BMC Neuroscience

RESEARCH

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Molecular assessment of NMDAR subunits and neuronal apoptosis in the trigeminal ganglion in a model of male migraine-induced rats following *Moringa oleifera* alcoholic extract administration

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Abstract

Introduction Migraine, a common disorder marked by severe and repetitive headaches, has been linked to the involvement of the NMDA receptor (NMDAR), a receptor responsible for glutamate signaling. *Moringa oleifera* (*M. oleifera*), recognized for its anti-inflammatory properties and therapeutic potential in various conditions, has been investigated. This study aims to assess the efficacy and precise mechanisms of *M. oleifera* for the treatment of migraine, for which evidence is limited.

Methods Rats were stratified into four distinct groups. The control group did not undergo the migraine-induction protocol. Post-induction, the "sumatriptan" group was administered sumatriptan injections, the "treatment" group received oral *M. oleifera* extract, and the "vehicle" group was provided with oral solvent treatment. Behavioral evaluations encompassing Von Frey's and hot plate assessments, in addition to qPCR analysis targeting Nr2a, Nr2b, Bax, Bcl-2, and Caspase-3, were conducted.

Results Von Fery's and hot plate tests revealed a notable decrease in triggering pressure and temperature within the vehicle group when compared to the other groups (both ps < 0.001). The Nr2a expression levels in both the vehicle and treatment cohorts exhibited significantly higher values than those observed in the control group ($p < 0.001$, $p = 0.001$) and the sumatriptan group ($p < 0.001$, $p = 0.002$). Conversely, no substantial alterations in Nr2b or Bcl-2 expression levels were observed across the study groups ($p = 0.404$, $p = 0.976$). Notably, heightened expressions of Caspase-3 and Bax were evident in the vehicle group relative to the other groups ($p = 0.013$, $p = 0.010$).

Conclusions Moringa oleifera extract appears to mitigate symptoms of migraine by inhibiting apoptosis, suggesting potential efficacy in migraine treatment; however, additional research investigating a wider range of pathways is necessary.

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Clinical trial number Not applicable.

Keywords *Moringa Oleifera*, Nr2a, Nr2b, Caspase-3, Bcl-2, Bax, Hot plate test, Von Frey's test

Background

Migraine represents a prevalent neurological disorder marked by severe and recurring headaches. An estimated one-third of individuals with migraine encounter aura, defined as transient neurological symptoms that precede the onset of the headache [1]. Cortical spreading depression (CSD) is presumed to be the fundamental mechanism driving the manifestation of a migraine aura [2]. CSD is a wave of neuronal and glial depolarization that gradually propagates throughout the cortex, disrupting brain activity as it traverses. Studies indicate that CSD triggers the activation of the trigeminovascular system, thereby precipitating migraine headache pain. The visual disturbances such as scotomas and zigzag patterns frequently described by individuals with migraines are probable outcomes of the cortical spreading depression wave traversing the visual cortex [3]. Delving into the mechanisms of CSD and migraine aura could offer valuable insights for the development of novel migraine prevention strategies.

Glutamate emerges as the predominant excitatory neurotransmitter within the nervous system. Within the trigeminal ganglion (TG), every neuronal subtype is equipped to both store and liberate glutamate following activation [4]. The N-methyl-D-aspartate receptor (NMDAR) receptor plays a crucial role in processes such as learning, memory, pain perception, and the development of central sensitization. Additionally, it is associated with cognitive and emotional disruptions in stress-related conditions [5, 6]. The NMDAR could potentially play a role in cortical spreading depression by triggering neuronal hyperexcitability alongside other underlying mechanisms [6, 7]. The initiation and propagation of CSD require NMDAR activation, whereas AMPA activation, a different gated glutamate receptor, is not essential for this process [8]. The decrease in NMDAR levels has been demonstrated to offer protection against migraine [6, 8]. It has been noted that multiple NMDAR antagonists, including MK-80, exhibit the ability to block CSD. However, their non-discriminatory actions and diverse side effect profiles render them unsuitable for clinical use [7, 9–11]. In the chick retina devoid of blood vessels, the inhibition of CSD by an NR2A antagonist suggests that the suppressive outcomes are mediated via neuronal and glial processes [10].

Moringa oleifera, a fast-growing, drought-resistant tree native to the Indian subcontinent, exhibits neuroprotective effects. Its extracts demonstrate antioxidant properties in an Alzheimer's disease rat model and regulate inflammation, oxidative stress, and apoptosis in a Parkinson's disease mouse model [12–15]. *M. oleifera* promotes neuronal outgrowth, differentiation, and synaptogenesis in a concentration-dependent manner, improves spatial memory, and mitigates neurodegeneration in hippocampal regions [12, 15–17]. The neuroprotective mechanisms of moringa involve the NF-kB/Nrf2/HO-1 signaling pathway [18]. *M. oleifera* seed extract has been shown to prevent glutamate-induced DNA damage in primary retinal ganglion cells in rats [19]. Furthermore, its extracts and components show protection against oxidative stress by reducing reactive oxygen species (ROS) formation and modulating antioxidant protein expression [20, 21]. The plant's antioxidant effects were shown to prevent di(2-ethylhexyl) phthalate (DEHP)-induced cell apoptosis in human neuroblastoma cells [21]. We conducted this study to assess the efficacy and precise mechanisms of *M. oleifera* leaf preparations for the treatment of migraines, specifically exploring whether these mechanisms affect apoptotic pathways and/or glutamate receptor expression levels, as evidence on these aspects is lacking in the scientific literature.

Methods

Animals

Within this investigation, 48 adult male Wistar rats weighing between 250 and 300 g were employed. The animals were obtained from the Institut Pasteur in Iran. The experimental setting entailed subjecting the animals to a light-dark cycle of 12 h each, regulated by an electric timer. Environmental factors such as temperature (ranging from 18 to 24 degrees Celsius) and humidity (maintained between 45% and 50%) were carefully controlled. The rats were housed in specialized plastic enclosures with ad libitum access to water and food, with drinking water sourced from the tap. Each animal underwent testing once daily. Euthanasia procedures, detailed in the trigeminal ganglion sampling segment, were followed by appropriate disposal of the corpses using lime. The entire experimental protocol adhered to the guidelines set forth by the National Institutes of Health (NIH) [22] and was approved by the Ethics Committee of AJA University of Medical Sciences (Ethics code: IR.AJAUMS.REC.1401.040).

Solutions

The pharmaceutical compounds and solutions employed in this research were all ACS-certified and stored as per the manufacturer's recommendations. Nitroglycerin (Sigma-Aldrich, 1466506) and Sumatriptan (Sigma-Aldrich, PHR2579) underwent dilution in 0.9% saline,

whereas *M. oleifera* extract was diluted in 5% Tween 80 (Sigma-Aldrich, P8192).

Study groups

We divided the animals into four groups, each comprising 12 rats. The "control" group included rats that did not undergo the migraine-induction procedure. In the "sumatriptan" group, rats received an intraperitoneal injection of sumatriptan at a dosage of 1 mg/kg from day 10 to day 24 after nine days of migraine induction [23]. In the "treatment" group, rats were orally administered *M. oleifera* extract dissolved in Tween 80 at a dosage of 10 ml/kg from day 10 to day 24 post-induction. Rats in the "vehicle" group received an oral treatment of Tween 80 at the same dosage as the treatment group following the induction phase.

Preparation of *M. oleifera* extract

The plants used in this study were obtained from a cultivation farm, and Dr. Yasaman Hosseini verified the identification of the plant material. Three hundred fifty grams of powdered aerial parts of the plant were placed in a shaker with one liter of 96% ethanol for three consecutive days in an Erlenmeyer flask. The extraction process involved ultrasound assistance (35 min, 35 degrees Celsius, 37 kHz). The solution was then filtered using Whatman No. 1 filter paper in a centrifuge at 3500 rpm (1008 g) for 10 min. The resulting extract was obtained using a vacuum evaporator. The extract was stored in dark glass containers in a refrigerator until further testing. 5% Tween 80 was utilized as a solvent for animal oral treatment [24].

Pseudo-migraine modeling

Nitroglycerin was administered to rats at a dosage of 10 mg/kg for nine consecutive days, with injections given every other day, resulting in a total of 5 injections on days 1, 3, 5, 7, and 9 to induce migraine-like symptoms [25]. Nitroglycerin was diluted with 0.9% normal saline solution to create a uniform white emulsion for migraine modeling at a dose of 10 mg/kg [26]. The identifiable manifestations of a seizure, such as an ear reddening, head scratching, cage climbing, and body tremors, were documented. Subsequently, these indicators were analyzed to determine the effective induction of the migraine model [27]. This model induces both acute and chronic migraines. Acute migraines manifest around two hours subsequent to each injection, whereas chronic migraines endure for weeks following the final injection [28].

Table 1 Primers used in this study

Gene		Primer
Bax	F	5′-GATGGCCTCCTTTCCTACTTC-3′
	R	5′-CTTCTTCCAGATGGTGAGTGAG-3′
Bcl-2	F	5′-GGAGGATTGTGGCCTTCTTT-3′
	R	5′-GTCATCCACAGAGCGATGTT-3′
Caspase-3	F	5′-CCACGGAATTTGAGTCCTTCT-3′
	R	5′-CCACTCCCAGTCATTCCTTTAG-3′
Nr2a	F	5′-GGAGGAGGTTGGGTCATTTAT-3′
	R	5′-AGTAGGCACTTGGGACTTTAC-3′
Nr2b	F	5′-CACCCGTATCTGCGATCTTATG-3′
	R	5′-GGTGAGAGTCTGAGCAGAAATG-3′
Gapdh	F	5′-ACTCCCATTCTTCCACCTTTG-3′
	R	5′-CCCTGTTGCTGTAGCCATATT-3′

Trigeminal ganglion sampling

Animals were fully anesthetized using an intraperitoneal injection of ketamine HCl (50 mg/kg) and chlorpromazine HCl (10 mg/kg). The animals were then positioned on a dissecting board with their motor organs secured in a supine posture. Surgical incisions were made in the mid-line sternum and the lateral sides of the chest to expose the live heart. The pericardial sac was carefully opened, and a needle was inserted into the left ventricle, followed by a larger incision in the right atrium for blood drainage. Cold saline was then infused into the bloodstream until the fluid ran clear. Following the animal's expiration [29], the right trigeminal ganglion was excised and preserved for subsequent qPCR analysis.

Quantitative PCR

After the animal was deceased, the right trigeminal ganglion was isolated on a cold surface. Tissue samples were rapidly frozen in liquid nitrogen and stored at -80 °C for several days before PCR tests were conducted. Following centrifugation at a temperature of 4 degrees Celsius for 10 min at 3000 g, supernatants were obtained from these samples, and the expression levels of Caspase-3, Bax, Bcl-2, as well as Nr2a and Nr2b subunits of the NMDA receptor, were assessed using the quantitative PCR technique [29–33]. Four animals from each group were randomly selected to undergo qPCR testing.

The total RNA was extracted from cells utilizing Trizol reagent, followed by reverse transcription of one microgram of the total RNA into cDNA using commercially available kits. The resulting cDNA was employed for quantitative real-time PCR on a Rotorgene 3000 thermocycler, with each sample being subjected to triplicate reactions. mRNA expression levels were quantified utilizing the $2^{-\Delta\Delta Ct}$ method, which calculates the fold-change in gene expression normalized to a housekeeping gene (gapdh), with $\Delta CT = CT$ (a target gene) – CT (a reference gene) and $\Delta\Delta CT = \Delta CT$(a target sample)–$\Delta CT$(a reference sample) = (CTD – CTB)–(CTC – CTA) [34]. The primers were designed using the PrimerQuest tool (Integrated DNA Technologies) and are provided in Table 1.

Behavioral examinations

The study assessed the adverse effects of migraine on pain perception, hypersensitization, and allodynia by conducting von Fery's and hot-plate tests on days 25 and 26.

Von Fery The von Frey filament test was employed to assess mechanical withdrawal thresholds in the hind paw. Von Frey filaments from Bioseb, USA, with escalating force levels, were administered to the hind paw five times. The mechanical threshold was characterized as the minimal filament force in grams that triggered a withdrawal response in a minimum of four out of the five instances. This measurement was documented as the mechanical threshold in grams [35].

Hot plate Animals were placed on a hot plate (Sorel Hot Plate model DS37, Ugo Basile, Italy) set to 52 ± 0.2 °C to assess heat sensitivity. The time taken from initial contact with the hot surface to the first observed sign of heat sensitivity, such as jumping or paw licking, was recorded. A 20-second cutoff was implemented to prevent tissue damage. The recorded latency time was the duration between contact and the first heat-induced response or the 20-second threshold if no reaction occurred [36].

Statistical analysis

The data was assessed for normal distribution using the Shapiro-Wilk test, and the homogeneity of variances was assessed using Levene's test. The evaluation of gene expression fold changes across control, sumatriptan, vehicle, and treatment groups was conducted using one-way ANOVA under the assumption of normality. Post hoc Tukey's HSD tests were employed following the omnibus test to identify significant group discrepancies. In cases where normality assumptions were violated, the Kruskal–Wallis test was applied, and post-hoc pairwise Wilcoxon rank-sum tests with Bonferroni's correction were implemented. Similar procedures were implemented to examine response pressure in the Von Frey test and reaction latencies in the hot plate test across the groups. Statistical significance was defined as $p < 0.05$. The statistical analyses were executed within the R open-source environment (R Foundation for Statistical Computing, Vienna, Austria).

Results

M. oleifera extract reduces thermal and mechanical allodynia associated with migraine

The results obtained from the hot plate test exhibited a normal distribution with consistent variances across groups. In contrast, the data derived from the von Frey test demonstrated a departure from the expected normal distribution pattern. The omnibus tests revealed statistically significant differences among the study groups for both the hot plate ($P < 0.001$) and von Frey tests ($P < 0.001$). Significant reductions in the pressure threshold for eliciting a response were observed when von Frey filaments were applied to the hind paws in the vehicle group, in contrast to the control, sumatriptan, and treatment groups ($P = 0.002$, $P = 0.002$, $P = 0.006$, respectively). Moreover, the latency to respond to heat-induced nociceptor stimulation was significantly shorter in the vehicle group compared to the control, sumatriptan, and treatment group ($P < 0.001$, $P < 0.01$, $P < 0.01$, respectively) (Fig. 1).

M. oleifera did not reduce the increased Nr2a expression in trigeminal ganglions associated with migraine

In relation to NMDAR subunits, the omnibus tests revealed a significant distinction in the Nr2a ($P < 0.001$) gene across the various study groups. Subsequent post hoc analyses indicated significantly elevated Nr2a expression levels in the vehicle group compared to both the control ($P < 0.001$) and sumatriptan ($P < 0.001$) groups. Additionally, rats in the treatment group exhibited higher Nr2a expressions than the control ($P = 0.001$) and sumatriptan ($P = 0.002$) groups but comparable to the vehicle group ($P = 0.714$) (Fig. 2a). No significant alterations in Nr2b expression levels were observed among the study groups ($P = 0.404$) (Fig. 2b).

M. oleifera attenuated increased apoptotic gene expression in trigeminal ganglions associated with migraine

The data for the apoptotic gene expression levels conformed to a normal distribution and exhibited homogeneous variances across the study groups. Omnibus tests revealed significant differences in the expression levels of the apoptotic genes Caspase-3 and Bax among the study groups ($P = 0.013$ and $P = 0.010$, respectively). However, no significant variation was observed for the Bcl-2 gene expression levels ($P = 0.976$). Caspase-3 demonstrated significantly higher expression in the vehicle group in comparison to the control, sumatriptan, and treatment groups ($P = 0.019$, $P = 0.049$, $P = 0.024$, respectively) (Fig. 2c). Conversely, Bcl-2 expression did not show significant differences among the study groups (Fig. 2d). Additionally, Bax gene expression was significantly elevated in the vehicle group compared to the control, sumatriptan, and treatment groups ($P = 0.022$, $P = 0.020$, $P = 0.026$, respectively) (Fig. 2e). These results suggest an increase in the BAX/BCL-2 ratio specifically observed in the vehicle group, a pattern that was not observed in the other study groups.

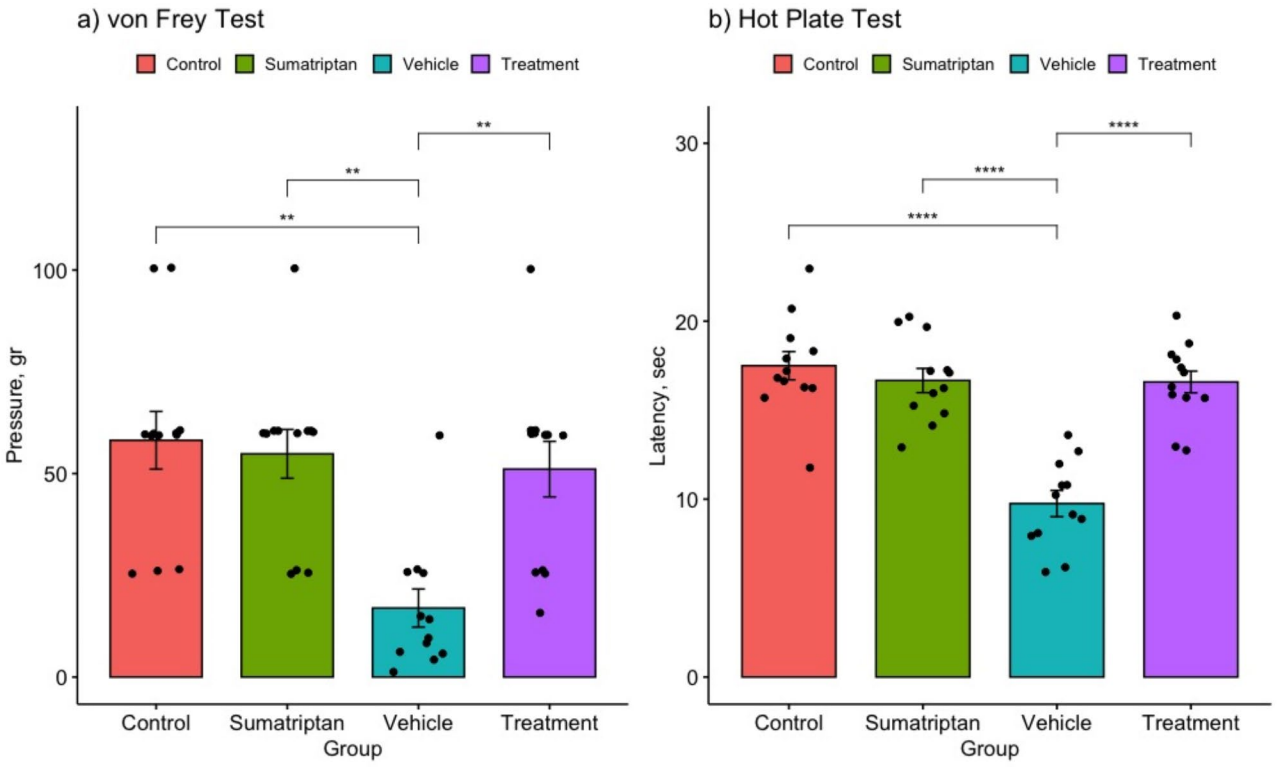

Fig. 1 Comparative analysis of migraine behavioral tests. The minimum force required to elicit a reaction in rats during the von Frey test and the time taken to elicit a response in the hot plate test. Results are displayed as mean values accompanied by error bars denoting the standard deviation across groups. $n = 12$, for all groups; one-way ANOVA with Tukey's correction for hot plate test data and Kruskal-Wallis test with post hoc Wilcoxon tests and Bonferroni's correction for von Frey's test data analysis; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

Discussions

The study observed the presence of allodynia and hypersensitization following GTN-induced migraine, which were absent in the treatment group. This suggests that *M. oleifera* extract may have a potential alleviating effect on migraine symptoms. Additionally, elevated expression levels of NR2A subunit of NMDAR, Bax, and Casp3 apoptotic genes were noted in the vehicle group but not in the treatment group. These results suggest a compelling role for *M. oleifera* extract in anti-migraine mechanisms, potentially involving the inhibition of apoptosis and modulation of NMDAR subunits, particularly NR2A.

Previous research has highlighted the critical role of glutamate receptors in pain processing [37]. The expression of several glutamate receptor subunits in various locations known to play a significant role in pain perception has been documented [37]. NMDAR functions as a gated glutamate receptor and ion channel, primarily facilitating the passage of Ca2 + ions. The receptor comprises an essential subunit, NR1, along with regulatory subunits, NR2 and NR3, which are capable of interchanging roles [38, 39]. Calcitonin gene-related peptide (CGRP) stands as a crucial neuropeptide associated with migraine pathogenesis. It is expressed extensively in both the peripheral and central nervous systems, including in neurons of the trigeminal ganglion [40]. Studies have indicated a correlation between plasma levels and headache intensity, demonstrating a reduction post-sumatriptan administration subsequent to a nitroglycerin-induced migraine episode [41]. Kynurenic acid analog 1, a substance with NMDAR antagonistic effects, alleviates hyperalgesia and decreases mRNA levels of CGRP, nNOS, and cytokines in the trigeminal ganglia [42]. The NR2A antagonists decreased both the frequency and amplitude of CSD following application to the contralateral internal cerebral vein [43]. The increase in CGRP levels within the rat amygdala subsequent to repetitive CSD was mitigated by inhibiting NR2A using NVP-AAM077, delivered via the contralateral internal cerebral vein [43]. Nitric oxide (NO), given its varied vascular and neurophysiological functions, holds a prominent position in the pathophysiology of migraine [44]. Tyrosine phosphorylation of NR2B and the modulatory subunit of NMDAR induces calcium influx, which, upon reaching a critical concentration, activates calmodulin to stimulate nitric oxide synthase (NOS), subsequently generating NO [6].

It has been demonstrated that there was an increase in NR2B expression levels in the trigeminal ganglion subsequent to GTN-induced migraine. Additionally,

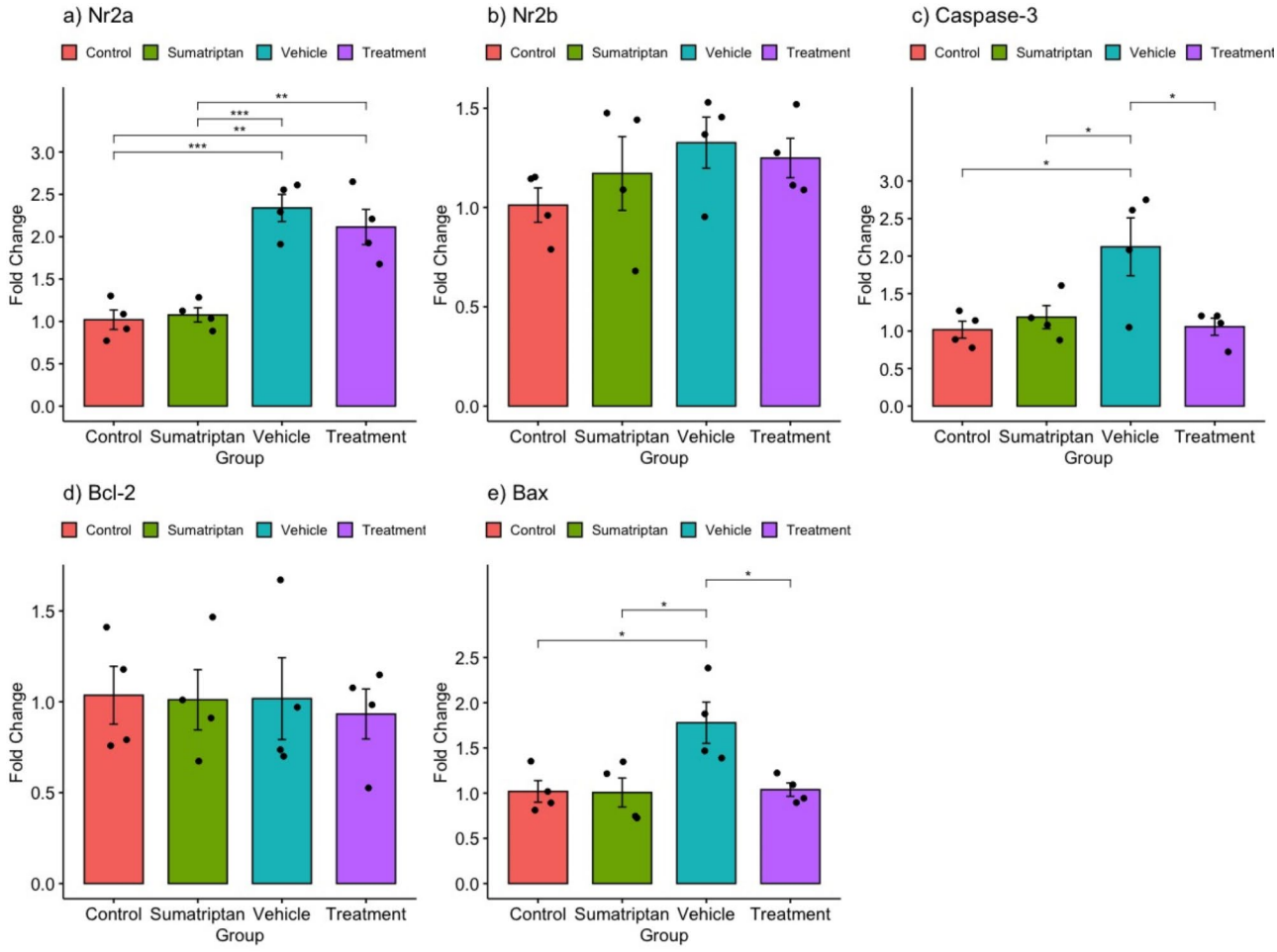

Fig. 2 Comparative analysis of implicated gene expression levels. The variations in expression levels are indicated as fold changes of the genes Nr2a, Nr2b, Capase-3, Bcl-2, and Bax. Results are displayed as mean values accompanied by error bars denoting the standard deviation across groups. $n=4$ for all groups; one-way ANOVA with Tukey's correction for multiple comparisons for all data analyses; * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$

levels of NOS, NO content, and tactile threshold were diminished by Adenovirus-expressing siPTEN [6]. Conversely, the 2-week ethological stress paradigm results in elevated hippocampal NR2A expression levels and an increased NR2A/NR2B ratio without impacting NR2B expression levels [5]. It has been demonstrated that the inflammatory soup in rats induces dural nociception, potentially contributing to impairments in spatial cognition, which may be linked to a reduced NR2A/2B ratio [45]. Masseter inflammation did not induce any variations in the expression levels of NR2A or NR2B within the trigeminal ganglia of rats [37]. An increase in NR2B expression levels was observed in the vehicle group, albeit not reaching statistical significance, whereas a significant elevation in NR2A expression levels was noted in the same group, persisting without reduction in the treatment group. The incongruent findings could possibly be attributed to the diverse stressors and inducers applied to the animals, as well as the specific tissue analyzed.

Apoptosis and cell death may be intricately involved in the pathogenesis of migraines. Notably, mitochondrial events and proteins exhibit significant influence on the apoptotic mechanisms within the trigeminal ganglia, irrespective of the presence of conventional apoptotic morphological indicators [46]. The influx of Ca2 + has been demonstrated to trigger a series of oxidative stress reactions, resulting in neuronal apoptosis within the trigeminal ganglion. This process can be mitigated by NMDA antagonists [47]. The heightened production of mitochondrial reactive oxygen species (mROS) and cytosolic reactive oxygen species (cROS) in migraine conditions induces the upregulation of TRPM2 proteins, consequently instigating apoptosis [48]. TRPM2 has been shown to facilitate an excessive influx of Ca2+, which increases the production of mROS and cROS, ultimately leading to neuronal death within the trigeminal ganglion during GTN-induced migraines in mice [49]. Research findings highlight that repetitive induction of migraines

via dural IS stimulation elevates P2 × 7R expression, initiates NLRP3 inflammasome activation, induces proinflammatory cytokine release (IL-1β and IL-18), and triggers the pyroptotic cell death pathway. Furthermore, neuronal degeneration and cognitive deficits were also observed in the study [50]. BAX and BCL2, key members of the Bcl-2 gene family, are localized in mitochondria and are pivotal in the regulation of apoptosis. BCL-2 inhibits apoptosis by entrapping activated BAX, preventing its oligomerization [51]. Also, the BCL-2 BH4 domain has been found to inhibit BAX activation through a noncanonical interaction mechanism [51]. Additionally, CASP3, a protein implicated in both apoptosis and proptosis, demonstrates heightened expression within the trigeminal ganglion of GTN-induced mice [49]. In the Fas apoptotic pathway, caspase-3 is activated downstream of caspase-8 and caspase-10, leading to the cleavage of cytoskeletal and nuclear proteins [52]. We detected heightened expression levels for BAX and CASP3 proteins in the vehicle group, providing evidence for the involvement of cell death in migraine. These elevations were completely mitigated in the intervention group, indicating that *M. oleifera* acts, at least partially, by stabilizing cells and preventing apoptosis and proptosis. Although there are reports of elevated apoptosis levels induced by *M. oleifera* extracts, their pro-apoptotic effects have been shown to target tumor cells while sparing cells from healthy donors [53].

Allodynia characterizes the sensation of pain triggered by typically non-painful stimuli. This process often entails central hypersensitization and involvement of the trigeminovascular system. Prolonged exposure to painful stimuli can amplify the excitability and synaptic efficacy within the nociceptive pathways of the central nervous system [54]. The throbbing pain observed in migraine patients arises from the hypersensitization of meningeal nerves influenced by the trigeminovascular system. This heightened sensitivity renders the nerves responsive even to arterial pulses, which under normal circumstances are considered non-nociceptive stimuli [54]. The prevalence of allodynia in patients with migraine ranges from 0.25 to 0.8, with a higher incidence among female patients [55, 56]. Moreover, studies have demonstrated a correlation between allodynia and aura, disease duration, depression, and elevated BMI [57–59]. Moreover, allodynia was demonstrated as a predictive factor for the elevation in the number of migraine days [60]. Allodynia is commonly correlated with migraine, whereas patients experiencing cluster headaches usually do not display allodynia unless they have a personal or familial background of migraine [56]. Previous studies indicate that the presence of allodynia independently contributed to the exacerbation of migraines during the COVID-19 pandemic, specifically when utilizing face masks and disinfectants [61]. There are three distinct types of allodynia: thermal, static mechanical (pressure), and dynamic mechanical (brush) [62]. In our investigation utilizing hot plate and Von Frey's tests, we examined thermal and mechanical allodynia and hypersensitization. Our findings indicate that thermal and mechanical allodynia were mitigated in the treatment group, highlighting the potential impact of *Moringa oleifera* extract in reducing hypersensitivity and subsequently improving the quality of life for migraine patients.

Our study has several limitations that should be acknowledged. Our relatively small sample size may limit the generalizability of our findings to broader populations. A larger sample size would enhance the statistical power, allowing for a better understanding of variability in response to treatment across different subgroups. Additionally, while we adjusted for known confounding factors, there may be other unmeasured variables, such as variations in diet, stress levels, or other comorbid conditions, that could influence the observed treatment effects. Furthermore, although we explored mechanisms related to apoptotic pathways and glutamate receptor expression levels, further mechanistic studies are needed to fully elucidate how *M. oleifera* exerts its effects. The short duration of follow-up in our study prevented us from assessing the long-term effects and safety of the treatment. Longer follow-up studies would be beneficial to evaluate the sustained impact of *M. oleifera* and identify potential delayed adverse effects. Lastly, the controlled experimental setting of the study may not fully capture real-world conditions, where various external factors could influence outcomes. Also, our investigation focused solely on two subunits of NMDAR. Existing literature highlights the essential contribution of other subunits to receptor functionality, where antagonists targeting specific subunits demonstrate only partial inhibition of CSD [7]. Also, the examination centered on the trigeminal ganglion unilaterally, highlighting the potential for contrasting insights when considering bilateral analysis. It is essential to acknowledge the potential divergence in the pathophysiological mechanisms underlying migraine symptoms between human and rodent models. Furthermore, it is plausible that the induction of migraines by GTN may engage distinct mechanisms and pathways compared to those involved in typical migraine episodes.

Conclusions

Moringa oleifera alleviates migraine-associated allodynia and hypersensitivity, at least partly by reducing CASP3 expression and the BAX/BCL-2 ratio, and stabilizing cells to prevent death in the CNS, demonstrating its potential in migraine management.

Acknowledgements
Not applicable.

Author contributions
All coauthors contributed to the conceptualization of the study. A.VN led the design and conceptualization of the study. He performed the experiments, collected and analyzed the data, wrote the manuscript, and prepared figures. A.V also contributed to the manuscript prepration, data collection and study design. Y.H helped conceive and design the study methodology. She oversaw the experiments and data gathering. Y.H, M.R.P, M.C, and M.M supervised the study. All coauthors reviewed the final manuscript draft prior to submission.

Funding
None.

Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The entire experimental protocol adhered to the guidelines set forth by the National Institutes of Health (NIH) [22] and was approved by the Ethics Committee of AJA University of Medical Sciences (Ethics code: IR.AJAUMS.REC.1401.040). We confirm that the animals used in this study were not privately owned by another institution, individual, or farm. They were obtained from an established laboratory animal facility for research purposes

Consent for publication
All authors declare their consent for the publication of the current manuscript in the journal *BMC Neuroscience*

Competing interests
The authors declare no competing interests.

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Received: 8 April 2024 / Accepted: 17 January 2025
Published online: 04 February 2025

References

1. Lucas C. Migraine with aura. Rev Neurol (Paris). 2021;177(7):779–84.
2. Lauritzen M. Cortical spreading depression in migraine. Cephalalgia. 2001;21(7):757–60.
3. Schott GD. Exploring the visual hallucinations of migraine aura: the tacit contribution of illustration. Brain. 2007;130(6):1690–703.
4. Fernández-Montoya J, Buendia I, Martin YB, Egea J, Negredo P, Avendaño C. Sensory input-dependent changes in glutamatergic neurotransmission-related genes and proteins in the adult rat trigeminal ganglion. Front Mol Neurosci. 2016;9.
5. Costa-Nunes J, Zubareva O, Araújo-Correia M, Valença A, Schroeter CA, Pawluski JL, et al. Altered emotionality, hippocampus-dependent performance and expression of NMDA receptor subunit mRNAs in chronically stressed mice. Stress. 2013;17(1):108–16.
6. Qin G, Fan X, Chen L, Shen C, Gui B, Tan G, et al. Preventive effects of AdR-siPTEN through the regulation of NMDA receptor NR2B subunit in trigeminal ganglia of migraine rats. Neurol Res. 2013;34(10):998–1006.
7. Peeters M, Gunthorpe MJ, Strijbos PJ, Goldsmith P, Upton N, James MF. Effects of pan- and subtype-selective N-methyl-D-aspartate receptor antagonists on cortical spreading depression in the rat: therapeutic potential for migraine. J Pharmacol Exp Ther. 2007;321(2):564–72.
8. Nellgard B, Wieloch T. NMDA-receptor blockers but not NBQX, an AMPA-receptor antagonist, inhibit spreading depression in the rat brain. Acta Physiol Scand. 1992;146(4):497–503.
9. Marrannes R, De Prins E, Willems R, Wauquier A. NMDA antagonists inhibit cortical spreading Depression, but accelerate the onset of neuronal Depolarization Induced by Asphyxia. Mechanisms of cerebral hypoxia and stroke. Advances in behavioral Biology.1988;303–4.
10. Bu F, Du R, Li Y, Quinn JP, Wang M. NR2A contributes to genesis and propagation of cortical spreading depression in rats. Sci Rep. 2016;6:23576.
11. Fatma K, Thumpati P, Panda D, Velayutham R, Dash J. Selective recognition of c-KIT 1 G-Quadruplex by Structural tuning of Heteroaromatic scaffolds and Side Chains. ACS Med Chem Lett. 2024;15(3):388–95.
12. Kou X, Li B, Olayanju JB, Drake JM, Chen N. Nutraceutical or pharmacological potential of Moringa oleifera Lam. Nutrients. 2018;10(3).
13. Ganguly R, Guha D. Alteration of brain monoamines & EEG wave pattern in rat model of Alzheimer's disease & protection by Moringa oleifera. Indian J Med Res. 2008;128(6):744–51.
14. Giacoppo S, Rajan TS, De Nicola GR, Iori R, Rollin P, Bramanti P, et al. The Isothiocyanate isolated from Moringa oleifera shows potent anti-inflammatory activity in the treatment of Murine Subacute Parkinson's Disease. Rejuvenation Res. 2017;20(1):50–63.
15. Srivastava G, Ganjewala D. An update on the emerging neuroprotective potential of Moringa oleifera and its prospects in complimentary neurotherapy. Phytomedicine Plus. 2024;4(2).
16. Hannan MA, Kang JY, Mohibbullah M, Hong YK, Lee H, Choi JS, et al. Moringa oleifera with promising neuronal survival and neurite outgrowth promoting potentials. J Ethnopharmacol. 2014;152(1):142–50.
17. Sutalangka C, Wattanathorn J, Muchimapura S, Thukham-mee W. Moringa oleiferaMitigates Memory Impairment and Neurodegeneration in Animal Model of Age-Related Dementia. Oxidative Med Cell Longev. 2013;2013:1–9.
18. Mundkar M, Bijalwan A, Soni D, Kumar P. Neuroprotective potential of Moringa oleifera mediated by NF-kB/Nrf2/HO-1 signaling pathway: a review. J Food Biochem. 2022;46(12).
19. Amina M, Bhat RS, Al-Dbass AM, Musayeib NM, Fahmy R, Alhadlaq L, et al. The protective effect of Moringa oleifera plant extract against glutamate-induced DNA damage and reduced cell viability in a primary retinal ganglion cell line. PeerJ. 2021;9:e11569.
20. Vafaei A, Vafaeian A, Iranmehr A, Nassireslami E, Hasannezhad B, Hosseini Y. Effects of beta-sitosterol on anxiety in migraine-induced rats: the role of oxidative/nitrosative stress and mitochondrial function. CNS Neurosci Ther. 2024;30(9):e14892.
21. Amara I, Ontario ML, Scuto M, Lo Dico GM, Sciuto S, Greco V et al. Moringa oleifera protects SH-SY5YCells from DEHP-Induced endoplasmic reticulum stress and apoptosis. Antioxidants. 2021;10(4).
22. Guide for the. Care and Use of Laboratory Animals2011.
23. King M, Marsden C, Fone K. A role for the 5-HT1A, 5-HT4 and 5-HT6 receptors in learning and memory. Trends Pharmacol Sci. 2008;29(9):482–92.
24. Bakre AG, Aderibigbe AO, Ademowo OG. Studies on neuropharmacological profile of ethanol extract of Moringa oleifera leaves in mice. J Ethnopharmacol. 2013;149(3):783–9.
25. Pradhan AA, Smith ML, McGuire B, Tarash I, Evans CJ, Charles A. Characterization of a novel model of chronic migraine. Pain. 2014;155(2):269–74.
26. Sun S, Zheng G, Zhou D, Zhu L, He X, Zhang C, et al. Emodin interferes with Nitroglycerin-Induced migraine in rats through CGMP-PKG pathway. Front Pharmacol. 2021;12:758026.
27. Wen W, Chen H, Fu K, Wei J, Qin L, Pan T, et al. Fructus Viticis methanolic extract attenuates trigeminal hyperalgesia in migraine by regulating injury signal transmission. Exp Ther Med. 2020;19(1):85–94.
28. Moye LS, Pradhan AAA. Animal Model of Chronic Migraine-Associated Pain. Curr Protoc Neurosci. 2017;80:9601–99.
29. Underwood W, Anthony R. AVMA guidelines for the euthanasia of animals: 2020 edition. Retrieved March. 2020;2013(30):2020–1.
30. Kamat PK, Rai S, Swarnkar S, Shukla R, Nath C. Mechanism of synapse redox stress in Okadaic acid (ICV) induced memory impairment: role of NMDA receptor. Neurochem Int. 2014;76:32–41.
31. Alsharafi WA, Xiao B, Li J. MicroRNA-139-5p negatively regulates NR2A-containing NMDA receptor in the rat pilocarpine model and patients with temporal lobe epilepsy. Epilepsia. 2016;57(11):1931–40.

32. He X, Sun J, Huang X. Expression of caspase-3, Bax and Bcl-2 in hippocampus of rats with diabetes and subarachnoid hemorrhage. Exp Ther Med. 2017;15(1):873–877.
33. Ding Y, Zhu W, Kong W, Li T, Zou P, Chen H. Edaravone attenuates neuronal apoptosis in hippocampus of rat traumatic brain injury model via activation of BDNF/TrkB signaling pathway. Archives Med Sci. 2021;17(2):514–22.
34. Rao X, Huang X, Zhou Z, Lin X. An improvement of the 2^(-delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. Biostat Bioinforma Biomath. 2013;3(3):71–85.
35. Pitcher GM, Ritchie J, Henry JL. Paw withdrawal threshold in the Von Frey hair test is influenced by the surface on which the rat stands. J Neurosci Methods. 1999;87(2):185–93.
36. Bannon AW, Malmberg AB. Models of nociception: hot-plate, tail-flick, and formalin tests in rodents. Curr Protoc Neurosci. 2007;41:8.9.1–8.9.16.
37. Lee J, Ro JY. Differential regulation of glutamate receptors in trigeminal ganglia following masseter inflammation. Neurosci Lett. 2007;421(2):91–5.
38. Ye R, Kong X, Han J, Zhao G. N-methyl-D-aspartate receptor antagonists for migraine: a potential therapeutic approach. Med Hypotheses. 2009;72(5):603–5.
39. Paoletti P, Bellone C, Zhou Q. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat Rev Neurosci. 2013;14(6):383–400.
40. Iyengar S, Johnson KW, Ossipov MH, Aurora SK. CGRP and the Trigeminal System in Migraine. Headache. 2019;59(5):659–81.
41. Juhasz G, Zsombok T, Jakab B, Nemeth J, Szolcsanyi J, Bagdy G. Sumatriptan causes parallel decrease in plasma calcitonin gene-related peptide (CGRP) concentration and migraine headache during nitroglycerin induced migraine attack. Cephalalgia. 2005;25(3):179–83.
42. Greco R, Demartini C, Zanaboni AM, Redavide E, Pampalone S, Toldi J, et al. Effects of kynurenic acid analogue 1 (KYNA-A1) in nitroglycerin-induced hyperalgesia: targets and anti-migraine mechanisms. Cephalalgia. 2016;37(13):1272–84.
43. Bu F, Yuan M, Ma D, Zhu Y, Wang M. Inhibition of NR2A reduces calcitonin gene-related peptide gene expression induced by cortical spreading depression in rat amygdala. Neuropeptides. 2020;84.
44. Karsan N, Gosalia H, Goadsby PJ. Molecular mechanisms of Migraine: nitric oxide synthase and neuropeptides. Int J Mol Sci. 2023;24(15).
45. Zhang M, Liu Y, Hu G, Kang L, Ran Y, Su M et al. Cognitive impairment in a classical rat model of chronic migraine may be due to alterations in hippocampal synaptic plasticity and N-methyl-D-aspartate receptor subunits. Mol Pain. 2020;16.
46. Rusu MC, Mănoiu VS, Vrapciu AD, Hostiuc S, Mirancea N. Altered mitochondrial anatomy of trigeminal ganglia neurons in diabetes. Anat Rec. 2016;299(11):1561–70.
47. Abushik PA, Niittykoski M, Giniatullina R, Shakirzyanova A, Bart G, Fayuk D, et al. The role of NMDA and mGluR5 receptors in calcium mobilization and neurotoxicity of homocysteine in trigeminal and cortical neurons and glial cells. J Neurochem. 2014;129(2):264–74.
48. Yüksel E, Nazıroğlu M, Şahin M, Çiğ B. Involvement of TRPM2 and TRPV1 channels on hyperalgesia, apoptosis and oxidative stress in rat fibromyalgia model: protective role of selenium. Sci Rep. 2017;7(1).
49. Yazğan Y, Nazıroğlu M. Involvement of TRPM2 in the neurobiology of experimental migraine: focus on oxidative stress and apoptosis. Mol Neurobiol. 2021;58(11):5581–601.
50. Wang Y, Shan Z, Zhang L, Fan S, Zhou Y, Hu L, et al. P2X7R/NLRP3 signaling pathway-mediated pyroptosis and neuroinflammation contributed to cognitive impairment in a mouse model of migraine. J Headache Pain. 2022;23(1):75.
51. Barclay Lauren A, Wales Thomas E, Garner Thomas P, Wachter F, Lee S, Guerra Rachel M, et al. Inhibition of pro-apoptotic BAX by a Noncanonical Interaction mechanism. Mol Cell. 2015;57(5):873–86.
52. Wang Y, Sun L-G, Xia C-H. Caspase-mediated Fas apoptotic pathway. World Chin J Digestology. 2006;14(36).
53. Potest M, Minutolo A, Gismondi A, Canuti L, Kenzo M, Roglia V et al. Cytotoxic and apoptotic effects of different extracts of Moringa Oleifera Lam on lymphoid and monocytoid cells. Experimental Therapeutic Med. 2019;18(1):5–17.
54. Aguggia M. Allodynia and migraine. Neurol Sci. 2012;33(Suppl 1):S9–11.
55. Burstein R, Yarnitsky D, Goor-Aryeh I, Ransil BJ, Bajwa ZH. An association between migraine and cutaneous allodynia. Ann Neurol. 2000;47(5):614–24.
56. Villar-Martinez MD, Goadsby PJ. Pathophysiology and therapy of Associated features of Migraine. Cells. 2022;11(17).
57. Eidlitz-Markus T, Shuper A, Gorali O, Zeharia A. Migraine and cephalic cutaneous allodynia in pediatric patients. Headache. 2007;47(8):1219–23.
58. Bigal ME, Ashina S, Burstein R, Reed ML, Buse D, Serrano D, et al. Prevalence and characteristics of allodynia in headache sufferers: a population study. Neurology. 2008;70(17):1525–33.
59. Lipton RB, Bigal ME, Ashina S, Burstein R, Silberstein S, Reed ML, et al. Cutaneous allodynia in the migraine population. Ann Neurol. 2008;63(2):148–58.
60. Louter MA, Bosker JE, van Oosterhout WP, van Zwet EW, Zitman FG, Ferrari MD, et al. Cutaneous allodynia as a predictor of migraine chronification. Brain. 2013;136(Pt 11):3489–96.
61. Yuksel H, Kenar SG, Gursoy GT, Bektas H. The impacts of masks and disinfectants on migraine patients in the COVID-19 pandemic. J Clin Neurosci. 2022;97:87–92.
62. Young WB, Richardson ES, Shukla P. Brush allodynia in hospitalized headache patients. Headache. 2005;45(8):999–1003.

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