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RESEARCH ARTICLE

Dermatoprotective effect of *Moringa oleifera* leaf extract on sodium valproate-induced skin damage in rats

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ABSTRACT

Valproic acid is an antiepileptic drug associated with skin-related issues like excessive hair growth, hair loss, and skin rashes. In contrast, *Moringa oleifera*, rich in nutrients and antioxidants, is gaining popularity worldwide for its medicinal properties. The protective properties of *M. oleifera* extract against skin-related side effects caused by valproic acid were investigated. Female rats were divided into control groups and experimental groups such as moringa, sodium valproate, and sodium valproate + moringa groups. A 70% ethanolic extract of moringa (0.3 g/kg/day) was given to moringa groups, and a single dose of sodium valproate (0.5 g/kg/day) was given to valproate groups for 15 days. In the skin samples, antioxidant parameters (such as glutathione, glutathione-S-transferase, superoxide dismutase, catalase, and total antioxidant capacity), as well as oxidant parameters representing oxidative stress (i.e. lipid peroxidation, sialic acid, nitric oxide, reactive oxygen species, and total oxidant capacity), were examined. Additionally, boron, hydroxyproline, sodium-potassium ATPase, and tissue factor values were determined. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was also carried out for protein analysis in the skin samples. The results showed that moringa could increase glutathione, total antioxidant capacity, sodium-potassium ATPase, and boron levels, while decreasing lipid peroxidation, sialic acid, nitric oxide, total oxidant capacity, reactive oxygen species, hydroxyproline, and tissue factor levels. These findings imply that moringa possesses the potential to mitigate dermatological side effects.

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Introduction

The skin is a multifunctional organ that goes beyond its role as a protective barrier. Its ability to adapt to different environments and respond to various stimuli is essential for overall health and survival. Proper skin care and protection are important to maintain its functions and prevent skin-related health issues. Skin health can be impacted by various factors, including medications, inadequate dietary habits, hormonal fluctuations, advancing age, individual skin type, ethnicity, inherent skin tone, stress levels, sleep patterns, hydration levels, environmental conditions, lifestyle choices, and the application of cosmetics (Daly 1982; Lopez-Ojeda et al., 2023).

Valproic acid/sodium valproate (VA), derived from valeric acid, is a widely used anticonvulsant drug for epilepsy and other seizure disorders (Ghodke-Puranik et al., 2013). It also finds applications in treating migraine, manic-depressive disorders, anxiety, and related psychiatric conditions. While generally well-tolerated, it can have a range of side effects, including indigestion, weight gain, dysphoria, fatigue, dizziness, drowsiness, hair loss, headache, nausea, vomiting, sedation, and tremors (Kostrouchova et al., 2007). Additionally, skin reactions such as hirsutism, hair loss, and skin rashes (though rare) can occur (Dreifuss and Langer 1988). Besides, when VA is combined with other antiepileptic drugs like lamotrigine, severe skin conditions like Stevens-Johnson syndrome and toxic epidermal necrolysis, while uncommon, can be life-threatening (Oflaz et al., 2011; Öner et al., 2012). Long-term use of VA has been linked to lichen planus (a mucocutaneous inflammation), and rarely nail anomalies leading to onychomadesis, even in childhood (Piraccini et al., 2010).

Moringa oleifera (*M. oleifera*) is the most recognized genus among the 13 tree and shrub species within the Moringaceae family. Originating in India, this crucial plant is cultivated globally, cherished for its resilience against drought and frost, and deeply rooted in traditional herbal medicine (Dhakad et al., 2019). Moringa species have multifaceted applications, finding utility in medicine, culinary, cosmetics, and oil production. Its nickname, the "Miracle Tree" underscores its reputation for combating serious societal ailments (Goyal et al., 2007). *M. oleifera* owes its therapeutic potential to phytochemicals such as alkaloids, flavonoids, carotenoids, tannins, anthraquinones, anthocyanins, and proanthocyanidins (Abdul Razis et al., 2014).

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Certain components of *M. oleifera* exhibit diverse pharmacological activities, including anti-cancer (Parvathy & Umamaheshwari, 2007), antibacterial (van den Berg & 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) and hepatoprotective (Buraimoh et al., 2011) properties. The leaves of *M. oleifera* are rich in protein, calcium, potassium, zinc, magnesium, iron, copper, polyphenols, beta-carotene, and vitamins C and E (Gopalakrishnan et al., 2016; Hukkeri et al., 2006; Leone et al., 2016). They are a natural source of antioxidants and have been used in traditional medicine for wound healing. *M. oleifera* has been shown to promote wound healing by stimulating the proliferation and migration in human fibroblast cells, and protecting skin keratinocytes from oxidative stress through an enhanced antioxidant defense systems (Gothai et al., 2016; Islam et al., 2005; Zhou et al., 2018). *M. oleifera* leaves and roots, along with other plant components, have been used in the formulation of medications to treat skin infections, superficial acne, and various skin conditions (Paliwal et al., 2011). Additionally, *M. oleifera* seed oil is a valuable cosmetic product in skin care routines due to its moisturizing properties for both hair and body (Maryam & Manzoor, 2023).

There is a study in the literature examining the effect of edaravone, not moringa, on the skins of rats given valproic acid (Tunali-Akbay et al., 2017). A literature search revealed no studies that have examined on biochemical parameters of *M. oleifera* in ameliorating VA-induced skin side effects. Therefore, there is a need to search for natural products that can reduce side effects.

In the present study, the possible protective effects of 70% ethanolic leaf extract of *M. oleifera* on skin tissues in VA-treated rats were investigated. Various biochemical parameters related to oxidative stress in rat skin homogenates were examined.

Materials and methods

Chemicals

The chemicals used in this study were of analytical purity and were obtained from Merck, Sigma Aldrich, and Fluka companies. Sodium valproate was purchased from Sigma Aldrich and the CAS number is 1069-66-5.

Plant materials

Fresh *M. oleifera* leaves were collected from farms in Sokoto town of Sokoto State, Nigeria in 2019. At the Botany Unit of Biological Sciences Department, Usmanu Danfodiyo University Sokoto, the plant was identified and authenticated by a taxonomist; Umar Abdullahi (PhD), followed by deposition of voucher specimens in the university herbarium (Herbarium number: UDUS/VS/2011/31).

Preparation of moringa leaf extract

M. oleifera leaves were dried in the shade 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 put 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. The solvent was removed by an evaporator. The resulting residue was weighed, transferred to labeled Eppendorf tubes, and stored at −20 °C until use.

Animals

Three-month-old Sprague Dawley female rats weighing 250–300 g were used. The study was approved by the Marmara University Experimental Animals Ethics Committee (Decision No: 77.2021mar, date:12.10.2021). The experimental animals were housed in the animal room which has optimum temperature (20 °C ± 2), humidity, and light/dark (12 h light/12 h dark) conditions. The rats were kept in their cages during the experiment. All rats were orally fed with fresh tap water and normal pellet-type rat food for their daily dietary needs under laboratory conditions.

Groups

The rats were divided into four groups (8 animals per group) as control (C) (0.9% NaCl given orally for 15 days), moringa (M) (a single dose of *M. oleifera* 70% ethanolic leaf extract (0.3 g/kg/day) given for 15 days), sodium valproate (VA) (a single dose of sodium valproate (0.5 g/kg/day) given orally for 15 days) and sodium valproate + moringa (VM) (sodium valproate + *M. oleifera* 70% ethanolic leaf extract given at the same dose and for the same duration). On the 16th day, fasted rats were sacrificed under anesthesia. After the back skin was cleaned of hair, approximately 2 × 2 mm^2 samples were taken and weighed. According to the weight of samples, their volumes were completed to obtain 10% w/v homogenate. Then they were homogenized with NaCl (0.9%) for 5 min at 15 000 rpm using a mechanical homogenizer (Janke & Kunkel Ultra t-Turraxt 25). Each homogenate (10% w/v) was divided into small aliquots separately and stored in a deep freezer (at −80 °C) until the biochemical analysis.

Biochemical parameters

The homogenates of skin samples were centrifuged and the clear supernatants were used for all biochemical parameters' analysis except tissue factor (TF). The skin homogenate was used directly for TF analysis.

The total protein (TP) levels in the skin homogenates were determined using the method of Lowry et al. (1950), and bovine serum albumin was used as the protein standard. Copper ions were applied to the rats' skin samples in an alkaline environment. They were then reduced with a phosphomolybdic-phosphotungstic acid reagent (Folin reagent). At 500 nm, the intensity of the blue color was evaluated spectrophotometrically. The intensity of the blue color formed is proportional to the protein concentration.

The antioxidant status of the skin samples was assessed by measuring glutathione (GSH), glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC).

For GSH determination, the absorbance of the colored product of the reaction of the Ellman separator, 5–5′dithiobis(2-nitrobenzoic acid] (DTNB), and sulfhydryl groups were spectrophotometrically recorded at 412 nm and calculated using the extinction coefficient (1.36×10^4 $M^{-1}cm^{-1}$) (Beutler, 1975).

GST activity was measured according to the spectrophotometric determination of the 340 nm absorbance belonging to the product formed by the conjugation of GSH with 1-chloro-2,4-dinitro-benzene (CDNB) (Habig & Jakoby, 1981).

SOD activities were determined by riboflavin-sensitized photooxidation of ortho-dianisidine. Ortho-dianisidine oxidation, by the reaction with riboflavin, is induced by SOD. The increase in absorbance depends on the SOD concentration. Finally, the colored product's absorbance was determined using spectrophotometry at 460 nm (Mylroie et al., 1986).

CAT activity was determined based on the H_2O formation reaction from H_2O_2. It was noted by the decrease in absorbance obtained at 240 nm (Aebi, 1984).

TAC is used to provide insights into the development and treatment of oxidative stress-related disorders. Antioxidants in the sample reduce dark blue-green colored 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical to the colorless reduced ABTS form. The change of absorbance at 660 nm is related to the total antioxidant level of the sample. The assay is calibrated with a stable antioxidant standard solution which is traditionally named Trolox Equivalent which is a vitamin E analog (Erel, 2004).

Oxidant status was evaluated by analyzing lipid peroxidation (LPO), sialic acid (SA), nitric oxide (NO), total oxidant capacity (TOC), and reactive oxygen species (ROS).

The absorbance of the pink color produced at the end of the reaction between the LPO product malondialdehyde (MDA) and thiobarbituric acid was evaluated spectrophotometrically to determine LPO (Ledwozyw et al., 1986).

The levels of SA were evaluated using the method of Warren. For the assay, the skin samples were first incubated at 80 °C for 1 hour with 0.1 N H_2SO_4 and the hydrolysate was used for analysis. The absorbances obtained from the samples were determined at 549 nm (Warren, 1959).

To measure NO levels, nitrate was converted to nitrite with vanadium (III) chloride. The complex diazonium compound was produced based on the reaction of nitrite sulfanilamide with N-(1-Naphthyl) ethylenediamine dihydrochloride in an acidic medium. This colored complex formed was measured spectrophotometrically at 540 nm (Miranda et al., 2001).

TOC provides information about the level of oxidants and ROS in a biological sample. Total oxidants present in the sample oxidize the ferrous ion–chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with a chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv./L) (Erel, 2005).

ROS determination in skin tissues was performed using Zhang et al.'s method. The amount of ROS was calculated using 2′,7′-dichlorofluorescein diacetate compound dissolved in 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES) buffer (Zhang et al., 2018).

Ridderstap and Bonting methods were used for the determination of sodium-potassium ATPase (Na^+/K^+-ATPase) activity in skin tissue with the help of the determination of Mg^{2+}-ATPase in the presence of ouabain and ATP in acidic medium (Ridderstap & Bonting, 1969).

Hydroxyproline (HP) levels were measured via collagen status, a major skin protein (Reddy & Enwemeka, 1996). The samples were hydrolyzed in the acidic medium and then neutralized. The neutralized samples were mixed with chloramine-T reagent. The chromophore was then developed with the addition of Ehrlich's reagent, and the absorbance of the reddish-purple complex was measured at 550 nm.

Additionally, skin boron levels were assessed according to the carminic acid method of Hatcher and Wilcox (Hatcher & Wilcox, 1950) with a few modifications (Kuru et al., 2019) to monitor its essential role in skin health and aging. First, samples were burned in a high-temperature furnace. The boron determination was carried out with a spectrophotometric method based on the measurement of the absorbance at 585 nm of the colored complex formed as a result of the reaction of boron and carminic acid in the presence of sulfuric acid. Boric acid was used as boron standard.

Tissue factor (TF) activity as an indicator of tissue damage in the skin was evaluated according to the method of Ingram and Hills (1976). In Quick's one-stage method, the TF activity of homogenate was reacted by pooled plasma obtained from healthy subjects. Since the clotting time is inversely proportional to the activity of TF, the lengthening of the clotting time shows decreased TF activity.

Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (1970). The skin homogenates were mixed in SDS gel loading buffer (Tris–HCl, SDS, mercaptoethanol, bromophenol blue, glycerol). Then, the samples were heated for five minutes in a boiling water bath, and equivalent protein concentrations (20 μg total protein) per lane of the gel were loaded on 7.5% SDS polyacrylamide gels (with 4.5% separating gel). Protein size in kilo Daltons (kDa) was determined by comparing the relative mobility of each band with the banding pattern of a colored protein standard of known molecular size. The mixture of bovine albumin (66 kDa), egg albumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle, 36 kDa), and bovine carbonic anhydrase (29 kDa) as protein molecular weight markers were used as standard protein mixture. Electrophoresis was carried out at 30 mA/gel for 1 h at 20 °C using Bio-Rad Mini-PROTEAN Tetra Vertical Electrophoresis Cell. For quantitative measurements,

high-resolution test photographs were taken using a Canon EOS 700D camera with an 18–55 lens for evaluation of protein bands after electrophoresis, and the images were exported as JPEG files. Densitometric plots of protein bands were drawn using ImageJ software (Schneider et al., 2012).

Statistical analyses

Biochemical results were statistically evaluated via GraphPad Prism 9.0. The values were expressed as means ± standard error. Assuming a normal distribution, the results were evaluated using an unpaired t-test and analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The value of $p < 0.05$ was considered statistically significant.

Results

The effects of VA and moringa treatments on TP, GSH, GST, SOD, CAT, and TAC parameters

Table 1 shows the values of antioxidant parameters (GSH, GST, SOD, CAT, TAC) examined in skin homogenates. TP levels of skin homogenates were not significantly different between groups ($p > 0.05$). They were 11.08 ± 0.84, 12.62 ± 0.86, 11.02 ± 0.93, and 11.21 ± 0.54 mg per gram tissue in the C, M, VA, and VM groups respectively. When C and VA groups were compared, it was observed that GSH and TAC values decreased significantly ($p < 0.0001$ and $p < 0.01$, respectively), SOD and CAT did not change significantly ($p > 0.05$), while GST increased significantly ($p < 0.0001$) in the VA group compared to the C group. Administration of moringa extract significantly increased GSH, GST, and TAC values in the VM group compared to the VA group ($p < 0.05$, $p < 0.05$ and $p < 0.01$, respectively). In the M group, TAC values increased significantly compared to the C group ($p < 0.0001$).

The effects of VA and moringa treatments on LPO, SA, NO, TOC and ROS parameters

The oxidant parameters; LPO, SA, NO, TOC, and ROS values increased significantly in the VA group compared to the C group ($p < 0.05$, $p < 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.0001$, respectively). In the VM group, LPO, SA, NO, TOC, and ROS all decreased significantly compared to the VA group ($p < 0.05$, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$, respectively). In the M group, LPO, NO and TOC values also decreased significantly when compared to the C group ($p < 0.05$, $p < 0.01$, and $p < 0.05$, respectively). When comparing the VM and M groups in terms of these parameters, only LPO was significantly higher, and SA was significantly lower in the VM group than in the M group ($p < 0.05$ and $p < 0.0001$ respectively). There was no significant difference between the NO, TOC, and ROS values of these groups ($p > 0.05$) (Table 2).

The effects of VA and moringa treatments on Na^+/K^+-ATPase and TF activities, boron, and HP levels

The Na^+/K^+-ATPase and TF activities, HP, and boron levels of all groups are presented in Table 3. Administration of moringa extract significantly reduced Na^+/K^+-ATPase activity of the M group as compared to the C and VM groups ($p < 0.001$, $p < 0.0001$, respectively). Administration of moringa extract to the VA group significantly increased Na^+/K^+-ATPase activities in the VM group as compared to the C and VA groups ($p < 0.0001$, $p < 0.0001$, respectively).

The HP levels of the M group were reduced in a significant manner as compared to the C, VA, and VM groups ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$, respectively). The HP levels of the VM group were significantly diminished as compared to the C and VA groups ($p < 0.05$, $p < 0.0001$, respectively).

Boron values in skin homogenates decreased significantly in the VA group compared to the control groups (C and M) ($p < 0.05$, $p < 0.05$). In the VM group, it increased significantly compared to the VA group ($p < 0.05$).

In skin homogenates, TF values increased significantly in the VA and M groups compared to the C group ($p < 0.001$, $p < 0.01$, respectively), and decreased significantly in the VM group compared to the VA group ($p < 0.0001$). Prolonging the time means that TF activity decreases, and shortening the time means that TF activity increases (Table 3).

SDS-polyacrylamide gel electrophoresis results

The protein bands obtained by SDS-PAGE were at the same position for each sample, and molecular weights ranged from 200–29 kDa in all skin samples. There were no significant differences between the groups in terms of the intensity

Table 1. The antioxidant parameters of the control and experimental groups and their comparison.

		Experimental groups		
	C ($n = 8$)	M ($n = 8$)	VA ($n = 8$)	VM ($n = 8$)
GSH (μg/gT)	23.00 ± 0.43	25.19 ± 1.29	15.96 ± 0.94^{d,h}	19.98 ± 0.65^{f,k}
GST (U/gT)	2.50 ± 0.14	2.92 ± 0.24	3.75 ± 0.17^{d,f}	3.07 ± 0.14^{k}
SOD (U/gT)	7.00 ± 0.16	8.33 ± 0.39	7.56 ± 0.35	7.24 ± 0.53
CAT (kU/gT)	27.62 ± 0.58	27.40 ± 0.75	28.35 ± 0.43	26.88 ± 0.40
TAC (μmol/gT)	1.47 ± 0.03	2.00 ± 0.12^{d}	1.07 ± 0.036^{b,h}	1.51 ± 0.07^{l,g}

Values were given as mean ± standard error. T: tissue, GSH: glutathione; GST: glutathione-S-transferase; SOD: superoxide dismutase; CAT: catalase; TAC: total antioxidant capacity, C: control group, M: moringa extract given control group; VA: sodium valproate given group; VM: moringa extract given sodium valproate group.
$^{b}p < 0.01$, $^{d}p < 0.0001$ vs C; $^{f}p < 0.01$, $^{g}p < 0.001$, $^{h}p < 0.0001$ vs M; $^{k}p < 0.05$, $^{l}p < 0.01$vs VA.

Table 2. The oxidant parameters of the control and experimental groups and their comparison.

		Experimental groups		
	C ($n = 8$)	M ($n = 8$)	VA ($n = 8$)	VM ($n = 8$)
LPO (nmol MDA/gT)	27.03 ± 2.14	18.33 ± 2.38^{a}	36.07 ± 2.59^{a,h}	27.34 ± 1.30^{e,k}
SA (mg/gT)	2.68 ± 0.13	2.96 ± 0.13	3.12 ± 0.10^{a}	2.15 ± 0.06^{b,o,h}
NO (nmol/gT)	11.33 ± 0.30	7.94 ± 0.39^{d}	12.97 ± 0.22^{a,h}	8.02 ± 0.43^{d,o}
TOC (μmol/gT)	0.062 ± 0.001	0.049 ± 0.002^{a}	0.076 ± 0.004^{b,h}	0.055 ± 0.004^{o}
ROS (ROS/gT)	92.50 ± 7.01	135.60 ± 4.44	342.00 ± 14.51^{d,h}	143.20 ± 4.72^{b,o}

Values were given as mean ± standard error. T: tissue; LPO: lipid peroxidation; SA: sialic acid; NO: nitric oxide; TOC: total oxidant capacity; ROS: reactive oxygen species; C: control group, M: moringa extract given control group; VA: sodium valproate given group; VM: moringa extract given sodium valproate group.
$^{a}p < 0.05$, $^{b}p < 0.01$, $^{d}p < 0.0001$ vs C; $^{e}p < 0.05$, $^{g}p < 0.001$, $^{h}p < 0.0001$ vs M; $^{k}p < 0.05$, $^{o}p < 0.0001$ vs VA.

of the protein bands evaluated with the ImageJ program. When compared to the C group, a decrease in the band density of the protein with a molecular weight of 200 kDa, coupled with an increase in the band intensities of the proteins with 75 kDa and of the proteins with a molecular weight lower than 66 kDa was observed in the M group. In the VA and VM groups, a decrease was observed in the band density of the protein with a molecular weight of 200 kDa as compared to the C group. An increase in the band density of the proteins with a molecular weight lower than 66 kDa in the M group compared to the C group was noticed. There was no significant difference between the VA and VM groups (Figure 1A and B).

Table 3. The Na^+/K^+-ATPase and TF activities, boron and HP levels of the control and experimental groups, and their comparison.

		Experimental groups		
	C ($n=8$)	M ($n=8$)	VA ($n=8$)	VM ($n=8$)
Na^+/K^+-ATPase (mmolPi/gT × h)	7.55 ± 0.24	4.92 ± 0.31^{c}	3.36 ± 0.16^{d,f}	14.30 ± 0.49^{d,h,o}
HP (µg/gT)	30.33 ± 1.67	9.40 ± 1.50^{d}	65.41 ± 2.62^{d,h}	15.31 ± 1.02^{a,h,o}
Boron (ppm)	135.90 ± 1.56	134.60 ± 1.24	128.90 ± 1.38^{a,e}	134.30 ± 0.73^{o}
TF (s)	204.00 ± 3.41	178.00 ± 1.98^{b}	174.00 ± 8.35^{c}	212.00 ± 2.46^{g,o}

Values were given as mean ± standard error. T: tissue; Na^+/K^+-ATPase: sodium-potassium ATPase; Pi: inorganic phosphorous; h: hour; s: second; HP: hydroxyproline; TF: tissue factor; C: control group; M: moringa extract given control group; VA: sodium valproate given group; VM: moringa extract given sodium valproate group.
$^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, $^{d}p < 0.0001$ vs C; $^{e}p < 0.05$, $^{f}p < 0.01$, $^{g}p < 0.001$, $^{h}p < 0.0001$ vs M; $^{o}p < 0.0001$ vs VA.

Discussion

Various factors can influence skin health. Oxidative stress is the deterioration of the oxidative balance as a result of the increase in ROS such as hydroxyl, superoxide radicals, and hydrogen peroxide. ROS occurs as a result of metabolic events in the cell, and the insufficiency of antioxidant systems responsible for their detoxification (Altobelli et al., 2020). Although VA is widely used for treatments, it increases oxidative stress and produces free radicals that aggravate tissue and organ damage (Turkyilmaz et al., 2020). Antioxidants from natural sources may provide new possibilities for the treatment and/or prevention of oxidative stress-mediated diseases (Rahaman et al., 2023). Supplements, specifically dietary botanicals, possessing anti-inflammatory, immunomodulatory, and antioxidant properties are among the promising group of compounds that can be exploited as ideal chemo-preventive agents for a variety of skin disorders (Afaq et al., 2007; Katiyar, 2007). The presence of phytochemicals makes moringa a good medicinal agent (Adusei et al., 2022; Gopalakrishnan et al., 2016; Islam et al., 2021).

GSH is the most important immune system antioxidant produced metabolically (Averill-Bates, 2023). Protecting cells against toxic compounds, radiation, and oxidative damage, taking part in the inactivation of some drugs, aiding amino acid transport across the plasma membrane, regenerating some important antioxidants, and regulating vitamins C and E are among the functions of GSH (Kurutas, 2016). In the present study, GSH level in skin homogenates decreased

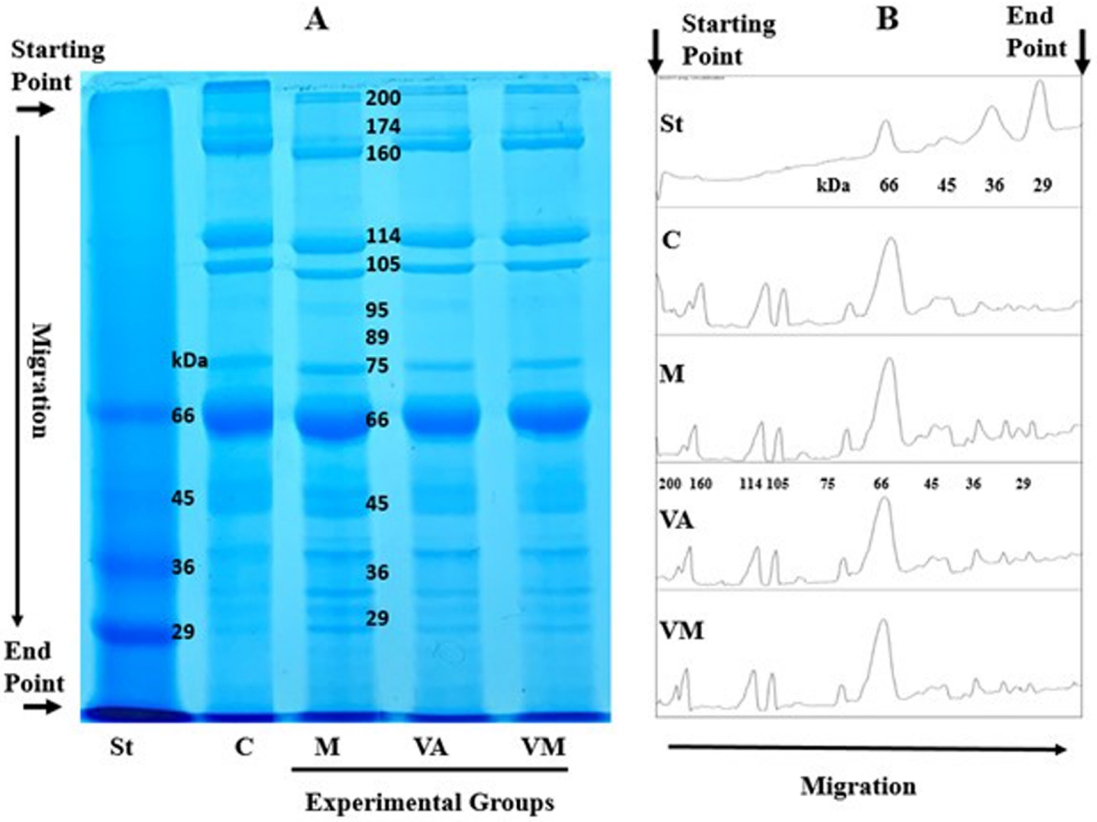

Figure 1. The SDS-PAGE bands of skin proteins (a), graphs of SDS-PAGE bands scanned with ImageJ program (b). St: standard protein mixture (bovine albumin: 66 kDa; egg albumin: 45 kDa; glyceraldehyde-3-phosphate dehydrogenase: rabbit muscle, 36 kDa, and bovine carbonic anhydrase: 29 kDa); MW: molecular weight; C: control group; M: moringa extract given group; VA: sodium valproate given group; VM: moringa extract given sodium valproate group.

significantly in the VA group compared to the control groups (C and M) and increased significantly in the VM group compared to the VA group owing to the effect of *M. oleifera*. These findings are consistent with the studies indicating that *M. oleifera* acts as a protective agent by increasing GSH levels in the liver (Abdel-Daeem et al., 2018) and muscle (Ertik et al., 2023). The reduction of GSH in the skin can be attributed to oxidative damage caused by VA; the increase in GSH is thought to be due to the antioxidants contained in the leaves of *M. oleifera*.

GST is an enzymatic antioxidant that catalyzes the conjugation of glutathione with toxic metabolites, minimizing oxygen damage and helping signal transduction (Richard, 2023). GST values in skin homogenates increased significantly in the VA group compared to the C groups and decreased significantly in the VM group compared to the VA group in the current study. These results were similar to that of the Ertik et al. study (2023) in which muscle GST values increased significantly in the valproic acid group compared to the control groups and decreased significantly in the valproic acid-moringa compared to the valproic acid group. It was suggested that this effect was probably due to moringa's antioxidant properties and hepatoprotective effect. Since GST is one of the GSH-dependent enzymes when the levels of GSH are altered, the activity of GST can be affected (Krishnamurthy and Wadhwani, 2012). Moringa extract may improve the oxidative metabolism of skin; its beneficial effects on the antioxidant enzyme systems boost antioxidant enzyme capacity.

Several enzyme families such as SOD and CAT contribute specifically to skin defense. SOD is a typical enzymatic eliminator of $O_2^{\bullet-}$. CAT and glutathione peroxidase decompose and eliminate H_2O_2. CAT is one of the crucial antioxidant enzymes that strongly mitigates oxidative stress by decomposing cellular H_2O_2 into water. A deficiency or the malfunction of CAT has been implicated in the pathogenesis of many age-associated degenerative diseases (Altobelli et al., 2020). No significant difference was found between the groups in terms of SOD and CAT activities in skin homogenates. Accordingly, the effect of VA or *M. oleifera* may not be related to the $O_2^{\bullet-}$ and H_2O_2 elimination mechanisms.

TAC and TOC levels in biological samples are useful biomarkers of antioxidant status and the extent of oxidative damage. Due to oxidative stress, the level of total antioxidants in tissues decreases, and that of total oxidants increases. The findings of the present study indicated increased TAC levels in the VM and M groups as compared to the VA and C groups respectively. On the other hand, TOC levels increased significantly in the VA group when compared to the control groups (C and M). It decreased significantly in the VM group when compared to the VA group, and in the M group compared to the C group. The positive effect of *M. oleifera* on antioxidant parameters is mostly due to the antioxidant properties of the plant leaves. Santos et al. (2012) have earlier reported that ethanol and saline extracts of *M. oleifera* leaves contain flavonoids that have excellent antioxidant properties.

Oxidative stress-related skin metabolism and the role of ROS have been implicated in various skin diseases, but the underlying mechanisms have not yet been elucidated. The proper usage of antioxidant drugs is still unknown. There is a lack of adequate knowledge/studies concerning the physiological roles of free radicals and ROS in skin homeostasis. Previous studies reported both increases or decreases in the metabolites of ROS, ROS generators, and antioxidants in skin diseases, with abnormal ROS generation being suggested to play a role in their pathogenesis (Nakai and Tsuruta, 2021). In the present study, ROS levels increased significantly in the VA group compared to the C group. In the VM group, ROS levels decreased significantly compared to the VA group due to the effect of *M. oleifera* extract. It is difficult to measure ROS directly in the skin and blood of patients with skin diseases. As an alternative, MDA (a major product of lipid oxidation) has been used (Gutteridge, 1995). Furthermore, nitrite and nitrate have been measured as direct metabolites of •NO (Green et al., 1982). In the present study, MDA and NO levels were evaluated in skin homogenates. LPO is a process in which free radical species confiscate electrons from lipids, thereby producing more reactive intermediates. In the case of oxidative stress induced by VA, LPO levels rise and cell membrane deterioration occurs (Abdelkader et al., 2020; Ourique et al., 2016). In the present study, skin LPO levels increased in the VA group compared to the C group. This outcome is in line with the findings of previous reports (Abdel-Daeem et al., 2018; Ertik et al., 2023). The use of *M. oleifer*a extract significantly reduced the LPO level in the skin tissue. The plant might have reversed and attenuated these defects via its antioxidant activity.

NO is synthesized by nitric oxide synthase (NOS) (Nakai and Tsuruta, 2021). It has important and varied functions in numerous cellular processes. Inadequate production of NO can have adverse effects on regular skin functioning, while an excess of NO can result in tissue damage and contribute to the onset of various diseases (Belmont et al., 1997; Kim and Choi, 2023; Pelegrino et al., 2020; Sirsjo et al., 1996; Stallmeyer et al., 1999). NO is a possible free radical that plays a role in the inflammatory process (Korde Choudhari et al., 2013). In the present study, the administration of VA led to an increase in NO levels, which was consistent with previous findings reported in the literature (Çelik et al., 2022). Moreover, the present study showed that *M. oleifera* extract appeared to counteract the elevated NO levels. This observation points to the extract's versatile biochemical effects, suggesting a potential role in alleviating inflammation. This outcome is supported by the study of Cuellar-Nunez et al. (2021), which indicated that ethanol extract from *M. oleifera* leaves alleviates inflammation and associated inflammatory responses.

SA is a structural component of proteins and lipids. It can act as a scavenger of ROS. Therefore, it has antioxidant properties (Iijima et al., 2004; Yadav et al., 2020). An increase in the levels of SA due to the disintegration of protein and lipid structure can arise in cases of infection, or increased biosynthesis may occur during oxidative stress. In the current study, SA levels in skin homogenates increased due to oxidative stress induced by VA. Its level decreased significantly when *M. oleifera* extract was administered to the VA group. The antioxidant activity of *M. oleifera* components must have reduced and or mopped up ROS from the tissue. The present findings are similar to the findings of previous studies (Abdel-Daeem et al., 2018; Ertik et al., 2023).

Na^+/K^+-ATPase, a pump required for cell survival (Hosseinzadeh et al., 2020), is also an enzyme located in phospholipids-rich portions of the plasma membrane. It maintains an intracellular electrochemical gradient by transporting Na^+ and K^+ across the cell membrane. It is highly sensitive to structural changes and, hence, a good marker of oxidant-induced tissue damage (Schwinger, 2021). Alozie et al. demonstrated that the methanolic extract of M. *oleifera* increased the blood Na^+/K^+-ATPase activity of diabetic rats (Alozie, 2021). Activation of Na^+/K^+-ATPase on the vascular smooth muscle cell mediates hyperpolarization, thereby modulating the regulation of vasodilation (Fujii et al., 2021). In the present study, the skin Na^+/K^+-ATPase activity of the VA group was significantly decreased as compared to the C group. Administration of moringa significantly increased Na^+/K^+-ATPase activity of M and VM groups compared to C and VA groups respectively. This effect is possibly due to the antioxidant activity of the glucosinolates, phenolic acids, and flavonoid compounds in *M. oleifera* leaves (Alozie, 2021; Bennett et al., 2003; Fujii et al., 2021). Moreover, Na^+/K^+-ATPase is a possible receptor for cardiac glycosides and may have a potential role in the cardiovascular system (Obradovic et al., 2023). The *M. oleifera* should be examined further for its potential in cardiovascular health.

TF is a cell membrane component constitutively expressed in a variety of body cells. It plays an important role in the activation of coagulation and inflammation (Siddhuraju and Becker, 2003; Vazhappilly et al., 2019). The shortened clotting time is indicative of increased TF activity. In the present study, TF activity in skin homogenates increased significantly in the VA group compared to the C group. However, it decreased significantly in the VM group compared to the VA group. The increased TF activity in tissue samples contributes to cellular damage (Levi et al., 2006). Oxidative stress has been shown to convert inactive TF to active TF (Oztay et al., 2015).

Boron is known to have important roles in mineral and hormone metabolism, bone development, antioxidant defense, and immune system, wound healing, and energy metabolism (Pizzorno, 2015). It is also beneficial for maintaining healthy skin by improving collagen production. It also lessens the risk of skin conditions such as eczema and psoriasis by reducing inflammation in the skin. Furthermore, boron has been found to have antimicrobial properties; it prevents skin infections and promotes overall skin health (Nielsen, 2000). The present study is one of the rare studies in which boron determination was performed on the skin. Boron levels in skin homogenates decreased significantly in the VA group compared to the control groups (C and M) but increased significantly in the VM group compared to the VA group. In a study on salivary glands, boron concentration decreased in the group given VA; It was observed that the concentration of boron increased significantly following the administration of moringa extract (unpublished data). This may be due to the high amount of boron content of *M. oleifera*.

Many proteins with different molecular weights have been identified in studies examining skin proteins (Tunali et al., 1998). The structural proteins of the skin are mainly collagen, keratin, elastin, and reticulin (Hoath et al., 1989). These proteins (especially collagen) are key components in connective tissues, providing support and structure. Excessive collagen production can lead to fibrosis. Therefore, controlling collagen production is very important. Various approaches, including drugs, gene therapy, and lifestyle changes affect skin components and texture. VA is known to reduce collagen in a variety of tissues via an unclear mechanism (Seet et al., 2016). Analysis indicates that there were no significant differences between the groups in terms of the intensity of the protein bands obtained by electrophoresis in the current study. A decrease in the band density of the protein with a molecular weight of 200 kDa was observed in the M group compared to the C group. An increase in the band densities of the protein with 75 kDa and of proteins with a molecular weight lower than 66 kDa was observed. In the VA and VM groups, a decrease was observed in the band density of the protein with a molecular weight of 200 kDa when compared to the C group, while a decrease in the band density of the proteins with a molecular weight lower than 66 kDa was observed in the M group. These components might be collagen β-chains (~215 kDa) (Inanc et al., 2017), keratin (~67 kDa), trichohyalin (~200 kDa) (Senshu et al., 1995), heat shock protein 70 (~72 kDa) (Souil et al., 2001). The protective effect of moringa extract against VA-induced effects may have accounted for the normal skin protein architecture that gave rise to no significance. The VA dose and administration time used in the present study may also have not induced pronounced/visible effects on protein components of the skin.

Conclusion

The 70% ethanolic extract of the moringa leaves partly protected against VA-induced oxidative stress and restored some antioxidant levels in the skin of VA-given rats. Therefore, *M. oleifera* may be useful in preventing and/or reducing the side effects of VA on the skin. To validate these encouraging preliminary findings and ascertain the clinical applicability of moringa in mitigating dermatological side effects in humans, further comprehensive investigations, including clinical trials, are required. These findings offer a promising foundation for potential strategies to bolster skin health in individuals undergoing antiepileptic drug treatment.

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Author contributions

GE: Methodology, Validation, Investigation, Data Curation, Writing-review and editing, Visualization, Project administration. SO: Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing-review and editing, Visualization. IBT: Methodology, Validation, Investigation, Data Curation, Writing-original draft preparation, Writing- review and

editing. BAT: Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing-review and editing, Visualization. UFM: Methodology, Validation, Investigation, Data Curation, Writing-review and editing. OS: Validation, Formal analysis, Resources, Data Curation, Writing- review and editing, Supervision. RY: Conceptualization, Validation, Formal analysis, Resources, Data Curation, Writing-review and editing, Supervision. AY: Conceptualization, Validation, Formal analysis, Resources, Data Curation, Writing-original draft preparation, Writing-review and editing, Supervision, Project administration, Funding acquisition.

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Data availability statement

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

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