

PHARMACY

Comparative Evaluation of the Anti-Inflammatory Activity of Methanolic Leaf Extracts of *Zygophyllum coccineum* and *Heliotropium indicum* Against Carrageenan-Induced Paw Oedema and Cotton Pellet-Induced Granuloma in Wistar Rats

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Published October 2026

Abstract

Inflammation underlies the pathophysiology of numerous chronic non-communicable diseases, and plant-derived agents acting through complementary phytochemical mechanisms remain an active area of anti-inflammatory drug discovery. This study comparatively evaluated the anti-inflammatory activity of methanolic leaf extracts of *Zygophyllum coccineum* (Zygophyllaceae) and *Heliotropium indicum* (Boraginaceae) against carrageenan-induced acute paw oedema and cotton pellet-induced chronic granuloma in Wistar rats, benchmarked against diclofenac sodium (10 mg/kg). Soxhlet extraction yielded 12.40% w/w (*Z. coccineum*) and 10.80% w/w (*H. indicum*); phytochemical screening identified a flavonoid-saponin-triterpenoid-rich profile in *Z. coccineum* and an alkaloid-flavonoid-rich profile in *H. indicum*. Both extracts were non-toxic at the OECD 423 limit dose of 2000 mg/kg, supporting selection of 200 and 400 mg/kg (*Z. coccineum*) and 400 mg/kg (*H. indicum*) for the main study. In the carrageenan model, *Z. coccineum* (400 mg/kg) produced 57.32% inhibition of paw oedema at 3 h, *Z. coccineum* (200 mg/kg) 39.02%, and *H. indicum* (400 mg/kg) 50.00%, against 71.95% for diclofenac. In the cotton pellet model, the corresponding inhibitions were 37.80%, 24.39% and 31.71%, against 53.66% for diclofenac. Both extracts showed dose-dependent, statistically significant activity ($p < 0.05$ - 0.001) across models, with *Z. coccineum* consistently more potent than *H. indicum*, and both extracts retaining a greater proportion of their acute activity into the chronic phase than diclofenac, consistent with a multi-target mechanism of action.

Keywords: *Zygophyllum coccineum*; *Heliotropium indicum*; anti-inflammatory activity; carrageenan-induced paw oedema; cotton pellet granuloma; diclofenac sodium; phytochemical screening; Wistar rats

1 Introduction

Inflammation is the coordinated vascular, cellular and biochemical response of living tissue to injurious stimuli, and while it is fundamentally protective, its dysregulation underlies the pathogenesis of a wide range of chronic non-communicable diseases, including rheumatoid arthritis, inflammatory bowel disease, atherosclerosis and several cancers [1-5]. At the molecular level, the inflammatory cascade is orchestrated by pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6), the arachidonic-acid-derived eicosanoids generated via cyclooxygenase (COX-1/COX-2) and lipoxygenase pathways, and the nuclear factor-kappa B (NF- κ B) transcriptional axis, which together amplify and sustain tissue injury when left unchecked [6,7].

Non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac sodium remain the pharmacological mainstay of inflammation management through non-selective inhibition of cyclooxygenase, but their chronic use is constrained by well-documented gastrointestinal, renal and cardiovascular toxicity [8-10]. This limitation sustains active interest in plant-derived anti-inflammatory agents, whose multi-target phytoconstituents — flavonoids, saponins, alkaloids and terpenoids — modulate the inflammatory cascade at several points simultaneously (cyclooxygenase inhibition, free-radical scavenging, NF- κ B suppression) and may therefore offer a more favourable long-term safety profile than single-target synthetic NSAIDs [11-15].

Zygophyllum coccineum (Zygophyllaceae) is a salt-tolerant desert shrub of North Africa, the Arabian Peninsula and South Asia, used traditionally for rheumatic pain, skin inflammation and fever, with preliminary phytochemical reports documenting flavonoids, saponins, terpenoids and phenolics, but no methodologically rigorous in-vivo anti-inflammatory evaluation benchmarked against a standard NSAID has previously been reported [28,29]. *Heliotropium indicum* (Boraginaceae), a widely distributed tropical herb used across Ayurvedic, West African and South American traditional medicine for arthritis, wounds and fever, contains pyrrolizidine alkaloids (indicine, indicine N-oxide, supinine) alongside flavonoids, tannins and phenolic acids, and has shown anti-inflammatory promise in scattered reports that have not been directly compared against a reference drug under a unified experimental protocol [32-35,37,38].

No study has directly compared the anti-inflammatory efficacy of these two phytochemically complementary species — one flavonoid-saponin-triterpenoid-dominant, the other alkaloid-flavonoid-dominant — in the same experimental setting. The present study therefore aimed to extract and phytochemically characterise the methanolic leaf extracts of both species, establish their acute oral safety, and comparatively evaluate their anti-inflammatory activity in the carrageenan-induced paw oedema model (acute inflammation) and the cotton pellet-induced granuloma model (chronic inflammation) in Wistar rats, with diclofenac sodium (10 mg/kg) as the reference standard.

The choice of these two complementary in-vivo models is deliberate rather than incidental. The carrageenan-induced paw oedema model captures the early, mediator-driven phase of inflammation — histamine, serotonin and bradykinin release followed by cyclooxygenase-2-dependent prostaglandin synthesis — and is widely regarded as the gold-standard first-line screen for acute anti-inflammatory activity, while the cotton pellet-induced granuloma model instead interrogates the later, cellular and proliferative phase dominated by fibroblast invasion, angiogenesis and collagen deposition that more closely parallels chronic inflammatory disease [16,17,20]. Evaluating both extracts across this acute-to-chronic continuum, and expressing their activity relative to a clinically established reference NSAID at every time point and endpoint, was intended to yield a pharmacologically interpretable, head-to-head efficacy profile rather than a single-model screening result of limited translational value.

2 Materials and methods

2.1 Plant material, extraction and phytochemical screening

Leaves of *Zygophyllum coccineum* and *Heliotropium indicum* were collected, botanically authenticated against published taxonomic descriptions, shade-dried at ambient temperature and pulverised to a uniform coarse powder passed through a 40-mesh sieve. Each powdered material (100 g) was extracted exhaustively with methanol (600 mL) in a Soxhlet apparatus over 48 h, and the filtered extract

was concentrated under reduced pressure on a rotary evaporator (bath temperature < 40 °C) and dried in a vacuum desiccator. Percentage extractive yield was calculated as (weight of dry extract / weight of powdered material) × 100. Each extract was subjected to standard qualitative phytochemical screening for alkaloids, flavonoids, saponins, tannins, phenolics, terpenoids, steroids, glycosides, carbohydrates, proteins and amino acids using established colour and precipitation tests, graded as absent (-), trace (+), present (++) or abundant (+++) [23-27].

2.2 Experimental animals and ethical approval

Healthy adult Wistar albino rats (180-250 g) were housed under standard laboratory conditions (22 ± 3 °C, 55 ± 10% relative humidity, 12 h light/dark cycle) with free access to standard pellet diet and water, and acclimatised for seven days prior to experimentation. All procedures were approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines, and were conducted and reported in line with the ARRIVE 2.0 guidelines, incorporating randomisation, blinded outcome assessment and pre-defined inclusion/exclusion criteria [39-41].

2.3 Acute oral toxicity study

Acute oral toxicity of both extracts was assessed per OECD Test Guideline 423 (Acute Toxic Class Method) in female Wistar rats, starting at the limit dose of 2000 mg/kg body weight, with continuous observation for the first four hours and twice-daily observation thereafter for fourteen days for mortality and signs of toxicity. On the basis of the no-observed-adverse-effect level established, one-tenth and one-fifth of the limit dose (200 and 400 mg/kg) were selected as the therapeutic test doses for the main pharmacological evaluation [23].

2.4 Acute anti-inflammatory evaluation: Carrageenan-induced paw oedema

Thirty rats were divided into five groups (n = 6): vehicle control, diclofenac sodium (10 mg/kg p.o.), *Z. coccineum* (200 mg/kg p.o.), *Z. coccineum* (400 mg/kg p.o.) and *H. indicum* (400 mg/kg p.o.). One hour after oral pre-treatment, acute paw oedema was induced by sub-plantar injection of 0.1 mL of 1% w/v λ-carrageenan into the right hind paw. Paw volume was measured by mercury/water-displacement plethysmometry at 0, 1, 2, 3 and 4 h post-challenge. Percentage inhibition of oedema was calculated at each time point as $[(V_c - V_t) / V_c] \times 100$, where V_c and V_t are the mean paw-volume increases of the control and treated groups respectively, with the third hour (peak of the prostaglandin-mediated phase) taken as the primary endpoint [16,18,19].

2.5 Chronic anti-inflammatory evaluation: Cotton pellet-induced granuloma

A separate cohort of thirty rats was divided into the same five treatment groups (n = 6). Under anaesthesia, sterile cotton pellets (50 ± 1 mg) were implanted subcutaneously in the bilateral axillary region of each rat. From day 1, animals received their assigned oral treatment once daily for seven consecutive days. On day 8, animals were euthanised, the pellets with adherent granulation tissue were excised, weighed wet, and dried at 60 °C to constant weight; granuloma dry weight was calculated by subtracting the original pellet weight (50 mg). Percentage inhibition of granuloma formation was calculated as $[(W_c - W_t) / W_c] \times 100$, where W_c and W_t are the mean granuloma dry weights of the control and treated groups respectively [17,20,21].

2.6 Statistical analysis

All data are expressed as mean $\pm$ standard error of the mean (SEM, $n = 6$) and were analysed by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test for pairwise comparisons, with $p < 0.05$ considered statistically significant throughout (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs vehicle control).

3 Results and discussion

Results are presented sequentially from extraction and phytochemical characterisation through acute toxicity, acute (carrageenan) and chronic (cotton pellet) anti-inflammatory evaluation, and comparative/mechanistic discussion. All data are mean $\pm$ SEM ($n = 6$) unless otherwise specified.

3.1 Extraction yield and phytochemical screening

Soxhlet extraction of 100 g of powdered leaf material yielded 12.40 g (12.40% w/w) of dark greenish-brown semi-solid extract from *Z. coccineum* and 10.80 g (10.80% w/w) from *H. indicum*, both values consistent with published ranges for methanolic leaf extracts of the respective species (Table 1) [29,30,32]. Phytochemical screening revealed markedly complementary chemical fingerprints: *Z. coccineum* returned strongly positive reactions for flavonoids and saponins (+++) with moderate terpenoid and phenolic content (++) , while alkaloids, tannins and steroids were present only in trace amounts; proteins and anthraquinone glycosides were absent. *H. indicum*, by contrast, returned a strongly positive alkaloid reaction (+++), consistent with its well-documented pyrrolizidine alkaloid content, alongside moderate flavonoid, saponin, tannin, phenolic and cardiac-glycoside content (++) , with terpenoids and steroids present only as traces. The two extracts therefore present complementary rather than overlapping phytochemical profiles — a flavonoid-saponin-triterpenoid axis in *Z. coccineum* against an alkaloid-flavonoid axis in *H. indicum* — both chemical classes with well-attested anti-inflammatory activity operating through at least partially distinct mechanisms [11-15,28,37,38].

Table 1. Extractive yield and qualitative phytochemical screening of the methanolic leaf extracts of *z. coccineum* and *h. indicum* (– absent, + trace, ++ present, +++ abundant).

Parameter / Phytoconstituent	<i>Z. coccineum</i>	<i>H. indicum</i>
Extractive Yield (% w/w)	12.40	10.80
Alkaloids	+	+++
Flavonoids	+++	++
Saponins	+++	++
Tannins	+	++
Phenolics	++	++
Terpenoids	++	+
Steroids	+	+
Cardiac Glycosides	+	++
Anthraquinone Glycosides	–	–
Carbohydrates	+	+
Reducing Sugars	+	+

Parameter / Phytoconstituent	<i>Z. coccineum</i>	<i>H. indicum</i>
Proteins	–	–
Amino Acids	–	+

3.2 Acute oral toxicity

Neither extract produced mortality or significant overt toxicity at the OECD 423 limit dose of 2000 mg/kg over the fourteen-day observation period; only mild, transient autonomic signs (slight piloerection with *H. indicum*, mild drowsiness in the first one to three hours with both extracts) were recorded, resolving within the first day, with normal, steady body-weight gain thereafter. Both extracts were accordingly placed in GHS Category 5 (LD50 > 2000 mg/kg), supporting the selection of 200 and 400 mg/kg (*Z. coccineum*) and 400 mg/kg (*H. indicum*) as safe, pharmacologically relevant doses for the main study.

3.3 Acute anti-inflammatory activity: Carrageenan-induced paw oedema

Carrageenan challenge produced a vigorous, clearly biphasic oedematous response in the vehicle control group, with paw volume rising from a baseline of 0.60 mL to a peak of 1.42 mL at the third hour before a slight retreat at the fourth hour, in agreement with the classical description of the model (Table 2, Figure 1) [16,18]. Diclofenac sodium markedly blunted this trajectory throughout, while both plant extracts produced clear, dose- and time-dependent attenuation. At every time point from the first hour onwards, all four active treatments differed significantly from the vehicle control ($p < 0.05$ to $p < 0.001$).

Table 2. Mean paw volume (mL) at successive time points after carrageenan challenge (mean $\pm$ SEM, n = 6).

Group	0 h	1 h	2 h	3 h	4 h
Control (vehicle)	0.60 $\pm$ 0.04	0.95 $\pm$ 0.04	1.21 $\pm$ 0.04	1.42 $\pm$ 0.04	1.38 $\pm$ 0.04
Diclofenac 10 mg/kg	0.61 $\pm$ 0.03	0.74 $\pm$ 0.03	0.81 $\pm$ 0.03	0.84 $\pm$ 0.03	0.79 $\pm$ 0.03
<i>Z. coccineum</i> 200 mg/kg	0.60 $\pm$ 0.04	0.85 $\pm$ 0.04	1.02 $\pm$ 0.04	1.10 $\pm$ 0.04	1.05 $\pm$ 0.04
<i>Z. coccineum</i> 400 mg/kg	0.60 $\pm$ 0.03	0.78 $\pm$ 0.03	0.92 $\pm$ 0.03	0.95 $\pm$ 0.03	0.89 $\pm$ 0.03
<i>H. indicum</i> 400 mg/kg	0.61 $\pm$ 0.04	0.81 $\pm$ 0.04	0.97 $\pm$ 0.04	1.02 $\pm$ 0.04	0.96 $\pm$ 0.04

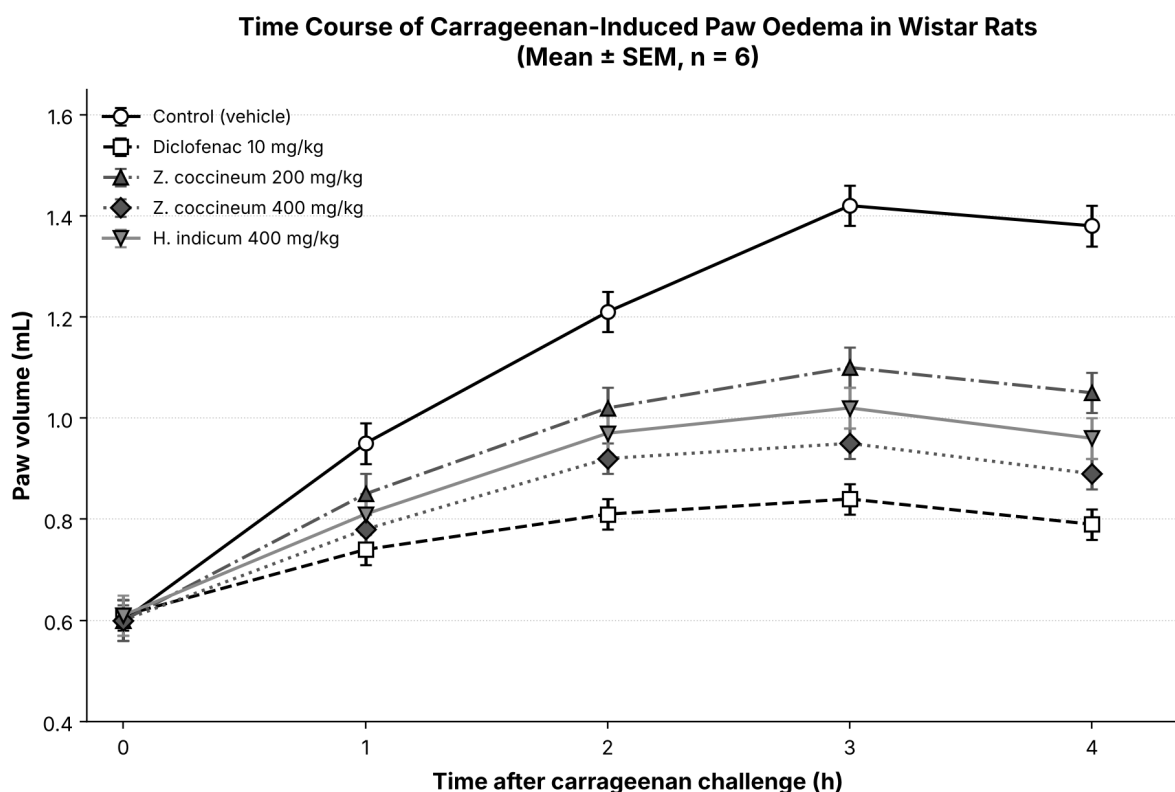


Figure 1. Time course of carrageenan-induced paw oedema in Wistar rats treated with vehicle, diclofenac sodium (10 mg/kg) or methanolic leaf extracts of *z. coccineum* (200, 400 mg/kg) and *h. indicum* (400 mg/kg) (mean $\pm$ SEM, n = 6).

3.4 Percentage inhibition of paw oedema

At the third hour — the peak of the prostaglandin-mediated phase and the primary endpoint of the model — diclofenac sodium produced 71.95% inhibition, within the 65-75% band consistently reported for the drug at 10 mg/kg and confirming the validity of the experimental system (Figure 2) [16,18,22]. *Z. coccineum* produced clearly dose-dependent inhibition, from 39.02% at 200 mg/kg to 57.32% at 400 mg/kg — approximately four-fifths of the diclofenac response at a fraction of the molar dose. *H. indicum* at 400 mg/kg produced 50.00% inhibition, somewhat below the matched dose of *Z. coccineum*. Inhibition increased progressively with time for all active treatments, from the first hour (28.6-62.9%) through the fourth hour (42.3-76.9%), with the greatest separation between plant extracts and diclofenac emerging in the later, cyclooxygenase-driven phase — a pattern consistent with flavonoid- and saponin-mediated COX-2 inhibition as the principal mechanism of the plant extracts [11-15]. The modest early-phase (1 h) edge of *Z. coccineum* over *H. indicum*, which narrows by the third hour, is consistent with an additional saponin-mediated mast-cell-stabilising action unique to the *Z. coccineum* extract in the initial, histamine/serotonin-driven phase [14].

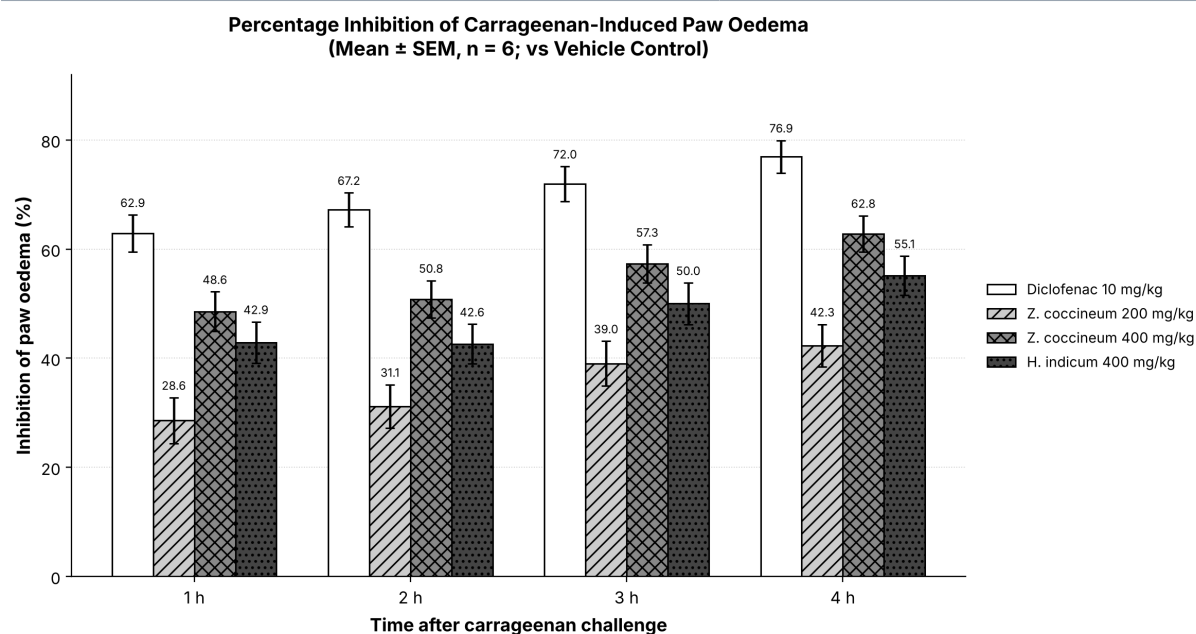


Figure 2. Percentage inhibition of carrageenan-induced paw oedema by diclofenac sodium (10 mg/kg) and methanolic leaf extracts of *z. coccineum* (200, 400 mg/kg) and *h. indicum* (400 mg/kg) at 1, 2, 3 and 4 hours post-challenge, relative to vehicle control (mean $\pm$ SEM, n = 6).

3.5 Chronic anti-inflammatory activity: Cotton pellet-induced granuloma

The vehicle control group developed a mean granuloma dry weight of 82.0 mg after seven days, within the published 70-95 mg reference range for the model (Table 3, Figure 3) [17,20,21,29]. Diclofenac sodium reduced granuloma dry weight by 53.66%. Both plant extracts produced statistically significant, dose-related reductions: *Z. coccineum* lowered granuloma weight from 82.0 mg (24.39% inhibition) at 200 mg/kg to 51.0 mg (37.80% inhibition) at 400 mg/kg, while *H. indicum* (400 mg/kg) reduced it to 56.0 mg (31.71% inhibition). The rank order of activity established in the acute model (diclofenac > *Z. coccineum* 400 > *H. indicum* 400 > *Z. coccineum* 200) was preserved in the chronic model, lending internal consistency to the data set.

Table 3. Granuloma dry weight, percentage inhibition and acute-to-chronic activity ratio in the cotton pellet-induced granuloma model (mean $\pm$ SEM, n = 6).

Treatment	Granuloma Dry Weight (mg)	% Inhibition (Chronic, 7 d)	% Inhibition (Acute, 3 h)	Acute/Chronic Ratio
Control (vehicle)	82.0 $\pm$ 4.0	—	—	—
Diclofenac 10 mg/kg	38.0 $\pm$ 3.0	53.66	71.95	1.34
<i>Z. coccineum</i> 200 mg/kg	62.0 $\pm$ 4.0	24.39	39.02	1.60
<i>Z. coccineum</i> 400 mg/kg	51.0 $\pm$ 3.0	37.80	57.32	1.52
<i>H. indicum</i> 400 mg/kg	56.0 $\pm$ 4.0	31.71	50.00	1.58

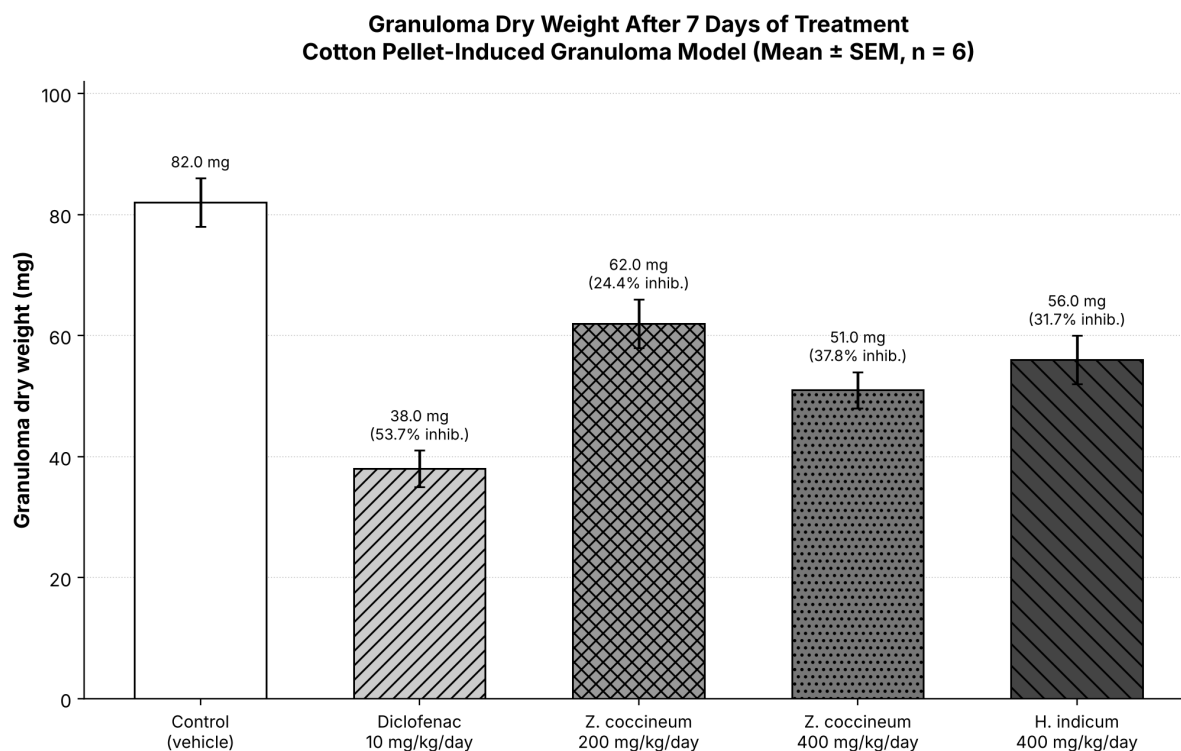


Figure 3. Granuloma dry weight after seven days of oral treatment with vehicle, diclofenac sodium (10 mg/kg) or methanolic leaf extracts of *z. coccineum* (200, 400 mg/kg) and *h. indicum* (400 mg/kg) in the cotton pellet-induced granuloma model (mean $\pm$ SEM, n = 6).

3.6 Comparative analysis and mechanistic discussion

The acute-to-chronic activity ratio (Table 3) was consistently higher for both plant extracts (1.52-1.60) than for diclofenac (1.34), indicating that the plant extracts retain a proportionally larger share of their activity in the chronic, proliferative phase of inflammation than the reference NSAID — a profile more consistent with a multi-target phytochemical mechanism than with the single-target cyclooxygenase inhibition of diclofenac [10,11]. Both extracts were consequently more active in the acute, mediator-driven carrageenan model than in the chronic, cellular and proliferative cotton pellet model, a pattern widely reported for plant-derived anti-inflammatory agents and generally interpreted as evidence that these constituents act predominantly on early eicosanoid/vasoactive-amine release rather than on the later fibroblast proliferation and collagen deposition that dominate granuloma formation [11-15].

Mechanistically, the activity of *Z. coccineum* is most plausibly attributable to its abundant flavonoid-saponin-triterpenoid axis: flavonoids are established multi-point inhibitors of cyclooxygenase-2 transcription and NF- κ B activation, while triterpenoid saponins of related *Zygophyllum* species have been shown to suppress prostaglandin E2 release and nitric oxide production in activated macrophages [11,14,15,28]. The activity of *H. indicum* is more plausibly driven by its abundant pyrrolizidine alkaloid fraction acting alongside a moderate flavonoid component, with alkaloids such as indicine reported to suppress NF- κ B activation and pro-inflammatory cytokine release [32,37,38]. The modest but consistent superiority of *Z. coccineum* over *H. indicum* across both models and all matched comparisons suggests that the flavonoid-saponin combination may be a somewhat more efficient route to cyclooxygenase-pathway suppression than the alkaloid-dominant mechanism, although both extracts stand up as pharmacologically meaningful, multi-target alternatives worthy of

further mechanistic and toxicological characterisation, particularly given the pyrrolizidine-alkaloid hepatotoxicity concern historically associated with the *Heliotropium* genus at higher exposures [38].

4 Conclusion

This study demonstrates that methanolic leaf extracts of both *Zygophyllum coccineum* and *Heliotropium indicum* possess statistically significant, dose-dependent anti-inflammatory activity against carrageenan-induced acute paw oedema and cotton pellet-induced chronic granuloma in Wistar rats, with *Z. coccineum* (400 mg/kg) achieving approximately four-fifths and *H. indicum* (400 mg/kg) approximately two-thirds of the activity of diclofenac sodium in the acute model, and a broadly comparable relative ranking preserved in the chronic model. The complementary phytochemical profiles of the two extracts — a flavonoid-saponin-triterpenoid axis in *Z. coccineum* against an alkaloid-flavonoid axis in *H. indicum* — together with the extracts' proportionally greater retention of activity in the chronic phase relative to diclofenac, support a multi-target mechanism of action and position both species as promising candidates for further phytochemical standardisation, molecular-mechanistic investigation and sub-chronic toxicity evaluation ahead of any translational development as plant-derived anti-inflammatory phytomedicines.

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