

Protective Effect of *Cuscuta reflexa* Extract Against Monosodium Urate-Induced Gouty Arthritis in Pigeons

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Abstract: Gouty arthritis is an inflammatory joint disorder caused by monosodium urate (MSU) crystal deposition, which triggers NLRP3 inflammasome activation and pro-inflammatory cytokine release. This study evaluated the protective effect of *Cuscuta reflexa* aqueous extract against MSU-induced gouty arthritis in pigeons (*Columba livia*). Healthy pigeons were divided into three groups: a normal control, an MSU-induced toxic control, and a group pre-treated with *C. reflexa* extract (400 mg/kg, 1 mL/kg intramuscularly) daily for seven days before MSU challenge on day 7. Serum uric acid, xanthine oxidase activity, TNF- α levels, and one-leg standing time- a functional pain-behaviour marker- were assessed. MSU administration significantly elevated uric acid (14.08 ± 0.3 mg/dL), xanthine oxidase activity (4.7 ± 0.9 mU/mL) and TNF- α (28.05 ± 0.2 pg/mL) relative to normal control ($P < 0.001$). Pre-treatment with *C. reflexa* extract significantly reduced uric acid (7.3 ± 0.2 mg/dL), xanthine oxidase activity (1.9 ± 0.4 mU/mL) and TNF- α (10.4 ± 0.3 pg/mL) relative to the toxic control ($P < 0.001$), and shortened the protective one-leg standing duration, indicating reduced joint pain. These findings support a dual xanthine oxidase-inhibitory and anti-inflammatory mechanism, plausibly attributable to the extract's flavonoid content (kaempferol, quercetin, luteolin), positioning *Cuscuta reflexa* as a promising natural adjunct for the management of gout.

Keywords: *Cuscuta reflexa*; gouty arthritis; monosodium urate; xanthine oxidase; TNF- α ; NLRP3 inflammasome; pigeon model

1. Introduction

Gout is one of the oldest recorded inflammatory joint diseases, resulting from the deposition of monosodium urate (MSU) monohydrate crystals in articular and periarticular tissues once serum urate exceeds its solubility threshold [1]. Contemporary reviews frame gout as a multifactorial disease shaped by hyperuricemia, chronic kidney disease, genetic susceptibility, alcohol use, dietary purine load, diuretic therapy, hypertension, obesity and metabolic syndrome, and note that affected patients carry an elevated risk of all-cause mortality, particularly from cardiovascular disease [2]. Only a minority of hyperuricemic individuals progress to clinical gout, indicating that elevated serum urate is necessary but not sufficient, and that the transition to overt disease depends on crystal formation and the host's inflammatory response to those crystals [3]. Mechanistically, MSU crystals are now understood to act as a "danger signal" recognised by innate immune sensors. Crystal deposition triggers Toll-like receptor engagement and assembly of the NLRP3 inflammasome in synovial macrophages, activating caspase-1, which cleaves pro-IL-1 β into its mature, secreted form and drives the cardinal features of an acute flare- rapid-onset pain, erythema, swelling and warmth [4,5]. This inflammasome-centred understanding has reframed gout as an autoinflammatory disease rather than a purely metabolic one, and has redirected therapeutic interest toward agents that interrupt either urate production, through xanthine oxidase inhibition, or the downstream inflammatory cascade, rather than relying on uricosuric or purely analgesic strategies alone [6]. Avian species offer a biologically distinct but mechanistically relevant model for studying urate-related pathology. Birds are uricotelic- lacking the hepatic enzyme uricase that converts uric acid to the more soluble allantoin- so nitrogenous waste is excreted almost entirely as poorly

soluble uric acid, and any impairment of renal handling produces pronounced hyperuricemia far more readily than in ureotelic mammals. This makes the pigeon a physiologically apt, cost-effective and comparatively humane model for intra-articular and intramuscular MSU challenge, while preserving the same crystal-triggered inflammatory cascade seen in human gout.

Current first-line pharmacotherapy for gout- xanthine oxidase inhibitors such as allopurinol and febuxostat, uricosuric agents and NSAIDs- carries recognised limitations. Allopurinol at standard fixed doses achieves target urate control in only 20–40 percent of patients and is associated with hypersensitivity reactions, renal toxicity and, rarely, Stevens–Johnson syndrome in susceptible individuals; febuxostat improves response rates but remains a synthetic small molecule with its own tolerability profile [7,8]. This gap has driven sustained pharmacognostic interest in plant-derived xanthine oxidase inhibitors and anti-inflammatory agents, particularly flavonoid-rich botanicals, as safer adjunctive or alternative therapies [9,10]. *Cuscuta reflexa* (family Convolvulaceae), a leafless holoparasitic climbing vine known traditionally as dodder, akashbel or witch's hair, has a longstanding presence in Ayurvedic and folk medicine for hepatic, renal and inflammatory conditions. Its phytochemical profile- rich in flavonoids including kaempferol, kaempferol-3-O-glucoside, quercetin and luteolin, together with the triterpenoid lupeol- aligns closely with compound classes independently shown to inhibit xanthine oxidase and suppress NLRP3-driven cytokine release in other systems [11]. Despite this plausibility, no prior study has directly evaluated *C. reflexa* in an MSU-induced gouty arthritis model. The present study was therefore designed to evaluate the protective effect of *C. reflexa* aqueous extract against MSU-induced gouty arthritis in pigeons, assessing serum uric acid, xanthine oxidase

activity, TNF- α , and functional pain behaviour as combined biochemical and clinical endpoints.

2. Materials and Methods

1) Plant Material and Authentication

Whole aerial parts of *Cuscuta reflexa* Roxb. were collected locally from Madurai district, Tamil Nadu, India, and authenticated by a qualified botanist in the Department of Botany at an affiliated academic institution, confirming the species and its placement within the family Convolvulaceae.

2) Extraction

Five kilograms of fresh whole plant material was cleaned, shade-dried at room temperature and powdered. The powder was cold-macerated in water at room temperature for 15 days with intermittent agitation. The aqueous macerate was filtered sequentially through muslin cloth and Whatman No. 1 filter paper, and the combined filtrate was evaporated to dryness under ambient shade conditions over approximately 40 days to yield a crude aqueous extract (*Cuscuta reflexa* aqueous extract, CRAE), which was reduced to powder to facilitate accurate dosing.

3) Preliminary Phytochemical Screening

The aqueous extract was screened qualitatively for flavonoids using the Shinoda test and the Vitali–Morin test for catechins and leucoanthocyanins, for tannins using the matchstick test, and for cardiac glycosides using the Keller–Killiani test. These screens confirmed the presence of flavonoid and polyphenolic constituents consistent with the compound classes previously reported for this species.

4) Experimental Animals

Healthy adult pigeons (*Columba livia*, 240–380 g, either sex) were acclimatised under standard laboratory housing at ambient temperature (25–30 °C) with a 12:12 h light/dark cycle, and given free access to standard grain feed and water. All procedures were conducted in accordance with internationally accepted principles for the care and use of laboratory animals.

5) Gouty Arthritis Induction and Treatment Protocol

Animals were randomly assigned to three groups. Group 1 (normal control) received 0.05 mL of normal saline by intra-articular injection into the left intertarsal joint on days 1 to 7. Group 2 (toxic/disease control) received monosodium urate by the intramuscular route as a single administration on day 7 to induce gouty arthritis. Group 3 (extract-treated) received *Cuscuta reflexa* aqueous extract at 400 mg/kg (1 mL/kg, 1% w/v) by the intramuscular route daily from day 1 to day 7, followed by MSU challenge on day 7. Behavioural changes were monitored through the remainder of day 7 following MSU administration. At evening on day 7, blood was collected from the wing vein of all animals without euthanasia and processed for biochemical analysis.

6) Biochemical Estimations

Serum uric acid, xanthine oxidase activity and TNF- α were quantified using standard biochemical assay and ELISA-based methods, following the manufacturer's protocols for each kit.

7) Functional Pain Assessment- One-Leg Standing Time

Because MSU-induced joint inflammation produces pain and mechanical discomfort, affected animals characteristically shift weight onto the unaffected limb. One-leg standing time- the latency to onset and the duration for which an animal voluntarily bore weight on the non-inflamed limb before returning both feet to the ground- was recorded as a validated behavioural proxy for pain severity and functional impairment. A longer standing duration indicates greater pain avoidance on the affected limb, and its reduction following treatment indicates improved joint comfort.

8) Statistical Analysis

Data are expressed as mean $\pm$ SEM and were analysed by one-way ANOVA followed by the Newman–Keuls multiple range post-hoc test. A value of $P < 0.001$ was considered the threshold for statistical significance throughout, with group differences flagged as significant versus normal control (a*) or versus toxic control (b*) as appropriate.

3. Results

Induction of gouty arthritis with MSU crystals produced significant derangement across all three biochemical markers and the functional pain-behaviour assay relative to normal control, confirming successful disease induction. Pre-treatment with *Cuscuta reflexa* extract produced significant, partial-to-near-complete normalisation across all endpoints relative to the untreated disease group.

1) Serum TNF- α

MSU administration produced a marked, statistically significant elevation in serum TNF- α in the disease-control group (28.05 ± 0.2 pg/mL) relative to normal control (3.2 ± 0.1 pg/mL; $P < 0.001$), consistent with NLRP3-driven cytokine release following crystal deposition. Pre-treatment with *C. reflexa* extract reduced TNF- α to 10.4 ± 0.3 pg/mL ($P < 0.001$ versus disease control)- a reduction of approximately 63 percent relative to the untreated disease state, though levels remained above the normal-control baseline, indicating partial rather than complete suppression of the inflammatory cascade at this dose and duration (Fig. 1).

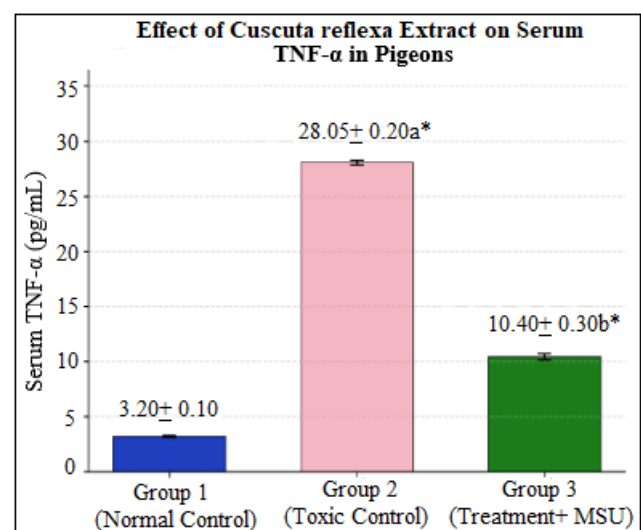


Figure 1: Effect of *Cuscuta reflexa* extract on serum TNF- α in pigeons. a* $P < 0.001$ vs. normal control; b* $P < 0.001$ vs. toxic control

2) Serum Uric Acid

Serum uric acid rose sharply following MSU challenge, from 6.01 ± 0.1 mg/dL in normal controls to 14.08 ± 0.3 mg/dL in disease controls ($P < 0.001$). Extract pre-treatment lowered uric acid to 7.3 ± 0.2 mg/dL ($P < 0.001$ versus disease control), a value close to the normal-control range and representing an approximate 48 percent reduction relative to the disease state, consistent with meaningful inhibition of urate accumulation rather than a marginal effect (Fig. 2).

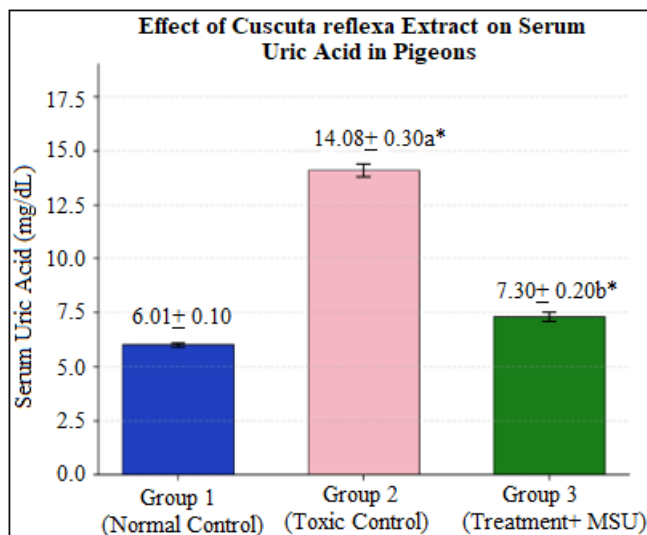


Figure 2: Effect of *Cuscuta reflexa* extract on serum uric acid in pigeons. a* $P < 0.001$ vs. normal control; b* $P < 0.001$ vs. toxic control

3) Xanthine Oxidase Activity

Xanthine oxidase activity increased more than three-fold following MSU induction (4.7 ± 0.9 mU/mL versus 1.4 ± 0.3 mU/mL in normal control; $P < 0.001$). Extract-treated animals showed activity of 1.9 ± 0.4 mU/mL ($P < 0.001$ versus disease control), close to baseline and supporting a direct or indirect xanthine oxidase-inhibitory action of the extract, in line with the flavonoid-driven xanthine oxidase inhibition documented for structurally related compounds in the wider phytochemical literature [10] (Fig. 3).

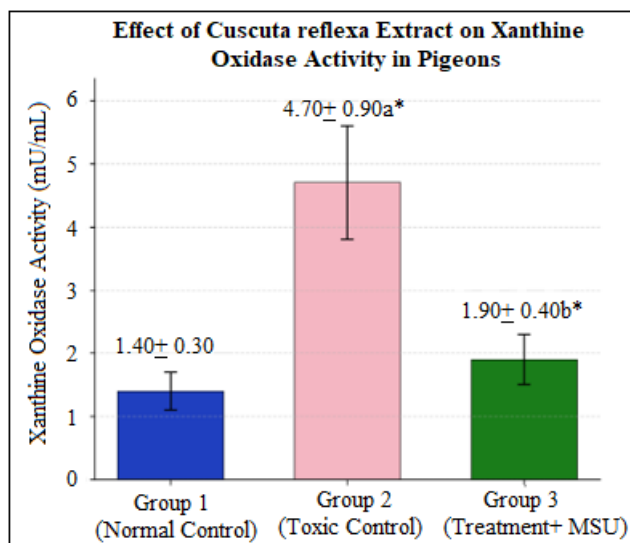


Figure 3: Effect of *Cuscuta reflexa* extract on xanthine oxidase activity in pigeons. a* $P < 0.001$ vs. normal control; b* $P < 0.001$ vs. toxic control

4) Functional Pain Behaviour- One-Leg Standing Time

Disease-control animals exhibited markedly prolonged one-leg standing duration (mean 86.0 ± 13.0 s across individual animals, range 72–98 s) with short onset latency (mean 4.7 ± 0.6 s), reflecting acute pain-avoidance behaviour on the affected limb. Extract-treated animals showed both delayed onset (mean 16.3 ± 1.5 s) and substantially shortened duration (mean 25.0 ± 5.6 s), indicating meaningfully reduced joint discomfort and improved weight-bearing tolerance relative to the untreated disease group (Fig. 4 and Table I)- a functional correlate consistent with the biochemical improvements described above.

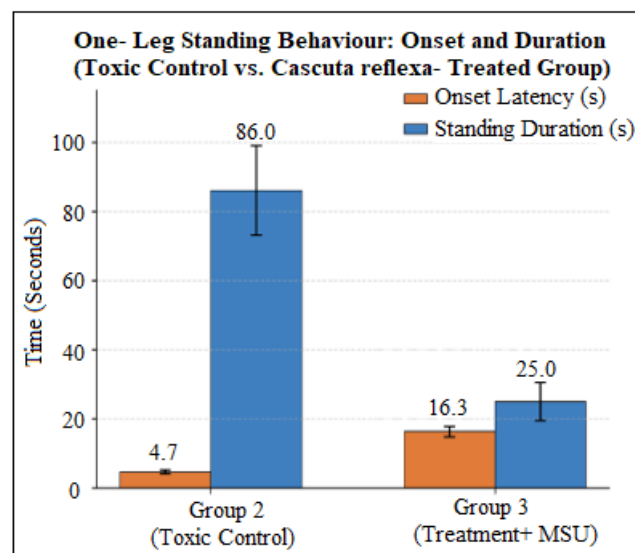


Figure 4: One-leg standing onset latency and duration in toxic control vs. *Cuscuta reflexa*-treated pigeons (mean $\pm$ SD, $n = 3/\text{group}$)

Table I: One-Leg Standing Time of Pigeons Across Groups

Group	Animal	Onset Latency	Standing Duration
Toxic control	1	5 s	1 min 12 s
Toxic control	2	4 s	1 min 28 s
Toxic control	3	5 s	1 min 38 s
Treatment + MSU	1	15 s	20 s
Treatment + MSU	2	18 s	24 s
Treatment + MSU	3	16 s	31 s

Collectively, all four endpoints moved concordantly: MSU produced significant derangement in every biochemical and functional marker measured, and *C. reflexa* pre-treatment produced significant, partial-to-near-complete normalisation across all of them, with the strongest relative effect on uric acid and xanthine oxidase and a somewhat more modest, though still highly significant, effect on TNF- α .

4. Discussion

The concordant pattern across uric acid, xanthine oxidase, TNF- α and functional pain behaviour supports a dual mechanism of protection: suppression of urate production at the enzymatic level, and attenuation of the downstream inflammatory response to whatever urate crystal burden remained. This is mechanistically coherent with current understanding of gout pathophysiology, in which xanthine oxidase governs the metabolic supply of urate while the NLRP3 inflammasome and its cytokine output govern the

clinical inflammatory phenotype [4,5]; an agent acting on only one arm would be expected to produce a more incomplete or one-sided biochemical signature than was observed here.

The magnitude of xanthine oxidase suppression is plausibly attributable to the extract's flavonoid content. Kaempferol, kaempferol-3-O-glucoside, quercetin and luteolin- all previously isolated from *C. reflexa* [11]- belong to exactly the flavonoid subclasses identified as the most potent xanthine oxidase inhibitors among dietary and medicinal polyphenols screened to date [10], providing a plausible phytochemical basis for the uric acid and xanthine oxidase findings rather than an unexplained empirical effect. The TNF- α reduction is similarly consistent with the broader natural-product literature on NLRP3/NF- κ B-targeted botanicals, several of which report comparable cytokine suppression in MSU-stimulated systems [13,14], and with lupeol's independently documented capacity to modulate T-helper cytokine output.

The partial rather than complete normalisation of TNF- α -remaining roughly three-fold above normal-control levels despite treatment- is a meaningful limitation to note rather than gloss over. It suggests that, at the dose and duration tested, the extract attenuates but does not fully abolish the acute inflammatory cascade triggered by MSU crystals already present at the time of challenge, consistent with a preventive/attenuating rather than curative mechanism, and mirroring the partial response rates seen even with standard xanthine oxidase inhibitors in clinical populations [7]. The concordant improvement in one-leg standing time strengthens confidence that the biochemical changes translate into a functionally meaningful reduction in pain and disability, rather than representing a biomarker shift without clinical correlate- an important distinction, since biochemical improvement without functional correlate is a common limitation in preclinical phytotherapy studies.

Several limitations warrant explicit acknowledgment. The avian intramuscular/intra-articular MSU model, while mechanistically relevant given birds' uricotelic physiology, differs from the classical rodent intra-articular air-pouch or paw-oedema models most commonly used in gout pharmacology, which may affect direct comparability with the wider preclinical literature. The study duration of seven days and the single extract dose tested limit conclusions about dose-response relationships and chronic safety. Histopathological examination of the affected joint tissue was not performed in this study, and its inclusion in future work would allow direct structural confirmation of the biochemical and behavioural improvements reported here. Likewise, mechanistic confirmation- for instance, direct in vitro xanthine oxidase inhibition assays or NLRP3/caspase-1 pathway markers- was not performed here and would strengthen the causal interpretation offered above. These are appropriate directions for follow-up work rather than weaknesses that undermine the present findings.

5. Conclusion

Cuscuta reflexa aqueous extract demonstrated significant protective activity against monosodium urate-induced gouty arthritis in pigeons, evidenced by concordant reductions in

serum uric acid, xanthine oxidase activity and TNF- α , alongside functional improvement in pain-related one-leg standing behaviour. These findings are consistent with a dual xanthine oxidase-inhibitory and anti-inflammatory mechanism plausibly mediated by the plant's flavonoid constituents (kaempferol, quercetin, luteolin) and are, to this study's knowledge, among the first to test *C. reflexa* specifically against an intact MSU-crystal challenge model rather than isolated enzymatic or cytokine assays.

Given the partial nature of the TNF- α response and the absence of direct histopathological and mechanistic confirmation, prospective work should prioritise dose-ranging studies, histological examination of joint tissue, in vitro xanthine oxidase and NLRP3-pathway assays, and extended-duration safety evaluation before *C. reflexa* can be positioned as a candidate adjunct therapy for gout. Subject to that validation, the present findings support its further investigation as a natural, potentially better-tolerated complement to existing xanthine oxidase-inhibitor pharmacotherapy.

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