Journal of Young Pharmacists, 2025; 17(3):653-660.
https://www.jyoungpharm.org

Original Article

Evaluation of Anti-Gout Effect of Polyherbal Formulation Acupen in Monosodium Urate Crystal-Induced Gouty Arthritis via Anti-Inflammatory Action in Experimental Animal Model

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ABSTRACT

Aim: This study aimed to evaluate the anti-gout activity of ACUPEN formulation using the Monosodium Urate (MSU)-induced gouty arthritis model and assess its Xanthine Oxidase (XO) inhibitory activity. **Materials and Methods:** Wistar albino rats were divided into six groups. The control group received normal saline, while the other groups were administered 50 µL of MSU crystals (25 mg/mL) intra-articularly into the right ankle joint under anesthesia. The study analyzed the swelling ratio, inflammation index, dysfunction index, gait analysis, and serum uric acid levels. Histopathological studies of the liver, kidney, and joints were also performed. The xanthine oxidase inhibitory activity of ACUPEN tablets was measured spectrophotometrically by quantifying the conversion of xanthine to uric acid and comparing it with the activity of allopurinol. **Results:** Treatment groups showed a significant reduction in inflammation and swelling at 12 and 24 hr. The high-dose ACUPEN group (600 mg/kg) demonstrated a significant improvement in inflammation at 48 hr. The XO *in vitro* study revealed an IC_{50} value of 24.8 for ACUPEN. Gait scores in treatment groups decreased, indicating symptom alleviation. No abnormalities were observed in liver and kidney biopsies. **Conclusion:** ACUPEN tablets exhibited significant XO inhibitory activity *in vitro* and demonstrated improvements in inflammation, dysfunction, and gait. These findings suggest that ACUPEN may effectively manage gouty arthritis by reducing hyperuricemia and inflammation. Further clinical studies are needed to confirm these effects *in vivo* and evaluate their safety and efficacy in humans.

Keywords: ACUPEN, Gouty Arthritis, Monosodium Urate Model, Polyherbal Formulation.

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Received: 27-02-2025;
Revised: 09-05-2025;
Accepted: 18-07-2025.

INTRODUCTION

Gouty arthritis is a chronic inflammatory condition characterized by intense pain and swelling in the joints due to the deposition of Monosodium Urate (MSU) crystals. These crystals form from excessive nucleic acid breakdown, triggering an inflammatory response. MSU crystals activate the immune system, leading to leukocyte infiltration and phagocytosis by macrophages and monocytes. Additionally, the generation of Reactive Oxygen Species (ROS) activates the Nuclear Factor Kappa B (NF-κB) pathway, releasing pro-inflammatory cytokines that further amplify the inflammatory process. If left untreated, this may lead to the degradation of joint tissues (Hidayat *et al*., 2020; Widyaningsih *et al*., 2017; Yang *et al*., 2018).

The current therapeutic approach for managing gouty arthritis includes the use of colchicine, corticosteroids, and Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), such as naproxen and indomethacin, to reduce pain and inflammation (Parisa *et al*., 2020). Furthermore, Urate-Lowering Medications (ULMs) like xanthine oxidase inhibitors (e.g., allopurinol) and uricosuric agents are utilized to lower uric acid levels. Xanthine oxidase inhibitors are particularly beneficial for patients with overproduction of urate, while uricosurics are recommended for those with impaired renal excretion (Yao *et al*., 2020). However, ULMs can cause adverse effects such as hepatotoxicity, hypersensitivity reactions, and renal dysfunction (Abu Bakar *et al*., 2018). Allopurinol, in particular, is linked to side effects like liver dysfunction and gastrointestinal issues (Widyaningsih *et al*., 2017).

DOI: 10.5530/jyp.20251785

Given these challenges, there is a pressing need for safer, more effective treatments that offer anti-inflammatory, analgesic, and urate-lowering properties without significant side effects (Hidayat *et al.*, 2020).

ACUPEN (300 mg), a poly-herbal formulation by ATBU Harita Pharmaceuticals Pvt. Ltd., is aimed at treating joint pain and inflammation in conditions like gouty arthritis, rheumatoid arthritis, and osteoarthritis. Its individual components, as shown in Table 1, have demonstrated potential in managing gouty arthritis (Khan and Khan, 2018; Hiruma-Lima *et al.*, 2000; Su *et al.*, 2011), but the synergistic effects are not well-established (Rajeshkumar *et al.*, 2013; Salehi *et al.*, 2019). This study aims to evaluate the anti-gout effects of ACUPEN in Monosodium Urate (MSU) crystal-induced gouty arthritis models through anti-inflammatory mechanisms (Sarup *et al.*, 2015; Singh, 2016; Sabina and Rasool, 2008).

MATERIALS AND METHODS

All experimental procedures for evaluating the anti-gout activity of the polyherbal formulation ACUPEN in an experimental animal model were carried out after obtaining approval from the Institutional Animal Ethics Committee (IAEC) of ROFEL Shri G. M. Bilakhia College of Pharmacy, with approval number ROFEL/IAEC/2022/032.

Preparation of MSU suspension

Add 0.5 mL of sterile Phosphate-Buffered Saline (PBS) to one vial containing 5 mg of MSU crystals and mix thoroughly to obtain a 10 mg/mL suspension.

Monosodium urate-induced gouty arthritis (Chen *et al.*, 2013)

Six *Wistar albino* rats were randomly assigned to six groups, with treatments administered orally once daily for five days. The control group received normal saline, while the other groups were injected with 50 µL of 25 mg/mL Monosodium Urate (MSU) crystals intra-articularly into the right ankle joint under anesthesia, 1 hr after the final dose. Paw thickness was measured at predefined time points over three days using a vernier caliper to assess inflammation. After 72 hr, the rats were euthanized, and blood samples were collected for serum uric acid analysis. Several parameters were evaluated during the study.

Assessment of swelling ratio (Yao *et al.*, 2020)

The width of the right ankle joint was measured using a vernier caliper at various time intervals: before injection, and at 12, 24, 48, and 72 hr post-injection. The diameters of the ankle joint, including lateral, medial, and anteroposterior dimensions (denoted as *a* and *b*), were recorded. Ankle joint volume was calculated using the following formula:

$$\text{Ankle Joint Volume} = \tfrac{1}{2} \times a \times b^2$$

Inflammation Index and Dysfunction Index

These indices were used to assess the macroscopic changes in the ankle joint in response to MSU crystal-induced inflammation. Evaluations were conducted at 12, 24, 48, and 72 hr after MSU injection. The scoring system was based on inflammation severity and was independently assessed by two observers (Yao *et al.*, 2020; Pereira *et al.*, 2019; Tungmunnithum *et al.*, 2021).

Grade 1: Skin erythema and moderate swelling, with visible bony landmarks in the joint.

Grade 2: Redness and swelling in the joint, disappearance of bony marks, and swelling confined to the joint.

Grade 3: Severe swelling beyond the joint, a stronger inflammatory response, and impaired foot mobility, with the foot often lifted off the ground.

This method is commonly used in gouty arthritis models (Roddy and Doherty, 2010) and aligns with the MSU crystal-induced peritonitis model (Spalinger and Scharl, 2018) for *in vivo* inflammation assessment.

Serum Uric Acid Measurement

On day eight, blood samples were collected, left at room temperature for 30 min, and then centrifuged at 3000 rpm for 15 min. Serum uric acid levels were measured using a commercial test kit and the phosphotungstic acid method (Yao *et al.*, 2020; Kapse *et al.*, 2022).

Gait Analysis

Gait was evaluated by applying black ink to the ventral surface of the rats' hind feet. The rats were then allowed to move freely on white paper, and the footprints of the injected leg were compared to those of the un-injected leg to assess weight-bearing during movement (Roddy and Doherty, 2010; Spalinger and Scharl, 2018).

Xanthine Oxidase Assay

Xanthine Oxidase (XO) inhibitory activity was assessed using a commercially available Xanthine Oxidase Assay Kit (Cat# 10010895), as described by Bustanji *et al.*, (2011). Enzyme and substrate solutions were freshly prepared, and the reaction was incubated at room temperature for 20 min. Absorbance was measured at 570 nm, and the starting rate was calculated. Quercetin served as the positive control. XO inhibitory activity was expressed as percent inhibition. IC_{50} values were determined using a dose-response curve generated in GraphPad Prism software (GraphPad Prism, USA).

Statistical Analysis

Data were expressed as Mean±Standard Deviation (SD) and analyzed using GraphPad Prism 6.0 software. One-way Analysis of Variance (ANOVA) followed by Student-Newman-Keuls test

was applied. Histological results and inflammation/dysfunction indices were evaluated using the rank-sum test in GraphPad Prism. A *p* value of <0.05 was considered statistically significant.

RESULTS

Xanthine oxidase assay

The Xanthine Oxidase (XO) inhibitory activity of ACUPEN was assessed using a dose-response curve fitted with a three-parameter logistic model. The IC_{50} value for the ACUPEN formulation was calculated to be 24.8 µg/mL, indicating notable XO inhibition. The regression model demonstrated a good fit with an R^2 value of 0.7580. For comparison, the standard formulation produced a linear equation of y=0.103x+0.9198 with a higher R^2 of 0.9688. A summary of the regression output is provided in Tables 2 and 3 and Figure 1.

Assessment of ankle swelling

MSU crystal injection into the right ankle joints of rats caused significant redness, swelling, and deformity at 12 and 24 hr post-injection in the disease control group. In contrast, the normal control group exhibited no signs of inflammation. Ankle joint volume measurements taken at 12, 24, 48, and 72 hr post-injection confirmed the successful establishment of the gout model. ACUPEN treatment significantly reduced ankle joint volume, particularly at 12 hr, 24 hr, and 48 hr, compared to the disease control group (Table 4).

Inflammation Index

Following MSU injection, the inflammation index scores in the disease control group were significantly higher than those in the healthy control group. ACUPEN treatment reduced inflammation scores at 12 hr, 24 hr, 48 hr, and 72 hr, with the highest dose showing a substantial therapeutic effect at 48 hr (Figure 2).

Dysfunction index

Behavioral assessments revealed significantly higher dysfunction scores in the disease control group compared to the normal control group. Although the high-dose ACUPEN group showed observable improvements at 48 hr and 72 hr, these differences were not statistically significant (Figure 3).

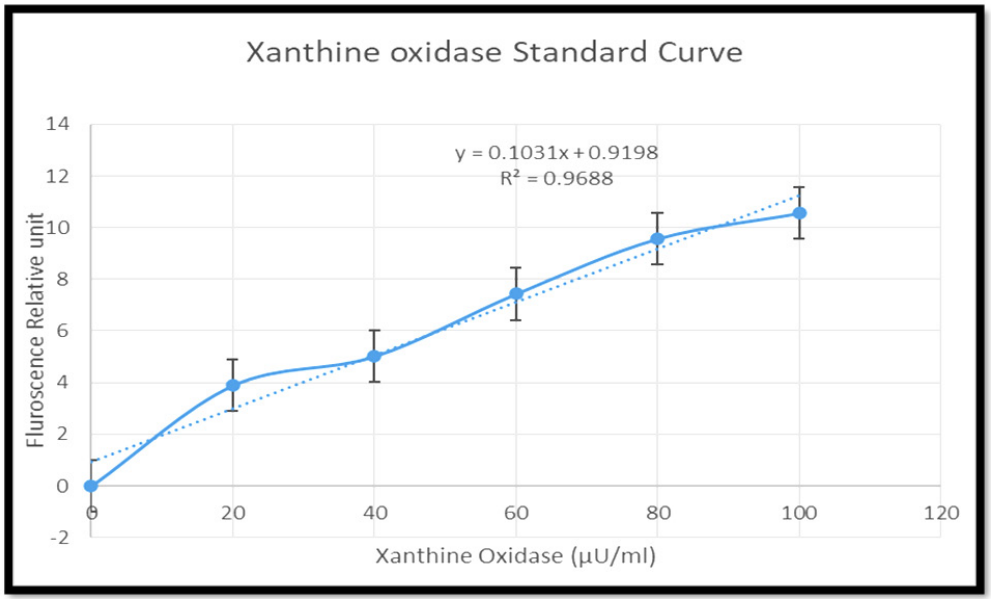

Figure 1: Standard curve for xanthine oxidase activity measured using spectrophotometric assay at 570 nm. Data expressed as mean of three replicates (*n*=3). Equation: y=0.103x + 0.9198; R^2=0.9688.

Observation of Inflammation Index at 12h							Observation of Inflammation Index at 24h					Observation of Inflammation Index at 48h					Observation of inflammation index at 72h				
Group	Unit	Grade 0	Grade 1	Grade 2	Grade 3	p value	Grade 0	Grade 1	Grade 2	Grade 3	P value	Grade 0	Grade 1	Grade 2	Grade 3	Pvalue	Grade 0	Grade 1	Grade 2	Grade 3	P value
Control	Pcs	6	0	0	0		6	0	0	0		6	0	0	0		6	0	0	0	
Disease control	Pcs	0	1	4	1	#	0	0	3	3	#	0	0	1	5	#	0	1	2	3	#
Standard	Pcs	0	2	3	1	*	0	1	4	1		0	2	3	1		2	2	1	0	
T1-Low dose	Pcs	0	1	4	1		0	1	3	2		0	2	2	2		1	1	3	1	
T2-Mid dose	Pcs	0	2	3	1		0	1	4	1		0	2	3	1		2	2	1	0	
T3-High dose	Pcs	0	3	2	1	*	0	2	3	1		0	3	2	1		3	2	1	0	

Measurement data represent the inter quartile range of 6 animals. The rank-sum test for multiple sets of independent samples was used for statistical analysis. #P<0.05 compared with the normal control group;
∗P < 0.05 compared with the disease control group

Figure 2: Inflammation index measured at 0, 12, 24, 48, and 72 hr after MSU injection. Values represent interquartile range (*n*=6). Statistical analysis by rank-sum test; *p*<0.05 vs. disease control, # *p*<0.05 vs. normal control.

Gait analysis

Gait scores increased at 12 hr and 24 hr post-MSU injection, indicating impaired movement due to arthritis (Figure 4). ACUPEN at all tested doses, as well as colchicine, significantly reduced gait scores, suggesting an improvement in arthritis-related symptoms.

Serum uric acid levels

Serum uric acid levels were significantly higher in the hyperuricemic rats than in the healthy control group. ACUPEN at doses of 150, 300, and 600 mg/kg effectively lowered serum uric acid levels and prevented excessive elevation following MSU injection (Table 5).

Histopathology Study

Histopathological examination of joint tissues in the disease control group revealed mild to moderate mononuclear cell infiltration, urate crystal deposits, and slight tissue damage (Figure 5). In contrast, all treatment groups showed no significant pathological alterations. Histological analysis of liver and kidney tissues confirmed mononuclear infiltration in the liver of the disease control group, whereas ACUPEN-treated groups exhibited no significant histopathological changes (Figure 6).

DISCUSSION

Gouty arthritis is characterized by recurrent episodes of intense joint pain and inflammation resulting from the deposition of Monosodium Urate (MSU) crystals. These crystals initiate an inflammatory cascade involving neutrophil infiltration, Reactive Oxygen Species (ROS) production, and activation of Nuclear Factor-Kappa B (NF-κB), which upregulates pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6. The present study demonstrates the efficacy of ACUPEN, a polyherbal formulation, in ameliorating MSU-induced gouty arthritis in rats by addressing both hyperuricemia and inflammation.

		Observation of Dysfunction Index at 12 h					Observation of Dysfunction Index at 24 h					Observation of Dysfunction Index at 48 h					Observation of Dysfunction Index at 72 h				
Group	Unit	Grade 0	Grade 1	Grade 2	Grade 3	p value	Grade 0	Grade 1	Grade 2	Grade 3	P value	Grade 0	Grade 1	Grade 2	Grade 3	Pvalue	Grade 0	Grade 1	Grade 2	Grade 3	P value
Control	Pcs	6	0	0	0		6	0	0	0		6	0	0	0		6	0	0	0	
Disease cc	Pcs	0	3	3	0	#	0	1	2	3	#	0	0	2	4	#	0	1	3	2	#
Standard	Pcs	0	4	2	0		0	3	2	1		0	1	3	2		2	2	1	1	
T1-Low dc	Pcs	0	3	3	0		0	2	2	2		0	1	2	3		1	1	2	1	
T2-Mid do	Pcs	0	4	2	0		0	2	3	1		0	3	1	2		2	2	1	1	
T3-High d	Pcs	0	4	2	0		0	3	2	1		0	2	2	2		1	3	1	0	

Measurement data represent the inter quartile range of 6 animals. The rank-sum test for multiple sets of independent samples was used for statistical analysis. #P<0.05 compared with the normal control group;

∗P < 0.05 compared with the disease control group

Figure 3: Dysfunction index from 0 to 72 hr post MSU injection. Data shown as interquartile range (*n*=6). Rank-sum test applied; $p<0.05$ *vs.* disease control, $\#p<0.05$ *vs.* normal control.

Table 1: Composition of the polyherbal formulation ACUPEN.

Sl. No.	Constituents	Content in mg	Parts Used
1	*Aloevera*	50	Leaf
2	*Commiphora mukul*	40	Gum
3	*Apium leptophyllium*	30	Fruit
4	*Ricinus communis*	30	Root
5	*Myristica fragrans*	20	Seed
6	*Boerhavia diffusa*	60	Root
7	*Triphala*	50	-
8	Excipients	20	-

Table 2: Standard readings for xanthine oxidase activity.

Standard	µU/mL	RFU 1	RFU 2				
A	0	0.1935	0.16	0	0	0	0
B	20	4.15	3.989	3.9565	3.829	3.89275	0.031875
C	40	5.284	5.104	5.0905	4.944	5.01725	0.036625
D	60	7.548	7.667	7.3545	7.507	7.43075	0.038125
E	80	9.661	9.816	9.4675	9.656	9.56175	0.047125
F	100	10.52	10.94	10.3265	10.78	10.55325	0.113375

Table 3: Xanthine oxidase inhibition by ACUPEN at different concentrations.

Concentration. (µg/mL)	Rfu 1	Rfu 2	Rfu 3	Xanthine oxidase (µU/mL)			Xanthine oxidase inhibitory activity (%)				
				1	2	3	1	2	3	Mean	SD
0.00	2.217	1.960	2.081	25.16	20.18	22.53	0.00	0.00	0.00	0.00	0.00
2.50	2.125	2.012	2.069	23.38	21.19	22.29	-3.34	6.35	1.46	1.49	4.84
5.00	2.201	1.298	1.749	24.85	7.34	16.09	-9.86	67.57	28.90	28.87	38.71
12.50	1.781	1.632	1.707	16.71	13.82	15.27	26.15	38.93	32.50	32.53	6.39
25.00	1.307	1.582	1.445	7.51	12.85	10.19	66.80	43.22	54.97	54.99	11.79
50.00	1.020	1.214	1.265	1.94	5.71	6.70	91.41	74.77	70.40	78.86	11.08
Quercetin (100 µg/mL)	0.050	0.018	0.342	-16.87	-17.50	-11.21	174.55	177.33	149.54	167.14	15.30

Values represent mean of three replicates (n=3). Percent inhibition calculated relative to control (0 µg/mL). SD=standard deviation. Quercetin used as positive control.

Table 4: Effect of ACUPEN on ankle swelling in monosodium urate-induced gouty arthritis in rats.

Group	Before injection of MSU (0 hr)	12 hr	24 hr	48 hr	72 hr
Control	86.62±7.181	87.20±5.58	87.40±5.56	89.40±5.69	90.30±5.70
Disease Control	84.23±4.242#	103.45±9.63#	131.28±19.49#	145.75±22.80#	134.54±16.27 #
Standard	80.08±6.342*	86.32±5.02*	115.57±11.14*	101.06±4.43 *	98.36±5.42 *
T1-low dose	80.65±4.64 *	85.03±12.80 *	88.02±12.18 *	91.65±5.42 *	81.99±5.30 *
T2-Mid dose	81.99±2.68 *	89.11±4.74 *	92.90±8.67 *	92.72±6.11 *	84.20±6.77 *
T3-High dose	82.97±12.45 *	90.73±12.65 *	96.81±12.96 *	90.80±6.34 *	83.40±2.47 *

Measurement data represent the Mean±standard deviation of 6 animals. One-way ANOVA followed by Student's Newman-Keul's test was used for statistical analysis. #p<0.05 compared with the normal control group; ∗p<0.05compared with the disease control group.

Table 5: Effect of ACUPEN on serum uric acid levels in rats.

Group	Before Injection uric acid (mg/dL)	After Injection uric acid (mg/dL)
Normal Control	1.33±0262	-
Disease control	1.13±0.125 #	2.4±0.245 #
Standard	1.2±0 *	1.46±0.047 *
T1	1.3±0.141 *	1.7±0.141 *
T2	1.13±0.047 *	1.43±0.125 *
T3	1.4±0.011*	1.63±0.188 *

Measurement data represent the Mean±standard deviation of data obtained from 6 animals. One-way ANOVA and Student's Newman-Keul's test was used for statistical analysis. #p<0.05 compared with the normal control group; *p<0.05 compared with the disease control group.

The Xanthine Oxidase (XO) assay revealed that ACUPEN possesses potent XO inhibitory activity, with an IC_{50} value of 24.8 µg/mL, indicating its potential to reduce uric acid levels by inhibiting its production. XO is a key enzyme in purine metabolism, and its inhibition is a well-established therapeutic strategy in the management of gout. These findings align with previous studies reporting that plant-derived bioactive compounds, especially flavonoids and polyphenols, inhibit XO activity (Bustanji *et al.*, 2011; Salehi *et al.*, 2019).

The MSU-induced model employed in this study successfully reproduced the pathophysiological features of acute gout, including joint swelling, inflammation, and impaired mobility. ACUPEN administration significantly reduced ankle swelling and joint inflammation as early as 12 hr post-injection, with the highest dose demonstrating sustained anti-inflammatory effects up to 48 hr. These results support the anti-inflammatory potential of the formulation, likely due to its components such as *Commiphora mukul, Aloe vera*, and *Ricinus communis*, which are known to modulate prostaglandin synthesis, reduce leukocyte infiltration, and suppress COX and NF-κB pathways (Sarup *et al.*, 2015; Su *et al.*, 2011).

Gait analysis and dysfunction index scores provided behavioral evidence of ACUPEN's therapeutic effect. ACUPEN-treated rats exhibited improved weight-bearing ability and mobility, indicating reduced joint pain and inflammation. Although the improvements in dysfunction scores were not statistically significant, the observed trends, particularly at 48 and 72 hr, suggest potential analgesic efficacy.

Serum uric acid levels were significantly reduced in all ACUPEN-treated groups, confirming the formulation's

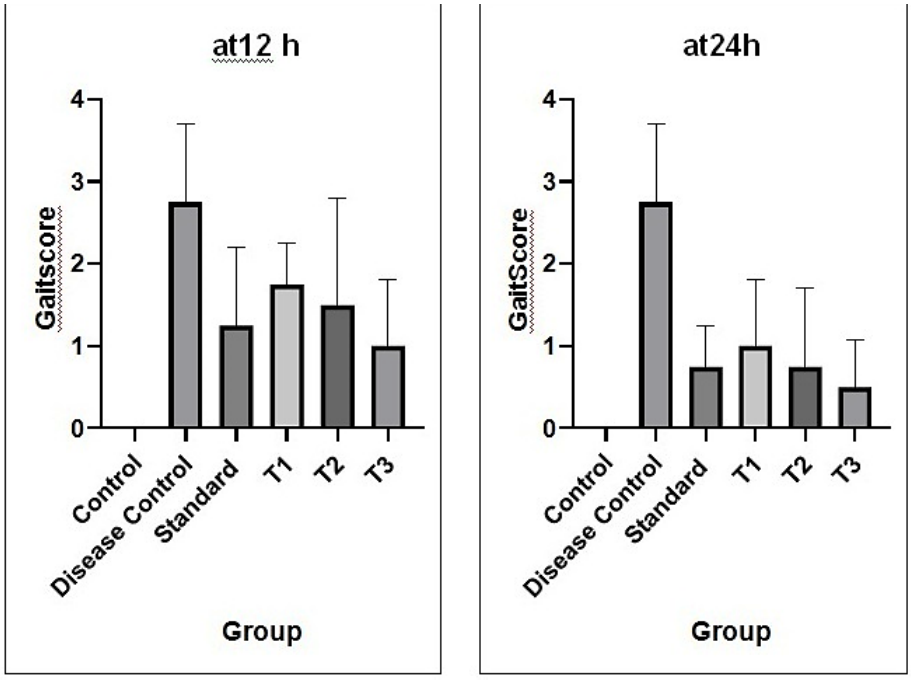

Figure 4: Gait score evaluated 12 and 24 hr after MSU injection using ink-footprint test. Values reflect improvement in movement post-ACUPEN treatment (n=6). p<0.05 vs. disease control, #p<0.05 vs. normal control.

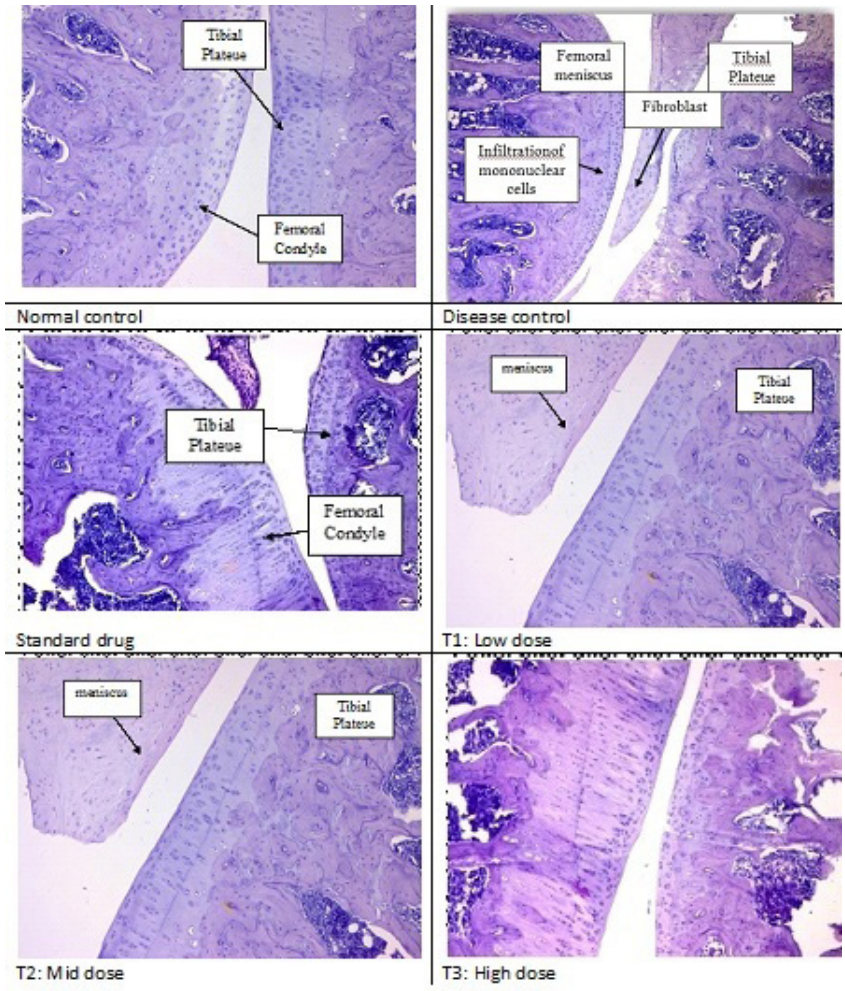

Figure 5: Histological sections of rat ankle joint (H&E stain, 400×). Disease control shows mononuclear infiltration and urate crystal deposits. ACUPEN-treated groups exhibit reduced inflammation and preserved tissue architecture.

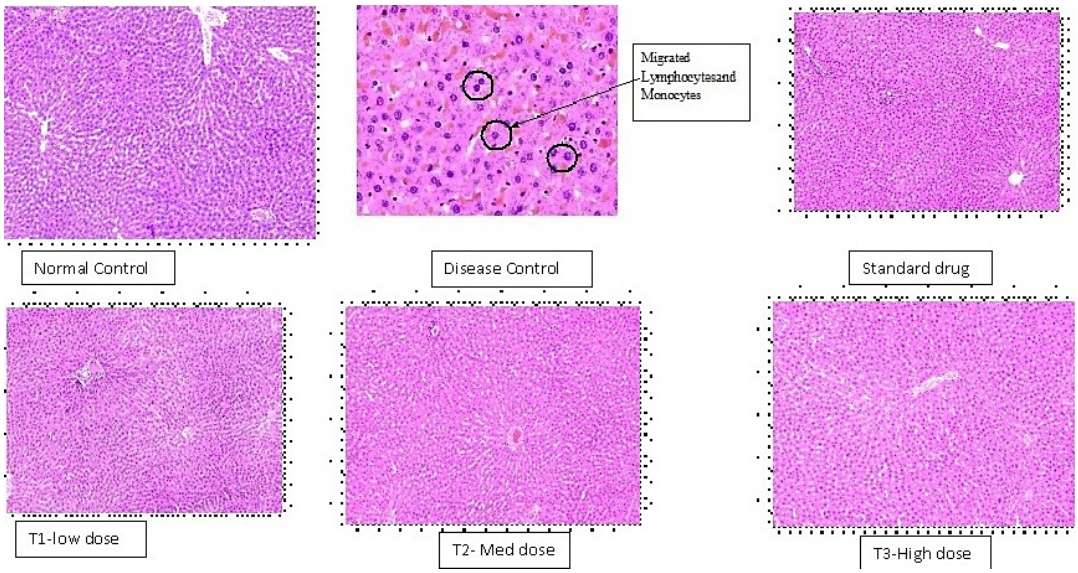

Figure 6: Liver histology (H&E stain, 400×). Control rats show normal architecture; disease control shows mononuclear infiltration. ACUPEN groups show no abnormal liver histopathology, suggesting hepatoprotection.

urate-lowering capability. This dual mechanism-reducing both uric acid production and inflammatory response-offers a potential advantage over monotherapy agents such as allopurinol, which primarily reduce uric acid synthesis but lack anti-inflammatory properties.

Histopathological analysis of joint tissues revealed decreased mononuclear infiltration and crystal deposition in ACUPEN-treated groups, indicating preservation of joint integrity. Importantly, no pathological alterations were observed in liver or kidney tissues, confirming the safety of ACUPEN at all tested doses. This is particularly important for chronic conditions like gout that may require long-term treatment.

Together, the findings of this study suggest that ACUPEN exerts a multi-targeted therapeutic effect via:

1. XO inhibition and uric acid reduction.
2. Anti-inflammatory activity through cytokine suppression and immune modulation.
3. Analgesic effects reflected in improved gait and functional scores.
4. Tissue protection as observed in histopathological evaluations.

The individual herbs in ACUPEN may act synergistically. For example, *Apium leptophyllum, Boerhavia diffusa*, and *Triphala* possess diuretic and antioxidant properties that aid in uric acid elimination and ROS neutralization. *Commiphora mukul* and *Ricinus communis* are known for their anti-inflammatory and analgesic activities (Yadav, 2021). This phytochemical synergy could explain the comprehensive therapeutic benefits observed in this study (Khan and Khan, 2018; Singh, 2016). While the results are promising, the study has several limitations. It evaluates only the acute effects of ACUPEN. Long-term efficacy, pharmacokinetics, and sub-chronic toxicity studies are necessary to support further development. In addition, clinical trials are essential to validate the safety and efficacy of ACUPEN in human subjects.

CONCLUSION

This study demonstrated that ACUPEN, a polyherbal formulation, exhibits significant anti-inflammatory, analgesic, and uric acid-lowering effects in a Monosodium Urate (MSU) crystal-induced gout model. ACUPEN inhibited xanthine oxidase activity, reduced ankle swelling, inflammation index scores, and serum uric acid levels, and improved joint function and gait in a dose-dependent manner. Histopathological analysis revealed no significant tissue damage in the treated groups, supporting the safety and potential therapeutic efficacy of ACUPEN. Although the differences from the disease control group were not always statistically significant-possibly due to the self-healing capacity of rats-ACUPEN shows promise as a candidate for managing hyperuricemia and gout. Further clinical studies are warranted to validate its effectiveness and safety in human populations.

ACKNOWLEDGEMENT

The authors sincerely thank ATBU Harita Pharmaceuticals Pvt. Ltd., for providing ACUPEN samples and support during the study.

ABBREVIATIONS

ROS: Reactive Oxygen Species; **NF-κB:** Nuclear Factor Kappa B; **NSAIDs:** Nonsteroidal Anti-Inflammatory Drugs; **ULMs:** Urate-Lowering Medications; **PBS:** Phosphate Buffered Saline; **IC_{50}:** Half Maximal Inhibitory Concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Cite this article: Desai A, Desai H, Desai R, Patel A, Desai C, Merai A, *et al*. Evaluation of Anti-Gout Effect of Polyherbal Formulation Acupen in Monosodium Urate Crystal-Induced Gouty Arthritis VIA Anti-Inflammatory Action in Experimental Animal Model. J Young Pharm. 2025;17(3):653-60.