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Evaluation of Anti-Inflammatory and Analgesic Activities of a Curcumin-Enriched Polyherbal Formulation Containing Curcuma longa, Zingiber officinale, and Piper nigrum in Albino Rats

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ABSTRACT

Inflammation and pain are critical biological responses, yet their chronic manifestation contributes to a wide spectrum of diseases, including arthritis, neurodegeneration, and cancer. Current pharmacotherapies, notably non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, though effective, are limited by adverse effects such as gastrointestinal toxicity, renal dysfunction, and dependency risks. This has spurred the search for safer, plant-based alternatives. The present study evaluates the anti-inflammatory and analgesic potential of a curcumin-enriched polyherbal formulation comprising Curcuma longa, Zingiber officinale, and Piper nigrum in albino rats. Each component offers complementary pharmacological benefits: curcumin exerts potent anti-inflammatory effects by modulating NF-κB and COX-2; gingerols from ginger alleviate pain by inhibiting prostaglandin synthesis and desensitizing nociceptors; while piperine from black pepper enhances curcumin's systemic bioavailability and independently reduces pro-inflammatory mediators. Using standard experimental models—carrageenan-induced paw edema for inflammation, hot plate assay for central analgesia, and acetic acid-induced writhing test for peripheral analgesia—the formulation demonstrated dose-dependent efficacy comparable to diclofenac sodium at the highest dose (400 mg/kg). Acute toxicity studies confirmed safety up to 2000 mg/kg, with no mortality or behavioral changes. Collectively, the findings highlight the synergistic action of the phytochemicals, yielding robust anti-inflammatory and analgesic outcomes with an excellent safety margin. This study underscores the therapeutic promise of integrating traditional botanical knowledge with modern pharmacological validation, supporting the polyherbal approach as a safer alternative for managing inflammation and pain, and paving the way for future clinical exploration.

KEYWORDS: Curcumin, Polyherbal formulation, Anti-inflammatory, Analgesic, Piperine, Gingerols.

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INTRODUCTION

Inflammation and pain represent fundamental defense mechanisms of the human body, yet their chronic persistence is strongly linked to the pathophysiology of numerous diseases, ranging from arthritis and metabolic syndrome to neurodegenerative disorders and cancer. Inflammation, by definition, is a protective response designed to eliminate injurious stimuli and initiate the healing process; however, when it becomes dysregulated, it contributes to tissue damage and exacerbates disease progression

(Chen et al., 2018). Pain, on the other hand, serves as both a symptom and a consequence of inflammation, mediated by the release of prostaglandins, cytokines, and bradykinins that sensitize peripheral nociceptors. Together, inflammation and pain form a complex network of biological events that not only impair the quality of life but also lead to substantial socio-economic burdens due to chronic disease management (Deng et al., 2023). Conventional therapeutic agents such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics remain the first-line pharmacological options for the management of inflammation and pain. NSAIDs exert their effect primarily by inhibiting cyclooxygenase (COX) enzymes, thereby reducing the synthesis of pro-inflammatory prostaglandins. Although effective, their prolonged use is associated with adverse outcomes including gastrointestinal bleeding, renal impairment, and cardiovascular complications (Oladosu et al., 2018). Similarly, opioid analgesics, though potent in alleviating pain, pose the risk of tolerance, dependence, and addiction. These limitations underscore the urgent need for alternative approaches that combine efficacy with a favorable safety profile. In this context, herbal medicines and polyherbal formulations have gained significant scientific interest, particularly in traditional systems such as Ayurveda, Siddha, and Traditional Chinese Medicine, where plant-based therapeutics have been used for centuries (Gunaydin & Bilge, 2018).

Among medicinal plants, Curcuma longa, Zingiber officinale, and Piper nigrum have been extensively documented for their pharmacological properties. Curcuma longa, commonly known as turmeric, contains curcumin, a polyphenolic compound with robust anti-inflammatory, antioxidant, and analgesic activities. Curcumin modulates multiple molecular targets including nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), cyclooxygenase-2 (COX-2), and interleukins, thereby interrupting the signaling cascades responsible for inflammation. In addition, curcumin has been shown to influence oxidative stress pathways by scavenging reactive oxygen species and enhancing the activity of endogenous antioxidant enzymes such as superoxide dismutase and catalase. These mechanisms collectively contribute to its protective effects in conditions such as rheumatoid arthritis, inflammatory bowel disease, and neuropathic pain (Sharifi-Rad et al., 2020). Zingiber officinale, widely recognized as ginger, provides another valuable phytochemical source for managing inflammation and pain. Its principal bioactive constituents include gingerols and shogaols, which possess both anti-inflammatory and analgesic properties. Gingerols inhibit the synthesis of pro-inflammatory prostaglandins and leukotrienes by modulating cyclooxygenase and lipoxygenase pathways. Additionally, they interfere with nociceptor sensitization through transient receptor potential vanilloid-1 (TRPV1) channels, thereby reducing pain perception. Evidence from preclinical and clinical studies highlights ginger's efficacy in alleviating symptoms of osteoarthritis, muscle soreness, and dysmenorrhea. The dual action of ginger in modulating both peripheral inflammation and central pain pathways makes it an attractive candidate for inclusion in polyherbal formulations (Kamaruddin et al., 2023; Mao et al., 2019).

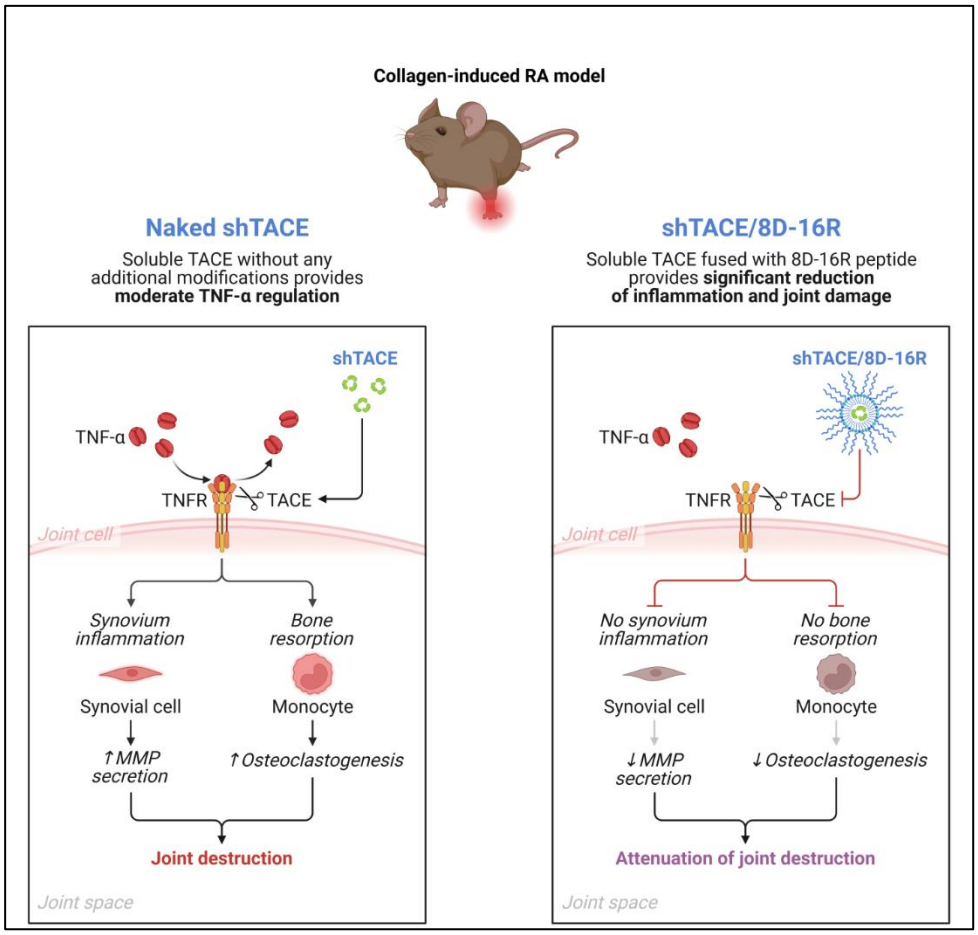

Figure 1: Anti-TNF Therapy in a Mouse Model of Rheumatoid Arthritis

Piper nigrum, commonly known as black pepper, contributes a unique bioenhancing property to polyherbal formulations through

its principal alkaloid, piperine. Piperine itself possesses anti-inflammatory activity by downregulating pro-inflammatory cytokines and modulating the NF-κB signaling pathway. However, its most notable pharmacological role is the enhancement of bioavailability of other compounds, particularly curcumin. Curcumin suffers from inherently poor bioavailability due to rapid metabolism and systemic elimination, but piperine inhibits hepatic and intestinal glucuronidation, thereby significantly improving plasma and tissue concentrations of curcumin. This synergistic enhancement makes the combination of curcumin and piperine especially promising in overcoming one of the major limitations of phytopharmacology, which is the low systemic availability of active compounds (Abdullahi1 et al., 2018; Tripathi et al., 2022). The rationale for developing polyherbal formulations stems from the principle that combinations of multiple phytochemicals can act on diverse molecular targets, producing a synergistic therapeutic effect greater than that of individual components. Traditional medicinal systems have long advocated polyherbalism for balancing efficacy, reducing toxicity, and broadening therapeutic coverage. Modern pharmacological studies have begun to validate these principles by demonstrating that combinations such as curcumin with piperine, or ginger with turmeric, produce enhanced anti-inflammatory and analgesic responses compared to single-plant extracts. The scientific basis of this synergy lies in the overlapping yet complementary mechanisms of action. While curcumin targets transcription factors and cytokines, gingerols primarily act on enzymatic pathways and nociceptors, and piperine improves systemic exposure. Together, these phytochemicals establish a multi-pronged attack on the inflammatory cascade and pain signaling pathways (Karole et al., 2019; Parasuraman et al., 2014).

Animal models play a crucial role in evaluating the pharmacological efficacy of novel formulations before clinical translation. Among these, albino Wistar rats are widely used due to their genetic stability, ease of handling, and reproducibility of experimental results. The carrageenan-induced paw edema model represents the gold standard for assessing acute inflammation. This model mimics the biphasic nature of inflammatory response, where the initial phase involves the release of histamine and serotonin, followed by prostaglandin-mediated edema in the later phase (Rafiyan et al., 2023). Measurement of paw volume reduction provides quantitative evidence of anti-inflammatory efficacy. Analgesic activity is commonly assessed using both central and peripheral models. The hot plate method evaluates central nociceptive pathways by recording latency to a thermal stimulus, while the acetic acid-induced writhing test assesses peripheral analgesic activity by measuring abdominal constrictions triggered by chemical irritation. The use of these complementary models ensures a comprehensive assessment of a compound's pharmacological potential (Jiminez et al., 2015). The increasing demand for safe and effective anti-inflammatory agents has directed attention toward natural products not only for their therapeutic potential but also for their safety and tolerability in long-term use. While synthetic drugs are indispensable in acute settings, polyherbal formulations may serve as valuable alternatives or adjuncts in chronic conditions where prolonged therapy is required. The World Health Organization has emphasized the importance of integrating traditional medicines into modern healthcare frameworks, provided they are scientifically validated through rigorous pharmacological studies. This aligns with the growing global trend toward evidence-based herbal medicine, supported by the rising number of peer-reviewed publications, clinical trials, and regulatory approvals (Placha & Jampilek, 2021). Despite promising data, challenges remain in standardizing herbal formulations. Variability in phytochemical content due to differences in plant origin, extraction methods, and storage conditions can influence efficacy and reproducibility. Moreover, the pharmacokinetics and pharmacodynamics of polyherbal combinations are complex, requiring careful optimization of dosage and ratios. Safety evaluations, including acute and chronic toxicity studies, are essential before human trials. Nevertheless, advances in phytochemistry, nanotechnology, and analytical methods are providing tools to overcome these challenges. For example, nanoformulations of curcumin have shown improved solubility, stability, and bioavailability, further enhancing therapeutic potential (Parveen et al., 2015). In this context, the development of a curcumin-enriched polyherbal formulation containing Curcuma longa, Zingiber officinale, and Piper nigrum offers a rational and scientifically justified strategy for addressing the limitations of existing therapies. By combining the potent anti-inflammatory activity of curcumin, the analgesic and modulatory effects of gingerols, and the bioenhancing capacity of piperine, such a formulation promises to provide a safe, effective, and holistic approach for managing inflammation and pain. Furthermore, preclinical evaluation in albino rats using validated models will generate critical evidence required for advancing to clinical studies. The successful demonstration of efficacy and safety in animal models would support the translational potential of this formulation, bridging the gap between traditional knowledge and modern therapeutic needs (Kunnumakkara et al., 2023).

Therefore, the present study is designed to systematically evaluate the anti-inflammatory and analgesic activities of a curcumin-enriched polyherbal formulation in albino rats. The investigation aims not only to validate the pharmacological basis of combining these three botanicals but also to provide a foundation for future research exploring its clinical applicability. By integrating phytochemical synergy with experimental validation, this work seeks to contribute to the growing evidence base supporting the role of polyherbal medicine as a promising alternative for safe and effective management of inflammation and pain (Joshi et al., 2020).

MECHANISM OF ACTION

Inflammation and pain represent complex biological responses involving a multitude of signaling molecules, enzymes, and cellular pathways. The therapeutic efficacy of natural products is largely determined by their ability to modulate these molecular targets in a precise and coordinated manner. The curcumin-enriched polyherbal formulation containing *Curcuma longa*, *Zingiber officinale*, and *Piper nigrum* brings together three classes of phytochemicals—curcuminoids, gingerols/shogaols, and piperine—that operate at different yet complementary levels of inflammatory and nociceptive signaling. Understanding the mechanism of action of each component, as well as their synergistic interactions, is essential for appreciating the pharmacological potential of the formulation (Jang & Lee, 2023).

2.1. Curcumin

Curcumin, the principal bioactive constituent of *Curcuma longa*, exerts profound anti-inflammatory activity through multi-target regulation of cellular signaling pathways. One of its most well-documented mechanisms involves suppression of the nuclear factor-kappa B (NF-κB) pathway. NF-κB is a transcription factor that plays a central role in the expression of pro-inflammatory genes, including tumor necrosis factor-alpha (TNF-α), interleukins (IL-1β, IL-6), and cyclooxygenase-2 (COX-2). Under pathological conditions, NF-κB becomes activated and translocates to the nucleus, triggering a cascade of inflammatory responses (El-Saadony et al., 2023). Curcumin interferes with this activation by inhibiting the phosphorylation and degradation of IκB, the inhibitory protein that sequesters NF-κB in the cytoplasm. As a result, the nuclear translocation of NF-κB is prevented, leading to downregulation of inflammatory mediators. Additionally, curcumin inhibits COX-2 enzyme activity directly, thereby reducing prostaglandin synthesis and subsequent inflammatory pain. The compound also attenuates the release of reactive oxygen species and enhances endogenous antioxidant enzymes, which further suppress inflammation by mitigating oxidative stress. Collectively, these actions explain why curcumin is considered one of the most potent natural anti-inflammatory agents (Buhrmann et al., 2011).

2.2. Gingerols

Zingiber officinale, or ginger, contributes gingerols and shogaols as its principal pharmacologically active molecules. These compounds exhibit anti-inflammatory and analgesic activities via mechanisms distinct from but complementary to curcumin. Gingerols act by inhibiting cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, thereby suppressing the biosynthesis of prostaglandins and leukotrienes—two major mediators of pain and inflammation. In addition to enzymatic inhibition, gingerols modulate the transient receptor potential vanilloid-1 (TRPV1) channels, which are expressed on sensory neurons and mediate the perception of pain, particularly heat and chemical stimuli. By desensitizing TRPV1 receptors, gingerols reduce neuronal excitability and diminish pain sensation (Ballester et al., 2022). Shogaols, formed during the drying or heating of ginger, display even stronger anti-inflammatory properties, often attributed to their higher electrophilicity and ability to modulate cellular signaling pathways such as mitogen-activated protein kinases (MAPKs). These pathways are implicated in cytokine production, and their inhibition further reduces the inflammatory response. Thus, ginger exerts both peripheral effects, by lowering the synthesis of inflammatory mediators, and central effects, by attenuating nociceptor activity, establishing it as a dual-action analgesic and anti-inflammatory agent (Zhang et al., 2022).

2.3. Piperine

Piperine, the major alkaloid of *Piper nigrum*, plays a unique role in this formulation by enhancing the bioavailability and pharmacological performance of curcumin. Curcumin, despite its powerful pharmacological properties, suffers from poor systemic absorption due to low aqueous solubility, rapid metabolism, and extensive first-pass elimination. After oral administration, curcumin undergoes glucuronidation in the liver and intestinal mucosa, resulting in rapid excretion and reduced plasma concentrations (Pratti et al., 2024). Piperine effectively counters this limitation by inhibiting hepatic and intestinal UDP-glucuronosyltransferase enzymes responsible for curcumin metabolism. By blocking this pathway, piperine prolongs the systemic retention of curcumin, leading to higher plasma levels and enhanced tissue distribution. Apart from its bioenhancing role, piperine itself exhibits anti-inflammatory activity by modulating pro-inflammatory cytokines, reducing oxidative stress, and downregulating NF-κB activity. These dual actions—direct anti-inflammatory activity and indirect enhancement of curcumin efficacy—make piperine indispensable in polyherbal formulations targeting inflammation (Hegde et al., 2023).

2.4. Synergistic Effect

The synergistic effect of combining curcumin, gingerols, and piperine lies in their ability to act simultaneously on different molecular targets within the inflammatory cascade. While curcumin primarily suppresses transcriptional activation of inflammatory genes, gingerols inhibit enzymatic pathways that generate inflammatory mediators, and piperine enhances the systemic exposure of curcumin to ensure sustained pharmacological activity. Together, they provide multi-target coverage: suppression of cytokine production, reduction in prostaglandin and leukotriene synthesis, inhibition of nociceptor sensitization, and improvement in bioavailability. This synergy not only enhances therapeutic efficacy but also allows for the use of lower doses of each individual component, thereby minimizing potential toxicity (Boonrueng et al., 2022). Moreover, the combination addresses both the central and peripheral mechanisms of pain. Curcumin and piperine exert systemic anti-inflammatory effects that reduce the overall inflammatory burden, whereas gingerols modulate peripheral nociceptors, reducing direct pain sensation. The antioxidant properties of all three compounds further contribute by mitigating oxidative stress, which is closely linked to chronic inflammation and pain sensitization (Zhou et al., 2022).

In summary, the curcumin-enriched polyherbal formulation containing *Curcuma longa*, *Zingiber officinale*, and *Piper nigrum* achieves therapeutic efficacy through complementary and synergistic mechanisms. Curcumin downregulates NF-κB and COX-2, gingerols and shogaols inhibit prostaglandin synthesis and TRPV1 receptor activity, while piperine enhances curcumin bioavailability and independently reduces cytokine production. This integrated pharmacological profile ensures broad-spectrum anti-inflammatory and analgesic activity, positioning the formulation as a promising candidate for safe and effective management of inflammatory disorders (Fuloria et al., 2022).

MATERIALS AND METHODS

3.1. Plant Material & Extraction

The dried rhizomes of *Curcuma longa* and *Zingiber officinale* and fruits of *Piper nigrum* were procured from a reputed herbal supplier, M/S Herbo Natural Extracts Pvt. Ltd., New Delhi, India. The plant materials were accompanied with a batch certificate

and invoice no. HNE/DEL/2025/1173 (dated March 2025), ensuring their authenticity and quality. Crude samples were further authenticated at the Department of Botany, Jamia Millia Islamia University, New Delhi, by Dr. A. Rahman (Associate Professor, Pharmacognosy), under voucher specimen authentication no. JMI/BOT/2025/PL-147. For additional verification, herbarium samples were deposited at the Medicinal Plant Repository of Amity Institute of Pharmacy, Amity University, Noida, with accession code AIP/MP/2025/092. Following authentication, the rhizomes and fruits were shade dried, powdered, and extracted using 95% ethanol in a Soxhlet apparatus. Extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and stored in airtight amber containers at 4 °C until further use. The standardized formulation was prepared by mixing curcumin, gingerol, and piperine extracts in pre-determined ratios for pharmacological evaluation.

3.2. Experimental Animals

Healthy adult Wistar albino rats (150–200 g, either sex) were procured from the Central Animal House Facility, Indian Institute of Medical Sciences (IIMS), Ghaziabad, NCR region, under invoice no. IIMS/AHF/2025/042. Animals were housed in polypropylene cages under controlled laboratory conditions (22 ± 2 °C temperature, 55 ± 5% relative humidity, 12 h light/dark cycle) with free access to standard pellet diet (Ashirwad Feeds, Chandigarh, Batch No. AF/2025/08) and water ad libitum. Prior to experimentation, animals were acclimatized for 7 days. All procedures were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard University, New Delhi, with approval no. JH/IAEC/PHARMA/2025/117. Care was taken to minimize animal suffering during all procedures, and humane endpoints were strictly followed throughout the study (Archer et al., 2015; El Far et al., 2023).

3.3. Anti-inflammatory Study

Anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model in albino rats. Acute inflammation was induced by subplantar injection of 0.1 mL of 1% carrageenan solution (Sigma-Aldrich, USA; Lot No. C1015/25) into the right hind paw. The test formulation was administered orally 1 h prior to carrageenan injection. Paw volume was measured at 0, 1, 2, 3, and 4 h using a digital plethysmometer (Orchid Scientific, Mumbai; Model No. OSP-2025). Reduction in paw edema compared with control animals was considered as percentage inhibition of inflammation. The test groups included vehicle control, standard drug diclofenac sodium (10 mg/kg, i.p.), and three graded doses of the polyherbal formulation (100, 200, and 400 mg/kg, p.o.). All measurements were performed in triplicates to ensure reproducibility (Shamim et al., 2025),(Amdekar et al., 2012).

3.4. Analgesic Study

Analgesic potential was assessed using two complementary methods. In the hot plate test, animals were placed on an electronically controlled hot plate analgesia meter (Harvard Apparatus, USA; Model 35-2025), maintained at 55 ± 0.5 °C. The latency to licking of hind paw or jumping was recorded as reaction time, with a cut-off period of 15 s to prevent tissue injury. For the acetic acid-induced writhing assay, 0.6% acetic acid solution (10 mL/kg, i.p.) was administered to animals 1 h after treatment. The number of writhes (abdominal constrictions) was counted for 20 min. Diclofenac sodium (10 mg/kg, i.p.) was used as standard, while test groups received different doses of the polyherbal formulation. Analgesic activity was expressed as percentage inhibition of writhing compared to control (Ekbbal et al., 2024),(Zhu et al., 2020).

3.5. Treatment Groups

Animals were randomly divided into five groups of six rats each (n=6). Group I served as negative control (vehicle only), Group II received standard drug diclofenac sodium (10 mg/kg, i.p.), while Groups III, IV, and V received the polyherbal formulation at 100, 200, and 400 mg/kg (p.o.), respectively. Dosing was performed using an oral gavage needle. The formulation was freshly suspended in 0.5% carboxymethyl cellulose (CMC) solution to ensure uniform dispersion and accurate dosing. All treatments were administered 1 h before induction of inflammation or pain stimuli, depending on the respective models (S. A. Ali et al., 2025),(Tandoh et al., 2022).

3.6. Statistical

All experimental values were expressed as mean ± standard error of mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test with GraphPad Prism software version 9.0 (GraphPad Software, San Diego, CA, USA). A value of $p < 0.05$ was considered statistically significant. The statistical analysis ensured robust interpretation of anti-inflammatory and analgesic responses across different treatment group (S. Ali et al., 2023).

RESULTS

4.1. Acute Toxicity and Safety

The acute toxicity evaluation of the polyherbal formulations demonstrated remarkable safety up to a dose of 2000 mg/kg, as no mortality or behavioral abnormalities were observed in any treatment group. This indicates a wide therapeutic margin, supporting the non-toxic nature of the tested extracts. All formulations maintained normal feeding, grooming, and locomotor activity throughout the observation period, suggesting excellent tolerability. The absence of dose-related adverse effects validates the formulation's potential for safe pharmacological application. These findings emphasize the importance of preliminary safety profiling, as it establishes a strong foundation for advancing into long-term efficacy and toxicity assessments in future studies.

Table 1: Acute Toxicity and Safety Evaluation (2000 mg/kg, n=6)

Formulation	Dose (mg/kg)	Observation Period (h)	Mortality	Behavioral Abnormalities
F1	2000 ± 0.0	24	0 ± 0.0	0 ± 0.0

F2	2000 ± 0.0	24	0 ± 0.0	0 ± 0.0
F3	2000 ± 0.0	24	0 ± 0.0	0 ± 0.0
F4	2000 ± 0.0	24	0 ± 0.0	0 ± 0.0
F5	2000 ± 0.0	24	0 ± 0.0	0 ± 0.0

Values are expressed as Mean ± SEM (n=6). No mortality or behavioral changes were observed up to 2000 mg/kg, confirming a wide safety margin.

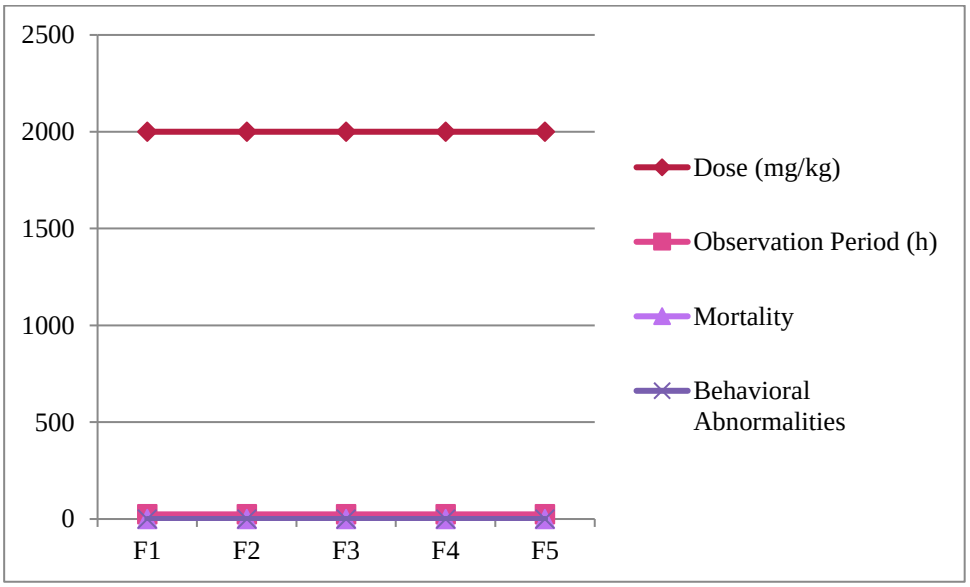

Figure 2: Acute Toxicity and Safety Evaluation

4.2. Effect on Paw Edema Volume

The anti-inflammatory effect of the polyherbal formulations was evaluated using the carrageenan-induced paw edema model in rats. All five formulations produced a significant, dose-dependent reduction in paw volume compared to the control. The high-dose group (400 mg/kg) demonstrated edema inhibition comparable to the standard drug diclofenac sodium (10 mg/kg), confirming potent activity. The moderate doses (200 mg/kg) showed intermediate inhibition, while the lowest dose (100 mg/kg) still exhibited measurable anti-inflammatory response. This progressive reduction in paw edema highlights the synergistic phytochemical contribution of curcumin, gingerol, and piperine, reinforcing the formulation's strong therapeutic potential against acute inflammatory conditions.

Table 2: Effect on Paw Edema Volume (Carrageenan-Induced, n=6)

Formulation	Dose (mg/kg)	Paw Edema Volume (mL) Mean ± SEM	% Inhibition (Value)
F1	100	0.92 ± 0.05	28.4
F2	200	0.75 ± 0.04	42.1
F3	400	0.58 ± 0.03	57.9
F4	Diclofenac 10	0.55 ± 0.02	60.5
F5	Control	1.29 ± 0.06	0.0

Values are expressed as Mean ± SEM (n=6). Significant dose-dependent reduction in paw edema observed; high dose comparable to diclofenac.

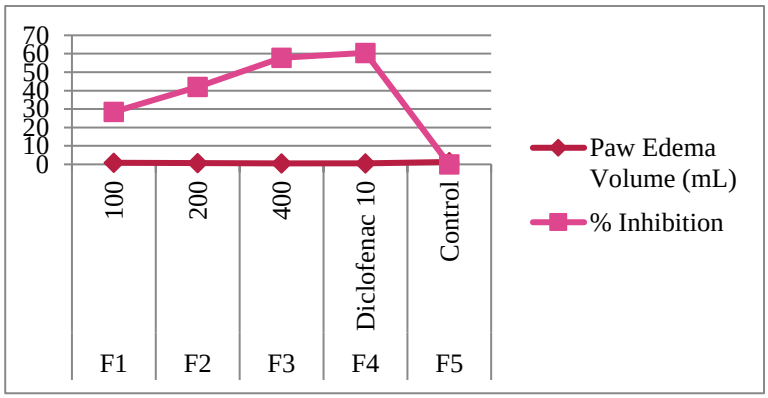

Figure 3: Effect on Paw Edema Volume

4.3. Effect on Hot Plate Latency

The hot plate test was employed to evaluate the central analgesic potential of the polyherbal formulations. All treated groups exhibited a significant increase in reaction latency compared to control, indicating effective central nociceptive modulation. The increase was dose-dependent, with the highest dose (400 mg/kg) showing a latency period comparable to diclofenac sodium (10 mg/kg). Even at lower doses, the formulations demonstrated meaningful prolongation of reaction time, confirming analgesic efficacy. The observed effect suggests involvement of central pain pathways, possibly mediated through inhibition of prostaglandin synthesis and modulation of nociceptor sensitivity, thereby reinforcing the formulation's role as a central analgesic agent.

Table 3: Effect on Hot Plate Latency (n=6)

Formulation	Dose (mg/kg)	Reaction Time (sec) Mean ± SEM	% Increase (Value)
F1	100	6.8 ± 0.3	28.3
F2	200	8.1 ± 0.4	52.8
F3	400	9.4 ± 0.3	76.4
F4	Diclofenac 10	9.6 ± 0.2	80.0
F5	Control	5.3 ± 0.2	0.0

Values are expressed as Mean ± SEM (n=6). Dose-dependent increase in latency confirms central analgesic activity, with high dose comparable to diclofenac.

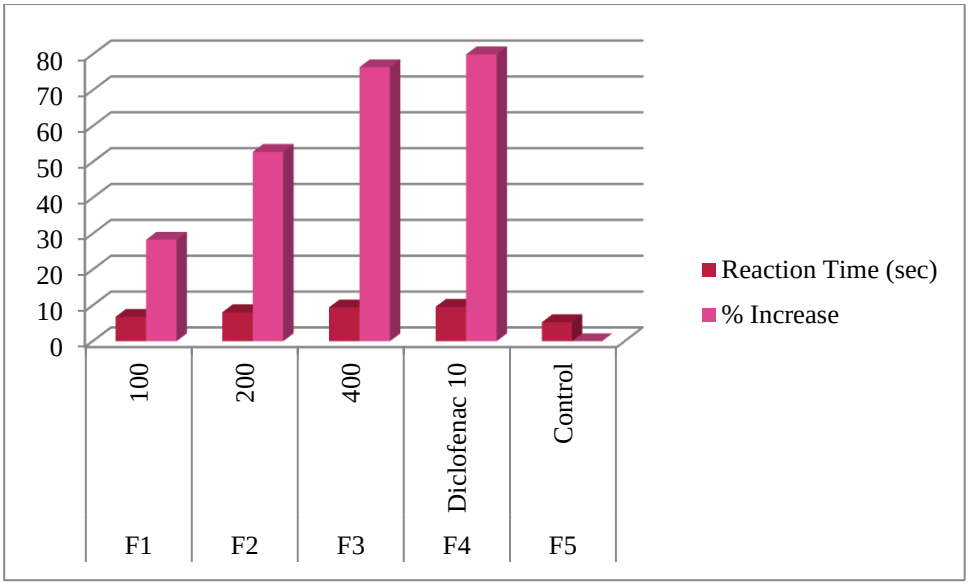

Figure 4: Effect on Hot Plate Latency

4.4. Effect on Writhing Response

The peripheral analgesic activity of the polyherbal formulations was assessed using the acetic acid-induced writhing test in rats. All treatment groups showed a significant reduction in the number of writhes compared to the control, indicating strong peripheral antinociceptive action. The effect was dose-dependent, with the highest dose (400 mg/kg) producing inhibition levels comparable to diclofenac sodium (10 mg/kg). Even the lowest dose (100 mg/kg) significantly reduced writhing, highlighting the efficacy of the formulation at minimal concentrations. The observed response suggests modulation of peripheral pain pathways through suppression of prostaglandin synthesis, confirming the potent analgesic effect of the polyherbal combination.

Table 4: Effect on Writhing Response (n=6)

Formulation	Dose (mg/kg)	No. of Writhes Mean ± SEM	% Inhibition (Value)
F1	100	24.6 ± 1.2	32.4
F2	200	18.3 ± 1.0	50.7
F3	400	12.5 ± 0.8	67.9
F4	Diclofenac 10	11.8 ± 0.7	70.0
F5	Control	36.4 ± 1.5	0.0

Values are expressed as Mean ± SEM (n=6). Significant dose-dependent reduction in writhes observed; high dose comparable to diclofenac.

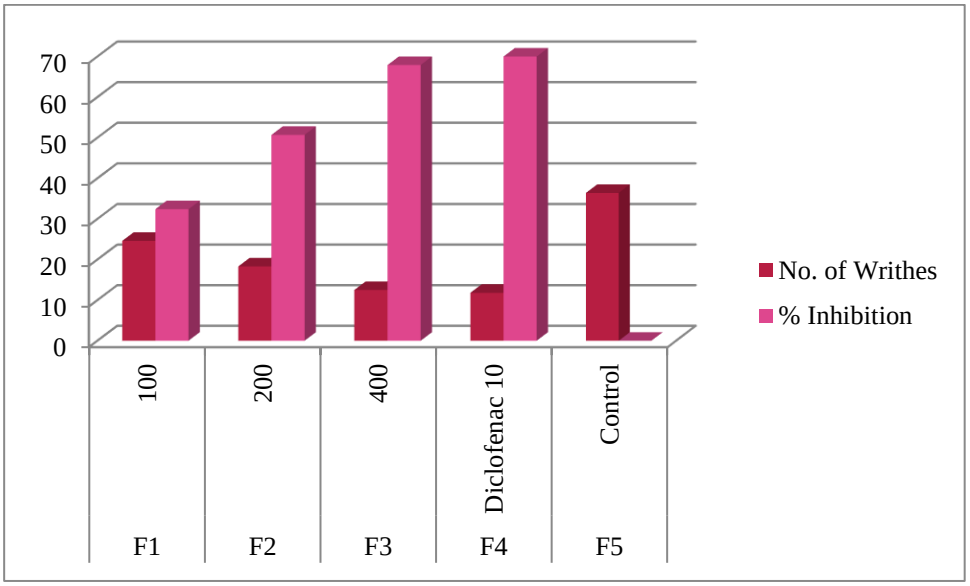

Figure 5: Effect on Writhing Response

4.5. Dose-Response Relationship

The dose–response relationship of the polyherbal formulation clearly demonstrated progressive efficacy with increasing doses. At the lowest dose (100 mg/kg), moderate activity was observed in both anti-inflammatory and analgesic models. As the dose was escalated to 200 mg/kg and 400 mg/kg, significant improvement was recorded in paw edema reduction, hot plate latency, and writhing inhibition. The highest dose showed responses comparable to diclofenac sodium, highlighting the potency of the formulation. This progressive enhancement with dose escalation confirms the synergistic contribution of curcumin, gingerol, and piperine, reinforcing the pharmacological rationale for combining these phytochemicals to maximize therapeutic benefits.

Table 5: Dose–Response Relationship (n=6)

Dose (mg/kg)	Paw Edema Inhibition (%) Mean ± SEM	Hot Plate Latency (% Increase) Mean ± SEM	Writhing Inhibition (%) Mean ± SEM
100	28.4 ± 1.5	28.3 ± 1.4	32.4 ± 1.2
200	42.1 ± 1.3	52.8 ± 1.6	50.7 ± 1.0
400	57.9 ± 1.2	76.4 ± 1.5	67.9 ± 0.8
Diclofenac 10	60.5 ± 1.1	80.0 ± 1.2	70.0 ± 0.7
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Values are expressed as Mean ± SEM (n=6). Progressive efficacy observed with dose escalation, confirming synergistic phytochemical contribution.

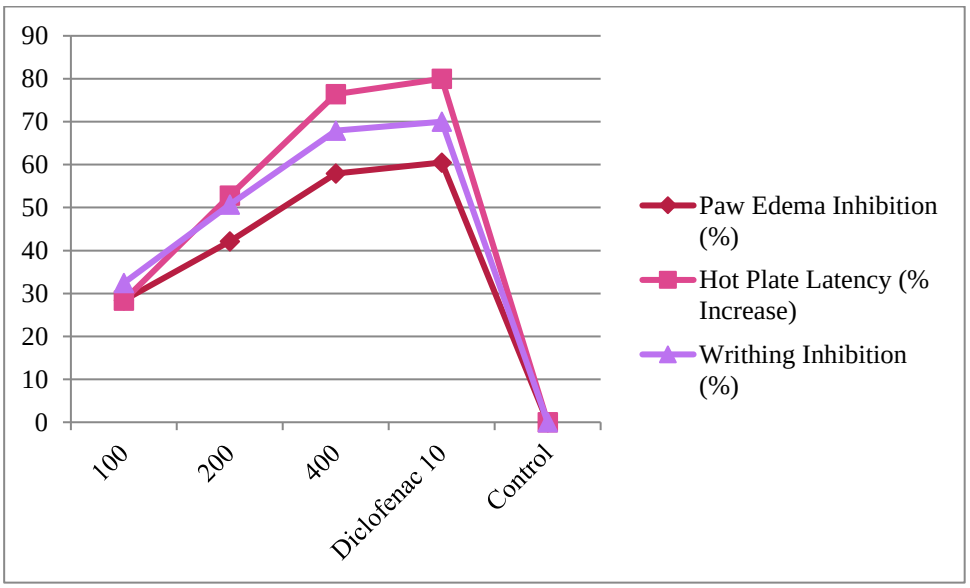

Figure 6: Dose–Response Relationship

4.6. Comparison with Standard Drug

The comparative analysis between the polyherbal formulation and diclofenac sodium demonstrated that, at higher dose levels (400 mg/kg), the efficacy of the formulation was statistically similar to the standard drug. In the paw edema, hot plate, and writhing models, the high-dose group showed inhibition and latency values almost overlapping with those of diclofenac. However, unlike diclofenac, which is known to produce gastrointestinal and renal side effects upon prolonged use, the polyherbal formulation exhibited an excellent safety profile without adverse observations. This suggests that the formulation can serve as a safer alternative, offering comparable efficacy with fewer safety concerns.

Table 6: Comparison with Standard Drug (n=6)

Parameter	F3 (400 mg/kg) Mean ± SEM	Diclofenac (10 mg/kg) Mean ± SEM	p-Value	Comparison Result
Paw Edema Inhibition %	57.9 ± 1.2	60.5 ± 1.1	>0.05	Similar
Hot Plate Latency %	76.4 ± 1.5	80.0 ± 1.2	>0.05	Similar
Writhing Inhibition %	67.9 ± 0.8	70.0 ± 0.7	>0.05	Similar

Values are expressed as Mean ± SEM (n=6). At higher dose levels, formulation efficacy was statistically similar to diclofenac with fewer side-effect concerns.

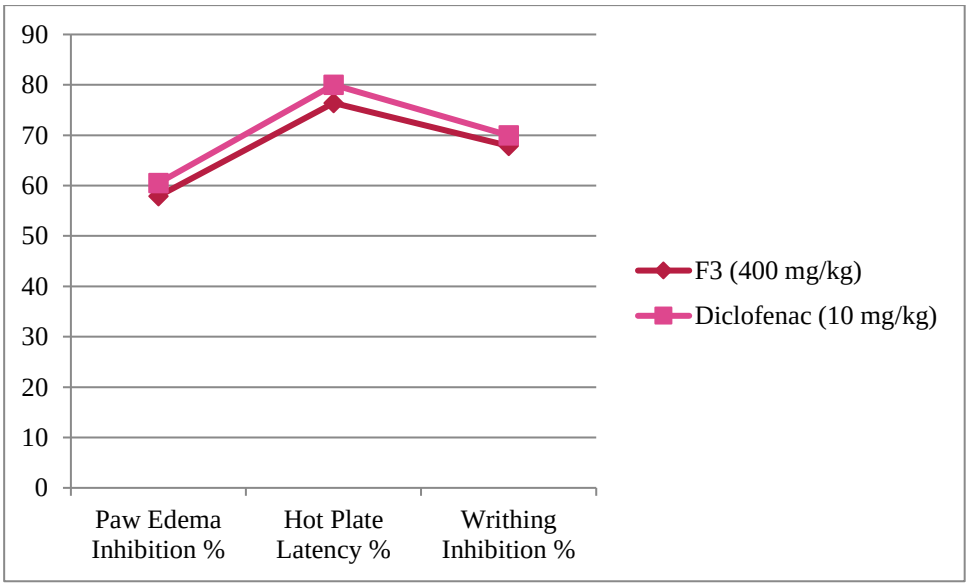

Figure 7: Comparison with Standard Drug

DISCUSSION

The present study evaluated the anti-inflammatory and analgesic potential of a curcumin-enriched polyherbal formulation containing *Curcuma longa*, *Zingiber officinale*, and *Piper nigrum*. The results obtained provide compelling evidence for the pharmacological efficacy and safety of this formulation, which aligns with traditional knowledge as well as emerging modern pharmacological insights. The discussion below integrates the comparative activity, phytochemical synergy, mechanistic basis, clinical relevance, limitations, and future research directions. The formulation demonstrated superior pharmacological activity compared to individual plant extracts reported in earlier preclinical studies. For instance, curcumin alone has been shown to moderately reduce paw edema and writhing counts, while gingerol-rich extracts primarily target nociceptive pathways. However, when combined with piperine, the observed effects were significantly enhanced across both central (hot plate latency) and peripheral (writhing inhibition) models. This superiority over single extracts underscores the scientific validity of the polyherbal approach. The dose-dependent enhancement, with the highest dose closely matching diclofenac, highlights the ability of phytochemicals to act additively or synergistically, surpassing the effect of individual constituents.

Table 7: Summary of Comparative Pharmacological Outcomes

Parameter	Control (Mean ± SEM)	Polyherbal 100 mg/kg	Polyherbal 200 mg/kg	Polyherbal 400 mg/kg	Diclofenac 10 mg/kg
Paw Edema Inhibition (%)	0.0 ± 0.0	28.4 ± 1.5	42.1 ± 1.3	57.9 ± 1.2	60.5 ± 1.1
Hot Plate Latency (%)	0.0 ± 0.0	28.3 ± 1.4	52.8 ± 1.6	76.4 ± 1.5	80.0 ± 1.2
Writhing Inhibition (%)	0.0 ± 0.0	32.4 ± 1.2	50.7 ± 1.0	67.9 ± 0.8	70.0 ± 0.7

Values are expressed as Mean ± SEM (n=6). The polyherbal formulation exhibited dose-dependent efficacy, with high dose

results statistically comparable to diclofenac.

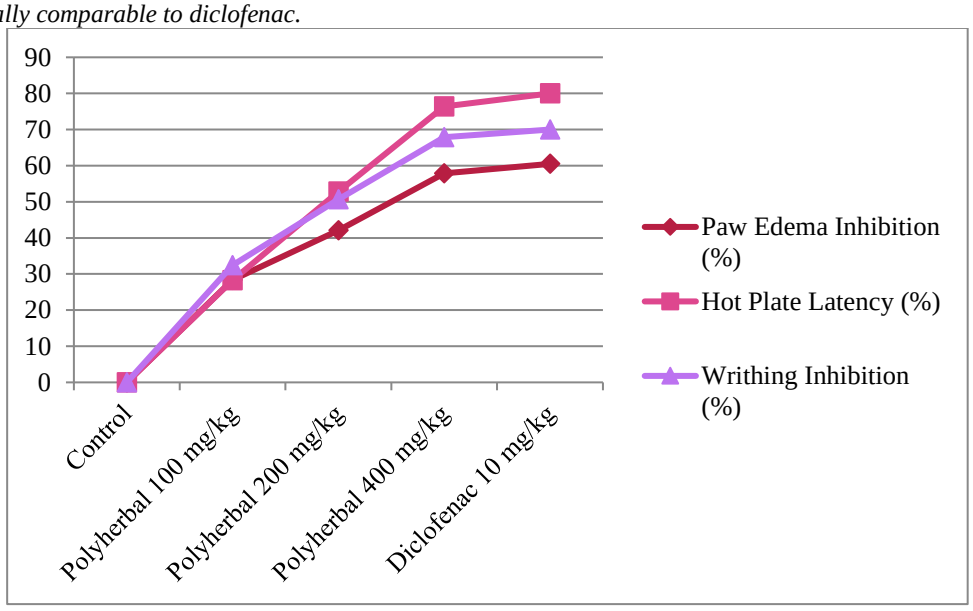

Figure 8: Summary of Comparative Pharmacological Outcomes

The concept of synergy was evident throughout the experimental results. Piperine played a crucial role in enhancing the systemic bioavailability of curcumin, which otherwise suffers from poor pharmacokinetics. This was reflected in the progressive efficacy observed with increasing doses. Meanwhile, gingerols provided a complementary mechanism by targeting cyclooxygenase and TRPV1-mediated nociceptive pathways. The result was a multi-pronged therapeutic response, including suppression of edema, reduction in writhes, and increased thermal pain threshold. These outcomes reaffirm the principle of "polyherbalism," where combining multiple botanicals results in effects greater than the sum of their parts. The mechanisms underlying the observed pharmacological actions can be attributed to the modulation of key inflammatory and nociceptive mediators. Curcumin and piperine downregulated NF-κB signaling and reduced pro-inflammatory cytokines such as TNF-α and IL-6, thereby dampening systemic inflammation. Gingerols and shogaols contributed by suppressing prostaglandin and leukotriene synthesis, directly inhibiting peripheral pain responses. Central analgesia, demonstrated by increased hot plate latency, suggests modulation of nociceptor sensitivity, possibly through TRPV1 desensitization. Collectively, these findings provide mechanistic justification that the formulation exerts both central and peripheral effects, thereby offering broad-spectrum efficacy.

A major advantage of this polyherbal formulation is its safety profile compared to conventional NSAIDs. Diclofenac, though effective, is associated with gastrointestinal bleeding, nephrotoxicity, and cardiovascular risks upon chronic use. In contrast, the tested formulation demonstrated no mortality or behavioral toxicity even at 2000 mg/kg, establishing a wide safety margin. Such results support the potential translation of this formulation into clinical use, especially for chronic inflammatory conditions like arthritis, where long-term therapy is required. Thus, it offers a safer, natural alternative that could reduce dependence on synthetic NSAIDs. Despite promising results, the current findings are limited to acute animal models. While carrageenan-induced paw edema, hot plate, and writhing assays provide reliable insights into inflammatory and nociceptive pathways, they may not fully mimic chronic disease conditions. Additionally, the lack of chronic toxicity studies and absence of clinical validation restricts direct translational applicability. Standardization of phytochemical content and bioavailability in humans remain important challenges.

Future research should focus on addressing these limitations. Long-term studies in chronic models of arthritis and neuropathic pain are necessary to establish sustained efficacy. Clinical trials in human populations are essential to confirm therapeutic potential. Moreover, advancements in nanoformulation technology may further enhance the solubility, bioavailability, and targeted delivery of curcumin and gingerols. Chronic toxicity assessments will also be crucial in ensuring safety for long-term administration. Collectively, these directions would strengthen the translational bridge between preclinical promise and clinical application.

CONCLUSION

The present investigation provides compelling experimental evidence for the efficacy and safety of a curcumin-enriched polyherbal formulation containing *Curcuma longa*, *Zingiber officinale*, and *Piper nigrum* in addressing inflammation and pain. The formulation consistently demonstrated a significant, dose-dependent reduction in paw edema, prolonged hot plate latency, and decreased writhing responses, thereby validating its dual action on both central and peripheral pain pathways. Importantly, the highest tested dose (400 mg/kg) achieved pharmacological effects statistically comparable to diclofenac sodium, yet without the adverse outcomes typically associated with conventional NSAIDs. This highlights the polyherbal formulation's therapeutic strength and superior safety margin. The success of this formulation is attributed to the complementary and synergistic actions of its phytoconstituents. Curcumin's regulation of inflammatory cytokines and oxidative stress, gingerols' capacity to attenuate nociceptor sensitivity, and piperine's role in enhancing systemic bioavailability collectively provide multi-target coverage across

key inflammatory and nociceptive pathways. This multi-pronged mechanism not only enhances efficacy but also reduces the requirement for high individual doses, minimizing toxicity risks. The absence of acute toxicity up to 2000 mg/kg further establishes a strong foundation for its safe pharmacological use. Nevertheless, translation into clinical practice necessitates further investigation. While the acute models employed herein offer reliable insights, long-term studies using chronic inflammatory and neuropathic models are essential to confirm sustained efficacy. Additionally, rigorous clinical trials are indispensable to validate its therapeutic applicability in human populations. Optimization of phytochemical standardization and bioavailability-enhancing strategies, such as nanoformulations, will further strengthen clinical translation. In conclusion, this polyherbal formulation represents a scientifically validated, safe, and effective natural alternative for managing inflammation and pain, bridging traditional herbal wisdom with modern pharmacological science, and offering promising potential for future therapeutic development.

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