Research Article

www.ijrap.net

(ISSN Online:2229–3566, ISSN Print:2277–4343)

EXPERIMENTAL VALIDATION OF THE ANTI-INFLAMMATORY EFFICACY OF BILWADI AGAD AGAINST CARRAGEENAN-INDUCED PAW EDEMA IN WISTAR ALBINO RATS

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Received on: 04/7/26 Accepted on: 31/7/26

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DOI: 10.7897/2277-4343.174139

ABSTRACT

Background: Inflammation is a protective response to tissue injury and infection; however, persistent inflammation contributes to the development of various chronic diseases. Although conventional anti-inflammatory drugs are effective, their prolonged use is associated with several adverse effects, necessitating the search for safer therapeutic alternatives. Bilwadi Agad, a classical polyherbal formulation, contains medicinal plants with reported anti-inflammatory properties. The present study was undertaken to evaluate its anti-inflammatory efficacy in an experimental model. Objective: To evaluate the anti-inflammatory efficacy of Bilwadi Agad against carrageenan-induced paw edema in Wistar albino rats. Methods: Eighteen Wistar albino rats were randomly divided into three groups (n = 6): control (0.25% CMC), Bilwadi Agad (200 mg/kg, p.o.) and indomethacin (10 mg/kg, p.o.). Acute inflammation was induced by subplantar injection of 1% carrageenan, and paw edema was measured using a digital plethysmometer at predetermined time intervals. Results: Treatment with Bilwadi Agad significantly reduced carrageenan-induced paw edema compared with the control group. The reduction became evident from the 2nd hour and continued up to the 9th hour, indicating significant anti-inflammatory activity. Conclusion: The present study demonstrated significant anti-inflammatory activity of Bilwadi Agad in the carrageenan-induced paw edema model in Wistar albino rats.

Keywords: Bilwadi Agad, Anti-inflammatory activity, Carrageenan, Wistar albino rats, Digital plethysmometer.

INTRODUCTION

Inflammation is a protective biological response of the body to harmful stimuli such as infection, tissue injury, or toxic agents. It involves a series of vascular and cellular events mediated by inflammatory mediators, including histamine, serotonin, prostaglandins and cytokines. Although inflammation plays an essential role in host defense and tissue repair, an excessive or prolonged inflammatory response can lead to tissue damage and contribute to the development of several acute and chronic inflammatory disorders. [1]

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat inflammatory conditions because of their effectiveness in relieving pain and suppressing inflammation. However, their prolonged use is associated with adverse effects such as gastrointestinal ulceration, renal impairment, and cardiovascular complications. These limitations have prompted continued research into safer and effective therapeutic alternatives, particularly those derived from medicinal plants and traditional formulations. [2]

Bilwadi Agad is a classical polyherbal formulation described in Ayurvedic literature for the management of various poisoning conditions.[3,4] The formulation comprises several medicinal plants with diverse pharmacological properties. Several studies have reported that the individual ingredients of Bilwadi Agad possess anti-inflammatory, antioxidant and immunomodulatory activities. The combined action of these phytoconstituents may contribute to the therapeutic potential of the formulation. However, despite its long-standing traditional use, scientific evidence supporting the anti-inflammatory activity of Bilwadi Agad as a complete formulation remains limited.[5-7]

The carrageenan-induced paw edema model is one of the most widely accepted experimental models for evaluating acute inflammation and screening potential anti-inflammatory agents. The model is characterized by biphasic inflammatory responses mediated by different chemical mediators, making it suitable for assessing the efficacy of test formulations. [8]

Therefore, the present study was undertaken to evaluate the anti-inflammatory efficacy of Bilwadi Agad against carrageenan-induced paw edema in Wistar albino rats.

MATERIALS AND METHODS

Preparation of the Test Drug

The raw ingredients of Bilwadi Agad were procured, authenticated by experts from the Department of Dravyaguna, Rishikul Campus, Uttarakhand Ayurved University, Haridwar (Authentication Certificate No. DG/RC/UAU-278), and cleaned before formulation preparation. Bilwadi Agad was prepared according to the classical method described in Ashtanga Hridaya

(Uttarasthana). All herbal ingredients were taken in equal proportions, shade-dried, powdered separately and passed through sieve No. 80 to obtain a fine powder. The powdered ingredients were mixed thoroughly and subjected to three successive Bhavana (trituration) processes using fresh Basta Mutra (Goat Urine) as the levigating medium. The formulation was shade-dried after each Bhavana. The dried material was finally pulverized, sieved, and stored in an airtight container until further use. The prepared Bilwadi Agad was evaluated for organoleptic characters, physicochemical parameters, HPTLC fingerprinting, aflatoxin content and total microbial count. The physicochemical analysis was carried out according to the standards prescribed in the Ayurvedic Pharmacopoeia of India (API).[9]

Experimental Animals

Healthy male Wistar albino rats weighing 150–250 g were procured from the Animal House Facility, Gurukul Kangri (Deemed to be University), Haridwar, India. Before the commencement of the study, the animals were acclimatized to laboratory conditions for seven days. The experimental protocol was approved by the Institutional Ethics Committee of Uttarakhand Ayurved University, Dehradun (Ref. No. UAU/RC/IEC/2025/PG/269) and the Institutional Animal Ethics Committee (IAEC), Gurukul Kangri (Deemed to be University), Haridwar (Ref. No. GKV/AHF/66/2025), in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India.[10]

Housing and Environmental Conditions

The animals were housed in polypropylene cages under standard laboratory conditions with a 12 h light/dark cycle, a temperature of 22 ± 3°C, and a relative humidity of 50–70%. Standard pellet diet and water *ad libitum* were provided throughout the experimental period. [10]

Dose Selection

The dose of Bilwadi Agad (200 mg/kg, p.o.) used in the present study was selected based on the findings of a preliminary acute oral toxicity study conducted according to OECD Guideline 423**.** [11] Indomethacin (10 mg/kg, p.o.) was used as the standard anti-inflammatory drug, while 0.25% CMC suspension (10 mL/kg, p.o.) served as the vehicle control.

Experimental Design

Eighteen healthy male Wistar albino rats were randomly allocated into three groups comprising six animals each. Individual animals were identified using picric acid markings throughout the experimental period.

Group I (Control): Received 0.25% CMC suspension (10 mL/kg, p.o.) once daily for seven consecutive days.

Group II (Test): Received Bilwadi Agad (200 mg/kg, p.o.) once daily for seven consecutive days.

Group III (Standard): Received indomethacin (10 mg/kg, p.o.) once daily for seven consecutive days.

All treatments were administered orally using a 16 G gastric oral gavage catheter attached to a graduated syringe. [12]

Carrageenan-induced Paw Edema Model

Carrageenan-induced paw edema was employed to evaluate the anti-inflammatory activity of Bilwadi Agad. On the seventh day, one hour after administration of the respective treatments, acute paw edema was induced by subplantar injection of 0.1 mL of freshly prepared 1% carrageenan suspension in sterile normal saline into the left hind paw of each rat. Basal paw volume was measured using a digital plethysmometer before carrageenan injection (0 h) and subsequently at 1, 2, 3, 6, and 9 hours after carrageenan administration. The increase in paw volume from the baseline value was considered as the measure of inflammation.[8]

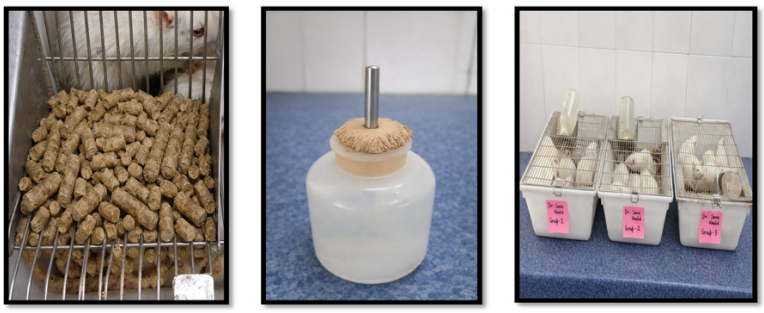

Figure 1: Animal housing and husbandry

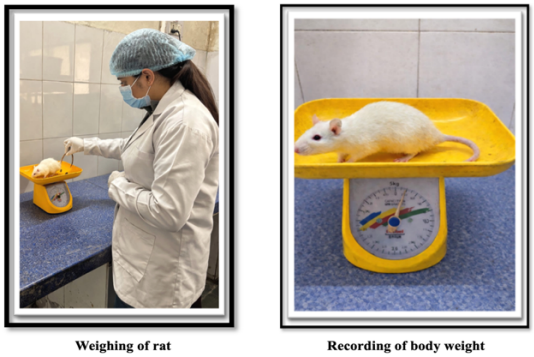

Figure 2: Body Weight Measurement of Experimental Animals

Figure 3: Oral pre-treatment using oral gavage before carrageenan injection

Figure 4: Carrageenan-induced Paw Edema Model

Table 1: Effect of Bilwadi Agad on Carrageenan-induced Paw Edema in Wistar Albino Rats (Mean ± SEM, n = 6)

Observation Time	Control Group Mean ± SEM	Test Group (Bilwadi Agad) Mean ± SEM	Standard Group (Indomethacin) Mean ± SEM
0 hr.	1.098 ± 0.016	1.082 ± 0.012	1.082 ± 0.013
1st hr	1.587 ± 0.016	1.565 ± 0.020	1.520 ± 0.018
2nd hr	1.935 ± 0.015	1.747 ± 0.028	1.658 ± 0.013
3rd hr	1.895 ± 0.013	1.655 ± 0.023	1.507 ± 0.019
6th hr	1.900 ± 0.012	1.422 ± 0.016	1.320 ± 0.013
9th hr	1.883 ± 0.010	1.260 ± 0.012	1.198 ± 0.026

Table 2: Two-way ANOVA followed by Dunnett's Multiple Comparison Test Showing Comparison of Treatment Groups with Control Group

Dunnett's multiple comparisons test	Mean Difference	95.00% CI of difference	Significant?	Summary	Adjusted P value
0 hr					
Group 1 vs Group 2	0.016	-0.088 to 0.120	No	ns	>0.05
Group 1 vs Group 3	0.016	−0.086 to 0.118	No	ns	>0.05
1st hr					
Group 1 vs Group 2	0.022	-0.051 to 0.095	No	ns	>0.05
Group 1 vs Group 3	0.067	0.006 to 0.128	Yes	*	<0.05
2nd hr					
Group 1 vs Group 2	0.188	0.052 to 0.324	**Yes**	*	<0.05
Group 1 vs Group 3	0.277	0.116 to 0.438	Yes	**	<0.01
3rd hr					
Group 1 vs Group 2	0.240	0.058 to 0.422	Yes	*	<0.05
Group 1 vs Group 3	0.388	0.172 to 0.604	Yes	**	<0.01
6th hr					
Group 1 vs Group 2	0.478	0.332 to 0.624	Yes	***	<0.01
Group 1 vs Group 3	0.580	0.424 to 0.736	Yes	***	<0.01
9th hr					
Group 1 vs Group 2	0.623	0.472 to 0.774	Yes	***	<0.01
Group 1 vs Group 3	0.685	0.508 to 0.862	Yes	***	<0.01

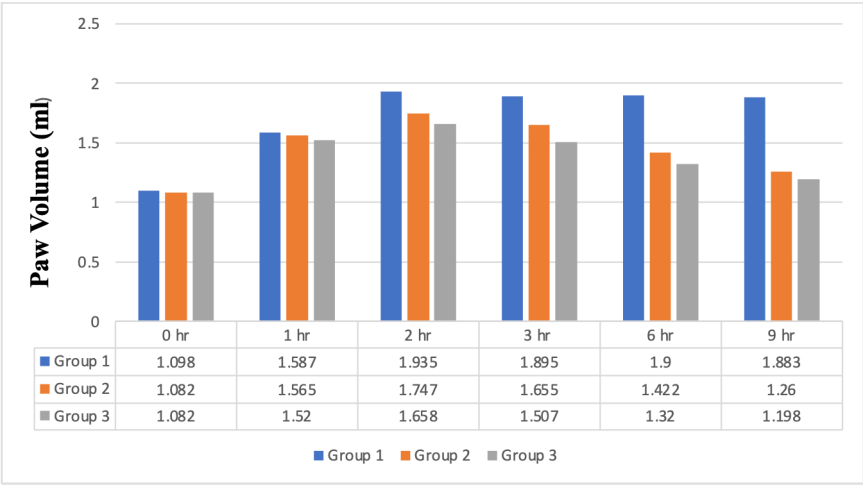

Figure 5: Effect of Bilwadi Agad and Indomethacin on Carrageenan-induced Paw Edema in Wistar Albino Rats

Table 3: Percentage Inhibition of Carrageenan-induced Paw Edema by Bilwadi Agad and Indomethacin

Observation time	Test group (Group-2)	Standard group (Group- 3)
0 hr	1.46%	1.46%
1st hr	1.39%	4.22%
2nd hr	9.71%	14.32%
3rd hr	12.66%	20.47%
6th hr	25.16%	30.53%
9th hr	33.08%	36.38%

Statistical Analysis

The data were expressed as mean ± SEM. Statistical analysis was performed using two-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. A p-value of < 0.05 was considered statistically significant.

RESULTS

Effect of Bilwadi Agad on Carrageenan-induced Paw Edema in Wistar Albino Rats

Carrageenan administration resulted in a progressive increase in paw volume in the control group, with the highest mean paw volume observed at the 2nd hour. Thereafter, paw edema gradually declined but remained higher than the baseline throughout the observation period. Rats treated with Bilwadi Agad showed a lower increase in paw volume at all observation intervals compared with the control group. A similar trend was observed in the indomethacin-treated group. The mean paw volume of all experimental groups at different time intervals is presented in Table 1, and the corresponding graphical representation is shown in Figure 5.

The results of two-way ANOVA followed by Dunnett's multiple comparison test showed a statistically significant reduction in paw edema in both the treatment groups when compared with the control group. In the Bilwadi Agad-treated group, a significant reduction was observed from the 2nd hour onwards ($P < 0.05$), with highly significant inhibition at the 6th and 9th hours ($P < 0.01$). The indomethacin-treated group showed significant inhibition from the 1st hour, with highly significant differences during the later observation intervals. The detailed statistical analysis is presented in Table 2.

The percentage inhibition of carrageenan-induced paw edema increased progressively with time in both the treatment groups. Bilwadi Agad produced a maximum inhibition of 33.08% at the 9th hour, while indomethacin showed a maximum inhibition of 36.38% at the same time point. The percentage inhibition of paw edema is presented in Table 3.

DISCUSSION

Carrageenan-induced paw edema is one of the most widely accepted experimental models for evaluating acute anti-inflammatory activity. The inflammatory response occurs in two distinct phases. The early phase is primarily mediated by histamine, serotonin and bradykinin, whereas the late phase is predominantly associated with the release of prostaglandins and other inflammatory mediators. Hence, this model is widely employed for screening potential anti-inflammatory agents.[8]

In the present study, carrageenan administration successfully induced acute inflammation in all experimental groups, as evidenced by an increase in paw volume. However, pretreatment with Bilwadi Agad and indomethacin significantly reduced paw edema compared with the control group. Bilwadi Agad showed a significant effect from the 2nd hour onwards, and the inhibitory effect increased progressively during the subsequent observation period, reaching a maximum percentage inhibition of 33.08% at the 9th hour. The anti-inflammatory response produced by Bilwadi Agad was comparable to that of the standard-treated group, indicating its promising anti-inflammatory potential. The inhibitory effect increased progressively during the subsequent observation period, reaching a maximum percentage inhibition of 33.08% at the 9th hour. The anti-inflammatory response produced by Bilwadi Agad was comparable to that of the standard-treated group, indicating its promising anti-inflammatory potential.

The progressive reduction in paw edema during the later hours of observation suggests that Bilwadi Agad may exert its anti-inflammatory effect predominantly by modulating mediators involved in the late phase of carrageenan-induced inflammation. This pattern of response indicates its potential to suppress the inflammatory process associated with acute inflammation.

Bilwadi Agad is a classical polyherbal formulation composed of medicinal ingredients traditionally indicated for the management of various toxic and inflammatory conditions. Previous studies have reported anti-inflammatory and antioxidant activities of several of its constituent drugs.[13,14,15] The observed anti-inflammatory activity in the present study may therefore be attributed to the combined and synergistic pharmacological actions of these ingredients, resulting in attenuation of carrageenan-induced paw edema.[13-15]

The findings of the present study demonstrate that Bilwadi Agad possesses significant anti-inflammatory activity in the carrageenan-induced paw edema model in Wistar albino rats. These findings provide experimental support for its traditional therapeutic use.

CONCLUSION

The present study demonstrated that Bilwadi Agad possesses significant anti-inflammatory activity against carrageenan-induced *paw* edema in Wistar albino rats. Oral administration of the formulation for seven consecutive days produced a significant reduction in paw edema, with a progressive increase in percentage inhibition throughout the observation period. The findings indicate the anti-inflammatory potential of Bilwadi Agad in acute inflammation and provide experimental evidence supporting its traditional therapeutic use. The results of the present study suggest that Bilwadi Agad may be considered a promising polyherbal formulation for the management of inflammatory conditions.

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Cite this article as:

Qazi Sana Khalid, Bhawana Mittal, Ramesh Chandra Tiwari and Vipin Kumar. Experimental validation of the anti-inflammatory efficacy of Bilwadi Agad against carrageenan-induced paw edema in Wistar albino rats. Int. J. Res. Ayurveda Pharm. 2026;17(4):69-73 doi: http://dx.doi.org/10.7897/2277-4343.174139

Source of support: Nil, Conflict of interest: None Declared