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RESEARCH ARTICLE

Evaluation of the Anti-Inflammatory Activity of Ethanolic Rhizome Extract of *Curcuma Longa* in Wistar Albino Rats Using the Formalin-Induced Paw Edema Model

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Abstract: Acute and sub-acute inflammatory conditions present substantial clinical challenges, driving the scientific search for safer phytotherapeutic alternatives to conventional non-steroidal anti-inflammatory agents. Rhizomes of *Curcuma longa* contain bioactive polyphenols with potent therapeutic actions. This research evaluates the anti-inflammatory activity of an ethanolic extract of *Curcuma longa* rhizomes compared to the standard cyclooxygenase inhibitor, ibuprofen, in healthy adult Wistar albino rats. Subplantar injection of 1% formalin successfully establishes localized, acute inflammatory edema in the hind paw of experimental animals. Prior oral administration of the ethanolic extract at a high dose of 100 mg/kg results in a highly significant, time-dependent reduction in paw thickness, presenting a peak edema inhibition of 83.0% at the fifth hour. This therapeutic effect is close to the 94.3% edema inhibition shown by ibuprofen at 40 mg/kg. In contrast, a lower extract dose of 50 mg/kg produces a moderate anti-inflammatory effect, achieving 12.2% edema inhibition by the end of the monitoring period. Phytochemical screening of the crude ethanolic extract confirms the presence of abundant curcuminoids, phenolic compounds, terpenoids, tannins, and steroidal structures. These bioactive components act synergistically to suppress the cascade of inflammatory mediators, particularly during the late phase of formalin-induced edema. The evidence confirms the traditional application of turmeric in managing inflammatory conditions and establishes its potential as a highly effective, dose-dependent natural therapeutic agent.

Keywords: *Curcuma longa*; Formalin; Paw Edema; Ibuprofen; Anti-inflammatory Activity.

1. Introduction

Inflammation is a highly coordinated physiological response of vascularized connective tissues to harmful exogenous or endogenous stimuli, such as pathogenic invasion, chemical irritants, mechanical trauma, or cellular necrosis [1]. The primary physiological purpose of this phenomenon is to isolate, neutralize, and eliminate the noxious agent while initiating the intricate cascades necessary for tissue regeneration [2]. Despite its protective nature under acute conditions, dysregulated or prolonged inflammatory activation can lead to cellular damage and the pathogenesis of diverse chronic disorders, including rheumatoid arthritis, diabetic microangiopathy, atherosclerosis, and oncological malignancies [3]. The classical clinical manifestations like redness, heat, swelling, pain, and loss of function are caused by rapid hemodynamic changes and microvascular alterations [3]. Localized tissue injury triggers the immediate release of primary vasoactive amines, chiefly histamine and serotonin, which induce transient vasodilation of local arterioles and increase capillary permeability, leading to the extravasation of plasma fluid and the development of inflammatory edema [4].

As the inflammatory response progresses from the immediate acute phase to the sub-acute phase, a complex array of chemical mediators is produced *de novo* [3]. These include bradykinin, a potent nonapeptide that stimulates nociceptors, and various pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) [4]. Crucially, the activation of phospholipase A2 enzymes cleaves membrane phospholipids to release arachidonic acid, which serves as the direct precursor for eicosanoid biosynthesis [5]. The action of cyclooxygenase (COX) enzymes on arachidonic acid yields prostanoids, predominantly prostaglandin E2 (PGE2) and prostacyclin (PGI2), which act as potent vasodilators, amplify edema formation, and sensitize peripheral afferent nociceptors to pain-inducing stimuli [5]. Non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen are widely prescribed to manage these conditions by inhibiting COX enzymes and halting prostaglandin

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production [6, 7]. However, prolonged systemic administration of classical NSAIDs is frequently associated with dose-limiting toxicities, most notably gastric mucosal erosion, gastrointestinal hemorrhage, and renal impairment due to the concomitant block of the cytoprotective COX-1 pathway [8, 9, 10].

These significant clinical drawbacks highlight the urgent therapeutic necessity to characterize novel, effective anti-inflammatory compounds derived from natural sources that possess a superior safety profile [11]. Among the most thoroughly used species is *Curcuma longa* Linn, a perennial herb of the Zingiberaceae family whose dried rhizomes (turmeric) have been utilized for millennia in traditional Ayurvedic, Siddha, and Unani medicine to treat localized wound healing and systemic joint inflammation [12]. The pharmacological efficacy of *Curcuma longa* is due to curcuminoids like curcumin, demethoxycurcumin, and bisdemethoxycurcumin alongside a volatile essential oil fraction rich in anti-inflammatory sesquiterpenes such as alpha-turmerone and ar-turmerone [13, 14]. Curcumin directly suppresses the activation of nuclear factor kappa B (NF-ϰB), thereby halting the downstream expression of pro-inflammatory cytokines and inducible nitric oxide synthase (iNOS), while also inhibiting the COX-2 pathway without causing gastric mucosal damage [15, 16].

This study was designed to evaluate and compare the *in vivo* anti-inflammatory efficacy of the crude ethanolic rhizome extract of *Curcuma longa* at two distinct oral dose levels (50 mg/kg and 100 mg/kg) against the standard reference drug, ibuprofen (40 mg/kg), in adult Wistar albino rats. Localized inflammation was chemically induced in the rodent subjects using the standardized formalin-induced paw edema model, which initiates a highly reproducible, biphasic neurogenic and inflammatory swelling response [17-19]. The specific objectives of this investigation are to extract the active phytoconstituents via cold maceration, conduct qualitative phytochemical screening to confirm the presence of therapeutic metabolites, establish the localized formalin model, and quantitatively measure the hourly changes in rat hind paw thickness (in millimeters) using a calibrated digital Vernier caliper over a five-hour monitoring window [20, 21]. This approach facilitates a precise mathematical analysis of the dose-dependent and time-dependent percentage of edema inhibition, providing robust scientific validation for the traditional use of turmeric.

2. Materials and Methods

2.1. Plant Material and Extraction

The dried rhizomes of *Curcuma longa* Linn were obtained from an authorized local botanical supplier in Bhimavaram, Andhra Pradesh, India. The taxonomic identity of the plant material was botanically authenticated at the Department of Pharmacognosy, K.G.R.L. College of Pharmacy, where a voucher specimen (Voucher No: KGRL/COG/2025/CL-09) was deposited for future reference. The rhizomes were thoroughly washed with distilled water to remove adhering soil and organic debris, sliced into uniform pieces, and subjected to complete shade drying at room temperature (25 ± 2 °C) for a period of fourteen days [22]. The completely dried rhizome slabs were then pulverized into a coarse powder utilizing a mechanical grinder. The resulting powder was passed through a mesh sieve (Sieve No. 40) to ensure uniform particle size distribution and stored in an airtight, light-resistant container.

To prepare the crude extract, the dry powder was subjected to cold maceration, a standard extraction methodology as per literature [23, 24]. 100g of the coarse rhizome powder was weighed and soaked in a sealed glass vessel containing 500 mL of 95% v/v ethanol as the extraction menstruum. The mixture was kept at room temperature for 72 hours with periodic, manual agitation at regular intervals of 6 hours to facilitate maximal cellular lysis and dissolution of the lipophilic curcuminoids and essential oils [23]. After the maceration period, the mixture was filtered through a double layer of sterile muslin cloth, followed by filtration through Whatman No. 1 filter paper. The filtrate was collected and concentrated under reduced pressure at a controlled temperature of 40 ± 1°C utilizing a rotary vacuum evaporator. The remaining solvent was evaporated to dryness in a water bath to yield a dark orange-yellow, semi-solid, highly aromatic crude ethanolic extract (percentage yield: 12.4% w/w) [25, 26]. The dry extract was stored in a vacuum desiccator until further use.

2.2. Qualitative Phytochemical Screening Tests

The crude ethanolic extract of *Curcuma longa* was subjected to preliminary qualitative chemical assays to identify the major classes of secondary metabolites responsible for the anti-inflammatory activity, following methods described by Turner [18] and Chanda [27]:

2.2.1. Phenolic Compounds (Ferric Chloride Test)

Following standard chemical screening methods [27], a small quantity of the crude extract (50 mg) was dissolved in 5 mL of distilled water and filtered. To the filtrate, 2-3 drops of a freshly prepared 5% w/v ferric chloride ($FeCl_3$) solution were added. The immediate appearance of a deep blue-black or greenish-black coloration indicated the presence of phenolic compounds, which are known to inhibit prostaglandin production and neutralize localized reactive oxygen species.

2.2.2. Curcuminoids (Alkali Test)

This assay is highly specific for the diarylheptanoid skeleton of curcumin as validated by Ammon et al [12]. A small aliquot of the extract was treated with 2 mL of a 10% w/v sodium hydroxide (NaOH) solution. The development of an intense, stable reddish-brown coloration confirmed the presence of curcuminoids. Upon subsequent acidification of the mixture with dilute hydrochloric acid (HCl), the immediate disappearance of the reddish-brown color to restore the original yellow hue validated the test.

2.2.3. Terpenoids (Salkowski Test)

According to the phytochemical identification methods of Vogel [19], approximately 100 mg of the extract was dissolved in 3 mL of chloroform in a clean test tube. Along the side of the test tube, 2 mL of concentrated sulfuric acid (H_2SO_4) was carefully added to form a distinct layer. The formation of a persistent, deep reddish-brown ring at the junction of the two liquid phases confirmed the presence of terpenoid structures.

2.2.4. Tannins (Lead Acetate Test)

Following established screening methodologies [27], a portion of the extract (50 mg) was boiled in 5 mL of distilled water, cooled, and filtered. The filtrate was treated with 1 mL of a 10% w/v lead acetate solution. The formation of a dense white precipitate indicated the presence of tannins, which contribute to membrane stabilization and anti-edematous actions.

2.2.5. Steroids (Liebermann-Burchard Test)

To identify steroidal structures, the classical assay outlined by Kulkarni [20] was performed. The crude extract was dissolved in 2 mL of chloroform in a dry test tube. To this solution, 1 mL of acetic anhydride was added, followed by the cautious addition of 1 mL of concentrated sulfuric acid (H_2SO_4) along the tube wall. A gradual color change from reddish-brown to blue-green indicated the presence of steroidal compounds.

2.3. Experimental Animals

The pharmacological study was carried out using twelve healthy adult Wistar albino rats of either sex, weighing between 150 g and 200 g. The animals were sourced from an authorized laboratory animal facility and housed in clean, standard polypropylene cages lined with sterile paddy husk. The animal room was maintained under strictly controlled laboratory conditions, including a 12-hour light-dark cycle, a temperature of 22 ± 3 °C, and relative humidity of 55 ± 5% [28]. The animals were fed a standardized commercial pellet diet and had unrestricted access to water *ad libitum*. Prior to the commencement of the experimental protocol, the rats were allowed to acclimate to the laboratory environment for a period of seven days. All animal handling, care, and experimental interventions were performed in strict compliance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), India, and OECD guidelines [21, 28]. The experimental protocol received formal approval and authorization from the Institutional Animal Ethics Committee (IAEC) of K.G.R.L. College of Pharmacy, Bhimavaram (Protocol Approval No: IAEC/KGRL/2025/141).

2.4. Formulation of Drug Suspensions

A 1% w/v sodium carboxymethyl cellulose (CMC) solution was prepared in double-distilled water to serve as the homogenous suspending vehicle [20].

2.4.1. Standard Drug Suspension

Commercial ibuprofen tablets (Brufen, 200 mg, Abbott India Ltd.) were selected as the standard reference. The outer film coating of the tablets was carefully peeled off using a sterile scalpel. The tablets were weighed, transferred to a clean porcelain mortar, and pulverized into an extremely fine powder. A calculated quantity of the tablet powder was suspended in a small volume of 1% w/v CMC, triturated thoroughly to form a smooth, lump-free paste, and diluted gradually to achieve a final concentration suitable for delivering a dose of 40 mg/kg body weight in a standard oral dosing volume of 10 mL/kg [20].

2.4.2. Test Extract Suspensions

The dry crude ethanolic extract of *Curcuma longa* was suspended in the 1% w/v CMC vehicle following standardized formulation principles [20]. Two distinct formulations were prepared: a low-dose suspension calibrated to deliver 50 mg/kg body weight, and a high-dose suspension calibrated to deliver 100 mg/kg body weight, maintaining a uniform oral administration volume of 10 mL/kg.

All formulations were freshly prepared on the day of the experiment and shaken thoroughly before administration to ensure uniform dosing.

2.5. Experimental Design and Formalin Induction of Paw Edema

The acclimated Wistar albino rats were randomly allocated into four distinct experimental groups, with each cohort consisting of three animals (n = 3), following standard pre-clinical screening guidelines [19, 20]:

- Group I (Disease Control): Received the vehicle orally (1% w/v CMC, 10 mL/kg) and was challenged with 1% w/v formalin.
- Group II (Standard Group): Received the standard reference drug orally (Ibuprofen, 40 mg/kg) and was challenged with 1% w/v formalin.
- Group III (Test Group - Low Dose): Received the *Curcuma longa* extract orally (50 mg/kg) and was challenged with 1% w/v formalin.
- Group IV (Test Group - High Dose): Received the *Curcuma longa* extract orally (100 mg/kg) and was challenged with 1% w/v formalin.

Exactly 60 minutes following the oral administration of the respective pre-treatments, acute localized inflammatory edema was induced in all animals. The rats were gently restrained, and 0.1 mL of a freshly prepared 1% w/v formalin solution in normal saline was injected into the subplantar aponeurosis of the right hind paw [16, 18].

To quantify the progression and resolution of the inflammatory edema, paw thickness was measured in millimeters (mm) using a high-precision digital Vernier caliper (Mitutoyo, Japan) as described by Vogel [19] and Kulkarni [20]. Measurements were recorded at 0 hour (immediately prior to the subplantar formalin injection to establish the normal baseline thickness of each individual animal) and sequentially at 1, 2, 3, 4, and 5 hours post-injection. The digital Vernier caliper was applied gently across the dorsoplantar axis of the right hind paw, taking extreme care to avoid exerting excessive mechanical pressure that could temporarily displace the localized fluid accumulation. For each animal, the absolute increase in paw thickness (Δ T) at each hourly interval was calculated as follows:

$$\Delta T = T_t - T_0$$

where T0 is the baseline paw thickness (mm) at 0 hour and Tt is the paw thickness (mm) at time t. The percentage inhibition of inflammatory edema was computed for each treated group relative to the disease control group using the relation [1, 16, 19]:

$$\% \text{ Edema Inhibition} = [\{\Delta T_{control} - \Delta T_{treated}\} / \Delta T_{control}] * 100$$

where $\Delta T_{control}$ represents the mean increase in paw thickness of the disease control group (Group I) and $\Delta T_{treated}$ represents the mean increase in paw thickness of the treated groups (Groups II, III, or IV).

3. Results And Discussion

3.1. Phytochemical Screening

Qualitative phytochemical screening of the crude ethanolic extract of *Curcuma longa* revealed a rich presence of primary secondary metabolites. The ferric chloride test produced an immediate, intense blue-black coloration, confirming the abundance of phenolic compounds. The alkali test produced a deep reddish-brown coloration that reverted to yellow upon acidification, verifying the presence of curcuminoids. The Salkowski test provided a distinct reddish-brown ring at the chloroform-acid interface, confirming the presence of terpenoids. Additionally, the lead acetate and Liebermann-Burchard assays confirmed the presence of tannins and steroidal structures, respectively. These secondary metabolites indicate a therapeutic matrix capable of acting on multiple inflammatory targets.

3.2. Evaluation of Anti-Inflammatory Activity

Subplantar injection of 1% w/v formalin into the rat hind paw produced rapid, localized edema in all experimental groups. In the disease control group (Group I), paw thickness increased progressively, rising from a baseline of 3.30 ± 0.03 mm to a peak of 6.38 ± 0.10 mm at the fifth hour post-challenge, confirming the successful induction of sub-acute localized inflammation. This continuous swelling is driven by the sustained release of pro-inflammatory mediators.

Prior oral administration of standard ibuprofen (40 mg/kg) significantly suppressed paw thickness throughout the monitoring period. The standard group presented a baseline thickness of 3.30 ± 0.01 mm, which reached 4.12 ± 0.04 mm at 2 hours and steadily resolved to 3.48 ± 0.03 mm by the fifth hour. This translates to an edema inhibition of 48.6% at the second hour and 94.3% at the fifth hour, confirming the rapid and powerful action of this non-selective cyclooxygenase inhibitor.

The test extract of *Curcuma longa* showed clear dose-dependent and time-dependent anti-inflammatory activity. The low-dose group (50 mg/kg) exhibited only a mild inhibitory effect, with paw thickness reaching 6.01 ± 0.09 mm at the fifth hour, representing a peak edema inhibition of 12.2%. In contrast, the high-dose group (100 mg/kg) showed potent anti-inflammatory effects. The mean paw thickness of the high-dose group peaked at 4.42 ± 0.04 mm at 2 hours and steadily declined to 3.82 ± 0.05 mm at 5 hours. This represents an edema inhibition of 29.8% at the second hour and 83.0% at the fifth hour, closely approaching the efficacy of standard ibuprofen.

3.2.1. Raw Paw Thickness

The detailed, animal-specific changes in paw thickness recorded across all experimental groups are shown in Table 1.

Table 1. Effects of *Curcuma longa* Extract and Ibuprofen on Rat Paw Thickness (mm)

Experimental Group	Animal ID	Baseline (0 hr)	1 hr	2 hr	3 hr	4 hr	5 hr
Group I (Disease Control)	1	3.25	4.10	4.80	5.40	5.90	6.20
	2	3.35	4.25	5.00	5.65	6.20	6.55
	3	3.30	4.20	4.90	5.50	6.05	6.40
	Mean ± SD	3.30 ± 0.05	4.18 ± 0.08	4.90 ± 0.10	5.52 ± 0.13	6.05 ± 0.15	6.38 ± 0.18
Group II (Standard Ibuprofen)	1	3.28	3.95	4.10	3.95	3.70	3.45
	2	3.32	4.00	4.15	4.00	3.75	3.50
	3	3.30	3.98	4.12	3.98	3.72	3.48
	Mean ± SD	3.30 ± 0.02	3.98 ± 0.03	4.12 ± 0.03	3.98 ± 0.03	3.72 ± 0.03	3.48 ± 0.03
Group III (Extract 50 mg/kg)	1	3.24	4.05	4.70	5.25	5.65	5.85
	2	3.36	4.22	4.92	5.50	5.92	6.15
	3	3.30	4.15	4.82	5.38	5.80	6.02
	Mean ± SD	3.30 ± 0.06	4.14 ± 0.09	4.81 ± 0.11	5.38 ± 0.13	5.79 ± 0.14	6.01 ± 0.15
Group IV (Extract 100 mg/kg)	1	3.25	4.00	4.35	4.30	4.05	3.75
	2	3.35	4.15	4.50	4.45	4.20	3.90
	3	3.30	4.08	4.42	4.38	4.12	3.82
	Mean ± SD	3.30 ± 0.05	4.08 ± 0.08	4.42 ± 0.08	4.38 ± 0.08	4.12 ± 0.08	3.82 ± 0.08

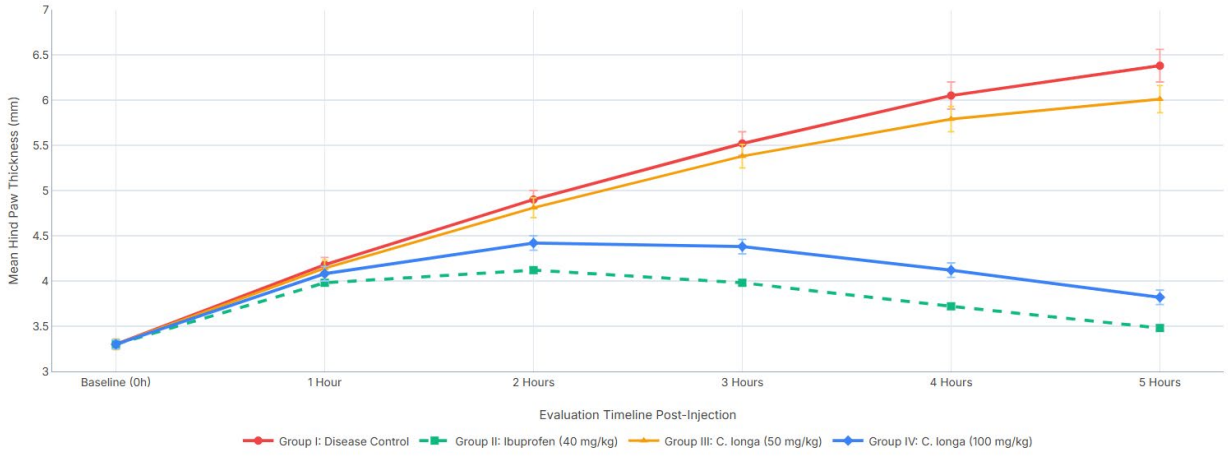

Figure 1. *In vivo* anti-inflammatory activity of *Curcuma longa*

3.2.2. % Edema Inhibition

The percent edema inhibition across the experimental groups are presented in Table 2.

Table 2. Percent Edema Inhibition Relative to Disease Control

Time Interval (Hours)	Group II (Standard Ibuprofen)	Group III (Extract 50 mg/kg)	Group IV (Extract 100 mg/kg)
1 Hour	23.3%	4.9%	12.0%
2 Hours	48.6%	5.4%	29.8%
3 Hours	69.5%	6.3%	51.4%
4 Hours	84.6%	9.5%	70.1%
5 Hours	94.3%	12.2%	83.0%

3.3. Discussion

The formalin-induced paw edema model serves as a good *in vivo* system that mimics acute clinical inflammatory processes [15, 16]. Subplantar administration of 1% formalin triggers a biphasic inflammatory response [16]. The early phase (0 to 1 hour) is primarily neurogenic, initiated by direct chemical activation of sensory C-fibers and the subsequent release of neuropeptides like substance P and calcitonin gene-related peptide (CGRP) [17]. This is followed by a late phase (2 to 5 hours) dominated by localized tissue damage and the release of inflammatory mediators, including histamine, serotonin, bradykinin, and pro-inflammatory prostaglandins [18, 22].

The standard reference drug, ibuprofen, exhibited potent anti-inflammatory activity, achieving 94.3% edema inhibition by the fifth hour. This rapid action is mediated by the competitive inhibition of inducible COX-2, which prevents the conversion of arachidonic acid into pro-inflammatory prostaglandins (PGE2 and PGI2) [6, 7]. This block suppresses microvascular dilation, reduces plasma extravasation, and decreases paw thickness.

The ethanolic rhizome extract of *Curcuma longa* showed clear dose-dependent and time-dependent anti-inflammatory activity. While the low-dose extract (50 mg/kg) produced only a modest effect (12.2% inhibition), the high-dose extract (100 mg/kg) achieved a potent anti-inflammatory response, reaching 83.0% inhibition at the fifth hour. This high-dose response was particularly effective during the late, prostaglandin-dependent phase of formalin-induced inflammation.

This potent anti-inflammatory efficacy is driven by the synergistic action of the bioactive secondary metabolites present in the extract, notably curcuminoids and sesquiterpenes [12, 14]. Curcuminoids act as multi-target anti-inflammatory agents [13]. At the transcriptional level, curcumin blocks the activation of nuclear factor kappa B (NF-ϰB), a major transcription factor that regulates the expression of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) and inducible enzymes like COX-2 and iNOS [13, 29]. The extract directly inhibits prostaglandin and nitric oxide production during the late phase of formalin-induced edema by suppressing the transcription of these proteins [13, 30].

The lipophilic sesquiterpene fraction (including turmerones and zingiberene) acts synergistically with curcuminoids [12, 14]. These compounds stabilize cellular membranes, prevent neutrophil infiltration, and reduce the release of lysosomal enzymes that contribute to localized tissue damage [14, 23]. Additionally, the phenolic hydroxyl groups in the extract's polyphenols act as powerful free radical scavengers, neutralizing reactive oxygen species (ROS) produced by infiltrating neutrophils and preventing lipid peroxidation [14, 24]. Through these integrated mechanisms, the high-dose *Curcuma longa* extract effectively resolves localized edema, approaching the therapeutic efficacy of standard NSAIDs like ibuprofen without presenting their associated mucosal toxicities.

4. Conclusion

This work showed that the ethanolic rhizome extract of *Curcuma longa* possesses significant, dose-dependent anti-inflammatory activity in Wistar albino rats. Using the standardized formalin-induced paw edema model, prior oral administration of the extract at 100 mg/kg produced a powerful anti-inflammatory response, achieving an 83.0% inhibition of paw swelling by the fifth hour. This therapeutic effect was close to the efficacy of standard ibuprofen (40 mg/kg), which achieved a 94.3% inhibition. Phytochemical screening confirmed that this potent activity is driven by a rich matrix of curcuminoids, phenolics, terpenoids, and tannins that act synergistically to suppress pro-inflammatory cyclooxygenase pathways and localized free radical damage. These results provide strong scientific validation for the traditional use of *Curcuma longa* in the management of inflammatory conditions and suggest its potential as a highly effective, natural therapeutic alternative to conventional NSAIDs.

Compliance with ethical standards

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Conflict of interest statement

The authors declare that they have no competing interests. There are no financial, personal, or professional relationships with any individuals, institutions, or pharmaceutical manufacturers that could inappropriately influence or bias the outcomes, data, or conclusions presented in this manuscript. The authors also declare no conflicts of interest regarding any commercial products or therapeutic agents that compete directly or indirectly with those mentioned herein.

Statement of ethical approval

The *in-vivo* experimental protocol and animal handling procedures described in this study were formally reviewed, validated, and authorized by the Institutional Animal Ethics Committee (IAEC) of K.G.R.L. College of Pharmacy, Bhimavaram, Andhra Pradesh, India (Protocol Approval Number: IAEC/KGRL/2025/141). All animal interventions, housing maintenance, and post-injection monitoring were executed in strict adherence to the guidelines prescribed by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India.

Statement of informed consent

The present research work does not contain any studies involving human subjects, patient case profiles, clinical surveys, or personal interviews conducted by any of the authors. Consequently, the requirement for obtaining statement of informed consent is not applicable to this investigation.

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