were collected in 2019 from farms in Sokoto, Nigeria. They were authenticated by a taxonomist; Umar Abdullahi at the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto (Herbarium voucher number: UDUS/VS/2011/31).

2.3. Preparation of moringa leaf extract

Collected leaf samples were shade-dried before being pulverized. The resulting powdery plant leaves were packed in paper envelopes and stored in polyethylene bags to prevent contamination and moisture absorption. A 100 g of the plant leaves were placed into the Soxhlet device to prepare the extract. Then 150 mL of 70% ethyl alcohol was added and refluxed until at least 20 siphons were formed. A rotary evaporator was used to remove the solvent. The residue obtained was weighed and kept at −20 °C until use. During the experiment the 70% ethanolic extract of Moringa leaves was dissolved in water and given orally to rats.

2.4. Animals

Female Sprague Dawley rats (three-month-old) weighing between 250 and 300 g were used in this study. Animals were housed under optimal temperature (20 °C ± 2), humidity and 12 h light/12 h dark cycles. The rats were kept in their cages throughout the experiment and were provided with fresh tap water and standard pellet-type rat food to meet their daily dietary needs under laboratory conditions. The study protocol was reviewed and approved by Marmara University Experimental Animals Ethics Committee (Decision No: 39.2022mar, date:May 17, 2022).

2.5. Groups

The animals were divided into four groups of six rats each. The control group (C) received 0.9 g % NaCl orally for 15 days. The control + Moringa group (CM) received a single dose of *M. oleifera* 70% hydroalcoholic leaf extract (0.3 g/kg/day) orally for 15 days. The sodium valproate group (SV) received a single dose of SV (0.5 g/kg/day) orally for 15 days. The sodium valproate + Moringa group (SVM) received SV and *M. oleifera* 70% hydroalcoholic leaf extract at the same dose and for the same duration. On the 16th day, fasted animals were sacrificed after anesthesia. The collected ELGs were homogenized in normal saline. Each homogenate (5% w/v) was divided into small aliquots and stored at −80 °C until the experimental day.

2.6. Biochemical parameters

The supernatants obtained from ELG homogenates were used for biochemical analysis, except for tissue factor (TF). The ELG homogenate was used directly for TF analysis. Based on the method of Lowry et al. (1951), total protein (TP) levels were determined using bovine albumin as the protein standard. The antioxidant capacity of the ELG samples was assessed by measuring glutathione (GSH) (Beutler, 1975), superoxide dismutase (SOD) (Mylroie et al., 1986), catalase (CAT) (Aebi, 1984) and total antioxidant status (TAS) (Erel, 2004). In contrast, the oxidant capacity was evaluated by analyzing lipid peroxidation (LPO) (Ledwozyw et al., 1986), sialic acid (SA) (Warren, 1959), nitric oxide (NO) (Miranda et al., 2001), and total oxidant status (TOS) (Erel, 2005). Additionally, ELG boron levels were assessed to monitor its role in gland health, following the method outlined by Hatcher and Wilcox (1950) with modifications by Kuru et al. (2019). As an indicator of ELG damage, TF activity was evaluated using the method described by Ingram and Hills (1976).

The oxidative stress index (OSI), which allows for the evaluation of both antioxidant and oxidant activity simultaneously, was also calculated using the formula as follows: OSI (AU: Arbitrary Unit) = [TOS (μM)/TAS (μM)] × 100. A lower OSI value (<1) indicates a greater antioxidant effect (Sánchez-Rodríguez and Mendoza-Núñez, 2019).

2.7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the Laemmli method (Laemmli, 1970). The ELG samples were mixed with SDS gel loading buffer containing Tris-HCl, SDS, mercaptoethanol, bromophenol blue and glycerol. They were then heated in a boiling water bath for 5 min and 20 μg of total protein per lane was loaded onto 7.5% SDS polyacrylamide gels (with 4.5% separating gel). Using the banding pattern of labeled protein standards of known molecular size, the size of the loaded proteins (kDa) was evaluated by comparing the relative mobility of each band. The mixture of bovine albumin (66 kDa), egg albumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (from rabbit muscle, 36

kDa) and bovine carbonic anhydrase (29 kDa) was used as the standard protein mixture. Electrophoresis was performed at 30 mA/gel for 1 h at 20 °C using a Bio-Rad Mini-PROTEAN Tetra Vertical Electrophoresis Cell. For quantitative measurements, high-resolution test photographs were taken using a Canon EOS 700D camera equipped with an 18–55 mm lens to evaluate protein bands after electrophoresis, and the images were exported as JPEG files. Densitometric plots of protein bands were generated using ImageJ software (Schneider et al., 2012).

2.8. Statistical analyses

Biochemical parameters were expressed as mean ± standard error and statistically analyzed using GraphPad Prism 9.0. As the data showed a normal distribution, unpaired t-tests and analysis of variance (ANOVA) were used for statistical comparisons, followed by Tukey's multiple comparison tests. Statistical significance was defined as a p-value of less than 0.05.

3. Results

3.1. The effects of SV and moringa treatments on TP, GSH, SOD, CAT and TAS parameters

Table 1 shows the values of TP and antioxidant parameters (GSH, SOD, CAT, TAS) examined in ELG homogenates. A significant difference in TP levels was not observed between the experimental groups ($p > 0.05$). They were 46.97, 47.23, 46.03, and 44.75 mg per gram of tissue in the C, CM, SVP and SVM groups, respectively. In the SV group, significantly lower GSH, SOD, CAT and TAC values were observed compared to the C group ($p < 0.0001$, $p < 0.05$, $p < 0.0001$, and $p < 0.05$ respectively). Moringa extract treatment in the SVM group significantly increased SOD, CAT and TAS values in comparison to the SV group ($p < 0.01$, $p < 0.0001$ and $p < 0.0001$, respectively). GSH values were not significantly different between the SV and SVM groups. In the CM group, CAT and TAS values increased significantly compared to the C group ($p < 0.01$, $p < 0.05$, respectively). When comparing the CM and SVM groups, a decrease in the GSH and CAT values and an increase in TAS values were observed in the SVM groups ($p < 0.0001$, $p < 0.01$, $p < 0.0001$ respectively). In the SVM group, there was a decrease in GSH values and an increase in TAS values compared to the C group ($p < 0.0001$). When comparing the CM and SV groups, it was observed that GSH, SOD, CAT and TAS values were significantly decreased in the SV group ($p < 0.0001$, $p < 0.001$, $p < 0.001$, and $p < 0.0001$ respectively).

Table 1
The values of TP and antioxidant parameters of ELG samples.

	C (n = 6)	CM (n = 6)	SV (n = 6)	SVM (n = 6)
TP (mg/gT)	46.97 ± 1.110	47.23 ± 0.580	46.03 ± 0.850	44.75 ± 0.580
GSH (mg/gT)	32.23 ± 0.670	30.97 ± 0.850	20.67 ± 1.040^{d,h}	22.73 ± 1.200^{d,h}
SOD (U/gT)	0.08 ± 0.006	0.10 ± 0.004	0.06 ± 0.004^{a,g}	0.09 ± 0.008^{m}
CAT (kU/gT)	1.40 ± 0.020	1.58 ± 0.030^{c}	1.15 ± 0.010^{d,g}	1.41 ± 0.040^{f,o}
TAS (mmol/gT)	3.09 ± 0.190	3.83 ± 0.150^{a}	2.24 ± 0.080^{d,h}	5.81 ± 0.250^{d,o,h}

Values were given as mean ± standard error. ELG: Extraorbital lacrimal gland, T: Tissue, TP: Total protein, GSH: Glutathione, SOD: Superoxide dismutase, U: Unit, CAT: Catalase, kU: Kilo unit, TAS: Total antioxidant status, C: Control group, CM: Moringa extract given control group, SV: Sodium valproate given group, SVM: Moringa extract and SV given group. $^{a}p<0.05$, $^{c}p < 0.01$, $^{d}p < 0.0001$ versus C, $^{f}p < 0.01$, $^{g}p < 0.001$, $^{h}p < 0.0001$ versus CM, $^{m}p < 0.01$, $^{o}p < 0.0001$ versus SV.

3.2. The effects of SV and moringa treatments on LPO, SA, NO and TOS parameters

Table 2 shows the values of oxidant parameters (LPO, SA, NO, and TOS) examined in ELG homogenates. No significant differences were observed between the SA values of the groups ($p > 0.05$). Compared to the control group, LPO, NO, and TOS values were significantly elevated in the SV group ($p < 0.01$, $p < 0.05$, and $p < 0.0001$, respectively). Due to the effect of Moringa extract, LPO, NO, and TOS levels were significantly decreased in the SVM group compared to the SV group ($p < 0.05$, $p < 0.05$, and $p < 0.0001$). When comparing the SVM and CM groups on these parameters, the changes were not statistically different ($p > 0.05$). In the SV group, there was an increase in LPO and TOS values in relation to the CM group ($p < 0.001$, $p < 0.0001$ respectively).

3.3. The effects of SV and moringa treatments on boron, TF and OSI parameters

Boron, TF and OSI values are presented in Table 3. TF activity was not meaningfully altered between groups ($p > 0.05$). Compared to control rats, OSI level was significantly increased, whereas a decrease in the boron level was seen in the SV group ($p < 0.0001$, $p < 0.05$ respectively). Moringa extract treatment noticeably reduced OSI in the SVM group ($p < 0.0001$). The OSI value was reduced more in the SVM group than in the CM group ($p < 0.01$).

3.4. SDS-PAGE results

The SDS-PAGE protein bands were observed in the same position for each sample and the molecular weights ranged from 150 to 25 kDa in all ELG samples (Fig. 1A). The ImageJ program showed that the intensity of the protein bands did not differ between the study groups (Fig. 1B). In the SV group, proteins with molecular weights of 90, 83 and 51 kDa exhibited decreased band densities compared to C, CM, and SVM (Fig. 1C).

4. Discussion

SV, an antiepileptic drug used to treat various seizure disorders, is linked to many side effects including hepatotoxicity, cardiotoxicity, nephrotoxicity and neurotoxicity. Metabolic changes related to antioxidant balance, inflammation, amino acids, fats and energy, as well as alterations in certain metabolite levels, are widely recognized as prominent mechanisms contributing to the toxic effects of SV (Gao et al., 2022). At physiological concentrations, reactive oxygen species (ROS) play an essential role as signaling molecules. On the contrary, an excess of ROS causes oxidative stress, leading to the degradation of DNA, proteins, and other intracellular molecules. This oxidative stress can

Table 2
The values of oxidant parameters of ELG samples.

	C (n = 6)	CM (n = 6)	SV (n = 6)	SVM (n = 6)
LPO (nmol MDA/gT)	0.16 ± 0.005	0.15 ± 0.006	0.19 ± 0.007^{b,g}	0.16 ± 0.005^{k}
SA (mg/gT)	1.60 ± 0.060	1.58 ± 0.030	1.61 ± 0.030	1.46 ± 0.060
NO (nmol/gT)	0.51 ± 0.003	0.56 ± 0.010	0.58 ± 0.010^{a}	0.52 ± 0.020^{k}
TOS (μmol/gT)	0.21 ± 0.020	0.25 ± 0.010	0.53 ± 0.020^{d,h}	0.19 ± 0.030^{o}

Values were given as mean ± standard error. ELG: Extraorbital lacrimal gland, T: Tissue, LPO: Lipid peroxidation, MDA: Malondialdehyde, SA: Sialic acid, NO: Nitric oxide, TOS: Total oxidant status, C: Control group, CM: Moringa extract given control group, SV: Sodium valproate given group, SVM: Moringa extract and SV given group. $^{a}p<0.05$, $^{b}p < 0.01$, $^{d}p < 0.0001$ versus C, $^{g}p < 0.001$, $^{h}p < 0.0001$ versus CM, $^{k}p < 0.05$, $^{o}p < 0.0001$ versus SV.

Table 3
Boron levels, TF activities and OSI values of ELG samples.

	C (n = 6)	CM (n = 6)	SV (n = 6)	SVM (n = 6)
Boron (ppm)	11.02 ± 0.2300	11.04 ± 0.2300	9.91 ± 0.1800[a,e]	10.44 ± 0.3000
TF (sec)	97.17 ± 0.9600	94.00 ± 2.1000	95.67 ± 1.4800	93.33 ± 1.2800
OSI (AU)	0.007 ± 0.0010	0.007 ± 0.0004	0.024 ± 0.0010[d,h]	0.0033 ± 0.0005[b,f,o]

Values were given as mean ± standard error. ELG: Extraorbital lacrimal gland, ppm: Parts per million, TF: Tissue factor, sec: Second, OSI: Oxidative stress index, AU: Arbitrary unit, C: Control group, CM: Moringa extract given control group, SV: Sodium valproate given group, SVM: Moringa extract and SV given group. [a]$p<0.05$, [b]$p < 0.01$, [d]$p < 0.0001$ versus C, [e]$p < 0.05$, [f]$p < 0.01$, [h]$p < 0.0001$ versus CM, [o]$p < 0.0001$ versus SV.

induce functional damage and cell death, contributing to the pathogenesis of numerous diseases (Lemos et al., 2022). In the lacrimal gland, inflammation and structural changes can arise due to exposure to various external factors, coupled with an imbalanced immune system associated with an inadequate response to stressors. Oxidative stress within the gland disrupts tear film functionality, thus initiates the dry eye syndrome and other related changes (de Souza et al., 2021). Hence, the importance of any compounds capable of reducing SV-induced oxidative stress when administered together cannot be overstated. The identification of the antioxidant, anti-inflammatory and anti-apoptotic effects of natural plants has led to the discovery of effective disease-modifying agents. *M. oleifera* has diverse defensive properties that counteract oxidative stress-mediated diseases *in vitro* and *in vivo* (Pareek et al., 2023).

Literature reviews show that *M. oleifera* leaf extracts are commonly used at doses ranging from 100 to 300 mg/kg/day for an initial treatment period of 2–8 weeks, and the extracts show therapeutic effects within these dose ranges (Mthiyane et al., 2022) and are considered to be within the safe dosage range (El-Hadary and Ramadan, 2019). In the present study, the moringa dose was used as 300 mg/kg. According to the Oztopuz et al. (2020) study sodium valproate was used at a dose of 500 mg/kg.

This study is the first to investigate LPO, SA, NO, TOS and OSI values in the assessment of SV-induced damage in ELG. In comparison with the C group, a significant rise in LPO, NO, TOS, and OSI values was seen in the SV group. It has been demonstrated that malondialdehyde levels in the lacrimal gland are significantly increased in diabetic models compared to control and insulin-treated diabetics after 10 weeks of the disease. This condition has been attributed to the failure of the antioxidant mechanism (Módulo et al., 2009). In another study, radioiodine therapy was associated with an increase in TOS levels and a decrease in TAS concentrations. This confirms the robust inflammatory stimulus of radioiodine in the lacrimal glands (Koca et al., 2021).

Another unique contribution of this research is the first use of Moringa as an antioxidant to reduce the oxidant load of ELG on SV-induced oxidative damage. Significantly lower OSI values were seen in the SVM group compared to SV rats. Histological evaluation was not performed in the present study. However, the histology of the lacrimal gland has been reported to be improve when Moringa leaf extract is given to a mouse model of Sjögren's syndrome (Susanto et al., 2022). *M. oleifera* leaves are rich in bioactive compounds, especially phenols, which might explain the decrease in the oxidant parameters of the ELGs. This reduction is likely due to the antioxidant activity of the phytochemicals in Moringa, which help to scavenge ROS. *M. oleifera* has over 40 natural antioxidants and is known for its ability to eliminate free radicals by increasing the expression of SOD, GPx (glutathione peroxidase) and CAT. It also reduces lipid peroxidation levels and increases FRAP (ferric ion reducing antioxidant power) levels (Xiao et al., 2020).

GSH is often referred to as the body's main antioxidant because of its crucial role in cellular defense against oxidative stress and in detoxification processes. This tripeptide has other roles in numerous tissues and organs throughout the body, including the ELG (Böhm et al., 2023). Overall, GSH plays a crucial role in protecting, maintaining, and regulating the function of the ELG. In this study, lower GSH levels were observed in the SV and SVM groups compared to the C group. The reason for the decrease in GSH in ELGs may be the conversion of harmful compounds into GSH derivatives in the GSH-based detoxification pathway. This finding is consistent with the increased LPO and TOS (indicators of oxidative stress) observed in the SV group and their reduction in the SVM group. In addition to decreased GSH levels, there is evidence of increased ROS concentrations during valproic acid treatment (Tong et al., 2005). Furthermore, co-administration of Moringa extract with valproic acid attenuates GSH depletion and reduces both LPO and AOPP (Advanced oxidation protein products) levels in the lens (Dağsuyu et al., 2023).

Cells have a complex antioxidant defense system to neutralize ROS. This system comprises the enzymatic and non-enzymatic mechanisms. SOD is a key component of the antioxidant system and is able to dismutate $O_2^{-\cdot}$. CAT catalyzes the conversion of H_2O_2 into water and O_2 (Paulsen et al., 2004). SOD knockout mice exhibit reduced size, inflamed, and aging accelerated lacrimal glands. These alterations are characterized by the accumulation of oxidized substances in the lacrimal gland, decreased tear production, blockage of secretory vesicles in the acinar cells and the development of keratitis (Lemos et al., 2022). In the present study, the co-administration of SV with Moringa extract resulted in increased SOD, CAT activities and TAS level. These changes might be an adaptation arising from Moringa extract enhanced antioxidant system in response to SV toxicity; primarily credited to the potent antioxidant properties of the extract. Consistent with these findings, it has been demonstrated that Moringa extract leads to increased SOD, GPx, GR (glutathione reductase) and GST activities in response to SV treatment in the lens (Dağsuyu et al., 2023).

The lacrimal gland is a source of ocular mucins, and SA is a common feature of normal secreted ocular mucins, which are known as sialoglycoproteins (Hsueh et al., 2022). Decreased levels of SA were detected in SVM groups, however these results were statistically insignificant. Oxidative stress enhanced the hydrolysis of SA residues of membrane proteins (López-Contreras et al., 2020), and Moringa may have mitigated this effect.

NO is a small gaseous molecule that plays a dual role in promoting cell death or cell survival. The lacrimal gland is a major source of NO in tears. Inducible NO synthase (iNOS) in inflammatory cells such as macrophages synthesizes NO, which could play a role in immune and inflammatory responses; it reduces ocular surface inflammation. However, prolonged NO overproduction by iNOS can produce highly toxic and reactive peroxynitrites. In such scenarios, NO acts as a pro-inflammatory mediator, triggering oxidative tissue injury and cell death. NO is critical for lacrimal gland function, tear production and ocular surface health. It is important to note that the effect of NO on the lacrimal gland is complex and can differ based on factors such as NO concentration, duration of exposure and cellular context (Tummanapalli et al., 2021). Compared to the C group, NO levels were elevated in the SV group in the present study, but decreased in the SVM group compared to the SV group. While the data presented in this study does not clearly prove prolonged overproduction of NO, it remains a possibility and Moringa extract may have prevented NO production. More investigation is needed to fully understand the exact mechanisms by which NO affects lacrimal gland function and the implications for ocular health and disease.

Boron is a trace element that occurs naturally in many foods and in the environment. It plays various roles in biological systems, including bone health, wound healing and metabolism (Pizzorno, 2015). However, its presence and function in the lacrimal gland has not been extensively studied compared to its role in other tissues and systems. Research on boron's effects on ocular health often focuses on its potential benefits for conditions such as dry eye syndrome or corneal

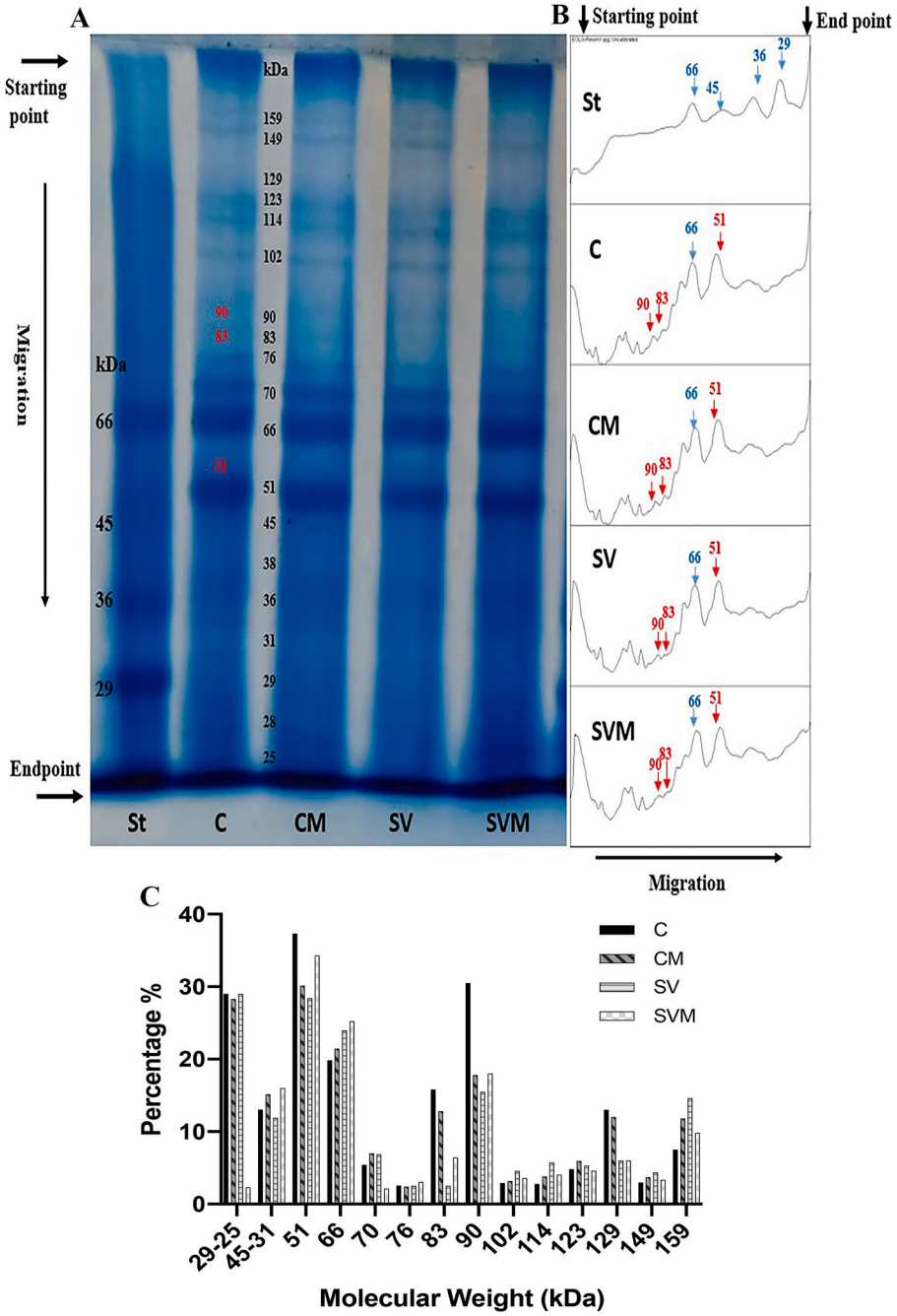

Fig. 1. The SDS-PAGE bands of ELG proteins (A); Graphs of SDS-PAGE bands scanned with ImageJ program (B); Percent intensities of the ELG protein bands after SDS-PAGE (C). In Fig. 1C, to improve visibility, the percent intensities of the 25–29, 83, 90 and 159 kDa bands have been magnified 10 times, while the 129 kDa band has been magnified 100 times. St: Standard protein mixture (bovine albumin: 66 kDa; egg albumin: 45 kDa; glyceraldehyde-3-phosphate dehydrogenase, from rabbit muscle: 36 kDa and bovine carbonic anhydrase: 29 kDa), MW: Molecular weight, C: Control group, CM: Moringa extract given control group, SV: Sodium valproate group, SVM: Moringa extract and SV given group, ELG:Extraorbital lacrimal gland, SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

wound healing, rather than its specific concentration within lacrimal gland tissues (Nagai et al., 2010; Avetisov et al., 2014). It is worth noting that boron is considered an essential micronutrient, meaning that it is required in small amounts for normal physiological functions. Nevertheless, the exact optimal concentration of boron in the lacrimal gland, or whether it accumulates there significantly compared to other tissues, is not well documented in the scientific literature. In malathion-induced changes, boron levels are found to decrease in plasma and in tissues such as the kidney, brain and liver (Coban et al., 2015). In another study, a high-fat diet decreased boron levels in the lens, while *Myrtus communis* L. extract and apocynin increased boron levels (Kuru Yaşar et al., 2021). Similar to these studies, the SV group in the present study had reduced ELG boron levels compared to the control group. Additionally, boron's involvement in various metabolic pathways and cellular processes suggests that it may play a role in maintaining the function of lacrimal gland cells (Coban et al., 2015). While it can be inferred that boron is likely to be found in the ELG due to its essential nature and distribution in the body, detailed information regarding its specific concentration or importance in lacrimal gland physiology requires further investigation and research.

TF is the primary initiator of the coagulation system necessary for the formation of a proper clot, which is essential for the normal process of

wound healing. A previous study showed that SV and Moringa did not differ between groups in terms of TF activity in the salivary glands (Cergel et al., 2024). Similarly, no difference was found between the groups regarding TF activities in ELG.

The ELG contains various proteins that are essential for its structure, function and regulation. These proteins include collagen (~300 kDa), elastin (~60–72 kDa), aquaporin-5 (~22 kDa), mucins such as MUC5AC and MUC7 (~600 kDa), immunoglobulins (150–970 kDa), lipocalin proteins (20–22 kDa), lysozyme (~15 kDa), cytokines (e.g., interleukins (~15–75 kDa), tumor necrosis factor ~17–26 kDa) and growth factors (e.g., epidermal growth factor (~6 kda)), lipases (~50 kDa), superoxide dismutase (~22–89 kDa), catalase (~240 kDa), glutathione peroxidase (~22 kDa), lactoferrin (~80 kDa), and thioredoxin reductase (~55 kDa) (Walcott, 1998; Remington and Nelson, 2002; Verkman, 2003; Kawashima et al., 2012; Batista et al., 2012; Karnati et al., 2013; Aluri et al., 2015; Fini et al., 2020; Lemos et al., 2022). These structural and functional proteins work together to ensure the proper functioning of the ELG and help maintain a healthy ocular environment. Any disruption in the expression or regulation of these proteins can lead to ocular surface disorders such as dry eye syndrome, inflammation or increased susceptibility to infection. In the present study, decreases in the density of protein bands with molecular weights of 51, 83, and 90 kDa were seen in SV given rats compared to all other groups (Fig. 1C). These proteins may include thioredoxin reductase, lactoferrin, lipase and, superoxide dismutase. SV-induced damage to the ELG may have resulted in diminished levels of these proteins, which suppress and prevent oxidative stress.

5. Conclusion

The findings obtained in this study revealed that *M. oleifera* extracts have antioxidant effects and are able to suppress the oxidative damage caused by SV in the ELGs of rats. The use of Moringa extract as an alternative therapy may offer benefits for individuals undergoing sodium valproate treatment who suffer from ELG problems.

5.1. Limitations

The first limitation of this study is that all rats were of the same sex, that is, female. Future research should investigate the impact of gender differences on this phenomenon. The second one is that there is no any of dosing studies for ELG in point of SV and Moringa extract. The third one is the lack of accuracy of the results of SDS-PAGE analysis with other methods such as real-time PCR, immunohistochemistry or Western blots showing selected markers of oxidative stress and antioxidant factors due to financial problems.

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

CRediT authorship contribution statement

Burcin Alev-Tuzuner: Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Sehkar Oktay:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Eda Cergel:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Data curation. **Gulsum Elik:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Data curation. **Umar Faruk Magaji:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Ozlem Sacan:** Writing – review & editing, Validation, Supervision, Resources, Formal analysis, Data curation. **Refiye Yanardag:** Writing – review & editing, Validation, Supervision, Resources, Formal analysis, Data curation, Conceptualization. **Aysen Yarat:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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