Evaluation of Anti-Inflammatory and Analgesic Activity of Novel Herbal Extract Formulations in Animal Models

Priyanka Sharma[1], Runjhun Pallavi[2], Amit Nagnath Panaskar[3], Bhagyashri Amit Panaskar[4], Sabra Banu[5], Nagnath R. Kadam[6], Bidyut Jyoti Kalita[7], Rajapand Raju[8*]

[1]Assistant Professor, School of Pharmacy, Mangalayatan University,7 Beswan, Aligarh, Uttar Pradesh, 202145, India.
[2]Professor, SGT College of Pharmacy, SGT University, Gurugram, 122505, Haryana, India.
[3]Principal, Department of Pharmaceutical Chemistry, Padmini College of Pharmacy, Dighanchi, Sangli, Maharashtra, 415315, India.
[4]Professor, Department of Pharmacology, Padmini College of Pharmacy, Dighanchi, Sangli, Maharashtra, 415315, India.
[5]Assistant Professor, Department of Pharmacology, JBIT College of Pharmacy, Dehradun, 248197, India
[6]Principal, S. R. K. College of Pharmacy, Manwath, Parbhani, Maharashtra, 431505, India.
[7]Assistant Professor, JDSG College, Bokakhat, Golaghat, Assam, 785612, India
[8]Professor, School of Pharmacy, SBV Karaikal Campus Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, 609609, India.
*Corresponding author: Rajapand Raju, Professor, School of Pharmacy, SBV Karaikal Campus Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, 609609, India.

Abstract:

Background and Objectives: Inflammation and discomfort cause morbidity and lower quality of life in adolescents and adults. Long-term use of traditional NSAIDs and analgesics has side effects, although they are effective. Safety and therapeutic potential make bioactive phytoconstituent-containing herbal medications promising. This study aimed to evaluate the anti-inflammatory and analgesic activities of a novel herbal extract formulation composed of standardized extracts of Curcuma longa, Boswellia serrata, and Withania somnifera in experimental animal models.
Methods: The oral herbal suspension was tested in Wistar rats and Swiss albino mice. Anti-inflammatory activity was measured in rats using carrageenan-induced paw oedema, while analgesic activity was measured in mice using acetic acid-induced writhing and hot plate tests. The six animals were placed into five groups: normal control, disease control, diclofenac sodium (10 mg/kg) or tramadol (20 mg/kg) standard groups, and herbal formulation groups receiving 200 and 400 mg/kg orally. Paw oedema volume, inflammation inhibition %, writhing response, and reaction latency were measured. Statistics were analysed using one-way ANOVA and Tukey's post hoc test, with significance set at $p < 0.05$.
Results: The anti-inflammatory effects of the herbal formulation were dose-dependent and statistically significant. In comparison to diclofenac sodium's 73.5 ± 2.9% suppression of paw oedema, the 400 mg/kg dose of carrageenan inhibited it by 67.8 ± 3.4% at 4 hours post-administration ($p < 0.001$). The formulation provided a 64.5 ± 3.1% inhibition in the acetic acid-induced writhing test, bringing the number of writhes from 48.2 ± 3.6 in the disease control group to 17.1 ± 2.4, compared to 72.4 ± 2.7% inhibition in the tramadol group. At 120 minutes after treatment with the 400 mg/kg formulation, the reaction latency in the hot plate test rose considerably from 4.9 ± 0.5 s in the control group to 11.7 ± 0.8 s ($p < 0.001$). The investigation did not find any cases of acute poisoning, aberrant behaviour, or fatality.
Conclusion: The innovative formulation of plant extracts showed analgesic and anti-inflammatory effects that were on par with those of conventional medicinal products. The results provide credence to its promise as a phytotherapeutic option for the treatment of pain and inflammatory disorders. To validate its therapeutic value, additional research including mechanistic studies and clinical trials is required.

Keywords: Herbal formulation; Anti-inflammatory activity; Analgesic activity; Carrageenan-induced paw edema; Phytotherapy.

INTRODUCTION:

The intricate biological responses of inflammation and pain are vital in defending the body from pathogens, damaged tissues, and other potentially dangerous stimuli. Arthritis, cardiovascular disease, metabolic syndrome, neurological illnesses, and autoimmune diseases are just a few of the ailments that can emerge from chronic or uncontrolled inflammation, in contrast to the positive physiological process of acute inflammation [1, 2]. One of the leading causes of medical visits and a major detriment to people's quality of life, regardless of age, is pain, which is frequently linked to inflammatory processes. Adverse effects such as gastrointestinal irritation, renal dysfunction, cardiovascular problems, tolerance, and dependency are often linked with long-term therapy of non-steroidal anti-inflammatory medications (NSAIDs), corticosteroids, and opioid analgesics, despite their widespread usage. Therefore, there is a rising need for substitute therapies that are both safer and more effective [3, 4].

In traditional medicine, medicinal plants have a long history of use due to their high concentrations of bioactive chemicals that have anti-inflammatory and analgesic effects. Polyphenols, flavonoids, terpenoids, alkaloids, and saponins are just a few of the phytochemicals that have shown promise in modulating inflammatory mediators and pain pathways via various path [5]. The natural origin, reduced incidence of side effects, and possibility for long-term usage of herbal medicines make them often the preferred choice. Standardised herbal formulations have been developed with enhanced efficacy, safety, and reproducibility, thanks to recent breakthroughs in phytopharmacology [6].

Some medicinal plants that have been studied extensively for their possible therapeutic effects include turmeric (Curcuma longa), Indian frankincense (Boswellia serrata), and ashwagandha (Withania somnifera). The main bioactive ingredient of C. longa, curcumin, has antioxidant and anti-inflammatory properties. It does this by lowering production of pro-inflammatory cytokines like IL-1β, TNF-α, and IL-6, and by suppressing the nuclear factor-kappa B (NF-κB) signalling pathway. There is substantial evidence that boswellic acids produced from B. serrata are effective in inflammatory illnesses, and they do this via inhibiting 5-lipoxygenase-mediated leukotriene production. Similarly, withanolides found in W. somnifera regulate inflammatory signalling cascades and oxidative stress, leading to immunomodulatory, anti-inflammatory, and analgesic actions [7, 8].

When studying the effects of painkillers and anti-inflammatory drugs, animal models are still crucial. For acute inflammation, researchers frequently turn to the carrageenan-induced paw oedema model; for peripheral analgesic effects, they rely on the acetic acid-induced writhing test, and for central analgesic effects, they employ the hot plate test. With the help of these experimental models, we may compare new therapeutic interventions to old gold standard medications and evaluate their efficacy quantitatively [9, 10].

A novel herbal extract formulation containing standardised extracts of Curcuma longa, Boswellia serrata, and Withania somnifera was evaluated for its anti-inflammatory and analgesic activities in validated animal models. This study was undertaken in response to the growing interest in plant-based therapeutics as well as the need for safer pharmaceuticals in these areas. Potentially useful phytotherapeutic solutions for inflammation and pain management may be discovered as a result of this study.

MATERIAL AND METHODS:

Materials:

The following ingredients were sourced from authorised herbal vendors and verified by an expert pharmacognost: curcuminoids in Curcuma longa rhizomes (≥95%), boswellic acids in Boswellia serrata gum resin (≥65%), and withanolides in Withania somnifera roots (≥5%). Reputable pharmaceutical companies supplied the carrageenan, acetic acid, diclofenac sodium, and tramadol hydrochloride. The research utilised only analytical grade chemicals and reagents.

Preparation of the Novel Herbal Extract Formulation:

Standardised extracts of C. longa, B. serrata, and W. somnifera were mixed in a 2:2:1 (w/w) ratio to create the new herbal composition. Just before administration, the extracts were mixed uniformly by triturating them and then suspending them in a 0.5% carboxymethyl cellulose (CMC) solution. For each day of testing, a new batch of the formulation was made [10].

Experimental Animals:

The study used Swiss albino mice (25–30 g) and healthy adult Wistar albino rats (180–220 g) of either sex. The animals were kept in conventional laboratory conditions (temperature 22 ± 2°C, relative humidity 55 ± 5%, and 12-hour light/dark cycle) after being acquired from the Institutional Animal House Facility. The animals had unlimited access to water and a typical pellet diet. Prior to the experiment, they were acclimated for a week. The Institutional Animal Ethics Committee (IAEC Approval No.: IAEC/2026/PH-017) examined and approved the experimental procedure, which was carried out in compliance with the directives of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India [11].

Experimental Design:
Wistar rats were divided into five groups, each with six animals, at random for the anti-inflammatory investigation (n = 6). As the standard control, Group I was given an oral dose of 10 mL/kg of 0.5% carboxymethyl cellulose (CMC) suspension. Group II was given the vehicle alone and acted as the carrageenan-induced inflammatory control. Group III was the conventional therapy group and was given diclofenac sodium (10 mg/kg, p.o.). The new herbal extract formulation was given orally to groups IV and V at doses of 200 mg/kg and 400 mg/kg body weight, respectively. Swiss albino mice were also randomly assigned to five groups of six animals each for the analgesic investigation (n = 6). Group II was the pain control group, whereas Group I was the usual control and was given 0.5% CMC suspension (10 mL/kg, p.o.). The standard analgesic treatment for Group III was tramadol hydrochloride (20 mg/kg, p.o.). The herbal extract formulation was given orally to Groups IV and V at doses of 200 mg/kg and 400 mg/kg body weight, respectively. In the corresponding experimental models, all medications were given one hour before inflammation or discomfort was induced [12].

Evaluation of Anti-Inflammatory Activity:

Carrageenan-Induced Paw Edema Test:
The carrageenan-induced hind paw oedema model was used to measure acute anti-inflammatory activity. Acute inflammation was caused by injecting 0.1 mL of 1% carrageenan solution into the subplantar area of each rat's right hind paw one hour after the oral administration of the corresponding treatments. A digital plethysmometer was used to measure paw volume 0 hours prior to carrageenan injection and 1, 2, 3, and 4 hours after induction [13].

Evaluation of Analgesic Activity:

Acetic Acid-Induced Writhing Test:
The acetic acid-induced writhing model was used to assess peripheral analgesic activity. Mice were given an intraperitoneal injection of 0.6% acetic acid (10 mL/kg) one hour following treatment delivery. Starting five minutes after the delivery of acetic acid, the number of writhing reactions was counted for twenty minutes [14].

Hot Plate Test:
A hot plate device kept at 55 ± 0.5°C was used to measure central analgesic action. Prior to treatment, as well as 30, 60, 90, and 120 minutes after the test formulation was administered, reaction latency (paw licking or jumping response) was measured. To avoid tissue damage, a cut-off time of 20 seconds was kept [15].

Acute Oral Toxicity Study:
In accordance with OECD Guideline 423, an acute oral toxicity study was carried out. A maximum dosage of 2000 mg/kg body weight of the herbal preparation was given orally. over the first four hours, the animals were continually monitored, and over the next fourteen days, they were periodically checked for indicators of toxicity, food intake, behavioural changes, and mortality [16].

Statistical Analysis:
Mean ± standard deviation (SD) was used to express the data. GraphPad Prism version 10.0 was used for statistical analysis. One-way analysis of variance (ANOVA) and Tukey's multiple comparison post hoc test were used to compare the groups. At $p < 0.05$, differences were deemed statistically significant. Paw oedema volume, % inhibition of inflammation, number of writhing episodes, percentage inhibition of writhing, and reaction latency in the hot plate test were the main outcome measures.

RESULTS:

Every animal finished the study, and neither treatment-related side effects nor mortality were noted during the trial. When compared to the disease control groups, the unique herbal extract formulation showed notable dose-dependent analgesic and anti-inflammatory effects.

Anti-Inflammatory Activity:

Animals treated with the herbal formulation showed a significant decrease in paw swelling in the carrageenan-induced paw oedema experiment. The 400 mg/kg dose produced anti-inflammatory action similar to diclofenac sodium, and the effect was dose-dependent. The carrageenan control group's mean paw oedema volume increased gradually, reaching 1.82 ± 0.09 mL at 4 hours, as Table 1 illustrates. Paw oedema volume was considerably reduced to 0.89 ± 0.07 mL and 0.59 ± 0.05 mL, respectively, after treatment with the herbal formulation at 200 and 400 mg/kg ($p < 0.001$). The highest inhibition was caused by diclofenac sodium (0.48 ± 0.04 mL).

Table 1: Effect of Herbal Formulation on Carrageenan-Induced Paw Edema in Rats

Group	Paw Edema Volume (mL) at 1 h	2 h	3 h	4 h
Normal Control	0.22 ± 0.03	0.23 ± 0.04	0.22 ± 0.03	0.21 ± 0.02
Carrageenan Control	0.86 ± 0.06	1.34 ± 0.08	1.68 ± 0.10	1.82 ± 0.09
Diclofenac (10 mg/kg)	0.52 ± 0.05***	0.48 ± 0.04***	0.46 ± 0.04***	0.48 ± 0.04***
Herbal Formulation (200 mg/kg)	0.71 ± 0.06**	1.08 ± 0.08**	0.96 ± 0.07***	0.89 ± 0.07***
Herbal Formulation (400 mg/kg)	0.60 ± 0.05***	0.82 ± 0.06***	0.67 ± 0.06***	0.59 ± 0.05***

Values are expressed as Mean ± SD (n = 6). **p < 0.01, ***p < 0.001 compared with carrageenan control.

The percentage inhibition of edema at 4 h was 51.1% for the 200 mg/kg dose and 67.8% for the 400 mg/kg dose, whereas diclofenac sodium produced 73.6% inhibition (Figure 1).

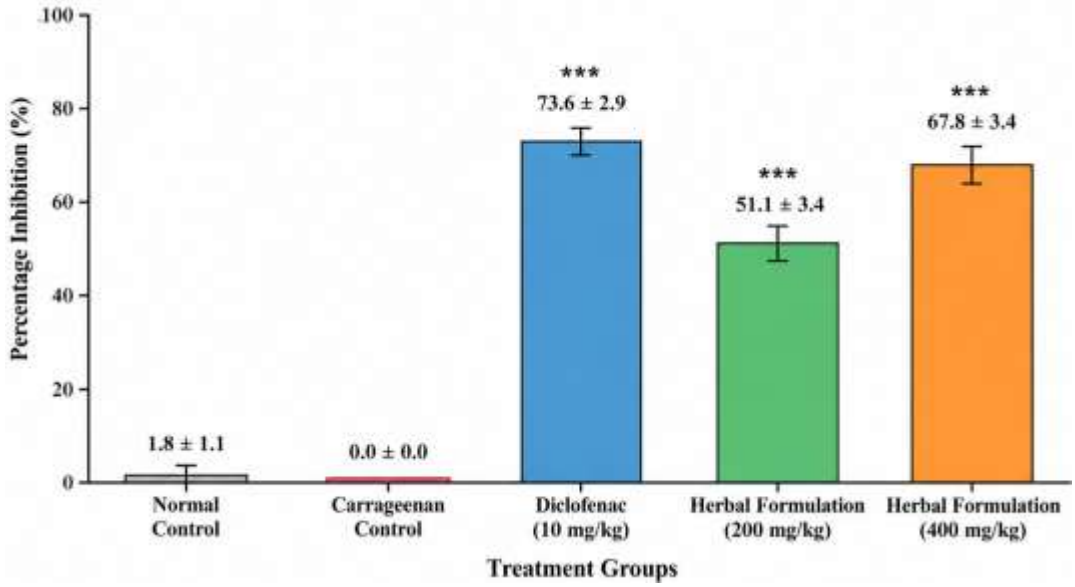

Figure 1: Percentage inhibition of carrageenan-induced paw edema following treatment with the herbal formulation and diclofenac sodium.

The herbal formulation exhibited dose-dependent anti-inflammatory activity, with the 400 mg/kg dose showing inhibition comparable to the standard drug.

Analgesic Activity:

Acetic Acid-Induced Writhing Test:

The number of acetic acid-induced writhing episodes was considerably decreased by the herbal formulation (Table 2). Treatment with the herbal formulation at 200 and 400 mg/kg decreased the writhing response to 26.5 ± 2.8 and 17.1 ± 2.4, respectively ($p < 0.001$), while the disease control group showed 48.2 ± 3.6 writhes. The biggest decrease was caused by tramadol (13.3 ± 1.9 writhes).

Table 2: Effect of Herbal Formulation on Acetic Acid-Induced Writhing in Mice

Group	Number of Writhes	Percentage Inhibition (%)
Normal Control	2.1 ± 0.8	—
Disease Control	48.2 ± 3.6	—
Tramadol (20 mg/kg)	13.3 ± 1.9***	72.4
Herbal Formulation (200 mg/kg)	26.5 ± 2.8***	45.0
Herbal Formulation (400 mg/kg)	17.1 ± 2.4***	64.5

Values are expressed as Mean ± SD (n = 6). ***$p < 0.001$ compared with disease control.

The reduction in writhing episodes observed with the herbal formulation indicates significant peripheral analgesic activity (Figure 2).

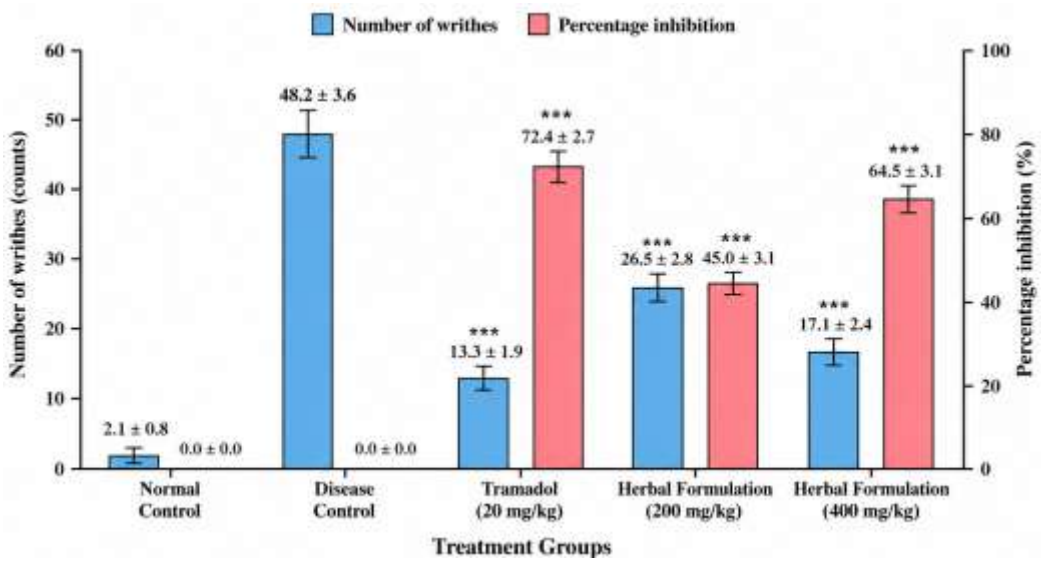

Figure 2: Effect of the herbal formulation on acetic acid-induced writhing in mice. A significant dose-dependent decrease in writhing response was observed, with the 400 mg/kg dose achieving 64.5% inhibition.

Hot Plate Test:

After the herbal formulation was administered, the hot plate test showed a significant increase in reaction latency, indicating central analgesic efficacy. Reaction latency in the 400 mg/kg group increased from a baseline value of about 5 seconds to 11.7 ± 0.8 seconds at 120 minutes, as shown in Table 3. The highest latency following tramadol administration was 13.8 ± 0.9 seconds.

Table 3: Effect of Herbal Formulation on Hot Plate Reaction Latency (Seconds)

Group	0 min	30 min	60 min	90 min	120 min
Normal Control	5.0 ± 0.4	5.1 ± 0.4	5.0 ± 0.3	5.2 ± 0.4	5.1 ± 0.3
Tramadol (20 mg/kg)	5.1 ± 0.4	8.4 ± 0.7***	10.7 ± 0.8***	12.9 ± 0.9***	13.8 ± 0.9***
Herbal Formulation (200 mg/kg)	4.9 ± 0.4	6.4 ± 0.5**	7.9 ± 0.6***	8.7 ± 0.7***	9.3 ± 0.7***
Herbal Formulation (400 mg/kg)	5.0 ± 0.5	7.5 ± 0.6***	9.6 ± 0.7***	10.8 ± 0.8***	11.7 ± 0.8***

Values are expressed as Mean ± SD (n = 6). **p < 0.01, ***p < 0.001 compared with control.

The increase in reaction latency was more pronounced at higher doses, demonstrating significant central analgesic effects of the herbal formulation (Figure 3).

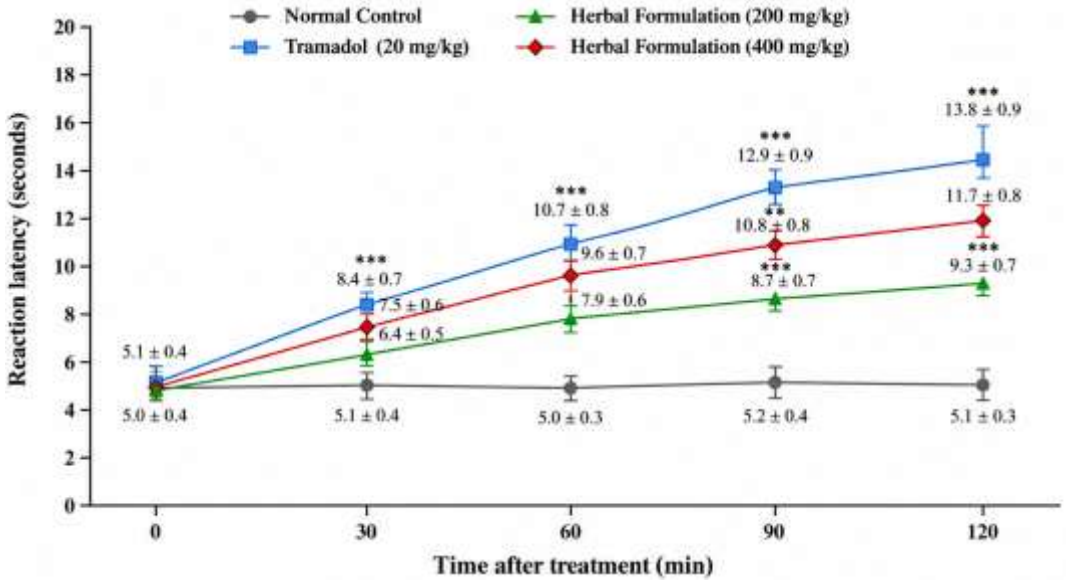

Figure 3: Time-dependent changes in hot plate reaction latency following oral administration of the herbal formulation. The 400 mg/kg dose produced a significant increase in latency throughout the observation period, approaching the effect of tramadol.

Acute Oral Toxicity Study:

Oral administration of the herbal formulation at 2000 mg/kg did not result in any mortality, behavioural abnormalities, symptoms of toxicity, changes in food intake, or loss of body weight. Thus, under the experimental conditions used, the formulation was deemed safe (Table 4).

Table 4: Acute Oral Toxicity Findings

Parameter	Observation
Mortality	None
Behavioral Changes	None
Tremors/Convulsions	None
Food Consumption	Normal
Water Intake	Normal
Body Weight Change	No significant change
Gross Toxicity Signs	None

DISCUSSION:

In established animal models of inflammation and pain, a novel herbal extract formulation including standardised extracts of Curcuma longa, Boswellia serrata, and Withania somnifera was tested for anti-inflammatory and analgesic properties. Significant dose-dependent anti-inflammatory and analgesic properties supported the formulation's use as a phytotherapeutic option for inflammatory illnesses and pain [17].

Histamine, serotonin, bradykinin, prostaglandins, and other inflammatory mediators are released biphasically in carrageenan-induced paw oedema, a popular experimental model for acute inflammation. The herbal formulation considerably reduced paw oedema volume at all observation times, with the 400 mg/kg dose inhibiting 67.8% after 4 hours compared to 73.6% with diclofenac sodium. Suppressing inflammatory mediator release and inhibiting prostaglandin production reduced paw oedema significantly. Curcuminoids, boswellic acids, and withanolides exhibit synergistic effects on COX, LOX, and NF-κB signalling pathways, leading to observed activity [18].

Acetic acid-induced writhing is a sensitive paradigm for peripheral analgesic action and increases peritoneal prostaglandins and other algogenic chemicals. Treatment with the herbal formulation dose-dependently reduced writhing episodes. Writhing was inhibited 64.5% at 400 mg/kg, similar to tramadol 72.4%. This suggests that the formulation reduces peripheral nociceptive responses by inhibiting inflammatory mediators that cause pain [19].

Hot plate testing assessed central analgesic activity. In formulation-treated mice, reaction latency increased significantly, suggesting CNS pain-modulating mechanisms. At 120 minutes, the reaction latency rose to 11.7 ± 0.8 seconds in the 400 mg/kg group and 13.8 ± 0.9 seconds in the tramadol group. The formulation's ability to increase pain threshold in this model shows that phytoconstituents may affect central neurotransmitter systems and pain-processing pathways in addition to peripheral anti-inflammatory actions [20].

This study's significant pharmacological action may be due to herbal components' complimentary processes. Curcumin from Curcuma longa reduces oxidative stress and inhibits pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6. Boswellia serrata boswellic acids reduce inflammation by inhibiting 5-lipoxygenase-mediated leukotriene production. Similarly, Withania somnifera withanolides are anti-inflammatory, immunomodulatory, antioxidant, and analgesic. Synergistic effects from these bioactive substances may have improved formulation efficacy [21, 22].

With no mortality, behavioural abnormalities, or toxicity at 2000 mg/kg, the acute oral toxicity trial proved the formulation's safety. These results indicate a high safety margin and support the formulation's pharmacological development. Limitations exist in this investigation. Only acute inflammation and pain models were used, and the molecular underpinnings of the apparent pharmacological effects were not examined. Future studies should use chronic inflammatory models, biomarker analysis, histological examination, and mechanistic investigations to understand pathways. Human clinical trials are needed to confirm the formulation's efficacy and safety [23-29].

CONCLUSION:

This study showed that the unique herbal extract formulation with standardised Curcuma longa, Boswellia serrata, and Withania somnifera extracts has anti-inflammatory and analgesic properties in animal models. The formulation reduced carrageenan-induced paw oedema dose-dependently, inhibiting 67.8% at 400 mg/kg, comparable to diclofenac sodium. The acetic acid-induced writhing and hot plate tests showed significant analgesic effect, demonstrating peripheral and central pain-modulating mechanisms. The formulation's safety was validated by the absence of mortality or adverse symptoms at 2000 mg/kg oral dose. The synergistic effects of curcuminoids, boswellic acids, and withanolides on inflammatory mediators and nociceptive pathways may explain the reported pharmacological effects. These data suggest the formulation could be a safe and effective phytotherapeutic option for inflammatory disorders and pain. Further mechanistic and clinical studies are needed to prove its therapeutic efficacy and human healthcare applicability.

Funding:
Not applicable.

Conflict of interest:
The authors declare that they have no conflict of interest.

REFERENCES:

1. Singh A, Malhotra S, Subban R. Anti-inflammatory and analgesic agents from Indian medicinal plants. International journal of integrative biology. 2008;3(1):57-72.
2. Saha A, Ahmed M. The analgesic and anti-inflammatory activities of the extract of Albizia lebbeck in animal model. Pakistan journal of pharmaceutical sciences. 2009 Jan 1;22(1):74.
3. Tamrat Y, Nedi T, Assefa S, Teklehaymanot T, Shibeshi W. Anti-inflammatory and analgesic activities of solvent fractions of the leaves of Moringa stenopetala Bak.(Moringaceae) in mice models. BMC complementary and alternative medicine. 2017 Sep 29;17(1):473.
4. Shojaii A, Motaghinejad M, Norouzi S, Motevalian M. Evaluation of anti-inflammatory and analgesic activity of the extract and fractions of Astragalus hamosus in animal models. Iranian journal of pharmaceutical research: IJPR. 2015;14(1):263.
5. Eum HA, Lee WY, Kim SH, Kim JY, Park SW, Lee JS, Choi SM, Pyo S, Kang MJ, Min YD, Shim SH. Anti-inflammatory activity of CML-1: an herbal formulation. The American journal of Chinese medicine. 2005;33(01):29-40.
6. Jami MS, Sultana Z, Ali ME, Begum MM, Haque MM. Evaluation of analgesic and anti-inflammatory activities on ethanolic extract of Terminalia chebula fruits in experimental animal models. American journal of plant sciences. 2014;5(1):63-9.
7. Ranjith D, Nisha AR, Nair SN, Litty M, Rahman M, Juliet S. Evaluation of analgesic and anti-inflammatory activity of herbal formulation used for mastitis in animals. International Journal of Applied Science and Engineering. 2018;6(1):37-48.
8. Ganga B, Wadud A, Jahan N, Makbul SA. Anti-inflammatory and analgesic activity of Habbe Gule Aakh, A polyherbal Unani formulation in animal models. Journal of Ayurveda and integrative medicine. 2021 Jan 1;12(1):9-12.
9. Wong YF, Zhou H, Wang JR, Xie Y, Xu HX, Liu L. Anti-inflammatory and analgesic effects and molecular mechanisms of JCICM-6, a purified extract derived from an anti-arthritic Chinese herbal formula. Phytomedicine. 2008 Jun 20;15(6-7):416-26.
10. Wong YF, Zhou H, Wang JR, Xie Y, Xu HX, Liu L. Anti-inflammatory and analgesic effects and molecular mechanisms of JCICM-6, a purified extract derived from an anti-arthritic Chinese herbal formula. Phytomedicine. 2008 Jun 20;15(6-7):416-26.
11. Dey D, Chaskar S, Athavale N, Chitre D. Inhibition of LPS-induced TNF-α and NO production in mouse macrophage and inflammatory response in rat animal models by a novel ayurvedic formulation, BV-9238. Phytotherapy Research. 2014 Oct;28(10):1479-85.
12. Cheng J, Ma T, Liu W, Wang H, Jiang J, Wei Y, Tian H, Zou N, Zhu Y, Shi H, Cheng X. In in vivo evaluation of the anti-inflammatory and analgesic activities of compound Muniziqi granule in experimental animal models. BMC complementary and alternative medicine. 2015 Dec;16(1):20.
13. Sulaiman MR, Zakaria ZA, Bujarimin AS, Somchit MN, Israf DA, Moin S. Evaluation of Moringa oleifera aqueous extract for antinociceptive and anti-inflammatory activities in animal models. Pharmaceutical biology. 2008 Jan 1;46(12):838-45.
14. Anyasor GN, Okanlawon AA, Ogunbiyi B. Evaluation of anti-inflammatory activity of Justicia secunda Vahl leaf extract using in vitro and in vivo inflammation models. Clinical phytoscience. 2019 Dec 27;5(1):49.
15. Paschapur MS, Patil MB, Kumar R, Patil SR. Evaluation of anti-inflammatory activity of ethanolic extract of Borassus flabellifer L. male flowers (inflorescences) in experimental animals. Journal of Medicinal Plants Research. 2009 Feb 1;3(2):49-54.
16. Umara MI, Altafa R, Iqbalb MA, Sadiqc MB. In vivo experimental models to investigate the anti-inflammatory activity of herbal extracts. Sci. Int.(Lahore). 2010;22(3):199-203.

17. Sharma S, Khare S, Dubey BK, Joshi A, Jain A. Analgesic activity of poly herbal formulation in experimental rats by acetic acid induced writhing test model and Hot plate model. Journal of Drug Delivery & Therapeutics. 2019 Mar 2;9:276.
18. Aiyalu R, Govindarjan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Brazilian Journal of Pharmaceutical Sciences. 2016 Sep;52(03):493-507.
19. Sharma MC, Sharma S, Kohli DV. Formulation and evaluation of analgesic activity, anti-inflammatory and anti-anxiety activity of using plant extracts. Digest Journal of Nanomaterials and Biostructures. 2010 Mar 1;5:147-51.
20. Aiamsa-Ard T, Monton C, Jongcharoenkamol J, Wunnakup T, Navabhatra A, Suksaeree J, Chankana N, Sudsai T. In Vitro and In Vivo Activity Evaluations, LC-MS Profiling, Stability Testing, and Capsule Formulation of a Thai Folk Analgesic Herbal Formula. Trends in Sciences. 2025 Aug 10;22(11):10865-.
21. Calixto JB, Scheidt C, Otuki M, Santos AR. Biological activity of plant extracts: novel analgesic drugs. Expert opinion on emerging drugs. 2001 Oct 1;6(2):261-79.
22. Kola-Mustapha AT, Yohanna KA, Ghazali YO, Ayotunde HT. Design, formulation and evaluation of Chasmanthera dependens Hochst and Chenopodium ambrosioides Linn based gel for its analgesic and anti-inflammatory activities. Heliyon. 2020 Sep 1;6(9).
23. Mo J, Panichayupakaranant P, Kaewnopparat N, Nitiruangjaras A, Reanmongkol W. Topical anti-inflammatory and analgesic activities of standardized pomegranate rind extract in comparison with its marker compound ellagic acid in vivo. Journal of ethnopharmacology. 2013 Jul 30;148(3):901-8.
24. Abotsi WK, Lamptey SB, Afrane S, Boakye-Gyasi E, Umoh RU, Woode E. An evaluation of the anti-inflammatory, antipyretic and analgesic effects of hydroethanol leaf extract of Albizia zygia in animal models. Pharmaceutical Biology. 2017 Jan 1;55(1):338-48.
25. Gupta, J., Sharma, B., Sorout, R., Singh, R. G., & Sharma, M. C., Ginger (Zingiber officinale) in traditional Chinese medicine: A comprehensive review of its anti-inflammatory properties and clinical applications. Pharmacological Research-Modern Chinese Medicine, 2025, 14, 100561.
26. Sharma, M., Maheshwari, A., & Bindal, N. A Novel Procedure to Synthesize 3-Chloro-1-(4a, 10b-diazaphenanthrene-2-yl)-4-phenyl Azetidin-2-ones and Exploration of Their Anti-Inflammatory Activity. Journal of Heterocyclic Chemistry, 2023, 50(S1), E116-E120.
27. Narayan, Sailesh, Barun Ranjan Sarkar, Dashrath Singh, and Suraj Sharma. "A research on the therapeutic potential of novel chemical entities utilized in an animal model of Alzheimer's disease." 2022: 29-34.
28. Keservani, RK., Sharma, AK., Ramteke, S., Novel vesicular approach for topical delivery of baclofen via niosomes, Lat Am J Pharm 29 (8), 1364-1370.
29. BD Adi, KK Raj, SK Anil, KK Rajesh, HM Gulam, Formulation and in vitro characterization of metoprolol tartrate loaded chitosan microspheres, ARS Pharmaceutica, 2012.