RESEARCH PAPER

EVALUATION OF ANTIGOUT ACTIVITY OF *ACHYRANTHES ASPERA* LEAF EXTRACTS ON POTASSIUM OXONATE – INDUCED GOUT MODEL.

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Short title: Antigout Activity of *Achyranthes aspera* Leaf Extract

ABSTRACT

Gout is an inflammatory joint disorder associated with hyperuricemia and urate crystal deposition. This study evaluated the antihyperuricemic and anti-gout activity of chloroform extract of *Achyranthes aspera* leaves in potassium oxonate-induced hyperuricemic rats. The extract was prepared by 72 h maceration and showed a 5.26% w/w yield. Thirty rats were divided into five groups and treated with 200 and 400 mg/kg extract, with Allopurinol as the standard. Serum uric acid, paw thickness, and radiographic changes were evaluated. The extract showed improvement in serum uric acid, paw thickness, and radiographic appearance, particularly at the higher dose. No mortality or toxicity was observed. The study suggests that
A. aspera may possess potential antihyperuricemic and anti-gout activity.

KEYWORDS: *Achyranthes aspera*; Chloroform extract; Hyperuricemia; Gout; Potassium oxonate; Serum uric acid; Paw edema; Anti-gout activity; Antihyperuricemic activity; Phytochemical screening.

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INTRODUCTION

Gout is one of the oldest known joint diseases in human history. The term gout is derived from the Latin word "gutta," meaning "a drop." [1][2] Since ancient times, gout has been called the "disease of kings" because it was associated with lifestyle factors. The main cause of gout pathogenesis is elevated serum uric acid, or hyperuricemia, which is considered the primary cause of monosodium urate crystal deposition. The development of hyperuricemia is influenced by genetic, metabolic, and environmental factors. [3]



FIG 1. Gout-Affected Hind Paw.[4]

Stages of Gout

Gout is classified into four stages: Asymptomatic deposits**,** Acute gout**,** Intercritical**,** Chronic gout [5]

In India, gout prevalence is rising due to lifestyle factors including reduced physical activity, obesity, protein-rich diets, alcohol consumption, and the use of medications such as thiazide diuretics.[6][7] In southern India, about 18% of gout cases occur before 30 years of age, often with acute polyarthritis, less frequent podagra, and early tophi formation. [8]

Age	Male		Female		Total (male and female)	
	No.	(%)	No.	(%)	No.	(%)
<30	2	7.14	2	9.09	4	8
30-39	1	3.57	2	9.09	3	6
40-49	9	32.14	5	22.72	14	28
50-59	9	32.14	1	4.54	10	20
60-69	6	21.42	9	40.90	15	30
>70	1	3.57	3	13.63	4	8
Total No.	28	56	22	44	50	100

Table 1. Epidemiology, Comorbidities and Clinical Features of Gout in Southern Part of India.[9]

Medicines for Gout

Gout treatment includes drugs for acute attacks: such as NSAIDs, colchicine, corticosteroids, and ACTH—to reduce inflammation and pain. For long-term control, urate-lowering medications like Allopurinol, Probenecid, Sulfinpyrazone, Febuxostat, and Pegloticase are used to lower serum uric acid and prevent flare-ups.[10]

Monosodium Urate (MSU) Crystal Deposition

Gout is the most common form of arthritis caused by crystal deposition and is also the leading type of

inflammatory arthritis overall.[11] In gout, monosodium urate (MSU) crystals accumulate in the joints and surrounding connective tissues, causing significant inflammation, though this inflammation usually remains confined to the affected area.[12] Serum uric acid > 6.8 mg/dL → the risk of MSU crystals forming increases → gout develop

Risk Factors

Gouty arthritis results from intense inflammation caused by monosodium urate (MSU) crystal deposition in joints. MSU crystals trigger inflammation, causing pain, swelling, redness, and acute arthritis. Factors favouring crystal deposition include changes in pH (e.g., perioperative ketosis), lower body temperature, and joint dehydration. Risk factors include a high-purine diet, alcohol use, obesity, and diuretic therapy. Triggers for acute attacks include infection, intravenous contrast media, acidosis, trauma, surgery, psoriasis flares, Triggers for acute attacks include infection, intravenous contrast media, acidosis, trauma, surgery, psoriasis flares, chemotherapy initiation, diuretic therapy, and starting or stopping allopurinol.[13] [14] [15]

Management and Treatment of Gout

Gout is a rheumatic disease that can be controlled and even cured in many cases with proper treatment. This leads to severe pain, swelling, and redness in the affected joints.[16][17] Considering its health and economic burden, several treatment guidelines have been developed. However, their proper implementation in clinical practice remains limited.[18] The unnecessary use of allopurinol in asymptomatic hyperuricemia and the lack of regular monitoring in urate-lowering therapy are common issues.[19][20][21]

Future Prospects

Recent progress in treating gout has introduced febuxostat, is an effective alternative to allopurinol, patients that cannot tolerate allopurinol, or kidney problems, or cannot use uricosuric drugs. Febuxostat is processed by the liver. It lowers uric acid levels better than allopurinol, but does not reduce gout flare-ups more effectively.[22] [23] Another new treatment option is recombinant uricase, known as pegloticase. This therapy helps break down uric acid into allantoin, which dissolves easily in water and is removed from the body. Pegloticase is mainly used to treat tumor lysis syndrome but might help patients with severe gout that does not respond to other treatments.[24][25]

Experimental Animal Models

- Potassium Oxonate – Induced Animal Model
- Monosodium Urate (MSU) Crystal – Induced Animal Model
- Acute Gouty Arthritis with Hyperuricemia Animal Model
- Diet-Induced Hyperuricemia Animal Model.[26]

Potassium Oxonate [27]

- Molecular formula - $C_4H_2KN_3O_4$
- IUPAC Name - potassium 4,6-dioxo-1H-1,3,5-triazine-2-carboxylate
- Target - Xanthine oxidase, Molecular weight - 195.17 g/mol
- In solvent - 80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture)
- synonyms - Oxonic acid, potassium salt, Potassium oxonate, Oxonate potassium

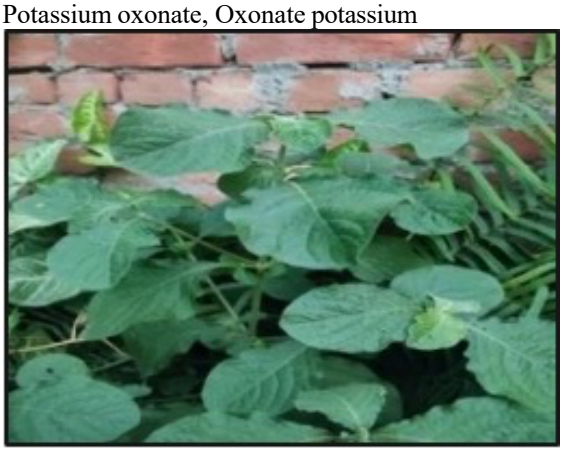


FIG 2. Structure - potassium oxonate (**$C_4H_2KN_3O_4$**).

ACHYRANTHES ASPERA -

Achyranthes aspera Linn., belongs to a member of the family Amaranthaceae, is known as Rough Chaff Flower. It is widely distributed throughout India. Various parts of the plant are used in traditional medicine or in combination with other herbal remedies.[28]

FIG 3. Plant – *Achyranthes Aspera.*

MATERIALS AND METHODS

Plant Material and Authentication

The *A. Aspera* leaf was used as a plant material. According to the thesis record, the material was authenticated by Dr. Alok Shrivastava, Professor, Department of Plant Science, Mahatma Jyotiba Phule Rohilkhand University, Bareilly, Uttar Pradesh. A voucher specimen was deposited in the departmental herbarium under voucher number RU/PS/PS/2025-23.

Extraction of *Achyranthes Aspera*

Achyranthes aspera leaves were cleaned, shade-dried, powdered, and sieved. The powder was macerated with chloroform for 72 hours with occasional stirring. The extract was filtered, concentrated below 50°C, and dried in a desiccator. The dried extract was weighed and stored at 4°C for further evaluation. From 1000g of *Achyranthes aspera*, 52.6 g of dried chloroform extract was obtained, giving a percentage yield of 5.26% w/w.

Preliminary Phytochemical Screening

The chloroform extract was screened using the qualitative procedure described in the thesis for alkaloids, glycosides, saponins, tannins, flavonoids, phenolic compounds, terpenoids, amino acids. The results reported in the thesis indicates the presence of alkaloids, flavonoids, tannins, phenolic compounds, glycosides.

Preparation of *Achyranthes Aspera*

The leaves of *A. aspera* were cleaned, shade-dried, powdered, and passed through sieve No.

40. About 1000 g of powder was macerated with chloroform for 72 h with intermittent stirring. The extract was filtered, concentrated below 50°C using a rotary vacuum evaporator, and dried to constant weight in a desiccator. The dried extract was stored in an airtight amber-colored container at 4°C for further evaluation.

Chemical Reagent Used

Sr. No.	Chemical/ Reagent	Purpose
1.	*A. aspera* leaf powder	Preparation of plant extracts
2.	Chloroform	Defatting before extraction
3.	Methanol	Extraction of plant material
4.	Potassium Oxonate	Induction of hyperuricemia in experimental rats
5.	Allopurinol	Standard anti-gout drug
6.	Tween 80 (Polysorbate 80)	Preparation of extract suspension for oral administration
7.	Normal Saline (0.9% NaCl)	Preparation of Potassium Oxonate solution
8.	Distilled Water	Preparation of solutions and reagents
9.	Uric Acid Estimation Kit	Estimation of serum uric acid
10.	Blood Urea Nitrogen (BUN) Kit	Estimation of blood urea nitrogen
11.	Serum Creatinine Kit	Estimation of serum creatinine

Table 2. Chemical And Reagents used for Extraction and Pharmacological Evaluation

Instruments and Equipment Used

Sr. No.	Instrument	Purpose
1.	Analytical Balance	Weighing of chemicals and plant material
2.	Animal Weighing Balance	Measurement of body weight of experimental animals
3.	Mechanical Grinder	Powdering of dried plant material
4.	Maceration Assembly	Preparation of plant extract
5.	Rotary Vacuum Evaporator	Concentration of plant extract
6.	Water Bath	Heating and concentration of extract
7.	Centrifuge	Oral administration of plant extract and allopurinol
8.	Syringes (1, 2 and 5 mL)	Retro-orbital blood collection
9.	Oral Gavage Feeding Needle	Oral administration of plant extract and allopurinol
10.	Capillary Tubes (Heparinized)	Retro-orbital blood collection
11.	Digital X-ray Machine	Radiographic evaluation of rat hind paw
12.	Plethysmometer	Measurement of paw edema volume
13.	Refrigerator (2–8°C)	Storage of reagents and serum samples
14.	Micropipettes	Accurate measurement of reagents and samples

Table 3. Instruments and Equipment Used in the Study

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the chloroform extract of *Achyranthes aspera* leaves showed the presence of alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, steroids, and terpenoids, while proteins and amino acids were absent.

Phytoconstituent	Test	Observation	Result
Alkaloids	Mayer's test	Cream precipitate	++
Alkaloids	Dragendorff's test	Orange-red precipitate	+++
Flavonoids	Alkaline reagent test	Intense yellow colour	++
Flavonoids	Lead acetate test	Yellow precipitate	++
Tannins	Ferric chloride test	Greenish-black colour	+++
Phenolics	Ferric chloride test	Bluish-green colour	+
Saponins	Foam test	Persistent froth	+++
Glycosides	Keller–Killiani test	Brown ring	++
Steroids	Liebermann–Burchard test	Green colour	+
Terpenoids	Salkowski test	Reddish-brown interface	+
Proteins	Biuret test	Negative	-
Amino acids	Ninhydrin test	Negative	-

Table 4. Preliminary phytochemical screening of chloroform extract of *A. aspera* leaves.

Preparation and Physical Evaluation of Test Drug

The chloroform extract was dark greenish-brown, semi-solid, homogeneous, and had a characteristic odour. It was stored at 4°C in an airtight amber-coloured container. For oral administration, the extract was freshly dispersed in 3% Tween 80 and administered at doses of

200 and 400 mg/kg body weight. The prepared suspension was uniform, smooth, and free from visible particles.

Parameter	Observation
Vehicle	3% Tween 80
Colour	Dark greenish – brown
Appearance	Uniform suspension
Homogeneity	Uniform
Visible particles	Absent
Stability	Stable during dosing
Route	Oral (p.o.)
Dose	200 and 400 mg/kg

Table 5. Physical characteristics of the prepared chloroform extract suspension.

Approval from IAEC Committee for Experimental Study

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), BIU College of Pharmacy, Bareilly International University, Bareilly. The study was conducted in accordance with CPCSEA guidelines, with appropriate measures to minimize pain, stress, and discomfort. Healthy adult Sprague-Dawley rats weighing 170–220 g were housed under standard laboratory conditions and provided standard pellet diet and water ad libitum. The number of animals was kept to the minimum required for statistically valid results, following the principles of the 3Rs (Replacement, Reduction, and Refinement). The potassium oxonate-induced hyperuricemia study was performed under appropriate animal handling and aseptic conditions. At the end of the study, animals were humanely sacrificed as per approved guidelines. Blood samples were collected for serum uric acid estimation, and radiographic examination of the hind paw/ankle joint was performed to assess joint changes.

Experimental Design

Normal control	vehicle only	6
Disease control	potassium oxonate only	6
Standard drug-treated	Allopurinol + Potassium oxonate	6
Low dose extract	*A. aspera* extract + Potassium oxonate	6
High dose extract	*A. aspera* extract + Potassium oxonate	6

Table 6. Experimental Grouping and Treatment Schedule of Animals

Day-Wise Experimental Design

Day	Experimental Procedure
Day 1	Induction + Treatment: Group I received normal control. Groups II–V received Potassium oxonate (250 mg/kg, i.p.). After 1 hour, Groups III, IV and V received Allopurinol (50 mg/kg), Achyranthes aspera 200 mg/kg and 400 mg/kg p.o., respectively.
Day 2	Treatment: All groups received their respective oral treatments.
Day 3	Induction + Treatment: Potassium oxonate (250 mg/kg, i.p.) was administered to Groups II–V, followed after 1 hour by their respective oral treatments.
Day 4	Treatment: All groups received their respective oral treatments only.
Day 5	Treatment: All groups received their respective oral treatments only.
Day 6	Treatment: All groups received their respective oral treatments only.
Day 7	Final Treatment & Assessment: Potassium oxonate (250 mg/kg, i.p.) was administered to Groups II–V, followed after 1 hour by respective oral treatments. Blood samples were collected under light diethyl ether anesthesia. Paw edema was assessed, and paw joint X-ray examination was conducted at IVRI.

Table 7. Day-wise Experimental Design

Preparation and Administration of Drug/Dose

A. Potassium Oxonate Solution

Prepared by dissolving 2.5 g of potassium oxonate in normal saline to a final volume of 10 mL, resulting in a concentration of 250 mg/ml. This stock solution was administered intraperitoneally to rats at a dose of 250 mg/kg body weight.

B. Allopurinol Solution

A 50 mg allopurinol tablet was dissolved in 10 mL distilled water, resulting in a concentration of 5 mg/mL. The solution was given orally to rats once daily at a dose of 5 mg/kg body weight.

C. *Achyranthes aspera* Extract

Administered at doses of 200 mg/kg or 400 mg/kg, prepared in 3% Tween 80 as the solvent.

RESULTS

Extraction and phytochemical screening

Preparation of Chloroform Extract

Dried powdered leaves of *A. aspera* were extracted with chloroform by maceration for 72 h at room temperature with occasional stirring. The extract was filtered through Whatman No.1 filter paper, concentrated under reduced pressure using rotary vacuum evaporator below 50°C,
and dried in a desiccator. The resulting dark

greenish-brown, sticky semi-solid extract with characteristic odour was stored in an airtight amber-colored container at 4°C until further use.

Parameter	Observation
Plant material	*Achyranthes aspera* leaves
Method	Maceration
Solvent	Chloroform
Duration	72 h
Appearance	Semi-solid
Colour	Dark greenish-brown
Odour	Characteristic
Storage	Amber container at 4°C

Table 8. Organoleptic Characteristics of Chloroform Extract of *A. aspera* Leaves

Percentage Yield of Chloroform Extract

A total of 1000 g of shade-dried powdered *Achyranthes aspera* leaves was extracted with chloroform by maceration for 72 h. The filtered extract was concentrated under reduced pressure below 50°C and dried to constant weight. A dark greenish-brown semi-solid extract weighing 52.6 g was obtained, corresponding to a percentage yield of 5.26% (w/w).

Calculation:

Percentage yield -(Weight of dried extract / Weight of crude drug) × 100 - (52.6 / 1000) × 100

- 5.26%

Parameter	Observation
Crude drug taken	1000 g
Solvent	Chloroform
Dried extract obtained	52.6 g
Percentage yield	5.26% (w/w)

Table 9. Percentage Yield of Chloroform Extract of *A. aspera* Leaves

Preparation and Physical Evaluation of Test Drug

The chloroform extract of *A. aspera* leaves was evaluated for its physical characteristics. The extract was dark greenish-brown, semi-solid, sticky, homogeneous, and free from visible foreign matter. It was stored in an airtight amber-colored container at 4°C until further use. For oral administration, the extract was freshly dispersed in 3% Tween 80 and mixed thoroughly to obtain a uniform suspension. The prepared suspension was smooth, homogeneous, and free from visible aggregates and was administered orally at doses of 200 and 400 mg/kg body weight to the experimental animals.

In-vivo Study

The anti-hyperuricemic and anti-gout activity of the chloroform extract of *Achyranthes aspera* leaves were evaluated using a potassium oxonate-induced hyperuricemia model in Sprague-Dawley rats. Hyperuricemia was induced by potassium oxonate (250 mg/kg, i.p.). Thirty rats were randomly divided into five groups (n = 6): normal control, disease control, Allopurinol-treated (50 mg/kg, p.o.), and *A. aspera* extract-treated groups receiving 200 and 400 mg/kg, p.o., respectively. The effects were assessed by measuring body weight, serum uric acid, paw volume, and radiographic changes, along with general health observations.

Body Weight

Animal No.	Normal Control	Positive Control (Allopurinol)	Disease Control	Low Test (200 mg/kg)	High Test (400 mg/kg)
1.	174	177	203	183	185
2.	171	181	206	186	188
3.	175	184	209	189	191
4.	179	187	212	192	194
5.	183	190	215	195	197
6.	187	193	218	198	200

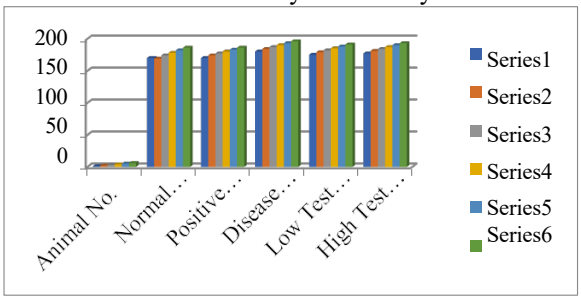
Table 10. Body weight was recorded on Day 0 before the initiation of treatment and used as the (170–220 g) baseline value

Statistical Analysis –

Statistical analysis of the body weight data showed a highly significant difference among the experimental groups ($p < 0.001$). This indicates that the differences in body weight observed between groups were statistically significant and were unlikely have occurred due to chance.

Animal No.	Normal Control	Positive Control (Allopurinol)	Disease Control	Low Test (200 mg/kg)	High Test (400 mg/kg)
1.	170	170	180	175	177
2.	169	174	184	179	181
3.	174	177	187	182	184
4.	178	180	190	185	187
5.	182	183	193	188	190
6.	186	186	196	191	193

Table 11. Final body weight of all experimental rats was recorded on the last day of the study



Statistical Analysis –

Statistical analysis of the body weight data showed a statistically significant difference among the experimental groups ($p = 0.0134$). This indicates that the observed variation in body weight among the groups was statistically significant.

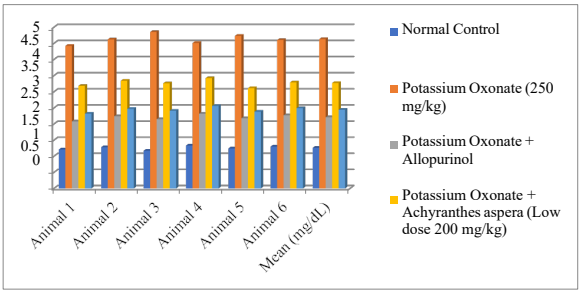
Blood Test (Uric Acid Estimation)

At the end of the experimental period, blood samples were collected and serum uric acid levels were

estimated using a commercial diagnostic kit according to the manufacturer's instructions. Serum uric acid was used as the primary biochemical parameter for evaluating the antihyperuricemic activity of the chloroform extract of *Achyranthes aspera* leaves.

Group	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Animal 6	Mean (mg/dL)
Normal Control	1.21	1.28	1.17	1.33	1.24	1.30	1.26
Potassium Oxonate (250 mg/kg)	4.42	4.62	4.85	4.51	4.73	4.60	4.63
Potassium Oxonate + Allopurinol	2.08	2.24	2.15	2.32	2.18	2.27	2.21
Potassium Oxonate + *Achyranthes aspera* (Low dose 200 mg/kg)	3.18	3.34	3.26	3.42	3.11	3.29	3.27
Potassium Oxonate + *Achyranthes aspera* (High dose 400 mg/kg)	2.32	2.47	2.41	2.56	2.38	2.49	2.44

Table 12. Effect of Chloroform Extract of *A. aspera* Leaves on Serum Uric Acid Level



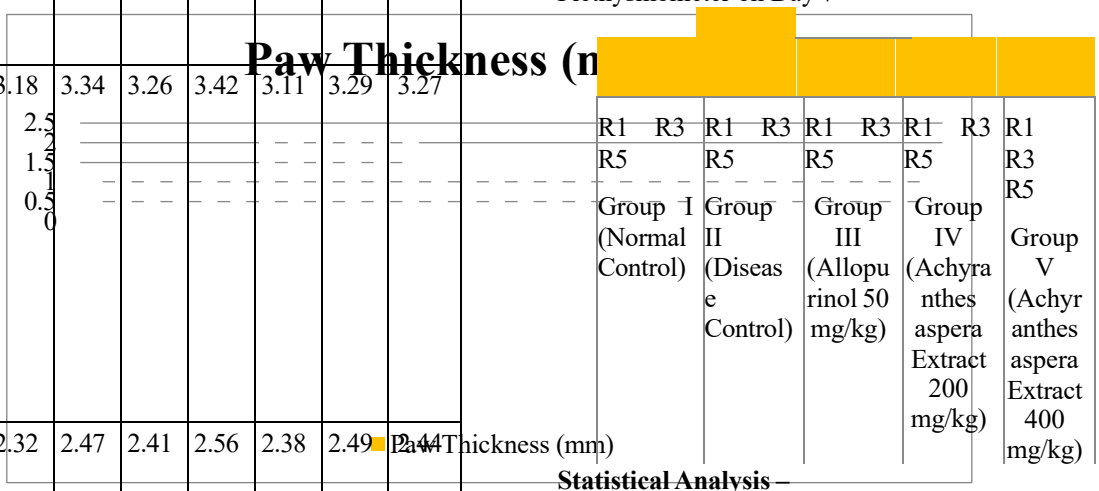
Statistical Analysis –

The serum uric acid levels showed a highly statistically significant difference among the experimental groups ($p < 0.001$). This indicates that the observed differences in serum uric acid levels between the treatment groups were unlikely to have occurred by chance and suggest a significant effect of the treatments on hyperuricemia.

Paw Volume Measurement (Plethysmometer)

Group	Mean Paw Thickness (mm)
Group I – Normal Control	1.19
Group II – Disease Control	2.17
Group III – Allopurinol 50 mg/kg	1.43
Group IV – *A. aspera* 200 mg/kg	1.71
Group V – *A. aspera* 400 mg/kg	1.33

Table 13. Group-wise paw thickness measurements of experimental rats recorded using a Plethysmometer on Day 7



Statistical Analysis –

Statistical analysis of paw thickness data revealed a highly statistically significant difference among the experimental groups ($p < 0.001$). This indicates that the observed differences in paw thickness between the groups were statistically significant.

Radiographic Examination (X-ray)

Group	Radiographic Observation	Paw Image
Group I (Normal Control)	Normal joint architecture with no soft tissue swelling or bone erosion.	
Group II (Potassium Oxonate)	Marked soft tissue swelling, joint space narrowing, and mild bone erosion.	

Group III (Allopurinol)	Considerable reduction in swelling with near-normal joint architecture.	
Group IV (Extract 200 mg/kg)	Mild soft tissue swelling with partial restoration of joint space.	
Group V (Extract 400 mg/kg)	Minimal swelling and almost normal joint structure with no obvious bone erosion.	

Table 14. Radiographic Observations of Experimental Groups

General Observation and Safety

Animals were monitored daily throughout the experimental period for general behaviour, food and water intake, locomotor activity, signs of toxicity, and mortality. No abnormal behavioural changes, signs of toxicity, or mortality were observed, indicating good general tolerability of the treatment.

Parameter	Observation
General behaviour	Normal
Food Intake	Normal
Water Intake	Normal
Locomotor Activity	Normal
Signs of Toxicity	Absent
Mortality	No mortality observed

Tabel 15. General Observations During the Experimental Period

Statistical Analysis

Data were expressed as Mean ± SD. Statistical analysis was performed using GraphPad Prism (Version 8.0 or later). Differences among groups were analysed using one-way ANOVA followed by Tukey's multiple comparison test. A P value < 0.05 was considered statistically significant.

Discussion

The present study evaluated the antihyperuricemic and anti-gout activity of the chloroform extract of *Achyranthes aspera* leaves in potassium oxonate-induced hyperuricemic rats. The extract showed a 5.26% w/w yield and contained alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, steroids, and terpenoids, which may contribute to its pharmacological activity.

Potassium oxonate produced a marked increase in serum uric acid and paw thickness in the disease control group. Treatment with *A. aspera* extract reduced serum uric acid levels, with mean values of 3.27 mg/dL and 2.44 mg/dL at 200 and 400 mg/kg, respectively, compared with 4.63 mg/dL in the disease control group. Paw thickness was also reduced from 2.17 mm in the disease control to 1.71 and 1.33 mm at 200 and 400 mg/kg, respectively.

Radiographic examination showed marked swelling and joint changes in the disease control group, whereas extract-treated groups showed improvement, particularly at 400 mg/kg. The absence of visible toxicity or mortality during the study further indicated good tolerability of the extract.

Overall, the findings suggest that *A. aspera* chloroform extract possesses antihyperuricemic and anti-gout potential, with greater effects observed at the 400 mg/kg dose. Further studies are required to establish its active constituents and mechanism of action.

Conclusion

The present study demonstrated that the chloroform extract of *Achyranthes aspera* leaves exhibited antihyperuricemic and anti-gout activity in potassium oxonate-induced hyperuricemic rats. The extract reduced serum uric acid and paw thickness and showed improvement in radiographic joint changes, particularly at 400 mg/kg. The extract was also well tolerated during the experimental period. These findings suggest that *A. aspera* may have potential as herbal candidate for further investigation in hyperuricemia and gout.

ETHICS STATEMNET

The animal study was approved by the IAEC, BIU College of Pharmacy, Bareilly International University, under approval no……, and conducted according to CPCSEA guidelines. Sprague-Dawley rats were used to evaluate the antihyperuricemic activity of *Achyranthes aspera* leaf extract using potassium oxonate (250 mg/kg, i.p.), *Achyranthes aspera* extract (200 and 400 mg/kg, p.o.) and Allopurinol (50 mg/kg, p.o.). No mortality occurred during the study; therefore, no euthanasia procedure was performed.

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CONFLICT OF INTEREST

A conflict-of-interest declaration was not specified in the source thesis and should be confirmed by all authors before submission.

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