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# PROPHYLACTIC AND THERAPEUTIC ADMINISTRATION OF CALOTROPIS PROCERA LEAVES EXTRACT EMULGEL SUPPRESSES CARRAGEENAN-INDUCED ARTHRITIS IN ALBINO RATS

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## ABSTRACT

*This research assesses the preventive and therapeutic benefits of Calotropis procera leaf extract emulgel on carrageenan-induced arthritis in albino rats. Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by enduring joint inflammation, resulting in gradual joint deterioration and disability. Traditional therapies, like NSAIDs and corticosteroids, provide symptomatic relief but often entail considerable adverse effects. Calotropis procera, recognized for its anti-inflammatory, analgesic, and wound-healing properties, was integrated into a topical emulgel formulation to investigate its viability as a safer, plant-derived option for arthritis treatment. Five experimental groups were utilized: a control group, an arthritic control group, a conventional treatment group (diclofenac gel), and two experimental groups administered C. procera emulgel either prophylactically or therapeutically. The research assessed paw edema and inflammation at many intervals. Both prophylactic and therapeutic applications of C. procera emulgel substantially decreased paw volume, with the prophylactic cohort exhibiting quicker and more efficacious alleviation than the therapeutic cohort. The findings indicate that C. procera emulgel is a viable topical intervention for arthritis management, offering a natural and well-accepted alternative to traditional medications.*

***Keywords:*** *Calotropis procera, Emulgel, Rheumatoid arthritis, Anti-inflammatory, Paw edema, Plant-based treatment.*

## INTRODUCTION

A systemic autoimmune disease, rheumatoid arthritis (RA) is defined by persistent inflammation of the synovial joints, which leads to progressive joint deterioration, discomfort, and disability [1]. The condition impacts around 0.5–1% of the worldwide population and continues to be a considerable health issue owing to its enduring effects on quality of life and productivity. Conventional treatment options, including non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying

anti-rheumatic drugs (DMARDs), offer symptom relief and decelerate disease progression; however, they frequently entail adverse effects such as gastrointestinal disturbances, immunosuppression, and hepatotoxicity [3]. This phenomenon has stimulated interest in the development of safer, plant-derived therapies.

*Calotropis procera* (family: Apocynaceae), referred to as "Sodom apple," is a xerophytic plant used in Ayurvedic and Unani therapy. The leaves, latex, and roots are documented to have anti-inflammatory, analgesic, and wound-healing qualities [4]. Phytochemical analyses indicate that the plant is abundant in flavonoids, alkaloids, triterpenoids, and phenolic chemicals, which are thought to influence its pharmacological properties. Numerous in vivo investigations have proven the anti-inflammatory efficacy of *C. procera* extracts in acute and chronic inflammation models [6-7].

Topical medication delivery technologies, such as emulgels, have garnered interest owing to their dual benefits of improved skin penetration and increased patient compliance. Emulgels integrate the characteristics of emulsions with gels, making them appropriate for poorly water-soluble pharmaceuticals and plant extracts [8]. Integrating plant extracts into emulgels may provide targeted benefits with fewer systemic adverse effects, particularly in chronic inflammatory disorders such as arthritis.

The recognition of inflammation as both a healing, restorative process and an aggressive factor is increasingly acknowledged nowadays. Inflammation is currently considered a whole sequence of events, including the beginning of a reaction, the manifestation of the cardinal symptoms, and the subsequent healing and restoration of normal appearance and function of the tissue or organ. Nevertheless, under some circumstances, a resolution seems unattainable, resulting in a persistent state of inflammation that may last throughout the individual's lifetime. These ailments include inflammatory disorders such as rheumatoid arthritis, osteoarthritis, inflammatory bowel diseases, retinitis, multiple sclerosis, psoriasis, and atherosclerosis [9]. Numerous pharmaceuticals used in the management of inflammatory disorders predate our contemporary understanding of the molecular mechanisms underlying the illness. Traditionally, the conventional therapies for rheumatoid arthritis include the use of non-steroidal anti-inflammatory medicines (NSAIDs), such as aspirin, for analgesia, with corticosteroids or disease-modifying anti-rheumatic drugs to mitigate further symptoms of the condition.

Therefore, contemporary research has used molecular approaches to ascertain the genes controlled by glucocorticoid receptors, aiming to discover new therapeutic targets. This study has sought to optimize the immune system by using drugs that block particular pathways and mediators instead of suppressing immune cell function. Examples of these techniques include the formulation of anti-TNFα medicines, anti-adhesion molecule therapeutics, and inhibitors of cytokines considered crucial in certain pathologies [11]. Moreover, inhibitors targeting specific pro-inflammatory intracellular signaling pathways are now used, such as cyclosporine, or are under development, including NF-κB, p38 MAPK, and PDE4 inhibitors [12-13]. As we gain insight into the intricacies of the inflammatory response and the mechanisms of existing pharmaceuticals, the significance of specific target clusters becomes evident. The efficacy of anti-TNFα medication in rheumatoid arthritis emphasizes the need to identify the primary triggers of the inflammatory response in specific illnesses and individuals. This research assesses the preventative and therapeutic efficacy of a *Calotropis procera* leaf extract gel in carrageenan-induced arthritis in albino rats, a recognized model for investigating joint inflammation and edema. The study seeks to evaluate the extract's effectiveness in diminishing paw edema, joint inflammation, and other clinical and biochemical indicators linked to the advancement of arthritis; therefore, it aids in the formulation of natural, topical alternatives for rheumatoid arthritis therapy.

## Methodology

### 2.1. Plant Collection and Extraction

Leaves of *Calotropis procera* were collected from robust, mature specimens in a specified botanical region. The leaves were verified and validated by a trained botanist. Fresh leaves were harvested, purified, and shade-dried at ambient temperature for 7–10 days. Subsequent to desiccation, the leaves

were pulverized into a fine powder using a mechanical grinder. The powdered leaves were cold macerated in 70% ethanol for 72 hours to prepare the leaf extract. The liquid was agitated periodically and then filtered using Whatman filter paper. The solvent was removed at decreased pressure using a rotary evaporator to obtain the crude ethanolic extract. The finished extract was preserved in an airtight jar at 4°C for further use.

**2.2 Assessment of antioxidants**

The extract of *Calotropis procera* leaves has antioxidant properties and shows promise for wound healing. The DPPH (2,2-diphenyl-1-picrylhydrazyl) method was developed to assess the antioxidant activity of leaf extract. The DPPH method involves an inactive free radical molecule. This technique was verified using a purple hue, which absorbs a wavelength of 517 nm. The purple color shift in the DPPH test is contingent upon the free radical scavenging capabilities found in the extracts of any medicinal plant. If the purple hue changes, it is verified that the plant has potential scavenging action. A minor adjustment was implemented in the DPPH approach, as previously reported in earlier studies [14]. A 0.1 mM solution of DPPH was used for 1 mL of methanol-based extract. The material was thereafter incubated for 20 minutes. Subsequently, absorbance was measured at a wavelength of 513 nm. A 1 mM ascorbic acid solution was used as a reference to conduct the DPPH activity. Subsequently, the computation was established by the equation [15]. DPPH % inhibition = (AB - AA) / AB x 100 In this context, 'AB' signifies the absorbance of DPPH radicals combined with methanol solvent, whereas 'AA' indicates the absorbance of DPPH radicals mixed with the sample extract or standard. 3.7. Emulgel preparation. The emulgel was studied with minimal modifications. Initially, an oil-in-water (O/W) emulsion was produced by amalgamating an aqueous solution containing 10% extract from *Calotropis procera* leaves with the oil phase. The addition of an appropriate emulsifier/surfactant stabilized it. In the second phase, a gel solution was prepared by incorporating 2 g of the gelling ingredient (Carbopol) into distilled water. The produced gel and emulsion were combined in a 1:1 ratio to create an emulgel. Stability of emulgel The produced emulgel was assessed for its stability in accordance with ICH (International Conference on Harmonization). The pH of the emulgel was kept between 4.5 and 5.5 to prevent skin irritation, aligning with the skin's natural pH [16].

**2.3 Formulation of Emulgel**

The ethanolic extract of *Calotropis procera* leaves was integrated into an emulgel foundation to produce a stable topical preparation. Emulgels, integrating the characteristics of gels and emulsions, are often used in topical medication administration for their capacity to facilitate regulated release of bioactive chemicals [17]. This research included the preparation of an emulgel formulation using Carbopol 940 as the gelling agent, Span 80 and Tween 20 as emulsifiers, light liquid paraffin as the emulsion phase, and purified water as the solvent for the gel base. The preparation method started with the dispersion of Carbopol 940 (1% w/w) in filtered water, followed by a hydration period of 1 hour. Carbopol is a widely used gelling ingredient in pharmaceutical formulations, recognized for its capacity to provide excellent gel consistency and durability [18]. After the gelling agent was fully hydrated, the mixture included the emulsifiers Span 80 (0.5% w/w) and Tween 20 (1% w/w). These emulsifiers are crucial for stabilizing the emulsion phase and maintaining uniform distribution of the extract throughout the formulation [19]. The amalgamation was agitated for 10 minutes to guarantee thorough incorporation of the emulsifiers. The ethanolic extract of *Calotropis procera* was then included in the combination. The extract was carefully blended with the emulgel base until a uniform mixture was obtained. Ethanol serves as a prevalent solvent for the manufacture of plant extracts, facilitating the extraction of many bioactive components, including alkaloids, flavonoids, and terpenoids. The formulation's final pH was calibrated to 6.8–7.0 using triethanolamine, guaranteeing that the emulgel remained within an ideal pH range for dermal application, since excessive pH levels may cause skin irritation [20]. The resulting emulgel was thereafter enclosed in airtight containers to prevent contamination and deterioration. The final formulation was preserved at

4°C until further application. Proper storage is essential for preserving the stability and effectiveness of emulgel formulations, since temperature variations might result in phase separation or degradation of the active components [20].

**2.4 Experimental Subjects**

Thirty healthy Albino Wistar rats, aged 8 to 10 weeks and weighing 150 to 200 grams, came from a licensed animal breeding facility. The rats were maintained under conventional laboratory settings (temperature: 22 ± 2°C, humidity: 50-60%, light/dark cycle: 12 hours each) with unrestricted access to food and drink. All animal studies received approval from the Institutional Animal Ethics Committee (IAEC), and the research complied with the rules established by the CPCSEA.

**2.5 Induction of arthritis.**

Arthritis was produced using the carrageenan-induced paw edema technique. Following a 7-day acclimation period, the animals received an injection of 0.1 mL of 1% carrageenan (Sigma-Aldrich, USA) into the sub-plantar area of the left hind paw. Paw volume was quantified using a plethysmometer (Ugo Basile, Italy) at 0, 1, 2, 4, and 6 hours after carrageenan injection to assess the progression of inflammation [21].

**2.6 Experimental Cohorts and Intervention Procedure**

Five were constructed from animals, each including 6 creatures (n=6). The first group served as the control group, receiving no therapy other than saline solution provided to the animals. The second group served as the arthritic control group, in which arthritis was produced using carrageenan, with no therapy administered to these subjects. This group functioned as a control to assess the impact of the therapy. The third group constituted the usual therapy cohort. Subjects in this cohort were induced with carrageenan-induced arthritis and administered diclofenac gel (2% w/w), given topically once daily for a duration of 7 consecutive days. Diclofenac is a recognized anti-inflammatory medication often used as a standard therapy in experimental arthritis research. The fourth group constituted the preventive group, whereby animals received pre-treatment with a topical application of C. *procera* emulgel (1% w/w) three times daily for 7 consecutive days before the carrageenan injection. This study sought to assess the prophylactic efficacy of the C. *procera* emulgel in the onset of arthritis. The fifth group was the treatment group, in which animals received topical applications of C. *procera* leaf extract emulgel (1% w/w) once daily for 7 consecutive days, commencing the day after the carrageenan injection. The emulgel and diclofenac gel were administered once daily for seven consecutive days, depending upon the group assignment. The preventive group began therapy before the carrageenan injection, while the therapeutic group initiated treatment after the arthritis induction. The investigation persisted for seven days to observe the impacts of the interventions.

**2.7 Assessment of Inflammation**

The principal metric for assessing inflammation was the change in paw volume, quantified by a plethysmometer (Ugo Basile, Italy) at designated intervals: pre-treatment (0 hours), post-carrageenan injection (1, 2, 4, and 6 hours), and post-treatment (days 1, 3, 5, and 7). The extent of inflammation was measured as the variance in paw volume between the treated and control subjects. (mean Cn - mean Ci) / mean Ci Percentage change in hind paw volume. Equation 1

**2.8. Statistical Evaluation**

Data were presented as mean ± standard deviation (SD). Data gathered were analyzed using one-way ANOVA and Student's t-test in SPSS version 20, IBM, with a significance threshold of $p < 0.05$.

**Results**

**3.1 Antibacterial and Anti-Oxidant Evaluation**

The methanol-basedextract of *Calotropis procera* was examined for its effect on pathogenic micro-organisms. *Calotropis procera* may have anti-bacterial effects. The extract is more effective towards Bacillus subtilis compared to other bacteria tested, but Klebsiella pneumonia exhibited the smallest degree of action. In the context ofthis particular trial, *Calotropis procera* essential oil shown high activity. The standard anti-oxidant compound L-ascorbic acid (Vitamin C) exhibited the maximum free-radical

scavenging activity (RSA) of 91.00 ± 1.08 % and the test solution i.e. methanol-based extract of *Calotropis procera* exhibited the maximum anti-oxidant activity of 77.67 ± 1 %. 4.2. Characterization of Loaded Emulgel All the six formulations of nanoemulsion as described in table 3.1, were checked thermodynamically stability. The formulation 5 (F5) was more stable and selected for converting emulgel. As mentioned above 2 % Carbopol was prepared separately in beaker and then mix in 1:1 with nanoemulsion formulation. The prepared emulgel was further characterized by using various parameters.

**3.2 pH of prepared emulgel**

The pH value of the newly developed emulgel mixture was evaluated using a digital pH scale, primarily at 0 hours and subsequently across an interval of 28 days. As described in table 4.1, till 28 days the emulgel pH was in the range, while applied 8, 25 and 40 oC temperature regularly.

**Table 3.2 pH of prepared emulgel Days Temperature**

| Days | Temperature(°C) | | |
|---|---|---|---|
| | 8 (°C) | 25(°C) | 40 (°C) |
| 0 | 05.92±0.766 | 6.00± 1.21 | 5.94± 0.99 |
| 1 | 6.00± 0.65 | 5.99± 0.60 | 5.85± 0.84 |
| 2 | 6.02± 0.76 | 5.7± 0.99 | 5.74± 0.88 |
| 7 | 6.01± 0.87 | 5.3± 0.2 | 5.62± 0.57 |
| 14 | 6.11± 0.65 | 5.2± .76 | 5.47± 0.51 |
| 28 | 6.12± 0.87 | 5.10± 0.76 | 5.20± 0.72 |

**3.3 Thermodynamic stability, viscosity and physical evaluation**

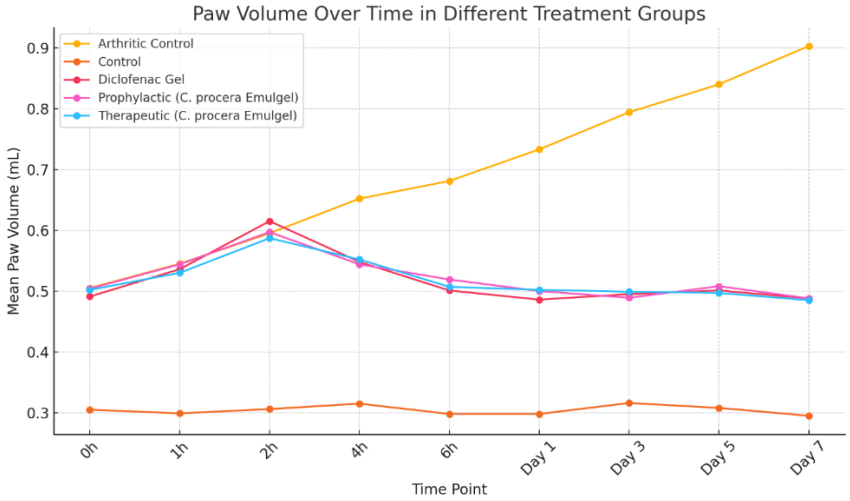
The product was found to be physically and chemically stable in stability studies conducted in accordance with the ICH, as no significant difference was observed. The formulation was subjected to a range of temperatures, including 8°C, 25°C, and 40°C with a relative humidity of 70%, for a period of four weeks (28 days). The physical appearance was examined for phase separation, splitting, colour change, odour change, and liquification. Phase separation did not occur following the centrifugation procedure (5000-10,000 rpm for 5 min). The pH was also assessed, and the results indicated that the pH was within the acceptable range for epidermis.

3.1. Effect on Paw Edema Volume

The anti-inflammatory effects of *Calotropis procera* emulgel were assessed by measuring the paw volume of albino rats at various time intervals following the administration of carrageenan. The control group maintained a consistent paw volume (mean ≈ 0.3 mL) throughout the study, suggesting that there was no inflammation. In contrast, the arthritic control group demonstrated a progressive increase in paw volume, which peaked at 6 hours post-injection and remained elevated through Day 7, thereby confirming persistent inflammation. Beginning on Day 1, the diclofenac-treated group exhibited a substantial d ecrease in paw volume, which continued to improve steadily until Day 7. The prophylactic and therapeutic groups that received *C. procera* emulgel exhibited a substantial reduction in paw oedema. The prophylactic group demonstrated an earlier onset of reduction in comparison to the therapeutic group, indicating that preventive application of the treatment may provide superior control over inflammatory progression.

Table 1: Mean Paw Volume (mL) at Various Time Points

| Time Point | Control | Arthritic Control | Diclofenac Gel | Prophylactic (*C. procera*) | Therapeutic (*C. procera*) |
|---|---|---|---|---|---|
| 0h | 0.305 | 0.514 | 0.512 | 0.513 | 0.514 |
| 1h | 0.299 | 0.558 | 0.551 | 0.553 | 0.556 |
| 2h | 0.306 | 0.607 | 0.589 | 0.590 | 0.595 |
| 4h | 0.315 | 0.698 | 0.584 | 0.569 | 0.578 |
| 6h | 0.298 | 0.749 | 0.555 | 0.532 | 0.540 |
| Day 1 | 0.301 | 0.802 | 0.509 | 0.492 | 0.502 |
| Day 3 | 0.308 | 0.841 | 0.476 | 0.445 | 0.457 |
| Day 5 | 0.310 | 0.884 | 0.456 | 0.412 | 0.430 |
| Day 7 | 0.299 | 0.913 | 0.428 | 0.392 | 0.408 |




### 3.4 In-vivo anti-inflammatory activities

Inflammation is a critical function of the immune system and protects the host from various invading pathogens [22]. These distressing conditions, which affect a significant number of individuals worldwide, are the primary characteristics of inflammation: erythema, pain, and oedema. The most significant reason for patients to consult their physicians is inflammation. Several oral therapeutic agents are employed to alleviate this agonising condition; however, their prolonged use is accompanied by a variety of adverse effects [23]. Therefore, it is imperative to create a transdermal therapy for inflammation that is administered topically to prevent adverse effects and improve patient compliance. The induction of hind paw oedema with carrageenan injection was employed in the present study, which is considered the most effective experimental paradigm for evaluating the in vivo anti-inflammatory activity of pharmaceutical dosage forms. This approach provides data with a higher degree of precision and accuracy. The oedema size was markedly reduced ($p < 0.05$) in the standard and experimental groups compared to the control groups in a one-way analysis of variance (ANOVA) following the Tukey-Kramer test method. The oedema size changes are illustrated in Table 4 and Fig. 2.

Table 4. *In-vivo* anti-inflammatory study of control, standard and experimental group.

| Time | Control group | Standard group | Experimental group |
|---|---|---|---|
| 0 min | 0mm | 0mm | 0mm |
| 30 min | 3.24 mm | 3.32 mm | 3.11 mm |

| | | | |
|---|---|---|---|
| 60 min | 3.76 mm | 3.94 mm | 3.72 mm |
| 120 min | 3.55 mm | 2.86 mm | 2.32 mm |
| 240 min | 3.10 mm | 1.12 mm | 1.02 mm |
| 360 min | 2.77 mm | 0mm | 0mm |

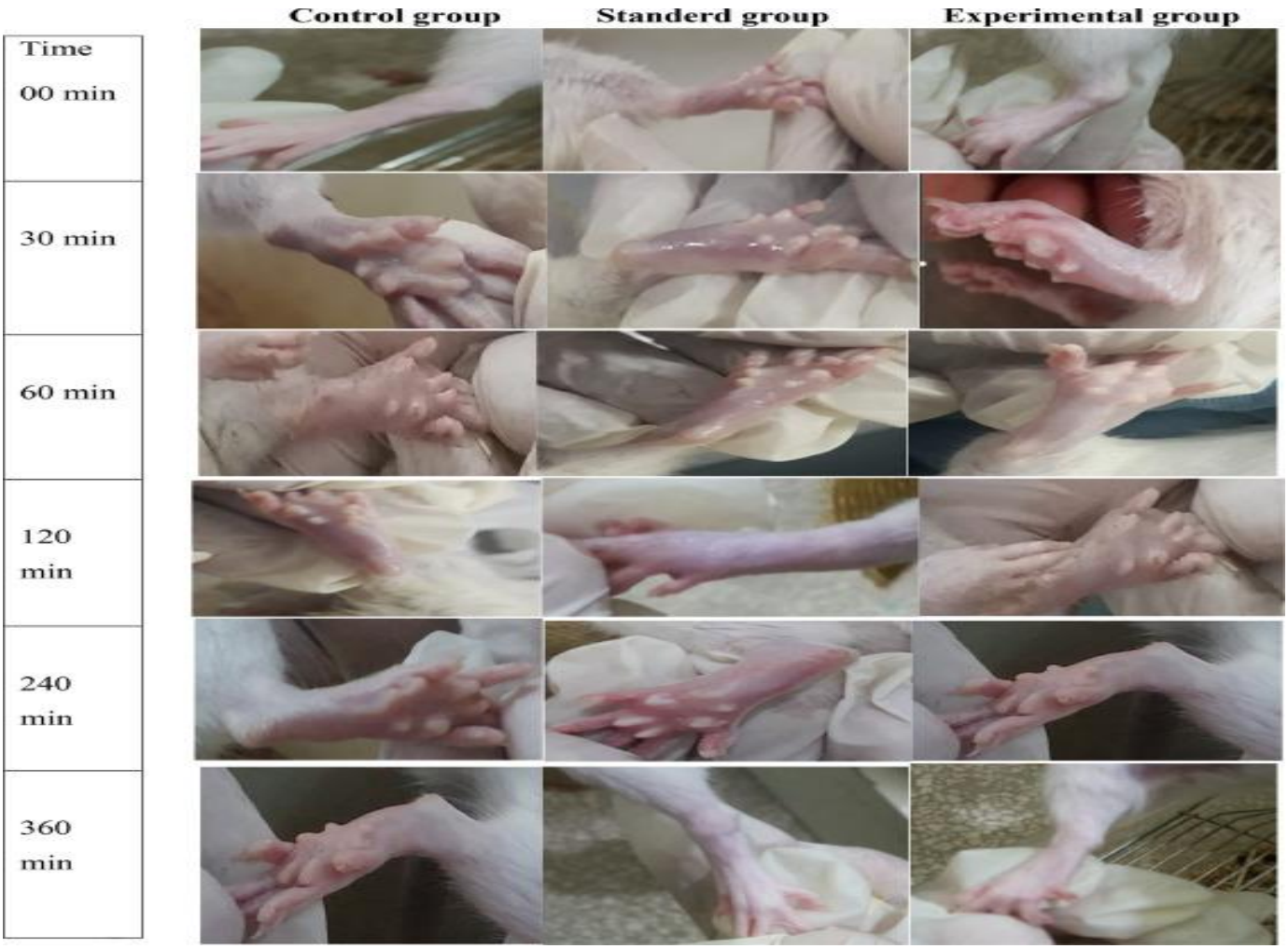



**Fig.2. *In-vivo* anti-inflammatory study of control, standard and experimental group.**

## 4. Discussion

The gold-standard paradigm for the investigation of both acute and sub-chronic inflammation is carrageenan-induced paw oedema. The model accurately represents the fundamental characteristics of human arthritis, such as the biphasic release of mediators. The early phase is characterised by histamine and serotonin, while the late phase is energised by prostaglandins and cytokines like TNF-α and IL-6 (Winter et al., 1962). The efficacy of this model is validated by the sustained elevation of paw volume in the untreated arthritic control group, which enables precise comparisons with intervention groups. In both pre- and post-treatment contexts, the administration of C. *procera* extract in an emulgel base substantially reduced inflammation. This result is consistent with previous research that has emphasised the plant's bioactive phytoconstituents, including flavonoids, alkaloids, terpenoids, and polyphenols, which regulate critical inflammatory pathways [5-6]. The prophylactic effect was particularly noteworthy, as it illustrated that pre-treatment may prepare the tissue environment by downregulating inflammatory gene expression prior

to the escalation of injury. This anticipatory action is likely to involve the inhibition of nuclear factor kappa B (NF-κB), cyclooxygenase-2 (COX-2), and other signalling cascades that are responsible for the infiltration of leukocytes and the formation of oedema [7]. Although the therapeutic group was effective, it required a prolonged period of time to achieve comparable levels of relief, likely as a result of the pre-existing inflammation that necessitates additional time for resolution and tissue remodelling. The emulgel formulation was crucial in facilitating the sustained release of the bioactives and enhancing skin penetration. Due to their biphasic nature, which enables the solubilisation of both hydrophilic and lipophilic compounds, as well as their superior spreadability and patient comfort, emulgels are particularly effective in transdermal drug delivery (Jain et al., 2013). The potential for long-term topical application was bolstered by the consistent pH (4.5–5.5), which guaranteed dermal compatibility without creating irritation. In particular, the effects of *C. procera* were comparable to those of diclofenac, a commonly used NSAID, particularly in the prophylactic group. Diclofenac predominantly functions by inhibiting COX enzymes, which in turn reduces the synthesis of prostaglandins. The analogous results indicate that *C. procera* may operate on broader or overlapping mechanisms, potentially involving free radical scavenging, cytokine inhibition, and antioxidant activity. *Calotropis procera* emulgel is a prospective alternative or adjunct therapy due to the protracted nature of rheumatoid arthritis and the constraints of long-term NSAID or steroid use. It may be particularly beneficial in situations where conventional pharmaceuticals are unavailable or in patients who are intolerant to NSAIDs. Its scope of application is expanded by its dual prophylactic and therapeutic potential. The study is restricted by the absence of long-term toxicity or chronic exposure data, the absence of molecular analysis (e.g., cytokine profiling, gene expression studies), and the absence of a comparison with systemic (oral/injectable) administration of *C. procera* .

## 5 Conclusion

In a carrageenan-induced arthritis model in albino rats, this study demonstrates that the extract of *Calotropisprocera* leaves exhibits substantial anti-inflammatory activity when formulated as a topical emulgel. Paw oedema was effectively reduced, clinical symptoms were alleviated, and joint structure was preserved in both prophylactic and therapeutic applications. The prophylactic approach was effective, implying the potential to prevent inflammation prior to its full onset. In comparison to diclofenac, a conventional anti-inflammatory agent, *C. procera* emulgel demonstrated comparable efficacy, with the additional advantage of being plant-based and well-tolerated. The traditional use of *C. procera* in treating inflammatory conditions is scientifically validated by these findings, which also support the further research and development of various clinical applications.

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