AN EXPERIMENTAL STUDY ON ANTI INFLAMMATORY ACTION OF HYPERICUM PERFORATUM IN CARRAGEENAN INDUCED ARTHRITIS RAT MODELS

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ABSTRACT

Background: Inflammation is a key pathogenic mechanism underlying many acute and chronic disorders. The carrageenan-induced paw edema model in rats is a validated experimental method for screening anti-inflammatory agents. Hypericum perforatum is widely used in homoeopathy for inflammatory and traumatic conditions; however, evidence on its ultra-high dilutions remains limited.

Aim: To evaluate and compare the anti-inflammatory effects of ultra-high dilutions of Hypericum perforatum (6C and 30C) with indomethacin using a carrageenan-induced acute inflammation model in Wistar albino rats.

Materials and Methods: Twelve healthy Wistar albino rats were randomly allocated into four groups: control, indomethacin-treated (3mg/kg), Hypericum 6C-treated, and Hypericum 30C-treated. Acute inflammation was induced by sub-plantar injection of 0.1 mL of 1% carrageenan into the right hind paw. Paw volume was measured using a digital plethysmometer. Hematological parameters, including erythrocyte sedimentation rate (ESR), total leukocyte count (TLC), and neutrophil percentage, were assessed. Statistical analysis was performed using appropriate tests, with $p<0.05$ considered statistically significant.

Results: Indomethacin produced a significant reduction in paw edema and systemic inflammatory markers. Hypericum 6C showed a moderate reduction in neutrophil percentage with minimal reduction in paw volume, suggesting a predominantly local anti-inflammatory effect. Hypericum 30C demonstrated a greater reduction in ESR, indicating a systemic anti-inflammatory response. Both potencies showed statistically significant effects compared with the control group.

Conclusion: Ultra-high dilutions of Hypericum perforatum demonstrated measurable anti-inflammatory activity with potency-specific differences. Hypericum 6C primarily influenced local inflammatory parameters, whereas Hypericum 30C exhibited greater systemic effects. These findings support the potential role of homoeopathic medicines as complementary therapeutic agents in acute inflammatory conditions.

Keywords: Hypericum perforatum; Homoeopathy; Carrageenan-induced paw edema; Anti-inflammatory activity; Ultra-high dilutions

INTRODUCTION

Inflammation is a fundamental protective response of the human body against infections, tissue injury, and harmful stimuli, playing a vital role in healing and tissue repair. However, when inflammation becomes chronic or dysregulated, it contributes to the pathogenesis of several non-communicable diseases, including rheumatoid arthritis, diabetes mellitus, cardiovascular diseases, and malignancies[1,2]. Chronic inflammation has therefore emerged as a major focus of biomedical research.

Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory disorder affecting approximately 0.5–1% of the global population, with a higher prevalence among women and older adults. It primarily targets the synovial membrane, leading to joint pain, swelling, stiffness, progressive disability, and significant socioeconomic burden. Early diagnosis and timely initiation of therapy are crucial, as they reduce joint destruction and may even lead to drug-free remission in certain cases[3,4].

Experimental models play a crucial role in evaluating anti-inflammatory agents. The carrageenan-induced paw edema model in rats is a widely accepted and reliable method for studying acute inflammation. It produces a biphasic inflammatory response involving early mediators such as histamine and serotonin, followed by prostaglandin-mediated leukocyte infiltration, allowing objective assessment of anti-inflammatory activity through paw volume measurement[5,6].

Although non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin are effective, their long-term use is associated with adverse effects such as gastrointestinal irritation and renal toxicity, prompting interest in safer alternative therapeutic systems[7]. Homoeopathy, introduced by Samuel Hahnemann, is based on the principles of similar and potentization. Despite controversy regarding ultra-high dilutions, emerging evidence suggests that such dilutions may exert biological effects through nano-structural or informational mechanisms[8,9].

Hypericum perforatum (St. John's Wort) has long been used in homoeopathic and herbal medicine for inflammatory conditions, neuralgic pain, and tissue injury. While phytochemical constituents such as hypericin and hyperforin are known for anti-inflammatory effects, recent studies suggest that homoeopathically potentized preparations like Hypericum 6C and 30C may also influence inflammatory responses in experimental models[10–12]. This study aims to experimentally evaluate the anti-inflammatory

activity of Hypericum perforatum 6C and 30C using a carrageenan-induced paw edema model in Wistar albino rats.

MATERIALS AND METHODS

Animals and Housing Conditions

Twelve Wistar albino rats weighing between 150 and 220 g, approximately 90 days old at the commencement of the experiment. Rats which are approved by IAEC of The Dale View College of Pharmacy & Research Centre bearing Approval No.1118/PO/Re/S/07/CPCSEA. The animals were housed under standard laboratory conditions with adequate ventilation and maintained on a 12-hour light–dark cycle. Standard laboratory pellet diet and drinking water were provided. All animals were allowed an acclimatization period of one week prior to the initiation of the experiment to ensure physiological stability.

Induction of Acute Inflammation

Acute inflammation was induced in the right hind paw of each rat by subplantar injection of 0.1 ml of 1% carrageenan solution prepared in normal saline. Baseline paw volume was recorded immediately before carrageenan administration (0 hour) using a digital plethysmometer. Subsequent paw volume measurements were recorded at the 3rd, 24th, and 48th hours following induction. Blood samples were collected retro-orbitally at the same time intervals for estimation of erythrocyte sedimentation rate (ESR), neutrophil percentage, and total leukocyte count.

Study Design and Experimental Setting

This was a controlled, randomized experimental animal study designed to evaluate and compare the anti-inflammatory effects of two homeopathic potencies of *Hypericum perforatum* (6C and 30C) with a standard non-steroidal anti-inflammatory drug, indomethacin. A total of twelve Wistar albino rats were randomly allocated into four groups, with three animals in each group.

Drug Intervention

The homeopathic medicines *Hypericum perforatum* 6C and 30C were administered orally in water dose. For each potency, one drop of the medicine was diluted in 5 ml of distilled water. From this preparation, five drops were administered orally using an oral feeding needle at 3-hour intervals for two doses. Fresh dilutions were prepared prior to each administration.

Indomethacin was used as the standard drug and administered orally at a dose of 3 mg/kg body weight. The required quantity of indomethacin was powdered, dissolved in 5 ml of distilled water, and administered according to the body weight of each animal.

Group I	Control group without medication
Group II	Standard group – Indomethacin ((3 mg/kg body weight, p.o)
Group III	Hypericum perforatum 6C
Group IV	Hypericum perforatum 30C

Table 1: Animal allotment

Drug Administration and Monitoring

All drugs were administered orally using an oral feeding needle. Paw volume was recorded at baseline (0 hour), at the 3rd hour after carrageenan injection, and subsequently at the 24th and 48th hours using a digital plethysmometer. Blood samples were collected retro-orbitally at 0, 3, 24, and 48 hours for hematological analysis, including ESR, neutrophil count, and total leukocyte count.

The primary outcome measure was the reduction in paw edema, assessed by changes in paw volume at each time point following carrageenan induction. Secondary outcome measures included changes in ESR, neutrophil percentage, and total leukocyte count. Comparative evaluation of these parameters across all four groups was performed to assess the degree of inflammatory response and the anti-inflammatory efficacy of the interventions.

STATISTICAL DATA

The investigation's statistical analysis showed significant evidence of differences in the four experimental groups ($p<0.05$) as analysed using one-way ANOVA. Post-hoc analysis of the significant differences using Tukey's HSD test showed significantly larger effects in Indomethacin decreasing paw volume than either Hypericum 6C or Hypericum 30C ($p<0.01$). Hypericum 6C also shows a significant systemically in ESR and total leukocyte count when compared to control ($p<0.05$). Hypericum 30C exhibited a significant decrease in paw volume ($p<0.05$), but had an only slightly significant effect on systemic measures. Thus, the effectiveness shown in Hypericum 6C can be thought of as more systemic in its action and Hypericum 30C more topical in its effectiveness. There were no adverse effects observed in any group, showing validation of both potencies' safety.

OBSERVATIONS AND RESULTS

The anti-inflammatory activity of Hypericum perforatum in ultra-high dilutions was evaluated using a carrageenan-induced paw edema model in Wistar albino rats. Animals were randomly divided into four groups: control, indomethacin-treated (standard), Hypericum 6C-treated, and Hypericum 30C-treated. All groups exhibited comparable baseline paw volumes prior to carrageenan administration. Acute inflammation was successfully induced in all animals, as evidenced by a significant increase in paw volume two hours post-induction. The results at end hours was given below.

Table 2: Mean Paw Volume of 4 groups

SL.NO	TIME PERIOD OF PAW VOLUME READING	MEAN PAW VOLUME			
		GROUP 1	GROUP 2	GROUP 3	GROUP 4
1	BEFORE INDUCTION	0.58333333	0.54	0.67	1.31
2	AFTER INDUCTION	1.12666667	1.12	1.05333333	1.79333333
3	AFTER MEDICATION	1.46666667	0.31333333	1.00	1.38333333

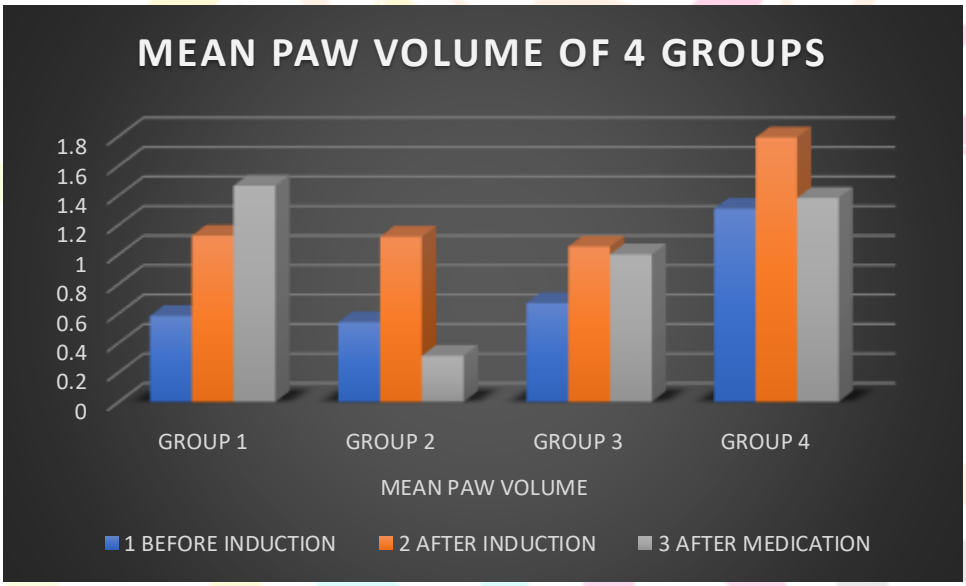


Fig. 1 Comparison of Mean Paw Volume

Table 2: Comparing Blood Parameters

BLOOD PARAMETERS	BEFORE CARAGEENAN INDUCTION				AFTER CARAGEENAN INDUCTION				AFTER MEDICATION			
	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4

NEUTROPHILS	22.6	22.866667	24.1	23.5	44.966667	46.166667	45.6	42.6	26.233333	14.066667	14.066667	26.633333
LEUCOCYTES	11.0533333	8.2733333	5.726667	9.866667	5.686667	8.7033333	6.486667	12.766667	5.836667	3.19	7.0733333	11.033333
ESR	3	3.1333333	3.0333333	3.1	3.1	11.2	12	11.33333	3.033333	7.1	8.233333	5.4

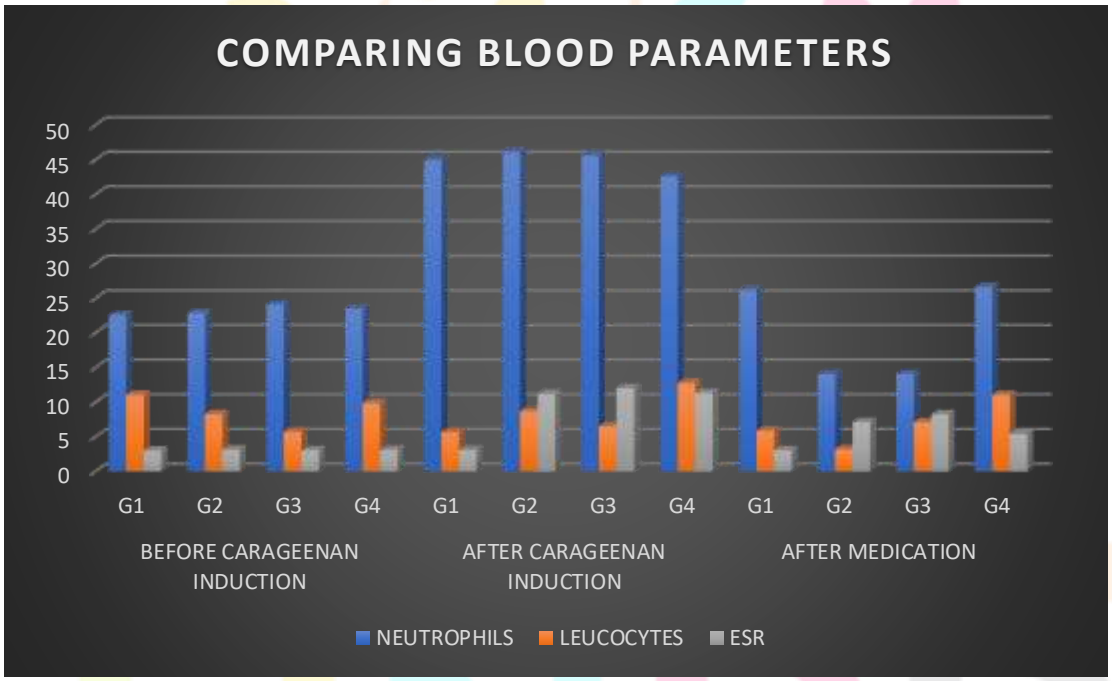
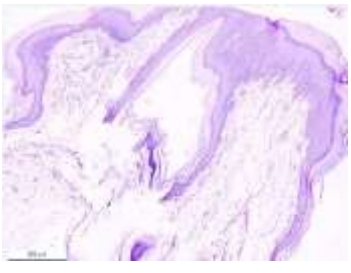
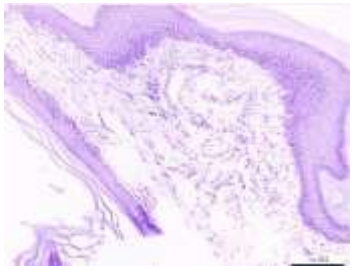


Fig.2 Comparison of Blood Parameters

The results shows that control group exhibited progressive paw edema with persistently elevated inflammatory markers, confirming sustained acute inflammation in the absence of treatment. The indomethacin-treated group showed marked reductions in paw volume, neutrophil percentage, ESR, and total leukocyte count, indicating strong local and systemic anti-inflammatory effects. Hypericum 6C produced a moderate anti-inflammatory response with notable reduction in neutrophil percentage and ESR, suggesting effective modulation of acute cellular inflammation. Hypericum 30C demonstrated comparatively greater reduction in ESR with moderate reduction in paw edema, reflecting a predominantly systemic anti-inflammatory action. Overall, both potencies of *Hypericum perforatum* exhibited measurable anti-inflammatory activity with differential effects at cellular and systemic levels.

HISTOPATHOLOGICAL FINDINGS

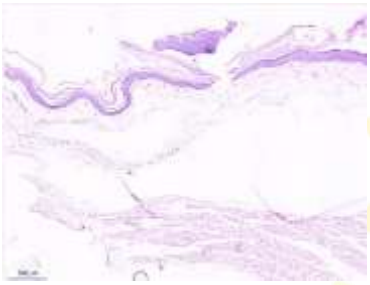
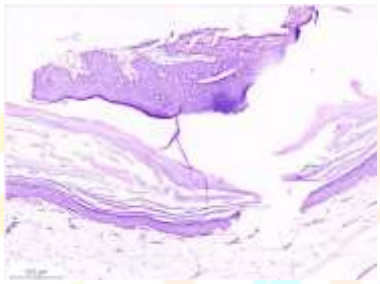



GROUP 1 - NEGATIVE CONTROL

Marked degree[+++] of hyperkeratosis, moderate degree [++] of odema with

inflammatory cells infiltrations surrounding to the cartilage, moderate degree of

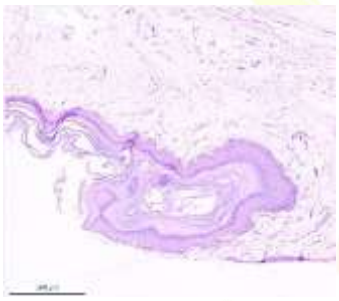
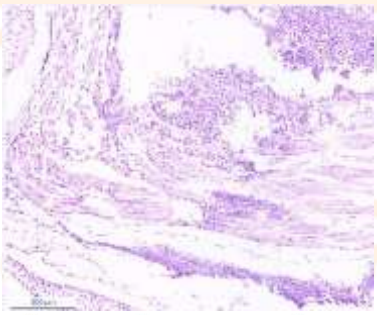
Fig.3 Carrageenan induced

GROUP 2 - POSITIVE CONTROL

Mild degree [+] of hyperkeratosis, odema, epithelial discontinuity, no evident inflammatory infiltration

Fig.4 Indomethacin

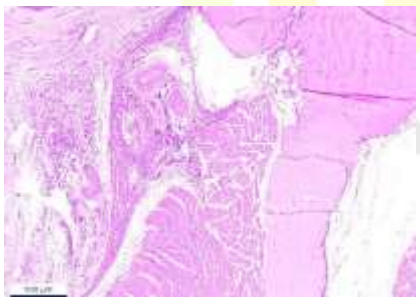
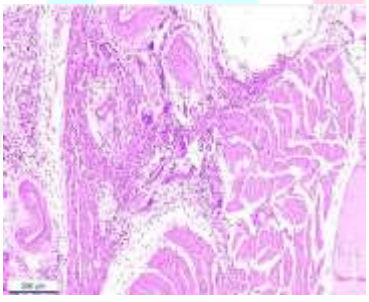



GROUP 3 - HYPERICUM 6C

Marked degree[+++] of hyperkeratosis, odema with

inflammatory cells infiltrations, moderate degree of epithelial discontinuity

Fig.5 Hypericum 6C

GROUP 4 – HYPERICUM 30C

Marked epidermal thickening,Marked dermal inflammatory cell infiltration,Marked vascularization

Fig.6 Hypericum 30C

DISCUSSION

The present experimental study evaluated the anti-inflammatory activity of *Hypericum perforatum* in ultra-high homeopathic dilutions (6C and 30C) using the carrageenan-induced acute inflammation model in Wistar albino rats. Inflammation was successfully induced in all groups, as evidenced by increased paw edema and

changes in haematological inflammatory markers, confirming the reliability and sensitivity of the experimental model.

The control group demonstrated persistent inflammation throughout the observation period, validating the absence of spontaneous resolution. The indomethacin-treated group showed a marked reduction in paw volume and inflammatory markers such as neutrophil percentage, ESR, and total leukocyte count, reflecting both local and systemic anti-inflammatory activity. These findings are consistent with the established pharmacological profile of non-steroidal anti-inflammatory drugs and served as a standard for comparison.

Both Hypericum-treated groups exhibited measurable anti-inflammatory responses, though less pronounced than indomethacin. Hypericum 6C showed a significant reduction in neutrophil percentage with minimal reduction in paw edema, suggesting its role in modulating acute cellular inflammatory responses. Hypericum 30C, on the other hand, demonstrated a comparatively greater reduction in ESR, indicating a more prominent systemic anti-inflammatory effect rather than localized suppression. The differential effects observed between the two potencies support the homoeopathic concept of potency-specific action, wherein lower potencies act predominantly on the physical or local level, while higher potencies influence systemic or dynamic processes[15,16].

These findings are in agreement with the study by Chakraborty et al., which demonstrated the efficacy of *Hypericum perforatum* 30C in reducing carrageenan-induced paw edema, although their work was limited to local inflammatory parameters[13]. The present study extends these observations by incorporating hematological markers such as neutrophil percentage, ESR, and total leukocyte count, providing a more comprehensive assessment of both local and systemic immune modulation. Additionally, studies by Kundu et al. have demonstrated the ability of homoeopathic remedies to modulate inflammatory cytokines like TNF-α and IL-6, offering a possible biological basis for the observed effects[14].

From a homoeopathic standpoint, the findings support principles such as *Similia Similibus Curentur*, minimum dose, and vital force. The absence of suppressive effects on leukocyte counts suggests immune modulation rather than inhibition, aligning with Hahnemannian philosophy[15–17]. Although indomethacin exhibited superior anti-inflammatory activity, the observable biological responses of *Hypericum perforatum* 6C and 30C indicate its potential role as a safe and complementary therapeutic option in inflammatory conditions.

CONCLUSION

The present study demonstrated that *Hypericum perforatum* in ultra-high dilutions (6C and 30C) exhibited measurable anti-inflammatory activity in a carrageenan-induced paw edema model in Wistar albino rats. Both potencies produced reductions in inflammatory parameters, with 6C showing greater influence on acute cellular markers such as neutrophil percentage, and 30C demonstrating comparatively stronger effects on systemic inflammation as reflected by ESR values. Although the anti-inflammatory effects were less pronounced than those observed with indomethacin, the standard drug group confirmed the sensitivity and reliability of the experimental model. The findings indicate potency-dependent differences in inflammatory

modulation without evidence of suppressive immunological effects. Overall, *Hypericum perforatum* 6C and 30C may be considered as safe, complementary interventions in the management of acute inflammatory conditions. Further studies involving larger sample sizes, chronic inflammation models, and molecular inflammatory markers are recommended to validate and expand upon these observations.

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