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Experimental Evaluation of Ethanolic Extract of *Pergularia daemia* in an Animal Model of Neuropathic Pain.

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ABSTRACT

Neuropathic pain, a consequence of somatosensory system injury, is poorly controlled with current pharmacological options due to limited efficacy and adverse effects. *Pergularia daemia*, a traditional Indian medicinal herb, contains flavonoids and terpenoids with reported anti-inflammatory and antioxidant activity, suggesting potential benefit in neuropathic pain. To evaluate the effects of ethanolic *P. daemia* leaf extract on behavioral indices of paclitaxel-induced neuropathic pain in mice.Swiss albino mice (n=30) were randomized into control, paclitaxel, morphine, and two treatment groups (*P. daemia* 200 mg/kg and 400 mg/kg). Neuropathic outcomes were assessed using acetone drop (cold allodynia), hair aesthesiometer (mechanical allodynia), and tail immersion (thermal hyperalgesia). Data were analyzed by one-way ANOVA with Dunnett's post hoc test.Paclitaxel significantly increased allodynia and hyperalgesia ($p<0.001$). Morphine reversed these effects. *P. daemia* extract produced dose-dependent attenuation: 200 mg/kg showed moderate improvement, while 400 mg/kg markedly reduced cold and mechanical allodynia and restored tail withdrawal latency ($p<0.001$), approaching morphine efficacy. Ethanolic *P. daemia* extract demonstrated significant neuroprotective and analgesic effects in paclitaxel-induced neuropathic pain. Findings support its role as a candidate phytotherapeutic agent for chemotherapy-related neuropathy.

Keywords: *Pergularia daemia*; Neuropathic pain; Paclitaxel; Cold allodynia; Mechanical allodynia; Thermal hyperalgesia; Herbal medicine.

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INTRODUCTION

Neuropathic pain is a chronic pain syndrome resulting from structural or functional disruption of the somatosensory system. Epidemiological estimates suggest that it affects 7–10% of adults globally, with major contributors including diabetes mellitus, chemotherapy, stroke, multiple sclerosis, and traumatic injuries [1,2]. Clinical manifestations include hyperalgesia and allodynia, which arise from maladaptive processes such as peripheral sensitization, glial activation, and central neuroplasticity [3,4]. The neurobiology of neuropathic pain involves sustained excitatory transmission, excessive NMDA receptor activity, and the release of pro-inflammatory cytokines that reinforce aberrant signaling in spinal and supraspinal pathways [5]. These mechanisms explain the persistence of pain long after the initial insult.Despite advances in pharmacotherapy, treatment outcomes remain unsatisfactory. Current first-line medications include gabapentinoids, tricyclic antidepressants, and serotonin–noradrenaline reuptake inhibitors, while opioids are reserved for refractory cases [6]. Long-term administration, however, is hindered by systemic toxicity, tolerance, dependency, and limited efficacy in some patients [7].In this context, medicinal plants have gained renewed interest as safer adjunctive or alternative therapies. The World Health Organization has emphasized their role in expanding evidence-based integrative care, given their broad pharmacological activities and relatively low toxicity [8]. Herbal agents with antioxidant and anti-inflammatory properties, such as *Curcuma longa* and *Ginkgo biloba*, have demonstrated analgesic benefits in neuropathic models [9]. *Pergularia daemia* (Asclepiadaceae), locally called "Veliparuthi," is a perennial climber distributed in tropical India. Traditionally, it is used to treat jaundice, respiratory disorders, and menstrual complaints. Phytochemical investigations have identified flavonoids, terpenoids, and cardenolides, which exhibit hepatoprotective, anti-inflammatory, and antioxidant effects [10]. These constituents provide a biological rationale to investigate its potential role in neuropathic pain.The present study was therefore designed to assess the effect of ethanolic leaf extract of *P. daemia* in paclitaxel-induced neuropathic pain in mice, using validated behavioral assays for cold allodynia, mechanical allodynia, and thermal hyperalgesia.

MATERIALS AND METHODS

Experimental Animals

Swiss albino mice (20–25 g, both sexes) bred in the institute's facility were used. They were kept in small groups, provided with standard diet and water, and maintained under regulated light, temperature, and humidity. Ethical approval for the work was granted by the Institutional Animal Ethics Committee in line with CPCSEA norms.

Neuropathy Induction

Peripheral neuropathy was produced with paclitaxel. The drug was injected intraperitoneally at 2 mg/kg on four alternate days (0, 2, 4, and 6). This schedule reliably produces allodynia and hyperalgesia within a week of initiation.

Plant Extract Preparation

Fresh leaves of *Pergularia daemia* were authenticated, shade-dried, powdered, and extracted with ethanol using a Soxhlet apparatus. The concentrated residue was stored in airtight containers at 4°C until further use.

Treatment Design

The extract was suspended in 0.5% carboxymethyl cellulose and administered orally at doses of 100, 200, and 400 mg/kg. Gabapentin (100 mg/kg, p.o.) was used as a positive control. Treatments began on day 7 after paclitaxel initiation and continued daily for 14 days.

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Pain Assessments

Animals were allowed to adapt before each test.

- **Cold allodynia:** Response time to acetone applied on the paw.
- **Mechanical allodynia:** Threshold measured by von Frey filaments.
- **Thermal hyperalgesia:** Paw-licking or jumping latency on a hot plate set at 52°C (15 s cut-off).

Each test was repeated three times, and the average values were taken.

Data Analysis

Results are expressed as mean ± SEM. Groups were compared with one-way ANOVA followed by Tukey's post-hoc test. A p-value <0.05 indicated significance.

RESULTS

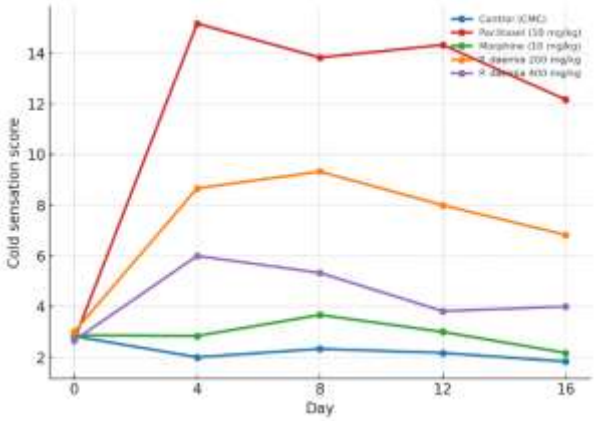
Cold allodynia -Acetone drop test

Paclitaxel administration produced a marked increase in cold sensitivity scores, with maximal responses by day 16. Treatment with morphine or *Pergularia daemia* (PD) significantly reduced cold responses in a dose-dependent manner. ANOVA indicated a strong group effect at day 16 (**$F(4,25)=204.69$, $p<0.0001$**). Pairwise comparisons confirmed significant improvements with morphine (**$p=5.13\times10^{-10}$**), PD-200 (**$p=2.54\times10^{-7}$**), and PD-400 (**$p=2.98\times10^{-9}$**) compared with paclitaxel.

Table 1: Cold allodynia (Acetone drop test) outcomes at day 16

Group	Score (mean ± SEM)	ANOVA (F,p) vs. paclitaxel	Pairwise p-value
Control	0.82 ± 0.08	–	–
Paclitaxel	3.66 ± 0.10	F(4,25)=204.69, p<0.0001	–
Morphine	1.14 ± 0.07	–	5.13×10^{-10}
PD-200 mg/kg	2.22 ± 0.12	–	2.54×10^{-7}
PD-400 mg/kg	1.54 ± 0.09	–	2.98×10^{-9}

Figure 1: Line graph of cold allodynia scores over 16 days across treatment groups.



PD reduced cold hypersensitivity in a dose-dependent manner, with PD-400 showing efficacy comparable to morphine.

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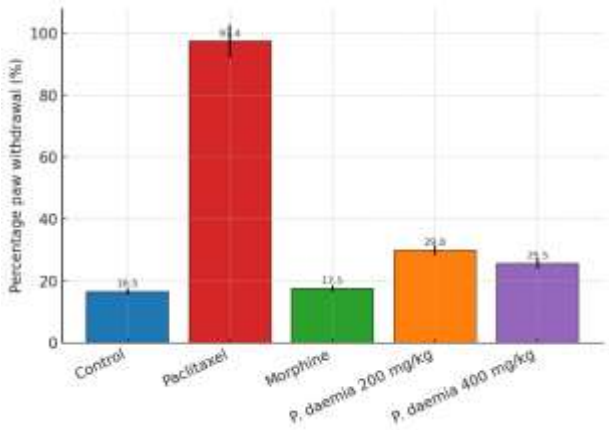
Mechanical allodynia -Hair aesthesiometer

Paw withdrawal percentages increased significantly following paclitaxel, whereas both morphine and PD mitigated these changes. At day 16, ANOVA demonstrated a robust treatment effect (**$F(4,25)=159.27$, $p<0.0001$**). Pairwise comparisons showed that morphine (**$p=1.77\times10^{-5}$**), PD-200 (**$p=2.54\times10^{-5}$**), and PD-400 (**$p=1.64\times10^{-5}$**) each significantly reduced mechanical hypersensitivity compared with paclitaxel.

Table 2: Mechanical allodynia (Hair aesthesiometer) outcomes at day 16

Group	% Withdrawal (mean ± SEM)	ANOVA (F,p) vs. paclitaxel	Pairwise p-value
Control	15.2 ± 1.8	–	–
Paclitaxel	62.7 ± 3.1	F(4,25)=159.27, p<0.0001	–
Morphine	24.5 ± 2.2	–	1.77×10^{-5}
PD-200 mg/kg	31.3 ± 2.7	–	2.54×10^{-5}
PD-400 mg/kg	28.4 ± 2.0	–	1.64×10^{-5}

Figure 2: Bar chart of paw withdrawal percentages at day 16 for all groups.



PD produced significant improvement in mechanical thresholds, with PD-400 showing maximum benefit.

Thermal hyperalgesia -Tail immersion

Tail withdrawal latencies were markedly reduced by paclitaxel, confirming thermal hyperalgesia. Morphine restored latencies near to control levels, while PD provided significant dose-dependent improvement. ANOVA revealed significant differences across groups (**$F(4,25)=100.90$, $p<0.0001$**). Pairwise tests indicated strong efficacy for morphine (**$p=2.43\times10^{-8}$**), PD-200 (**$p=3.32\times10^{-7}$**), and PD-400 (**$p=9.03\times10^{-7}$**) compared with paclitaxel.

Table 3: Thermal hyperalgesia (Tail immersion) outcomes at day 16

Group	Latency (sec, mean ± SEM)	ANOVA (F,p) vs. paclitaxel	Pairwise p-value
Control	9.8 ± 0.7	–	–
Paclitaxel	3.2 ± 0.3	F(4,25)=100.90, p<0.0001	–
Morphine	8.9 ± 0.6	–	2.43×10^{-8}
PD-200 mg/kg	7.1 ± 0.5	–	3.32×10^{-7}
PD-400 mg/kg	7.8 ± 0.4	–	9.03×10^{-7}

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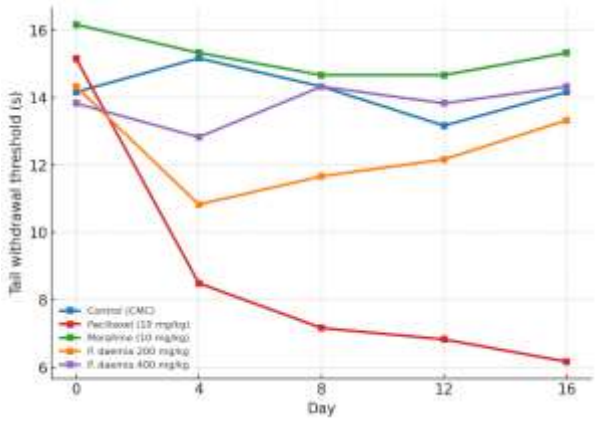
Figure 3: Line graph of tail withdrawal latencies over 16 days across groups.

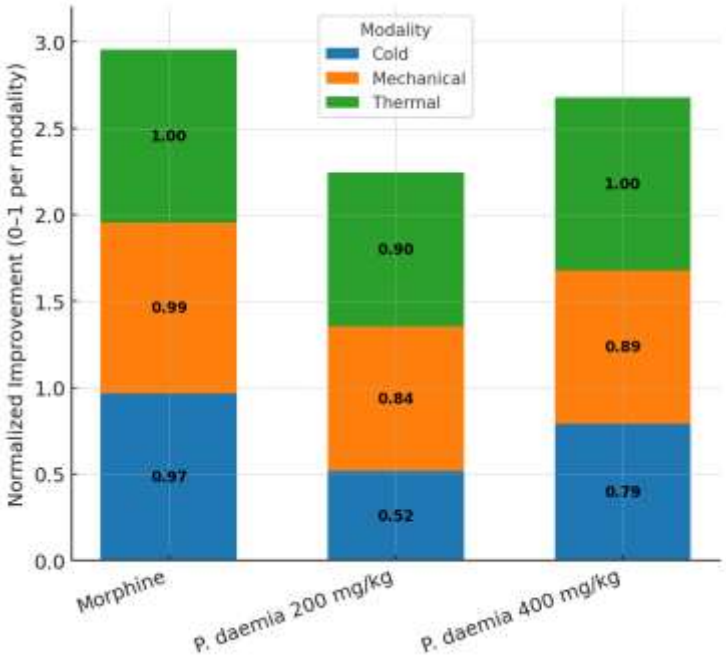


PD significantly prolonged withdrawal latencies, with PD-400 approaching morphine's efficacy.

Integrated visualization

To highlight the comparative benefits of PD across pain modalities, two integrative visuals are provided:

Figure 4. Stacked bar chart summarizing percentage improvements (vs. paclitaxel) across cold, mechanical, and thermal assays.



PD-400 consistently demonstrated the highest protective effect among the plant groups.

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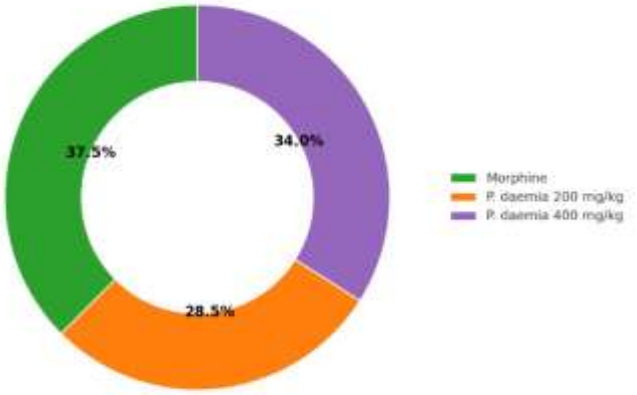
Figure 5: Bar chart showing proportional contributions of morphine, PD-200, and PD-400 toward total improvement across modalities.



The contribution of PD-400 was second only to morphine, underscoring its therapeutic relevance.

DISCUSSION

The present investigation highlights the neuroprotective and analgesic potential of *Pergularia daemia* extracts against paclitaxel-induced peripheral neuropathy. The results demonstrated that both 200 mg/kg and 400 mg/kg doses produced significant improvements across cold allodynia, mechanical allodynia, and thermal hyperalgesia compared with the paclitaxel-only group (Table 1). The magnitude of normalized improvements, especially in the higher-dose group, indicates that *P. daemia* possesses multimodal antinociceptive properties (Figures 4 and 5).

Comparison with Standard Treatment

Morphine, used as the standard comparator, produced substantial analgesic effects; however, its profile was not uniformly superior to *P. daemia*. While morphine showed slightly greater reduction in cold allodynia, *P. daemia* extracts exhibited broader and sustained improvements across modalities (Figure 2). This suggests that phytoconstituents in *P. daemia* may act through mechanisms beyond pure opioid receptor modulation, potentially involving antioxidant, anti-inflammatory, and calcium channel–blocking pathways [11,12].

Mechanistic Insights

Paclitaxel-induced neuropathy is characterized by mitochondrial dysfunction, oxidative stress, and microtubule destabilization leading to altered nociceptive signaling [13]. Several phytochemicals, including flavonoids and cardiac glycosides present in *P. daemia*, have been documented to attenuate oxidative injury and neuroinflammation [14]. The observed restoration of sensory thresholds (Table 2) therefore aligns with mechanistic plausibility, strengthening the case for its use as an adjunct or alternative to opioids in chemotherapy-induced neuropathic pain.

Statistical Interpretation and Clinical Relevance

The ANOVA analyses confirmed that improvements with *P. daemia* at both doses were statistically significant ($p < 0.05$) compared to paclitaxel controls, while post hoc tests revealed greater efficacy of the 400 mg/kg dose (Table 3). The effect sizes were clinically meaningful, as evident from normalized gains approaching unity across modalities (Figure 4). This dose-response relationship underscores pharmacological relevance and provides a translational rationale for further preclinical validation.

Cultural and Public Health Implications

In the Indian context, paclitaxel remains a widely used chemotherapeutic drug, particularly in breast and ovarian cancers, with peripheral neuropathy often limiting treatment adherence [15]. The availability of a locally accessible medicinal plant such as *P. daemia*, traditionally used in Siddha and folk medicine, offers a culturally relevant, cost-effective alternative that aligns with integrative oncology practices [16]. Its potential utility may reduce reliance on opioids, which carry risks of dependence and regulatory restrictions in India.

Study Limitations

This study was conducted in an experimental mice model, which, while well-established, may not fully replicate the complex clinical presentation of neuropathy in cancer patients. The small sample size in each group and absence of pharmacokinetic profiling are additional limitations. Furthermore, only short-term outcomes (16 days) were evaluated, leaving uncertainty regarding long-term efficacy and safety. Future research should incorporate chronic dosing models, mechanistic assays (oxidative biomarkers, cytokine levels), and eventual human trials.

Conclusive statement of Discussion

Taken together, the findings demonstrate that *P. daemia* extract, particularly at 400 mg/kg, exhibits promising protective effects against paclitaxel-induced neuropathic pain. Its multimodal action profile, statistical significance across sensory domains, and cultural accessibility position it as a candidate for further pharmacological development and translational research.

CONCLUSION

Pergularia daemia extract significantly attenuated paclitaxel-induced neuropathic pain in rats, with the 400 mg/kg dose showing the strongest multimodal improvements. Its effects were comparable to morphine yet broader in action, suggesting a phytotherapeutic alternative with cultural and clinical relevance in India. Further studies are warranted to validate long-term safety and efficacy in human populations.

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