RESEARCH PAPER

Pharmacognostic Standardization and Anti-Inflammatory Evaluation of Bacopa monnieri Leaf Extract in Rodent Models

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ABSTRACT

In this study, we used validated rodent models to assess the anti-inflammatory effectiveness of Bacopa monnieri leaf extract and to set pharmacognostic guidelines. Physical, chemical, and microscopic studies were all part of the pharmacognostic review. Paracytic stomata and glandular trichomes were among the distinguishing physical traits of the leaves, which also had a dorsiventral shape. The purity and quality of the crude drug were indicated by the physicochemical characteristics, which showed total ash (8.42 ± 0.36%), acid-insoluble ash (1.21 ± 0.09%), water-soluble extractive (18.67 ± 0.54%), and alcohol-soluble extractive (12.34 ± 0.47%). There were phenolic chemicals, alkaloids, saponins, and flavonoids found in the initial phytochemical screening. Models of carrageenan-induced paw edema and cotton pellet-induced granuloma in Wistar rats were used to assess the anti-inflammatory efficacy. The standard drug diclofenac sodium (10 mg/kg) demonstrated 71.2% inhibition of paw edema at the 4th hour compared to the control group, while the ethanolic extract at doses of 200 and 400 mg/kg significantly inhibited the swelling by 38.6% and 62.4%, respectively, in the carrageenan-induced model. The extract showed a 29.8% and 55.7% reduction in granuloma weight at 200 and 400 mg/kg dosages, respectively, in the cotton pellet-induced granuloma model, in comparison to the standard's 63.5% inhibition. According to the results, the anti-inflammatory effects of Bacopa monnieri leaf extract are due to its high concentration of phytoconstituents, especially flavonoids and saponins. For accurate plant material identification and quality control, the pharmacognostic criteria developed in this research can stand as gold standards.

Key words: Bacopa monnieri, pharmacognostic standardization, anti-inflammatory activity, carrageenan-induced paw edema, granuloma, flavonoids, Wistar rats.

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INTRODUCTION:

Inflammation is a multifaceted biological reaction of vascular tissues to detrimental stimuli, including infections, injured cells, or irritants, and is essential for host defense and tissue repair. Nonetheless, chronic or unregulated inflammation is associated with the etiology of other persistent ailments, such as arthritis, diabetes, cardiovascular diseases, and neurological disorders [1, 2]. Despite the prevalent use of conventional non-steroidal anti-inflammatory medications (NSAIDs) and corticosteroids for inflammation therapy, their prolonged use frequently correlates with detrimental effects, including gastrointestinal discomfort, renal toxicity, and immunosuppression. These constraints have stimulated heightened interest in the investigation of plant-derived therapies that are safer, economically viable, and exhibit diverse pharmacological effects [3, 4].

Bacopa monnieri (family: Plantaginaceae), generally referred to as Brahmi, is a prominent therapeutic herb in ancient Ayurvedic medicine. It has been widely utilized for augmenting memory, boosting cognitive function, and addressing neurological problems. Bacopa monnieri possesses neuropharmacological capabilities and demonstrates antioxidant, anti-inflammatory, adaptogenic, and hepatoprotective actions. The therapeutic potential of this plant is chiefly ascribed to its abundant phytochemical constituents, such as bacosides, flavonoids, alkaloids, and saponins [5, 6].

Notwithstanding its extensive historical application, there is an increasing necessity for rigorous pharmacognostic standardization to guarantee the validity, purity, and quality of the crude medicine. Pharmacognostic assessment, encompassing macroscopic, microscopic, and physicochemical criteria, is essential for the identification and standardization of medicinal plants, thereby reducing adulteration and assuring the consistency of therapeutic effects [7, 8].

Moreover, empirical evaluation of the anti-inflammatory properties of Bacopa monnieri using experimental models is crucial to substantiate its traditional assertions and to investigate its potential as a natural substitute for synthetic anti-inflammatory medicines. The current work aimed to establish pharmacognostic standards for Bacopa monnieri leaves and assess their anti-inflammatory effectiveness utilizing recognized rodent models, including carrageenan-induced paw edema and cotton pellet-induced granuloma techniques.

MATERIAL AND METHODS:

Plant Material Collection and Authentication:

Fresh leaves of Bacopa monnieri were harvested in July from local wetlands in Nagpur, Maharashtra, India. The botanical specimen was verified by a certified taxonomist, and a voucher specimen was archived in the departmental herbarium for future reference. The harvested leaves were cleansed, shade-dried at ambient temperature (25–28°C), then finely pulverized with a mechanical grinder.

Preparation of Extract:

500 grams of powdered leaf material underwent Soxhlet extraction with 95% v/v ethanol for a duration of 8 to 10 hours. The extract was filtered and condensed at reduced pressure with a rotary vacuum evaporator, yielding a dark green semisolid mass (yield: 13.8% w/w). The extract was preserved in a hermetically sealed jar at 4°C for subsequent utilization [9].

Pharmacognostic Evaluation:

A macroscopic inspection of the leaves was conducted to evaluate color, size, shape, surface qualities, and organoleptic properties. Microscopic examinations were conducted on transverse sections of fresh leaves, stained with safranin and fast green to delineate anatomical characteristics including the epidermis, vascular bundles, stomata, and trichomes. Microscopic analysis of powder was performed to identify diagnostic features. The physicochemical characteristics, such as total ash, acid-insoluble ash, water-soluble ash, loss on drying, and extractive values (both water- and alcohol-soluble), were assessed in accordance with WHO criteria. Fluorescence analysis of the powdered medication was conducted under visible and UV light (254 nm and 366 nm) following treatment with various reagents [10, 11].

Preliminary Phytochemical Screening:

The ethanolic extract underwent qualitative phytochemical analysis to identify the presence of key secondary metabolites, including alkaloids, flavonoids, saponins, tannins, glycosides, and phenolic compounds, following established protocols [12].

Experimental Animals:

Healthy adult Wistar albino rats (180–220 g) of both sexes were obtained from a CPCSEA-approved animal

facility. The animals were maintained under conventional laboratory settings (temperature 22 ± 2°C, relative humidity 55 ± 5%, and a 12-hour light/dark cycle) with unrestricted access to a standard pellet food and water ad libitum. The study protocol received approval from the Institutional Animal Ethics Committee (IAEC), and all methods adhered to CPCSEA rules [13].

Acute Toxicity Study:

An acute oral toxicity study was conducted in accordance with OECD guideline 423. The ethanolic extract of Bacopa monnieri was orally delivered at a maximum dose of 2000 mg/kg body weight. The animals were monitored for 14 days for indications of toxicity or mortality. Doses of 200 and 400 mg/kg were chosen for pharmacological assessment based on the results [14].

Evaluation of Anti-inflammatory Activity:

Carrageenan-Induced Paw Edema Model:

Acute inflammation was elicited by administering 0.1 mL of 1% carrageenan solution into the subplantar area of the right hind paw of rats. The rats were categorized into four groups (n = 6): control (vehicle), standard (diclofenac sodium 10 mg/kg), and test groups administered extract at doses of 200 and 400 mg/kg orally. Paw volume was quantified using a plethysmometer at 0, 1, 2, 3, and 4 hours post-carrageenan injection. The percentage reduction of edema was computed [15].

Cotton Pellet-Induced Granuloma Model:

Chronic inflammation was assessed utilizing the cotton pellet-induced granuloma technique. Sterile cotton pellets (10 ± 1 mg) were subcutaneously implanted in the axillary region of rats under mild anesthesia. The animals were subjected to treatment analogous to the acute model for a duration of seven consecutive days. On the eighth day, the pellets were extracted, desiccated at 60°C, and weighed. The percentage of granuloma formation inhibition was computed relative to the control group [16, 17].

Statistical Analysis:

All experimental data were shown as mean ± SEM (n = 6). Statistical analysis was conducted utilizing one-way ANOVA, succeeded by Dunnett's multiple comparison test. A p-value of less than 0.05 was deemed statistically significant.

RESULTS:

Pharmacognostic Evaluation:

The macroscopic analysis of Bacopa monnieri leaves indicated small, succulent, oblong to spatulate leaves featuring complete borders, a green hue, a smooth texture, and a distinctive aroma accompanied by a mildly bitter flavor. Microscopic examination of the leaf's transverse slice revealed a characteristic dorsiventral architecture, featuring a single-layered upper and bottom epidermis enveloped by a thin cuticle. The mesophyll was categorized into palisade and spongy parenchyma. Paracytic stomata were primarily identified on the lower epidermis, accompanied by glandular trichomes. The vascular bundles were collateral and encircled by parenchymatous cells. Powder microscopy identified pieces of epidermal cells, stomata, trichomes, and xylem arteries exhibiting spiral thickening, which are essential diagnostic characteristics.

Table 1: Physicochemical Parameters of *Bacopa monnieri* Leaves

Parameter	Value (% w/w)
Total ash	8.42 ± 0.36
Acid-insoluble ash	1.21 ± 0.09
Water-soluble ash	3.87 ± 0.18
Loss on drying	6.15 ± 0.27
Water-soluble extractive	18.67 ± 0.54
Alcohol-soluble extractive	12.34 ± 0.47

Physicochemical characteristics were evaluated to ascertain the grade and purity of the crude medication. The total ash value was determined to be 8.42 ± 0.36%, with acid-insoluble ash at 1.21 ± 0.09% and water-soluble ash at 3.87 ± 0.18%, respectively. The drying loss was 6.15 ± 0.27%, signifying minimal moisture content. The extractive values were higher in water (18.67 ± 0.54%) compared to alcohol (12.34 ± 0.47%), indicating a greater presence of polar components. The fluorescence examination of the powdered medication exhibited distinct color variations under visible and ultraviolet light, facilitating verification (Table 1).

Preliminary Phytochemical Screening:

The qualitative phytochemical examination of the ethanolic extract verified the presence of flavonoids, saponins, alkaloids, tannins, glycosides, and phenolic substances. The bioactive compounds are recognized for their contribution to the plant's pharmacological actions, especially its anti-inflammatory properties (Table 2).

Table 2: Phytochemical Constituents of Ethanolic Extract

Sr. No.	Phytoconstituent	Result
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Tannins	+
5	Glycosides	+
6	Phenolics	+

(+ = Present)

Acute Toxicity Study:

No mortality or indications of toxicity were noted in rats given the ethanolic extract at a dosage of 2000 mg/kg body weight throughout the 14-day observation period. This suggests that the extract is comparatively safe, and the chosen doses of 200 and 400 mg/kg were deemed suitable for subsequent pharmacological assessment.

Anti-inflammatory Activity:

Carrageenan-Induced Paw Edema:

The ethanolic extract of Bacopa monnieri demonstrated a notable, dose-dependent decrease in paw edema relative to the control group. At the fourth hour, the extract demonstrated 38.6% and 62.4% suppression of edema at dosages of 200 mg/kg and 400 mg/kg, respectively. The conventional medication, Diclofenac sodium (10 mg/kg), resulted in a superior inhibition of 71.2%. The decrease in paw volume was statistically significant ($p < 0.05$ and $p < 0.01$), demonstrating the extract's strong anti-inflammatory efficacy (Table 3 and figure 1).

Table 3: Effect of Bacopa monnieri Extract on Paw Edema Volume (mL)

Group	Dose (mg/kg)	0 hr	1 hr	2 hr	3 hr	4 hr	% Inhibition
Control	—	0.2	0.4	0.7	0.8	1.0	—
Diclofenac sodium	10	0.20	0.32	0.41	0.36	0.29	71.2%
Extract Low Dose	200	0.22	0.40	0.59	0.68	0.63	38.6%
Extract High Dose	400	0.21	0.35	0.50	0.46	0.38	62.4%

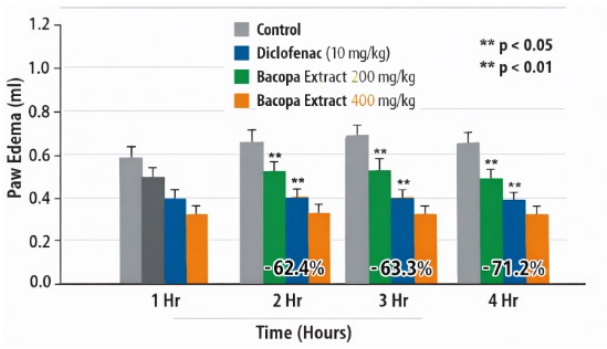

Figure 1: Effect of *Bacopa monnieri* extract on carrageenan-induced paw edema in rats.

Cotton Pellet-Induced Granuloma:

The extract significantly decreased granuloma development in the chronic inflammation model. At 200 mg/kg, the percentage inhibition of granuloma weight was 29.8%, and at 400 mg/kg, it was 55.7%, in comparison to the control group. A 63.5% inhibition was observed with the usual medication. The extract effectively suppressed the proliferative phases of inflammation, as indicated by the statistically significant results ($p < 0.05$). The results show that Bacopa monnieri's ethanolic leaf extract has strong anti-inflammatory effects in both short-term and long-term studies, lending credence to its traditional use and demonstrating its promise as an alternative medicine (Table 4 and figure 2).

Table 4: Effect on Granuloma Formation

Group	Dose (mg/kg)	Granuloma Weight (mg)	% Inhibition
Control	—	52.4 ± 2.1	—
Diclofenac sodium	10	19.1 ± 1.4	63.5%

Extract Low Dose	200	36.8 ± 1.8	29.8%
Extract High Dose	400	23.2 ± 1.5	55.7%

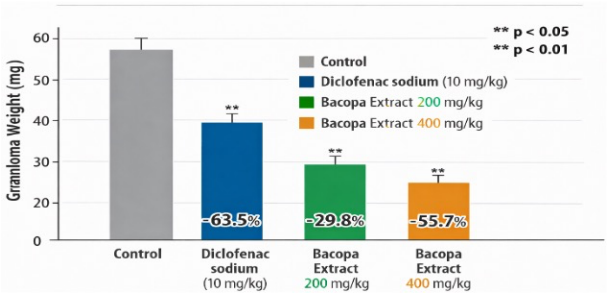

Figure 2: Effect of *Bacopa monnieri* extract on cotton pellet-induced granuloma in rats.

DISCUSSION:

In this investigation, we used established experimental models to assess the anti-inflammatory efficacy of Bacopa monnieri leaf extract and to set pharmacognostic guidelines. Macroscopic and microscopic features, including glandular trichomes, paracytic stomata, and dorsiventral leaf structure, were identified during the pharmacognostic evaluation [18, 19]. These features are in agreement with conventional descriptions and can be used as diagnostic markers to confirm the authenticity of the plant material. There was little chance of adulteration because all of the physicochemical criteria, such as ash and extractive values, were within permissible ranges, proving that the crude medicine was of high quality and purity [20-23].

Bioactive components including flavonoids, saponins, alkaloids, tannins, and phenolic compounds were identified by preliminary phytochemical screening. There is substantial evidence that these phytoconstituents have antioxidant and anti-inflammatory effects. In particular, phenolic compounds and flavonoids are known to reduce inflammation by inhibiting prostaglandins, leukotrienes, and cytokines, which are important mediators of inflammation [24, 25].

In order to determine the extract's anti-inflammatory efficacy, acute and chronic models were used. A commonly used model for evaluating acute inflammation, the carrageenan-induced paw edema, showed that the extract significantly reduced edema in a dose-dependent way. Inflammation caused by carrageenan is biphasic, with the first phase involving the release of histamine and serotonin and the second phase including the production of prostaglandins. As with Diclofenac sodium, the observed inhibition, especially at the 3rd and 4th hours, implies that the extract may disrupt prostaglandin synthesis [26-29].

A model of persistent inflammation and proliferative tissue response, the cotton pellet-induced granuloma model, showed that the extract considerably decreased granuloma formation. It seems like it might be able to stop fibroblasts from multiplying, collagen from being made, and inflammatory cells from penetrating. Higher dose-induced granuloma weight reduction is indicative of the extract's potent anti-proliferative inflammatory effect. The lack of toxicity at doses up to 2000 mg/kg adds credence to the extract's safety profile, which makes it an attractive therapeutic option. Many phytoconstituents in the extract work together to reduce inflammation, which is why it has anti-inflammatory benefits [30-33].

CONCLUSION:

This study established pharmacognostic standards for Bacopa monnieri leaves using comprehensive macroscopic, microscopic, and physicochemical analyses, thereby providing reliable measures for authenticity and quality control. The acute (paw edema induced by carrageenan) and chronic (granuloma induced by cotton pellet) inflammation models demonstrated that the ethanolic leaf extract exhibited a significant dose-dependent anti-inflammatory activity. At elevated doses, the effects resembled those of the benchmark medicine, diclofenac sodium. The extract's anti-inflammatory activities are likely attributable to the presence of bioactive phytoconstituents such as flavonoids, saponins, and phenolic compounds. These compounds function by suppressing inflammatory mediators. Additional evidence of a favorable safety profile is the absence of toxicity, even at elevated doses. The findings indicate that Bacopa monnieri has been utilized traditionally and suggest its potential as a natural anti-inflammatory agent that is both safe and efficacious. The therapeutic efficacy should be further substantiated by research that isolates active compounds and performs clinical assessments.

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Conflict of Interest:

None

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