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Original Research Article

Assessment of neuropharmacological effects of ethanolic leaf extract of *Pergularia daemia* in migraine induced rat

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ABSTRACT

Background: Migraine is a common neurological disorder. *Pergularia daemia* has reported anti-inflammatory and analgesic properties. We evaluated the neuropharmacological effects of its ethanolic leaf extract (EEPDP) in nitroglycerin (NTG)-induced migraine in rats.

Methods: Leaves were extracted with ethanol (Soxhlet), screened phytochemically and analysed by GC-MS. Thirty male Wistar rats formed five groups: normal control; disease control (NTG 10 mg/kg I.P., days 1, 4, 7, 10, 13); sumatriptan 1 mg/kg I.P.; and EEPDP 200 or 400 mg/kg P.O. for 14 days plus NTG. Migraine-like behaviour was scored (maximum 16), brain nitrite was estimated with Griess reagent, and hippocampal histology was examined. Scores were analysed by ANOVA with Dunnett's test.

Results: Yield was 28.78% w/w. Alkaloids, flavonoids, phenolics, tannins, steroids, carbohydrates and anthraquinone glycosides were detected; GC-MS tentatively identified 15 compounds, chiefly 3-O-acetyl-6-methoxy-cycloartenol (38.3% of area). The behavioural score was 9.40 ± 0.24 in disease control versus 2.40 ± 0.24 in normal control ($p < 0.0001$), and 3.60 ± 0.40 with sumatriptan, 2.80 ± 0.49 and 2.60 ± 0.24 with EEPDP 200 and 400 mg/kg (all $p < 0.0001$ versus disease control). Brain nitrite fell by 61% (sumatriptan), 26% and 29% (EEPDP 200 and 400 mg/kg), although most values exceeded the calibration range and are qualitative. Disease-control hippocampus showed neuronal loss and degeneration; treated groups appeared normal.

Conclusions: EEPDP attenuated NTG-induced behavioural changes, brain nitrite elevation and hippocampal alterations in rats, supporting further mechanistic evaluation as an anti-migraine candidate.

Keywords: *Pergularia daemia*, Migraine, Nitroglycerin, Nitric oxide, Sumatriptan, Hippocampus

INTRODUCTION

Migraine is a chronic, recurrent neurological disorder characterised by attacks of moderate to severe, often unilateral and pulsating headache accompanied by nausea, vomiting, photophobia and phonophobia, with or without aura.¹ Headache disorders, including migraine, rank among the leading causes of years lived with disability worldwide, and migraine is a major cause of disability in people younger than 50 years.² Migraine affects approximately 12-15% of the global population and is

more prevalent in women, particularly during the reproductive years, which points to a hormonal influence.³⁻⁵ Genetic susceptibility, triggers such as stress, sleep disturbance and diet, and psychiatric comorbidity further shape disease activity.⁶⁻⁹

Migraine is no longer regarded as a purely vascular disorder; it is understood as a neurovascular disorder of altered brain excitability and sensory processing.^{10,11} Activation of the trigeminovascular system releases vasoactive neuropeptides, notably calcitonin gene-related peptide (CGRP), substance P and neurokinin A, which

produce meningeal vasodilation, neurogenic inflammation and sensitisation of nociceptive pathways.^{10,12} Cortical spreading depression is believed to underlie aura and may activate trigeminal nociceptive pathways.^{13,14} Nitric oxide (NO) is a potent vasodilator that can activate these pathways.¹⁵ Despite advances such as CGRP-targeted therapies, migraine remains under-diagnosed and under-treated, and overuse of acute medication carries the risk of medication-overuse headache.^{6,9}

Several animal models are used to study migraine, including the NTG-induced, cortical spreading depression, inflammatory soup, CGRP, dural electrical stimulation, genetic and TRPA1/TRPV1 agonist models, each of which reproduces particular facets of the disorder.^{13,16-21} NTG, a NO donor, activates the trigeminovascular system, promotes neurogenic inflammation and CGRP release, and produces migraine-like behaviour in rodents; the model is simple and reproducible, although it mimics migraine-like symptoms rather than spontaneous attacks.¹⁶

Medicinal plants are of interest as sources of anti-migraine agents because their constituents may have anti-inflammatory, antioxidant, neuroprotective and vasomodulatory effects. For example, parthenolide from feverfew inhibits NTG-induced Fos activation in an animal model of migraine.¹⁶ *Pergularia daemia* (Forssk.) Chiov. (family Asclepiadaceae) is a twining perennial herb distributed through tropical and subtropical regions and used in traditional medicine for inflammation, joint pain, fever and related complaints. Extracts of the plant have been reported to show anti-inflammatory, analgesic, antioxidant and neuroprotective activity. In the literature we reviewed, its effect in experimental migraine had not been examined.

This study therefore evaluated the neuropharmacological EEPD at 200 and 400 mg/kg in NTG-induced migraine in rats, using behavioural scoring, brain nitrite estimation and hippocampal histopathology, with sumatriptan as the reference drug.

METHODS

Plant material and extraction

Leaves of *Pergularia daemia* were collected between November 2025 and January 2026 from cultivable land, roadsides and river banks around Vandavasi (604 408), Tamil Nadu. The specimen was authenticated as *P. daemia* (Forssk.) Chiov. (synonym *P. extensa*; family Asclepiadaceae) by Dr. V. Gangadevi, Department of Botany, Arignar Anna Government Arts College, Cheyyar (certificate no. AAA202603, dated 27 January 2026). The leaves were cleaned, shade-dried, coarsely powdered with a mechanical grinder and passed through a No. 10 sieve. Powdered material (500 g) was defatted with petroleum ether, and 120 g of the powder was extracted with ethanol in a Soxhlet apparatus. The extract was evaporated and

concentrated, and the percentage yield was calculated as (weight of extract/weight of powder taken) × 100.

Preliminary phytochemical screening

EEPD was screened by standard qualitative tests for carbohydrates (Molisch, Benedict and Fehling tests), alkaloids (Hager, Wagner, Mayer and Dragendorff tests), glycosides (Borntrager test for anthraquinone glycosides; Keller-Killiani and Legal tests for cardiac glycosides), steroids and triterpenoids (Salkowski and Liebermann-Burchard tests), phenolic compounds (ferric chloride test), tannins (gelatin and lead acetate tests) and flavonoids (ammonia test).

GC-MS analysis

GC-MS analysis was carried out on a Clarus 680 gas chromatograph fitted with an Elite-5MS fused-silica column (5% biphenyl, 95% dimethylpolysiloxane; 30 m × 0.25 mm ID, 0.25 µm film thickness). Helium was the carrier gas at a constant flow of 1 mL/min and the injector temperature was 260 °C. The oven was held at 60 °C for 2 min, raised to 300 °C at 10 °C/min and held for 6 min. The transfer line and ion source were maintained at 240 °C; electron impact ionisation was at 70 eV, scanning 40-600 Da (scan time 0.2 s, interval 0.1 s). Compounds were tentatively identified by comparing their mass spectra with the NIST (2008) library.

Animals and ethical approval

Male Wistar albino rats (120-150 g) were obtained from Mass Biotech, Pulipakkam, Chengalpattu, Tamil Nadu. Animals were housed at 22±2 °C and 55±5% relative humidity under a 12 h light/dark cycle, with pellet diet and water *ad libitum*. The protocol was approved by the Institutional Animal Ethics Committee of Adhiparasakthi College of Pharmacy (proposal no. APCP/IAEC/2025-2026/II/09; meeting held on 22 December 2025).

Experimental design

Thirty rats were allocated to five groups of six: Group I, normal control (no NTG); Group II, disease control (distilled water for 14 days plus NTG 10 mg/kg i.p. on days 1, 4, 7, 10 and 13); Group III, sumatriptan 1 mg/kg i.p. for 14 days plus NTG on the same days; Group IV, EEPD 200 mg/kg p.o. for 14 days plus NTG; and Group V, EEPD 400 mg/kg p.o. for 14 days plus NTG. At the end of the 14-day period the animals were sacrificed and the brains were removed for nitrite estimation and histopathology.

Behavioural scoring

Migraine-like behaviour was scored using an eight-item grimace-scale-based scoring sheet: head scratching, rolling, dizziness/ataxia, immobility, paw rubbing/licking, jumping, upward lying position and head rubbing. Each item was scored 0-2 (0, absent or normal; 1, occasional,

mild or reduced; 2, frequent, severe or continuous), giving a total of 0-16 per animal. Total scores were graded as 0-3 normal, 4-8 mild, 9-13 moderate and ≥ 14 severe.

Estimation of NO in brain tissue

Brain tissue (500 mg) was homogenised in 4.5 mL of 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 10-20 min at 4°C. The clear supernatant (2 mL) was mixed with 2 mL of freshly prepared Griess reagent, and the absorbance of the developed colour was read at 540 nm in a UV-visible spectrophotometer. Nitrite was calculated against a sodium nitrite standard curve (highest standard, 100 μ M).

Histopathology

Brain tissue was collected after sacrifice and processed for histopathological examination; hippocampal sections were examined microscopically and assessed qualitatively for neuronal number and morphology, the neurofibrillary matrix and blood vessels.

Statistical analysis

Behavioural scores are expressed as mean $\pm$ SEM and were analysed by one-way analysis of variance (ANOVA)

followed by Dunnett's multiple comparison test; $p < 0.05$ was considered significant. Nitrite values are presented descriptively.

RESULTS

Extractive yield and phytochemical screening

The ethanolic extract weighed 34.54 g from 120 g of powder, a yield of 28.78% w/w. Phytochemical screening showed carbohydrates, alkaloids, anthraquinone glycosides, steroids, phenolic compounds, tannins and the flavonoids; cardiac glycosides were not detected in Table 1.

GC-MS profile

GC-MS of EEPD showed 15 peaks that were matched to NIST library entries (Figure 1 and Table 2). The most abundant was 3-O-acetyl-6-methoxy-cycloartenol (retention time 31.18 min; 38.3% of the summed area of the listed peaks), a triterpenoid derivative, followed by 3,3-dimethyl-4-heptanol (19.43 min; 11.3%) and 2-(aminooxy) pentanoic acid (19.96 min; 7.1%). The remaining peaks, eluting mainly at 9-10 min, corresponded to low-molecular-weight oxygenated and nitrogenous compounds and a sugar-derived anhydro compound.

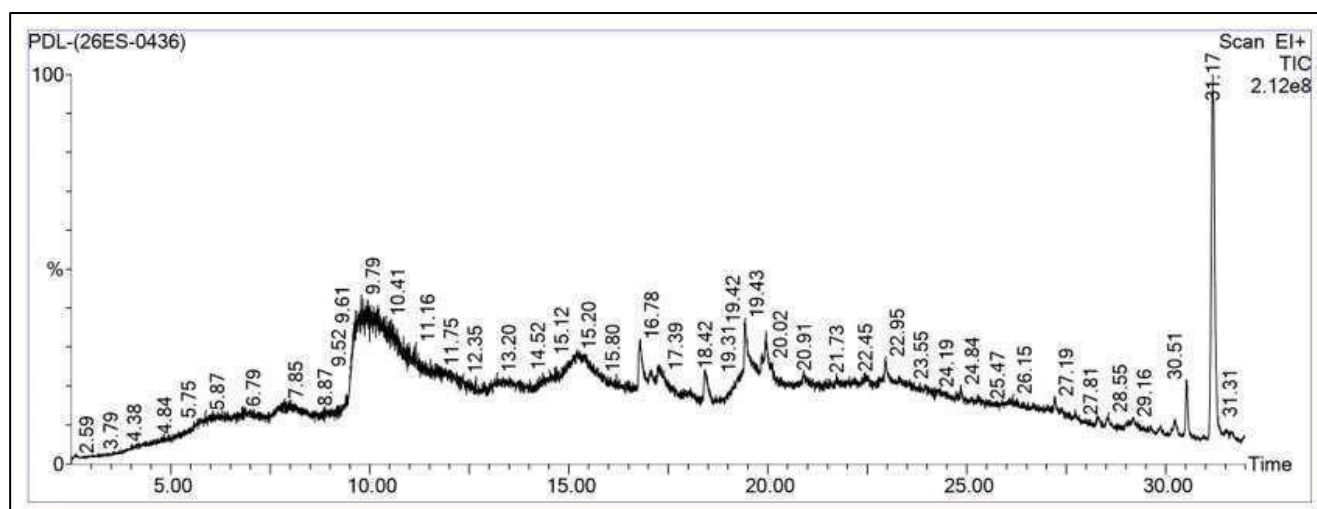


Figure 1: GC-MS total ion chromatogram of the ethanolic leaf extract of *P. daemia*.

Table 1: Preliminary phytochemical screening of the EEPD.

Constituents	Tests performed	Result
Carbohydrates	Molisch, Benedict, Fehling	Present
Alkaloids	Hager, Wagner, Mayer, Dragendorff	Present
Anthraquinone glycosides	Borntrager	Present
Cardiac glycosides	Keller-Killiani, Legal	Absent
Steroids	Salkowski, Liebermann-Burchard	Present
Phenolic compounds	Ferric chloride	Present
Tannins	Gelatin, lead acetate	Present
Flavonoids	Ammonia	Present

Table 2: Compounds tentatively identified in EEPD by GC-MS (NIST library match).

S. no.	RT (min)	Compound	MW (g/mol)	Peak area	Area (%)*
1	9.641	Propanal, 2,3-dihydroxy-, (S)-	90	5,863,668	11.26
2	9.786	Ethanimidic acid, ethyl ester	87	1,903,389	3.66
3	9.836	Formic acid hydrazide	60	1,048,587	2.01
4	9.941	Acetamide, N-(3-oxo-4-isoxazolidinyl)-, (R)-	144	2,181,880	4.19
5	10.016	Diglycolic acid	134	789,766	1.52
6	10.212	Ethanimidic acid, ethyl ester	87	1,499,001	2.88
7	16.794	1,6;2,3-Dianhydro-4-deoxy-beta-D-lyxohexopyranose	168	2,250,572	4.32
8	18.425	Propanoic acid, 2-(aminoxy)-	105	1,490,866	2.86
9	19.425	3,3-Dimethyl-4-heptanol	144	5,471,446	10.51
10	19.856	Pentanoic acid, 2-(aminoxy)-	133	1,158,044	2.22
11	19.956	Pentanoic acid, 2-(aminoxy)-	133	3,708,096	7.12
12	22.952	(S)-3-Ethyl-4-methylpentanol	130	1,877,139	3.61
13	30.225	2-Piperidinone, 6-methyl-	113	865,228	1.66
14	30.525	1-Pentene, 4-methyl-	84	2,006,110	3.85
15	31.180	3-O-Acetyl-6-methoxy-cycloartenol	498	19,948,816	38.32

*RT, retention time; MW, molecular weight. *Percentage of the summed area of the 15 listed peaks.

Behavioural scores

Repeated NTG administration raised the behavioural score from 2.40 ± 0.24 in normal control to 9.40 ± 0.24 in disease control ($p < 0.0001$), which falls in the moderate category. Sumatriptan (3.60 ± 0.40), EEPD 200 mg/kg (2.80 ± 0.49) and EEPD 400 mg/kg (2.60 ± 0.24) each reduced the score significantly relative to disease control ($p < 0.0001$), by 62%, 70% and 72%, respectively (Figure 2). Scores of individual treated animals ranged from 2 to 5, compared with 9-10 in disease control. The mean score of the 400 mg/kg group was numerically the lowest among the treated groups; the difference between the two extract doses was small and was not tested.

Nitric oxide

Brain nitrite in disease control ($260.7 \mu\text{M}$) was about 19 times that of normal control ($13.6 \mu\text{M}$). Treatment lowered the values to $100.8 \mu\text{M}$ with sumatriptan (61% below disease control), $192.9 \mu\text{M}$ with EEPD 200 mg/kg (26%) and $185.4 \mu\text{M}$ with EEPD 400 mg/kg (29%) (Table 3, Figure 3). The absorbance values of the disease control and both extract groups exceeded that of the highest sodium nitrite standard ($100 \mu\text{M}$), and no additional aliquots were available for dilution and repeat analysis. These values are therefore interpreted as indicating relative differences between groups rather than absolute concentrations, and no statistical comparison is claimed.

Hippocampus histopathology

Hippocampus sections of normal control rats showed normal neuronal number and morphology, a normal neurofibrillary matrix and normal blood vessels. Disease-control sections showed reduced neuronal numbers with some degenerating neurons, a tangled neurofibrillary

matrix and proliferation of blood vessels. Sections from the sumatriptan, EEPD 200 mg/kg and EEPD 400 mg/kg groups showed normal neuronal numbers and morphology, normal neurofibrillary matrix and normal blood vessels, indicating a good response to treatment (Figure 4).

Estimated concentrations for the disease control, EEPD 200 mg/kg and EEPD 400 mg/kg groups lie above the highest calibration standard ($100 \mu\text{M}$) and are approximate.

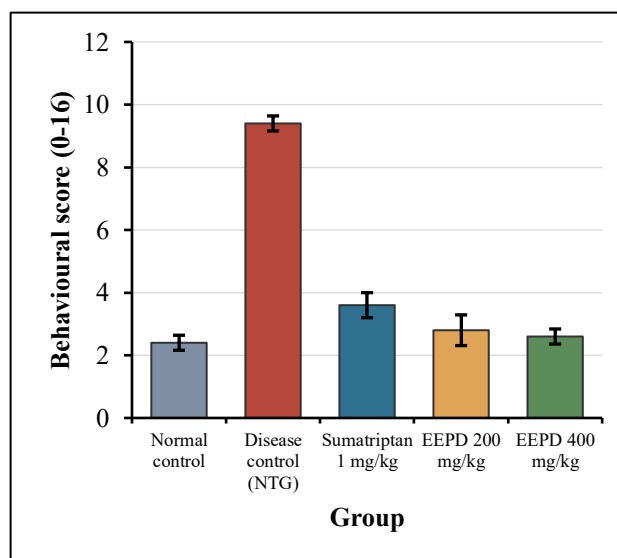


Figure 2: Effect of EEPD on the behavioural score in NTG-treated rats.

*Mean ± SEM (n=6 animals scored per group). **** $p < 0.0001$ versus normal control; #### $p < 0.0001$ for sumatriptan, EEPD 200 mg/kg and EEPD 400 mg/kg versus disease control (one-way ANOVA, Dunnett's test). NTG, nitroglycerin; EEPD, ethanolic extract of *P. daemia*.

Table 3: Brain nitrite (Griess assay) in the experimental groups.

Groups	Mean absorbance (540 nm)	Estimated NO (μ M)	Change vs disease control (%)
Normal control	0.071	13.6	-
Disease control (NTG)	1.361	260.7	-
Sumatriptan 1 mg/kg	0.526	100.8	-61.3
EEPD 200 mg/kg	1.007	192.9	-26.0
EEPD 400 mg/kg	0.968	185.4	-28.9

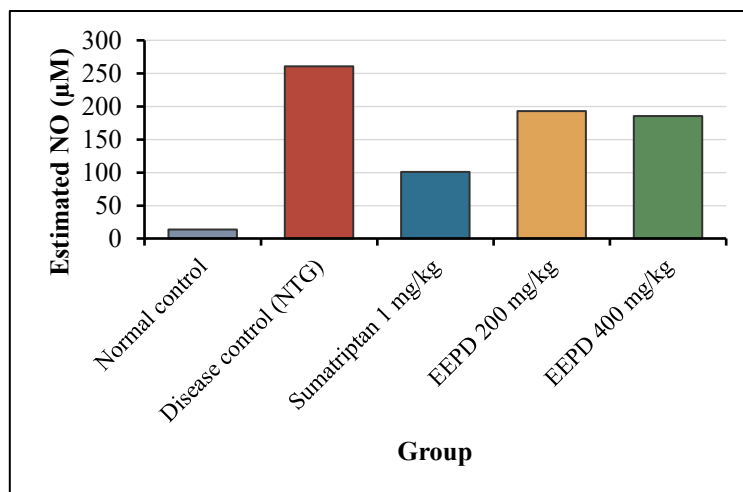


Figure 3: Effect of EEPD on brain NO (nitrite) levels in NTG-treated rats.

*Group means; values above 100 μ M exceed the calibration range and are approximate

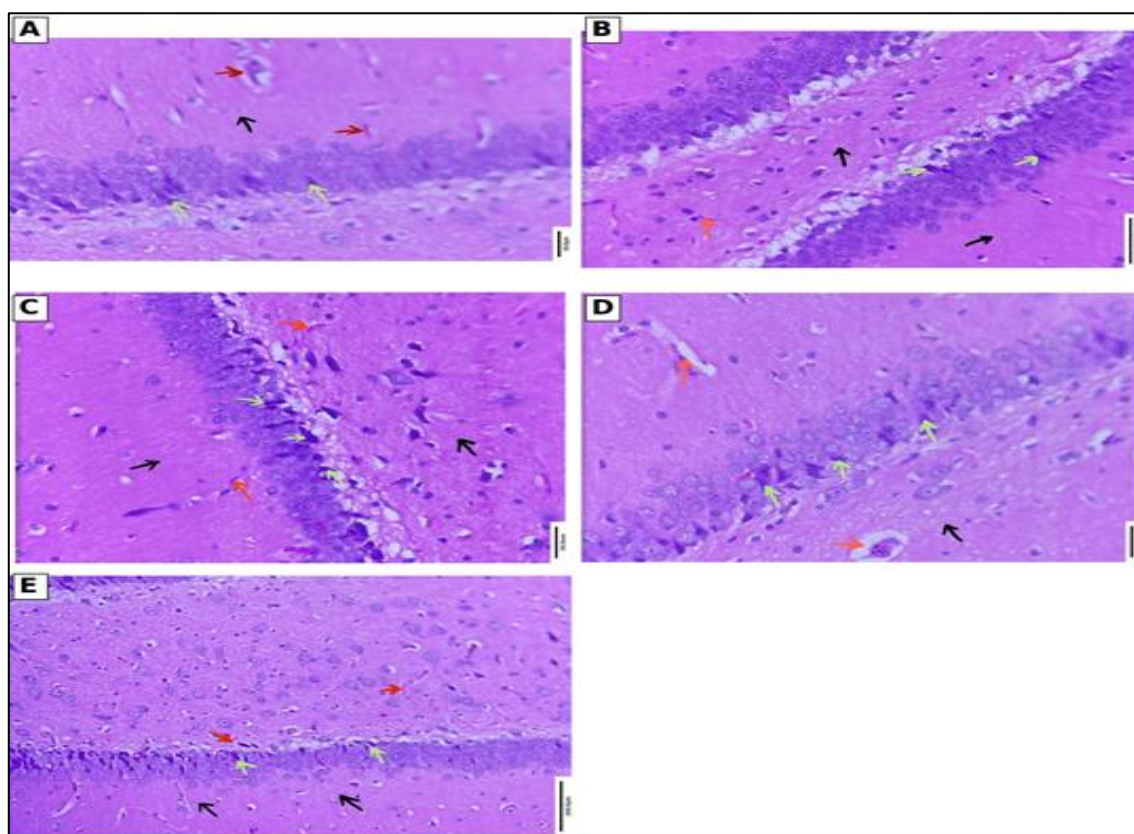


Figure 4 (A-E): Hippocampal histology. (A) Normal control; (B) disease control; (C) sumatriptan; (D) EEPD 200 mg/kg; (E) EEPD 400 mg/kg.

*Green arrows, neurons; black arrows, neurofibrillary matrix; red arrows, blood vessels. Scale bars are shown in each panel.

DISCUSSION

In this study, the ethanolic leaf extract of *P. daemia* reduced NTG-induced behavioural changes and lowered brain nitrite, and hippocampal architecture was preserved in treated rats. Within the literature we reviewed, this extract had not previously been examined in an NTG migraine model.

The extract gave a yield of 28.78% w/w and contained alkaloids, flavonoids, phenolic compounds, tannins, steroids, anthraquinone glycosides and carbohydrates. GC-MS tentatively identified 15 compounds; the dominant one, 3-O-acetyl-6-methoxy-cycloartenol (38.3% of the summed area), is a cycloartenol-type triterpenoid. Flavonoids, phenolics and tannins are classes widely associated with antioxidant and anti-inflammatory activity, and such properties are relevant to migraine, in which neurogenic inflammation and oxidative stress are involved. However, this study did not test which constituents account for the effect, and several of the low-molecular-weight GC-MS matches may reflect minor or artefactual components.

NTG is an NO donor, and repeated administration activates the trigeminovascular system and produces migraine-like behaviour in rodents.^{15,16} The disease-control score of 9.40 (moderate category) confirms that the model produced marked behavioural change. Pretreatment with sumatriptan, a 5-HT_{1B/1D} agonist, and with either dose of the extract brought mean scores to 2.60-3.60, close to the normal control value of 2.40. Numerically, the extract groups scored lower than the sumatriptan group, but the study was not designed to test equivalence or superiority to the reference drug, and the 200 and 400 mg/kg doses were not clearly separable, so a dose-response relationship cannot be concluded.

Brain nitrite in the disease control group was far higher than in normal control, in keeping with NTG acting as an NO donor. The extract lowered nitrite by roughly one quarter to three tenths, whereas sumatriptan lowered it by more than half; thus, on this measure the extract was less effective than the reference drug. Because the disease-control and extract-group readings exceeded the calibration range, these results are semi-quantitative. They are compatible with an effect of the extract on NO-related signalling, but this was not examined directly, and CGRP, c-Fos and trigeminal nucleus caudalis markers, which are established endpoints in the NTG model were not measured.¹⁶

Disease-control hippocampal sections showed neuronal loss with degenerative change, a tangled neurofibrillary matrix and vascular proliferation, whereas sections from the sumatriptan and extract groups appeared normal. The hippocampus is not the principal site of migraine pain generation, so these findings are best viewed as evidence of protection against NTG-associated neural injury rather

than of anti-migraine action in the trigeminovascular pathway. The assessment was descriptive and not scored.

This study has several limitations. First, only one model (repeated NTG) was used, which mimics migraine-like symptoms rather than spontaneous attacks, and the trigeminovascular endpoints (CGRP, c-Fos, facial allodynia) were not assessed. Second, the behavioural endpoint was a composite observational score, the group sizes were small (five scored animals per group), only male rats were studied, and no group received the extract alone, so intrinsic effects of the extract could not be separated from its effect on NTG responses. Third, nitrite values for most groups exceeded the calibration range and could not be re-assayed, so they are qualitative, and the histopathological assessment was descriptive and restricted to the hippocampus. Fourth, the extract was not standardised, GC-MS identification was tentative (library match without reference standards), the active constituents were not isolated, and the 200 and 400 mg/kg doses were not compared statistically. Finally, treatment lasted only 14 days and acute toxicity and pharmacokinetics were not studied.

CONCLUSION

The ethanolic leaf extract of *P. daemia* at 200 and 400 mg/kg reduced NTG-induced behavioural scores, lowered brain nitrite relative to untreated NTG-treated rats and preserved hippocampal architecture. These findings support further work, including CGRP and trigeminal endpoints, standardisation of the extract, and isolation and testing of its constituents, to establish its anti-migraine potential.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee (APCP/LAEC/2025-2026/II/09)

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