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Evaluation of Oral Acute Toxicity and Pharmacological Effects of DNC NAMCTGU Capsules in Mice: Analgesic, Anti-inflammatory, and Hypouricemic Activities

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ABSTRACT

The rising incidence of gout and the negative side effects associated with current treatments have sparked interest in safer herbal alternatives. This research evaluated the oral acute toxicity, pain-relieving, inflammation-suppressing and uric acid-reducing properties of DNC NAMCTGU capsules, a polyherbal formulation comprising extracts from *Cleistocalyx operculatus* buds, *Perilla frutescens* leaves, and *Curcuma longa* rhizomes. To assess these effects, mice were given daily oral doses of 400, 800, or 1200 mg/kg. During the writhing assay induced by acetic acid, DNC NAMCTGU at a dose of 800 mg/kg significantly decreased abdominal constrictions relative to the control group ($p < 0.05$), with a response similar to diclofenac (10 mg/kg). In models of inflammation caused by carrageenan, the same dose significantly decreased paw and joint swelling at various times ($p < 0.05$), demonstrating anti-inflammatory effects comparable to diclofenac (5-10 mg/kg. In experimental models of hyperuricemia established with potassium oxonate, this treatment reduced serum uric acid by 25% in the acute model and 28–58% in the chronic model versus the untreated group ($p < 0.05$), although the levels were still higher than those achieved with allopurinol ($p < 0.05$). No signs of toxicity were observed at any dose. These findings suggest that DNC NAMCTGU is a safe and effective polyherbal preparation with analgesic, anti-inflammatory, and hypouricemic properties, supporting its potential as an alternative treatment for gout and inflammatory disorders. Additional research is needed to determine its clinical applicability.

Keywords: DNC NAMCTGU, *Cleistocalyx operculatus*, *Perilla frutescens*, *Curcuma longa*, analgesic, anti-inflammatory, hypouricemic, acute toxicity

Introduction

Gout has emerged as a progressively important global health issue, largely resulting from the consumption of purine-rich foods driven by rapid economic growth and urbanization. This condition, one of the most prevalent types of inflammatory joint disease, arises from issues with purine metabolism or impaired kidney function in excreting uric acid. These disturbances result in increased levels of uric acid in the bloodstream and accumulation of monosodium urate crystals in the joints, which subsequently trigger both acute and chronic inflammatory responses.[1, 2] Data from the 2021 Global Burden of Disease project indicate that around 55.8 million individuals had gout in 2020. This figure is anticipated to rise by 72.6%, reaching an estimated 95.8 million cases by 2050, a trend primarily attributed to population expansion and increasing body mass index (BMI). Data from the year 2020 indicate a marked sex disparity in gout prevalence, with males exhibiting rates approximately 3.26 times higher than those observed in females, and the risk of developing the disease showed a steady increase with advancing age.[3,4] Clinically, gout is marked by sudden flare-ups, joint deformities, chronic joint damage, and kidney stones.

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Such manifestations can markedly impair quality of life and potentially result in long-term disability....[5,6] Furthermore, gout is often accompanied by major comorbidities, most prominently cardiovascular disorders, chronic kidney dysfunction, obesity, and type 2 diabetes mellitus, thereby highlighting the urgent need for integrated and effective management approaches.[7, 8]

Current treatment approaches for gout are mainly directed at lowering serum uric acid concentrations and alleviating inflammation through the administration of urate-reducing and anti-inflammatory agents. Xanthine oxidase inhibitors, including allopurinol and febuxostat, are commonly utilized to suppress the production of uric acid. In addition, uricase-based therapies, including pegloticase, rasburicase, and uricozyme, enhance the enzymatic breakdown of uric acid. Although these pharmacological options demonstrate substantial efficacy, their use is often associated with adverse reactions, including gastrointestinal upset, hypersensitivity responses, vasculitis, and, in certain cases, renal complications, which may compromise long-term adherence.[10, 11] Consequently, increasing attention has been directed toward exploring safer, plant-derived alternatives with fewer side effects for the sustainable management of gout.

Natural bioactive compounds can both reduce uric acid levels and modulate inflammation, with C. operculatus flower buds specifically targeting Nrf-2, NF-κB, COX-2, and IL-6 signaling pathways.[12] Moreover, curcumin, the principal bioactive constituent of C. longa rhizomes, has demonstrated notable analgesic and anti-inflammatory effects in both preclinical and clinical studies. Evidence from arthritis models suggests that curcumin mitigates inflammatory responses through suppression of neutrophil recruitment, inhibition of prostaglandin biosynthesis, and reduction of HMGB1 secretion from endothelial cells.[13, 14] Clinical trials have demonstrated that a 2 g dose of curcumin provides analgesic effects comparable to 500 mg of acetaminophen,

supporting its therapeutic potential for joint disorders.[15] The hypouricemic effect of *P. frutescens* leaves has been attributed to suppression of xanthine oxidase activity, highlighting their potential for use in treating hyperuricemia..[16, 17]

Despite these promising findings, studies on the acute toxicity and combined effects of these medicinal plants remain limited. No studies to date have thoroughly investigated the potential synergistic actions of *C. operculatus* flower buds, *P. frutescens* leaves, and *C. longa* rhizomes in gout management. Therefore, this work evaluated the effects of DNC NAMCTGU on pain relief, inflammation control, and serum uric acid reduction *in vivo*, a herbal formulation containing extracts from these three medicinal plants, administered orally at 400, 800, and 1200 mg/kg in mice.

Materials and Methods

Sample

DNC NAMCTGU capsules were manufactured by Research and Development Institute of Medicinal Plants - Nam Can Tho University. Each capsule contains 200 mg of combined extracts, comprising 100 mg of *C. operculatus* flower bud, 50 mg of *P. frutescens* leaves, and 50 mg of *C. longa* rhizome.

Animals

Mice of the *Swiss albino* strain, including both sexes and weighing between 20 and 25 g, at 6–7 weeks of age, were utilized in the experiments, sourced from IVAC, Nha Trang, Vietnam. Before experimentation, the animals were housed under standard laboratory conditions for a 5-day acclimatization period. Mice were housed in plastic cages (25 × 35 × 15 cm) with wood shavings as bedding and provided ad libitum access to commercial rodent feed and fresh water. Animals were housed under controlled conditions: 22–24 °C, 40–60% humidity, and a 12 h photoperiod of light and darkness. To promote welfare and minimize stress, cages were regularly cleaned and enriched with nesting materials. All animal procedures were approved by the Scientific Committee of Nam Can Tho University, Can Tho City, Vietnam, and complied with ethical regulations (Approval No. 159/QĐ-ĐHNCT, dated 28 March 2019; No. 222/QĐ-ĐHNCT, dated 22 May 2020) in compliance with the 3Rs (Replacement, Reduction, and Refinement) principle.

Chemicals

Carrageenan (Sigma-Aldrich, USA) was diluted to 1-2% in physiological saline. Waterproofing solution Ornano imbidente (Ugo Basile, Italy) was prepared by diluting 1 mL in 500 mL of distilled water with 250 mg NaCl. Potassium oxonate (Sigma-Aldrich, USA), acetic acid (Prolabo, France), and allopurinol (300 mg tablets; Stada Limited Company, Vietnam) were used in this study. A uric acid quantification kit was purchased from Erba (Italy). Diclofenac (Voltaren 50 mg tablets) was obtained from Novartis (Italy).

Acute Safety Assessment

The study examined the acute toxicological profile of DNC NAMCTGU capsules in mice in accordance with the Vietnamese Ministry of Health guidelines for preclinical and clinical studies of traditional medicines and natural products. Ten healthy adult mice (5 males and 5 females) were fasted for at least 12 hours prior to dosing, with water provided *ad libitum*. The mice were given a single dose of DNC NAMCTGU capsules orally, using a gavage needle. The maximal feasible dose was 50 mg/kg body weight. After administering the dose, the mice were closely monitored for any changes in behavior, signs of toxicity, and mortality during the first 4 hours, then again at 72 hours, and daily for the next 14 days. Upon completion of the experiment, animals were humanely euthanized in accordance with institutional ethical standards.

Analgesic Effect

Analgesic effects of DNC NAMCTGU capsules were determined using the acetic acid–writhing model. Forty healthy male mice were assigned to five groups of eight. The groups received oral treatments as follows: 10 mL/kg of distilled water administered orally (pathology control), 10 mg/kg diclofenac (positive control; Novartis, Switzerland), and DNC NAMCTGU capsules at three dose levels of 400, 800, and 1200 mg/kg. Thirty minutes after administration, all animals received an intraperitoneal injection of 0.7% (v/v) acetic acid (10 mL/kg; Prolabo, France) to elicit visceral nociception. Behavioral responses indicative of pain such as contracting their abdominal muscles, twisting their bodies, arching their backs, and extending at least one limb was recorded every 5 minutes for a total of 40 minutes. [20, 21,22]

Evaluation of Acute Anti-Inflammatory Effect

Mouse Paw Edema Model Induced by Carrageenan

Therapeutic Effects

For this assay, sixty healthy male mice underwent a 12-hour fasting period prior to the experiment. The mice were then assigned to six groups of ten. Group 1 (normal/physiological reference) and Group 2 (disease model) were administered distilled water orally at 10 mL/kg. Group 3 (positive reference) received diclofenac orally at 10 mg/kg (Novartis, Italy), while Groups 4, 5, and 6 were treated with DNC NAMCTGU capsules at 400, 800, and 1200 mg/kg, respectively. One hour following treatment administration, all groups - except the physiological control - were injected intraplantarly with 25 µL of a 1% carrageenan suspension (w/v, Sigma-Aldrich, USA) to induce paw inflammation. The physiological control group was treated with 0.9% NaCl at an equivalent dose volume. Paw volume was recorded using a plethysmometer at baseline (V_0) and then at 1, 3, 5, 24, 48, 72, 96, and 120 hours after carrageenan injection (V_t). Animals were re-administered treatments once daily following the 24, 48, 72, and 96-hour measurements. Paw edema was quantified as the percentage change in volume from baseline ($\Delta V_t = [(V_t - V_0)/V_0] \times 100\%$).[23]

Preventive Effects

Similarly, for the preventive effects test, sixty healthy male mice underwent a 12-hour fasting period prior to the experiment. The mice were then assigned to six groups of ten. Group 1 (normal/physiological reference) and Group 2 (disease model) were administered distilled water orally at 10 mL/kg. Group 3 (positive reference) received diclofenac orally at 10 mg/kg (Novartis, Italy), while Groups 4, 5, and 6 were treated with DNC NAMCTGU capsules at 400, 800, and 1200 mg/kg, respectively. Treatments were given once daily in the morning (between 9:00 and 11:00 AM) for seven consecutive days. On day 7, one hour following the final dose, animals (except for those in the physiological control group) were injected intraplantarly in the left hind paw with 25 µL of a 1% carrageenan suspension (w/v, Sigma-Aldrich, USA). The physiological reference group was administered 0.9% sodium chloride (NaCl) instead. Paw volume was recorded using a plethysmometer at baseline (V_0) and at 1, 3, 5, 24, 48, 72, and 96 hours after the carrageenan injection. After the measurements at 24, 48, 72, and 96 hours, the corresponding treatments were re-administered. Paw swelling was expressed as a percentage increase in volume compared to baseline ($\Delta V_t = [(V_t - V_0)/V_0] \times 100\%$).[24]

Evaluation of Therapeutic Effects on Arthritis

Acute Arthritis Model

The acute anti-arthritic activity of DNC NAMCTGU capsules, was evaluated in sixty healthy male mice that had been fasted for 12 hours prior to experimentation. Group 1 (normal/physiological reference) and 2 (arthritic control) were given distilled water orally at 10 mL/kg. Group 3 (positive reference) received diclofenac orally at 5 mg/kg (Novartis, Italy), while Groups 4, 5, and 6 were treated with DNC NAMCTGU capsules at 400, 800, and 1200 mg/kg, respectively. Initial knee joint diameters (V_0) were measured before inflammation was induced. After oral administration of the assigned treatments, and following the 1-hour interval, mice in Groups 2-6 received an intra-articular injection of 15 µL of 2% carrageenan into the right knee joint to elicit inflammation. The mice in Group 1 received 0.9% sodium chloride (NaCl) as a physiological control. Treatments were administered once daily throughout the study period. Joint swelling was assessed at 1, 24, and 48 hours post-induction, and the progression of inflammation was also monitored at multiple time points - 1, 3, 4, 24, 48, and 72 hours following carrageenan injection.[25]

Chronic Arthritis Model

To evaluate the long-term anti-inflammatory activity of DNC NAMCTGU capsules, sixty healthy male mice underwent a 12-hour fasting period prior to the experiment. Group 1 (normal/physiological

reference) and 2 (arthritic control) were given distilled water orally at 10 mL/kg once daily for 21 days. Group 3 (positive reference) received diclofenac orally at 5 mg/kg (Novartis, Italy). Groups 4 to 6 were treated with DNC NAMCTGU capsules at 400, 800, and 1200 mg/kg, respectively, also administered once daily in the morning throughout the 21-day study. Chronic arthritis was induced in Groups 2 to 6 via intra-articular injection of 15 µL of 2% carrageenan solution (prepared in 0.9% NaCl) into the right knee joint every three days over a 19-day period (total of 7 injections). Mice in Group 1 received intra-articular injections of 0.9% NaCl as a control. Paw volume was recorded before the first induction (V_0), and subsequently on days 3, 6, 9, 12, 15, and 18. The degree of joint inflammation was assessed by monitoring changes in knee joint diameter throughout the experimental period.[26, 27]

Evaluation of the Hypouricemic Effect
Acute Hyperuricemia Model
Forty-eight healthy male mice were randomly assigned to six groups of eight. Group 1 (normal/physiological reference) and Group 2 (disease model) were administered distilled water orally at 10 mL/kg. Group 3 (positive reference) was given allopurinol (10 mg/kg, Stada, Vietnam) by oral gavage, while Groups 4, 5, and 6 were treated with DNC NAMCTGU capsules at 400, 800, and 1200 mg/kg, respectively. With the exception of the normal control group, hyperuricemia was induced in all animals by intraperitoneal injection of potassium oxonate (300 mg/kg; Sigma-Aldrich, USA) suspended in 0.5% carboxymethyl cellulose-sodium (CMC-Na, 10 mL/kg). The normal control group instead received 0.9% saline at the same volume. Two hours after oxonate administration, blood samples were withdrawn, anticoagulated with EDTA, and centrifuged at 3000 rpm for 10 min at ambient temperature (25 °C). Plasma uric acid concentrations were determined using a commercial assay kit (Erba, Italy) following the manufacturer's instructions.[28, 29]

Chronic Hyperuricemia Model
Forty-eight healthy male mice were randomly assigned to six groups of eight. Group 1 (normal/physiological reference) and and Group 2 (disease model) were administered distilled water orally at 10 mL/kg. Group 3 (positive reference) was given allopurinol (10 mg/kg, Stada, Vietnam) on days 7 and 15; on the remaining days, this group received distilled water. Test groups (Groups 4, 5, and 6) were treated with DNC NAMCTGU capsules at 400, 800, and 1200 mg/kg, respectively, administered daily for 15 consecutive days. All groups, with the exception of the normal control, were rendered chronically hyperuricemic through intraperitoneal administration of potassium oxonate (Sigma-Aldrich, USA). The dosing schedule consisted of 300 mg/kg on day 1, followed by 250 mg/kg on day 3, 200 mg/kg on day 5, and 150 mg/kg on days 7, 9, 11, 13, and 15. Oral treatments (distilled water, allopurinol, or DNC NAMCTGU) were given one hour after each potassium oxonate injection in accordance with the assigned group regimen. Blood collection was performed from the tail vein on days 7 and 15, two hours after oral administration, following a minimum 12-hour overnight fasting period. Plasma uric acid concentrations were subsequently measured to evaluate the effect of the interventions.[28, 29]

Statistical Analysis
Statistical analyses were carried out with SPSS software (version 22). Data are reported as mean ± standard deviation (SD). Group differences were first assessed using the Kruskal–Wallis test, and when appropriate, post hoc pairwise comparisons were conducted with the Mann–Whitney U test. A p-value < 0.05 was considered statistically significant.

Results and Discussion

Acute Safety Assessment
Acute oral toxicity testing of DNC NAMCTGU capsules was performed in Swiss albino mice, and the findings are summarized in Table 1. Each animal received a single oral dose of DNC NAMCTGU hard capsules at a concentration of 0.64 g/kg, administered in a volume of 50 mL/kg, which corresponds to an effective dose of 32.05 g/kg. Within the first hour following administration, the mice exhibited transient signs such as reduced locomotor activity, sluggish movement, mild tremors, and accelerated respiration. These symptoms were temporary, and the animals gradually returned to normal behavior.

No mortality was observed within the initial 72 hours post-treatment. The mice were continuously monitored over a 14-day period under standard housing and care conditions. Throughout this observation phase, no deaths occurred, and no noticeable abnormalities were detected in terms of behavior, fur appearance, appetite, or excretory patterns.
Based on these findings, the LD_{50} could not be determined, as the formulation did not induce lethal or toxic effects at the administered dose. The experimental protocol followed a modified Behrens method, and the tested dose - equivalent to 3 to 6 times the expected therapeutic level - showed no adverse outcomes. These results suggest a therapeutic index of approximately 40, demonstrating a considerable margin of safety for DNC NAMCTGU capsules and highlighting their potential for future therapeutic development or clinical application.

Analgesic Effect of DNC NAMCTGU Capsules in Mice
As illustrated in Table 2, diclofenac (10 mg/kg) markedly attenuated the acetic acid–induced abdominal writhing response in mice, showing a reduction ranging from approximately 45% to 83% relative to the untreated pathology control ($p < 0.05$). Oral exposure to DNC NAMCTGU capsules at 400, 800, and 1200 mg/kg led to a dose-related suppression of writhing behavior in mice ($p < 0.05$). Although DNC NAMCTGU demonstrated analgesic properties, its effect was less potent than that of diclofenac throughout all observed time intervals. Interestingly, comparison of the 800 and 1200 mg/kg treatments revealed no meaningful difference in analgesic activity ($p > 0.05$), suggesting that efficacy did not increase beyond the 800 mg/kg dose.
These findings are in agreement with earlier reports. For instance, a previous study evaluating the analgesic effect of *C. longa* rhizome extract in male Wistar rats, with the acetic acid–evoked abdominal constriction model, demonstrated that a 400 mg/kg dose markedly decreased the number of abdominal contractions relative to the control group.[30] In another model of pain, an ethanolic extract of *C. longa* at the same dosage produced pain-relieving effects comparable to those of aspirin in a thermal nociception assay.[31] Moreover, leaf extracts of *P. frutescens* leaves exhibited pronounced antinociceptive activity in both chemical-induced abdominal constriction and thermal nociception assays.[32] Clinical investigations further validated these results, showing that patients with mild knee osteoarthritis who received *P. frutescens* extract for eight weeks experienced significant reductions in pain scores, as evaluated by the VAS and WOMAC, relative to the placebo group. These data collectively support the preclinical and clinical analgesic potential of *P. frutescens*.[33]

Anti-inflammatory Effect of DNC NAMCTGU Capsules in Mice
Evaluation of Acute Inflammation in a Mouse Paw Edema Model Induced by Carrageenan
Table 3 presents the therapeutic responses of the test formulations on carrageenan-induced acute inflammation. Compared with the normal (physiological) control, the untreated inflammation group (pathology group) showed a marked rise in paw edema at all observation time points ($p < 0.01$). Oral administration of diclofenac (10 mg/kg) produced a marked reduction in paw edema, with decreases ranging from 35% to 80% relative to the pathology control group ($p < 0.05$). Likewise, oral administration of DNC NAMCTGU capsules at 400, 800, and 1200 mg/kg resulted in a notable decrease in paw edema, with reductions of approximately 15% to 40% observed within the 1–120 hour window post-carrageenan challenge ($p < 0.05$). However, during the first 24 hours following induction, the paw swelling in all DNC NAMCTGU-treated groups remainded significantly greater than that observed in the diclofenac group ($p < 0.05$). Overall, although DNC NAMCTGU capsules demonstrated anti-inflammatory activity, their effect was less pronounced than that of diclofenac at equivalent time points. Among the different doses tested, the 800 mg/kg dose yielded the most significant therapeutic effect in reducing acute inflammation.
Table 4 describes the preventive anti-inflammatory effects of the tested formulations. In the pathology group, paw edema peaked at 3 hours post-carrageenan injection and subsequently declined, though it remained significantly elevated relative to the physiological group at all time points beyond 3 hours ($p < 0.01$). Diclofenac at 5 mg/kg significantly reduced paw swelling compared with the pathology group at several observation periods ($p < 0.05$). Among the DNC

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Table 1: Acute oral toxicity of DNC NAMCTGU capsules in mice

Test Mice		1	2	3	4	5	6	7	8	9	10
Sex		♂	♂	♂	♂	♂	♀	♀	♀	♀	♀
Weight at dosing (g)		29.1	27,5	27	30.6	29.2	23.9	23.2	22.9	24	23.6
Dose volume (mL)		1.46	1.38	1.35	1.53	1.46	1.2	1.16	1.15	1.2	1.18
Total weight (g)		261.0									
Total volume (mL)		13.07									
Total capsule weight (g)		8.3648									
Dose administered (g/kg)		32.05									
Number of test mice		10									
Number of mice dead after 72 hours		0									
Number of mice dead after 14 days		0									
Weight of mice (g) after DNC NAMCTGU administration	7 days	33.9	33.4	32.7	37.6	35.7	32.4	32.5	31.9	33.2	32.2
	14 days	34.2	34.8	34.5	32.4	34.5	36.6	37.1	35.9	41.8	40.5

Table 2: Analgesic effects on acetic acid-induced rithing response in mice

Group (n = 8)	Mouse abdominal writhing response (seconds) (Mean±SEM)								
	0-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	Total
Pathology	10.9 ± 3.0	20.1 ± 1.8	18.8 ± 2.6	16.8 ± 1.8	15.6 ± 2.1	11.1 ± 1.9	7.5 ± 1.1	8.5 ± 1.7	109.3 ± 10.6
Diclofenac 10 mg/kg	3.1 ± 1.1	7.1 ± 1.9$^{\#\#}$	10.4 ± 2.9	6.6 ± 1.3$^{\#\#}$	3.9 ± 0.8$^{\#\#}$	3.6 ± 1.0$^{\#\#}$	2.4 ± 0.6$^{\#\#}$	1.0 ± 0.8$^{\#\#}$	38.6 ± 7.3$^{\#\#}$
DNC NAMCTGU 400 mg/kg	6.0 ± 1.2	15.5 ± 1.4$^{\$}$	16.1 ± 2.2	13.9 ± 2.2$^{\$\$}$	9.9 ± 1.4$^{\$\$}$	8.5 ± 1.3$^{\$\$}$	6.3 ± 1.7$^{\$}$	5.6 ± 1.3$^{\$}$	81.8 ± 8.0$^{\$\$}$
DNC NAMCTGU 800 mg/kg	6.8 ± 2.5	14.3 ± 2.9	13.1 ± 2.4	9.6 ± 2.2$^{\#}$	11.3 ± 1.8$^{\$\$}$	5.8 ± 1.0	4.6 ± 1.2	3.6 ± 0.6$^{\#\#\$}$	69.0 ± 9.5$^{\#\#}$
DNC NAMCTGU 1200 mg/kg	6.8 ± 1.6	10.3 ± 2.3$^{\#}$	10.0 ± 1.0$^{\#\&}$	9.6 ± 1.5$^{\#}$	11.0 ± 0.9$^{\$\$}$	8.5 ± 1.9$^{\$}$	5.3 ± 0.9$^{\$}$	2.9 ± 0.4$^{\#\#\$\&}$	64.3 ± 4.3$^{\#\#\$}$

$^{*}p < 0.05$ and $^{**}p < 0.01$: Compared to physiological group at the same time point of observation
$^{\#}p < 0.05$ and $^{\#\#}p < 0.01$: Compared to pathology group at the same time point of observation
$^{\$}p < 0.05$: Compared to diclofenac 5 mg/kg group at the same time point of observation
$^{\&}p < 0.05$: Compared to DNC NAMCTGU 400 mg/kg group at the same time point of observation

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Table 3: Acute therapeutic anti-inflammatory effect of DNC NAMCTGU capsules in carrageenan-induced paw edema in mice

Group (n = 10)	Footpad swelling (% mean ± SEM)						
	$\triangle V_{1h}$	$\triangle V_{3h}$	$\triangle V_{5h}$	$\triangle V_{24h}$	$\triangle V_{48h}$	$\triangle V_{72h}$	$\triangle V_{96h}$
Physiology	13.54 ± 3.37	11.51 ± 1.64	8.43 ± 1.58	8.38 ± 2.40	4.36 ± 1.10	3.09 ± 1.11	2.05 ± 1.12
Pathology	21.77 ± 2.21	47.90 ± 4.34**	33.26 ± 5.47**	37.46 ± 3.18**	31.38 ± 4.21**	24.58 ± 2.78**	17.17 ± 1.22**
Diclofenac 5 mg/kg	16.20 ± 1.31	31.32 ± 4.14$^{**\#}$	21.08 ±1.86**	20.78 ± 2.29$^{**\#\#}$	15.81 ± 2.59$^{**\#\#}$	11.83 ± 1.93$^{**\#\#}$	6.89 ± 1.70$^{\#\#}$
DNC NAMCTGU 400 mg/kg	17.29 ± 3.98	50.13 ± 4.24$^{**\$}$	27.00 ± 3.06**	37.19 ± 4.52$^{**\$}$	26.13 ± 5.37**	19.16± 4.78**	14.27 ± 3.82**
DNC NAMCTGU 800 mg/kg	24.56 ± 3.95	41.04 ± 2.96**	30.53 ± 4.74**	23.52± 5.36	16.19 ± 3.06$^{**\#\#}$	10.68 ± 2.22$^{*\#\#}$	7.49 ± 1.87$^{*\#\#}$
DNC NAMCTGU 1200 mg/kg	21.8 ± 3.24	43.17 ± 5.86**	22.48 ± 3.94**	19.98 ± 2.87$^{*\#\#\&\&}$	13.95 ± 2.18$^{**\#\#}$	9.99 ± 3.87$^{\#\#}$	6.45 ± 2.13$^{\#\#}$

$\triangle$V: represents the degree of paw edema caused by inflammation at each time point, reflecting the anti-inflammatory effect of the treatment.
$^{*}p < 0.05$ and $^{**}p < 0.01$: Compared to physiological group at the same time point of observation.
$^{\#}p < 0.05$ and $^{\#\#}p < 0.01$: Compared to pathology group at the same time point of observation.
$^{\$}p < 0.05$: Compared to diclofenac 5 mg/kg group at the same time point of observation.

Table 4: Acute preventive anti-inflammatory effect of DNC NAMCTGU capsules in carrageenan-induced paw edema in mice

Group (n = 10)	Footpad swelling (% mean ± SEM)							
	$\triangle V_{1h}$	$\triangle V_{3h}$	$\triangle V_{5h}$	$\triangle V_{24h}$	$\triangle V_{48h}$	$\triangle V_{72h}$	$\triangle V_{96h}$	$\triangle V_{120h}$
Physiology	8.08 ± 1.69	7.37 ± 2.86	2.50 ± 1.27	2.50 ± 1.67	0.83 ± 0.83	0.83 ± 0.83	0.83 ± 0.83	0.83 ± 0.83
Pathology	64.04 ± 6.47**	88.11 ± 6.69**	76.66 ± 6.42**	74.15 ± 5.85**	56.71 ± 8.55**	35.96 ± 4.10**	38.97 ± 5.24**	34.63 ± 3.61**
Diclofenac 10 mg/kg	36.54 ± 5.27$^{**\#}$	56.87 ± 4.27$^{**\#}$	42.53 ± 3.86$^{**\#\#}$	37.55 ± 4.28$^{**\#\#}$	16.27 ± 5.04$^{**\#\#}$	15.81 ± 4.91$^{**\#}$	12.57 ± 3.95$^{**\#\#}$	7.45 ± 2.19$^{**\#\#}$
DNC NAMCTGU 400 mg/kg	43.65 ± 3.74$^{**\#}$	63.21 ± 1.23$^{**\#}$	65.04 ± 3.96$^{**\#\$\$}$	60.49 ± 3.41$^{**\#\$\$}$	55.53 ± 8.79$^{**\$\$}$	44.96 ± 9.57$^{**\$}$	30.87 ± 5.90$^{**\$}$	21.73 ± 2.70$^{**\#\#\$}$
DNC NAMCTGU 800 mg/kg	41.28 ± 4.27$^{**\#}$	62.99 ± 5.33$^{**\#}$	60.00 ± 2.19$^{**\#\$}$	55.07 ± 4.35$^{**\#\#\$\$}$	34.72 ± 6.87$^{**\#}$	28.46 ± 4.90**	25.87±5.37*	19.31 ± 4.99$^{**\#}$
DNC NAMCTGU 1200 mg/kg	43.38 ± 3.85$^{**\#}$	67.21 ± 5.31$^{**\#}$	63.39 ± 3.27$^{**\#\$\$}$	62.65 ± 4.43$^{**\#\$\$}$	38.01 ± 4.72$^{**\$}$	31.14 ± 4.32**	27.68 ± 4.49$^{**\$}$	21.18 ± 3.80$^{**\#\$}$

ΔV: the change in paw volume (edema) after inflammation induced by carrageenan, measured following treatment, compared to the baseline volume.
$^{*}p < 0.05$ and $^{**}p < 0.01$: Compared to physiological group at the same time point of observation.
$^{\#}p < 0.05$ and $^{\#\#}p < 0.01$: Compared to pathology group at the same time point of observation.
$^{\$}p < 0.05$: Compared to diclofenac 5 mg/kg group at the same time point of observation.
$^{\&}p < 0.05$: Compared to DNC NAMCTGU 400 mg/kg group at the same time point of observation.
$^{@}p < 0.05$: Compared to DNC NAMCTGU 800 mg/kg group at the same time point of observation.

NAMCTGU-treated groups, the 800 mg/kg dose produced the most consistent preventive effect, significantly reducing paw thickness at most time points ($p < 0.05$), except at 3 hours post-induction. Likewise, the 1200 mg/kg dose also produced a statistically meaningful reduction in inflammation beginning at 24 hours post-induction ($p < 0.05$). All tested doses of DNC NAMCTGU (400, 800, and 1200 mg/kg) demonstrated a preventive anti-inflammatory effect, with the 800 mg/kg dose showing the highest efficacy. Remarkably, its preventiveactivity was statistically indistinguishable from that of diclofenac at 5 mg/kg ($p > 0.05$).

These results are consistent with previous studies on the anti-inflammatory effects of *P. frutescens*, which showed significant inhibition of carrageenan-induced paw edema in animal models at doses of 2.5 and 5 mL/kg, supporting its strong pharmacological activity. These outcomes further reinforce the observations from our study and emphasize the therapeutic potential of *P. frutescens*-derived bioactive

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Table 5: The level of swelling of foot joints on acute arthritis model in mice

Group (n = 8)	Mouse foot joint swelling (% mean ± SEM)					
	$\triangle V_{1h}$	$\triangle V_{3h}$	$\triangle V_{5h}$	$\triangle V_{24h}$	$\triangle V_{1h}$	$\triangle V_{48h}$
Physiology	9.11 ± 1.01	11.93 ± 2.18	4.85 ± 1.38	1.46 ± 0.72	4.41 ± 1.60	5.35 ± 1.93
Pathology	17.48 ± 2.59*	29.17 ± 2.80**	25.28 ± 3.18**	14.64 ± 2.70**	11.36 ± 3.02	16.60 ± 3.31**
Diclofenac 5 mg/kg	16.27 ± 1.28*	12.29 ± 1.48$^{\#\#}$	10.88 ± 1.43$^{*\#\#}$	5.48 ± 1.08$^{**\#\#}$	2.52 ± 0.75$^{\#}$	3.22 ± 1.72$^{\#\#}$
DNC NAMCTGU 400 mg/kg	15.33 ± 2.27	10.83 ± 2.79$^{\#\#}$	8.47 ± 2.22$^{\#\#}$	8.47 ± 2.33*	7.99 ± 2.84	6.01 ± 2.15$^{\#}$
DNC NAMCTGU 800 mg/kg	13.16 ± 1.60*	11.58 ± 1.57$^{\#\#}$	7.13 ± 1.51 $^{\#\#}$	5.07 ± 0.99$^{*\#\#}$	7.15 ± 2.82	5.02 ± 1.66$^{\#}$
DNC NAMCTGU 1200 mg/kg	21.32 ± 2.00$^{**@@}$	19.17 ± 2.01$^{*\#\#\$\&@}$	12.15 ± 2.12$^{*\#\#}$	7.68 ± 2.95*	13.33 ± 4.27	4.65 ± 2.14$^{\#}$

△V: represents the change in joint diameter after inflammation was induced by carrageenan, compared to the baseline measurement (V_0).
$^{*}p < 0.05$ and $^{**}p < 0.01$: Compared to physiological group at the same time point of observation
$^{\#}p < 0.05$ and $^{\#\#}p < 0.01$: Compared to pathology group at the same time point of observation
$^{\$}p < 0.05$: Compared to diclofenac 5 mg/kg group at the same time point of observation
$^{\&}p < 0.05$: Compared to DNC NAMCTGU 400 mg/kg group at the same time point of observation
$^{@}p < 0.05$: Compared to DNC NAMCTGU 800 mg/kg group at the same time point of observation

Table 6: The level of swelling of the mouse foot joints on the chronic arthritis model in mice

Group (n = 8)	Mouse foot joint swelling (% mean ± SEM)					
	$\triangle V_{D3}$	$\triangle V_{D6}$	$\triangle V_{D9}$	$\triangle V_{D12}$	$\triangle V_{D15}$	$\triangle V_{D18}$
Physiological	5.35 ± 1.93	0.00 ± 0.00	4.43 ± 1.92	11.92 ± 2.26	10.44 ± 3.44	8.92 ± 2.73
Pathology	16.60 ± 3.31**	25.09 ± 2.91**	19.03 ± 2.83**	27.09 ± 2.48**	27.65 ± 1.58**	19.43 ± 1.93*
Diclofenac 5 mg/kg	3.22 ± 1.72$^{\#\#}$	6.56 ± 2.41$^{*\#\#}$	8.16 ± 1.88$^{\#\#}$	8.39 ± 1.96$^{\#\#}$	10.88 ± 2.96$^{\#\#}$	4.05 ± 1.37$^{\#\#}$
DNC NAMCTGU 400 mg/kg	6.01 ± 2.15$^{\#}$	1.28 ± 0.84$^{\#\#}$	8.34 ± 1.23$^{\#\#}$	13.73 ± 3.68$^{\#}$	7.58 ± 2.68$^{\#\#}$	4.63 ± 2.29$^{\#\#}$
DNC NAMCTGU 800 mg/kg	5.02 ± 1.66$^{\#}$	0.63 ± 0.63$^{\#\#@}$	12.09 ± 2.51*	14.89 ± 2.09$^{\#\#\$}$	8.05 ± 1.04$^{\#\#}$	6.17 ± 1.36$^{\#\#}$
DNC NAMCTGU 1200 mg/kg	4.65 ± 2.14$^{\#}$	6.68 ± 2.58$^{\#}$	16.87 ± 3.07$^{**\$\&}$	17.02 ± 4.92$^{\#}$	16.29 ± 4.63	11.68 ± 4.81$^{\$\&}$

ΔV: represents the change in knee joint volume (paw volume) following chronic inflammation induced by carrageenan, compared to the initial volume (V_0) measured before induction.
$^{*}p < 0.05$ and $^{**}p < 0.01$: Compared to physiological group at the same time point of observation.
$^{\#}p < 0.05$ and $^{\#\#}p < 0.01$: Compared to pathology group at the same time point of observation.
$^{\$}p < 0.05$: Compared to diclofenac 5 mg/kg group at the same time point of observation.
$^{\&}p < 0.05$: Compared to DNC NAMCTGU 400 mg/kg group at the same time point of observation.
$^{@}p < 0.05$: Compared to DNC NAMCTGU 800 mg/kg group at the same time point of observation.

Table 7: Uric acid concentration of test groups in model of acute hyperuricemia

Group (n = 8)	Physiological	Pathology	Allopurinol 10 mg/kg	DNC NAMCTGU 400 mg/kg	DNC NAMCTGU 800 mg/kg	DNC NAMCTGU 1200 mg/kg
Uric acid concentration (mg/dL) (Mean ± SEM)	2.44 ± 0.14	3.71 ± 0.27**	2.77 ± 0.16$^{\#}$	4.28 ± 0.23$^{**\$\$}$	2.69 ± 0.26$^{\#\&\&}$	5.08 ± 0.45$^{**\#\$\$@@}$

$^{*}p < 0.05$ and $^{**}p < 0.01$: Compared to physiological group at the same time point of observation.
$^{\#}p < 0.05$ and $^{\#\#}p < 0.01$: Compared to pathology group at the same time point of observation.
$^{\$}p < 0.05$: Compared to diclofenac 5 mg/kg group at the same time point of observation.
$^{\&}p < 0.05$: Compared to DNC NAMCTGU 400 mg/kg group at the same time point of observation.
$^{@}p < 0.05$: Compared to DNC NAMCTGU 800 mg/kg group at the same time point of observation.

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Table 8: Uric acid concentration of test groups in model of chronic hyperuricemia

Group (n = 8)		Physio-logical	Pathology	Allopurinol 10 mg/kg	DNC NAMCTGU 400 mg/kg	DNC NAMCTGU 800 mg/kg	DNC NAMCTGU 1200 mg/kg
Uric acid concentra-tion (mg/dL) (Mean ± SEM)	After 7 days	2.56 ± 0.30	5.86 ± 0.43**	3.33 ± 0.33$^{\#\#}$	6.50 ± 0.50$^{**\$\$}$	2.45 ± 0.29$^{\#\#\$\&\&}$	6.21 ± 0.21$^{**\$\$@@}$
	After 15 days	4.00 ± 0.29	5.83 ± 0.36**	1.63 ± 0.09$^{**\#\#}$	5.31 ± 0.34$^{*\$\$}$	4.08 ± 0.45$^{\#\$\$\&}$	5.82 ± 0.30$^{**\$\$@}$

$^{*}p < 0.05$ and $^{**}p < 0.01$: Compared to physiological group at the same time point of observation
$^{\#}p < 0.05$ and $^{\#\#}p < 0.01$: Compared to pathology group at the same time point of observation.
$^{\$}p < 0.05$: Compared to diclofenac 5 mg/kg group at the same time point of observation.
$^{\&}p < 0.05$: Compared to DNC NAMCTGU 400 mg/kg group at the same time point of observation
$^{@}p < 0.05$: Compared to DNC NAMCTGU 800 mg/kg group at the same time point of observation.

compounds in managing acute inflammatory conditions.[34] In addition, previous investigations identified dimethyl cardamonin, a constituent of *C. operculatus* flower buds, as having notable anti-inflammatory effects, primarily mediated through modulation of the PI3K-PDK1-PKC signaling pathway, underscoring its promise as a candidate anti-inflammatory agent.[35] Similarly, curcumin and its analogues have been extensively shown to attenuate carrageenan-induced paw edema in Swiss albino mice. Dose-dependent effects were observed across a range of 20, 40, 80, and 160 mg/kg, with the highest dose producing the most pronounced reduction in paw swelling.[36]

Evaluation of the Therapeutic Effects of DNC NAMCTGU on Arthritis in a Mouse Model Induced by Carrageenan

As shown in Table 5, administration of 2% carrageenan induced notable swelling in the foot joints of mice, with edema levels ranging from 11.4% to 29.2% relative to the normal reference group ($p < 0.05$), with the exception of time points beyond 48 hours, where no significant difference was observed. Diclofenac, administered orally at 5 mg/kg, effectively mitigated joint inflammation, producing a significant reduction in swelling by approximately 57% to 81% decrease in swelling in comparison with the pathology group. This anti-arthritic activity was apparent as early as 3 hours after carrageenan injection ($p < 0.05$). Treatment with DNC NAMCTGU capsules at 400, 800, and 1200 mg/kg likewise produced significant reductions in joint swelling at the 3-, 5-, and 72-hour assessments following a 1% carrageenan challenge ($p < 0.05$). Among the tested regimens, the 800 mg/kg dose showed the greatest therapeutic benefit, achieving decreases in inflammation comparable to those observed in the diclofenac-treated group ($p > 0.05$).

As presented in Table 6, the pathology developed marked foot swelling, with edema levels ranging from 16.6 - 27.7% which remained significantly higher than values observed in the physiological control group from day 3 to day 18 ($p < 0.05$). Oral administration of diclofenac (5 mg/kg) consistently reduced swelling across all evaluation points, with highly significant differences observed ($p < 0.01$). Likewise, treatment with DNC NAMCTGU capsules at 400 and 800 mg/kg resulted in foot joint swelling levels comparable to those observed in the diclofenac-treated group ($p > 0.05$). These results suggest that DNC NAMCTGU capsules, particularly at 400 and 800 mg/kg, exert therapeutic effects against chronic arthritis comparable to diclofenac at 5 mg/kg.

This is the first investigation to evaluate the combined *in vivo* anti-inflammatory effects of a formulation containing extracts from *C. operculatus* flower buds, *P. frutescens* leaves, and *C. longa* rhizomes. Within experimental models of carrageenan-induced inflammation, encompassing both acute and chronic phases, DNC NAMCTGU capsules significantly reduced paw edema, with the 800 mg/kg dose consistently demonstrating the best efficacy-comparable to diclofenac at 5-10 mg/kg.

The study's results are consistent with both traditional and experimental uses of the component herbs. For instance, an ethanol extract of *P. frutescens* (perilla leaf extract) was evaluated in patients with mild knee joint pain, where a daily dose of 600 mg significantly improved pain and joint function while maintaining a favorable safety profile throughout the stud.[37] Similarly, clinical data on curcumin, the principal bioactive compound of turmeric (C. longa), demonstrated anti-inflammatory efficacy at doses between 500 and 1000 mg/day, with notable reductions in markers such as VAS and WOMAC scores, along with improved joint function indices and minimal adverse effects.[38] The study examined the chemical makeup and biological effects of the flower buds of *C. operculatus*. It found that these flower buds have strong anti-inflammatory and antioxidant properties.Tests on mouse models showed that the extract from the flower buds could reduce inflammation and counteract the effects of free radicals, particularly through mechanisms such as suppressing the release of pro-inflammatory cytokines and mitigating intracellular oxidative stress.[39] The findings highlight the therapeutic potential of DNC NAMCTGU capsules and suggest that the observed anti-inflammatory effect may result from a synergistic interaction among the active constituents of the included medicinal plants.

Evaluation of the Hypouricemic Effect of DNC NAMCTGU Capsules in Mice

The hypouricemic activity of DNC NAMCTGU capsules was tested using both acute and chronic models of potassium oxonate-induced hyperuricemia. In the acute model, as shown in Table 7, administration of potassium oxonate (300 mg/kg, i.p.) led to a 1.5-times increase in serum uric acid levels compared with the normal control group ($p < 0.01$). Oral allopurinol at 10 mg/kg lowered serum uric acid by roughly 25% relative to the hyperuricemic group ($p < 0.05$). Among the tested DNC NAMCTGU doses, the 800 mg/kg treatment yielded a comparable 25% decline ($p > 0.05$), whereas the 400 mg/kg and 1200 mg/kg groups did not exhibit a significant decline.

In the chronic hyperuricemia model (Table 8), elevated serum uric acid levels persisted over the 15-day period. Mice in the pathology group showed approximately 2.2- and 1.46-fold increases in serum uric acid on days 7 and 15, respectively, relative to the normal control group ($p < 0.01$). Treatment with allopurinol (10 mg/kg) markedly lowered serum uric acid by 43% on day 7 and 72% on day 15 ($p < 0.01$), with day 7 levels returning close to physiological values ($p > 0.05$). Oral administration of DNC NAMCTGU at 800 mg/kg resulted in significant reductions of 28% and 58% on days 7 and 15, respectively ($p < 0.05$); however uric acid concentrations in the DNC NAMCTGU-treated groups remained notably elevated compared with the allopurinol group ($p < 0.05$), reflecting a comparatively milder but still meaningful hypouricemic effect.

These results indicate that DNC NAMCTGU capsules, particularly at 800 mg/kg, exhibit a moderate yet significant capacity to reduce serum uric acid in both acute and chronic hyperuricemia models. Although this effect is not as strong as allopurinol in the chronic model, the comparable performance in the acute model is encouraging. Previous studies have shown that standardized *P. frutescens* leaf extract, which is rich in rosmarinic acid, can significantly lower blood uric acid concentrations in potassium oxonate-treated hyperuricemic mice, with

reductions ranging from approximately 14% to 37% at doses of 0.5–1 g/kg, respectively, relative to the pathological control group ($p \leq 0.001$). These findings suggest that rosmarinic acid contributes substantially to the hypouricemic activity of *P. frutescens* leaves and may facilitate the development of herbal therapies for hyperuricemia.[40] In addition, curcumin administered at 50 mg/kg in hyperuricemic mice was shown to decrease serum uric acid, improve renal function, modulate gut microbiota, and reduce inflammation, highlighting its potential as an adjunctive treatment for uric acid-induced nephritis.[41] Furthermore, studies onn *C.longa*, *P. frutescens*, and *C. operculatus* have reported their ability to inhibit xanthine oxidase and possess antioxidant properties.[42,43,44] Collectively, these observations support the use of this multi-herbal formulation as a complementary approach for managing hyperuricemia and gout.

Conclusion

Preclinical studies demonstrated that DNC NAMCTGU capsules possess significant analgesic, anti-inflammatory, and hypouricemic activities. Oral administration at doses of 400, 800, and 1200 mg/kg produced effective pain relief, preventive and therapeutic anti-inflammatory effects in acute models, and conferred therapeutic benefits in chronic arthritis models. Among these, the 800 mg/kg consistently showed the most pronounced effects, effectively reducing joint inflammation and lowering serum uric acid levels. These findings support the potential of DNC NAMCTGU as a promising polyherbal formulation for gout prevention and management. Further studies are needed to clarify the underlying molecular mechanisms and to confirm its clinical effectiveness in human trials.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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