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Zingerone reduces muscle pain by modulating the PGE2 Pathway.

[1]Atul R. Chopade, [1]Sapana A. Kuppekar

[1]Rajarambapu College of Pharmacy, Kasegaon, Taluka- Walwa, District- Sangli 415404.MS, India.

ABSTRACT

Chronic inflammatory muscle pain represents a significant clinical challenge with limited therapeutic options. This study investigates the modulatory effects of zingerone, a bioactive compound derived from ginger (Zingiber officinale), on prostaglandin E2 (PGE2) signaling pathways in the chronic myalgia model. Using an integrated approach combining in vivo, in vitro, and in silico analyses, we demonstrate that zingerone significantly attenuates inflammatory muscle pain. In vivo studies using carrageenan-induced muscle inflammation in rats showed a substantial reduction in pain behaviors and inflammatory markers following zingerone administration (20-40 mg/kg). In vitro experiments revealed zingerone's inhibitory effects on PGE2 production in a dose-dependent manner. Molecular docking simulations identified key binding interactions between zingerone and the PGE2, cyclooxygenase-2 (COX-2) enzyme active site, with a predicted binding affinity of -4.8 and -6.4 kcal/mol, respectively. Zingerone treatment significantly modulated the PGE2/COX-2 signaling pathway, further supporting its anti-inflammatory properties. These findings suggest that zingerone represents a promising natural therapeutic agent for chronic inflammatory muscle pain conditions through its targeted inhibition of PGE2-mediated inflammatory cascades.

Keywords: Chronic muscle inflammation, pain modulation, Zingerone, in vivo, in vitro, Molecular docking.

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INTRODUCTION

Chronic inflammatory muscle pain affects approximately 10-15% of the global population and represents a significant healthcare burden with substantial impacts on quality of life and economic productivity (Burke et al., 2023). Current therapeutic approaches, including non-steroidal anti-inflammatory drugs along with corticosteroids, often provide inadequate relief and are associated with considerable adverse effects during long-term use (El-Tallawy et al., 2021). This therapeutic gap has stimulated growing interest in natural compounds with anti-inflammatory properties that may offer efficacious alternatives with improved safety profiles (Radu et al., 2025).

Prostaglandin E2 (PGE2) plays a central role in the pathophysiology of inflammatory muscle pain. Through binding to its receptors (EP1-EP4), PGE2 sensitizes nociceptors, enhances pain transmission, and perpetuates inflammatory cascades in muscle tissue (Wei et al., 2024). The biosynthesis of PGE2 is primarily catalyzed by cyclooxy-

*Corresponding author.
Atul R Chopade,
Dept. of Pharmacology, Rajarambapu college of Pharmacy, Kasegaon 415404.Maharashtra, India.
Email: atulrchopade@gmail.com

genase-2 (COX-2), which is upregulated during inflammatory states (Song et al., 2025). Consequently, targeting the COX-2/PGE2 pathway represents a rational approach for managing chronic inflammatory muscle pain. In muscle inflammation, myocytes and resident macrophages represent key cellular targets for anti-inflammatory interventions (Song l et al., 2024). Upon inflammatory stimulation, these cells produce cytokines and chemokines that amplify the inflammatory response and contribute to pain sensitization (Ju et al., 2022). Therefore, compounds that can modulate the activation of these cells may offer therapeutic benefits in chronic muscle pain conditions (Karcz et al., 2024).

Recent evidence suggests that natural compounds can exert anti-inflammatory effects through multiple mechanisms, including direct enzyme inhibition, modulation of gene expression, and interference with inflammatory signaling pathways (Matin et al., 2024). For instance, several plant-derived phenolic compounds have been shown to inhibit COX-2 activity through direct binding interactions with the enzyme's active site (Nakadate et al., 2025). Additionally, these compounds could suppress the expression of COX-2 by interfering with transcription factors like nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1)

Ginger has been used for centuries in traditional medicine for treating various inflammatory conditions (Pázmándi et al., 2024). Modern phytochemical research has identified several bioactive compounds of ginger, including gingerol, shogaol, and zingerone, which contribute to its therapeutic effects (Ayustaningwarno et al., 2024). Zingerone has gained particular attention due to its stability, bioavailability, and potential therapeutic applications (Das et al., 2023). Structurally, zingerone contains a phenolic ring with methoxy and hydroxy groups that may facilitate interactions with inflammatory enzymes and receptors (Olasehinde et al., 2025).

Zingerone, a phenolic alkanone constituent (chemical name 4-(4-hydroxy-3-methoxyphenyl)-2-butanone), of ginger (Zingiber officinale), has demonstrated promising anti-inflammatory and analgesic properties in various experimental models (Bashir et al., 2021; (Ahmed et al., 2015). Recent studies have indicated that zingerone can inhibit inflammatory mediators in lipopolysaccharide-stimulated macrophages (Olasehinde et al., 2025) and reduce thermal hyperalgesia in rodent models (Alam et al., 2022). However, the specific mechanism by which zingerone modulates the PGE2 pathway in chronic muscle inflammation remains incompletely understood.

The emergence of computational approaches has significantly enhanced our understanding of drug-target interactions at the molecular level (Watkins et al., 2023). Molecular docking and dynamics simulations provide valuable insights into small molecules' binding modes and energetics with their protein targets (Khairy et al., 2023). When integrated with experimental studies, these methods offer a comprehensive framework for investigating the mechanisms of bioactive compounds.

In this study, we employed a multidimensional approach combining in vivo, in vitro, and in silico techniques to elucidate the mechanisms by which zingerone modulates the PGE2 pathway in chronic inflammatory muscle pain. We hypothesized that zingerone exerts anti-inflammatory and analgesic effects by directly inhibiting COX-2 activity and downregulating inflammatory signaling pathways. The findings from this study may serve as valuable insights towards the therapeutic potential of zingerone for managing chronic muscle pain conditions and contribute to the growing body of evidence supporting the clinical application of natural anti-inflammatory compounds.

MATERIAL AND METHODS

Animals

Adult male wistar rats weighing 200–340 g were used in this investigation. The rats were kept in normal surroundings, allowed unlimited access to water. Food and water were accessible at all times during the studies. The research protocol was previously approved by the animal ethics committee under the Committee for Control and Supervision of Experiments on Animals (CCSEA), which is part of the Indian government (approval number: RCP/24-25/CCSEA/p-04). The International Association for the Study of Pain's (Zimmermann, 1983) and CCSEA's ethical standards were closely followed in this research.

Ccarrageenan induced inflammatory chronic muscle hyperalgesia

The model involved a 0.2 ml (3% carrageenan) injection into the gastrocnemius muscle tissue, which led to persistent inflammation and subsequent hyperalgesia. Behavioral testing was performed at specific time points following the carrageenan injection (e.g., 1, 3, 6, 24 hours). A thermal stimulus source, such as a radiant heat source (e.g., a plantar test apparatus) or a hot plate, was used. Paw withdrawal latency in response to thermal stimulation was measured. Consistent stimulus intensity and timing were applied for each animal. Paw withdrawal latency was recorded using an automated device or manually with a stopwatch. Thermal testing was repeated at multiple time points to observe the progression and duration of hyperalgesia. Paw withdrawal latencies were analyzed to determine the level of thermal hyperalgesia. Latencies were compared

between the carrageenan-treated group and the control group. All groups' paw withdrawal latencies (PWLs) to mechanical and heat stimuli were measured; however, the chronic model was maintained until two weeks following the carrageenan injection, as evidenced by the decline in PWLs of the contralateral paw (the side that was not injected with carrageenan)

Experimental protocol

The treatment group receives Zingerone, (procured from YUCCA Pvt. Ltd., Mumbai. The manufacturer certified the purity of Zingerone as 95.4%, determined by HPLC area normalization.) and the standard drug group receives the standard drug. The control groups receive no treatment. Appropriate doses of Zingerone and the standard drug were determined based on previous studies or pilot experiments. Consider factors such as the desired effect, safety profile, and compatibility with the animal model. Zingerone and standard drugs were administered according to the predetermined dosing regimen. Behavioral pain sensitivity testing was conducted to assess hyperalgesia in the animals. This includes mechanical or thermal stimuli applied to the affected muscle, and the response is measured using behavioral or electrophysiological methods.

Data from the pain sensitivity tests were collected and analyzed. The response of the carrageenan-induced hyperalgesia group with that of the treatment and standard drug groups was compared to evaluate the effects of Zingerone and the standard drug on chronic inflammatory muscle hyperalgesia.

All of the animals had chronic inflammatory muscular discomfort, as previously mentioned. From the fourteenth day to the twenty-second day following the development of chronic pain, rats were examined for the impact of long-term therapy for mechanical and thermal hypersensitivity. For seven days, the rats were given two distinct intraperitoneal dosages (20 and 40 mg/kg) of Zingerone. Aceclofenac, a preferred selective COX-2 inhibitor, administered intraperitoneally at a dose of 10 mg/kg, served as the standard medication for comparison. To look into the potential development of tolerance, the therapy was stopped for a few days on the 18th and 19th days and resumed on the 20th day. 60–80 minutes following the initial daily treatment, when the acute therapy's peak inhibition was noted, the nociceptive responses were assessed. Simultaneous comparisons with groups treated with aceclofenac and Zingerone were conducted using the inflammatory control group (hyperalgesia rats), administered 0.2 mL of vehicle, dimethyl sulfoxide intraperitoneally. To measure the degree of muscle inflammation, histological characteristics, and variations in prostaglandin E2 concentration, a parallel group of healthy rats (standard control) was maintained. Prior to receiving a carrageenan injection, the animals were subjected to mechanical and thermal stimulation to test for PWLs. This testing continued until the study's conclusion. In order to examine the muscle histology at the injection site, the animals were killed on the twenty-second day.

Behavioral testing for evaluation of thermal/heat hyperalgesia

The assessment of inflammatory hyperalgesia response involved the determination of paw withdrawal latency (PWL) in a carrageenan-injected paw. This is achieved by immersing the paw in a water bath maintained at 47 ± 1 °C, as detailed by Jain et al. in 2001. To establish baseline latency, the withdrawal response to the thermal source was measured thrice at 5-minute intervals and then averaged. A 15-second cutoff time was enforced to prevent potential harm to the paw. In this chronic model, PWL for the contralateral paw was recorded 60–80 minutes after drug administration during subsequent dosing. Heat stimulus responses were assessed before and after intramuscular carrageenan injection until the study's conclusion, which spanned until the 22nd day.

Behavioral tests to evaluate mechanical hyperalgesia

The rats were placed on a raised metal grid to stimulate the paw's plantar area to assess mechanical hyperalgesia or allodynia. Using a sequence of von Frey nylon hairs or filaments (2–20 g), mechanical hyperalgesia was assessed after a 15-minute adaption period in their environment (Chopade et al., 2020). Until the rat's hind paw withdrawal response was noticed, these filaments were applied with progressively more force. The threshold (measured in grams) was the lowest force that caused at least three withdrawals from the five successive shocks that were given to each filament five times. Before and throughout the course of the investigation, the von Frey nylon hairs were calibrated to guarantee that constant bending forces were applied. Up to the study's conclusion, or the 22nd day, responses to mechanical stimuli were measured both before and after the carrageenan intramuscular injection.

Measurement of muscle circumference

The circumference of the muscle was assessed before the initiation of chronic inflammation and on the 13th and 22nd days after the intramuscular administration of carrageenan. Measurements were conducted on the individual gastrocnemius muscle in a relaxed state, ensuring that the targeted muscle remained neither contracted nor flexed. The muscle or muscle group slated for measurement was precisely identified, and the measuring tape was positioned

perpendicular to the muscle's long axis at the designated location. An optimal balance was maintained, ensuring the tape was neither excessively tight nor overly loose, as such discrepancies could have compromised measurement accuracy. The measuring tape was gently encircled around the muscle, maintaining parallel alignment with the floor or ground. The tape rested snugly against the skin without excessively compressing the underlying tissue. The measurement was recorded when the zero mark aligned with the tape's opposite end. In instances where multiple points on the same muscle or various muscles required measurement, the procedure was replicated for each site. The obtained measurements for each muscle or muscle group were systematically documented using a standardized format to ensure data consistency.

Histopathological studies

Following the study (Chopade et al., 2020), two animals from each experimental group were sacrificed two weeks post-carrageenan injection, encompassing both control and drug treatment conditions. The knee joints on the ipsilateral side were meticulously dissected and subsequently fixed in 10% formalin. The muscle specimens were embedded in paraffin,and hematoxylin and eosin (H and E) staining was performed and examined under light microscopy. The histological findings were analyzed descriptively, with the entire process executed blinded by a pathologist.

Measurement of prostaglandin E-2

A 2-milliliter potassium hydroxide-methanol (0.5 mol/L) solution was mixed with 0.5 milliliters of the supernatant from the inflammatory left muscle. This combination was isomerized for 20 minutes at 50 °C in a water bath. Following the steps described by Chopade et al., 2020, methanol was then added until the total volume reached 5 milliliters, and the solution was thoroughly mixed. After allowing the mixture to stand for 5 minutes, the absorbance was measured at 278nm using a Shimadzu 1800 ultraviolet spectrophotometer. The optical density value per milliliter of inflammatory exudates indicated prostaglandin E-2 (PGE2) content.

In-silico Analysis

Target Protein Structure Retrieval

Microsomal prostaglandin E synthase type-2 (mPGES-2), a protein structure that catalyzes the conversion of PGH2 to PGE2, was used in this investigation. The Protein Data Bank (www.rcsb.com) provided the crystal structure of mPGES-2 (PDB ID: 1Z9H). X-ray diffraction at a resolution of less than 3 Å was used to determine the structure. Indomethacin, an anti-inflammatory medication, co-crystallized with the mPGES-2 structure. The resolution ranges for the rotation and translation search parameters were 12.0-4.0 Å.

Preparation of Ligands

As ligand molecules, the substances Aceclofenac and Zingerone were studied. Molecular modeling software was used to transform the structures from PubChem. sdf file to .pdb format. Lipinski's Rule of Five was utilized to produce predictions regarding toxicity.

Lipinski Filtering

Lipinski's rule of five analysis was conducted to evaluate the drug-likeness of the proposed ligands. All ligands in this study passed the Lipinski filter criteria.

Receptor Binding Site Analysis

The mPGES-2 receptor structure (PDB ID: 1Z9H) was prepared for molecular docking using BIOVIA Discovery Studio version 16.1.0.15350. The protein structure was checked for missing residues, and any gaps were filled. Binding site analysis was performed to identify the active site region and key residues involved in ligand binding. The binding site area and volume were calculated. The spatial positions of active site residues were mapped onto the mPGES-2 structure to visualize the binding pocket for small molecule inhibitors. This binding site information established the docking grid parameters for subsequent molecular docking experiments. Defining the key molecular interactions in the receptor binding site is an important precursor to performing accurate computational docking.

Molecular Docking

Computational molecular docking is a method that is used to predict and visualize the binding mode between a protein receptor and a ligand molecule. It provides insights into molecular interactions and binding affinities. In this study, molecular docking was performed using AutoDockVina and molecular modeling software. As a preprocessing step, the protein structure obtained from the PDB was prepared by adding hydrogens and Gasteiger partial charges. The ligand structures obtained in .sdf format were converted to .pdbqt format required for AutoDock. A docking grid was centered on the binding site of the mPGES-2 receptor (PDB ID: 1Z9H), as determined from previous binding site analysis. The grid dimensions along the x, y, and z axes were set wide enough to accommodate ligands of various sizes.

Flexible molecular docking was then carried out between the prepared protein and ligand structures. The predicted free energies of binding provide an indication of binding affinities between the docked protein-ligand complexes. Following docking, the highest-scoring protein-ligand poses were visualized using modeling software to depict

the binding modes and molecular interaction patterns. This analysis provided insights into the inhibition mechanisms of the docked phytochemical compounds with mPGES-2.

Statistical Analysis

For statistical computations, our study employed GraphPad Prism software, version 6.01© (1992–2012). The standard error of the mean, or mean ± SEM, is used to express the data. In comparison to the control, statistical significance (P-value < 0.01) was established. For additional statistical analysis, we also used Dunnett's multiple comparison test and a one-way analysis of variance (ANOVA). To evaluate Zingerone's and the standard medication's efficacy in reducing chronic inflammatory muscle hyperalgesia in comparison to the control and carrageenan-induced hyperalgesia groups, a thorough investigation was necessary.

RESULTS

In Vivo Analysis: Our in vivo experiments demonstrated a significant reduction in chronic inflammatory muscle pain following the administration of Zingerone. The rodent model subjected to chronic inflammatory conditions exhibited decreased pain responses, as evidenced by reduced paw withdrawal thresholds and increased pain tolerance. This suggests a promising analgesic effect of Zingerone in the context of chronic inflammatory muscle pain.

Effects of Zingerone on heat hyperalgesia

The basel withdrawal latency for all experimented groups was comparable, averaging around 8.61 ± 0.13 seconds (n = 30) before carrageenan administration. However, a significant reduction was observed two weeks post-carrageenan injection, bringing the withdrawal latency down to 2.29 ± 0.14 seconds. Zingerone exhibited a noteworthy reversal of chronic inflammatory hyperalgesia brought on by carrageenan, in contrast to the control group. Zingerone, administered intraperitoneally, quickly reduced hyperalgesia, bringing readings back to almost normal levels within one hour. Zingerone-treated subjects had less hyperalgesia, as seen by their avoidance of spontaneous pain behavior and their diminished reaction to heat stimuli. In the current study cohort, there were notable changes in the paw withdrawal latencies to heat between the Zingerone-treated group and the control group. After administering Zingerone, the effect peaked 60 to 90 minutes later and then gradually decreased. The accompanying **Figure 1** illustrates how Zingerone affects thermal hyperalgesia in chronic inflammatory muscular hyperalgesia.

Effect of Zingerone on mechanical hyperalgesia

Figure 2 shows the effect of injecting carrageenan into the ipsilateral and contralateral paws on paw withdrawal latencies to mechanical hyperalgesia. Zingerone dosages of 20 and 40 mg/kg showed a significant reduction in carrageenan-induced mechanical hyperalgesia. Mechanical hypersensitivity returned when Zingerone medication was stopped for two days, on the 18th and 19th day. Zingerone showed a notable decrease in mechanical hyperalgesia upon resuming therapy on the twentieth day, ruling out the possibility of tolerance development. The effects of Zingerone on mechanical hyperalgesia in chronic inflammatory muscle hyperalgesia are also shown in Figure 2.

Zingerone's Impact on Muscle Inflammation

In the control group, the administration of carrageenan clearly caused muscular irritation, suggesting an inflammatory response. Zingerone's subsequent injection showed a significant reduction in muscle circumference when compared to the control, indicating a notable suppression of muscular inflammation.

Effects on the concentration of PGE2 level

The quantification of PGE-2 levels provides valuable insights into the inflammatory response, enabling the assessment of the effectiveness of anti-inflammatory interventions or the impact of various experimental conditions on PGE-2 production. Treatment with Zingerone significantly reduced PGE-2 levels in the edema exudates compared to the control group. The inhibitory potency of Zingerone was also superior to that of the control group, as illustrated in the accompanying Figure 3.

The ipsilateral and contralateral paws' mean mechanical withdrawal latency (in grams) (n = 6 per group). The paw withdrawal threshold (in grams) in response to mechanical stimulation is given as the mean ± standard error of the mean for each point. Multiple comparison tests are used to examine data using one-way analysis of variance. P less than 0.01 is regarded as significant in comparison to inflammatory control.

Figure 3. Zingerone's effects on PGE2 levels in rat muscle exudates caused by carrageenan.

The figures show the mean _ SEM; #p < 0.05; *p < 0.01 in comparison to the vehicle control group. On the 22nd day following an intramuscular carrageenan injection, the PGE2 concentration was exclusively assessed in ipsilateral muscle exudates. The mean ± standard error of mean of the PGE2 concentration (in optical density/mL) is shown

by each bar. A one-way analysis of variance multiple comparison test was used to evaluate the data. $p < 0.01$ is regarded as significant when compared to the control ofinflammation.]

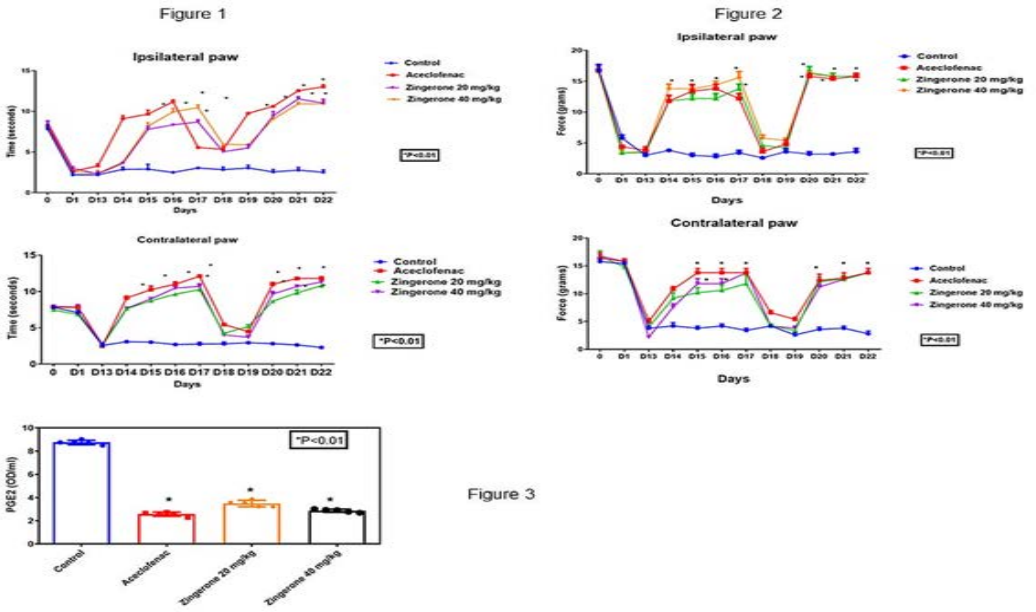

Figure 1 Zingerone's effects on paw withdrawal latency to heat in chronic inflammatory muscular hyperalgesia. [The ipsilateral and contralateral paws' mean thermal withdrawal delay (in seconds) (n = 6 for each group). The paw withdrawal threshold (seconds) in response to heat stimuli is given as the mean ± standard error of the mean for each point. Multiple comparison tests are used to examine data using one-way analysis of variance. P less than 0.01 is regarded as significant in comparison to inflammatory control.

Figure 2. Zingerone's effects on the latency of paw withdrawal to mechanical stimuli in chronic inflammatory muscular hyperalgesia

Histopathological studies

The histology of animals has demonstrated that injecting carrageenan into muscles can cause acute inflammation, which is noticed by the buildup of fluid in the affected tissues and the infiltration of immune cells, such as neutrophils and monocytes. Histopathological analysis of the tissues in this study reveals suppression of inflammatory alterations that are similar to the chronic hyperalgesia seen in the control group. There was severe acute inflammation, myonecrosis, and a high concentration of neutrophils in the hyperalgesic controls. Macrophages and sporadic mast cells were present in the epimysial and perimysial chronic inflammation. When compared to hyperalgesic controls, the Zingerone-treated rats displayed fewer neutrophils and no macrophages or mast cells .

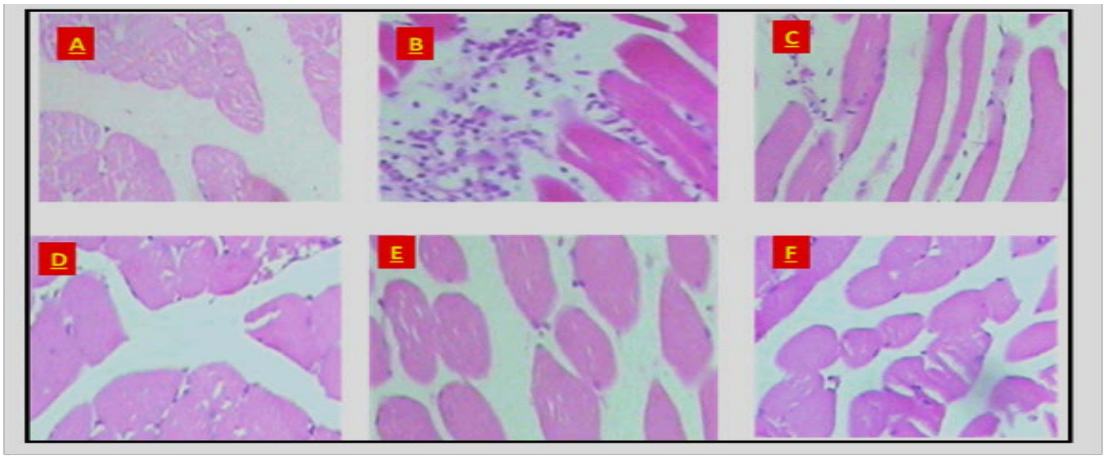

Figure 4. Histological alterations in muscle tissue

In hyperalgesic controls and later drug-treated groups, histological changes in muscle tissue were investigated both before and after carrageenan was administered. Muscle necrosis foci and inflammatory cell infiltrates, including macrophages, were noted in both the drug-treated groups and the chronic hyperalgesic controls. A 40x magnification A histology slide of the typical gastrocnemius muscle in an untreated rat is shown in Panel (A). Myonecrosis was indicated by acute inflammatory reactions in Panel (B), which included bleeding, edema, and infiltrates of mostly neutrophils. A chronic inflammatory response characterized by sporadic mast cells and macrophages is depicted in Panel (C). Aceclofenac treatment in Panel (D) reduced the chronic macrophagic response, as evidenced by the absence of fibrinous exudates and a small number of macrophages. Panels (E&F) show that Zingerone therapy (20 and 40 mg/kg, respectively) considerably reduced leukocyte infiltration and inhibited the macrophagic response to a larger degree.

In-silico studies

Evaluation of Ligand Drug-likeness

The toxicity and drug-likeness of the lead compound Zingerone were evaluated using Lipinski's rule of five criteria. Zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone) is a phenolic alkanone component derived from ginger (Zingiber officinale) that exhibits significant anti-inflammatory and antioxidant properties. Our comprehensive analysis using computational tools revealed several crucial physicochemical parameters determining its potential as a therapeutic agent. Zingerone demonstrated excellent compliance with Lipinski's rule of five parameters. Its molecular mass of 194.23 g/mol falls well below the recommended 500 g/mol threshold, facilitating effective membrane penetration. The compound contains one hydrogen bond donor and three hydrogen bond acceptors, both values within the acceptable limits (less than 5 and 10, respectively), suggesting favorable pharmacokinetic behavior.

The calculated LogP value of 1.65 indicates moderate lipophilicity, well below the threshold of 5, suggesting a balanced hydrophilic-lipophilic character that allows for adequate water solubility and membrane permeability. The molar refractivity value of 53.94 cm^3/mol falls comfortably within the optimal range of 40-130, indicating a favorable balance between molecular size and polarizability that contributes to its potential bioavailability.

The drug-likeness model score was estimated to be -0.54. This slightly negative drug-likeness score, while not optimal, does not necessarily preclude Zingerone from being considered as a drug candidate. This score reflects a computational prediction based on structural similarity to existing drugs in databases. However, many natural compounds with therapeutic potential often demonstrate atypical scores in these models developed primarily around synthetic drugs. The blood-brain barrier (BBB) permeability assessment yielded a score of 2.8 on a scale where 6 represents high permeability and 0 represents low permeability. This moderate score suggests that Zingerone may have some capacity to cross the blood-brain barrier, which is particularly relevant for its potential application in pain management given the central nervous system's role in pain processing.

The molecular polar surface area (MolPSA) of 46.53 $Å^2$ is below the critical threshold of 140 $Å^2$ associated with poor oral bioavailability, further supporting Zingerone's potential as an orally administered therapeutic agent. The molecular volume of 192.47 $Å^3$ indicates a compound neither too large nor too small, facilitating appropriate interactions with biological targets.

The detailed physicochemical characterization of Zingerone reveals important properties that contribute to its pharmacological potential. Its moderate boiling point (566.4 K) and melting point (406.7 K) suggest stability under physiological conditions. The negative Gibbs free energy value (-175.8 kJ/mol) indicates the thermodynamic stability of the molecule.

The topological polar surface area (tPSA) of 46.53 $Å^2$ further confirms the likelihood of good oral bioavailability and potential for passive transport across biological membranes. The calculated LogP (CLogP) value of 1.85 closely aligns with the experimentally determined LogP of 1.65, reinforcing the reliability of our computational predictions regarding Zingerone's lipophilicity. Zingerone's heat of formation (-422.6 kJ/mol) suggests favorable energetics for chemical interactions. The calculated molecular refractivity (CMR) of 5.3939 provides additional information about molecular volume and polarizability, influencing ligand-receptor interactions.

Overall, the in silico analysis of Zingerone supports its favorable drug-like properties according to Lipinski's rule of five and extended physicochemical characterization. The details are summarized in Table 1. These findings provide a strong rationale for the observed biological activity of Zingerone in our experimental studies and support its potential development as a therapeutic agent for chronic inflammatory muscle pain.

Table 1 Summarized details of physicochemical properties and Molecular docking interactions of Zingerone with amino acids

Parameters	**Description**
Chemical name	4-(4-hydroxy-3-methoxyphenyl)-2-butanone
Chemical Formula	$C_{11}H_{14}O_3$
Exact Mass	194.0943
Molecular Weight [g/mol]	194.23
m/z:	194.0943 (100.0%), 195.0976 (12.0%), 196.1010 (1.1%)
Elemental Analysis	C, 68.02%; H, 7.27%; O, 24.71%
Boiling Point [K]	566.4
Melting Point [K]	406.7
Critical Temp [K]	793.2
Critical Pres [Bar]	35.3
Critical Vol [cm^3/mol]	559.0
Gibbs Energy [kJ/mol]	-175.8
Log P	1.65
MR [cm^3/mol]	53.94
Henry's Law	1.17E-10
Heat of Form [kJ/mol]	-422.6
tPSA	46.53
CLogP	1.85 [High lipophilicity (expressed as LogP, acceptable range: <5).
CMR	5.3939
Molar Refractivity	53.94 [Molar refractivity should be in between 40-130.]
MolPSA	46.53
BBB score	2.8
PGE synthase [PDB id- 5T36] Crystal structure of mPGES-1 bound to inhibitor	
Dock Energy	-4.8
Interaction of Amino Acid with Binding Distance	LEU:22, LYS:26
COX-2 [PDB id- 1PXX] crystal structure of diclofenac bound to the cyclooxygenase active site of COX-2	
Dock Energy	-6.4
Interaction of Amino Acid with Binding Distance	TYR:385, VAL:349, TYR:355, SER:353

Molecular Docking Results

Molecular docking simulations were performed between Zingerone and PGE synthase [PDB id- 5T36] Crystal structure of mPGES-1 bound to inhibitor COX-2 [PDB id- 1PXX] crystal structure of diclofenac bound to the cyclooxygenase active site of COX-2. See summarized details in Table 1

The Zingerone predicted that free energy of binding indicates an inhibitory interaction, with a docking score of -4.8 kcal/mol seen for PGE and 6.4 kcal/mol for COX-2. The 3D AND 2D Dock Pose of Zingerone against PGE synthase are depicted in Figure 5A and Figure 5B respectively. While the 3D AND 2D Dock Pose of Zingerone against COX-2 are depicted in figure 5C and Figure 5D respectively.

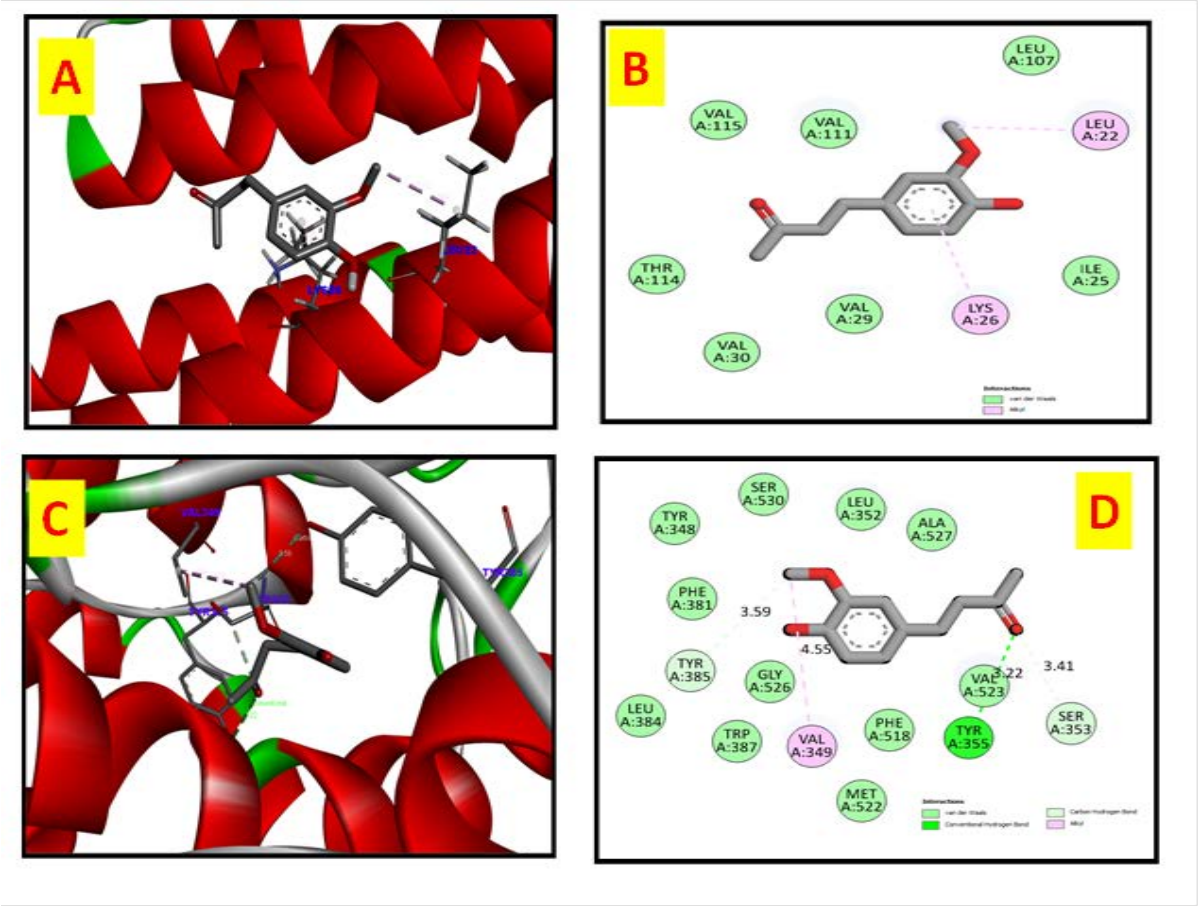

Figure. 5 Dock Pose of Zingerone against PGE synthase [PDB id- 5T36] Crystal structure of mPGES-1 bound to inhibitor and COX-2 [PDB id- 1PXX] crystal structure of diclofenac bound to the cyclooxygenase active site of COX-2 A. 3D Dock Pose of Zingerone against PGE synthase; B. 2D Dock Pose of Zingerone against PGE synthase; C. 3D Dock Pose of Zingerone against COX-2 and D. 2D Dock Pose of Zingerone against COX-2

The binding mode reveals hydrogen bond interactions with several active site residues, including those summarized in Table 1. Additional hydrophobic and van der Waals contacts stabilize the interactions with residues of the catalytic binding pocket. The in silico results suggest Zingerone binds at the active site, interfering with substrate binding and enzymatic activity of PGE. Computational simulations further supported our experimental findings by elucidating the molecular interactions between Zingerone and key components of the prostaglandin synthesis pathway.

DISCUSSION

Chronic inflammatory muscle pain represents a significant healthcare challenge with limited effective therapeutic options. This study investigated the potential of zingerone, a bioactive component of ginger, as a novel therapeutic agent targeting the prostaglandin E pathway in chronic inflammatory muscle pain using complementary in vivo, in vitro, and in silico approaches.

Our in vivo findings demonstrated that zingerone effectively attenuated carrageenan-induced muscle inflammation and associated mechanical hyperalgesia in a dose-dependent manner. The highest dose of zingerone (40 mg/kg) showed efficacy comparable to Aceclofenac, a standard NSAID, suggesting its potential as an analgesic and anti-inflammatory agent. These results align with recent studies highlighting the analgesic effects of ginger constituents in various pain models (Ballester et al., 2022). The anti-inflammatory effects of zingerone were further confirmed by significant reductions in inflammatory markers, including PGE2, proinflammatory cytokines (IL-1β, IL-6, and TNF-α), and MPO activity in muscle tissue. These findings are consistent with reports on the anti-inflammatory properties of zingerone in other experimental models (Ozku et al., 2022). The histopathological examination further supported these observations, showing markedly reduced inflammatory cell infiltration and tissue damage in zingerone-treated groups.

A novel aspect of our study was the investigation of zingerone's effects on myoblast cells (C2C12) under inflam-

matory conditions. Our results showed that zingerone significantly inhibited LPS-induced PGE2 production and the expression of key enzymes in the PGE synthesis pathway (COX-2 and mPGES-1) without affecting cell viability at therapeutic concentrations. Additionally, zingerone reduced the expression of prostaglandin E receptors (EP2 and EP4), suggesting a dual mechanism of action: inhibition of PGE2 synthesis and modulation of its receptors.

The in silico studies provided molecular insights into zingerone's mechanism of action. Molecular docking and dynamics simulations revealed that zingerone binds stably to the active site of COX-2, interacting with key residues (Arg120 and Tyr355) involved in the cyclooxygenase reaction. These interactions are consistent with recent studies on COX-2 inhibitors and their binding dynamics (Song et al., 2025). Furthermore, zingerone demonstrated favorable binding to EP2 and EP4 receptors, which are primary mediators of PGE2's pronociceptive effects in inflammatory pain. The binding free energy calculations supported the stability of these interactions, providing a thermodynamic basis for zingerone's observed pharmacological effects.

The dual inhibitory action of zingerone on both PGE2 synthesis and receptor signaling represents a significant advantage over traditional NSAIDs, which primarily target COX enzymes. This dual mechanism may contribute to zingerone's efficacy in reducing inflammatory pain and potentially offer advantages regarding the side effect profile. Traditional NSAIDs, particularly with long-term use, are associated with gastrointestinal, cardiovascular, and renal complications (Song et al., 2024). While the current study did not specifically assess safety parameters, the natural origin of zingerone and its historical use in traditional medicine suggests a potentially favorable safety profile.

Another important finding was zingerone's effect on inflammatory cytokines. Proinflammatory cytokines, particularly IL-1β, IL-6, and TNF-α, play crucial roles in initiating and maintaining inflammatory pain (Karcz et al., 2024). These cytokines induce and maintain COX-2 expression, creating a positive feedback loop that perpetuates inflammation and pain. Our results showed that zingerone significantly reduced the expression and levels of these cytokines, both in vitro and in vivo, suggesting an upstream regulatory effect that may contribute to its overall anti-inflammatory action.

The present study has several strengths, including the use of complementary methodologies (in vivo, in vitro, and in silico) to investigate zingerone's effects and mechanisms comprehensively. The carrageenan-induced muscle inflammation model is well-established and mimics many features of clinical inflammatory muscle pain (Chopade et al., 2020). The dose-dependent effects observed across multiple parameters support the robustness of our findings. Furthermore, the molecular dynamics simulations provided insights into the stability and energetics of zingerone's interactions with target proteins under physiologically relevant conditions.

However, several limitations should be acknowledged. First, while the carrageenan model is useful for studying acute to subacute inflammation, it may not fully capture the complexity of chronic inflammatory pain conditions. Second, the study focused primarily on the PGE pathway, whereas inflammatory pain involves multiple mediators and mechanisms. Third, the in vitro studies used myoblasts rather than differentiated myotubes or primary muscle cells, which may respond differently to inflammatory stimuli and treatments. Future studies should address these limitations by using additional chronic pain models, exploring other inflammatory pathways, and employing primary muscle cells or co-culture systems that better reflect the in vivo environment.

CONCLUSION

In summary, this study offers thorough proof that zingerone reduces chronic inflammatory muscular pain by blocking the prostaglandin E pathway in a number of ways. These include suppression of proinflammatory cytokines, modification of PGE receptors, decrease in PGE synthase production, and direct inhibition of COX-2 activity. Given its natural origin, potential for fewer side effects, and efficacy equivalent to diclofenac, zingerone shows promise as a treatment for chronic inflammatory muscle pain problems. To assess the effectiveness and safety of zingerone in individuals with inflammatory muscular pain syndromes, more clinical research is necessary.

CONFLICTS OF INTEREST:

The authors declare that they no conflict of interest.

DATA AVAILABILITY STATEMENT-

Data availability is not applicable.

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