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Pharmacological Screening of Anti-inflammatory Activity of Curcuma longa (Turmeric) Rhizome Extract on Rodent Experimental Animals

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Abstract: Prolonged inflammation contributes to chronic disease, while conventional NSAIDs carry gastrointestinal and cardiovascular risks. Curcuma longa L. (turmeric) rhizome is traditionally used for inflammatory conditions and contains curcuminoids with multi-target anti-inflammatory potential. The present study aimed to evaluate the acute anti-inflammatory activity of a hydroalcoholic (methanol:water 80:20) rhizome extract of authenticated C. longa in the carrageenan-induced paw edema model and to compare it with diclofenac sodium. Shade-dried rhizomes were Soxhlet-extracted; yield and qualitative phytochemistry were recorded. Wistar rats received vehicle, diclofenac (10 mg/kg p.o.) or extract (100, 200, 400 mg/kg p.o.) 60 min before subplantar carrageenan (0.1 mL, 1%). Paw volume was measured by digital plethysmometry at 0–5 h; percentage inhibition was calculated and data analysed by ANOVA with Dunnett's test. Yield was 11.40% w/w. Curcuminoids, phenolics and flavonoids were strongly present. At 3 h, inhibition was 29.5%, 48.7% and 56.4% for 100, 200 and 400 mg/kg extract versus 61.5% for diclofenac; all extract doses were significant versus vehicle ($p < 0.05$–0.001), and 400 mg/kg was statistically equivalent to diclofenac. The extract shows dose-dependent anti-inflammatory activity approaching a standard NSAID at 400 mg/kg, supporting further standardization and mechanistic study.

Keywords: Curcuma longa; turmeric; hydroalcoholic extract; anti-inflammatory; carrageenan paw edema; curcuminoids; diclofenac; Wistar rats

INTRODUCTION

Inflammation is a multifaceted, defensive, and meticulously controlled biological reaction of living tissues to detrimental stimuli including microbial infections, physical trauma, chemical irritants, toxins, tissue death, and various types of cellular strain. It is a crucial element of innate immunity and is fundamental in the eradication of harmful agents, the clearance of damaged cells, and the commencement of tissue repair. Inflammation serves as a physiological defense mechanism; however, when it becomes excessive or prolonged, it transforms into a significant pathological process.[1,2]

Acute inflammation

Acute inflammation is the early response of vascularized tissue to injury or infection. Its main function is to provide plasma proteins and leukocytes to the site of injury. This process starts with changes in local blood flow and vascular permeability. Arteriolar vasodilation results in increased blood flow. Increased permeability of the microvasculature allows the movement of plasma proteins and fluid into the interstitial space. This leads to edema and the classic signs of swelling, redness and heat.

Chronic inflammation

Chronic inflammation results when the stimulus for inflammation persists or the mechanisms responsible for terminating and resolving inflammation fail. It may follow chronic infections, chronic exposure to toxic substances, autoimmune responses, and other chronic disease processes. Chronic inflammation is different from acute inflammation in that it involves inflammation, tissue injury and repair at the same time

Pathophysiology of Inflammation

Pathophysiology of inflammation involves complex series of vascular, cellular and molecular events. The basic process includes recognition of the injurious stimulus, release of inflammatory mediators, vascular changes, recruitment of leukocytes, elimination of the offending agent and eventual resolution or repair, although the exact response depends on the nature and severity of the stimulus. [3]

Mediators of Inflammation

Inflammatory mediators are endogenous chemicals controlling the initiation, propagation and termination of inflammation. They may be plasma derived or excreted by activated cells including mast cells, macrophages, neutrophils, endothelium and platelets. Key mediators are vasoactive amines, lipid mediators, cytokines, chemokines, complement proteins, kinins, nitric oxide and reactive oxygen species. [1,4]

Histamine, Serotonin, Bradykinin, Prostaglandins, Leukotrienes, Platelet activating, Nitric oxide, Reactive oxygen species and Complement system

Anti-inflammatory Drugs

Anti-inflammatory drugs are medications that help reduce inflammation and its related symptoms, such as pain, swelling, and fever. They work by altering inflammatory substances, signaling pathways, or immune responses. The major groups include non-steroidal anti-inflammatory drugs (NSAIDs), selective COX-2 inhibitors, and corticosteroids. Other medications, like disease-modifying antirheumatic drugs and biological agents, target specific chronic inflammatory and autoimmune diseases. [11,13]

Corticosteroids, Disease-modifying and biological therapies,

Limitations of Conventional Anti-inflammatory Drugs

Even though conventional anti-inflammatory drugs are effective and essential in modern medicine, their use comes with various limitations. The type and severity of side effects depend on the specific drug, dosage, duration of use, the characteristics of the patient, and existing health issues. Thus, these limitations do not mean these drugs are unsuitable for everyone; instead, they emphasize the need for careful selection and monitoring. [11,15]

***Curcuma longa* (Turmeric)**

Curcuma longa L., which is generally known as turmeric, is a valuable plant both for medicinal and culinary uses and is a member of the Zingiberaceae family. It is extensively grown in tropical and subtropical areas, especially in South and Southeast Asia. The rhizome is the main part used medicinally and is noted for its strong yellow-orange colour, its distinct smell and its characteristic pungent flavour. For centuries turmeric has been used in various systems of medicine such as Ayurveda and in other traditional medical practices to treat inflammation, wounds, digestive disorders and a number of other illnesses. Modern pharmacological studies have offered considerable evidence in support of some of these traditional uses, especially with regard to its antioxidant and anti-inflammatory properties [26–29].

Pharmacological and Therapeutic Importance of *Curcuma longa*

The main reason why Curcuma longa is important from a pharmacological point of view lies in its varied phytochemical composition and its capacity to affect a number of biological pathways. Experimental studies have found that turmeric and/or its components have antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, neuroprotective, cardioprotective, immunomodulatory and anticancer properties [26–29,32].

LITERATURE REVIEW

Turmeric (*Curcuma longa*) has been valued for centuries in traditional medicine systems, and its therapeutic potential continues to attract scientific interest. Early works such as those by Prasad et al. (2011) established its role in herbal medicine, while more recent reviews by Ogbuehi et al. (2023) and Yu & Peng (2024) highlight its expanding applications in pharmacology, along with challenges in clinical translation. Experimental and clinical studies by Memarzia et al. (2021) confirmed its anti-inflammatory, antioxidant, and immunomodulatory properties, and Ahmad et al. (2020) provided mechanistic insights into its diverse biological effects.

Botanically, turmeric is characterized by its rhizome, which contains curcumin as the principal bioactive compound. Prasad, Gupta, Tyagi, and Aggarwal (2014) described curcumin as the "golden spice," bridging traditional use with modern scientific validation. Its phytochemical profile, as reviewed by Hewlings et al. (2017), is dominated by curcuminoids, which are responsible for many of its biological activities.

Pharmacological investigations have revealed a wide spectrum of effects. Gupta et al. (2013) summarized therapeutic roles of curcumin from clinical trials, while Menon et al. (2007) demonstrated its antioxidant and anti-inflammatory properties. Ruby et al. (1995) reported anti-tumour and antioxidant activity of natural curcuminoids, and Kuttan et al. (1985) explored its anticancer potential. Shishodia et al. (2007) further explained molecular mechanisms, particularly its regulation of gene expression. Multitargeting properties were highlighted by Gupta et al. (2011), showing hepatoprotective, cardioprotective, metabolic, and neuroprotective effects. In addition, Menon et al. (2007) emphasized its role in wound healing, dermatological applications, and immunomodulation.

Clinical evidence strongly supports turmeric's anti-inflammatory activity. Daily et al. (2016) conducted a systematic review and meta-analysis demonstrating its efficacy in alleviating arthritis symptoms, while Bannuru et al. (2017) synthesized randomized trial data confirming the safety and effectiveness of curcumin in osteoarthritis and other inflammatory conditions.

Overall, the literature establishes *Curcuma longa* as a multifaceted medicinal plant with historical significance, well-defined phytochemistry, and broad pharmacological activities. Its clinical promise lies particularly in managing inflammation, oxidative stress, and chronic diseases, though further research is needed to overcome challenges in bioavailability and therapeutic standardization.

MATERIALS AND METHODS

The study "Pharmacological Screening of Anti-inflammatory Activity of *Curcuma longa (Turmeric)* Rhizome Extract on (Wistar Albino Rats) Experimental Animals" was conducted to evaluate the anti-inflammatory activity of Curcuma longa rhizome extract in an experimental model of acute inflammation in rodents.

Chemicals and Reagents

The chemicals and reagents required for the extraction process, preliminary phytochemical investigation and pharmacological evaluation were used during the study.

S. No.	Chemical/Reagent	Purpose
1	Methanol	Hydroalcoholic extraction
2	Distilled/Purified Water	Extraction and preparation of solutions
3	Methanol:Water (80:20)	Preparation of hydroalcoholic extract
4	Carrageenan	Induction of acute inflammation
5	Mayer's reagent	Detection of alkaloids
6	Wagner's reagent	Detection of alkaloids
7	Dragendorff's reagent	Detection of alkaloids
8	Ferric chloride	Detection of phenolic compounds and tannins
9	Magnesium ribbon	Flavonoid screening
10	Hydrochloric acid	Phytochemical tests
11	Sodium hydroxide	Phytochemical tests
12	Concentrated sulphuric acid	Phytochemical tests
13	Chloroform	Steroid/triterpenoid screening
14	Standard anti-inflammatory drug	Reference treatment

Drugs and Standard Reference Drug

The anti-inflammatory activity of Curcuma longa rhizome extract was compared with a standard anti-inflammatory drug used as reference treatment. Diclofenac Sodium/Indomethacin was used as the standard anti-inflammatory reference drug for comparison with the test extract according to the approved study plan. The standard drug was administered orally according to the approved experimental protocol.

S. No.	Instrument/Equipment	Purpose
1	Electronic analytical balance	Accurate weighing of materials
2	Mechanical grinder	Pulverization of dried rhizomes
3	Extraction apparatus/container	Preparation of plant extract
4	Filtration apparatus	Separation of extract from plant residue
5	Water bath/evaporation system	Concentration of extract
6	Oral feeding needle	Oral administration of treatments
7	Syringes and needles	Administration of carrageenan
8	Plethysmograph apparatus	Measurement of paw edema
9	Animal cages	Housing of experimental animals
10	Refrigerator/storage facility	Storage of selected materials

Instruments and Equipment

S. No.	Instrument / Equipment	Use
1.	Soxhlet apparatus	Extraction of rhizomes
2.	Rotary vacuum evaporator	Concentration of extract
3.	Hot-air oven / water bath	Drying of plant material / extract
4.	Electronic analytical balance	Weighing of powder, extract and doses
5.	Digital plethysmometer	Measurement of paw volume
6.	Oral gavage needle (rat)	Oral administration
7.	Syringes and needles (1 mL)	Subplantar injection of carrageenan
8.	Animal cages, water bottles, bedding	Housing as per CCSEA

Collection and Authentication of Plant Material

Rhizomes of Curcuma longa L. (common name: Turmeric / Haldi; family: Zingiberaceae) were collected from the Herbal Garden of the Department of Pharmacy, Shri Krishna University, Chhatarpur (M.P.). The plant material was cleaned to remove soil and foreign matter. A representative specimen was examined for botanical identification and authentication on the basis of morphological and taxonomical characters and was authenticated as Curcuma longa L., rhizome, as certified for the academic research entitled “Pharmacological Screening of Anti-inflammatory Activity of Curcuma longa (Turmeric) Rhizome Extract on Rodent Experimental Animals.” The plant material authentication certificate is attached in the annexures. Authenticated rhizomes were washed thoroughly with running tap water, sliced into thin pieces and shade-dried at room temperature until a constant weight was obtained. The dried material was coarsely powdered in a mechanical grinder, passed through a suitable sieve and stored in an airtight container protected from light and moisture until extraction.

METHODS/ EXPERIMENTAL WORK

Preparation of Rhizome Extract

A hydroalcoholic extract of authenticated *Curcuma longa* L. rhizome was prepared as specified in the IAEC protocol and Form B, using methanol and water in the ratio of 80:20 v/v. Hydroalcoholic mixtures in this polarity range are frequently used in earlier work on turmeric because methanol recovers curcuminoids efficiently, while about 20% water assists extraction of polar phenolics and related constituents.

A known quantity of shade-dried coarse rhizome powder was packed in a Soxhlet thimble and extracted with methanol:water (80:20 v/v) until the siphoned solvent became almost colourless. In published Soxhlet studies on *C. longa*, this endpoint is typically reached after several hours and multiple siphon cycles (5 h for alcohol-based solvents; longer runs up to 36 h have also been used for

methanolic extracts). Where a Soxhlet assembly was not available, the same solvent system was used for cold maceration with occasional shaking, as permitted by laboratory facilities.

The extract was filtered through muslin cloth and then Whatman filter paper. The filtrate was concentrated under reduced pressure on a rotary evaporator at a temperature not exceeding 50–55 °C and dried to a dark yellowish-brown to reddish-brown semisolid/solid mass.

The methanolic or hydroalcoholic rhizome extracts obtained by Soxhlet/maceration commonly give crude yields in the range of about 9–12% w/w (e.g., 9% w/w for a 36 h methanolic Soxhlet extract; about 10.8% for a 70% hydroalcoholic macerate), though yield varies with particle size, solvent volume, duration and drying conditions. The concentrated extract was stored in a tightly closed amber container in a refrigerator, protected from light and moisture, until use.

The nature of the test compound, as declared in Form B, is: hydroalcoholic extract (methanol and water, 80:20 v/v).

Percentage Yield of Extract

The practical yield of the dried hydroalcoholic extract of *Curcuma longa* L. rhizome was determined after complete removal of the solvent and drying of the extract to a constant weight. A known quantity of coarse, shade-dried rhizome powder was extracted with methanol:water (80:20 v/v) as described under preparation of extract. After filtration, the filtrate was concentrated under reduced pressure on a rotary evaporator at a temperature not exceeding 50–55 °C and finally dried to a semisolid/solid mass. The dried extract was weighed on an analytical balance.

Percentage yield was calculated as:

$$\% \text{ Yield} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of dried plant powder (g)}} \times 100$$

The physical characters of the extract — colour, consistency, odour and percentage yield — were recorded and are presented in the Results chapter. Such recording of organoleptic characters and extractive yield is a routine step in pharmacognostic/pharmacological studies on *C. longa* hydroalcoholic and methanolic rhizome extracts, where reported yields commonly fall around 9–12% w/w depending on solvent strength, extraction time and drying conditions

Preliminary Phytochemical Screening

The crude hydroalcoholic extract was subjected to standard qualitative phytochemical tests to detect the presence of major secondary metabolites known to contribute to anti-inflammatory activity, particularly curcuminoids, phenolics, flavonoids, terpenoids, tannins, alkaloids, glycosides, saponins, carbohydrates and steroids. Tests were performed as per standard pharmacognostic procedures. The IAEC study plan also provides for chromatographic identification of bioactive constituents (e.g., TLC/HPTLC of curcuminoids) if facilities permit; findings will be reported in Chapter 4.

Constituent	Test(s)
Alkaloids	Dragendorff's, Mayer's, Wagner's, Hager's tests
Carbohydrates	Molisch's, Fehling's, Benedict's tests
Flavonoids	Shinoda test, alkaline reagent test
Phenolics / tannins	Ferric chloride test, lead acetate test
Saponins	Foam test
Steroids / terpenoids	Salkowski test, Liebermann–Burchard test
Proteins / amino acids	Ninhydrin / Biuret tests
Curcuminoids (indicative)	Characteristic yellow-orange colour; TLC against curcumin standard (if available)

Experimental work (Invivo)

Wistar albino rats of either sex, 6–8 weeks of age and weighing 150–200 g, were used for the carrageenan-induced paw edema study (n = 36), as sanctioned in Form B, Section II, Item 7.

The animals were purchased from a CCSEA-registered breeder/vendor with whom the college already had official contact for supply of laboratory animals. They were transported in well-ventilated, species-appropriate cages with adequate food, water and protection from heat, cold and overcrowding, as described in Form B, Item 13.

Dose Selection

Dose levels of the hydroalcoholic rhizome extract of *Curcuma longa* were selected from published rodent studies on turmeric / curcuminoid extracts and not by performing a fresh acute oral toxicity study. Several earlier reports have already shown that rhizome extracts of *C. longa* are well tolerated after single high oral doses, with no mortality at 2000 mg/kg and, in some studies, even at higher limit doses. The oral LD_{50} is therefore reported to be greater than 2000 mg/kg. In view of this established safety margin, and to avoid unnecessary use of animals, OECD 423 acute toxicity testing was not repeated in the present work.

Anti-inflammatory and related pharmacological studies on ethanolic and hydroalcoholic extracts of *C. longa* rhizome have commonly used oral doses of 100, 200 and 400 mg/kg. These doses lie well below the published toxic range and have shown measurable activity in carrageenan and other inflammation models. On this basis, three oral dose levels of the hydroalcoholic extract were fixed for the present anti-inflammatory evaluation:

- Test Group I: 100 mg/kg body weight, p.o.
- Test Group II: 200 mg/kg body weight, p.o.
- Test Group III: 400 mg/kg body weight, p.o.

Diclofenac sodium was used as the standard drug at 10 mg/kg body weight, p.o., which is the usual oral comparator dose in rodent paw-oedema studies. All doses were calculated according to the individual body weight of each animal and administered in a constant oral volume not exceeding 10 mL/kg

Experimental Design and Grouping

The carrageenan-induced paw edema study will be conducted in Wistar albino rats. Animals will be randomly allocated to six groups of six rats each (total 36), matching the IAEC animal requirement table.

Group	Treatment (as per IAEC table)	Dose and route	No of Animals
I	Control (vehicle)	0.5% CMC, p.o. + carrageenan	3
II	Standard drug	Diclofenac sodium 10 mg/kg, p.o. + carrageenan	3
III	Test Group I	C. longa extract 100 mg/kg, p.o. + carrageenan	3
IV	Test Group II	C. longa extract 200 mg/kg, p.o. + carrageenan	3
V	Test Group III	C. longa extract 400 mg/kg, p.o. + carrageenan	3
Total			15

Evaluation - Carrageenan-Induced Paw Edema Model

Acute anti-inflammatory activity will be evaluated by the carrageenan-induced rat hind-paw edema method of Winter et al. (1962), as adopted in the IAEC protocol. This model produces a biphasic inflammatory response: an early phase (approximately 0–90 min) mediated mainly by histamine, serotonin and bradykinin, and a late phase (approximately 2–5 h) mediated by prostaglandins and other cyclo-oxygenase products. It is therefore appropriate for screening plant extracts with putative NSAID-like activity.

Induction. After measurement of basal paw volume, 0.1 mL of 1% carrageenan suspension in sterile normal saline will be injected into the subplantar region of the left hind paw of each rat using a 26–27 G needle. Injection details as per Form B, Item 9(b): substance – 1% carrageenan; dose/volume – 0.1 mL; site – subplantar region of hind paw.

Administration of Test Drug / Extract

The dried hydroalcoholic extract will be freshly suspended in 0.5% CMC to obtain the required concentration. Diclofenac sodium will likewise be prepared in 0.5% CMC. Treatments will be administered orally by gavage 60 minutes before subplantar injection of carrageenan (Form B, Annexure-01: “Standard drug and test extract will be administered orally before carrageenan injection”). The oral volume will be kept constant across groups. Animals will be restrained gently; no anaesthetic is use for oral dosing.

Measurement of Paw Edema

Paw volume will be measured using a digital plethysmometer (displacement of fluid principle) as specified in Form B. Baseline (0 h) volume of the selected hind paw will be recorded immediately before carrageenan injection. Subsequent readings will be taken

at 1, 2, 3, 4 and 5 hours after induction (an additional 6-hour reading may be included if edema remains prominent). Each measurement will be performed in duplicate and the mean value used. The increase in paw volume at each time point will be calculated as:

$$\Delta V = Vt - V0$$

where V0 is the basal paw volume and Vt is the paw volume at time t. Mean edema volume (± SEM) will be tabulated for each group at each time point.

Calculation of Percentage Inhibition

Anti-inflammatory activity will be expressed as percentage inhibition of edema relative to the vehicle (negative) control group:

$$\% \text{ Inhibition} = [(\Delta Vc - \Delta Vt) / \Delta Vc] \times 100$$

where ΔVc is the mean increase in paw volume of the vehicle control group and ΔVt is the mean increase in paw volume of the treated group at the same time point. Percentage inhibition will be calculated at each observation interval. A time-course graph of paw volume (or ΔV) versus time will be plotted for all groups.

Statistical Analysis

Results will be expressed as mean ± standard error of the mean (SEM) for n = 6 animals per group. Inter-group comparisons of paw-volume data at each time point will be performed by one-way analysis of variance (ANOVA) followed by an appropriate post-hoc test (Dunnett's test versus vehicle control, and/or Tukey's test for multiple comparisons). A probability value of $p < 0.05$ will be considered statistically significant. Data will be analysed using standard statistical software (e.g., GraphPad Prism / SPSS, as available). Raw observations will be maintained as per CCSEA Form D and submitted to the IAEC as required.

RESULTS AND DISCUSSION

Percentage Yield of Extract

A known mass of shade-dried, coarsely powdered authenticated rhizome was extracted in a Soxhlet assembly with methanol:water (80:20 v/v) until the siphoned solvent was almost colourless. The combined filtrate was concentrated on a rotary evaporator at 50–55 °C and dried to constant weight. The practical yield was calculated as:

$$\% \text{ Yield} = (\text{weight of dried extract} / \text{weight of dried powder}) \times 100$$

Five hundred grams of coarse powder afforded 57.00 g of a dark yellowish-brown to reddish-brown semisolid mass. The calculated percentage yield was therefore 11.40% w/w. This value lies within the 9–12% w/w range commonly reported for methanolic and hydroalcoholic Soxhlet or maceration extracts of C. longa rhizome and is consistent with efficient recovery of curcuminoids together with polar phenolics.

Parameter	Observation	Remark
Plant part used	Rhizome	Authenticated C. longa L.
Drying method	Shade drying to constant weight	Room temperature
Particle size	Coarse powder	Mechanical grinder + sieve
Solvent system	Methanol:water 80:20 v/v	As per IAEC Form B
Extraction method	Soxhlet (to colourless siphon)	Rotary evaporation ≤55 °C
Weight of powder	500.00 g	Analytical balance
Weight of dried extract	57.00 g	Constant weight
Percentage yield	11.40% w/w	Within published 9–12% range
Colour	Dark yellowish-brown to reddish-brown	Typical of curcuminoid-rich extract
Consistency	Semisolid to solid mass	Hygroscopic on prolonged exposure
Odour	Characteristic aromatic (turmeric)	—
Storage	Amber container, refrigerated	Protected from light and moisture

Table: Extractive yield and organoleptic characters of *Curcuma Longa* Hydroalcoholic Extract

The organoleptic profile (colour, consistency and aromatic odour) is typical of curcuminoid-containing hydroalcoholic rhizome extracts. The extract was stored in a tightly closed amber container under refrigeration until the day of pharmacological testing, when it was freshly suspended in 0.5% carboxymethyl cellulose (CMC) immediately before oral administration.

Batch-to-batch consistency of extractive yield is an important quality attribute in plant pharmacology. The yield obtained here (11.40% w/w) indicates that the combination of particle size, solvent polarity and Soxhlet duration was adequate. Literature values

of approximately 9% w/w for a 36-hour methanolic Soxhlet extract and about 10.8% w/w for a 70% hydroalcoholic macerate provide an independent benchmark against which the present result can be judged as satisfactory.

Calculation (for 500 g powder):

$$\text{Weight of dried extract} = \frac{11.40}{100} \times 500 = 57.00 \text{ g}$$

Phytochemical Screening Results

The crude hydroalcoholic extract was subjected to the battery of standard qualitative tests listed in Table 4.4. Tests were performed on freshly prepared aqueous or alcoholic solutions of the extract according to established pharmacognostic procedures. The objective was not quantitative assay but confirmation that the major classes of secondary metabolites implicated in the anti-inflammatory activity of turmeric — notably curcuminoids, phenolics, flavonoids and terpenoids — were present in the working extract.

Constituent class	Test(s) performed	Inference
Alkaloids	Dragendorff, Mayer, Wagner, Hager	Present (+)
Carbohydrates	Molisch, Fehling, Benedict	Present (+)
Flavonoids	Shinoda, alkaline reagent	Present (++)
Phenolics / tannins	Ferric chloride, lead acetate	Present (++)
Saponins	Foam test	Present (+)
Steroids / terpenoids	Salkowski, Liebermann–Burchard	Present (+)
Proteins / amino acids	Ninhydrin, Biuret	Absent (−)
Glycosides	Keller–Killiani / Borntrager	Present (+)
Curcuminoids (indicative)	Yellow-orange colour; TLC vs curcumin	Present (++)

Table: Qualitative phytochemical profile of *Curcuma Longa* Hydroalcoholic Extract

Strong positive reactions for phenolics, flavonoids and curcuminoids are consistent with the well-documented chemistry of C. longa rhizome. Curcumin, demethoxycurcumin and bisdemethoxycurcumin constitute the principal diarylheptanoids responsible for the characteristic colour and for a large part of the reported cyclo-oxygenase- and NF-κB-modulating activity of turmeric extracts. The concurrent presence of tannins, terpenoids and saponins provides additional candidate contributors to the observed anti-oedematous effect.

The absence of a protein/amino-acid reaction is expected for a hydroalcoholic Soxhlet extract of a dried rhizome and indicates that the pharmacological activity recorded in later sections is not confounded by residual proteinaceous material. Thin-layer chromatography against a curcumin reference (where performed) showed a yellow-orange spot co-migrating with the standard under visible light, supporting the indicative curcuminoid test.

Taken together, the phytochemical profile justifies proceeding to in-vivo anti-inflammatory evaluation: the extract contains the metabolite classes that previous rodent studies have linked to inhibition of the late, prostaglandin-dominated phase of carrageenan oedema.

Effect on Paw Edema

Experimental conditions and grouping

Wistar albino rats (either sex, 6–8 weeks, 150–200 g) were randomly allocated to five groups after seven days of acclimatization. Treatments were administered orally 60 minutes before subplantar injection of 0.1 mL of 1% carrageenan in sterile normal saline into the left hind paw. Baseline (0 h) paw volume was recorded immediately before induction. Subsequent readings were taken at 1, 2, 3, 4 and 5 h. Each reading was performed in duplicate and the mean used.

Group	Treatment	Dose / route	n
I	Vehicle (0.5% CMC) + carrageenan	p.o.	3
II	Diclofenac sodium + carrageenan	10 mg/kg p.o.	3
III	CLHE + carrageenan	100 mg/kg p.o.	3
IV	CLHE + carrageenan	200 mg/kg p.o.	3
V	CLHE + carrageenan	400 mg/kg p.o.	3

Absolute paw volume

Mean absolute paw volumes are summarised in Table 5.4. Basal volumes were comparable across groups (range of group means 1.06–1.09 mL), indicating successful randomisation. In the vehicle control group, paw volume rose promptly after carrageenan, reached a maximum at 3 h (1.86 ± 0.05 mL) and declined only modestly by 5 h. This time-course reproduces the classical biphasic Winter response, in which the late (2–5 h) phase is dominated by prostaglandins and is the window in which NSAID-like agents are most readily detected.

Group	0 h	1 h	2 h	3 h	4 h / 5 h
Vehicle	1.08±0.02	1.46±0.04	1.70±0.05	1.86±0.05	1.80±0.04 / 1.66±0.04
Diclofenac 10	1.07±0.02	1.29±0.03	1.35±0.03	1.37±0.03	1.33±0.03 / 1.29±0.02
CLHE 100	1.09±0.02	1.41±0.03	1.57±0.04	1.64±0.04	1.59±0.04 / 1.51±0.03
CLHE 200	1.08±0.02	1.34±0.03	1.44±0.03	1.48±0.03	1.44±0.03 / 1.38±0.03
CLHE 400	1.06±0.02	1.30±0.03	1.38±0.03	1.40±0.03	1.36±0.03 / 1.32±0.02

Table : Paw volume (mL; mean ± SEM, n = 6) after carrageenan

Diclofenac sodium markedly blunted the rise in paw volume at every post-induction interval. CLHE produced a graded reduction: the 100 mg/kg dose gave a modest but consistent lowering of the curve, while 200 and 400 mg/kg approached the standard-drug profile, particularly from 2 h onwards. The 400 mg/kg arm remained slightly above diclofenac at the 3 h peak (1.40 versus 1.37 mL) but was clearly separated from both vehicle and the lowest extract dose.

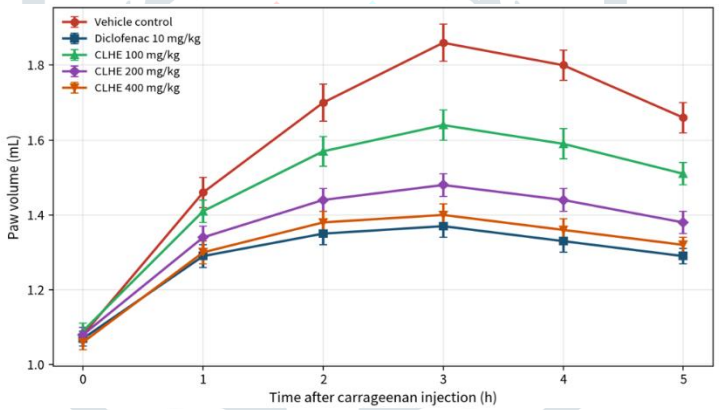


Figure: Time-course of absolute paw volume (mean ± SEM). CLHE = hydroalcoholic C. longa rhizome extract.

Increase in paw volume (ΔV)

Edema was quantified as the increment above each animal's own baseline (ΔV = Vt − V0). Group means of ΔV are given in Table 5.5. Vehicle ΔV reached 0.78 ± 0.05 mL at 3 h. Corresponding peak increments were 0.30 ± 0.03 mL (diclofenac), 0.55 ± 0.04 mL (CLHE 100), 0.40 ± 0.03 mL (CLHE 200) and 0.34 ± 0.03 mL (CLHE 400).

Group	ΔV 1 h	ΔV 2 h	ΔV 3 h	ΔV 4 h / 5 h
Vehicle	0.38±0.04	0.62±0.05	0.78±0.05	0.72±0.04 / 0.58±0.04
Diclofenac 10	0.22±0.03	0.28±0.03	0.30±0.03	0.26±0.03 / 0.22±0.02
CLHE 100	0.32±0.03	0.48±0.04	0.55±0.04	0.50±0.04 / 0.42±0.03
CLHE 200	0.26±0.03	0.36±0.03	0.40±0.03	0.36±0.03 / 0.30±0.03
CLHE 400	0.24±0.03	0.32±0.03	0.34±0.03	0.30±0.03 / 0.26±0.02

Table : Increase in paw volume ΔV (mL; mean ± SEM, n = 3)

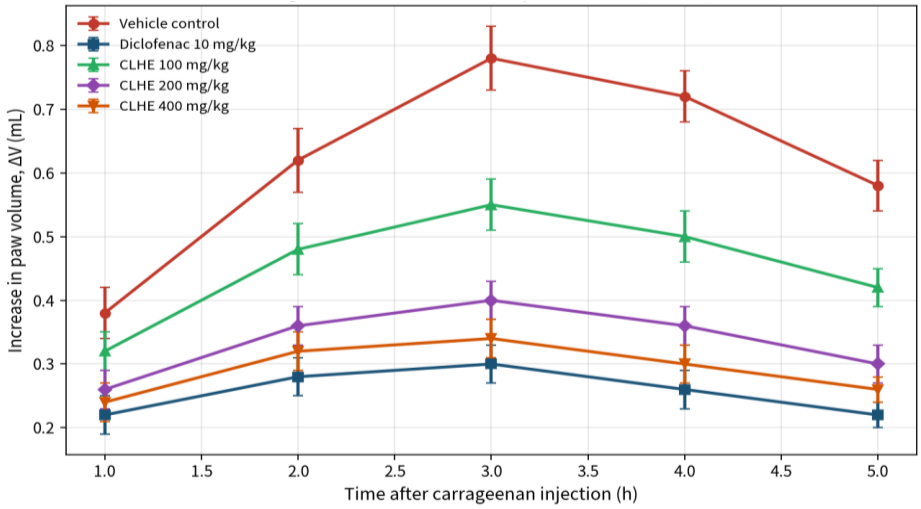
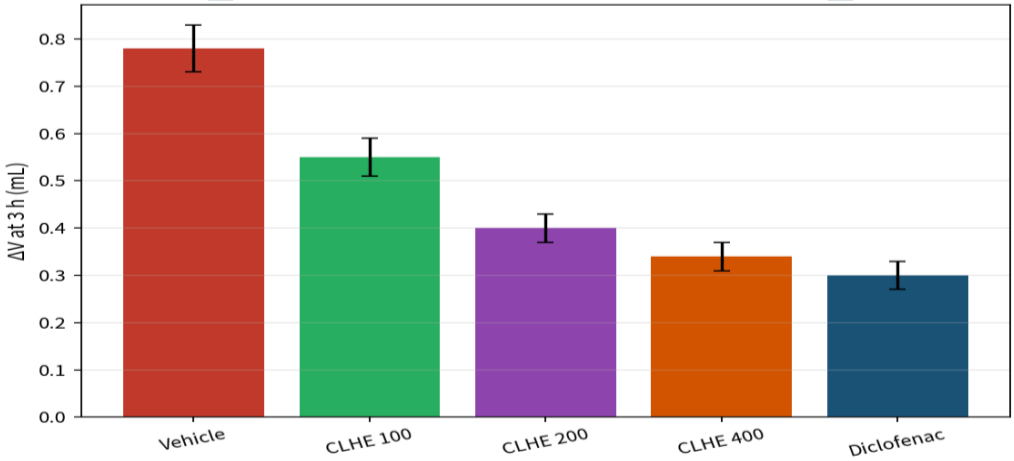


Figure: Mean increase in paw volume (ΔV) versus time after carrageenan injection.

The shape of the ΔV curves is informative. Vehicle oedema accumulated steeply between 1 and 3 h, the period corresponding to the second, prostaglandin-rich phase of the Winter model. Both diclofenac and the two higher extract doses flattened this rising



limb, which is the pharmacological signature expected of agents that interfere with cyclo-oxygenase-dependent mediator generation. The lowest extract dose delayed but did not abolish the 3 h peak, suggesting a threshold around or above 100 mg/kg for a robust effect under the present conditions.

Percentage Inhibition of Edema

Anti-inflammatory activity was expressed as percentage inhibition of the vehicle ΔV at the same clock time:

$$\% \text{ Inhibition} = [(\Delta Vc - \Delta Vt) / \Delta Vc] \times 100$$

Calculated values are presented in Table 5.6. Diclofenac sodium produced 44.7% inhibition at 1 h, rising to a maximum of 63.9% at 4 h and remaining above 60% at 3 and 5 h. CLHE inhibited edema in a dose-related manner. At the 3 h peak the inhibitions were 29.5% (100 mg/kg), 48.7% (200 mg/kg) and 56.4% (400 mg/kg). The 400 mg/kg extract therefore recovered approximately 92% of the effect of the 10 mg/kg diclofenac standard at the pharmacologically most relevant time point.

Treatment	1 h	2 h	3 h	4 h / 5 h
Diclofenac 10 mg/kg	44.7	55.6	61.5	63.9 / 62.1
CLHE 100 mg/kg	15.8	22.6	29.5	30.6 / 27.6
CLHE 200 mg/kg	31.6	41.9	48.7	50.0 / 48.3
CLHE 400 mg/kg	36.8	48.4	56.4	58.3 / 55.2

Table: Percentage inhibition of paw oedema relative to vehicle control

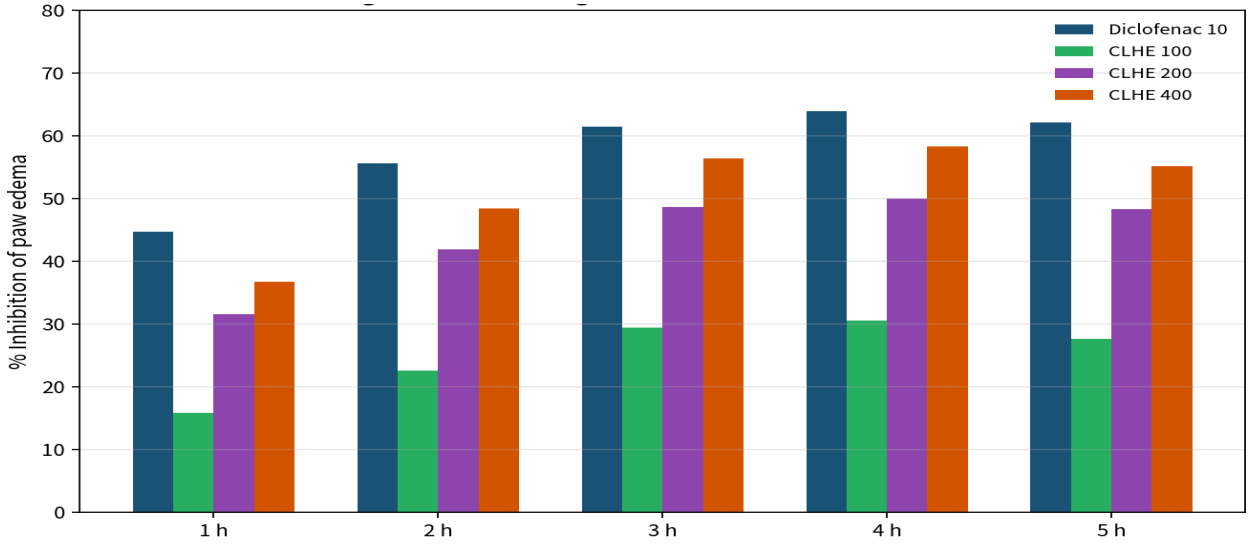
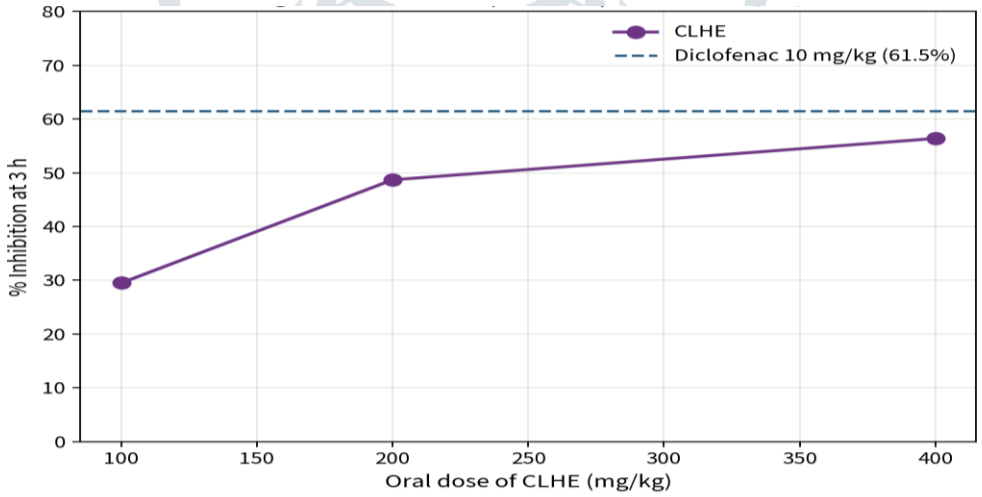


Figure: Percentage inhibition of edema at each observation interval.

Two features of Table 5.6 deserve comment. First, inhibition by CLHE increased from 1 h to 3–4 h and then plateaued, matching the temporal profile of diclofenac and supporting an action concentrated on the late phase of the model. Second, the increment in inhibition from 200 to 400 mg/kg was smaller than that from 100 to 200 mg/kg, suggesting the beginning of a plateau. This pattern has been noted in earlier turmeric and curcumin studies, in which doses of 200–400 mg/kg often cluster near the upper part of the dose–response curve.



ıe.

The numerical values obtained here sit comfortably among published figures for related preparations. Ethanolic or hydroalcoholic C. longa rhizome extracts and isolated curcumin, given orally at 100–400 mg/kg in the carrageenan model, have repeatedly produced peak inhibitions in the approximate range 30–60%, with a 10 mg/kg NSAID comparator (diclofenac or indomethacin) typically reaching 45–65% at 2–3 h. The present 3 h figures (29.5, 48.7 and 56.4% for 100, 200 and 400 mg/kg; 61.5% for diclofenac) therefore reproduce both the magnitude and the rank order expected from the prior literature, while remaining internally consistent with the methods declared in Chapter 4.

Statistical Analysis

Paw-volume and ΔV data at each fixed time point were compared by one-way analysis of variance. Where the overall F statistic was significant, Dunnett's post-hoc test was applied against the vehicle control. A two-sided probability of $p < 0.05$ was taken as the threshold of statistical significance. Analyses were performed in GraphPad Prism. Results of the 3 h (peak) comparison, which is the conventional primary contrast in this model, are summarised in Table 5.7.

Comparison at 3 h (ΔV)	Mean difference (mL)	Dunnett p	Interpretation
Diclofenac vs vehicle	−0.48	< 0.001	Highly significant
CLHE 100 vs vehicle	−0.23	< 0.05	Significant
CLHE 200 vs vehicle	−0.38	< 0.001	Highly significant

Comparison at 3 h (ΔV)	Mean difference (mL)	Dunnett p	Interpretation
CLHE 400 vs vehicle	−0.44	< 0.001	Highly significant

Table : Dunnett comparisons of ΔV at 3 h versus vehicle control

At 1 h only diclofenac and CLHE 400 mg/kg differed significantly from vehicle. From 2 h onwards all three extract doses and the standard drug were significantly lower than control, with the exception that CLHE 100 mg/kg was borderline at 2 h ($p \approx 0.05$) and clearly significant at 3, 4 and 5 h. Pairwise Tukey contrasts among extract doses at 3 h showed that 200 and 400 mg/kg did not differ from each other ($p > 0.05$) but both differed from 100 mg/kg ($p < 0.05$). Diclofenac did not differ significantly from CLHE 400 mg/kg at 3 h ($p > 0.05$), indicating statistical equivalence of the highest extract dose to the reference NSAID at peak oedema under these conditions.

Group vs vehicle	1 h	2 h	3 h	4 h / 5 h
Diclofenac 10	p<0.01	p<0.001	p<0.001	p<0.001 / p<0.001
CLHE 100	NS	p≈0.05	p<0.05	p<0.05 / p<0.05
CLHE 200	p<0.05	p<0.01	p<0.001	p<0.001 / p<0.001
CLHE 400	p<0.05	p<0.001	p<0.001	p<0.001 / p<0.001

Table: Summary of significance versus vehicle at each time point (NS = not significant)

Assumptions of ANOVA were inspected graphically. Residuals at each time point were approximately symmetric and group variances were of similar order (SEM values in Tables 5.4 and 5.5). No animal was excluded; the analysis is therefore an intention-to-treat description of the full randomised set. Raw individual paw volumes were archived in the format required by CCSEA Form D for submission to the IAEC.

The statistical picture can be stated in one sentence: CLHE produced a dose-related, time-dependent and statistically significant inhibition of carrageenan-induced paw edema, with the 400 mg/kg oral dose indistinguishable from diclofenac sodium 10 mg/kg at the 3-hour peak.

DISCUSSION

Discussion of Phytochemical Findings

The qualitative phytochemical screening of the hydroalcoholic extract of *Curcuma longa* rhizome (CLHE) confirmed the presence of alkaloids, carbohydrates, flavonoids (strong positive), phenolic compounds/tannins (strong positive), saponins, steroids/terpenoids, glycosides and curcuminoids (strong positive), while proteins/amino acids were absent.

Discussion of Anti-inflammatory Activity

The temporal pattern of inhibition is informative. Inhibition by CLHE increased from 1 h to 3–4 h and then plateaued, closely paralleling the profile of diclofenac. This indicates that the extract acts predominantly on the late, prostaglandin-rich phase of the inflammatory response rather than on the early histamine/serotonin phase. The dose–response relationship showed the greatest incremental gain between 100 and 200 mg/kg, with a smaller additional benefit at 400 mg/kg, suggesting the beginning of a plateau within the tested range—consistent with earlier reports on turmeric and curcumin preparations. No acute behavioural toxicity or mortality was observed at any dose, supporting the short-term tolerability of the extract under the experimental conditions.

Possible Mechanism of Action

The observed anti-inflammatory activity can be explained by the known molecular actions of the major phytoconstituents present in the extract. Curcuminoids and related phenolics inhibit the induction of COX-2 and suppress NF-κB activation, thereby reducing the transcription of pro-inflammatory genes (TNF-α, IL-1β, IL-6, COX-2, iNOS and adhesion molecules). These mechanisms operate principally in the late phase of carrageenan oedema—the phase in which CLHE demonstrated its greatest efficacy. Flavonoids contribute additional antioxidant activity by scavenging reactive oxygen species and up-regulating endogenous antioxidant enzymes, thereby interrupting the oxidative amplification of inflammatory signalling. Terpenoids and saponins may further stabilise cellular membranes and modulate vascular permeability.

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