RESEARCH PAPER

# Comparative Study of the Anti-inflammatory and Analgesic Effects of Selected Natural Flavonoids in Rodent Models

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**ABSTRACT**

The present study was designed to evaluate the analgesic and anti-inflammatory potential of a traditional folklore formulation and to develop a transdermal drug delivery system incorporating Zingiber officinale (ginger) extract and egg albumin. Ginger extract was prepared using ethanol by maceration and subjected to phytochemical screening, which confirmed the presence of flavonoids, phenolics, alkaloids, tannins, and volatile oils. The extract demonstrated significant in vitro antioxidant activity and notable inhibition of protein denaturation, indicating strong anti-inflammatory potential. A transdermal gel formulation was developed using Carbopol as a polymeric base and evaluated for physicochemical properties including pH, viscosity, spreadability, extrudability, and in vitro drug release. Among the developed formulations, batch F3 exhibited maximum drug release and was selected as the optimized formulation. Compatibility studies using FTIR and DSC confirmed the absence of significant interactions between the extract and excipients. Pharmacological evaluation revealed significant analgesic activity in the hot plate model, along with a reduction in inflammatory markers such as tumor necrosis factor-alpha (TNF-α). In vivo anti-inflammatory activity assessed using the carrageenan-induced paw edema model showed a significant reduction in inflammation. Molecular docking studies further supported the mechanism of action by demonstrating strong binding interactions of gingerol with inflammatory mediators. Overall, the findings suggest that the developed ginger-based transdermal formulation and folklore medicine possess significant analgesic and anti-inflammatory activity, supporting their traditional use and potential as safe and effective alternatives to conventional therapies.

**Keywords:** Zingiber officinale; Transdermal gel; Folklore medicine; Analgesic activity; Anti-inflammatory activity; TNF-α; Molecular docking; Antioxidant activity; Carbopol; Carrageenan-induced paw edema

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**Conflict of interest:** None

## Introduction

Inflammation is a protective biological response to injury, infection, or trauma, aimed at eliminating harmful stimuli and initiating healing. However, excessive or prolonged inflammation can lead to tissue damage and chronic diseases, presenting clinically as redness, swelling, pain, and loss of function.

Blunt trauma is a common cause of inflammation, resulting from internal tissue damage due to mechanical forces. This triggers the release of inflammatory mediators such as prostaglandins and cytokines, leading to pain and swelling. Although NSAIDs are widely used to manage such conditions, their long-term use is associated with adverse effects including gastrointestinal, renal, and cardiovascular complications. Due to these limitations, there is growing interest in herbal alternatives. Medicinal plants contain bioactive compounds like flavonoids and phenolics that exhibit antioxidant, anti-inflammatory, and analgesic effects through multiple mechanisms. Among them, Zingiber officinale (ginger) is well recognized for its therapeutic properties. Its active constituents, including gingerols and shogaols, inhibit inflammatory mediators such as TNF-α and COX enzymes while also providing antioxidant protection. These properties support its traditional use in managing pain and inflammation associated with trauma and musculoskeletal disorders.[1]

In addition to individual herbal components, traditional folklore medicine often utilizes combinations of natural substances to enhance therapeutic effects. One such commonly used remedy involves the topical application of ginger powder mixed with egg white. This formulation is widely used in various regions for the management of blunt trauma, bruises, and muscle pain. Ginger provides anti-inflammatory and analgesic effects, while egg white, rich in albumin, offers soothing and protective properties that enhance the stability and adherence of the formulation to the skin. The combination is believed to improve local circulation, reduce swelling, and accelerate healing. Despite its widespread traditional use, scientific

validation of this formulation remains limited, necessitating systematic investigation. [2,3]

The development of novel drug delivery systems has further enhanced the therapeutic potential of herbal medicines. Among these, transdermal drug delivery systems (TDDS) have emerged as an effective approach for delivering active compounds through the skin directly to the site of action. Transdermal systems offer several advantages, including avoidance of first-pass metabolism, sustained drug release, improved patient compliance, and reduced systemic side effects. In the context of inflammation and blunt trauma, transdermal formulations allow localized delivery of anti-inflammatory agents, resulting in higher drug concentration at the affected site and faster relief from symptoms.

Hydrogel-based transdermal formulations, particularly those using polymers such as Carbopol, are widely used due to their favorable physicochemical properties, including high water content, ease of application, and controlled drug release. Incorporation of herbal extracts into such formulations can enhance their stability, bioavailability, and therapeutic efficacy. Moreover, advances in formulation techniques and analytical tools, such as Fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC), enable the evaluation of drug–excipient compatibility and ensure the quality and safety of the final product. [4]

In light of the above considerations, the present study aims to evaluate the analgesic and anti-inflammatory potential of a folklore formulation based on *Zingiber officinale* and egg albumin, along with the development of a transdermal gel formulation. The study involves phytochemical analysis, in vitro antioxidant and anti-inflammatory evaluation, molecular docking studies, and in vivo pharmacological assessment using established experimental models. The findings are expected to provide scientific validation for traditional practices and contribute to the development of safe, effective, and plant-based therapeutic alternatives for the management of inflammation and pain associated with blunt trauma. [5]

**Materials and Methods [6-11]**

**Drug Profile:**

Ginger (*Zingiber officinale* Roscoe) is a perennial herbaceous plant. The rhizome, often known as ginger root, is widely renowned for its culinary and medicinal applications, with a history of use in traditional systems like Ayurveda, Chinese and Unani medicine. Biological Source: The dried rhizome of *Zingiber officinale* is the primary source of ginger used for therapeutic purposes.

Family: Zingiberaceae

Geographical Distribution: Ginger is indigenous to tropical regions, including India, China, and West Africa. This crop grows in different tropical and subtropical production zones today worldwide (Tarver, 2014).

**Macroscopic Characteristics:**

Plant: Grows to a height of 2-4 feet, featuring green-purple flowers arranged in terminal spikes.

Rhizome: Buff-colored with longitudinal striations, measuring around 2.75 to 6 inches in length and 1 to 1.5 cm in breadth. It possesses a characteristic aromatic odor and a pungent taste.

**Microscopic Characteristics:**

Ginger shows suberized cork tissue, parenchymatous cortex rich in starch and oleoresin, and closed collateral vascular bundles with non-lignified fibers. Its activity is mainly due to phenolic compounds like **gingerols, shogaols, and zingerone**. Gingerols provide pungency and convert to more active shogaols on drying, while zingerone is a milder compound with therapeutic benefits.

Zingerone: A degradation product of gingerols, zingerone is less pungent and has been confirmed for its potential therapeutic benefits.

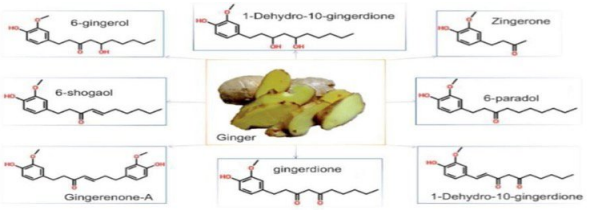



**Figure 1: Phyto-constituents of Ginger**

**METHODS**

**Collection of plant material**

Dried rhizomes of Ginger were collected from local Market of Pune, Maharashtra.

**Extraction of Ginger by maceration**

Ginger rhizomes were converted into coarse powder. Powder sieved through sieve no 43. Coarse powder (100 g) mixed with ethanol (95%v/v). Mixture was kept for 72 hrs at room temperature for maceration with shaking with stirrer at regular intervals. Filtered extract was concentrated with the help of distillation and evaporated to dryness by heating on electrically heated water bath at 60$^{o}$C. Successful multiple maceration cycles were carried out to get sufficient quantity of extract (GEE). [12]

**Organoleptic Evaluation of Extract**

Prepared GEE evaluated for colour, appearance, and odour.

**Phytochemical Analysis**

GEE screened for phytochemicals present in it by series of tests.

**Table 1: Phytochemical Screening of GEE**

| Phytoconstituent | Test Procedure |
|---|---|
| Tannins | GEE (0.5 g) + 2 mL of water then boiled in a water bath. Mixture filtered, + 1 mL of 10% FeCl3 solution to the filtrate. |
| Flavonoids | 5 mL of D/W+ 0.2 g of GEE + 1 mL of 1% AlCl3 solution. |
| Phenol | GEE (0.5 g) + 1 mL of 10% FeCl3 solution. |

| | |
|---|---|
| Saponin | + 4 mL D/W boil in waterbath. |
| Reducing sugar | GEE (0.2 g) + 2 mL D/W + 1 mL Fehling solution A + 1mL Fehling B. |
| Glycoside | GEE (0.2 g) + 2.5 mL of dil $H_2SO_4$ boiled for 15 minutes, cooled and neutralized. 5 mL of Fehling solution A and Fehling solution B was mixed to neutral solution. |
| Volatile oil | GEE (0.2 g) + 2 mL of ethanol + few drops of $FeCl_3$ solution was added. |
| Amino acid | GEE (0.2 g) + 5 mL of D/W allowed to stand for 3 h. Filtered 2 mL of the filtrate + 0.1 mL million reagent. |
| Anthraquinones | GEE (0.2 g) + 5 mL of $CHCl_3$ ---shaken together for 5 minutes---Filtered. To the filtrate + 2.5 mL of 10% ammonium hydroxide. |
| Steroids (Salkowaski test) | Alayer was formed by adding 0.2 g of GEE + 2 mL of $CHCl_3$, followed by 2 mL of strong $H_2SO_4$. |
| Alkaloids | Mayer's test: 2 ml GEE + Mayer's Reagent Hager's test: 2 ml of GEE + few drops of Hager's reagent |

$$Percent\ inhibition(\%) = \frac{(AbsControl - AbsSample)}{AbsControl} \times 100$$

**Analysis of Extract [13-17]**

**Determination of λmax**

100mg of GEE was accurately weighed and dissolved in ethanol to make volume of 100ml to produce stock solution. After proper dilutions the resulting solution was screened for a wavelength range 200 to 400nm to determine absorption maximum by UV spectrophotometer (Schimadzu)

$$Percent\ Antioxidant\ activity(\%) = \frac{(AbsControl - AbsSample)}{AbsControl} \times 100$$

**Determination of calibration curve**

GEE 10 mg was added with 10 mL ethanol to prepare stock solution. Serial dilutions were made from stock solution with various. Absorbance was recorded at 286 nm by UV spectrophotometer (Schimadzu).

**Determination of FTIR spectra**

FTIR spectroscopy was employed examine the sample's chemical structure and functional groups. The spectra were acquired at 4000-400 $cm^{-1}$ using an FTIR spectrometer (Bruker) at Bharati Vidyapeeth's College of Pharmacy, Mumbai. The obtained spectra were used to identify characteristic peaks, confirm the molecular structure by comparing with reference spectra from the library [10].

**Determination of Thermal Characteristics by DSC**

DSC (Differential Scanning Calorimetry) utilized for investigate of thermal properties of the sample, including melting point and thermal stability. Approximately 10mg dried GEE received a sealed placement inside an aluminum pan which contained an empty pan for comparison. The experiment was executed on a DSC instrument (Q20 V24.11) under nitrogen flow conditions at 50 mL/min. The study examined the thermal behavior of samples for a temperature range of 0 to 300°C, using scanning rate of 10°C per minute. The thermograms obtained were analyzed to identify phase transitions, polymorphism, and potential interactions between the drug and excipients [10].

***In vitro* anti-inflammatory Activity**

Using protein denaturation technique, GEE's anti-inflammatory effects were investigated. Distilled water was used to reconstitute GEE and provide stock solutions with the proper concentrations. 1% BSA solution was applied to a series of test tubes containing GEE (100, 200, 300, 400, and 500 mg/ml). The response blend was sequential dilutions of GEE and a 1 percent BSA aqueous solution having a pH adjusted with 1 N HCl. First, the mixes were incubated for 20 minutes at 37°C; mixture was heated for more 20 minutes at 51°C.

This resulted in protein denaturation, which mimicked inflammatory diseases. The samples' turbidity was detected at 660 nm following cooling. Triplicate readings were noted and the percent inhibition of protein denaturation determined [60]

Where AbsControl : absorbance of the control AbsSample : absorbance of sample containing GEE.

**In-vitro Antioxidant Activity DPPH Assay method [18]**

The diphenyl-2-picrylhydrazyl (DPPH) was used to assess free radical scavenging capacity of GEE. A 10-mg/ml stock solution of GEE was serially diluted to create a range of concentrations (500-100 µg/ml) in water. For working solution, 1 ml of 0.3 ml DPPH in absolute methanol was mixed with 2.5 ml of different aqueous dilutions of ginger extract. The solutions were kept in the dark at RT for 30 minutes. A spectrophotometer was used to evaluate the absorbance of several incubation solutions at 517 nm against absolute methanol. Ethanol was used to prepare the control instead of ginger extract. All experiments were performed in duplicate [60]. The percentage of free radical scavenging activity was calculated by

where AbsControl -- the absorbance of the control AbsSample - absorbance sample containing GEE.

**Development and Assessment of a Transdermal Gel Incorporating GEE [12-15]**

**Preformulation Studies**

**Study of Drug-Excipient Compatibility with FTIR**

To explore drug-excipient compatibility samples of GEE and a physical mixture of GEE with carbopol 934,

carbopol 940, PEG 400 and albumin were analysed by FTIR (Bruker).

**Drug-Excipient Compatibility study with DSC**

To explore thermal characteristics of drug and physical mixture of extract with carbopol934, carbopol940, PEG400 and albumin, samples were subjected for thermal analysis by DSC Q20 V24.11 controller. On sealed pans made of perforated aluminium, the sample to be examined was put. A temperature range of 0 to 300° C was covered by the investigation of the thermal behavior of samples at a scanning rate of 10°C/minute.

**Molecular Docking to study molecular interactions [13-17]**

**Protein-Ligand Docking**

**Target Protein Retrieval and Preparation**

The RCSB Protein Data Bank provided X-ray crystallographic information for ovalbumin structures under the PDB ID 1OVA. The validation process established through resolution and mutation analysis and wwPDB validation along with co-crystal ligand assessment and the Ramachandran plot assessment verified the results. The specific details given in Table 2.

**Table 2: Comparative analysis of retrieved protein with standard values and for validation**

| PARAMETERS | DETAILS | STANDARDS |
|---|---|---|
| Protein Id and | 1VOA | - |
| METHOD OF EXPERIMENT | X - RAY Diffraction | X - RAY Diffraction |
| PDB Validation | Better | Better |
| Resolution | 1.95 Å | Near about 3.00 Å |
| Mutation | No | No |

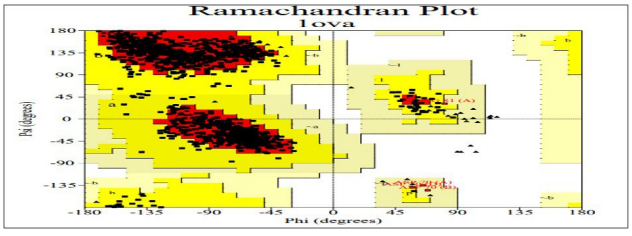



**Figure 2:** Ramachandran Plot 1OVA retrieved from server PROCHECK

CHIMERA v1.16 performed standard residues energy optimization with AM1-BCC force field while handling nonstandard residues through its force fields (AMBER ff14SB) so extra chains and cocrystal ligands and water molecules were removed from the proteins.

**Grid Generation**

The software CHIMERA v1.16 executed protein structural optimization and minimization through its built-in force fields that supported standard residues with nonstandard residues with AM1-BCC and AMBER ff14SB. The proteins underwent a process where researchers removed all nonstandard residues consisting of co-crystal ligands, additional chains and water molecules.

**Table 3: The amino acids active sites**

| Protein ID | AMINO ACIDS THE ACTIVE SITES |
|---|---|
| 1OVA | ALA28, ALA46, ALA115, ALA150, ALA197, ALA205, ALA332, ALA335, ALA343,ILE26, ILE50, ILE87, ILE99, ILE103, ILE168, ILE169, ILE340, LEU41, LEU84, LEU100, LEU114, LEU137, LEU156, LEU173, LEU193, LEU226, LEU260, MET31, MET63, MET206, MET291, MET293, PHE87, PHE112, PHE190, PHE198, PHE227, PHE244, PRO85, PRO106, PRO175, PRO207, PRO217, PRO243, PRO255, PRO369, PRO391,VAL90, VAL109, VAL172, VAL178, VAL189, VAL210, VAL218, VAL257, VAL302, VAL333, VAL347, VAL389, ASN47, ASN49, ASN91, ASN101, ASN107, ASN158, ASN166, ASN171, ASN298, ASN341, CYS87, GLN87, GLN102, GLN132, GLN152, GLN164, GLN174, GLN204, GLN213, GLN219, GLN331,GLY24, GLY27, GLY86, GLY87, GLY140, GLY141, GLY167, GLY246, GLY259, GLY344, SER25, SER59, SER89, SER94, SER95, SER111, SER113, SER116, SER159, SER163, SER176, SER177, SER215, SER245, SER258, SER301, SER390 THR88, THR104, THR203, THR211, ARG345, GLU346, CYS373, , ILE377, ALA378, THR379, ASN380, VAL382, TRP160, TYR110, TYR138, TYR297, ARG97, ARG139, ARG154, ARG170, ARG209, ARG228, ARG290, ARG387 , HIS44, HIS45, HIS337, PHE269HIS368, LEU131,LYS42, LYS375, HIS376, LYS105, LYS135, LYS191, LYS196, LYS199, LYS216, LYS292, LYS296 ASP98, ASP108, ASP151, ASP200, SER277,GLU40, GLU48, GLU136, GLU155, GLU162, GLU195, GLU201, GLU212, GLU214, GLU241, GLU256, GLU261, GLU264, GLU294, GLU295, GLU339, GLU342, ALA60, SER87, ALA87, ARG117, TYR119, GLU122, PRO128, LEU142, GLU143, PRO144, ILE145, ASN146, ALA149, ALA153, THR247, SER249, LEU251, , LEU272, THR273, THR276,SER278, ASN279, MET281, GLU281,. |

The enclosing box was minimized to align with the shape and characteristics of the protein's active site, ensuring compatibility with the ligands intended for docking.

**Table 4: Grid parameter**

| Sr. no. | Protein Id | Centre Coordinates | | | Size Coordinates | | |
|---|---|---|---|---|---|---|---|
| | | x | y | z | X | y | z |
| 1 | 1OVA | 1.773 | 49.846 | 39.916 | 35 | 35 | 35 |

**Ligands Preparation:** Ligands were imported into MarvinSketch after being extracted from ChemSpider and cleaned in two and three dimensions. The MMFF94 force field minimized clean structures to determine the lowest energy conformer before exporting it to MOL2 format.

**Molecular Docking of Target Protein with Ligands**

The Bash script was developed in-house through the combination of AutoDock Tools 1.5.6 for ligand preparation with ADFR Suite for protein processing. Both the ligand and the protein needed conversion to PDBQT format before initiating the docking process. The AutoDock Vina 12.3 software executed docking simulations where the receptor maintained a rigid structure but the ligands had full freedom to rotate their hydrogen bonds. The docking simulations were performed with AutoDock Vina version 1.2.3 running a grid system at 0.375 Å intervals. Placing the grid box directly at the active sites of target, improved the detection of potential binding sites between receptor and ligand. All parameters operated using default configurations. The docking simulations utilized the XYZ points defined in Table by setting their values to 23 and 32 respectively, with a total of nine docking modes and an energy range of three. The docking procedure was performed under the same conditions as the previous studies.

**Protein-Protein Docking:**

**Target Protein Retrieval and Preparation**

The research evaluated essential targets which demonstrate anti-inflammatory properties through examining TNF-α, Toll-like receptor 4 (TLR4-human), and tetragonal hen egg white lysozyme. Multiple Homo sapiens taxonomic protein sequences originated from the National Centre for Biotechnology Information (NCBI). A search platform called Basic Local Alignment Search Tool (BLAST) inside the Protein Data Bank identified biological sequences following their retrieval. The best ten sequence matches were chosen using both a query coverage test and percentage identity evaluation and E-value assessment. Toll-like receptor 4 (TLR4-human) and TNF- α along with tetragonal hen egg white lysozyme have their three-dimensional X-ray crystallographic structures accessible in the Protein Data Bank under specific accession numbers. Multiple quality and reliability parameters evaluated these structures through resolution analysis and mutation examinations as well as wwPDB validation checks for co-crystallized ligands and Ramachandran plot evaluations. Here are the specifics.

**Table 5:** Validation for protein selection in docking analysis occurred through standard reference value comparisons to the retrieved protein.

| **Parameters** | **Protein details** | | | **Standards** |
|---|---|---|---|---|
| Targets | TNF-α | TLR-4 | Hen egg white lysozyme | - |
| Protein id | 2AZ5 | 3FXI | 2YVB | - |
| Method of experiment | X-ray diffraction | X-ray diffraction | X-ray diffraction | X-ray diffraction |
| Mutation | No | No | No | No |
| Resolution | 2.10 Å | 3.10 Å | 1.62 Å | Near about 2.00 $a^0$ |
| Wwpdb validation | Better | Better | Better | Better |
| Co-crystal ligand | 307 | -- | -- | - |
| Ramchandran plot (by procheck server) Residues in favoured + allowed regions | 90.2% | 100% | 100% | >80 % |

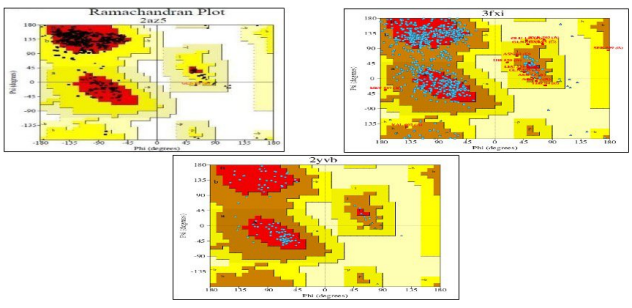



**Figure 3:** Ramachandran Plot 2AZ5, 3FXI & 2YVB obtained from PROCHECK server

Molecular optimization and minimization becomes essential prior to running docking studies and affects all types of molecules including macromolecules and macromolecules. Our research accuracy depends on initial

verification of binding pocket residues through PDBsum server analysis. A visual database of 3D structures in the Protein Data Bank (PDBsum) contains information about interactions with typical inhibitors and proteins. CHIMERA v1.16 was used to fix missing residues and build side chains for the proteins before docking investigations. The following stage incorporated optimization and minimization procedures. The optimization process involved performing 1000 Steepest Descent steps with a step size of 0.1 Å followed by 100 Conjugate Gradient steps also at 0.1 Å distance. All hydrogen atoms were included during the minimization while we accurately determined the protonation states of each histidine residue. The protein structures received their charges through the implementation of the AMBER ff14SB force field. We utilized Biovia Discovery Studio Visualizer V21.1.0.20298 to remove water molecules and excess chains that did not constitute standard residues after finishing the optimization and minimization procedures.

**Grid Generation**

Receptor grids were defined using AutoDockTools together with Chimera as well as Maestro. The estimation of grid volumes for proteins without bound ligands relied on CASTp (Computed Atlas of Surface Topography of Proteins) and SPPIDER and META-PPISP online tools. The determination of grid volume used ligand dimensions for cases where proteins included co-crystallized ligands but utilized online tools for proteins that did not bear co-crystallized ligands. The following are the details regarding the amino acids present in the grid pocket:

**Table 6: Active Sites Amino Acids 2AZ5**

| Protein | Active Sites Amino Acids |
|---|---|
| 2AZ5 | GLY121A, GLY121B, GLY122A, LEU55D, LEU57A, LEU57B, LEU120A, LEU120B, SER60A, SER60B, TYR59A, TYR59B, TYR119A, TYR119B, TYR151A, TYR151B |

The enclosed simulation space restricted itself to dimensions which matched the active site form of the protein while accommodating anticipated dockable ligands.

**Table 7: Grid Parameter 2AZ5**

| PDB ID | Centre co-ordinates | | | Size co-ordinates | | |
|---|---|---|---|---|---|---|
| | X | Y | Z | X | Y | Z |
| 2AZ5 | -19.41 | -74.65 | 33.85 | 25 | 25 | 25 |

**Ligand Preparation**

The selected molecule was first imported into MarvinSketch v21.13, where it was processed in both 2D and 3D formats. To enhance structural stability, energy minimization was performed using the MMFF94 force field. The conformer having least energy was then identified and preserved in the 3D mol2 file format for further analysis.

**Molecular Docking Procedure**

A Bash script developed in-house processed the ligand and protein structures which we acquired. AutoDock Tools version 1.5.7 generated ligand preparation steps and the ADFR Suite handled protein processing. The script processed the structures to produce PDBQT files which maintained ligand flexibility through preservation of all rotatable bonds while keeping the receptor structure fixed. The molecular docking analysis used AutoDock Vina 1.2.5 along with a grid spacing of 0.375 Å. AutoDock Vina performed docking inside the active site box to enhance the detection of receptor-ligand contacts while maintaining default parameter values for all other aspects. The docking procedure included nine modes with an energy range of three, and the CPU settings were configured to 23 and 32, respectively. Testing the redocked complex used the same settings for initial docking procedures to verify accurate and reliable outcomes

**Protein-protein docking:**

Molecular docking brings two distinct biomolecules together to form a complex structure that is biologically relevant and preferably similar to the native one. By specifying 3FXI as the receptor and 2YVB as the ligand, ClusPro is used to dock the Hen egg white lysozyme (2YVB) with TLR-4 (3FXI). Interface residues are defined in the docking servers to restrict protein-protein interactions to the interaction site.

**Experimental design and Formulation of Transdermal gel**

Carbopol 934 (0.25%, 0.5%, and 0.75% concentrations) and Carbapol 940 (0.25%, 0.5%, and 0.75% concentrations) were utilized at three distinct concentrations and combinations during the formulation process, resulting in a total of nine batches.

**Table 8: Formulation Table for Transdermal Gels**

| Composition | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---|---|---|---|---|---|---|---|---|---|
| GEE (g) | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Carbopol 934 (g) | 0.5 | 0.25 | 0.75 | - | - | - | - | - | - |
| Carbapol 940 (g) | - | - | - | 0.5 | 0.25 | 0.75 | - | - | - |
| Carbapol (934 + 940) (g) | - | - | - | - | - | - | 0.5 + 0.5 | 0.25 + 0.50 | 0.50 + 0.25 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Propylene glycol 400 (ml) | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Albumin (gm) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Triethanolamine q.s | pH 7 | pH 7 | pH 7 | pH 7 | pH 7 | pH 7 | pH 7 | pH 7 | pH 7 |
| Sodium benzoate (gm) | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 |
| D/W | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. |

**Preparation of Transdermal Gel:**

Carbopol was carefully weighed and dispersed in 50 ml of D/W in a beaker. Carbopol was allowed to swell in the beaker for half an hour before being manually stirred for another 30 minutes. In a separate container, 5 ml of propylene glycol and the required amount of GEE were combined, with weighed amount of Sodium Benzoate, and the combination was properly mixed. After Carbopol had been completely distributed, five grams of GEE and preservative solutions were added while continually stirring. The last ingredient added to the combination was egg albumin. To reach the appropriate gel consistency, Triethanolamine was progressively added into the formulations to desired the pH, and the remaining D/W was added to raise the total volume to 100 ml.

**Evaluation of Gel Formulations**

**Physical Appearance:** The physical attributes of gel formulations included examination of color, consistency, homogeneity and verification of phase separation absence.

**pH Measurement**

The study determined gel pH values through gel dissolving in 100 mL of distilled water which required a two-hour rest period. The pH measurement occurred through digital pH meter readings.

**Determination of Spreadability**

Spreadability was measured using an apparatus featuring a wooden block with a pulley at one end. They were assessed using the gels' slip and drag properties. Two grams of gel were deposited onto a ground slide in this device. Next, a second glass slide with dimensions that matched those of the fixed ground slide and a hook was placed on the gel. A one-kilogram weight was applied to eliminate air and ensure a uniform gel film between the two slides for five minutes on top. Spreadability was determined using the following formula:

$$S = M \times L \div T,$$

where S is spreadability, M is the weight in the pan (connected to the upper slide), L is the length moved by the slide, and T is the time (in seconds).

**Extrudability Assessment:**

The prepared gel formulations were sealed in standard collapsible aluminum tubes, which were then crimped at the ends. The initial weight of each tube was documented. To evaluate extrusion, the tubes were securely placed between two glass slides. After removing the cap, a 500-gram weight was applied over the slides. The extruded gel was collected, weighed, and the percentage of gel extruded was then calculated. (extrudability >90% = excellent, >80% = good, >70% = fair).

**Viscosity Determination**

We measured the viscosity of gel formulations by using Brookfield Viscometer (RVT) with spindle No. 7 suitable for semi-solid materials. The gel sample was transferred to a clean sample container, ensuring no air bubbles were present. The spindle was immersed in the gel, and the viscosity was determined at a temperature of 25°C ± 1°C at 12 rpm to study the shear-thinning behavior. The viscosity values were recorded in centipoise (CPs) and analyzed to assess the gel's rheological properties, measurements were conducted in triplicate, and the average values were recorded.

***In vitro* Drug Release Study**

The release profile assessments were done by using Franz diffusion cells. Among the components of the receptor media solution phosphate buffer served as the main constituent with pH setting at 7.4. The externally facing surface of the dialysis membrane consisted of recently isolated egg membrane material which maintained a secure position on the donor compartment. A volume of 100 mL of isotonic phosphate buffer (pH 7.4) served to fill the donor chamber before assembling the diffusion cell.

The scientists submerged 100 mL receptor medium which contained 1 g gel formulation while continuously stirring the solution through the egg membrane. The experiment was performed at a controlled 37 ± 1 ºC temperature level under which researchers extracted 1 mL solution samples for subsequent spectrophotometry evaluation at 286 nm.

**Optimization**

The physical qualities of all batches underwent examination through tests which assessed viscosity, pH, extrudability, spreadability and in vitro drug release measurements. Considering drug release of all formulations, formulation with maximum drug release considered as optimum, further utilized for in vivo studies.

**Stability Studies**

The selected gel formulation received storage treatment in collapsible aluminium tubes while stability examinations

occurred at room temperature throughout three months. Evaluation of physical characteristics, pH measurements, flow characteristics and drug quantitation and drug release testing were performed on collected samples month by month [28].

**Pharmacological Screening [18-22]**

The experiments were performed according to both OECD guidelines and CCSEA regulations as well as the evaluation criteria of the IAEC at Invitox R and D Institute, Pune with protocol approval number IRDI/2025-29/M6/07.

***In vivo* Analgesic Activity**

Animals were distributed into four groups of six animals in each group Group I: Control

Group II: Standard, Marketed formulation (Rumalaya Gel) Group III: Transdermal Gel **F3**

Group IV: Folklore Medicine (Ginger Powder + Egg White)

The instrument hot plate was adjusted to 55-56°C for this experiment. Wistar rats were placed on the plate, and a stopwatch was used to time how long it took for the rats to start licking their paws or jumping. Observations were made initially at zero time, then at 30, 60, 90, and 120 minutes of application for gel formulations and folklore medicine.

***Ex-vivo* Anti-inflammatory activity**

Inflammation was induced through a subcutaneous injection of 0.2 mL of 1% w/v carrageenan solution to plantar region of right hind paw of Wistar rats (190-210 g).

A total of 24 laboratory subjects received division into four distinct groups with six animals in each.

Group I: Control

Group II: Standard, Marketed formulation (Rumalaya Gel)

Group III: Transdermal Gel F3

Group IV: Folklore Medicine (Ginger Powder + Egg White)

**Assay of TNF-Alpha**

Blood sample was withdrawn from lateral tail vein at different time intervals as 15 min, 30min, 45min, 60min, 90min and 120min. A serum separation process occurred before ELISA (enzyme-linked immunosorbent assay) analysis. ELISA Rat TNF-alpha GenlisaTM kit used to assess the concentration of the inflammatory marker TNFα.

The lyophilized TNFα Vials received 730 ml of distilled water for the preparation of a standard solution with 2000pg/ml concentration. The standard dilutions were prepared at concentrations of 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25 pg/ml while absorbance was read at 450nm to construct the standard curve. Each well received 100µl of standard and rat isolated plasma then the plate was sealed during 2 hours of incubation at room temperature. The washing procedure ended and detection antibody solutions entered each well before a one-hour incubation. Each well received 100ml dimmed streptavidin-HRP solution for a 30-minute incubation period at room temperature. The TBM substrate was added to each well at 100 ml then kept in dark conditions for 30 minutes before adding stop solution at 100 ml per well to measure absorbance at 450mn within 30 min upon addition of stop solution [148].

***In vivo* Anti-inflammatory activity**

Mice received ginger extract through different preparation methods for anti-inflammatory testing using a protocol that induced carrageenan swelling in their hind paws. The administration of 0.1 mL carrageenan (1% w/v) solution at the right hind paw sub-plantar region induced edema in every rat. Six rats from a total of twenty-four animals received random assignment into the following four groups: Group I Positive control rats.

Group II received treatment with a commercial remedy, Rumalaya gel applied topically to their skin.

Group III topical treatment of ginger transdermal gel containing extract which was applied directly to their skin.

Group IV folklore medicine applied topically.

The formulations' anti- inflammatory effects were evaluated by measuring paw swelling using a vernier calliper.

The proportion of percent inhibition was determined as a measure of the immediate anti-inflammatory impact utilizing the formula below:

$$\% \text{ inhibition} = [(V_t - V_0) / V_t] \times 100$$

where $V_t$ is the paw volume at time (t), and $V_0$ is the paw volume at time zero.

**Statistical Analysis**

The experimental data for analgesic and anti-inflammatory activity were statistically analyzed to establish the significance of the results. Depending on the distribution of the data, either parametric or nonparametric tests were used. One-way ANOVA with Dunnett's test performed multiple comparisons of group data which was displayed through mean ± SD measures. The research indicates statistical significance when p- value reaches a value below 0.05. The statistical analyses for this study were performed through GraphPad Prism version 10.

**Results**

**Extraction and Evaluation of extract**

**Extraction of Ginger rhizomes**

Yield of ethanolic extract of Ginger (GEE) was found to be 1.625% w/w.

**Organoleptic Evaluation of Extract**

Prepared GEE appeared in brown in colour, fine powder and having characteristic odour.

**Phytochemical Analysis**

The alcoholic extract confirmed presence of different phytochemical components viz. table.

**Table 9: Phytochemical Screening of GEE**

| Phytoconstituent | Observation | Inference |
|---|---|---|
| Tannin | Bluish black color to solution indicated the presence of tannin | + |
| Flavonoid | Light yellow precipitate | + |
| Phenol | Deep bluish green colouration | + |
| Saponin | Formation of creamy, small bubbles (frothing). | + |
| Reducing sugar | A brick-red precipitate formed at the bottom of the test tube. | + |
| Glycoside | Formation of brick red precipitate | + |
| Volatile oil | Green colouration. | + |
| Amino acid | Yellow precipitate | + |
| Anthraquinones | A violet coloration was observed in the upper layer. | + |
| Steroids | Formation of a green ring at the interface. | + |
| Alkaloids | Mayer's Test: Formation of a white precipitate.<br>Hager's Test: Formation of a yellow precipitate | +<br>+ |

**Analysis of Extract**

**Determination of λmax:**

The UV-Visual spectral analysis of GEE revealed maximum absorption (λmax) at 281 nm was observed, suggesting the presence of conjugated phenolic compounds or other chromophoric constituents.

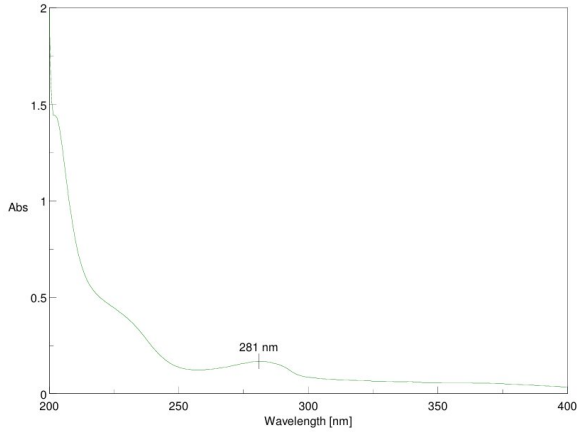



**Figure 1: Determination of λmax of Ginger extract**

**UV calibration Curve**:

The calibration curve of GEE, prepared in ethanol, and exhibited a linear relationship with a correlation coefficient ($R^2$) of 0.983, indicating a strong linearity between concentration and absorbance

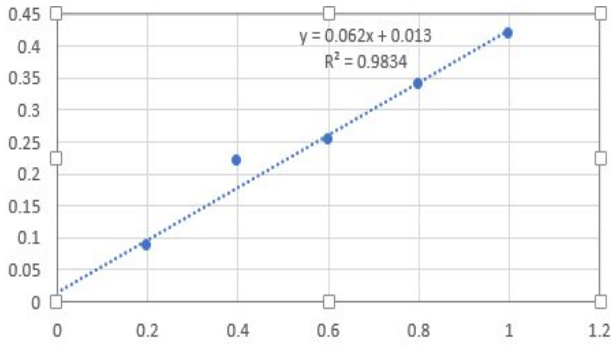



**Figure 2: Calibration Curve of GEE**

**Determination of FTIR spectra**

Infrared absorption spectrum showed characteristic absorption bands for GEE

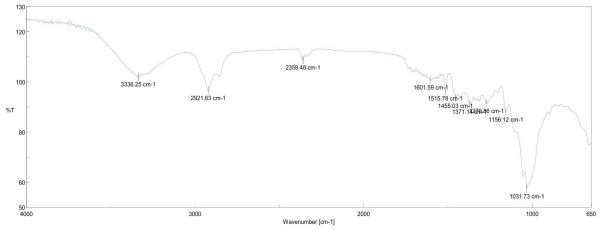


**Figure 3: IR spectra of GEE**

**Table 10: GEE FTIR Spectra Interpretation**

| Ginger extract | Functional Group | IR range | Stretching/ Bending |
|---|---|---|---|
| 3332.9 | O-H | 3200-3400 | Stretching |
| 2921.63 | C-H | 2700-3300 | Stretching |
| 1601.59 | C=O | 1600-1900 | Stretching |
| 1515.78 | C=C | 1475-1610 | Stretching |
| 1031.73 | C-O | 900-1300 | Stretching |

**Determination of Thermal Characteristics by DSC**

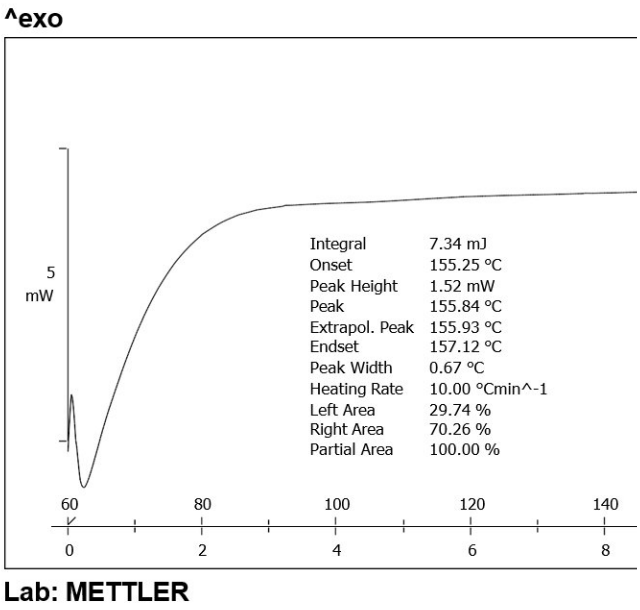



**Figure 4. DSC Thermogram of GEE**

The extract exhibits endothermic peaks around 155°C. The peak at 155°C may suggest the melting of a compound within the extract, possibly a partially crystalline phase.

***In-vitro* Anti-inflammatory Activity of GEE**

GEE showed notable anti-inflammatory effects, with 70.71% inhibition at 500 µg/ml. The extract's inhibition of protein denaturation was found to be concentration-dependent, suggesting that it may be used as an anti-inflammatory agent.

**Table 11. *In vitro* Anti-inflammatory study of GEE**

| GEE (µg/ml) |
|---|
| Control |
| 100 |
| 200 |
| 300 |
| 400 |
| 500 |
| Diclofenac 500 |

***In-vitro* Antioxidant activity of GEE**

GEE inhibited DPPH radicals with an IC50 of 280 µg/ml, indicating its antioxidant activity.

**Table 12: *In vitro* antioxidant activity of GEE**

| GEE (µg/ml) |
|---|
| Control |
| 100 |
| 200 |
| 300 |
| 400 |
| 500 |
| Ascorbic Acid 500 |

**Formulation of Transdermal Gel**

**Pre-formulation Studies**

**Compatibility Studies with FTIR**

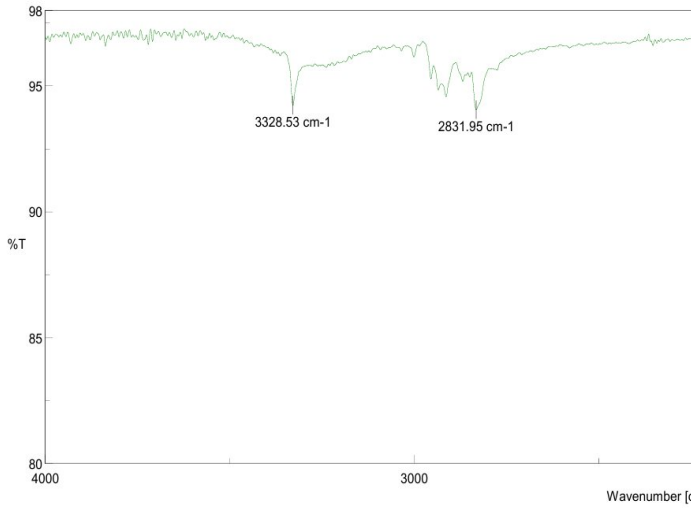



**Figure 13: IR spectra of Physical mixture of GEE and excipients**

Infrared absorption spectrum showed characteristic absorption bands for GEE and Physical mixture as described.

**Table : FTIR Spectra Interpretation**

| Ginger extract | Physical Mixture | Functional Group |
|---|---|---|
| 3328.53 | - | O-H |
| 2831.95 | 2926 | C-H |
| 1684.52 | 1697 | C=O |
| 1590.99 | 1516 | C=C |
| 1020.16 | 1035 | C-O |

The compatibility of GEE with the physical mixture of Carbopol and PEG was concluded by analysing the IR spectrum of the ethanolic extract with that of the physical mixture, showing no significant shifts of peaks.

**Molecular Docking Study**

**Protein Ligand Docking Visualization**

Autodock Vina processing results enabled the creation of a complex with the Biovia Discovery Studio visualizer which generated 2D and 3D complex images using Maestro 12.3 (academic edition).

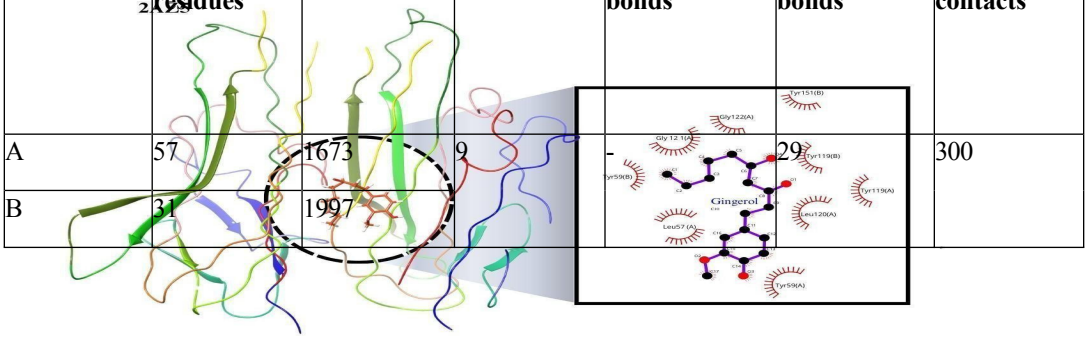


**Figure : Molecular docking 2D and 3D images of Hen egg white lysozyme (2YVB) with TLR-4 (3FXI).**

**Table . Interface statistics of Hen egg white lysozyme (2YVB) with TLR-4 (3FXI).**

| Chain | No. of interface residues | Interface area (Å$^2$) | No. of salt bridges | No of disulphide bonds | No. of hydrogen bonds | No of non-bonded contacts |
|---|---|---|---|---|---|---|
| A | 57 | 1673 | 9 | - | 29 | 300 |
| B | 31 | 1997 | | | | |

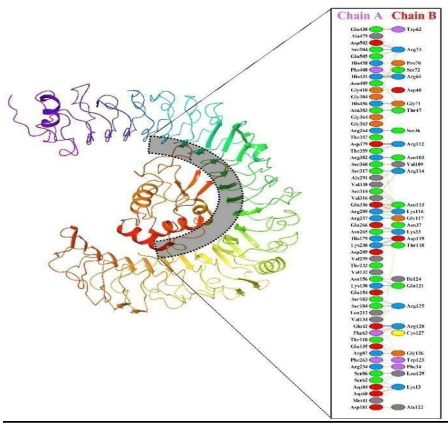
**Figure : 3D and 2D images of interaction of protein 1OVA with 6 gingerol**

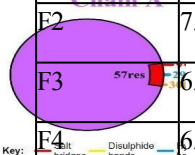
**Protein-Protein Docking**

The PDBsum server provided cluster score and binding energy data for hen egg white lysozyme binding to TLR-4 (3FXI) while the PLIP server generated such data for gingerol interacting with TNF-α (2AZ5). A mixture of hydrogen bonds, salt bridges and hydrophobic interactions can be found with additional data points listing residue IDs and distances.

The negative binding energy, the existence of a hydrogen bond and hydrophobic contacts, as well as the enhanced solubility and stability of gingerol with ovalbumin, all point to a strong and specific binding of 6 gingerol to ovalbumin.

The binding interactions between gingerol with hen egg white lysozyme (2YVB) and TNF-α (2AZ5) are primarily mediated through TLR-4 (3FXI) showing negative binding energy and hydrophobic contacts along with hydrogen bonds. Molecular docking analysis showed two important protein interactions between TNF-α and gingerol (-5.587 energy) and between hen egg white lysozyme and TLR-4 (-960.5 clusterscore).




The research revealed strong affinity levels together with potential inhibitory effects on the primary inflammatory pathways. The inflammatory cascade primarily depends on TNF-α along with TLR-4 as its essential mediators activating and perpetuating inflammatory reactions. The combination of 6-Gingirol with TNF-α and Hen egg white lysozyme with TLR-4 may indicate a dual mode of action in reducing inflammation. Gingerol's interaction with TNF-α may directly modulate this cytokine's function, potentially lowering pro-inflammatory signalling.

**Formulation and Evaluation of Transdermal Gel**

**Development of Transdermal Gel**:

As per the experimental design, a total of nine formulations were developed, incorporating two gelling agents, Carbopol 934 and Carbopol 940, as variable factors as per formulation Table.

Evaluation of Transdermal Gel Formulations

**Physical characteristics:**

All formulation batches were uniform, light brown gels.

**pH Measurement:**

The pH of all the formulated preparations observed within the range of 6.2-7.1 compatible to the pH of adult skin.

**Table : pH of Transdermal gel formulations**

| Formulation | pH |
|---|---|
| F1 | 7.0 |
| F2 | 7.0 |
| F3 | 6.9 |
| F4 | 6.8 |
| F5 | 7.1 |
| F6 | 7.6 |
| F7 | 6.8 |
| F8 | 6.2 |
| F9 | 6.5 |

**Formulations**

**Determination of Spreadability**:
All gel formulations showed good spreadability. Gels with Carbopol 934 showed better spreadability as compared to gels with Carbopol 940.

**Table : Spreadability of Transdermal formulations**

| Formulation | Weight applied (gm) | Spread diameter (cm) | Spreadability ($cm^2$) |
|---|---|---|---|
| F1 | 5 | 4.8 | 26.4 |
| F2 | 5 | 4.6 | 14.5 |
| F3 | 5 | 4.2 | 22.16 |
| F4 | 5 | 3.6 | 24.8 |
| F5 | 5 | 4.2 | 21.5 |
| F6 | 5 | 3.9 | 18.7 |
| F7 | 5 | 4.9 | 24.7 |
| F8 | 5 | 4.3 | 22.9 |
| F9 | 5 | 3.7 | 21.1 |

Lower polymer concentration (0.5%) results in higher spreadability, meaning the gel spreads more easily. Higher polymer concentration (1.5%) leads to lower spreadability, indicating increased gel viscosity. Carbopol 934 showed slightly better spreadability than Carbopol 940 at the same concentration.

**Extrudability Assessment**
The Carbopol 934 gels demonstrated good extrudability to permit the smooth extrusion of the gel from the tube.

**Table : Extrudability of Transdermal Gel**

| Batches | Weight of formulation (g) | % extruded | Extrudability |
|---|---|---|---|
| F1 | 16.65 | 86.06 | ++ |
| F2 | 17.02 | 89.18 | ++ |
| F3 | 17.51 | 81.66 | ++ |
| F4 | 16.85 | 86.23 | ++ |
| F5 | 17.43 | 81.23 | ++ |
| F6 | 17.39 | 88.67 | ++ |
| F7 | 16.00 | 84.23 | ++ |
| F8 | 17.20 | 82.79 | ++ |
| F9 | 16.82 | 84.42 | ++ |

**Viscosity Determination**:
Brookfield viscometer was utilised to measure the viscosity of the gel formulations. The viscosity of the transdermal gel formulations ranged from 20,232 to 34,004 cps. Formulations containing Carbopol 940 exhibited higher viscosity and a firmer consistency, whereas those with Carbopol 934 demonstrated lower viscosity with a softer consistency. The viscosity values for all formulations are summarized in Table below.

**Table 6.12: Viscosity of Transdermal Gel Formulations**

| Batches | Viscosity (Cps) |
|---|---|
| F1 | 20001 ±0.11 |
| F2 | 23532 ±0.43 |
| F3 | 34224 ±0.21 |
| F4 | 31308 ±0.69 |
| F5 | 35047 ±0.58 |
| F6 | 38305 ±0.25 |
| F7 | 39482 ±0.54 |
| F8 | 32906 ±0.75 |
| F9 | 34004 ±0.98 |

***In-vitro* drug release Study**:

In-vitro drug release assays showed % CDR through egg membrane for all gel formulations for 2 hours. Transdermal gel formulation F3 had a larger percentage release of gingerol content (92.23%) than other formulations. Gels made using carbopol-934 as a gelling agent released more polyphenolic content than other formulations.

**Table 6.13: *In vitro* Drug release of Transdermal gel formulations**

| Time (min) | % Cumulative drug release (with SD) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | **F3** | F4 | F5 | F6 | F7 | F8 | F9 |
| 15 | 9.2 ± 0.016 | 9.49 ± 0.051 | **11.78 ± 0.043** | 8.21 ± 0.11 | 8.38 ± 0.64 | 8.21 ±0.046 | 10.42 ±0.032 | 9.89 ±0.042 | 10.21 ±0.012 |
| 30 | 15.57 ± 0.091 | 18.39 ± 0.089 | **21.7.5 ±0.041** | 18.35 ±0.023 | 13.23 ±0.041 | 12.34 ±0.032 | 12.21 ±0.042 | 13.21 ±0.034 | 14,21 ±0.043 |
| 45 | 22.07 ± 0.047 | 27.37 ± 0.121 | **32.58 ±0.032** | 24.32 ±0.069 | 25.23 ±0.042 | 24.89 ±0.054 | 28.35 ±0.032 | 27.32 ±0.043 | 27.01 ±0.034 |
| 60 | 36.32 ± 0.012 | 37.24 ± 0.12 | **47.9 ±0.052** | 32.32 ±0.012 | 34.32 ±0.023 | 33.56 ±0.034 | 38.67 ±0.043 | 38.32 ±0.024 | 37.89 ±0.023 |
| 75 | 48.05 ±0.082 | 46.30 ±0.071 | **53.37 ±0.049** | 41.21 ±0.023 | 42.05 ±0.082 | 44.30 ±0.031 | 47.30 ±0.043 | 48.30 ±0.087 | 46.30 ±0.071 |
| 90 | 63.94 ±0.016 | 55.96 ±0.092 | **72.62 ±0.0524** | 52.12 ±0.043 | 53.84 ±0.016 | 55.96 ±0.042 | 54.96 ±0.031 | 55.26 ±0.032 | 55.12 ±0.022 |
| 105 | 71.63 ±0.014 | 69.27 ±0.011 | **88.02 ±0.061** | 64.21 ±0.067 | 67.63 ±0.014 | 66.27 ±0.016 | 67.27 ±0.021 | 64.27 ±0.016 | 69.05 ±0.08 |
| 120 | 78.70 ±0.012 | 74.82 ±0.023 | **92.23 ±0.041** | 71.23 ±0.045 | 78.70 ±0.012 | 87.82 ±0.023 | 74.82 ±0.023 | 74.82 ±0.023 | 84.76 ±0.021 |

**Optimization of Batch**: All formulations demonstrated satisfactory viscosity, spreadability, and extrudability. Furthermore, F3 exhibited highest *in vitro* drug release. Formulation F3 was considered as an optimum formulation and utilised for future tests, including *in vivo* analgesic activity, *in vivo* anti-inflammatory, and stability studies.

**Stability studies:**

At ambient circumstances the stability of the optimized formulation **F3** was investigated. Stability investigations revealed that the physical appearance, rheological characteristics, extrudability, and spreadability of the produced gel did not significantly change at the end of three months of storage at room temperature.

**Table :** Stability Studies of formulation F3

| Evaluation Parameters | Initial | After 1 month | After 2 months | After 3 months |
|---|---|---|---|---|
| pH | 6.9 | 6.9 | 6.89 | 6.9 |
| Viscosity (CPs) (±SD) | 34224 ±0.21 | 34224 ±0.21 | 34424 ±0.42 | 34624 ±0.34 |
| Spreadability (±SD) (gm.cm/sec) | 22.16 ±0.089 | 22.14 ±0.075 | 23.61 ±0.045 | 23.60 ±0.085 |
| Extrudability (%) (±SD) | 91.71 ±0.092 | 91.60 ±0.087 | 90.99 ±0.76 | 90.82 ±0.045 |
| *In vitro* drug release (% CDR) | 92.23 ±0.041 | 92.21±0.056 | 92.01 ±0.062 | 91.82 ±0.043 |

The final findings after three months indicated no statistically significant changes for color tests or pH readings Drug content tests and in vitro release examinations of the F3 served together with analytical evaluations. Three months marked the duration of stability maintained by the optimized formula according to results from the stability test.

**Pharmacological screening**

***In vivo* analgesic Activity**

The *in vivo* analgesic effect of an optimized gel formulation F3 and folklore and marketed Rumalaya gel was evaluated using Wistar rats by hot plate.

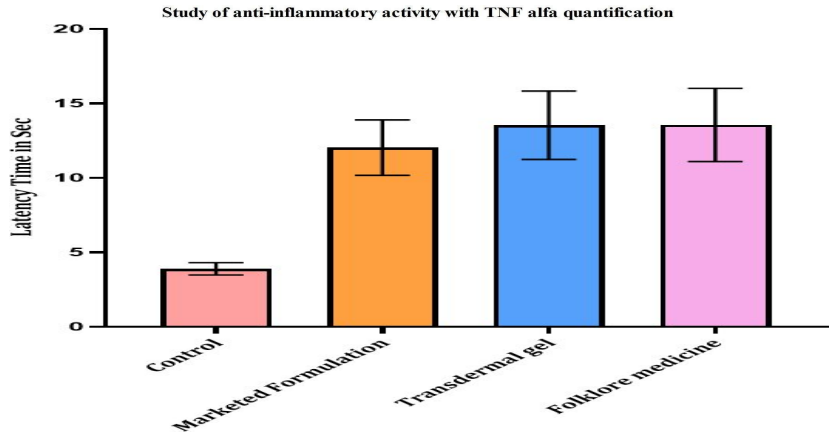



**Figure : Analgesic activity by Hot Plate method**

*** indicated statistically significance at level 1% (p <0.001)

The given bar graph represents the analgesic activity (pain relief) measured using the Hot Plate Method. The y-axis (Time in Seconds) indicates the reaction time (latency period) of the subject before responding to the heat stimulus (e.g., licking, jumping, or withdrawal of paws).

The control group has the lowest reaction time, meaning no significant analgesic effect was observed. The animals quickly responded to the heat, indicating normal pain sensitivity. The reaction time for group treated with marketed formulation was higher than the control group, indicating moderate analgesic activity. This suggests that the marketed formulation provides some pain relief, but the effect is not the highest among all groups. The reaction time of F3 is higher than both the control and marketed formulation, suggesting a strong analgesic effect.

The latency time for folklore medicine is slightly higher than the transdermal gel, suggesting a similar or even better analgesic effect.

***Ex vivo* Anti-inflammatory activity (Assay of TNF-α)**

ELISA by Rat TNF-alpha GenlisaTM kit exhibited decrease in TNFα in plasma of group III (F3) and G IV (Folklore Medicine) than in control group.

**Figure :** Quantification TNF α in rat blood

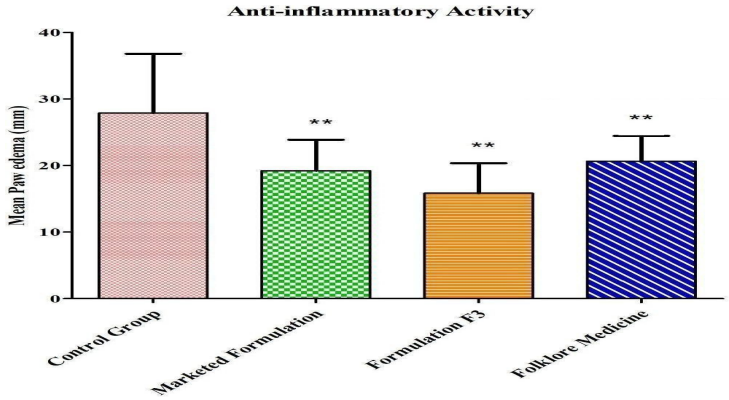
** indicated statistically significance at level 1% (p <0.01)

Control group exhibited highest TNF-alpha levels, indicating the presence of inflammation. The marketed formulation, transdermal gel, and folklore medicine all demonstrated a reduction in TNF-alpha levels, suggesting anti-inflammatory effects.

Among the treatments, transdermal gel and folklore medicine appear to be as effective as or slightly better than the Marketed Formulation. Ginger gel and folklore medicine showed better results than the marketed formulation.

***In vivo* Anti-inflammatory activity**:

The anti-inflammatory activity was evaluated using the carrageenan-induced hind paw edema model in rats. The control group showed a progressive increase in paw swelling, indicating maximum inflammation. The marketed formulation reduced paw edema compared to control, demonstrating moderate anti-inflammatory activity. The F3 transdermal gel showed a further reduction in inflammation, indicating improved efficacy. The folklore medicine exhibited the lowest paw swelling among all groups, suggesting a strong anti-inflammatory effect. Overall, both F3 gel and folklore medicine were more effective than the marketed formulation, with statistically significant results ($p < 0.01$).




** indicated statistically significance at level 1% ($p < 0.01$)

**Figure : *In vivo* Anti-inflammatory activity**

**Discussion**

The present study evaluated the analgesic and anti-inflammatory potential of *Zingiber officinale* formulations and validated a traditional folklore remedy using a transdermal approach. The results demonstrated significant pharmacological activity of the hydroalcoholic ginger extract, mainly due to the presence of flavonoids, phenolics, gingerols, and shogaols. Phytochemical screening confirmed these bioactive compounds, which are known for their antioxidant and anti-inflammatory properties. The extract showed strong in vitro antioxidant activity, indicating effective free radical scavenging. This suggests its ability to reduce oxidative stress, thereby playing an important role in controlling inflammation and preventing tissue damage. The in vitro anti-inflammatory activity, demonstrated through protein denaturation inhibition, indicates that the extract stabilizes proteins and prevents inflammatory processes. This mechanism is particularly important in conditions where denaturation of proteins contributes to inflammation. The results are consistent with previous studies reporting that natural phytoconstituents inhibit inflammatory mediators and protect cellular integrity.

The development of transdermal gel formulations using Carbopol polymers provided an efficient delivery system for the herbal extract. Among the developed formulations, batch F3 exhibited optimal physicochemical properties and maximum drug release, indicating its suitability for transdermal application. The compatibility studies using FTIR and DSC confirmed the absence of any significant interaction between the drug and excipients, ensuring formulation stability and efficacy. The in vivo pharmacological evaluation further strengthened the findings. The significant increase in reaction time observed in the hot plate test indicates strong analgesic activity of the formulation. This effect may be attributed to the inhibition of central pain mediators and modulation of nociceptive pathways. Additionally, the carrageenan-induced paw edema model demonstrated a marked reduction in inflammation, suggesting effective inhibition of inflammatory mediators such as prostaglandins and cytokines.

The reduction in TNF-α levels observed in ex vivo analysis provides further insight into the mechanism of action. TNF-α is a key pro-inflammatory cytokine involved in the initiation and progression of inflammation. The ability of the formulation to reduce TNF-α levels indicates its potential to modulate inflammatory signaling pathways, particularly those mediated by NF-κB and COX enzymes.

Molecular docking studies revealed strong binding interactions of gingerol with inflammatory targets such as TNF-α and TLR-4, supporting the experimental findings. These interactions suggest that gingerol may inhibit receptor activation and downstream signaling pathways, thereby reducing inflammation. The involvement of egg albumin as a carrier may also enhance drug stability and penetration, contributing to the observed synergistic effect.

The results of the present study also highlight the advantages of transdermal drug delivery over conventional oral therapy. Transdermal formulations provide localized action, avoid first-pass metabolism, and reduce systemic side effects, thereby improving patient

compliance and therapeutic efficiency. Moreover, the use of herbal formulations offers a safer alternative to synthetic drugs such as NSAIDs, which are associated with significant adverse effects upon prolonged use.

Overall, the findings of this study validate the traditional use of ginger and egg white in the management of pain and inflammation. The developed transdermal formulation demonstrated superior analgesic and anti-inflammatory activity compared to conventional approaches, indicating its potential as an effective and safer therapeutic option.

**Conclusion**

The present study successfully demonstrated the analgesic and anti-inflammatory potential of a traditional folklore formulation along with the development of a transdermal gel containing ginger extract (GEE). The optimized formulation (F3) exhibited desirable physicochemical properties, significant drug release, and superior pharmacological activity compared to the marketed formulation. Both the transdermal gel and folklore medicine effectively reduced inflammation and pain, as evidenced by decreased TNF-α levels. These findings validate the therapeutic relevance of traditional remedies and highlight the potential of herbal transdermal systems as safe, effective, and promising alternatives for the management of inflammatory conditions, particularly those associated with blunt trauma.

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