RESEARCH PAPER

Anti-Arthritic Activity Of Hamelia Patens Leaves Extracts In Experimental Animal Model

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ABSTRACT
The foremost objective of this investigation is to evaluate, anti-arthritic effect of Hamelia patens leaf extracts using the in vivo FCA-induced arthritis model and in vitro egg albumin denaturation assay method and in vivo activity was performed using Albino Wistar rats at doses of 200 and 400 mg/kg, with diclofenac sodium (20 mg/kg) used as the standard drug. Arthritis was induced using Complete Freund's Adjuvant (FCA), and disease progression was evaluated by measuring the rats' paw volume. The in-vitro activity was conducted by the egg albumin denaturation assay model, which displayed a significant, dose related reduction in the paw volume, followed by a decrease in the white cell count and an increase in red cell count. The in-vitro assay further confirmed that dose related inhibition effect of protein unfolding (denaturation) by the leaves extracts. Overall, Hamelia patens demonstrated the potent anti-arthritic activity by reducing the inflammation at the joints, which supports the plants potential effect, as natural remedial agent for the management of arthritis disease.
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INTRODUCTION

Rheumatoid arthritis is chronic (long-term) inflammation diseases, which mainly influence the joints and surrounding periarticular tissues. In rheumatoid arthritis, an aberrant immune response results in the production of harmful inflammatory mediators that cause continuous inflammation at synovial (1). The primary manifestations of rheumatoid arthritis include pain, swelling, and progressive damage of the cartilage and bone, which may ultimately result in the permanent disability of bones. Although the exact cause of the disease remains unclear, several hypotheses suggest that its onset is triggered by a combination of genetic susceptibility and exposure to the factors such as viral infections (2). The management of rheumatoid arthritis typically requires the uses of analgesics, non-steroidal anti-inflammatory drugs (NSAID's), glucocorticoids, and disease-modifying anti-rheumatic drugs also known as DMARD's. Despite of their therapeutic benefits, these agents are also related with the adverse effects. DMARD's, for example, it may produce mild side effects like nausea, but can also lead to serious complications such as hepatotoxicity, blood dyscrasias, and interstitial lung disease (3). Cytotoxic class of drugs possesses teratogenic effect and this should be strictly avoided during pregnancy (4). Moreover, prolonged administration of NSAID can adversely affect the hepatic and renal function and also associated with the cardiovascular risk (5). Medicinal plants are considered as the safe, economical, effective, and easily available for the treatment of various diseases. Owing to these advantages, they have been widely used by traditional medical practitioners in their day-to-day practice. *Hamelia patens Jacq* belongs to the family of **Rubiaceae** and possesses significant **traditional medicinal benefits (6).** *Hamelia patens* are rich in alkaloids, flavonoids, and oxindole alkaloids such as pteropodine and isopteropodine. These phytochemicals are previously have been reported that these are useful in the treatment of various disease conditions like depression, eating disorders, anxiety, obesity (7). In Mexico, *Hamelia patens* are traditionally employed for different medicinal purposes. It is especially used for stopping bleeding, healing sores, and treating menstrual disorders. Other reported used in the management of pimples, malaria, skin diseases, blisters, eczema, stomachache, athlete's foot, pain relief, skin lesions, rashes, insect bites, itching, headache, asthma, burns, scurvy, inflammation, rheumatism, nervous shock, postpartum pain, uterine and ovarian afflictions, scant menstruation, as well as expelling intestinal worms and treating the dysentery, (8) Based on the traditional claims,

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the present study aims to evaluate the anti-arthritic potential of *Hamelia patens*, as its efficacy against the arthritis has not yet been scientifically investigated.

METHODOLOGY

Collection, Extraction of *Hamelia patens* Jacq plant leaves

Collection and Authentication of Plant leaves

Hamelia patens Jacq leaves were collected from FRLHT (Foundation for Revitalisation of Health Traditions Ethno Medicinal Garden and Nursery) yalahanka, Bengaluru. The authentication process was done by Dr. V. Rama Rao (Research officer, of Botany, from Central Ayurveda Research Institute) as *Hamelia patens* Jacq belong to the family Rubiaceae (RRCBI-13045)

Plant leaves extract preparation

The collected leaves were separated, thoroughly washed, and shade-dried. The dried leaves then reduced to roughly powdered form (300 µm). Extraction was performed using a Soxhlet apparatus or continuous hot percolation method with 100 g of powdered leaves and 500 mL of 90% ethanol. The process was carried out at 50 °C for 12 hours. The obtained extract was concentrated by using hot water bath, and transferred to an airtight container for storage and further dosing

Experimental animals maintaining and approval from ethical for the in-vivo investigation

The in-vivo investigation study protocol was reviewed and approved by Animal Ethics Committee of Visveswarapura institute of pharmaceutical science under the protocol number VIPS/IAEC/03 08 2024/27 RN, and the experiments were conducted following ethical clearance. Adult Albino Wistar rats (9–12 weeks age, weighing about 180–250 g) were procured. They were housed in polypropylene cages under the condition of light, dark cycle, with the temperature condition at 22 ± 2 °C, and the moisture was maintained 60%. All the animals were provided with the standard pellet diet and water. To ensure the hygienic condition, bedding material was replaced every day by day, and cages were cleaned regularly.

In vivo pharmacological investigations

Acute oral toxicity study.

For the oral toxicity testing of *Hamelia patens*, the OECD Guideline 423 was employed. Six female *Albino Wistar* rats were used, with three animals per step; firstly the drug was tested at the dose of 2000 mg/kg body weight. The drug to be tested was dissolved in water and administered as a single oral dose to the overnight-fasted rats. Following administration, the animals were observed closely initially the first 30 minutes and periodically throught the first 24 hours, and thereafter on a daily basis. Observations included changes in skin, fur, and eyes, as well as clinical signs such as tremors, convulsions, and alterations in behavioral patterns were observed[9].

Freund's adjuvant induced arthritis (FCA)

All the Animals were classified into 5 groups (n = 6) in a random way. Group I marked as the normal control, Group II served as the disease control (FCA-induced), Groups III and IV were pre-treated with the ethanolic leaf extract of *Hamelia patens* at the doses of 200 mg/kg and 400 mg/kg body weight respectively, and Group V treated with the standard diclofenac (10 mg/kg). Arthritis was induced by CFA (0.1ml), the injection was administered into the sub-plantar region of the rats paws. Due to the viscous nature of CFA, rats were made anaesthetized by administration of pentobarbitone sodium (40 mg/kg) prior adjuvant administration. Drug treatment was initiated on day 0, 30 minutes before CFA injection, and continued once in a day, daily for 21 consecutive days. Paw edema was measured periodically up to 21 days using a Plethysmometer. At the end of experiment, all the animals were made anesthetized by pentobarbitone sodium (40 mg/kg, b.w), and blood samples were obtained from the retro-orbital plexus puncture for hematological parameter analysis, including hemoglobin (Hb) level, red blood cell (RBC) count, and white blood cell (WBC) count[10, 11].

Denaturation assay of egg albumin

5ml of reactant mixture was made to prepare, which consisted of 0.2 ml of albumin from fresh egg, 2.8 ml of phosphate-buffer saline of pH about 6.4, and the test drug of 2 ml in varying dose concentrations (100, 250, and 500 µg/ml). An equal amount of distilled water was used as the control. The reaction mixtures were incubated in an incubator at the temperature 37 ± 2 °C for 15 mins, followed by heating at 70 °C for 5 minutes. After the cooling of reaction mixture, absorbance reading was measured at 660 nm using a spectrophotometer. Diclofenac sodium (100, 250, and 500 µg/ml) was used as the standard drug. The protein denaturation percentage inhibition of test and standard was calculated by standard formula[12]

$$\% \text{ inhibition} = 100 \times [V t / V C - 1]$$

V t = test sample absorbance,

V c = control absorbance

Hematological examination

Following sacrifice by anesthesia overdose, blood specimens were collected via retro-orbital route into EDTA-coated tubes. Blood parameters including white blood cell (WBC) count, red blood cell (RBC) count, and hemoglobin (Hb) levels, was subsequently analyzed.

RESULTS

Percentage yield values

Extraction yield value of leaves Ethanolic extracts was calculated. The extractive value was found to be 15.1%

Results of acute oral toxicity study

From the evaluation of oral toxicity study, the ethanolic extract of *Hamelia patens* at the dose of 2000 mg/kg body weight, no toxicity or mortality signs were observed thought the dosing period, indicating the safety of the extract. For the present study, $1/10^{th}$ and $1/5^{th}$ were selected as study doses, corresponding to 200 and 400 mg/kg body weight, accordingly.

Freund's adjuvant induced arthritis

The values in table 1, shows the change in the paw volume after oral administration of *Hamelia patens* and standard diclofenac sodium. Edema rate of the induced foot pad was reached maximum in arthritic control (0.241) whereas the paw volume of diclofenac and ethanolic low dose and high dose of *Hamelia patens* was 0.27, 0.248, 0.263 at 7th day, after the treatment for next 14 days with diclofenac sodium , *Hamelia patens* low dose(200mg) and high dose (400mg), the paw volume was significantly decreased at 0.185 (***P<0.001), 0.225 (***P<0.00), 0.183 (***P<0.001) respectively, compared to the arthritic control. This indicates that the ethanolic extract at high dose was found to be more potent and it was comparable with the standard group

Table 1: Paw volume effects of the drugs by FCA induced arthritis method.

Groups n=6	Paw volume (ml)			
	Day 1	Day 7	Day 14	Day 21
Normal control	0.0450 ± 0.0022	0.046 ± 0.0021	0.056 ± 0.0021	0.056 ± 0.002
Arthritis control	0.1750 ± 0.0076$^{###}$	0.241 ± 0.0070$^{###}$	0.345 ± 0.007	0.360 ± 0.057$^{###}$
Hamelia patens extract 200mg/kg	0.171 ± 0.094	0.248 ± 0.0094	0.255 ± 0.007**	0.225 ± 0.005***
Hamelia patens extract 400mg/kg	0.170 ± 0.0577	0.263 ± 0.0042*	0.216 ± 0.004***	0.183 ± 0.004***
Diclofenac sodium 10mg/kg	0.176 ± 0.0071	0.270 ± 0.0057*	0.231 ± 0.004***	0.185 ± 0.0042***

Above values are expressed in mean ± SEM. One way ANOVA which is followed by Bonferroni multiple comparison's test, the standard and test groups compared to arthritic control. *P<0.05, **P<0.01, ***P<0.001.

Hematological parameters of FCA induced arthritis model

As showed in table 2 and figure 1, Disease control shown elevated levels of WBC count (11.28) and decreased levels of RBC and HB (5.93), (8.53) respectively. Whereas diclofenac sodium (10mg/kg) shown decreased levels of WBC (8.2), Increased levels of RBC (8.31) and increased levels of HB (13.13) which shown the significant difference (P<0.001, P<0.01 and P<0.01), respectively compared to arthritic control

Table 2: Effect of drug on hematological parameters of FCA induced arthritis model

GROUPS (n=6)	WBC (µl)	RBC (µl)	Hb (g/dl)
Normal Control	8.133 ± 0.1726	9.272 ± 0.0.0673	12.41 ± 1.671
Disease control	11.28 ± 0.11682$^{###}$	5.933 ± 0.286$^{###}$	8.53 ± 0.2737$^{###}$
Hamelia patens (200mg/kg)	8.060 ± 0.1984***	8.410 ± 0.0.1946**	11.18 ± 0.3642*
Hamelia patens 400mg/kg	8.92 ± 0.3005***	10.07 ± 0.3935**	12.73 ± 0.2722*
Diclofenac sodium (10mg/kg)	8.288 ± 0.0769***	8.317 ± 0.0.0872**	13.13 ± 0.0.1885**

Above values are expressed in mean ±Sem, one way ANOVA followed by Bonferroni multiple comparisons test all the groups were compared to disease control group *P<0.05, **P<0.01, ***P<0.001.

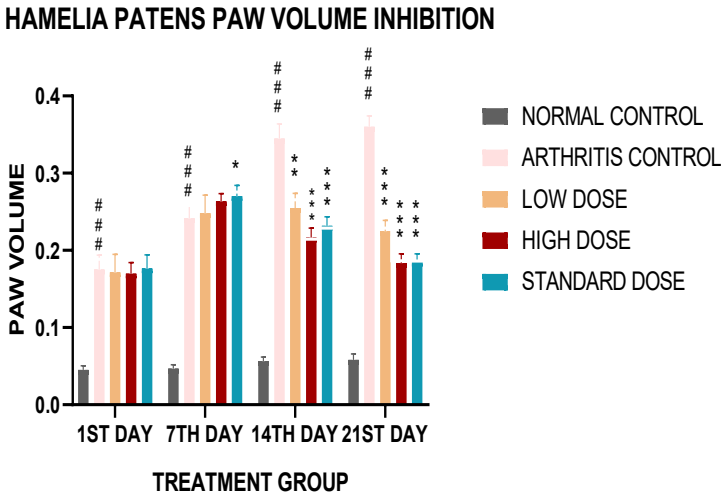

Figure 1: Effect of extracts on paw volume by FCA induced arthritis

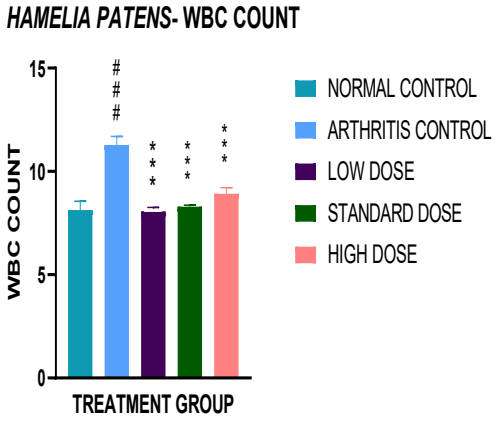

Figure 2: Effect of drug on WBC in FCA induced arthritis

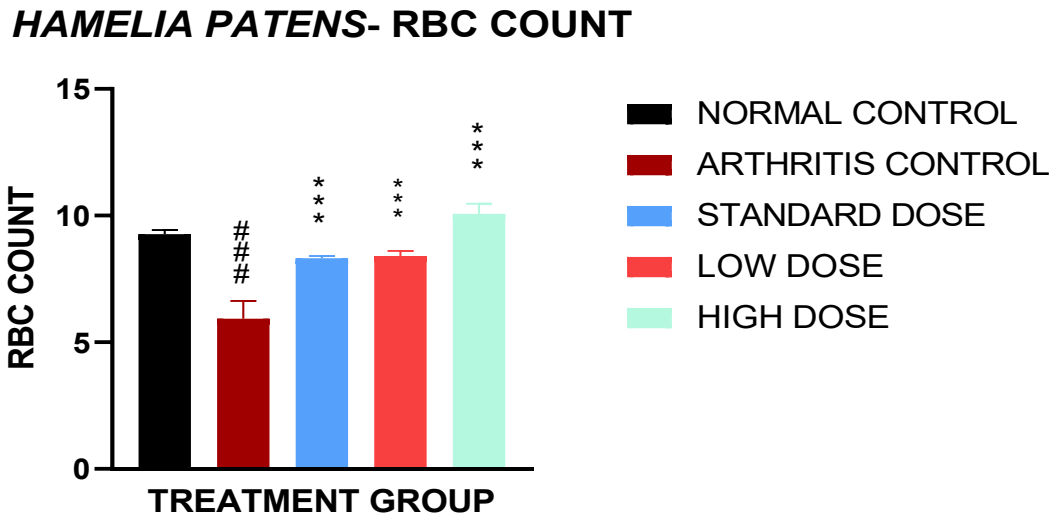

Figure 3: Effect of drug on RBC level by FCA-Induced arthritis model

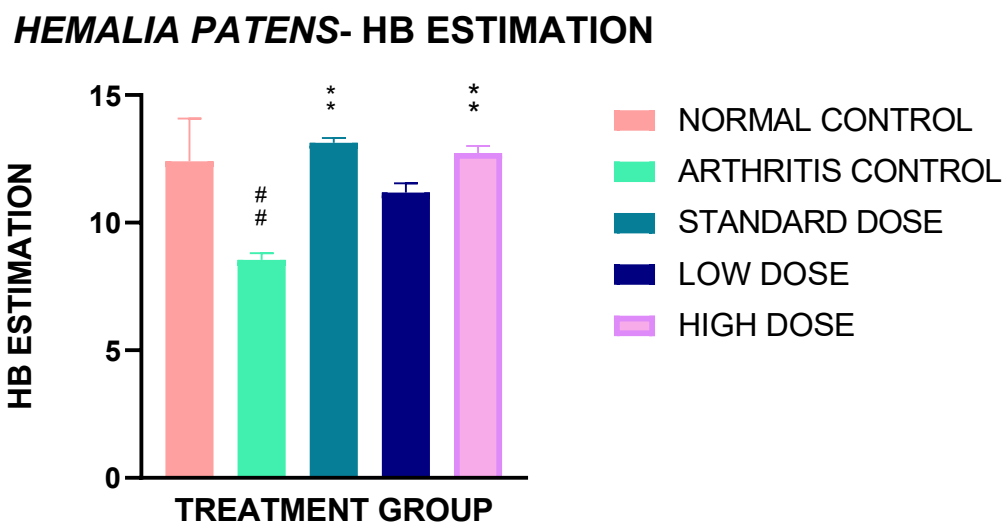

Figure 4: drugs effect on HB in FCA Induced arthritis model

Egg albumin denaturation method

In in-vitro anti-arthritic activity by Egg Albumin denaturation method, was screen different at concentration of 100, 250 and 500 mcg/ml. The ethanolic extract Exhibited 36.5, 46.34, and, 67.07 of % inhibition. Whereas, standard diclofenac at 100, 250 and 500 mcg/ml showed 13.41, 20.73 and 54.87 of inhibitory activity respectively, which is in dose dependent manner**.**

Table 4, Effect of drugs on protein denaturation

Treatment	**Concentration (µg/ml)**	**Percentage inhibition%**
Control	–	–
Hamelia patens	100 250 500	36.5 46.34 67.07
Diclofenac sodium	100 250 500	13.41 20.73 54.87

DISCUSSION

The present research investigated that the anti-arthritic activity of *Hamelia patens* by FCA-induced arthritis method. The findings demonstrated a significant anti-arthritic activity of *Hamelia patens*, as evidenced by a reduction in paw volume and improvement in other evaluated parameters. FCA, a potent immunological adjuvant widely used in experimental research, enhances antibody production and induces arthritis by triggering an immune response. Injection of killed *Mycobacterium tuberculosis* leads to the activation of inflammatory mediators like cytokines, interleukins, and tumor necrosis factor, resulting in chronic synovial inflammation and subsequent edema[13]. The present study evaluated the anti-arthritic activity by FCA induced and egg albumin denaturation in-vitro method. The in-vitro study demonstrated a dose-dependent percentage inhibition of protein denaturation. As dose increases, the protein denaturation inhibition also increases, which is indicating that the plant leaves have greater protective effect against protein denaturation, which is associated with anti-inflammatory conditions[14]. In the FCA-induced model, the rats were pre-treated with the *Hamelia patens* extract and diclofenac standard drug. Both treatments were showed that there was a significant reduction in the paw volume. On the initial day (day 1), similar paw volume measurements were observed across all the experimental groups. Although, by the 7th and 14th days, a marked enlarge in paw volume was noticed in the arthritic control group compared to the pre-treated groups. On the final day (day 21), the paw volume remained significantly higher in case of disease control group, whereas the test and standard-treated groups showed a considerable reduction. Notably, the paw volume reduction observed in the standard group was similar to that of the high dose ethanolic extract (400 mg/kg) was observed. These findings were further supported by hematological analysis, which revealed an increased WBC count along with decreased RBC count and hemoglobin levels in the arthritic control group, indicating the worsen of arthritic condition. In contrast, the pre-treated test and standard groups exhibited normalization of WBC, RBC,

and hemoglobin levels, demonstrating that the anti-arthritic potential of *Hamelia patens* leaf extracts. The high-dose extract showed greater efficacy compared to the low-dose treatment. The reduction in paw edema and inflammation may be due to the existence of bioactive phytochemicals like flavonoids and alkaloids, which are known to control the inflammation by inhibiting the release of inflammatory mediators15. Hence this drug might act as a potent anti-arthritis agent.

CONCLUSION

With the aim of evaluating of an anti-arthritic activity, the in-vitro study revealed that the anti-arthritic effect of extracts by inhibiting the protein denaturation effect, which were compared between the test and standard drugs, in which the plant exhibited potent anti-arthritic activity. From the in vivo study, the plant extracts showed anti-arthritic effect, as which was proven by a reduction in paw volume of rats. Overall, the findings suggest that *Hamelia patens* possess potent anti-arthritic effect. Further studies are necessary to identify the specific phytochemical which are responsible for its anti-arthritic property.

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