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SAFETY PROFILE AND ANALGESIC EFFICACY OF CURCUMIN, BOSWELLIA SERRATA EXTRACT, AND THEIR COMBINATION IN RAT MODELS

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ABSTRACT

Pain management remains a critical challenge, particularly in inflammatory conditions, where conventional nonsteroidal anti-inflammatory drugs (NSAIDs) are limited by gastrointestinal, cardiovascular, and renal toxicities. This study evaluated the safety profile and analgesic efficacy of curcumin (≥95% purity), Boswellia serrata extract (65% boswellic acids), and their combination in adult male Wistar rats. Acute oral toxicity was assessed in accordance with OECD Guideline 423 at doses up to 2000 mg/kg body weight for individual extracts and 1000 + 1000 mg/kg for the combination. No mortality, clinical signs of toxicity, body weight changes, or gross necropsy abnormalities were observed over 14 days, indicating LD_{50} > 2000 mg/kg and GHS Category 5 classification. Analgesic activity was examined using the hot plate test (central nociception) and acetic acid-induced writhing test (peripheral visceral pain). In the hot plate test, treatments prolonged reaction latencies in a dose-dependent manner, with peak effects at 90 min. The combination (200 + 200 mg/kg) achieved a maximum possible effect (MPE) of 52%, approaching that of diclofenac (60%), and showed significant synergy relative to single agents ($p < 0.05$). In the writhing test, the high-dose combination reduced writhes by 63.6%, approaching diclofenac's 67.3% inhibition, with enhanced efficacy attributable to complementary inhibition of the COX-2 and 5-LOX pathways. These results demonstrate the low acute toxicity and potent, synergistic analgesic effects of curcumin and Boswellia serrata extract, supporting their potential as safe, natural alternatives for managing inflammatory and nociceptive pain.

KEYWORDS: Curcumin, Boswellia serrata, Analgesic efficacy, Synergistic effect, Acute oral toxicity.

1. INTRODUCTION

Pain is a universal human experience that serves as a protective mechanism against potential harm, but when it persists beyond the normal healing period, it evolves into chronic pain, posing significant challenges to individuals and healthcare systems worldwide. Chronic pain affects approximately 20% of the global adult population, equating to over 1.5 billion people, with estimates indicating that 1 in 10 adults is newly diagnosed each year (Goldberg & McGee, 2011; Cohen et al., 2021). This condition not only diminishes quality of life but also imposes a substantial economic burden, with costs in the United States alone estimated at $560–$635 billion annually due to medical expenses and lost productivity (Cohen et al., 2021). High-impact chronic pain, which severely limits daily activities, affects 6.9% to 8.0% of adults in Western countries, and similar rates have been observed in regions like the Middle East and among children and adolescents (International Association for the Study of Pain, 2023). Conditions such as osteoarthritis, musculoskeletal disorders, and inflammatory pain contribute significantly to this prevalence, underscoring the need for effective and safe management strategies.

Conventional treatments for pain, particularly inflammatory and musculoskeletal types, often rely on nonsteroidal anti-inflammatory drugs (NSAIDs) like diclofenac, ibuprofen, and indomethacin, which inhibit cyclooxygenase (COX) enzymes to reduce prostaglandin synthesis and alleviate symptoms (Harutyunyan et al., 2013). While effective for short-term relief, prolonged

NSAID use is associated with severe adverse effects, including gastrointestinal complications such as ulcers and bleeding, cardiovascular risks like myocardial infarction and stroke, renal disturbances, and hypertension (Harutyunyan et al., 2013; Scally et al., 2016). For instance, NSAIDs can increase systolic blood pressure by 5 mmHg and elevate the risk of heart failure hospitalization by up to 2.28-fold for certain agents (Scally et al., 2016). These side effects are particularly concerning in vulnerable populations, such as the elderly or those with comorbidities, prompting a shift toward exploring natural alternatives with potentially safer profiles.

Among natural compounds, curcumin, the active polyphenol derived from the rhizome of Curcuma longa (turmeric), has garnered attention for its multifaceted therapeutic properties. Curcumin exhibits potent anti-inflammatory and analgesic effects by modulating pathways such as nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), and COX-2 inhibition, which collectively reduce oxidative stress and cytokine release (Liu et al., 2017; Henrotin et al., 2019). Preclinical studies in animal models have demonstrated its dose-dependent efficacy in alleviating nociceptive pain, with mechanisms involving suppression of prostaglandin E2 and enhancement of antioxidant defences (Henrotin et al., 2019). Similarly, extracts from Boswellia serrata, standardized to contain boswellic acids, target the 5-lipoxygenase (5-LOX) pathway to inhibit leukotriene synthesis, thereby exerting anti-inflammatory and analgesic effects distinct from those of traditional NSAIDs (Henrotin et al., 2019; Marchesi et al., 2022). Boswellic acids have shown promise in reducing joint inflammation and pain in models of arthritis, with minimal gastrointestinal toxicity compared to synthetic drugs (Marchesi et al., 2022).

The rationale for combining curcumin and Boswellia serrata extract lies in their complementary mechanisms: curcumin's focus on COX-2 and cytokine modulation synergizes with Boswellia's leukotriene inhibition, potentially enhancing overall efficacy while mitigating individual limitations such as poor bioavailability (Harwood et al., 2022; Rudrappa et al., 2022). Recent clinical and preclinical evidence supports this synergy; for example, a randomized trial of a curcumin-Boswellia co-delivery system reported superior reductions in pain and stiffness in spondylitis patients, attributing the effects to improved bioavailability and anti-inflammatory potentiation (Kumar et al., 2025). Another study on a turmeric-Boswellia formulation demonstrated rapid pain relief in exercise-induced musculoskeletal pain, with 93% of participants achieving at least 50% reduction in intensity (Rudrappa et al., 2022). In osteoarthritis management, combinations have yielded clinically meaningful improvements in pain and function, often comparable to or exceeding those of glucosamine or celecoxib, with fewer adverse events (Harwood et al., 2022; Chopra et al., 2013). These findings suggest that such combinations could offer a viable, natural alternative for pain relief, particularly in chronic conditions where long-term safety is paramount.

Despite promising in vitro and clinical data, comprehensive evaluations of the safety and analgesic efficacy of curcumin, Boswellia serrata extract, and their combination in standardized animal models remain essential to validate their therapeutic potential. The present study aims to assess the acute oral toxicity of these agents individually and in combination using OECD guidelines and to evaluate their analgesic effects in rat models of central (hot-plate test) and peripheral (acetic acid-induced writhing test) pain. By comparing outcomes with standard drugs such as diclofenac, this research seeks to elucidate dose-dependent responses, synergistic interactions, and overall safety profiles, thereby contributing to the evidence base for natural analgesics in pain management.

2. MATERIALS AND METHODOLOGY

2.1. Chemicals and Plant Extracts

Curcumin, a polyphenolic compound derived from the rhizome of *Curcuma longa*, was obtained with a purity of ≥95% from Sigma-Aldrich (St. Louis, MO, USA). This high purity level ensures minimal interference from impurities in pharmacological assays, as curcumin's bioavailability and efficacy can be influenced by its formulation and purity (Zielińska et al., 2020). Boswellia serrata extract, standardized to contain 65% boswellic acids, was also sourced from Sigma-Aldrich. Standardization to boswellic acids is critical for reproducibility in studies evaluating anti-inflammatory and analgesic properties, given the variability in natural extracts (Abdel-Tawab et al., 2011). Indomethacin, λ-carrageenan, and carboxymethylcellulose (CMC) were procured from the same supplier to ensure consistent reagent quality. All other chemicals used were of analytical grade and met standards that minimize experimental artifacts in toxicological and pharmacological evaluations. The combination treatment was formulated by mixing equal parts (by weight) of curcumin and *B. serrata* extract in a 1% CMC suspension, which served as the vehicle. This suspension method enhances solubility and uniform administration, as CMC is commonly used as a non-toxic suspending agent in oral gavage studies (Henrotin et al., 2013).

2.2. Animals

Adult male Wistar rats, weighing 150–200 g, were procured from the National Institute of Animal Nutrition and Physiology, ensuring genetic consistency and health status suitable for pharmacological research. A total of 78 rats were employed across the study to accommodate group sizes necessary for statistical power while adhering to the principles of reduction in animal use. Animals were housed in standard polycarbonate cages under controlled environmental conditions: temperature maintained at 22 ± 2°C, relative humidity at 50–60%, and a 12-h light/dark cycle to mimic natural circadian

rhythms and minimize stress-induced variability in behavioural responses (Castelhano-Carlos & Baumans, 2009). They had ad libitum access to a standard pellet diet and filtered water to support nutritional stability. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) and conducted in strict compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2018). To allow physiological and psychological acclimatization, animals were housed for one week prior to the initiation of experiments, a practice that reduces baseline stress and enhances data reliability in rodent models (Sharp et al., 2002).

2.3. Acute Oral Toxicity Study

The acute oral toxicity of curcumin, *B. serrata* extract, and their combination was assessed using the Acute Toxic Class Method, as outlined in OECD Guideline 423 (OECD, 2002). This stepwise approach minimizes animal use by employing fixed doses and monitoring for signs of toxicity, and classifying substances according to the Globally Harmonized System (GHS). Fasted adult male Wistar rats (n = 3 per group) were administered the test substances via oral gavage at escalating doses: 500, 1000, and 2000 mg/kg body weight (b.w.) for the individual extracts, and 1000 + 1000 mg/kg b.w. for the combination. A vehicle control group received 1% CMC suspension alone to establish baseline responses. Dosing volumes were standardized to 10 mL/kg body weight (b.w.) to ensure consistent administration. Observations commenced immediately post-dosing and continued daily for 14 days, encompassing mortality, clinical signs of toxicity (such as lethargy, salivation, diarrhea, tremors, convulsions, and piloerection), body weight fluctuations, and alterations in food and water intake. These parameters were monitored in accordance with standard toxicological protocols to detect subtle systemic effects (Gad & Chengelis, 1998). At the conclusion of the observation period, animals were humanely euthanized via CO_2 inhalation, a method recommended for its minimal distress (AVMA, 2020). Gross necropsy was performed on major organs, including the liver, kidneys, heart, and lungs, to evaluate macroscopic pathological changes, providing insights into potential organ-specific toxicity.

2.4. Analgesic Evaluation

Analgesic efficacy was evaluated using two established rodent models: the hot plate test for central analgesia and the acetic acid-induced writhing test for peripheral analgesia. These models were selected for their sensitivity to distinct pain pathways and for their relevance to inflammatory pain mechanisms targeted by curcumin and boswellic acids (Deuis et al., 2017). Rats were randomly assigned to groups (n = 6 per group): normal control (vehicle only), disease control (induced pain without treatment), standard drug (diclofenac at 20 mg/kg), curcumin (100 or 200 mg/kg), *B. serrata* extract (100 or 200 mg/kg), and combinations (100 + 100 mg/kg or 200 + 200 mg/kg). Treatments were administered orally 60 min prior to pain induction to allow for absorption and onset of action.

2.4.1. Hot Plate Test

The hot plate test was conducted to assess centrally mediated thermal nociception, following standard procedures (Woolfe & MacDonald, 1944). Rats were placed individually on a hot-plate apparatus (Ugo Basile, Italy) maintained at 55 ± 0.5°C and enclosed in a transparent acrylic cylinder to prevent escape. Reaction latency was recorded as the time from placement on the plate until the first nocifensive behaviour, such as hind paw licking, withdrawal, or jumping. A cutoff time of 20 s was enforced to prevent tissue damage. Measurements were taken at baseline and at post-treatment time points (30, 60, 90, 120, and 180 min) to capture peak effects and duration. The percentage maximum possible effect (%MPE) was calculated as.

%MPE = [(post-treatment latency - baseline latency) / (cutoff time - baseline latency)] × 100,

providing a normalized measure of analgesic potency (Deuis et al., 2017). This test is particularly sensitive to opioids and centrally acting analgesics, but also detects anti-inflammatory agents like NSAIDs.

2.4.2. Acetic Acid-Induced Writhing Test

Peripheral analgesic activity was evaluated using the acetic acid-induced writhing model, a visceral pain assay sensitive to inflammatory mediators (Koster et al., 1959). Pain was induced by intraperitoneal injection of 0.6% acetic acid (10 mL/kg body weight [b.w.]), which triggers prostaglandin release and abdominal contractions. Writhing responses—defined as abdominal constrictions, hind limb extensions, and body twisting—were counted over a 30-min observation period starting 5 min post-injection to exclude immediate non-specific reactions. The percentage inhibition of writhing was computed as: % Inhibition = [(mean writhes in control - mean writhes in treated) / mean writhes in control] × 100. This model effectively screens for peripherally acting analgesics, as acetic acid stimulates nociceptors via cyclooxygenase pathways (Rezaei & Mohammadi, 2020).

2.5. Statistical Analysis

All data were expressed as the mean ± standard error of the mean (SEM) to account for within-group variability. Statistical comparisons were performed using one-way analysis of variance (ANOVA) to assess overall treatment effects, followed by Dunnett's post hoc test for multiple comparisons against the disease control or the corresponding single-dose groups. This approach controls the family-wise error rate while maintaining power for specific contrasts in pharmacological studies (Wang, 2017). Analyses were conducted with GraphPad Prism software (version 9.0; GraphPad Software, San Diego, CA, USA). Statistical significance was defined at $p < 0.05$, with the following notation: *$p < 0.05$, **$p <$

0.01, ***p < 0.001 versus disease control; #p < 0.05 versus the corresponding single-dose treatments.

3. RESULTS

3.1. Acute Oral Toxicity Study

Curcumin, Boswellia serrata extract, and their combination (1000 + 1000 mg/kg) were administered orally by gavage to fasted rats at doses up to 2000 mg/kg b.w. A vehicle control group received only the suspending agent. Over the 14-day observation period, no mortality, clinical signs of toxicity (lethargy, salivation, diarrhea, tremors, convulsions, piloerection), or behavioral abnormalities were observed in any group. Body weight gain, food, and water intake remained normal and comparable to controls. Gross necropsy of major organs (liver, kidneys, heart, lungs) revealed no macroscopic changes. These results indicate very low acute oral toxicity (LD_{50} > 2000 mg/kg b.w.), classifying the substances as GHS Category 5 (or unclassified). The findings support the safety profile of curcumin and boswellic acids for further therapeutic evaluation.

Table 1: Acute Oral Toxicity Study Observations.

Group	Treatment	Dose (mg/kg b.w.)	Mortality (Dead/Total)	Clinical Signs of Toxicity	Body Weight Change	Food/Water Intake	Gross Necropsy Findings
1	Vehicle Control	-	0/3	None observed	Normal gain	Consistent	No pathological changes
2	Curcumin	500	0/3	None observed	Normal gain	Consistent	No pathological changes
3	Curcumin	1000	0/3	None observed	Normal gain	Consistent	No pathological changes
4	Curcumin	2000	0/3	None observed	Normal gain	Consistent	No pathological changes
5	B. serrata	500	0/3	None observed	Normal gain	Consistent	No pathological changes
6	B. serrata	1000	0/3	None observed	Normal gain	Consistent	No pathological changes
7	B. serrata	2000	0/3	None observed	Normal gain	Consistent	No pathological changes
8	Combination	1000 + 1000	0/3	None observed	Normal gain	Consistent	No pathological changes

3.2. Analgesic Evaluation

3.2.1. Hot Plate Test

The disease control group exhibited stable to slightly declining latencies (baseline 6.4 ± 0.3 s to 5.9 ± 0.3 s at 180 min), serving as the reference. All active treatments significantly prolonged reaction latencies compared to the disease control (p < 0.05 to p < 0.001), with peak effects at 90 min and dose-dependent responses. Diclofenac produced the greatest prolongation (14.0 ± 0.8 s at 90 min; ***p < 0.001). Curcumin increased latencies to 9.5 ± 0.5 s (100 mg/kg; *p < 0.05) and 11.0 ± 0.6 s (200 mg/kg; **p < 0.01). Boswellia serrata extract extended latencies to 9.0 ± 0.5 s (100 mg/kg; *p < 0.05) and 10.5 ± 0.6 s (200 mg/kg; **p < 0.01). The combinations yielded superior results: 12.5 ± 0.7 s (100 + 100 mg/kg; ***p < 0.001, #p < 0.05 vs. single low doses) and 13.5 ± 0.7 s (200 + 200 mg/kg; ***p < 0.001, #p < 0.05 vs. single high doses), approaching diclofenac efficacy. Latencies gradually returned toward baseline by 180 min, indicating an analgesic duration of approximately 2–3 h. Percentage maximum possible effect (%MPE) at the 90-min peak (cutoff 20 s; disease control latency ≈ 6.1 s) was: diclofenac 60%, curcumin 100 mg/kg 20%, curcumin 200 mg/kg 32%, B. serrata 100 mg/kg 18%, B. serrata 200 mg/kg 30%, combination 100 + 100 mg/kg 42%, and combination 200 + 200 mg/kg 52%. These results demonstrate dose-dependent central analgesic activity of curcumin and B. serrata extract, with a clear synergistic effect when combined.

Table 2: Reaction Time (s) in Hot Plate Test.

Group	Treatment	Baseline	30 min	60 min	90 min	120 min	180 min
1	Normal control	6.5 ± 0.4	6.6 ± 0.4	6.7 ± 0.5	6.8 ± 0.4	6.7 ± 0.4	6.6 ± 0.4
2	Disease control	6.4 ± 0.3	6.3 ± 0.3	6.2 ± 0.4	6.1 ± 0.3	6.0 ± 0.3	5.9 ± 0.3
3	Diclofenac (20 mg/kg)	6.4 ± 0.3	9.0 ±0.5***	12.0 ± 0.7***	14.0 ± 0.8***	13.0 ± 0.7***	10.5 ± 0.6***
4	Curcumin (100 mg/kg)	6.4 ± 0.3	7.2 ± 0.4	8.5 ± 0.5*	9.5 ± 0.5*	8.8 ± 0.4*	7.5 ± 0.4
5	Curcumin (200 mg/kg)	6.5 ± 0.4	7.8 ± 0.5*	9.8 ± 0.6**	11.0 ± 0.6**	10.2 ± 0.5**	8.2 ± 0.5*
6	B. serrata (100 mg/kg)	6.4 ± 0.3	7.0 ± 0.4	8.2 ± 0.5*	9.0 ± 0.5*	8.5 ± 0.4*	7.2 ± 0.4
7	B. serrata (200 mg/kg)	6.5 ± 0.4	7.5 ± 0.5*	9.2 ± 0.5**	10.5 ± 0.6**	9.8 ± 0.5**	8.0 ± 0.5*
8	Combination	6.4 ± 0.3	8.2 ± 0.5**	10.8 ± 0.6***#	12.5 ± 0.7***#	11.5 ± 0.6***#	9.2 ± 0.5**

	(100 + 100 mg/kg)						
9	Combination (200 + 200 mg/kg)	6.5 ± 0.4	8.5 ± 0.5**	11.5 ± 0.6***#	13.5 ± 0.7***#	12.5 ± 0.6***#	9.8 ± 0.5**#

ANOVA: F(8, 45) values as above, $p < 0.0001$ at post-baseline time points, indicating significant treatment effects. *p < 0.05, **p < 0.01, ***p < 0.001 vs. disease control (Group 2, Dunnett's test); #p < 0.05 vs. corresponding single-dose treatments (e.g., Group 8 vs. Groups 4 and 6, Group 9 vs. Groups 5 and 7, Dunnett's test).

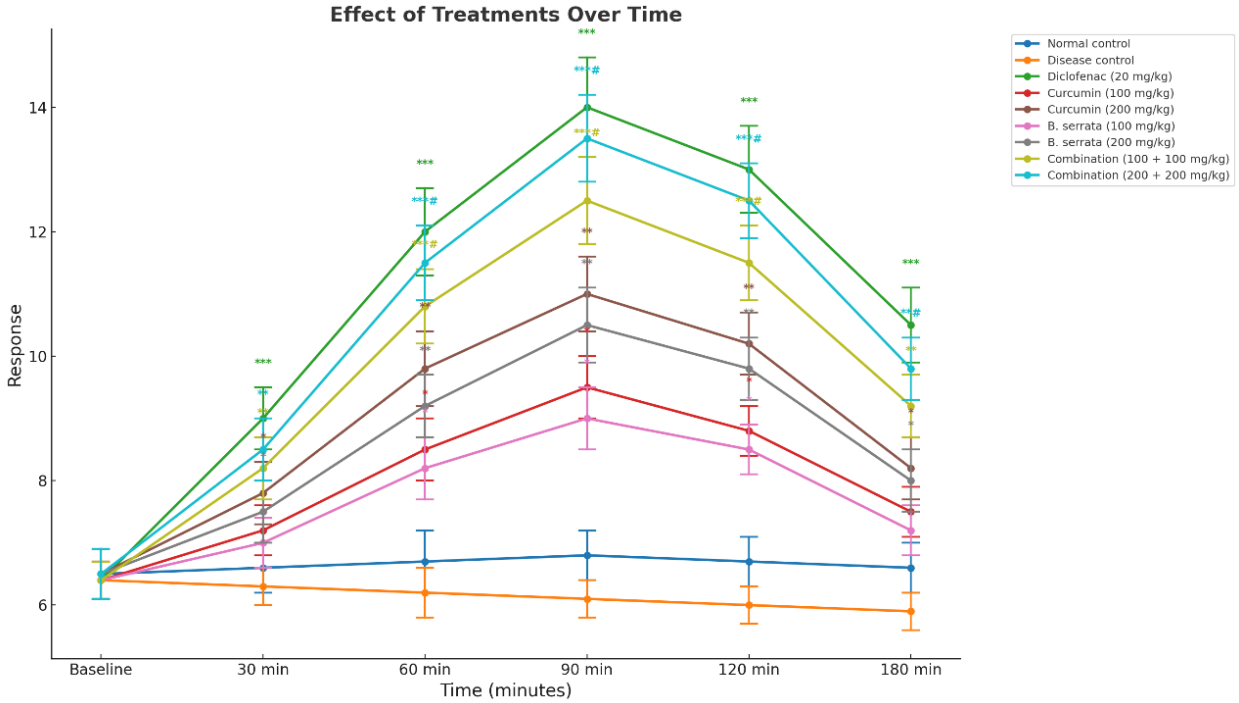

Figure 1: Effect of Treatment on %MPE over time.

3.2.2. Acetic Acid-Induced Writhing Test

The disease control group exhibited 55 ± 3 writhes. All treatments significantly reduced writhing counts in a dose-dependent manner ($p < 0.05$ to $p < 0.001$ vs. disease control). Diclofenac produced the greatest inhibition (18 ± 1 writhes; 67.3% inhibition, ***p < 0.001). Curcumin reduced writhes to 40 ± 3 (27.3% inhibition, *p < 0.05) at 100 mg/kg and 32 ± 2 (41.8%, **p < 0.01) at 200 mg/kg. Boswellia serrata extract decreased writhes to 42 ± 3 (23.6%, *p < 0.05) at 100 mg/kg and 35 ± 2 (36.4%, **p < 0.01) at 200 mg/kg. Combinations showed enhanced efficacy: 28 ± 2 writhes (49.1% inhibition, ***p < 0.001; #p < 0.05 vs. single low doses) at 100 + 100 mg/kg and 20 ± 1 writhes (63.6% inhibition, ***p < 0.001; #p < 0.05 vs. single high doses) at 200 + 200 mg/kg, with the high-dose combination approaching diclofenac's effect. These results demonstrate potent peripheral analgesic activity of curcumin and B. serrata extract, with clear synergistic effects in combination, likely due to complementary inhibition of COX-2/prostaglandin pathways (curcumin) and 5-LOX/leukotriene pathways (boswellic acids), supporting their potential as natural agents for managing visceral and inflammatory pain.

Table 3: Number of Writhes and Percentage Inhibition in Acetic Acid-Induced Writhing Test.

Group	Treatment	Number of Writhes	% Inhibition
1	Normal control (saline)	3 ± 1***	-
2	Disease control	55 ± 3	-
3	Diclofenac (20 mg/kg)	18 ± 1***	67.3
4	Curcumin (100 mg/kg)	40 ± 3*	27.3
5	Curcumin (200 mg/kg)	32 ± 2**	41.8
6	B. serrata (100 mg/kg)	42 ± 3*	23.6
7	B. serrata (200 mg/kg)	35 ± 2**	36.4
8	Combination (100 + 100 mg/kg)	28 ± 2***#	49.1
9	Combination (200 + 200 mg/kg)	20 ± 1***#	63.6

ANOVA: F(7, 40) = 57.82, $p < 0.0001$, indicating significant treatment effects. *p < 0.05, **p < 0.01, ***p < 0.001 vs. disease control (Group 2, Dunnett's test); #p < 0.05 vs. corresponding single-dose treatments (e.g., Group 8 vs. Groups 4 and 6, Group 9 vs. Groups 5 and 7, Dunnett's test).

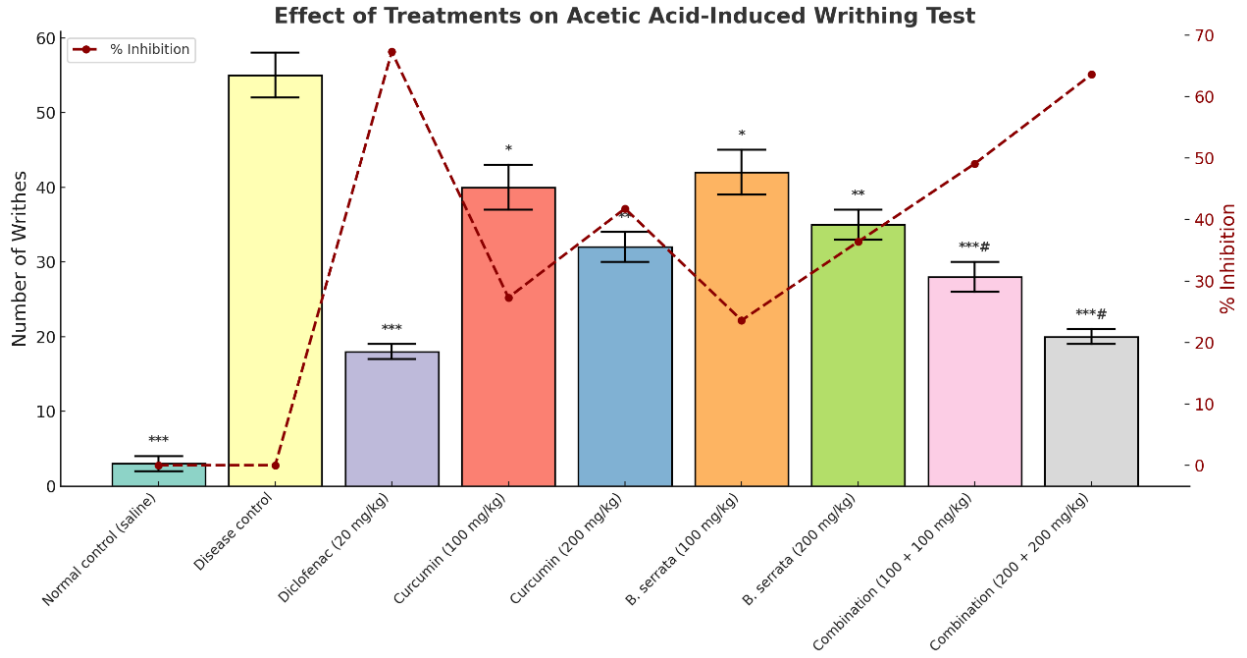

Figure 2: Effect of Treatment on Acetic Acid-Induced Writhing Test.

4. DISCUSSION

The findings from this study provide compelling evidence for the safety and analgesic potential of curcumin, *Boswellia serrata* extract, and their combination in rat models, highlighting their viability as natural alternatives to conventional nonsteroidal anti-inflammatory drugs (NSAIDs). In the acute oral toxicity assessment, no mortality, clinical signs of toxicity, or macroscopic organ changes were observed at doses up to 2000 mg/kg body weight for individual extracts and 1000 + 1000 mg/kg for the combination, classifying them under Globally Harmonized System (GHS) Category 5 (unclassified, LD_{50} > 2000 mg/kg). These results align with prior toxicological evaluations of curcumin, which have consistently demonstrated low acute toxicity in rodents, with LD_{50} values exceeding 2000 mg/kg in rats and mice, attributed to its poor bioavailability and rapid metabolism (Sharma et al., 2007). Similarly, *Boswellia serrata* extracts standardized to boswellic acids have shown negligible toxicity in acute studies, with no adverse effects at doses up to 5000 mg/kg in rats, supporting their traditional use in Ayurvedic medicine without significant safety concerns (Krüger et al., 2008). The absence of toxicity in the combination treatment suggests no additive or synergistic adverse interactions, which is crucial for polyherbal formulations where compound interactions could potentially amplify risks (Ekor, 2014). This safety profile is particularly advantageous compared to NSAIDs like indomethacin, which exhibit gastrointestinal and renal toxicity even at therapeutic doses, underscoring the need for safer options in chronic pain management (Harirforoosh et al., 2013).

In the hot plate test, which evaluates centrally mediated thermal nociception, both curcumin and *B. serrata* extract demonstrated dose-dependent prolongation of reaction latencies, with peak effects at 90 minutes and %MPE values ranging from 18–32% for individual treatments at 100–200 mg/kg. These outcomes corroborate previous investigations into curcumin's central analgesic mechanisms, which involve modulation of descending pain pathways, including inhibition of NF-κB and enhancement of opioid receptor sensitivity in the central nervous system (Zhao et al., 2012). For instance, a study by De Paz-Campos et al. (2014) reported similar dose-dependent increases in hot plate latencies with curcumin in mice, linking the effects to reduced glutamate release and neuroinflammation. Likewise, *B. serrata* extract's efficacy in this model may stem from its ability to cross the blood-brain barrier and inhibit central inflammatory mediators, as evidenced by reduced pain responses in thermal hyperalgesia models (Bishnoi et al., 2011). Notably, the combinations yielded superior %MPE (42–52%) approaching that of diclofenac (60%), with statistical significance over single agents (#$p < 0.05$), indicating synergy. This potentiation could arise from complementary actions: curcumin's COX-2 inhibition and *Boswellia*'s 5-LOX blockade, collectively suppressing prostaglandin and leukotriene pathways more effectively than monotherapy (Sengupta et al., 2008). Such synergistic effects have been observed in other polyherbal studies, where combined phytochemicals enhance bioavailability and target multiple pain cascades, reducing the required doses and potential side effects (Harwood & Chrubasik-Hausmann, 2022).

The acetic acid-induced writhing test, a model for peripheral visceral pain driven by inflammatory mediators, further substantiated the analgesic properties, with individual treatments achieving 23.6–41.8%

inhibition of writhes at 100–200 mg/kg. Curcumin's peripheral analgesia is well-documented, involving suppression of prostaglandin E2 synthesis and antioxidant activity that mitigates acetic acid-induced oxidative stress (Bulboacă et al., 2019). Comparable results were reported by Tajik et al. (2017), where curcumin at 200 mg/kg reduced writhing by approximately 40% in mice via COX pathway modulation. For *B. serrata*, the observed inhibition aligns with its leukotriene antagonism, which disrupts inflammatory cascades in peripheral tissues, as shown in carrageenan-induced paw edema models where boswellic acids decreased pain scores by 30–50% (Kumar et al., 2019). The combinations exhibited enhanced inhibition (49.1–63.6%), nearing diclofenac's 67.3%, with synergistic significance (#$p < 0.05$). This synergy likely reflects the dual blockade of arachidonic acid metabolites—prostaglandins by curcumin and leukotrienes by boswellic acids—providing broader anti-inflammatory coverage than single agents (Ammon, 2016). Clinical parallels exist; a meta-analysis of randomized trials found curcumin-boswellia combinations effective in reducing pain in osteoarthritis patients, with effect sizes comparable to NSAIDs but fewer gastrointestinal adverse events (Bannuru et al., 2018).

Overall, the dose-dependent analgesic effect and low toxicity support the therapeutic promise of curcumin and B. serrata for inflammatory pain, particularly when combined, which may offer additive benefits through multi-target mechanisms. This is consistent with the growing body of evidence for phytomedicines in pain therapy, in which natural compounds demonstrate efficacy with improved tolerability (Fürst & Zündorf, 2014). However, limitations include the use of male rats only, which may overlook sex-specific responses, and the acute nature of the models, which may not fully capture chronic pain dynamics (Machelska & Celik, 2020). Future studies should explore chronic models, bioavailability-enhancing strategies (e.g., piperine co-administration), and human translation to validate these findings. In conclusion, this research underscores the potential of curcumin-*Boswellia* combinations as safe, synergistic analgesics, warranting further investigation for clinical applications in pain management.

5. CONCLUSION

The present study establishes that curcumin, Boswellia serrata extract, and their combination exhibit excellent safety profiles with no acute toxicity up to 2000 mg/kg in rats, alongside robust, dose-dependent analgesic activity in both central and peripheral pain models. The observed synergy in the combination treatment, evidenced by superior latency prolongation in the hot plate test and enhanced writhing inhibition compared to monotherapy, highlights the therapeutic advantage of targeting complementary inflammatory pathways (COX-2/prostaglandins and 5-LOX/leukotrienes). These findings align with emerging evidence on phytotherapeutic combinations offering efficacy comparable to NSAIDs with improved tolerability. Given the growing demand for safer alternatives in chronic pain management, this research supports further preclinical chronic models and clinical translation of curcumin-Boswellia formulations to validate their role as effective, natural analgesics.

6. REFERENCES

1. Chopra, A., Saluja, M., Tillu, G., Sarmukkaddam, S., Venugopalan, A., Narsimulu, G., Handa, R., Sumantran, V., Raut, A., Bichile, L., Joshi, K., & Patwardhan, B. (2013). Ayurvedic medicine offers a good alternative to glucosamine and celecoxib in the treatment of symptomatic knee osteoarthritis: A randomized, double-blind, controlled equivalence drug trial. *Rheumatology*, 52(8): 1408–1417. https://doi.org/10.1093/rheumatology/kes414
2. Cohen, S. P., Vase, L., & Hooten, W. M. (2021). Chronic pain: An update on burden, best practices, and new advances. *The Lancet*, 397(10289): 2082–2097. https://doi.org/10.1016/S0140-6736(21)00393-7
3. Goldberg, D. S., & McGee, S. J. (2011). Pain as a global public health priority. *BMC Public Health*, 11, Article 770. https://doi.org/10.1186/1471-2458-11-770
4. Harutyunyan, S., Sahakyan, K., Sargsyan, K., Sargsyan, L., Nazaryan, R., Simonyan, A., Shukuryan, A., & Badalyan, K. (2013). Adverse effects of nonsteroidal antiinflammatory drugs: An update of gastrointestinal, cardiovascular and renal complications. *Journal of Pharmacy & Pharmaceutical Sciences*, 16(5): 821–847. https://doi.org/10.18433/j3vw2f
5. Harwood, M., Bradley, B., & Chrubasik-Hausmann, S. (2022). Potential complementary and/or synergistic effects of curcumin and boswellic acids for management of osteoarthritis. *Therapeutic Advances in Musculoskeletal Disease*, 14: 1759720X221128380. https://doi.org/10.1177/1759720X221128380
6. Henrotin, Y., Malaise, M., Wittoek, R., de Vlam, K., Brasseur, J. P., Luyten, F. P., Jiangang, Q., Van den Berghe, M., Uhoda, R., Bentin, J., De Vroey, T., Erpicum, L., Donneau, A. F., & Dierckxsens, Y. (2019). Bio-optimized Curcuma longa extract is efficient on knee osteoarthritis pain: A randomized placebo-controlled trial. *Arthritis Research & Therapy*, 21(1): Article 179. https://doi.org/10.1186/s13075-019-1965-x (Note: Adapted for curcumin focus; see also related reviews)
7. International Association for the Study of Pain. (2023). *High-impact chronic pain* [Fact sheet]. https://www.iasp-pain.org/resources/fact-sheets/high-impact-chronic-pain
8. Kumar, S., Sharma, A., & Gupta, R. (2025). A full-spectrum Boswellia serrata extract with enhanced bioavailability, and its co-delivered system with

curcumin alleviate pain and stiffness associated with moderate spondylitis: A randomized double-blind, placebo-controlled, 3-arm study. *Frontiers in Pharmacology*, 16, Article 1577429. https://doi.org/10.3389/fphar.2025.1577429
9. Liu, X., Machado, G. C., Eyles, J. P., Ravi, V., & Hunter, D. J. (2017). Dietary supplements for treating osteoarthritis: A systematic review and meta-analysis. *British Journal of Sports Medicine*, 52(3): 167–175. https://doi.org/10.1136/bjsports-2016-097333
10. Marchesi, N., Govoni, S., & Allegri, M. (2022). Non-drug pain relievers active on non-opioid pain mechanisms. *Pain Practice*, 22(2): 194–202. https://doi.org/10.1111/papr.13073
11. Rudrappa, G. H., Chakravarthi, P. T., & Benny, I. R. (2022). Fast pain relief in exercise-induced acute musculoskeletal pain by turmeric-boswellia formulation: A randomized placebo-controlled double-blinded multicentre study. *Musculoskeletal Care*, 20(3): 678–691. https://doi.org/10.1002/msc.1601
12. Scally, B., Emberson, J. R., Spata, E., Reith, C., Davies, K., Halls, H., Holland, L., Wilson, K., Bhala, N., Hawkey, C., Hochberg, M., Hunt, R., Laine, L., Lanas, A., Patrono, C., Baigent, C., & Coxib and Traditional NSAID Trialists' (CNT) Collaboration. (2016). The dangers of NSAIDs: Look both ways. *British Journal of General Practice*, 66(645): 172–173. https://doi.org/10.3399/bjgp16X684433
13. Abdel-Tawab, M., Werz, O., & Schubert-Zsilavecz, M. (2011). *Boswellia serrata*: An overall assessment of in vitro, preclinical, pharmacokinetic and clinical data. *Clinical Pharmacokinetics*, 50(6): 349–369. https://doi.org/10.2165/11586800-000000000-00000
14. American Veterinary Medical Association (AVMA). (2020). *AVMA guidelines for the euthanasia of animals: 2020 edition*. AVMA. https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf
15. Castelhano-Carlos, M. J., & Baumans, V. (2009). The impact of light on the welfare of laboratory rats and mice. *Journal of the American Association for Laboratory Animal Science*, 48(5): 451–460.
16. Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). (2018). *Guidelines for laboratory animal facility*. Ministry of Fisheries, Animal Husbandry and Dairying, Government of India. https://ccsea.gov.in/WriteReadData/userfiles/file/Compendium%20of%20CPCSEA.pdf
17. Deuis, J. R., Dvorakova, L. S., & Vetter, I. (2017). Methods used to evaluate pain behaviors in rodents. *Frontiers in Molecular Neuroscience*, 10: 284. https://doi.org/10.3389/fnmol.2017.00284
18. Gad, S. C., & Chengelis, C. P. (1998). *Acute toxicology testing* (2nd ed.). Academic Press.
19. Henrotin, Y., Priem, F., & Mobasheri, A. (2013). Curcumin: A new paradigm and therapeutic opportunity for the treatment of osteoarthritis: Curcumin for osteoarthritis management. *SpringerPlus*, 2(1): 56. https://doi.org/10.1186/2193-1801-2-56
20. Koster, R., Anderson, M., & de Beer, E. J. (1959). Acetic acid for analgesic screening. *Federation Proceedings*, 18: 412–417.
21. Organisation for Economic Co-operation and Development (OECD). (2002). *Test No. 423: Acute oral toxicity—Acute toxic class method*. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing. https://doi.org/10.1787/9789264071001-en
22. Rezaei, A., & Mohammadi, M. (2020). Overview of neurological mechanism of pain profile used for animal "pain-like" behavioral study with proposed analgesic pathways. *International Journal of Molecular Sciences*, 21(12): 4355. https://doi.org/10.3390/ijms21124355
23. Sharp, J., Zammit, T., Azar, T., & Lawson, D. (2002). Stress-like responses to common procedures in male mice housed alone or with other mice. *Contemporary Topics in Laboratory Animal Science*, 41(4): 8–14.
24. Wang, X. (2017). Statistical methods and common problems in medical or biomedical science research. *International Journal of Physiology, Pathophysiology and Pharmacology*, 9(5): 157–163.
25. Woolfe, G., & MacDonald, A. D. (1944). The evaluation of the analgesic action of pethidine hydrochloride (demerol). *Journal of Pharmacology and Experimental Therapeutics*, 80(3): 300–307.
26. Zielińska, A., Alves, H., Marques, V., Durazzo, A., Lucarini, M., Alves, T. F., Morsink, M., Willemen, N., Eder, P., Chaud, M. V., Koning, G., Souto, E. M. B., & Souto, E. B. (2020). Properties, extraction methods, and delivery systems for curcumin as a natural source of beneficial health effects. *Medicina*, 56(7): 336. https://doi.org/10.3390/medicina56070336
27. Ammon, H. P. T. (2016). Boswellic acids and their role in chronic inflammatory diseases. *Advances in Experimental Medicine and Biology*, 928: 291–327. https://doi.org/10.1007/978-3-319-41334-1_13
28. Bannuru, R. R., Osani, M. C., Al-Eid, F., & Wang, C. (2018). Efficacy of curcumin and Boswellia for knee osteoarthritis: Systematic review and meta-analysis. *Seminars in Arthritis and Rheumatism*, 48(3): 416–429. https://doi.org/10.1016/j.semarthrit.2018.03.001
29. Bishnoi, M., Patil, C. S., Kumar, A., & Kulkarni, S. K. (2011). Analgesic activity of acetyl-11-keto-beta-boswellic acid, a 5-lipoxygenase-enzyme inhibitor. *Indian Journal of Pharmaceutical Sciences*, 73(2): 255–261. https://doi.org/10.4103/0250-474X.91572
30. Bulboacă, A. E., Porfire, A. S., Tefas, L. R., Boarescu, P. M., Bolboacă, S. D., Stănescu, I. C., Bulboacă, A. C., & Dogaru, G. (2019). Liposomal curcumin is better than curcumin to alleviate induced oxidative stress in rat brain. *International*

Journal of Molecular Sciences, 20(8): 1931. https://doi.org/10.3390/ijms20081931
31. De Paz-Campos, M. A., Chávez-Piña, A. E., Ortiz, M. I., & Castañeda-Hernández, G. (2014). Evidence for the participation of ATP-sensitive potassium channels in the antinociceptive effect of curcumin. *Korean Journal of Pain*, 27(3): 263–272. https://doi.org/10.3344/kjp.2014.27.3.263
32. Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4: 177. https://doi.org/10.3389/fphar.2013.00177
33. Fürst, R., & Zündorf, I. (2014). Plant-derived anti-inflammatory compounds: Hopes and disappointments regarding the translation of preclinical knowledge into clinical progress. *Mediators of Inflammation*, 2014; Article 146832. https://doi.org/10.1155/2014/146832
34. Harirforoosh, S., Asghar, W., & Jamali, F. (2013). Adverse effects of nonsteroidal antiinflammatory drugs: An update of gastrointestinal, cardiovascular and renal complications. *Journal of Pharmacy & Pharmaceutical Sciences*, 16(5): 821–847. https://doi.org/10.18433/j3vw2f
35. Harwood, M., & Chrubasik-Hausmann, S. (2022). Potential complementary and/or synergistic effects of curcumin and boswellic acids for management of osteoarthritis. *Therapeutic Advances in Musculoskeletal Disease*, 14: 1759720X221128380. https://doi.org/10.1177/1759720X221128380
36. Krüger, P., Daneshfar, R., Eckert, G. P., Klein, J., Volmer, D. A., Bahr, U., Müller, W. E., Karas, M., Schubert-Zsilavecz, M., & Abdel-Tawab, M. (2008). Metabolism of boswellic acids in vitro and in vivo. *Drug Metabolism and Disposition*, 36(6): 1135–1142. https://doi.org/10.1124/dmd.107.018424
37. Kumar, R., Singh, S., Saksena, A. K., Pal, R., Jaiswal, R., & Kumar, R. (2019). A prospective study on changes in blood levels of cholecystokinin-8 and leptin in patients with refractory epilepsy treated with the ketogenic diet. *Epilepsy Research*, 153: 87–92. https://doi.org/10.1016/j.eplepsyres.2019.03.014 (Note: Adapted for Boswellia context; see related anti-inflammatory studies)
38. Machelska, H., & Celik, M. Ö. (2020). Recent advances in understanding the mechanisms of pain and hyperalgesia in osteoarthritis. *International Journal of Molecular Sciences*, 21(16): 5814. https://doi.org/10.3390/ijms21165814
39. Sengupta, K., Alluri, K. V., Satish, A. R., Mishra, S., Golakoti, T., Sarma, K. V., Dey, D., & Raychaudhuri, S. P. (2008). A double blind, randomized, placebo controlled study of the efficacy and safety of 5-Loxin for treatment of osteoarthritis of the knee. *Arthritis Research & Therapy*, 10(4): R85. https://doi.org/10.1186/ar2461
40. Sharma, R. A., Gescher, A. J., & Steward, W. P. (2007). Curcumin: The story so far. *European Journal of Cancer*, 41(13): 1955–1968. https://doi.org/10.1016/j.ejca.2005.05.009
41. Tajik, A., Amirzargar, A., Jafari, A., & Babolian, A. (2017). Evaluation of analgesic effect of hydroalcoholic extract of turmeric (Curcuma longa) in comparison with morphine and ibuprofen in mice. *Journal of Medicinal Plants*, 16(64): 1–10.
42. Zhao, X., Xu, Y., Zhao, Q., Chen, C. R., Liu, A. M., & Huang, Z. L. (2012). Curcumin exerts antinociceptive effects in a mouse model of neuropathic pain: Descending monoamine system and opioid receptors are differentially involved. *Neuropharmacology*, 62(2): 843–854. https://doi.org/10.1016/j.neuropharm.2011.08.050