

# DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF EMULGEL CONTAINING *OCIMUM SANCTUM* LEAVES EXTRACT FOR ANTI-INFLAMMATORY ACTIVITY

Kirti Shukla<sup>1\*</sup>, Dr. Manmohan Sharma<sup>2</sup>, Dr. Amit Kumar Sharma<sup>3</sup>

<sup>1</sup>Research Scholar, Dr. K N Modi University, Newai, (Rajasthan)-304021, India

<sup>2</sup>Professor, School of Pharmaceutical Studies Faculty of Health Science, Dr. K N Modi University, Newai, (Rajasthan)-304021, India

<sup>3</sup>Associate Professor, Department of Applied Sciences, Dr. K N Modi University, Newai, (Rajasthan)-304021, India

## Abstract:

The present study was undertaken to formulate and evaluate a herbal emulgel of *Ocimum sanctum* L. for anti-inflammatory activity. The ethanolic extract of *Ocimum sanctum* leaves was prepared by Soxhlet extraction using 70% ethanol, yielding 15.4% extract rich in flavonoids, alkaloids, tannins, saponins, phenolics, glycosides, and terpenoids. Preformulation studies including FTIR analysis, solubility studies,  $\lambda_{max}$  determination, and calibration curve analysis confirmed compatibility and suitability of the extract for formulation development. The  $\lambda_{max}$  was observed at 278 nm with good linearity ( $R^2 = 0.998$ ). Nine emulgel formulations (F1–F9) were prepared using suitable gelling agents, emulsifiers, and oil phase components and evaluated for physicochemical parameters such as appearance, pH, viscosity, spreadability, homogeneity, drug content, extrudability, in vitro drug release, and stability. All formulations showed satisfactory appearance, smooth texture, absence of phase separation, skin-compatible pH (5.82–6.21), and drug content between 94.12% and 98.42%. In vitro drug release studies demonstrated sustained release up to 24 h, with formulation F4 showing maximum cumulative drug release (99.94%). Anti-inflammatory activity evaluated by protein denaturation assay showed significant inhibition, with formulation F7 exhibiting 69.05% inhibition comparable to Diclofenac gel (74.44%). In vivo carrageenan-induced paw edema studies in Albino Wistar rats also confirmed significant anti-inflammatory activity. The developed *Ocimum sanctum* emulgel may serve as a safe and effective herbal topical therapy for inflammatory conditions.

**Keywords:** *Ocimum sanctum* L., Emulgel, Herbal formulation, Anti-inflammatory activity, Carrageenan-induced paw edema, Diclofenac gel, Topical drug delivery.

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## Introduction:

Inflammation is a protective biological response of the body triggered by injury, infection, or exposure to harmful stimuli.

While it is essential for tissue repair and defense against pathogens, prolonged or excessive inflammation may contribute to the onset and progression of various skin and systemic disorders. Common

inflammatory skin diseases such as psoriasis, eczema, dermatitis, acne, and allergic reactions are characterized by symptoms including redness, swelling, itching, pain, and impairment of the skin barrier. These conditions not only cause physical discomfort but also adversely affect emotional health, self-esteem, and overall quality of life. Consequently, there is an increasing demand for effective, safe, and patient-friendly anti-inflammatory treatments capable of providing sustained therapeutic benefits with minimal side effects [1,2].

Conventional anti-inflammatory agents such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are widely used in the management of inflammatory disorders because of their potent therapeutic efficacy. However, long-term and repeated administration of these medications is often associated with various adverse effects, including skin irritation, hypersensitivity reactions, tissue damage, and systemic complications. Prolonged use of topical corticosteroids, in particular, may result in skin atrophy, telangiectasia, and suppression of the hypothalamic–pituitary–adrenal (HPA) axis, especially when applied excessively or over extended durations. These limitations have encouraged the search for safer and more effective alternative therapies that can provide adequate anti-inflammatory action with improved tolerability and a lower incidence of side effects [2,3].

At the cellular and molecular level, inflammation is controlled by complex biochemical pathways involving several inflammatory mediators. These mediators include pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, as well as enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), and transcription factors such as nuclear factor-kappa B (NF- $\kappa$ B). Persistent activation of these mediators may lead to chronic inflammation, resulting in tissue damage

and progression of various diseases. Therefore, current anti-inflammatory research is mainly directed toward inhibiting or regulating these molecular targets to provide safe, effective, and sustained therapeutic benefits [4,5].

In recent years, herbal medicines and natural products have attracted significant interest as promising anti-inflammatory agents because of their therapeutic effectiveness and relatively safer nature. Numerous plant-derived bioactive constituents, such as flavonoids, phenolic compounds, terpenoids, and alkaloids, have shown potent anti-inflammatory activity by modulating multiple inflammatory pathways. In contrast to synthetic drugs that often act through a single target, phytoconstituents exert their effects through several mechanisms simultaneously. They reduce the production of inflammatory mediators, alleviate oxidative stress, and support tissue repair and regeneration, thereby providing a holistic and balanced approach for the treatment and management of inflammatory disorders [6,7].

*Ocimum sanctum*, commonly known as Tulsi or Holy Basil, is an important medicinal and aromatic herb belonging to the Lamiaceae family. It holds a significant position in traditional Indian medicinal systems, especially Ayurveda, where it has been extensively utilized for centuries for the prevention and treatment of various ailments. In Indian tradition, Tulsi is considered a sacred plant and is commonly cultivated in households and temples because of its religious and therapeutic significance. Ancient Ayurvedic literature describes Tulsi as a rejuvenating herb that enhances immunity, supports longevity, and contributes to overall physical and mental health [8,9].

An emulgel is an advanced topical drug delivery system formed by incorporating an emulsion into a gel base. In this system, a stable oil-in-water (O/W) or water-in-oil

(W/O) emulsion is initially prepared using appropriate quantities of oil and aqueous phases, which is subsequently mixed with a gel matrix to produce the final emulgel formulation. The formulation typically consists of oils, aqueous phase ingredients, emulsifying agents, gelling agents, and penetration enhancers that collectively contribute to its stability, viscosity, and enhanced drug delivery performance. In emulgels, the emulsion serves as an effective vehicle for solubilizing and transporting the drug through the skin, thereby improving topical drug administration [10,11].

Traditional topical dosage forms such as ointments, creams, lotions, suspensions, and pastes are associated with several disadvantages. Many of these formulations produce a greasy feeling on the skin, cause inconvenience during application, and exhibit poor spreadability, often requiring vigorous rubbing. To address these limitations, gel-based formulations have emerged as a preferred option in pharmaceutical and cosmetic fields due to their non-greasy texture, ease of application, improved spreadability, and enhanced patient compliance [12,13].

A gel is a semisolid dosage form composed of a three-dimensional network of macromolecules that retains a substantial quantity of liquid within its structure. Despite offering numerous advantages over conventional semisolid formulations, gels are generally unsuitable for the effective delivery of hydrophobic or poorly water-soluble drugs. To overcome this drawback, the emulgel system was introduced by integrating the characteristics of both emulsions and gels. The incorporation of an emulsion into a gel base facilitates efficient topical delivery of hydrophobic therapeutic agents while preserving the favorable properties of gels, including enhanced spreadability, improved stability, ease of application, and better patient acceptability [14,15].

## **Materials and Methods:**

Fresh leaves of *Ocimum sanctum* were collected and authenticated at the CSIR–National Institute of Science Communication and Policy Research (CSIR–NIScPR), Raw Materials Herbarium and Museum, Delhi (RHMD). The botanical identification of the plant material was carried out on the basis of macroscopic characteristics, detailed literature survey, and comparison with authenticated herbarium specimens available at RHMD. The specimen was authenticated under Authentication No. NIScPR/RHMD/Consult/2024/0781-34 dated 07/03/2024.

The collected leaves were thoroughly washed with running water to remove adhering dust, dirt, and other extraneous matter. The cleaned plant material was shade dried at room temperature for 7–10 days until complete drying was achieved. The dried leaves were then pulverized using a mechanical grinder to obtain a coarse powder, which was stored in airtight containers for subsequent experimental and formulation studies.

Healthy adult Albino Wistar rats of either sex, weighing between 150–200 g, were used for the experimental study. The animals were obtained from a registered animal house facility and maintained under standard laboratory conditions, including a temperature of  $25 \pm 2^\circ\text{C}$ , relative humidity of  $55 \pm 5\%$ , and a 12 h light/dark cycle throughout the study period.

## **Preparation of Plant Extract**

### **Extraction Procedure**

Fresh leaves of *Ocimum sanctum* were collected from a nearby medicinal plant garden and authenticated by a qualified botanist from the Department of Botany. The collected leaves were thoroughly washed with distilled water to eliminate dust, soil particles, and other impurities.

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After cleaning, the leaves were shade dried at ambient room temperature for about 8–10 days until a constant weight was obtained, indicating complete drying. The dried leaves were then ground into coarse powder using a mechanical grinder, and the powdered material was stored in airtight containers away from moisture and direct light until further use [16,17].

For extraction, nearly 250 g of the powdered plant material was packed into a Whatman filter paper thimble and subjected to Soxhlet extraction using 70% ethanol as the extraction solvent. The extraction procedure was continued for approximately 18–24 hours at a temperature corresponding to the boiling point of the solvent. Repeated extraction cycles were carried out until the solvent in the siphon tube became colorless, which confirmed the complete extraction of phytochemical constituents from the plant material [18].

The resulting extract was filtered and concentrated under reduced pressure using a rotary vacuum evaporator maintained at 40–45°C to remove the solvent efficiently. The concentrated extract was further dried in a vacuum desiccator to obtain a semisolid residue. The final dried extract was weighed, and the percentage yield was determined based on the initial quantity of powdered plant material used for extraction [19].

The prepared extract was finally stored in airtight amber-colored glass containers at 4°C to protect it from light and degradation until further studies such as phytochemical screening, calibration analysis, and formulation development were carried out [20,21].

**Table 1: Extraction Parameters**

S. No.	Extraction Parameter	Condition
1	Extraction	Soxhlet Extraction

	Method	
2	Plant Material	<i>Ocimum sanctum</i> L. leaves powder
3	Quantity of Drug	250 g
4	Solvent Used	70% Ethanol
5	Extraction Time	18–24 h
6	Extraction Temperature	Boiling point of solvent (40–45°C)
7	End Point	Siphon solvent became colorless
8	Concentration Method	Rotary vacuum evaporation
9	Drying Method	Vacuum desiccation
10	Storage Condition	Airtight amber-colored container at 4°C

**Method of Preparation of *Ocimum sanctum* Emulgel**

The emulgel formulations of *Ocimum sanctum* extract (F1–F9) were prepared using the emulsion incorporation technique. Initially, the gel base was prepared by dispersing the required amount of Carbopol 934 in distilled water with continuous stirring, followed by sufficient hydration to ensure complete swelling of the polymer. After proper hydration, propylene glycol was added slowly under constant stirring to obtain a smooth and homogeneous gel base [22].

In a separate process, the oil phase was prepared by dissolving Span 80 in liquid paraffin along with the accurately weighed quantity of *Ocimum sanctum* extract. Simultaneously, the aqueous phase was prepared by dissolving Tween 80 and methyl paraben in distilled water. Both the oil and aqueous phases were heated separately to a temperature range of 70–75°C. The oil phase was then gradually added to the aqueous phase under

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continuous mechanical stirring to produce a stable emulsion system [23,24].

The prepared emulsion was carefully incorporated into the gel base with gentle and continuous stirring until a uniform emulgel formulation was obtained. Finally, triethanolamine was added dropwise to adjust the pH of the formulation within the suitable skin-compatible range of 6.0–6.5 and to achieve the desired consistency. The formulated emulgels were packed in airtight containers and stored at room temperature for subsequent evaluation and characterization studies [25].

**Table 2: Composition of *Ocimum sanctum* L. Emulgel Formulations (F1–F9) (% w/w)**

S · N o ·	Ingr edien ts (% w/w)	Formulation Code								
		F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9
1	<i>Ocimum sanctum</i> L. extract	5	5	5	5	5	5	5	5	5
2	Carbopol 934	0 · 5 0	0 · 7 5	1 · 0 0	0 · 5 0	0 · 7 5	1 · 0 0	0 · 5 0	0 · 7 0	1 · 0 5
3	Liquid paraffin	2 · 5 0	2 · 5 0	2 · 5 0	3 · 0 0	3 · 0 0	3 · 0 0	3 · 5 0	3 · 5 0	3 · 5 0
4	Tween 80	0 · 5 0	0 · 5 0	0 · 5 0	0 · 7 5	0 · 7 5	0 · 7 5	1 · 0 0	1 · 0 0	1 · 0 0
5	Span 80	0 · 7 5	0 · 7 5	0 · 7 5	1 · 0 0	1 · 0 0	1 · 0 0	1 · 2 5	1 · 2 5	1 · 2 5

6	Propylene glycol	3 · 5 0	3 · 5 0	3 · 5 0	4 · 0 0	4 · 0 0	4 · 0 0	4 · 5 0	4 · 5 0	4 · 5 0
7	Methyl paraben	0 · 0 1	0 · 0 1	0 · 0 1	0 · 0 1	0 · 0 1	0 · 0 1	0 · 0 1	0 · 0 1	0 · 0 1
8	Distilled water QS	5 0	5 0	5 0	5 0	5 0	5 0	5 0	5 0	5 0
9	Triethanolamine	q · s ·	q · s ·	q · s ·	q · s ·	q · s ·	q · s ·	q · s ·	q · s ·	q · s ·

**Evaluation Parameters of *Ocimum sanctum* Emulgel**

The formulated *Ocimum sanctum* emulgels were subjected to comprehensive evaluation to assess their physicochemical properties, formulation stability, and suitability for topical drug delivery applications. These evaluation studies were carried out to ensure the quality, consistency, effectiveness, and patient acceptability of the developed formulations.

Various parameters were examined during the evaluation process, including physical appearance, pH, viscosity, spreadability, drug content uniformity, in vitro drug release behavior, extrudability, homogeneity, and stability studies. The results obtained from these investigations helped in determining the overall performance and therapeutic potential of the prepared emulgel formulations [26].

**Physical Appearance**

The prepared emulgel formulations of *Ocimum sanctum* were visually examined to evaluate their physical appearance, color, consistency, homogeneity, grittiness, and any signs of phase

separation. The assessment was carried out under normal daylight conditions to study the aesthetic quality and physical stability of the formulations. Particular emphasis was placed on the smoothness, uniformity, and overall texture of the emulgels.

Each formulation was carefully observed for the presence of particulate matter, lump formation, syneresis, or separation of phases. Formulations exhibiting a smooth texture, uniform consistency, and absence of visible instability or phase separation were considered satisfactory and selected for further evaluation studies [27,28].

### Determination of pH

The pH of the prepared Ocimum sanctum emulgel formulations was measured using a calibrated digital pH meter. Before analysis, the instrument was standardized using standard buffer solutions of pH 4.0 and 7.0 to ensure accurate readings. For the study, approximately 1 g of emulgel was dispersed in 25 mL of distilled water and allowed to equilibrate properly before measurement [29].

To prevent contamination and maintain accuracy, the pH meter electrode was thoroughly rinsed with distilled water before and after each reading. The electrode was then immersed into the prepared formulation dispersion, and the pH value was recorded once the reading became stable. All measurements were carried out in triplicate, and the average pH values were calculated. Maintaining the pH within the normal physiological range of the skin was considered important to reduce the risk of irritation and to enhance the suitability of the formulation for topical application [30,31].

### Viscosity Determination

The viscosity of the prepared Ocimum sanctum emulgel formulations was evaluated using a Brookfield viscometer fitted with an appropriate spindle. Before

analysis, the formulation samples were transferred into clean beakers and allowed to stabilize at room temperature ( $25 \pm 1^\circ\text{C}$ ) for nearly 30 minutes to ensure uniformity of the samples [32].

During the measurement, the spindle was carefully immersed into the emulgel without touching the sides or bottom of the container. The viscosity readings were recorded at a rotational speed of 50 rpm after the instrument displayed stable values. Each formulation was analyzed in triplicate to ensure accuracy and reproducibility of the results. The viscosity study was conducted to assess the consistency, flow characteristics, and overall rheological behavior of the developed emulgel formulations [33,34].

### Spreadability Study

The spreadability of the prepared Ocimum sanctum emulgel formulations was evaluated to assess the ease with which the formulation could be applied and distributed uniformly over the skin surface. For the study, a fixed quantity of emulgel was placed between two clean glass slides, and a specified weight was applied on the upper slide to achieve uniform spreading of the formulation [35].

After the application of the weight, the time taken for the two slides to separate under the influence of an applied force was carefully recorded. The spreadability study was performed to determine the consistency and ease of application of the emulgel formulations for topical use [36,37].

Spreadability was calculated using the following equation:

$$S = \frac{M \cdot L}{T}$$

Where:

- (S) = Spreadability
- (M) = Weight tied to upper slide
- (L) = Length of glass slide
- (T) = Time required to separate the slides

Higher spreadability values indicated better ease of application and uniform spreading characteristics of the emulgel formulation.

### **Drug Content Determination**

Drug content estimation was performed to evaluate the uniform distribution of Ocimum sanctum extract within the prepared emulgel formulations. For the analysis, an accurately weighed quantity of emulgel equivalent to 1 g was dissolved in ethanol and stirred continuously using a magnetic stirrer to ensure complete extraction of the drug from the formulation matrix.

The prepared solution was then centrifuged at 5000 rpm for 10 minutes to separate any insoluble components. From the obtained clear supernatant, 0.1 mL was carefully withdrawn and further diluted to 10 mL with ethanol. The absorbance of the diluted solution was measured using a UV-visible spectrophotometer at the predetermined wavelength of maximum absorbance ( $\lambda_{max}$ ). The study was conducted to determine the uniformity and consistency of drug distribution within the emulgel formulations [38,39].

**Drug content was calculated using the following equations:**

$$\text{Drug Content (\%)} = \frac{\text{Drug Content (mg)}}{\text{Label Claim (mg)}}$$

The study was performed in triplicate and average values were reported.

### **In Vitro Drug Release Study**

The in vitro drug release behavior of the Ocimum sanctum emulgel formulations was evaluated using a Franz diffusion cell apparatus. The diffusion system consisted of donor and receptor compartments separated by an egg membrane, which acted as the diffusion barrier. Throughout the study, the effective diffusion area and the volume of the receptor compartment were maintained under controlled conditions to ensure accurate and reproducible results [40].

The receptor compartment was filled with freshly prepared phosphate buffer saline (PBS, pH 7.4), which served as the diffusion medium. The medium was maintained at a constant temperature and continuously stirred using a magnetic stirrer to ensure uniform distribution of the released drug.

An accurately weighed quantity of the emulgel formulation was evenly spread over the membrane placed in the donor compartment. At specific predetermined time intervals, samples were withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh diffusion medium to maintain sink conditions throughout the experiment.

The collected samples were suitably diluted and analyzed using a UV-visible spectrophotometer at the selected wavelength of maximum absorbance ( $\lambda_{max}$ ). The cumulative percentage drug release was calculated over time, and the release patterns of different formulations were compared to evaluate their drug release performance [41].

### **Extrudability Study**

The extrudability of the prepared Ocimum sanctum emulgel formulations was evaluated using collapsible aluminum tubes. The developed formulations were

carefully filled into clean lacquered aluminum tubes and properly sealed to prevent leakage and contamination.

During the study, the amount of emulgel extruded from the tube under the application of a specified weight within a fixed period of time was determined. Formulations that exhibited smooth, uniform, and easy extrusion with minimal force were considered to possess satisfactory extrudability properties. This evaluation was carried out to assess the ease of handling, patient convenience, and suitability of the formulation for topical application [42].

### **Homogeneity**

The homogeneity of the prepared Ocimum sanctum emulgel formulations was assessed through visual and manual examination. A small quantity of the formulation was pressed between the thumb and index finger and also spread as a thin film over a clean glass slide to evaluate its uniformity and texture.

The formulations were carefully examined for smoothness, consistency, uniform appearance, and the absence of coarse particles, lumps, or aggregates. Emulgel formulations showing a smooth texture, consistent appearance, and no signs of grittiness were considered to possess good homogeneity [43].

### **Stability Study**

The stability study of the prepared Ocimum sanctum emulgel formulations was conducted to assess their physical stability under different storage conditions. The formulations were stored at refrigerated temperature ( $2 \pm 2^\circ\text{C}$ ), room temperature ( $25 \pm 2^\circ\text{C}$ ), and elevated temperature ( $37 \pm 2^\circ\text{C}$ ) for a duration of four weeks.

Throughout the storage period, the formulations were periodically examined

for any changes in physicochemical properties such as color, consistency, homogeneity, pH, spreadability, and phase separation. Formulations that retained their original characteristics without showing any significant variation during the study period were considered physically stable and suitable for further application [44].

### **In Vitro Anti-Inflammatory Activity of Ocimum sanctum Emulgel**

The anti-inflammatory potential of the prepared Ocimum sanctum emulgel formulations was evaluated using the in vitro protein denaturation method, with egg albumin employed as the protein source. Protein denaturation is recognized as one of the important factors involved in the inflammatory process, and substances capable of inhibiting protein denaturation are considered to possess anti-inflammatory activity. This study was therefore performed to determine the ability of the developed formulation to prevent heat-induced protein denaturation under controlled laboratory conditions [45].

### **Procedure**

The reaction mixture was prepared by combining 1 mL of freshly prepared 1% egg albumin solution with 1 mL of the Ocimum sanctum emulgel extract solution at an appropriate concentration. The pH of the mixture was carefully adjusted to 6.3 using 1N hydrochloric acid to maintain suitable experimental conditions.

The prepared reaction mixtures were incubated at  $37 \pm 1^\circ\text{C}$  for 20 minutes to allow proper interaction between the protein and the test formulation. After incubation, the mixtures were heated in a water bath at  $70^\circ\text{C}$  for 5 minutes to induce protein denaturation. The samples were then allowed to cool to room temperature.

The absorbance of the resulting solutions was measured at 660 nm using a UV-

visible spectrophotometer against a suitable blank. Simultaneously, a control sample containing all reagents except the test formulation was prepared and treated under identical experimental conditions for comparison of results [46].

The percentage inhibition of protein denaturation by the *Ocimum sanctum* L. emulgel formulation was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{(\text{Control} - \text{Sample})}{\text{Control}} \times 10$$

Where:

- Control = Absorbance of control reaction
- Sample = Absorbance of test sample

Higher percentage inhibition indicated greater anti-inflammatory potential of the prepared emulgel formulation.

### In Vivo Anti-Inflammatory Activity of *Ocimum sanctum* Emulgel

#### Experimental Animals:

Healthy adult Albino Wistar rats of either sex weighing between 150–200 g were selected for the study. The animals were procured from an approved animal house facility and maintained under standard laboratory conditions of temperature ( $25 \pm 2^\circ\text{C}$ ), relative humidity ( $55 \pm 5\%$ ), and 12 h light-dark cycle. The animals were housed in polypropylene cages with free access to standard pellet diet and water ad libitum.

Prior to initiation of the experiment, the animals were acclimatized to laboratory conditions for a period of one week. All experimental procedures were performed according to institutional ethical guidelines and approval obtained from the Institutional Animal Ethics Committee

(IAEC) constituted under CPCSEA regulations [47].

#### Grouping of Animals:

The experimental animals were divided randomly into different groups consisting of six animals in each group.

- **Group I** – Normal control group
- **Group II** – Carrageenan-induced inflammatory control group
- **Group III** – Standard treatment group receiving marketed anti-inflammatory formulation/drug
- **Group IV** – Test group treated with *Ocimum sanctum* L. emulgel formulation

**Table 3: Grouping of animals**

Group	Experimental Treatment	Description
Group I	Normal Control	Animals received no carrageenan injection and no treatment
Group II	Carrageenan-Induced Inflammatory Control	Animals received carrageenan injection to induce inflammation without any treatment
Group III	Standard Treatment Group	Animals treated with marketed anti-inflammatory formulation/drug following carrageenan administration
Group IV	Test Treatment Group	Animals treated with <i>Ocimum sanctum</i> L. emulgel formulation following

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		carrageenan-induced inflammation
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The treatment protocol was designed to compare the anti-inflammatory effect of the developed herbal emulgel with inflammatory control and standard treatment group.

**Induction of Paw Edema:**

Acute inflammation was induced by subplantar administration of carrageenan solution into the right hind paw of experimental rats. Freshly prepared 1% w/v carrageenan suspension in normal saline was used for induction of inflammation.

A volume of 0.1 mL carrageenan solution was injected into the subplantar region of the right hind paw of each rat except animals belonging to the normal control group. The carrageenan injection produced localized edema due to inflammatory response.

**Treatment Procedure:**

The developed *Ocimum sanctum* L. emulgel formulation was applied topically to the plantar surface of the inflamed paw of animals belonging to the test group. The standard group received a standard anti-inflammatory preparation, while the inflammatory control group received no therapeutic treatment except carrageenan administration.

The formulation was applied uniformly over the paw area approximately 30 min before carrageenan injection and continued according to the experimental protocol. Care was taken to ensure uniform application and absorption of the formulation.

**Measurement of Paw Volume:**

The paw volume of experimental animals was measured using a digital plethysmometer immediately before carrageenan administration to obtain initial paw volume. Subsequent paw volume measurements were recorded at predetermined time intervals such as 1, 2, 3, 4, and 5 h after carrageenan injection.

The increase in paw volume was considered as the degree of inflammation. The anti-inflammatory activity of the formulation was assessed by comparing paw edema volume of treated groups with inflammatory control group.

**Calculation of Percentage Inhibition of Edema:**

The percentage inhibition of paw edema produced by the *Ocimum sanctum* L. emulgel formulation was calculated using the following equation:

$$\% \text{ Inhibition of Edema} = \frac{(V_c - V_t)}{V_c} \times 100$$

Where:

- (V<sub>c</sub>) = Mean paw edema volume of control group
- (V<sub>t</sub>) = Mean paw edema volume of treated group

Higher percentage inhibition indicated significant anti-inflammatory activity of the developed emulgel formulation [48].

**Statistical Analysis:**

The experimental data obtained from in vivo anti-inflammatory studies were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using appropriate statistical software. The significance between different groups was evaluated using one-way analysis of variance (ANOVA) followed by suitable

post hoc test. A value of  $p < 0.05$  was considered statistically significant [49].

### **Ethical Considerations:**

All animal handling and experimental procedures were performed in accordance with CPCSEA guidelines for care and use of laboratory animals. Adequate precautions were taken to minimize pain and discomfort to experimental animals throughout the study period [50].

### **Result and Discussion:**

#### **Collection and Authentication of Plant Material**

Fresh leaves of *Ocimum sanctum* were successfully collected and authenticated at the CSIR–National Institute of Science Communication and Policy Research (CSIR–NIScPR), Raw Materials Herbarium and Museum, Delhi (RHMD). The submitted specimen was identified and confirmed as *Ocimum sanctum* (Tulsi) through detailed macroscopic evaluation, comparison with authenticated herbarium samples, and verification using standard taxonomical references. The authenticity of the plant material was certified under Authentication No. NIScPR/RHMD/Consult/2024/0781-34 dated 07/03/2024, confirming the purity and genuineness of the sample used in the present research work.

The collected leaves were observed to be fresh, healthy, and free from fungal growth, microbial contamination, and other foreign impurities. Following proper washing and shade drying, the leaves retained their characteristic aromatic odor and greenish appearance, suggesting minimal loss or degradation of active phytoconstituents during processing. The dried material was successfully pulverized into a coarse powder with uniform particle size suitable for extraction studies. The powdered drug was stored in airtight containers, where it remained stable

without noticeable moisture uptake or deterioration throughout the storage period.

Proper authentication and systematic processing of *Ocimum sanctum* leaves ensured the reliability, quality, and reproducibility of the plant material used for subsequent phytochemical extraction, formulation development, and pharmacological evaluation studies.

#### **Preparation of Plant Extract**

The ethanolic extract of *Ocimum sanctum* leaves was successfully prepared using the Soxhlet extraction technique with 70% ethanol as the extraction solvent. The extraction process yielded a dark greenish-brown semisolid extract possessing a characteristic aromatic odor and smooth consistency. Repeated extraction cycles facilitated efficient separation and recovery of phytoconstituents from the powdered plant material. During the extraction process, the solvent in the siphon tube gradually turned colorless after multiple cycles, indicating complete extraction of the active constituents from the leaves.

The collected leaves were thoroughly cleaned, shade dried, and pulverized to obtain a uniformly coarse powder suitable for extraction. Shade drying effectively reduced the moisture content while preserving the natural color and characteristic aroma of the plant material. The powdered drug remained stable during storage and showed no evidence of microbial growth or contamination.

The concentrated extract obtained after rotary vacuum evaporation exhibited good consistency and physical stability. Evaporation under reduced pressure at controlled low temperature helped preserve heat-sensitive phytoconstituents and minimized degradation of bioactive compounds. Subsequent drying in a vacuum desiccator produced a stable

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semisolid extract appropriate for further formulation development and pharmacological investigations.

The percentage yield obtained from the Soxhlet extraction process indicated that 70% ethanol was an effective solvent for extracting phytochemical constituents from *Ocimum sanctum* leaves. The prepared extract remained stable under refrigerated storage conditions without any noticeable alteration in its appearance or characteristic odor.

**Table 4: Observational of *Ocimum sanctum* L. Extract**

S. No.	Parameter Evaluated	Observation
1	Plant part used	Leaves
2	Extraction method	Soxhlet extraction
3	Solvent used	70% Ethanol
4	Quantity of powdered drug	250 g
5	Extraction duration	18–24 h
6	Color of extract	Dark greenish-brown
7	Nature of extract	Semisolid mass
8	Odor	Characteristic aromatic odor
9	Drying method	Vacuum desiccation
10	Storage condition	4°C in amber-colored container

**Table 5: Percentage Yield of *Ocimum sanctum* L. Extract**

S. No.	Parameter	Value
1	Weight of powdered leaves taken	250 g

2	Weight of dried extract obtained	38.5 g
3	Percentage yield	15.4%

The percentage yield was calculated using the following equation:

$$\% \text{ Yield} = \frac{\text{Weight of Extract Obtained}}{\text{Weight of Crude Drug Taken}} \times 100$$

The obtained extract was further utilized for phytochemical screening, analytical characterization, formulation development, and pharmacological evaluation studies.

#### Evaluation Parameters of *Ocimum sanctum* L. Emulgel:

The prepared *Ocimum sanctum* emulgel formulations (F1–F9) were subjected to comprehensive evaluation for various physicochemical and performance-related parameters, including physical appearance, pH, viscosity, spreadability, drug content, *in vitro* drug release, extrudability, homogeneity, and stability studies. The results obtained from these evaluations indicated that the developed formulations possessed satisfactory characteristics and were suitable for topical drug delivery applications.

#### Physical Appearance

All prepared *Ocimum sanctum* emulgel formulations were visually evaluated for parameters such as color, consistency, homogeneity, grittiness, and phase separation. The formulations showed a smooth texture, uniform appearance, and desirable consistency without the presence of visible particles or lump formation. During the evaluation, no signs of phase separation or syneresis were observed in any of the formulations. The overall

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findings suggested that the developed emulgels possessed good physical stability along with acceptable aesthetic and application characteristics suitable for topical use.

**Table 6: Physical Appearance of Ocimum sanctum L. Emulgel Formulations**

Formulation Code	Colour	Consistency	Homogeneity	Grittiness	Phase Separation
F1	Light green	Smooth	Good	Absent	Absent
F2	Light green	Smooth	Good	Absent	Absent
F3	Greenish cream	Smooth	Excellent	Absent	Absent
F4	Greenish cream	Smooth	Excellent	Absent	Absent
F5	Pale green	Slightly viscous	Good	Absent	Absent
F6	Pale green	Smooth	Excellent	Absent	Absent
F7	Greenish white	Smooth	Excellent	Absent	Absent
F8	Light cream	Smooth	Excellent	Absent	Absent

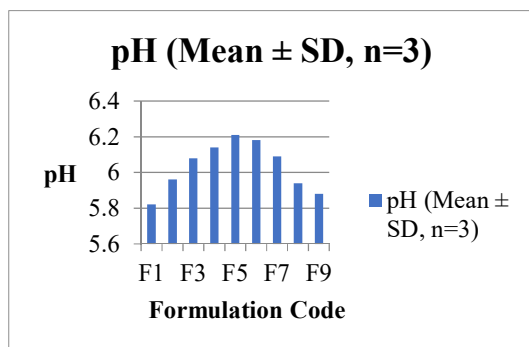
	green				
F9	Creamish green	Smooth	Excellent	Absent	Absent

**Determination of pH**

The pH values of all the developed Ocimum sanctum emulgel formulations were found to be within the suitable range for topical application. The recorded pH values ranged from 5.82 to 6.21, which closely corresponds to the normal physiological pH of the skin. This indicates that the formulations are appropriate for dermal use and are less likely to cause skin irritation or discomfort upon application.

**Table 7: pH of Ocimum sanctum L. Emulgel Formulations**

Formulation Code	pH (Mean ± SD, n=3)
F1	5.82
F2	5.96
F3	6.08
F4	6.14
F5	6.21
F6	6.18
F7	6.09
F8	5.94
F9	5.88



**Figure 1: pH of Ocimum sanctum L. Emulgel Formulations**

The results confirmed that the prepared formulations-maintained skin-compatible pH values and may minimize the possibility of skin irritation during topical administration.

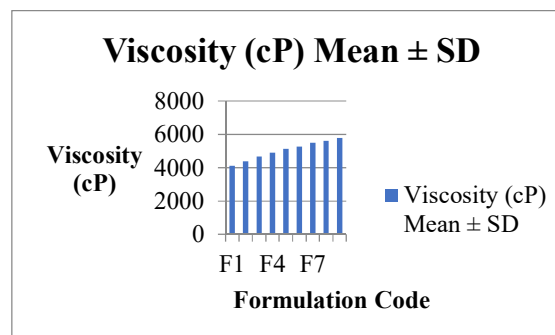
### Viscosity Determination

The viscosity studies indicated that all prepared Ocimum sanctum emulgel formulations possessed appropriate rheological properties suitable for topical application. It was observed that increasing the concentration of the polymer produced a corresponding increase in the viscosity of the emulgel system. The formulations exhibited satisfactory consistency and flow behavior, which are important for ensuring proper application and retention on the skin surface.

**Table 8: Viscosity of Ocimum sanctum L. Emulgel Formulations**

Formulation Code	Viscosity (cP) Mean ± SD
F1	4120
F2	4385
F3	4652
F4	4896
F5	5128
F6	5264
F7	5482

F8	5618
F9	5786



**Figure 2: Viscosity of Ocimum sanctum L. Emulgel Formulations**

Among all formulations, F9 showed maximum viscosity whereas F1 exhibited comparatively lower viscosity. The obtained viscosity values suggested good consistency and stability of the formulations.

### Spreadability Study

Spreadability studies indicated that all formulations possessed satisfactory spreading behavior and could be applied easily on the skin surface.

The spreadability was calculated using the following equation:

$$S = \frac{M \cdot L}{T}$$

**Table 9: Spreadability of Ocimum sanctum L. Emulgel Formulations**

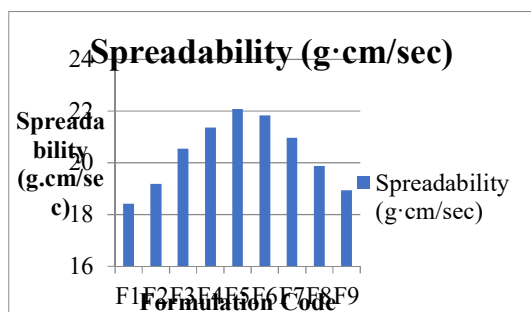
Formulation Code	Spreadability (g·cm/sec)
F1	18.42
F2	19.18

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F3	20.54
F4	21.36
F5	22.08
F6	21.84
F7	20.96
F8	19.88
F9	18.94

**Table 10: Drug Content of Ocimum sanctum L. Emulgel Formulations**

Formulation Code	Drug Content (%) Mean ± SD
F1	94.12
F2	95.48
F3	97.16
F4	98.42
F5	97.88
F6	98.16
F7	97.52
F8	96.84
F9	95.96



**Figure 3: Spreadability of Ocimum sanctum L. Emulgel Formulations**

Higher spreadability values were observed for F5 and F6, indicating improved ease of application and better patient compliance.

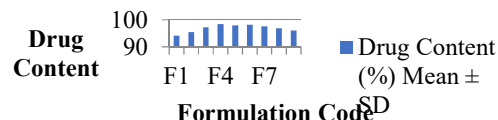
### Drug Content Determination

Drug content analysis demonstrated uniform distribution of Ocimum sanctum L. extract within all prepared emulgel formulations.

The percentage drug content was calculated using the following equation:

$$\text{Drug Content (\%)} = \frac{\text{Drug Content (mg)}}{\text{Label Claim (mg)}} \times 100$$

### Drug Content (%) Mean ± SD



**Figure 4: Drug Content of Ocimum sanctum L. Emulgel Formulations**

The drug content values ranged from 94.12% to 98.42%, indicating efficient incorporation and uniform distribution of the herbal extract in the emulgel matrix.

### In Vitro Drug Release Study

The in vitro drug release studies of the Ocimum sanctum emulgel formulations revealed a sustained and controlled release pattern over a duration of 24 hours. The cumulative percentage drug release from all formulations increased progressively with time, indicating continuous diffusion

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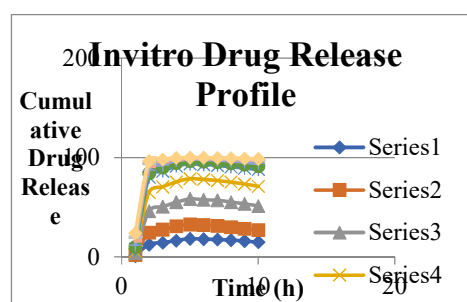
of the active phytoconstituents from the emulgel matrix.

The observed sustained release behavior suggested efficient permeation of the herbal extract through the diffusion membrane, which may contribute to prolonged therapeutic action following topical application. These findings indicate that the developed emulgel system has the potential to provide extended drug release and improved therapeutic effectiveness.

1	9	9	9	9	9	9	9	9	9
6	1.	5.	8.	9.	8.	8.	8.	7.	6.
	2	8	1	1	8	5	0	4	8
	4	6	4	2	4	2	6	2	4
2	9	9	9	9	9	9	9	9	9
0	4.	7.	9.	9.	9.	9.	8.	8.	7.
	1	2	0	6	4	1	8	4	9
	8	4	6	8	2	8	6	2	4
2	9	9	9	9	9	9	9	9	9
4	6.	8.	9.	9.	9.	9.	9.	8.	8.
	4	1	4	9	8	6	2	9	5
	2	6	2	4	2	4	8	6	4

**Table 11: In Vitro Drug Release Profile of Ocimum sanctum L. Emulgel Formulations**

T i m e (h r)	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9
1	1	1	1	1	1	1	1	1	1
2	2	2	3	3	3	3	2	2	2
4	4	5	5	5	5	5	5	5	5
6	6	7	7	7	7	7	7	7	7
8	8	8	9	9	9	9	9	8	8
10	10	10	10	10	10	10	10	10	10
12	12	12	12	12	12	12	12	12	12
14	14	14	14	14	14	14	14	14	14
16	16	16	16	16	16	16	16	16	16
18	18	18	18	18	18	18	18	18	18
20	20	20	20	20	20	20	20	20	20
22	22	22	22	22	22	22	22	22	22
24	24	24	24	24	24	24	24	24	24



**Figure 5: In Vitro Drug Release Profile of Ocimum sanctum L. Emulgel Formulations**

Among all the developed Ocimum sanctum emulgel formulations, formulation F4 showed the highest cumulative drug release of 99.94% after 24 hours, indicating excellent diffusion and sustained release properties. The optimized formulation exhibited a controlled and continuous drug release pattern throughout the study period, suggesting its suitability for prolonged topical therapeutic activity and improved treatment effectiveness.

**Extrudability Study**

Extrudability studies indicated satisfactory tube extrudability properties for all formulations. The formulations could be extruded smoothly with minimum force.

**Table 12: Extrudability of Ocimum sanctum L. Emulgel Formulations**

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Formulation Code	Extrudability
F1	Good
F2	Good
F3	Very Good
F4	Excellent
F5	Excellent
F6	Excellent
F7	Very Good
F8	Good
F9	Good

F4, F5, and F6 exhibited excellent extrudability, suggesting convenient handling and ease of application.

### Homogeneity Study

The homogeneity of the prepared Ocimum sanctum emulgel formulations was assessed visually to evaluate the uniform distribution of all ingredients within the formulation matrix. The formulations were carefully examined for parameters such as smoothness, consistency, appearance, and the presence of any coarse particles or aggregates.

All prepared emulgels showed a smooth and uniform texture with good consistency, without any signs of grittiness or phase separation. The findings confirmed proper blending of the formulation components and demonstrated satisfactory physical uniformity, indicating the suitability of the emulgels for topical application.

**Table 13: Homogeneity Evaluation of Ocimum sanctum L. Emulgel Formulations**

Formulation Code	Degree of Homogeneity	Surface Texture
F1	Good	Smooth
F2	Good	Smooth
F3	Excellent	Smooth and Uniform
F4	Excellent	Smooth and Uniform
F5	Excellent	Smooth and Uniform
F6	Excellent	Smooth and Uniform
F7	Excellent	Smooth and Uniform
F8	Good	Smooth
F9	Good	Smooth

The study confirmed that all formulations possessed satisfactory homogeneity and acceptable consistency. Formulations F3 to F7 showed comparatively superior uniformity and smooth texture, indicating efficient dispersion of Ocimum sanctum L. extract within the emulgel base.

### Stability Studies of Ocimum sanctum Emulgel

The optimized Ocimum sanctum emulgel formulation was subjected to stability studies to evaluate its physical stability and suitability for storage under different environmental conditions over time. The study was conducted in accordance with ICH guidelines to determine the influence of temperature on the physicochemical properties of the formulation.

The optimized formulation, F4, was filled into collapsible aluminum tubes and stored under various storage conditions, including refrigerated temperature ( $2 \pm 2^\circ\text{C}$ ), room

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temperature ( $25 \pm 2^\circ\text{C}$ ), and accelerated temperature conditions ( $37 \pm 2^\circ\text{C}$ ) for a period of four weeks. During the study period, the formulation was periodically examined for any changes in physical appearance, color, phase separation, pH, consistency, homogeneity, and spreadability.

The optimized *Ocimum sanctum* emulgel formulation remained smooth, homogeneous, and free from visible particulate matter throughout the entire study period. No significant alterations in color, texture, or phase separation were observed under refrigerated and room temperature storage conditions. Under accelerated temperature conditions, a slight increase in viscosity along with a minor change in appearance was noted; however, the formulation continued to remain physically stable without any signs of instability or phase separation.

The pH of the formulation showed only minimal variation during storage, indicating good stability of the emulgel system and compatibility among the formulation ingredients. In addition, the spreadability and consistency of the formulation remained satisfactory under all storage conditions, demonstrating the retention of desirable rheological properties.

The findings of the stability study confirmed that the optimized *Ocimum sanctum* emulgel formulation possessed good physical stability and successfully maintained its pharmaceutical characteristics throughout the storage period. The absence of significant physicochemical changes suggested that the developed formulation was stable, reliable, and suitable for topical therapeutic application.

**Table 14: Stability Study of Optimized *Ocimum sanctum* L. Emulgel Formulation (F4)**

Storage Condition	Color Change	Phase Separation	pH Change	Consistency	Overall Stability
$2 \pm 2^\circ\text{C}$	No change	Absent	Negligible	Stable	Stable
$25 \pm 2^\circ\text{C}$	No change	Absent	Negligible	Stable	Stable
$37 \pm 2^\circ\text{C}$	Slight change	Absent	Minor change	Slightly viscous	Stable

To further evaluate formulation stability, various physicochemical parameters were monitored periodically during the storage period.

**Table 15: Physicochemical Stability Data of Optimized *Ocimum sanctum* L. Emulgel Formulation**

Parameter	Initial	1 Week	2 Weeks	4 Weeks
Appearance	Smooth, homogeneous greenish emulgel	No change	No change	No change
pH	$6.82 \pm 0.03$	$6.79 \pm 0.04$	$6.76 \pm 0.05$	$6.73 \pm 0.04$
Spreadability (g·cm/s)	$18.42 \pm 0.31$	$18.35 \pm 0.28$	$18.21 \pm 0.33$	$18.06 \pm 0.30$
Viscosity (cP)	$21,420 \pm 48$	$21,360 \pm 52$	$21,245 \pm 56$	$21,110 \pm 61$
Homogeneity	Excellent	Excellent	Excellent	Excellent

Values are expressed as Mean  $\pm$  SD (n = 3).

The stability study results revealed that no significant changes occurred in the physicochemical properties of the optimized *Ocimum sanctum* emulgel formulation during the entire storage period. Minor reductions observed in parameters such as pH, spreadability, and viscosity remained within acceptable pharmaceutical limits and did not adversely influence the performance or stability of the formulation.

The overall findings demonstrated that the developed *Ocimum sanctum* emulgel formulation maintained satisfactory stability under refrigerated, room temperature, and accelerated storage conditions. Therefore, the optimized formulation can be considered pharmaceutically stable and appropriate for storage as well as topical therapeutic application.

#### **In Vivo Anti-Inflammatory Activity of *Ocimum sanctum* L. Emulgel by Carrageenan-Induced Paw Edema Method**

The in vivo anti-inflammatory activity of the developed *Ocimum sanctum* L. emulgel formulation was evaluated using the carrageenan-induced hind paw edema model in Albino Wistar rats. Carrageenan-induced paw edema is one of the most reliable and extensively employed experimental models for screening acute anti-inflammatory activity of synthetic as well as herbal formulations. The inflammatory response produced by carrageenan is biphasic in nature. The initial phase occurring within the first hour is mainly mediated through the release of histamine and serotonin, whereas the late phase occurring after 2–5 h is associated predominantly with prostaglandin synthesis and cyclooxygenase pathway activation. Therefore, inhibition of carrageenan-induced paw edema is considered indicative of anti-inflammatory potential of test formulations.

Following subplantar administration of carrageenan suspension into the right hind paw of experimental animals, significant edema formation was observed in the inflammatory control group. The paw swelling increased progressively with time and reached maximum intensity during the later phase of inflammation. In contrast, animals treated with the standard diclofenac gel and *Ocimum sanctum* L. emulgel exhibited marked reduction in paw edema volume throughout the experimental duration.

The normal control group maintained nearly constant paw thickness during the entire study period, confirming the absence of inflammatory response in untreated animals. However, the inflammatory control group demonstrated a rapid increase in paw thickness after carrageenan administration, confirming successful induction of acute inflammation.

The standard treatment group treated with diclofenac gel showed significant suppression of edema formation at all time intervals. The anti-inflammatory activity became more pronounced after the 3rd hour, which may be attributed to inhibition of prostaglandin synthesis by diclofenac sodium. Similarly, the test group treated with *Ocimum sanctum* L. emulgel demonstrated substantial inhibition of paw edema when compared with the inflammatory control group.

The anti-inflammatory effect of the developed herbal emulgel may be attributed to the presence of various bioactive phytoconstituents such as flavonoids, tannins, phenolic compounds, ursolic acid, rosmarinic acid, and eugenol present in *Ocimum sanctum* L. These phytochemicals are known to inhibit inflammatory mediator release, suppress cyclooxygenase pathway, reduce prostaglandin synthesis, and stabilize lysosomal membranes, thereby reducing inflammatory edema and tissue swelling.

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The anti-inflammatory activity of the herbal formulation increased progressively with time and maximum inhibition was observed at the 5th hour following carrageenan administration. The optimized *Ocimum sanctum* L. emulgel formulation showed anti-inflammatory activity comparable to the standard diclofenac gel, suggesting its promising therapeutic potential for topical inflammatory conditions.

**Table 16: Effect of *Ocimum sanctum* L. Emulgel on Carrageenan-Induced Paw Edema in Experimental Rats**

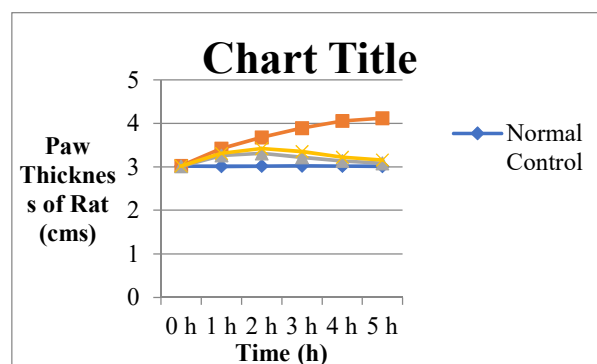
Experimental Group	0 h	1 h	2 h	3 h	4 h	5 h
Normal Control	3.02	3.01	3.02	3.03	3.02	3.01
Inflammatory Control	3.03	3.42	3.68	3.89	4.06	4.12
Standard Treatment (Diclofenac Gel)	3.01	3.26	3.31	3.22	3.14	3.08
Test Treatment ( <i>Ocimum sanctum</i> L. Emulgel)	3.02	3.31	3.42	3.35	3.22	3.16

Values are expressed as Mean  $\pm$  SD (n = 6).

The results presented in Table 16 demonstrated that carrageenan administration caused significant increase in paw thickness in the inflammatory control group throughout the experimental duration. The edema formation was maximum during the late phase of inflammation due to enhanced prostaglandin synthesis.

The standard diclofenac gel markedly reduced paw edema volume and exhibited maximum inhibition among all treatment groups. The developed *Ocimum sanctum* L. emulgel formulation also produced significant reduction in edema formation when compared with the inflammatory control group. The anti-inflammatory activity of the herbal formulation was found to be comparable with the standard preparation.

To further evaluate anti-inflammatory efficacy, percentage inhibition of paw edema was calculated relative to the inflammatory control group.



**Figure 6: Comparative Effect of *Ocimum sanctum* L. Emulgel and Diclofenac Gel on Carrageenan-Induced Paw Edema**

**Table 17: Percentage Inhibition of Paw Edema by *Ocimum sanctum* L. Emulgel and Standard Diclofenac Gel**

Treatment Group	Paw Thickness at 5 h	% Inhibition of Paw
Normal Control	3.01	0
Standard Treatment (Diclofenac Gel)	3.08	17.2
Test Treatment ( <i>Ocimum sanctum</i> L. Emulgel)	3.16	16.2

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	(cm)	Edema
Inflammatory Control	4.12	—
Standard Treatment (Diclofenac Gel)	3.08	54.26
Test Treatment ( <i>Ocimum sanctum</i> L. Emulgel)	3.16	50.38

Values are expressed as Mean  $\pm$  SD (n = 6).

The percentage inhibition data revealed that the standard diclofenac gel exhibited maximum inhibition of paw edema (54.26  $\pm$  0.42%), whereas the developed *Ocimum sanctum* L. emulgel formulation showed 50.38  $\pm$  0.36% inhibition. The comparatively small difference between the standard and test formulation indicates significant anti-inflammatory efficacy of the herbal emulgel.

The results suggest that the developed herbal formulation effectively suppressed carrageenan-induced acute inflammation and may act through inhibition of cyclooxygenase-mediated prostaglandin synthesis. The observed anti-inflammatory activity validates the traditional medicinal use of *Ocimum sanctum* L. in inflammatory disorders.

Statistical analysis using one-way ANOVA followed by suitable post hoc test demonstrated that the reduction in paw edema in the treated groups was statistically significant ( $p < 0.05$ ) when compared with the inflammatory control group. The findings confirmed that the developed *Ocimum sanctum* L. emulgel possesses considerable in vivo anti-inflammatory activity and may serve as a promising herbal topical anti-inflammatory formulation.

#### Interpretation of Figure

The graphical representation clearly demonstrates progressive increase in paw edema in the inflammatory control group following carrageenan administration. In contrast, treatment with diclofenac gel and *Ocimum sanctum* L. emulgel significantly reduced paw swelling throughout the study period. The herbal formulation exhibited anti-inflammatory activity comparable to the standard diclofenac gel, particularly during the later phase of inflammation. The results confirmed the effectiveness of the developed emulgel formulation in suppressing acute inflammatory response.

#### Discussion

The present study successfully developed and evaluated a topical herbal emulgel formulation of *Ocimum sanctum* L. for anti-inflammatory activity. The authenticated leaves were extracted using 70% ethanol, yielding 15.4% hydroalcoholic extract rich in alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenolic compounds, and other bioactive constituents responsible for anti-inflammatory effects. FTIR compatibility studies confirmed absence of drug–excipient interaction, while preformulation studies demonstrated suitable physicochemical properties for formulation development. The prepared emulgels showed acceptable appearance, homogeneity, skin-compatible pH, satisfactory viscosity, spreadability, drug content, and excellent stability under various storage conditions. In vitro drug release studies revealed sustained release behavior, with formulation F4 showing maximum cumulative drug release (99.94%) over 24 hours. The formulations also exhibited appreciable in vitro anti-inflammatory activity through inhibition of protein denaturation, with F7 showing maximum inhibition (69.05%), comparable to standard diclofenac gel. Furthermore, in vivo evaluation using carrageenan-induced paw edema model demonstrated significant reduction in inflammation, where the herbal emulgel

produced 50.38% inhibition of paw edema compared to 54.26% by diclofenac gel. The anti-inflammatory activity was attributed to phytoconstituents such as eugenol, flavonoids, ursolic acid, rosmarinic acid, and phenolic compounds that inhibit inflammatory mediators and prostaglandin synthesis. Overall, the study confirmed that the developed *Ocimum sanctum* emulgel is a stable, effective, and promising natural topical anti-inflammatory formulation with potential for long-term management of inflammatory skin disorders.

### Conclusion

The present study was carried out to formulate and evaluate an herbal emulgel of *Ocimum sanctum* L. for anti-inflammatory activity. The ethanolic extract of *Ocimum sanctum* leaves was prepared successfully by Soxhlet extraction using 70% ethanol. From 250 g of powdered leaves, 38.5 g of extract was obtained with a percentage yield of 15.4%. Preliminary phytochemical screening confirmed the presence of important bioactive constituents such as flavonoids, alkaloids, tannins, saponins, phenolic compounds, glycosides, and terpenoids. The UV spectrophotometric study showed  $\lambda_{\max}$  at 278 nm with good linearity ( $R^2 = 0.998$ ). Nine emulgel formulations (F1–F9) were prepared and evaluated for physicochemical parameters. All formulations showed satisfactory appearance, homogeneity, and stability without phase separation. The pH of formulations ranged from 5.82 to 6.21, indicating suitability for topical application. Drug content was found between 94.12% and 98.42%. In vitro drug release studies demonstrated sustained release behavior for 24 h. Among all formulations, F4 showed maximum cumulative drug release of 99.94% and was selected as the optimized formulation. Stability studies confirmed that the optimized formulation remained stable under different storage conditions. The in

vitro anti-inflammatory study revealed significant inhibition of protein denaturation. Formulation F7 showed maximum inhibition (69.05%), which was comparable with standard Diclofenac gel (74.44%). The in vivo anti-inflammatory activity was evaluated using carrageenan-induced paw edema model in rats. The inflammatory control group showed maximum paw edema (4.12 cm at 5 h), whereas diclofenac gel and *Ocimum sanctum* emulgel reduced paw thickness to 3.08 cm and 3.16 cm, respectively. The percentage inhibition of paw edema was found to be 54.26% for diclofenac gel and 50.38% for *Ocimum sanctum* emulgel. Overall, the developed *Ocimum sanctum* L. emulgel exhibited satisfactory physicochemical properties, good stability, controlled drug release, and significant anti-inflammatory activity comparable with standard diclofenac gel. The study concluded that the herbal emulgel may serve as a promising natural topical anti-inflammatory formulation for the management of inflammatory conditions.

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