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Research Article

NEUROPHARMACOLOGICAL EVALUATION OF SEDATIVE HYPNOTIC AND ANXIOLYTIC EFFECTS OF ETHANOLIC LEAF EXTRACT OF *PLUMERIA PUDICA* IN EXPERIMENTAL MICE

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ABSTRACT

The present study evaluated the phytochemical constituents and sedative-hypnotic activity of the ethanolic leaf extract of *Plumeria pudica* Jacq. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, phenolic compounds, tannins, and glycosides. Acute oral toxicity was assessed according to OECD guideline 423 and experimental doses were selected accordingly. Sedative hypnotic activity was evaluated in Swiss albino mice using phenobarbitone- and thiopental-induced sleep models, while locomotor and anxiolytic activities were assessed using open field, elevated plus maze, and rota-rod tests. The extract produced a dose-dependent reduction in sleep onset latency and an increase in sleep duration, along with decreased locomotor activity and anxiolytic effects. The findings support the traditional use of *Plumeria pudica* in sleep-related disorders and indicate its potential as a natural sedative hypnotic agent.

Keywords: *Plumeria pudica*, Insomnia, Sedative-hypnotic activity, Phytochemical screening, Anxiolytic activity.

INTRODUCTION

Insomnia is one of the most common sleep disorders, characterized by difficulty in initiating or maintaining sleep, leading to impaired daytime functioning and reduced quality of life (Morin & Benca, 2012). Chronic insomnia is associated with neuropsychiatric disorders, cardiovascular complications, metabolic disturbances, and increased healthcare burden. Although non-pharmacological interventions such as cognitive behavioural therapy are considered first-line management, pharmacological treatment remains necessary in many patients with persistent or severe symptoms (Morin & Benca, 2012; Roehrs & Roth, 2012). Conventional sedative-hypnotic drugs, including benzodiazepines and non-benzodiazepine hypnotics, are effective in the short-term management of insomnia. However, their long-term use is limited by adverse effects such as tolerance, dependence, withdrawal symptoms, cognitive impairment, and residual daytime sedation (Roehrs & Roth, 2012). These limitations have stimulated increasing interest in identifying safer alternatives with fewer side effects for the management of sleep disorders (WHO, 2013).

Medicinal plants have been traditionally used for the treatment of insomnia and other central nervous system disorders due to their perceived safety and multi-target therapeutic effects. Several plant-derived phytoconstituents, including flavonoids, alkaloids, saponins, and phenolic compounds, have been reported to exert sedative, anxiolytic, and hypnotic activities, often through modulation of the GABAergic neurotransmission pathway (Singh & Sharma, 2018; Rang & Dale, 2019). Despite their widespread traditional use, many medicinal plants lack systematic pharmacological validation. *Plumeria pudica* Jacq. is an ornamental plant traditionally used in folk medicine for the management of anxiety, inflammation, and sleep-related disturbances. Preliminary phytochemical studies have indicated the presence of bioactive constituents that may contribute to central nervous system depressant activity. However, scientific evidence supporting its sedative-hypnotic potential remains limited (Choudhary et

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al., 2015; Mukherjee & Kevalia, 2025). Therefore, the present study was undertaken to evaluate the phytochemical profile and sedative-hypnotic activity of the ethanolic extract of *Plumeria pudica* leaves using established experimental models in mice.

MATERIALS AND METHODS

Plant material and extraction

Leaves of *Plumeria pudica* Jacq. were collected from Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, Andhra Pradesh and authenticated by a qualified botanist. The collected leaves were washed, shade-dried at room temperature, and coarsely powdered. The powdered material was subjected to ethanolic extraction using the maceration method. The extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain a semisolid mass, which was stored in an airtight container until further use.

Phytochemical screening

Preliminary phytochemical screening of the ethanolic extract was carried out using standard qualitative tests to identify the presence of major phytoconstituents such as alkaloids, flavonoids, saponins, tannins, phenolic compounds, glycosides, and terpenoids (Nadendla, 2016).

Experimental animals

Healthy adult Swiss albino mice of either sex, weighing 20-30 g, were used for the study. Animals were housed under standard laboratory conditions with a 12 h light-dark cycle, controlled temperature (22-25°C), and free access to standard pellet diet and water. All experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Ethical approval

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee under CCSEA guidelines (Approval No 1048/PO/Re/S/07/CCSEA). All efforts were made to minimize animal suffering and the number of animals used.

Acute oral toxicity study

Acute oral toxicity of the ethanolic extract of *Plumeria pudica* was evaluated according to OECD guideline 423. Mice were administered the extract orally and observed for signs of toxicity and mortality for 14 days. Based on the results, experimental doses of 200, 400, and 600 mg/kg were selected for pharmacological evaluation (OECD, 2001).

Experimental design

Animals were randomly divided into groups, each consisting of six mice. Group I. Control group received normal saline. Group II. Standard group received diazepam (4 mg/kg). Groups III-V. Test groups received ethanolic extract of *Plumeria pudica* at doses of 200, 400, and 600 mg/kg orally

Evaluation of sedative-hypnotic activity

Sedative-hypnotic activity was assessed using phenobarbitone- and thiopental-induced sleeping time tests. Sleep onset latency and total sleep duration were recorded following administration of the test extract or standard drug (Kulkarni, 2012; Vogel, 2008).

Evaluation of anxiolytic and locomotor activity

Anxiolytic and locomotor activities were evaluated using the open field test, elevated plus maze, hole-board test, and rota-rod test according to standard experimental procedures (File & Wardill, 2001; Kulkarni, 2012). Parameters such as locomotor activity, time spent in open arms, head-dipping behavior, and motor coordination were recorded (Pal & Dutta, 2011).

Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnett's post-hoc test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Acute oral toxicity studies conducted according to OECD guideline 423 showed no mortality or significant behavioral changes in mice up to the tested dose level. Based on these findings, doses of 200, 400, and 600 mg/kg were selected for further pharmacological evaluation. Preliminary phytochemical screening of the ethanolic extract of *Plumeria pudica* leaves revealed the presence of flavonoids, alkaloids, saponins, phenolic compounds, tannins, and glycosides. Evaluation of motor coordination using the rota-rod test showed a mild reduction in fall-off time in extract-treated groups at higher doses when compared to the control group. In the open field test, mice treated with the ethanolic extract showed a significant reduction in locomotor activity, as indicated by a decrease in the number of lines crossed when compared to the control group. The reduction in activity was dose dependent.

Table 1. Phytochemical screening of *Plumeria pudica* leaves.

S. No	Test Performed	Interference
1.	Alkaloids	-
2.	Carbohydrates	+
3.	Cardiac glycosides	-

4.	Proteins and amino acids	+
5.	Tannins and Phenolic compounds	+
6.	Steroids and sterols	+
7.	Triterpenoids	-
8.	Saponins	+

Table 2. Acute toxicity effects of ethanolic extract of *Plumeria pudica Jacq* in mice at various doses.

Treatment	Dose (mg/kg)	Mortality after 72 hours	Percent Mortality (%)
Saline	0.9%	---	----
Ethanolic extract of *P lumeria pudica Jacq*	250	0/6	0
	500	0/6	0
	1000	0/6	12.34
	1500	1/6	16.66
	2000	1/6	16.66

Table 3. Central / Skeletal Muscle Relaxant Property of leaves of *Plumeria pudica Jacq*. Cass in mice.

Group Dose mg/kg(p.o.)	Treatment (mg/kg), p.o.	Time spent on revolving rod (in seconds)
Normal Control	Saline	207.3±2.35
Positive Control	Diazepam (4mg/kg) i.p	13.64±0.49
Ethanolic extract of *Plumeria pudica Jacq*	200 mg/kg	36.01±2.34
	400 mg/kg	89.26±1.29
	600mg/kg	45.29±1.27

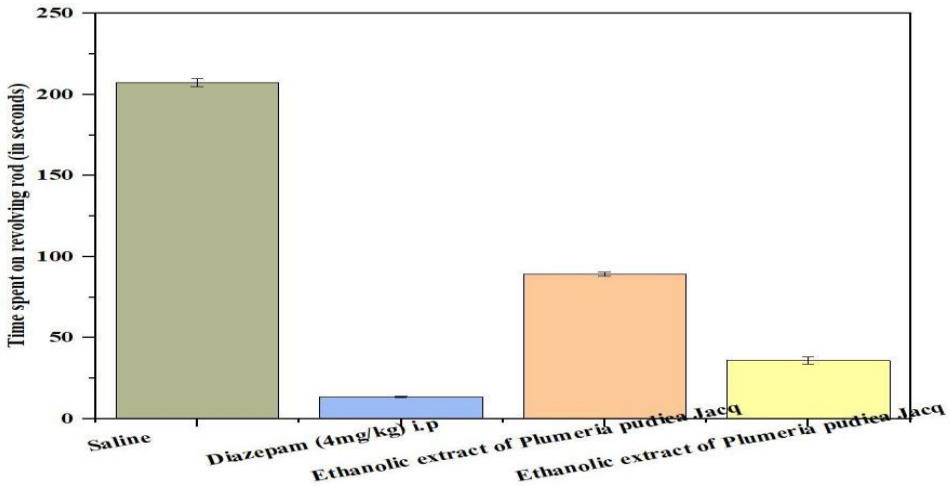

Figure 1. Skeletal Muscle Relaxant Property of leaves of *Plumeria pudica Jacq.* Cass in mice.

Administration of the ethanolic extract of *Plumeria pudica* produced a dose-dependent reduction in sleep onset latency and a significant increase in total sleeping time in phenobarbitone- and thiopental-induced sleep models when compared with the control group. The effects observed at higher doses were statistically significant and comparable to the standard drug diazepam.

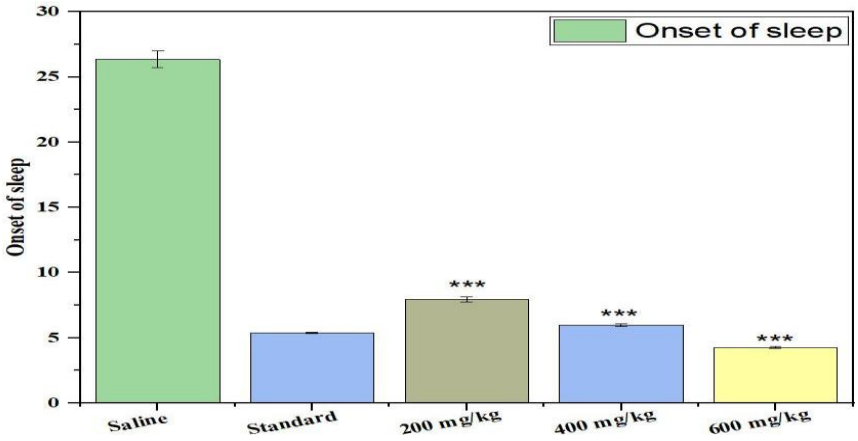

Figure 2. Effect of the ethanolic extract of *Plumeria pudica* Jacq. on (A) sleep onset latency and (B) total sleeping time in thiopental sodium-induced sleep-in mice. Values are expressed as mean ± SEM (n = 6). Control group received saline (10 mL/kg), standard group received diazepam, and test groups received ethanolic extract of *Plumeria pudica* Jacq. at doses of 200, 400, and 600 mg/kg.

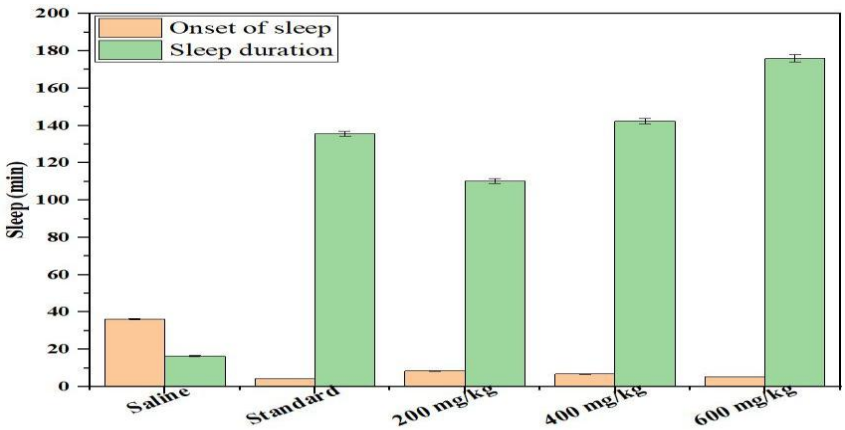

Figure 3. Effect of the ethanolic extract of *Plumeria pudica* Jacq. on (A) sleep onset latency and (B) total sleeping time in phenobarbitone-induced sleep-in mice. Values are expressed as mean ± SEM (n = 6). Control group received saline (10 mL/kg), standard group received diazepam, and test groups received ethanolic extract of *Plumeria pudica* Jacq. at doses of 200, 400, and 600 mg/kg.

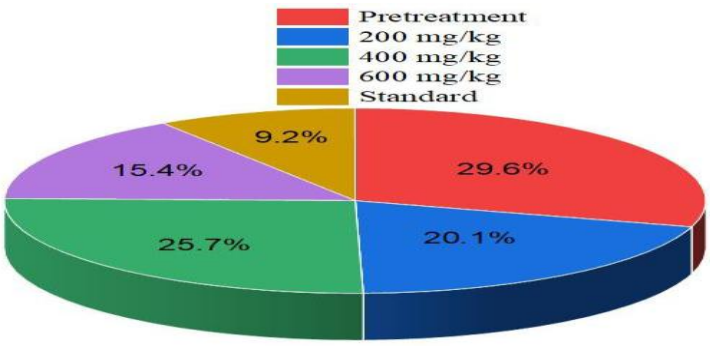

Figure 4. Effect of the ethanolic extract of *Plumeria pudica* Jacq. on motor coordination in mice assessed using the rota-rod test. Animals were evaluated 30 min after administration of the ethanolic extract or vehicle and 15 min after administration of diazepam. Fall-off time was recorded during a 180 s observation period. Values are expressed as mean ± SEM (n = 6).

Table 4. Effect of ethanolic extract of *Plumeria pudica Jacq* in open field test in experimental animals.

Treatment	Dose (mg/kg)	No. of Lines Crossed in 5 min
Saline	10 (mL/kg)	28.39±0.64
Standard	4	14.57±0.24***
Extract	200	20.46±0.95**
	400	28.94±1.02**
	600	15.43±0.88***

In the elevated plus maze test, treatment with the ethanolic extract resulted in a significant increase in the time spent in the open arms compared to the control group. These effects were dose dependent and statistically significant at higher doses.

Table 5. Anxiolytic activity of leaves extract of Plumeria pudica Jacq by elevated plus maze apparatus in mice.

Group	Treatment (mg/kg), p.o.	Time Spent in Closed Arm (Sec.) (2nd Day)	Time Spent in Closed Arm (Sec.) (7th Day)	Time Spent in Open Arm (Sec.) (2ndDay)	Time Spent in Open Arm (Sec.) (7th Day)
Normal Control	Saline	269.35±1.25	264.31±3.27	45.26±0.69	39.52±0.36
Positive control	Diazepam (4mg/kg)	145.28±3.62	110.45±2.61	95.68±1.24	46.28±0.52
Plumeria pudica Jacq	200 mg/kg	201.34±1.02	215.84±1.05	53.21±3.25	39.46±0.14
	400 mg/kg	225.49±2.04	236.19±2.04	88.49±1.26	49.57±0.58
	600 mg/kg	195.36±1.24	203.16±1.22	75.94±2.14	40.21±0.39

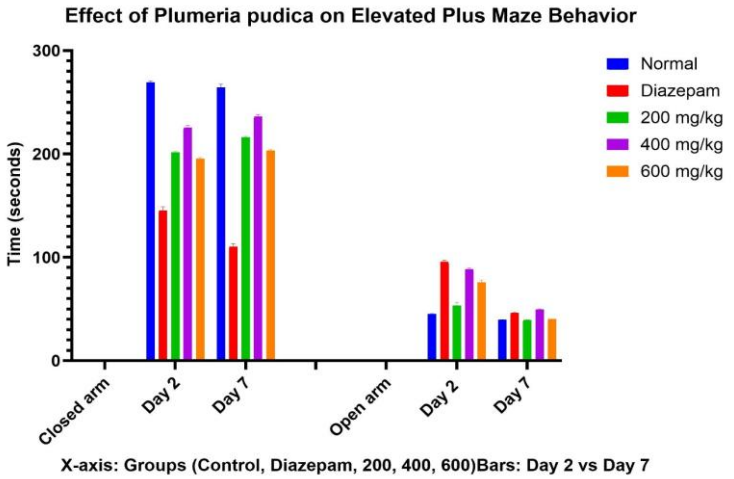

Figure 5. Effect of *Plumeria pudica* extract on time spent in closed arms (seconds) on Day 2 and Day 7 in Elevated Plus Maze model. Data expressed as Mean ± SEM (n=6). Effect of *Plumeria pudica* extract on time spent in open arms (seconds) on Day 2 and Day 7 in Elevated Plus Maze model. Data expressed as Mean ± SEM (n=6).

The present study evaluated the phytochemical composition and sedative-hypnotic activity of the ethanolic extract of *Plumeria pudica* leaves using established experimental models in mice. The findings demonstrate that the extract possesses significant sedative, hypnotic, and anxiolytic activities, as evidenced by prolonged sleeping time, reduced sleep latency, decreased locomotor activity, and increased exploratory behavior in anxiety-related tests. The sedative-hypnotic effect observed in phenobarbitone- and thiopental-induced sleep models suggests a central nervous system depressant action of the extract. Barbiturate-induced sleep models are widely used to assess sedative and hypnotic agents, as enhancement of sleep duration and reduction of sleep onset indicate potentiation of inhibitory neurotransmission in the brain. The dose-dependent effects observed in the present study further support the pharmacological relevance of the extract. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, phenolic compounds, tannins, and glycosides in the ethanolic extract of *Plumeria pudica*. Flavonoids and alkaloids are known to exert sedative and anxiolytic effects through interaction with the

GABA_A receptor complex and modulation of neurotransmitter release. Phenolic compounds and saponins have also been reported to contribute to central nervous system depressant activity and antioxidant protection, which may indirectly influence sleep regulation.

The reduction in locomotor activity observed in the open field test indicates sedative properties of the extract, while increased time spent in the open arms of the elevated plus maze suggests anxiolytic activity. These findings are consistent with previous reports on plant-derived compounds exhibiting CNS depressant and anxiolytic effects. The mild impairment observed in the rota-rod test at higher doses further supports the muscle relaxant and sedative action of the extract. Overall, the results of the present study provide scientific support for the traditional use of *Plumeria pudica* in the management of sleep-related disorders. However, the exact mechanisms underlying its sedative-hypnotic effects remain to be elucidated. Further studies involving isolation of active constituents, receptor-binding assays, and molecular mechanism investigations are required to substantiate these findings and to explore the therapeutic potential of *Plumeria pudica* in insomnia management.

CONCLUSION

The present study demonstrates that the ethanolic extract of *Plumeria pudica* leaves possesses significant sedative-hypnotic and anxiolytic activities in experimental mice. These effects are supported by behavioral and pharmacological findings and may be attributed to the presence of bioactive phytoconstituents such as flavonoids and alkaloids. The results provide scientific validation for the traditional use of *Plumeria pudica* in sleep-related disorders. Further investigations involving isolation of active compounds and detailed mechanistic studies are warranted to confirm its therapeutic potential in the management of insomnia.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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