e - ISSN - 2249-7668
Print ISSN - 2249-7676

International Journal of
Pharmacology & Toxicology

www.ijpt.org

EXPLORING THE ANTI-INFLAMMATORY EFFICACY OF PAVONIA PROCUMBENS METHANOL EXTRACT IN PRECLINICAL MODELS

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ABSTRACT

Natural products have long been a cornerstone of medicine, with their relevance persisting into modern pharmacology, especially for addressing chronic and inflammatory conditions. This study evaluates the anti-inflammatory potential of the methanol extract of *Pavonia procumbens* leaves using the formalin-induced paw edema model in Swiss albino mice. The phytochemical screening revealed significant phenolic content (243.94 ± 53.28 mg/g), known for modulating inflammatory pathways. Acute toxicity studies demonstrated the extract's safety at a dose of 2000 mg/kg with no observed adverse effects. Anti-inflammatory activity was assessed in two doses (200 mg/kg and 400 mg/kg), compared against diclofenac (10 mg/kg) as a standard. The extract showed a dose-dependent reduction in paw edema, with the higher dose achieving a 61.72 ± 0.19% inhibition of edema at 240 minutes, comparable to diclofenac's 64.84 ± 0.13%. The anti-inflammatory effects are attributed to bioactive compounds such as phenolics, flavonoids, and tannins, which likely inhibit pro-inflammatory mediators like prostaglandins, cytokines, and nitric oxide. These findings highlight the therapeutic potential of *Pavonia procumbens* as a natural alternative to synthetic anti-inflammatory drugs, offering comparable efficacy with fewer side effects. Further research is warranted to elucidate the extract's molecular mechanisms and pharmacokinetics to ensure clinical applicability. With continued study, *Pavonia procumbens* could become a valuable addition to plant-based anti-inflammatory therapies

Keywords: *Pavonia procumbens*, anti-inflammatory, phenolic content, paw edema, natural therapeutics.

INTRODUCTION

Natural products have been essential in the evolution of medicine, providing therapeutic solutions for centuries. Despite the shift toward synthetic drugs during the Industrial Revolution, natural products remain significant, particularly for chronic and inflammatory diseases. Approximately 25% of prescription drugs worldwide are derived from natural sources, such as digoxin, quinine, vincristine, and morphine. Additionally, many synthetic drugs rely on natural precursors, highlighting the enduring relevance of natural compounds in modern pharmacology. Herbal medicine, a key component of traditional medical systems like Ayurveda, Siddha, Unani, and Traditional Chinese Medicine (TCM), continues to play a vital role in healthcare, especially in rural areas where it is often the primary resource. India's biodiversity supports over 45,000 plant species, with around 8,000 having documented medicinal properties [1]. These plants are widely used due to their perceived safety, efficacy, and affordability, forming the foundation for traditional and modern treatments. Inflammation, a complex physiological response to injury or infection, is involved in various chronic conditions such as arthritis, diabetes, and cardiovascular diseases. While synthetic drugs like NSAIDs and corticosteroids are effective, their prolonged use is associated with adverse effects. This has driven interest in exploring natural alternatives with fewer side effects. Plants like *Curcuma longa*, *Boswellia serrata*, and *Phyllanthus emblica* are known for their anti-inflammatory properties, attributed to bioactive

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compounds like alkaloids, flavonoids, and polyphenols [2]. This study evaluates the anti-inflammatory potential of the methanol extract of *Pavonia procumbens* leaves using the formalin-induced paw edema model in Swiss albino mice. It aims to extract the leaves, determine the phenolic content, conduct acute toxicity studies, and assess the extract's efficacy. By integrating traditional knowledge with scientific inquiry, this research seeks to develop safer, natural anti-inflammatory alternatives, advancing the field of natural product-based drug discovery.

MATERIALS AND METHODS

Plant Material Collection and Authentication

The leaves of *Pavonia procumbens* were collected from Tirumala, Andhra Pradesh, India. The plant was identified and authenticated by Dr. K. Madhava Chetty, Principal Scientist (Economic Botany), National Bureau of Plant Genetic Resources, Hyderabad. The leaves were washed, dried in the shade at room temperature, and stored in an airtight container until use.

Preparation of Methanol Extract

The dried leaves were powdered and sieved through a 60-mesh sieve. About 50 g of the powdered leaves were dewaxed with petroleum ether to remove chlorophyll. Subsequently, the powder was extracted with methanol using a Soxhlet apparatus for 72 hours. The methanol extract was evaporated to dryness using a rotary evaporator at a temperature not exceeding 60°C and stored in a desiccator until further use [3].

Phytochemical Screening

The methanol extract was subjected to preliminary phytochemical screening for various bioactive compounds such as carbohydrates, proteins, tannins, flavonoids, saponins, and steroids using standard qualitative tests [4].

Determination of Total Phenolic Content

Total phenolic content was estimated colorimetrically using the Folin-Ciocalteu reagent. Quercetin was used as the standard for calibration. For sample preparation, 0.5 g of the methanol extract was dissolved in water, and 0.1 ml of this solution was mixed with 1.25 ml of Folin-Ciocalteu reagent. After five minutes, 2.5 ml of 20% sodium carbonate solution was added, and the mixture was allowed to react for 30 minutes. The absorbance was measured at 765 nm, and total phenolic content was expressed as quercetin equivalents [5].

Animals and Housing

Swiss albino mice (6-8 weeks old, 20-25 g) were used for the study. Animals were housed in groups under standard laboratory conditions: a temperature of 22°C ± 3°C, relative humidity of 50-60%, and a 12-hour light/dark cycle [6]. They were provided with standard laboratory diets and water ad libitum. The experimental protocol adhered to ethical guidelines for animal care and use.

Acute Oral Toxicity Study

The acute oral toxicity study was conducted according to OECD Guideline 423. Mice were fasted overnight and administered a single oral dose of the methanol extract (2000 mg/kg) using a gavage needle. Observations for toxic effects, including changes in skin, fur, behavior, and mortality, were recorded over 14 days. Body weights were monitored on days 1, 7, and 14. Food and water intake were also recorded [7].

Formalin-Induced Paw Edema Model

The anti-inflammatory activity of the methanol extract was evaluated using the formalin-induced paw edema model in Swiss albino mice. Acute inflammation was induced by subplantar injection of 0.05 ml of 10% formalin in the right hind paw of each mouse [8]. The animals were divided into five groups:

1. Normal group: Received normal saline (0.9% p.o).
2. Control group: Received formalin only.
3. Standard group: Received formalin and diclofenac sodium (10 mg/kg p.o).
4. Test-1 group: Received formalin and methanol extract (200 mg/kg p.o).
5. Test-2 group: Received formalin and methanol extract (400 mg/kg p.o).

The paw volume was measured using a plethysmometer at 0, 1, 2, 3, and 4 hours post-formalin injection. The percentage inhibition of paw edema was calculated using the following formula:

$$\%\text{Inhibition} = \left(\frac{\Delta\text{Control} - \Delta\text{Test}}{\Delta\text{Control}}\right) \times 100$$

Statistical Analysis

Data were expressed as mean ± SEM. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. A p-value of <0.05 was considered statistically significant. The analysis was performed using Graph Prism 5.0 software [9].

RESULTS

Total Phenol Content

The methanol extract of *Pavonia procumbens* leaves exhibited a phenol content of 243.94 ± 53.28 mg/g of extract, as determined by the Folin-Ciocalteu method. This high phenolic content suggests the presence of bioactive compounds contributing to its anti-inflammatory properties.

Acute Toxicity Study

In the acute toxicity study, mice administered with 2000 mg/kg of the methanol extract showed no mortality or significant behavioral changes. There was no statistical difference in body weight, food intake, or water

consumption compared to the control group, indicating that the extract is safe for oral administration.

Formalin-Induced Paw Edema Model

The anti-inflammatory activity of the methanol extract was assessed in the formalin-induced paw edema model in Swiss albino mice. Two doses of the extract (200 mg/kg and 400 mg/kg) were tested against a control group (formalin only) and a standard group treated with diclofenac (10 mg/kg).

Paw Diameter

Table 1 summarizes the changes in paw diameter over time. The control group showed a significant increase in paw diameter, peaking at 120 minutes (4.45 ± 0.028 mm). In contrast, the standard group and the high dose of the extract (400 mg/kg) significantly reduced paw diameter, showing near-normal values at 240 minutes (2.12 ± 0.025 mm and 2.15 ± 0.028 mm, respectively). The low dose (200 mg/kg) also demonstrated a reduction in paw diameter but to a lesser extent than the high dose or diclofenac.

Table 1: Effect of MPP on paw diameter of mice

Group	0 min	60 min	120 min	180 min	240 min
Normal	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
Control	2.15 ± 0.028	4.05 ± 0.064	4.45 ± 0.028	4.25 ± 0.047	3.70 ± 0.040
Standard	2.20 ± 0.040***	2.65 ± 0.047***	2.40 ± 0.040***	2.25 ± 0.028***	2.12 ± 0.025***
Test-1 (200 mg/kg)	2.27 ± 0.025***	3.32 ± 0.070***	3.25 ± 0.075***	3.17 ± 0.062***	3.0 ± 0.040***
Test-2 (400 mg/kg)	2.20 ± 0.040***	2.75 ± 0.085***	2.55 ± 0.064***	2.25 ± 0.025***	2.15 ± 0.028***

Values are expressed as mean ± SEM (n = 6). ***p<0.001 compared to the control group.

Paw Edema Volume

The effect of the extract on paw edema is presented in Table 2. Both doses of the extract significantly reduced paw edema compared to the control group. The high dose (400 mg/kg) showed results comparable to diclofenac, with paw edema at 240 minutes measuring 0.30 ± 0.040 ml versus 0.275 ± 0.025 ml for the standard group.

Table 2: Effect of MPP on paw edema of mice

Group	0 min	60 min	120 min	180 min	240 min
Normal	0.2 ± 0.00	0.2 ± 0.00	0.2 ± 0.00	0.2 ± 0.00	0.2 ± 0.00
Control	0.225 ± 0.025	0.70 ± 0.040	0.875 ± 0.025	0.75 ± 0.028	0.65 ± 0.028
Standard	0.25 ± 0.028***	0.50 ± 0.040***	0.425 ± 0.025***	0.35 ± 0.228***	0.275 ± 0.025***
Test-1	0.275 ± 0.025*	0.75 ± 0.028*	0.75 ± 0.028*	0.575 ± 0.047*	0.475 ± 0.047*
Test-2	0.225 ± 0.025***	0.60 ± 0.040***	0.50 ± 0.040***	0.40 ± 0.040***	0.30 ± 0.040***

Values are expressed as mean ± SEM (n = 6). ***p<0.001, **p<0.01, *p<0.05 compared to the control group.

Percentage Inhibition of Paw Edema

The high dose of the extract showed significant inhibition of paw edema, with a percentage inhibition of 61.72 ± 0.19% at 240 minutes, comparable to diclofenac's 64.84 ± 0.13%. The low dose demonstrated moderate inhibition, reaching 47.18 ± 0.26% at 240 minutes.

Table 3: Effect of MPP on the % inhibition of paw edema

Groups	% inhibition of edema				
	0min	60min	120min	180min	240min
Diclofenac	46.18±0.25	54.83±0.25	63.46±0.36	64.49±0.13	64.84±0.13
Test 1	41.10±0.27***	44.80±0.22***	46.97±0.27***	43.63±0.36***	47.18±0.26***
Test 2	44.49±0.42***	45.87±0.36***	63.18±0.39^{ns}	64.22±0.18^{ns}	61.72±0.19***

The methanol extract of *Pavonia procumbens* exhibited significant anti-inflammatory activity in the formalin-induced paw edema model. Both doses reduced paw edema and diameter compared to the control, with the high dose showing comparable effects to diclofenac. These results suggest the extract's potential as a natural anti-inflammatory agent.

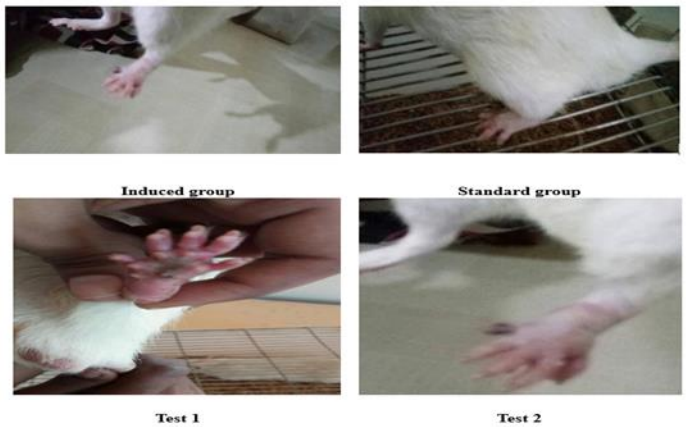

Figure 1: Representative images of paw edema reduction in different groups.

DISCUSSION

The present study evaluated the anti-inflammatory potential of the methanol extract of *Pavonia procumbens* leaves using the formalin-induced paw edema model in Swiss albino mice. The results demonstrated significant anti-inflammatory activity, attributed to the extract's rich phytochemical composition, particularly its high phenolic content of 243.94 ± 53.28 mg/g [10]. Phenolic compounds, recognized for their antioxidant and anti-inflammatory properties, likely modulate inflammatory pathways by inhibiting pro-inflammatory mediators like prostaglandins, cytokines, and nitric oxide. The acute toxicity study confirmed the safety of the extract at a dose of 2000 mg/kg, with no mortality or adverse effects observed. In the formalin-induced paw edema model, the methanol extract significantly reduced paw edema volume and diameter in a dose-dependent manner [11]. The high dose (400 mg/kg) exhibited comparable efficacy to diclofenac, a standard NSAID, achieving 61.72 ± 0.19% inhibition of paw edema at 240 minutes. This suggests the extract's ability to inhibit both histamine and serotonin release (early phase) and prostaglandin synthesis (late phase) [12]. The observed anti-inflammatory effects are likely due to the synergistic action of phytoconstituents like phenolic compounds, flavonoids, and tannins, which scavenge free radicals, inhibit COX enzymes, and downregulate inflammatory cytokines [13, 14]. The findings emphasize *Pavonia procumbens* as a promising natural alternative to synthetic anti-inflammatory drugs, offering comparable efficacy with fewer side effects [15, 16]. Further studies, including molecular and pharmacokinetic evaluations, are recommended to elucidate its precise mechanisms and ensure clinical applicability. With continued research, *Pavonia procumbens* has the potential to meet the demand for safer, plant-based anti-inflammatory therapies.

CONCLUSION

This study highlights the significant anti-inflammatory potential of the methanol extract of *Pavonia procumbens* leaves, evaluated using the formalin-induced paw edema model in Swiss albino mice. The high phenolic content of the extract (243.94 ± 53.28 mg/g) suggests the presence of bioactive compounds that effectively modulate inflammatory pathways. Phenolic compounds are well known for their ability to inhibit pro-inflammatory mediators, reduce oxidative stress, and enhance overall anti-inflammatory responses. The extract exhibited no signs of acute toxicity at a dose of 2000 mg/kg, indicating a wide safety margin and feasibility for therapeutic application. In the formalin-induced paw edema model, both tested doses (200 mg/kg and 400 mg/kg) demonstrated significant reductions in paw edema and diameter in a dose-dependent manner. The high dose (400 mg/kg) showed efficacy comparable to diclofenac, a standard NSAID, suggesting the extract's potential as an alternative to synthetic drugs. The results also highlight the dose-dependent anti-inflammatory effects of the extract, with the higher dose achieving 61.72 ± 0.19% inhibition of paw edema, close to diclofenac's 64.84 ± 0.13%. These findings support the traditional use of *Pavonia procumbens* in managing inflammatory conditions and provide a strong foundation for its development as a plant-based therapeutic agent. Further studies are needed to elucidate the molecular mechanisms of action, perform long-term safety evaluations, and explore the pharmacokinetics of the bioactive compounds. With continued research, *Pavonia procumbens* could become a valuable addition to the arsenal of natural anti-inflammatory therapies, offering a safer alternative to conventional treatments.

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