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Comparative Assessment of the Anti-Inflammatory Activity of Parthenium Hysterophorus and Aspirin Using Cotton Pellet-Induced Granuloma Method in Albino Wistar Rats

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KEYWORDS

Anti-Inflammatory Activity, Parthenium hysterophorus, Cotton Pellet-Induced Granuloma Method, Albino Wistar Rats

ABSTRACT:

Background: The increasing interest in plant-based therapies has brought attention to Parthenium hysterophorus, a traditionally used herb reported to exhibit analgesic, antipyretic, and wound-healing properties. However, its anti-inflammatory efficacy requires further experimental validation.

Objective: To evaluate and compare the anti-inflammatory activity of hydro-alcoholic extract of Parthenium hysterophorus with aspirin using the cotton pellet-induced granuloma model in albino Wistar rats.

Methods: Rats were divided into five groups: control (distilled water), three groups receiving PH extract (200, 400, and 800 mg/kg orally), and a standard group receiving aspirin (630 mg/kg). The degree of inflammation was assessed by measuring the wet and dry weights of implanted cotton pellets. Percentage inhibition of granuloma formation was calculated relative to the control group.

Results: PH extract demonstrated a dose-dependent reduction in both wet and dry granuloma weights. Maximum inhibition was observed at 800 mg/kg, with 27.78% inhibition (wet) and 30.00% inhibition (dry), compared to 50.00% and 60.00% respectively in the aspirin group. Statistical analysis confirmed significant anti-inflammatory activity in all treatment groups versus control ($p < 0.05$ to $p < 0.0001$).

Conclusion: Parthenium hysterophorus extract exhibits significant anti-inflammatory activity in vivo, particularly at higher doses. Although its efficacy was lower than aspirin, the findings support its potential as a natural anti-inflammatory agent. Further studies are warranted to isolate active constituents and elucidate underlying mechanisms.

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Introduction

India's longstanding tradition of herbal medicine, deeply embedded in cultural and historical contexts [1]. It represents one of the most diverse and ancient systems of therapeutic knowledge globally [2]. From the classical the times of Charaka and Sushruta [3] to contemporary folk practices, plant-based remedies have served as critical resources in indigenous healthcare. The growing global reliance on traditional medicine (approximately 80% of the world's population as per World Health Organization) underscores the importance of scientifically validating these practices [4].

Parthenium hysterophorus stands out due to its dual reputation as both an invasive weed and a plant with untapped pharmacological potential [5]. Native to the Americas but now widespread in India, Parthenium hysterophorus has traditionally been used to manage various ailments (fever, gastrointestinal disturbances, and inflammatory skin conditions) [6]. Recent studies suggest that its active phytochemical, parthenin, may offer medicinal properties, especially analgesic and antimicrobial effects [7]. Recently, it has been also explored for its potential anticancer applications [8].

However, despite anecdotal and preliminary evidence, the systematic pharmacological evaluation of Parthenium hysterophorus is still limited [9]. The present study aims to explore the anti-inflammatory activity of Parthenium hysterophorus, contributing to a broader understanding of its therapeutic applications that may support development of low-cost, plant-based medicinal alternatives.

Materials & Methods

Ethical clearance was obtained from the Institutional Ethics Committee (IEC), Mahatma Gandhi Institute of Medical Sciences (MGIMS), Sewagram, Wardha, Maharashtra, India before the commencement of this study. Fresh Parthenium hysterophorus was collected and authenticated by the local botanist. The aerial parts were shade-dried and then powdered. The powdered aerial parts were macerated for 24 hours in 70 % ethanol. The hydro-alcoholic extracts were obtained by percolation using 70% ethanol as a solvent. The percolated solution was again shade-dried, and finally the extract thus obtained was stored. A fresh solution was prepared by dissolving the extract in distilled water before each experiment. For the oral administration, Parthenium hysterophorus extract used in this study was prepared by dissolving the extract in distilled water before each experiment. The Figure 1, 2 and 3 shows the photograph of Parthenium hysterophorus plant, the instrument used as a percolator in our study and a sample of Parthenium hysterophorus extract prepared in our departmental experimental laboratory, respectively.

Albino Wistar rats were procured after obtaining the permission from Institutional Ethics Committee (IEC). The animals were caged in the polyvinyl wire mesh cages at the Animal House of our institute. They were maintained under standard laboratory condition (a 12 hour light and dark cycle with a Temperature of 22^0C ± 3^0C and Humidity of 60% ± 10%) with access to food and water ad libitum according to the guidelines from Organization for Economic Co-operation and Development (OECD) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India [10]. The animals were allowed to adapt to the new surrounding by giving a rest period of one week before subjecting them to the experimentation. Healthy adult Albino Wistar rats of either sex weighing 150-250 g were used for the Cotton pellet-induced granuloma model [11].

On the first day of the experiment, 5mg (±1mg) sterile cotton pellets were inserted in the inner side of the right thigh of each rat with all the aseptic precautions, under the Pentobarbitone (30mg/kg, Intraperitoneal (i.p.)) anesthesia [12]. The animals were given test and standard drug orally once daily for 7 days.

Table 1 shows the details of 5 groups of the 30 rats included in our study.

The pellets had remained in the bodies of the rats for 7 days [10,11]. On the 8th day, the rats were sacrificed and the wet cotton pellets containing the granuloma was removed. The weight of these cotton pellets was measured. Then they were dried in hot-air oven. Again, the weights were measured. The change in the granuloma weight was calculated by subtracting the weight of the original cotton pellet.

The percentage inhibition of Granuloma by the drugs in rats was calculated using the following formula [13]:

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% Inhibition of granuloma = [(Control - Test)/ Control] × 100

All the results were expressed as Mean ± Standard Deviation (SD). The differences between the experimental groups were compared by the one-way Analysis of Variance (ANOVA) test followed by Dunnett's post hoc test. After the comparison between the study groups, we classified as statistically Significant ($p < 0.05$), Very significant ($p< 0.01$), and Highly significant ($p< 0.001$).

Results

The table 2 shows the anti-inflammatory activity in terms of percentage inhibition based on the comparison of weights of cotton pellets in the different groups of Albino Wistar rats i.e. the group receiving Distilled water (Control group), the Test (Parthenium Hysterophorus) and Standard drug (Aspirin).

Figure 4 highlights the anti-inflammatory activity in terms of differences in granuloma weight in different groups (the Control Group, the Groups receiving Test Drug i.e. Parthenium Hysterophorus in different concentrations and the Group receiving the Standard Drug i.e. Aspirin) of Albino Wistar rats using the Cotton pellet-induced granuloma method.

Figure 5 demonstrates the anti-inflammatory activity in terms of percentage inhibition in different groups (the Control Group, the Groups receiving Parthenium Hysterophorus at different concentrations and the Group receiving Aspirin) of Albino Wistar rats using the Cotton pellet-induced granuloma method.

Table 2, Figure 4 and Figure 5, indicate that Parthenium hysterophorus extract demonstrates dose dependent anti-inflammatory activity. The observed reductions in both wet and dry granuloma weights suggest its efficacy. The 800 mg/kg dose of PH extract exhibited the most robust anti-inflammatory effects among test groups, approaching those of the standard drug.

These observations suggest ethnopharmacological use of Parthenium hysterophorus in managing inflammatory conditions and provides a scientific basis for further investigation.

Discussion

Parthenium hysterophorus extract at the doses of 200, 400 and 800 mg/kg showed significant inhibition of granuloma as compared to control. The findings clearly demonstrate that PH extract exerts a dose-dependent anti-inflammatory effect, with the highest dose (800 mg/kg) approaching the efficacy of aspirin, a standard reference drug. It should, however, be noted that the anti-inflammatory activity was not as strong as the standard drug, i.e., aspirin. The extract decreased both dry and wet weight of the cotton pellet granuloma in rats, indicating its anti-inflammatory activity. Winter & Porter evaluated the anti-inflammatory activity of Parthenium hysterophorus by the same cotton pellet granuloma model. They proposed that Parthenium hysterophorus extract significantly reduces granuloma formation, indicating effectiveness in chronic inflammation models [14]. They suggested that reduction in both wet and dry granuloma weight suggests inhibition of collagen fiber formation and mucopolysaccharide synthesis, similar to the action of non-steroidal anti-inflammatory drugs (NSAIDs) [15].

Parthenium hysterophorus is often regarded as an environmental nuisance due to its allergenic properties [3]. However, its medicinal potential, particularly its anti-inflammatory activity, has been explored in several studies [3, 4, 15- 19]. This plant contains various bioactive compounds that contribute to its pharmacological effects. Studies have identified many sesquiterpene lactones from Parthenium hysterophorus, including parthenolide, luteolin, reynosin, santamarin, and apigenin, which exhibit anti-inflammatory effects [16]. The various mechanisms proposed to be responsible for anti-inflammatory effects of Parthenium hysterophorus are as follows: inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes (due to parthenolide and other sesquiterpene lactones that suppress prostaglandin and leukotriene synthesis), suppression of pro-inflammatory cytokines (lower levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6, and interleukin (IL)-1β), and antioxidant activity (due to flavonoids and polyphenols) [16].

Research on Parthenium hysterophorus has demonstrated significant anti-inflammatory effects across multiple experimental models, highlighting its potential as a medicinal plant. Venkataiah et al. (using carrageenan-

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induced paw edema model) have demonstrated that the ethanolic extract of Parthenium hysterophorus provides dose-dependent reduction in paw swelling, comparable to conventional NSAIDs [17]. These findings suggest inhibition of histamine-, serotonin-, and prostaglandin-mediated inflammation [18]. Patel et al. tested Parthenium hysterophorus for skin irritation and allergic reactions using a topical anti-inflammatory gel model. Results confirmed that the gel did not trigger inflammation, making it a promising topical anti-inflammatory agent [19]. Zhou et al. used a lipopolysaccharide-induced inflammation model and concluded that Parthenium hysterophorus extract reduces levels of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, highlighting its therapeutic potential in autoimmune disorders and chronic inflammatory diseases [20].

Our study was based on the cotton pellet granuloma model. It is a time-proven experimental method used to assess the anti-inflammatory effects of pharmacological agents [21]. The strength of our study due to this model are as follows: reliable induction of inflammation and its quantifiable results, strict adherence to the standardized procedure, and prompt evaluation of anti-inflammatory efficacy. However, there are some drawbacks of this model: limited representation of inflammation, influence of fluid absorption, ethical concerns, time-consuming procedures, and potential for infection. Despite the promising results observed in our study, several challenges remain: toxicity concerns, standardization issues, and-most importantly-the need for clinical validation.

Conclusions

Based on the findings of our study, it can be concluded that Parthenium hysterophorus extract exhibits dose-dependent anti-inflammatory activity in albino Wistar rats. However, further comprehensive research is required to better understand the underlying mechanisms responsible for its analgesic, anti-inflammatory, antipyretic, and wound-healing properties across different experimental animal models. If the anti-inflammatory efficacy and safety profile of Parthenium hysterophorus extract are validated through additional preclinical studies, well-designed clinical trials may be undertaken in the future to evaluate its potential therapeutic use in human subjects.

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Figure 1: Photograph of Parthenium Hysterophorus plant

Figure 2: Photograph of Percolator used for the preparation of Parthenium Hysterophorus extract

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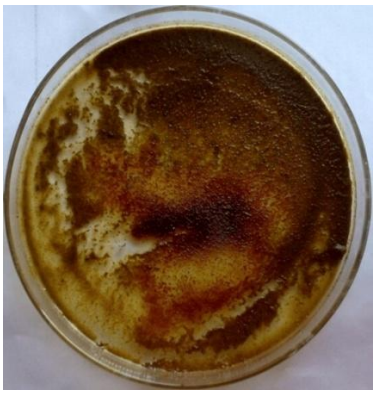
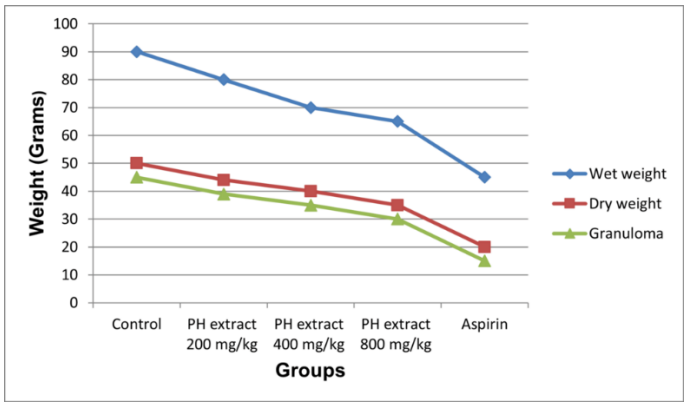
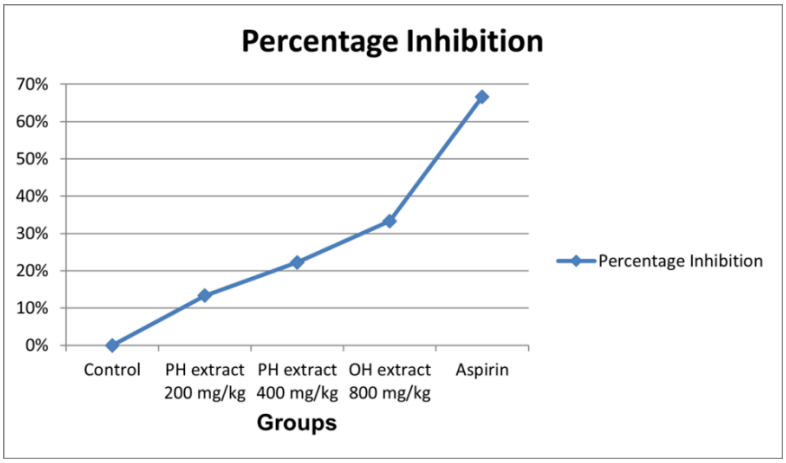


Figure 3: Photograph of Parthenium Hysterophorus extract



PH - Parthenium Hysterophorus, mg – Mili Gram, KG – Kilo Gram)

Figure 4: Anti-inflammatory activity in terms of difference in weight of granulomas in different groups (the Control group, the groups receiving Test drug in different concentration and the group receiving Standard drug) of Albino Wistar rats by the Cotton pellet-induced granuloma method



(PH - Parthenium Hysterophorus, mg – Mili Gram, KG – Kilo Gram)

Figure 5: Anti-inflammatory activity in terms of percentage inhibition in different groups (the Control group, the groups receiving Test drug in different concentration and the group receiving Standard drug) of Albino Wistar rats by the Cotton pellet-induced granuloma method

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Sr. No.	Name of the group	Treatment Given	Number of rats enrolled
1	Group 1 *	Distilled water	6
2	Group 2 #	PH extract 200mg/kg orally	6
3	Group 3 #	PH extract 400mg/kg orally	6
4	Group 4 #	PH extract 800mg/kg orally	6
5	Group 5 ##	Standard drug aspirin 630mg/kg orally	6

(PH - Parthenium Hysterophorus, Mili Gram, KG – Kilo Gram)

[#Groups of Rats receiving the test drug in different doses, ##Group of Rats receiving the Standard drug, *Control group]

Table 1: Details of Albino Wistar rats and Parthenium Hysterophorus/Aspirin extract dosage

Sr. No.	Group	Dose (mg/kg)	Wet Weight (mg) (Mean ± SD)	% Inhibition (Wet)	Dry Weight (mg) (Mean ± SD)	% Inhibition (Dry)
1	Control (Vehicle)	2ml	90 ± 3.35	–	50 ± 3.85	–
2	PH Extract	200	80 ± 3.16	11.11%	44 ± 3.87	12.00%
3	PH Extract	400	70 ± 3.44	22.22%	40 ± 3.03	20.00%
4	PH Extract	800	65 ± 2.93	27.78%	35 ± 4.56	30.00%
5	Aspirin (Standard Drug)	630	45 ± 2.28	50.00%	20 ± 3.45	60.00%

(% - Percent, PH - Parthenium Hysterophorus, SD - Standard Devaition)

Table 2: Anti-inflammatory activity of Parthenium Hysterophorus extract on Albino Wistar rats by the Cotton pellet-induced granuloma method

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Azmat Kamal Ansari, Shabana Andleeb Ansari, Prafulla Thaware, Sushil Varma

Acquisition, analysis, or interpretation of data: Azmat Kamal Ansari, Shabana Andleeb Ansari, Prafulla Thaware, Sushil Varma

Drafting of the manuscript: Azmat Kamal Ansari, Prafulla Thaware

Critical review of the manuscript for important intellectual content: Azmat Kamal Ansari, Shabana Andleeb Ansari, Sushil Varma

Supervision: Sushil Varma

Ethics Statement and Conflict of Interest Disclosures

Human subjects: All authors have confirmed that this study did not involve human participants or tissue.

Animal subjects: ETHICS COMMITTEE, MAHATMA GANDHI INSTITUTE OF MEDICAL SCINCES, SEWAGRAM. WARDHA, MAHARASHTRA Issued protocol number MGIMS/IEC/PHARM/13/2011.

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Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following:

1. **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work.
2. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work.
3. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.