



## A brief review on in vivo models for Gouty Arthritis

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### ABSTRACT

Gout is more common in men than in women, by a factor of 3.1–10.1. Gout prevalence and incidence have increased in recent decades, with prevalence reaching 11–13% and incidence reaching 0.4% in people over the age of 80. Age-related renal impairment, altered drug distribution, and increased prevalence of comorbidities have significant consequences for safe and effective gout pharmacotherapy. The Discovery of Fruitful in-vivo animal models needs the effective screening of drugs or formulations used in the treatment of gout. In vivo animal models of Gouty arthritis are extensively used to investigate pathogenic mechanisms governing inflammation-driven bone and cartilage damage. Four commonly utilized models include the Potassium oxonate induced hyperuricemic model, MSU crystals induced gouty arthritis animal model, Animal Model of Acute Gouty Arthritis with Hyperuricemia, and Diet-induced hyperuricemia. These offer unique advantages for correlating different aspects of gouty arthritis with human disease. In-vivo animal models served as testing beds for novel biological therapies, including cytokine blockers and small molecule inhibitors of intracellular signaling that have revolutionized gouty arthritis treatment. This review highlights a brief overview of in vivo experimental models for assessment of hypouricemic, anti-inflammatory, as well as renal protective effects of test compounds with some evaluation parameters in detail.

### 1. Introduction

The most prevalent form of inflammatory arthritis is Gout, which results from hyperuricemia. Hyperuricemia is described by the elevated level of serum uric acid. The saturation level of SUA (Serum Uric Acid) at 37 °C and pH (power of hydrogen) 7 is 6.8 mg/dL (Milligrams per Deciliter). Above the saturation level, inflammatory MSU (monosodium urate crystals) are formed in the synovium and joint. Patients are classified as Hyperuricemic if their SUA level is greater than 7 mg/dL in men and 6 mg/dL in women [1]. The frequency and severity of gout are found to be between 1–4% and 0.1–0.3%. Gout is more common in men than in women, by a factor of ratio 3.1 to 10.1. The prevalence of gout reported by the national BJD (Bone and Joint Decade) India COPCORD (Community-oriented program from the control of rheumatic diseases) survey of 2006–2011 is 0.05% [2]. Gout incidence and prevalence have been increased in recent decades, with prevalence reaching 11–13% and incidence reaching 0.4% in people over the age of 80. Gout is more common in ethnic minorities in the United States, Han Chinese, New Zealand Maori, and some Asian racial groups [3]. Gout can affect the quality of life and sometimes lead to irreversible joint damage. Gouty arthritis is normal in people with gout. Concurrent occurrence of

multiple medical conditions at the same time of Gouty arthritis is very common in individuals with gout, and it can make management and treatment of Gout more difficult. Many studies support the connection between the recognized metabolic precursor of gout, hyperuricemia, and hypertension [4,5]. The Discovery of Fruitful in-vivo animal models needs the effective screening of drugs or formulations used in the treatment of gout. Several animal models have been developed to investigate the basic mechanisms for hyperuricemia. This review gives an overview of in-vivo models of hyperuricemia leading to gouty arthritis and the relevance of these models to the human pathogenesis of gout. Animals are exposed to certain environmental conditions, such as dietary induction or with the help of some pharmaceuticals. Based on the pathogenesis of gout there are several in-vivo animal models are available for screening of plant-based as well as some promising pharmaceuticals in hyperuricemia and gout (see Table 1).

#### 1.1. Pathophysiology of hyperuricemia

Hyperuricemia is described by the elevated level of serum uric acid. Supersaturation of uric acid above 6.8 mg/dL leads to the formation of uric acid crystals. Uric acid crystals are also known as MSU crystals. The inflammatory reaction of host tissue to accumulated MSU crystals

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List of abbreviations	
BJD	(Bone and Joint Decade)
BSA	(Bovine serum albumin)
COPCORD	(Community-oriented program from the control of rheumatic diseases)
DTNB	(5,5'-dithiobis-(2-nitrobenzoic acid)
ELISA	(Enzyme-Linked Immunosorbent Assay)
GSH	(Glutathiones)
GLDH	(Glutamate dehydrogenase)
HGPRT	(Hypoxanthine-guanine phosphoribosyl transferase)
H2O2	(Hydrogen peroxide)
HCL	(Hydrochloric acid)
IL-1	(Interleukin 1)
IL-6	(Interleukin 6)
IL-8	(Interleukin 8)
LTB4	(Leukotriene B4)
mg/dL	(Milligrams per Deciliter)
MSU	(monosodium urate crystals)
M	(molar)
mL/100 g	(Milliliter/100 g)
mol/L	(moles per liter)
µl	(microliter)
mg/mL	(milligrams per milliliter)
mg/kg/day	(Milligram per Kilogram per Day)
Malondialdehyde	(MDA)
m	(meter)
NLRP3	(Nod-like receptor protein 3)
N	(Normality)
NaOH	(sodium hydroxide)
Nm	(Nanometer)
pH	(Power of hydrogen)
PGE2	(Prostaglandin E2)
PBS	(phosphate buffer solution)
Rpm	(revolutions per minute)
SUA	(Serum Uric Acid)
SOD	(Superoxide Dismutase)
TNF-β	(tumor necrosis factor-beta)
TNF-α	(tumor necrosis factor-alpha)
TLR	(Toll-Like Receptor)
UA	(Uric acid)
39. XO	(Xanthine Oxidase)

initiates the clinical Gout disease. Cell regeneration, food intake, and de novo synthesis are the leading causes of increased urate level, which is a degradation agent of purines. MSU crystals preferentially deposit in joints and articular tissues, such as the first metatarsophalangeal joint, the midfoot, and the knee, excruciatingly painful inflammatory arthritis in these areas.

The occurrence of an increased supersaturation of uric acids may be caused by a variety of factors [6,7].

- Purine absorption is changed or increased.

- Reduced uric acid excretion
- Increased uric acid output

In certain cases, the cause of increasing uric acid levels is unclear.

#### 1. Increased consumption

The elevated absorption of purine is mostly due to the patient's increased consumption of purine-rich foods such as asparagus, meat broths, mushroom, liver, kidney, and sweetbreads. Guanosine, a purine

**Table 1**  
Models comparison.

Models	Potassium oxonate induced animal model	MSU crystals induced animal model	Acute Gouty Arthritis with Hyperuricemia animal model	Diet induced hyperuricemia Animal model
Dose	250 mg/kg	50 µl MSU crystals (25 mg/ml)	Potassium oxonate (1.5 g/kg/day) + 50 µl MSU crystals (25 mg/ml)	Hypoxanthine 200 mg/kg/day + yeast extract 30 mg/kg/day + potassium oxalate 200 mg/kg/day
Characteristics	Uricase inhibition	NLRP3 inflammasome activation	Uricase inhibition and NLRP3 inflammasome activation	Increase source of purine
Route of Administration	Intraperitoneal	Intraarticular	Intragastric + Intraarticular	Intragastric
Treatment period	3 Days	7 Days	28 Days	8 weeks
Similarities to human Gout	1. Uric acid level Increase 2. No synovial inflammation 3. No bone and cartilage Destruction	1. Synovial inflammation 2. Bone and cartilage Destruction	1. Synovial inflammation 2. Bone and cartilage Destruction	1. Synovial inflammation 2. Bone and cartilage Destruction
Pain mechanism	Release of cytokines	Release of cytokines	Release of cytokines	Release of cytokines
Pros	1. Gout-like pathogenesis 2. It is simple to carry out 3. Less time consuming	1. Gout-like pathogenesis 2. Parameters derived from working with this animal model are of sufficient use as reference values. 3. Easy to study the inflammatory mechanism 4. Less time-consuming.	1. Gout-like pathogenesis 2. Parameters derived from working with this animal model are of sufficient use as reference values. 3. Easy to study the inflammatory mechanism	1. Gout-like pathogenesis 2. It is simple to carry out
Cons	1. Mice and rats have low sensitivity to drugs. 2. Parameters derived from working with this animal model are of limited use as reference values 3. Difficult to study the inflammatory mechanism	1. It is difficult to carry out, as it requires additional skill for intraarticular injection of MSU crystals into the knee joint of mice and rats	1. It is difficult to carry out, as it requires the additional skill of intraarticular injection of MSU crystals into the knee joint of rodents 2. More time consuming	1. Parameters derived from working with this animal model are of limited use as reference values 2. Difficult to study inflammatory mechanism 3. More time consuming

nucleotide, is especially abundant in beer.

2. Excessive enzyme function
  - Phosphoribosyltransferase overactivity
  - HGPRT (Hypoxanthine-guanine phosphoribosyl transferase) deficiency
  - HGPRT absence [8].
3. Renal excretion is decreased/reduced.

The most frequent cause of hyperuricemia is impairment of renal excretion. The following are some of the reasons that contribute to its decreased elimination:

- Renal activity that has been compromised by hereditary factors (Reduced glomerular filtration rate)
- Use of diuretics [9].
- Alcohol consumption [10].
- Drugs that are harmful to the renal tubules, such as cyclosporine which cause a reduction in uric acid removal and increases urate retention.

Purine is converted to hypoxanthine, which is then oxidized by XO (xanthine-oxidase) to form xanthine. Uric acid is formed when xanthine is oxidized again by xanthine oxidase. Fig. 1 shows the enzymatic pathway of purine to the uric acid end product [11].

### 1.2. Pathophysiology of Gouty Arthritis

Inflammation of Gouty Arthritis is initiated by the deposition of UA (Uric acid) crystals in the joint space. When these crystals are engulfed by synovial cells, they allow lysosomal enzymes to be released and activates inflammatory chemokines, which start the inflammatory process [12,13]. The release of mast cells and monocytes, as well as the activation of neutrophils, is linked to gouty arthritis. These crystals can be contained by well-differentiated macrophages without triggering an inflammatory response [14].

Mast cells also play an important role in triggering acute gouty attacks by releasing histamine and IL-1(Interleukin), as a result of this Vascular permeability and vasodilation increase [15]. Chemotactic

factors generated by monocytes and mast cells, as well as local vasodilation, contribute to neutrophil chemotaxis. The recruitment of endothelial cells also exacerbates the inflammatory reaction and neutrophil migration. This causes an excess of neutrophils in the region [16]. The 90% of neutrophil activation and acute inflammation exacerbation is caused by an excess of chemotactic factors such as leukotrienes, platelet-activating agent, and interleukins, especially IL-8, within the synovium. As a result, focusing on IL-8 could be a successful strategy for preventing acute gout attacks. The acute gout attack is normally self-limiting. It goes away within a few hours or days after it appears. By clearing cellular apoptotic remnants, macrophages also help in the slowing of the inflammatory cascade. TNF - $\beta$  (tumor necrosis factor-beta) is also secreted by macrophages, which inhibits IL-1, a major player in the inflammatory process [17].

Chronic gouty arthritis is caused by systemic inflammation that occurs as a result of repeated gout attacks. Chronic gout is marked by severe synovitis, bony erosions, cartilage destruction, and tophi growth. This may be due to a variety of factors. Chondrocytes are activated to release inflammatory cytokines, matrix metalloproteases, and nitric oxide when urate crystals are found in the synovium, causing cartilage damage and the initiation of bone erosions [18,19].

Anti-inflammatory cytokines are essential regulators of the inflammatory response. Proteolysis of proinflammatory cytokines, as well as decreased expression of TNF- $\alpha$  (tumor necrosis factor-alpha) and interleukin receptors on the surface of leukocytes, are both involved in resolving the acute attack. Vasodilation and increased vascular permeability are required for macrophage extravasations into the synovial fluid to clear the inflammatory regions (Fig. 2) [17].

### 1.3. NLRP3 inflammasome activation in MSU crystal induced gouty arthritis

NLRP3 (Nod-like receptor protein) inflammasome plays an important role in the onset of acute inflammation of gout. ASC (adaptor molecule apoptosis-associated speck-like protein containing a CARD) is an important linker between NLRP3 protein and pro-caspase-1 protease in joint inflammation of acute gout. Martinon et al. [20]. When monocytes ingest MSU crystals, Toll-like receptors TLR2 and TLR4 activate the NLRP3 inflammasome. ASC adaptor protein promotes hydrolysis of

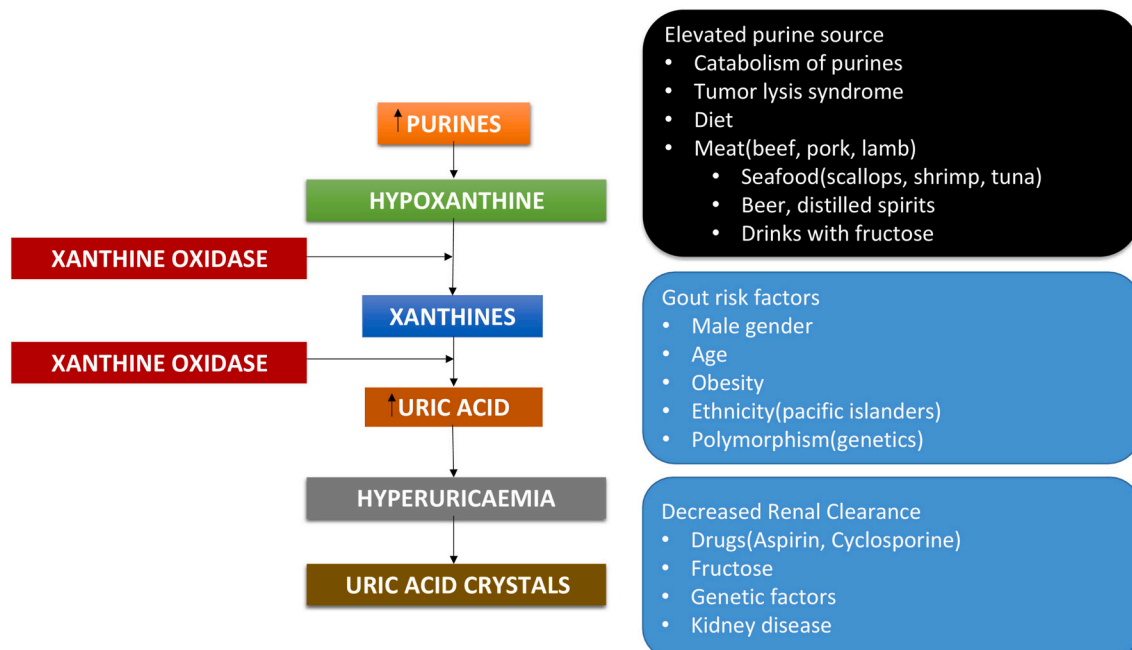


Fig. 1. Enzymatic pathway of purine to uric acid end product and risk factors of elevated uric acid level.

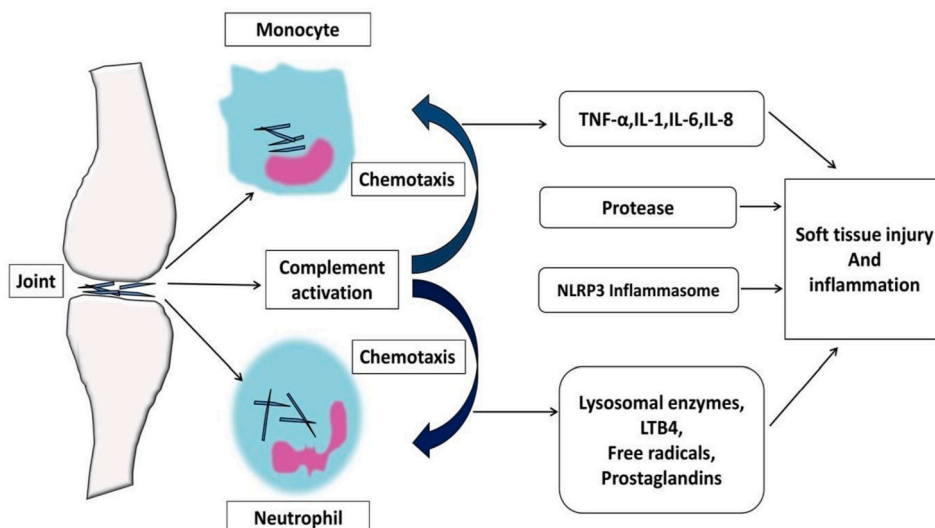


Fig. 2. Pathophysiology of Gouty Arthritis inflammation. (IL-1: Interleukin 1, IL-6: Interleukin 6, IL-8: Interleukin 8, LTB4: Leukotriene B4, NLRP3: Nod-like receptor protein 3, TNF-α: tumor necrosis factor-alpha).

pro-caspase-1 protease and converts pro-caspase-1 protease to active caspase-1 protease [21]. Resulting in the development of pro-inflammatory IL-1 β and mature IL-1 β after cleavage by active caspase-1, eventually leading to a strong inflammatory response in gout patients [22] (see Fig. 3).

2. Experimental in-vivo models for gout

Several animal models of hyperuricemia and gouty arthritis have been reported, for in vivo assessment of hypouricemic, anti-inflammatory as well as renal protective effects of the compound. The injection of MSU-crystals into various anatomical structures to induce crystal-induced inflammation has been proposed; however, only a few of these models accurately reflect the joint microenvironment in which an acute gouty attack occurs.

2.1. Potassium oxonate induced hyperuricemia model

Potassium oxonate, a competitive uricase inhibitor, produces hyperuricemia in rodents [23]. The end product of purine nucleotide catabolism is Uric acid. Adenine and guanine, two purine nucleic acids, are converted to uric acid with the help of the xanthine oxidase enzyme.

Xanthine oxidase is the last enzyme in the purine nucleotide catabolism pathway of humans [24].

The purine metabolism pathway is different in rodents and humans. Metabolites of purine are excreted through renal as urine and water solubility of uric acid is low so it tends to deposit in the body especially in joints. In animal uricase, an enzyme is present instead of a xanthine oxidase enzyme. The uricase enzyme, also known as uric oxidase, transforms uric acid to allantoin, a water-soluble material that allows uric acid to be excreted more readily through the urine [25]. The basic principle of increasing the source of uric acid, reducing uric acid excretion, and inhibiting uricase is used to establish the rodent model of hyperuricemia. Potassium oxonate is the agent that inhibits the role of uricase and produces hyperuricemia in rats, mice, rabbits, dogs, and pigs [24].

An experimental animal model of hyperuricemia initiated by the administration of potassium oxonate and used to determine the anti-hyperuricemic effect of test compounds. To summarise, per animal, except those in the normal control group, received 250 mg/kg potassium oxonate dissolved in 0.9% saline solution intraperitoneally 1 h before oral administration of test compounds, once a day, for 3 days of the experiment. 1 h after the final drug administration, mice are anesthetized with ketamine and xylazine (100 mg/kg and 20 mg/kg, respectively) to collect blood from the abdominal aorta. The blood is enabled to clot for around 1 h at room temperature before being centrifuged for 10 min at 2500 rpm (revolutions per minute). The serum is isolated and deposited at 20 °C before the uric acid assay is performed [26].

2.2. MSU crystals induced gouty arthritis animal model

The formation of MSU crystals is the first critical step in the progression of gout. MSU crystals trigger inflammatory cellular responses. Monosodium urate crystals formation in synovial joints was first discovered in 1961. The presence of MSU crystals is believed to be a requirement for diagnosing gout [27]. Synovial cells, monocytes-macrophages, and neutrophils are stimulated by MSU crystals, which allow them to release cytokines such as IL-1 (interleukin-1), TNF (tumor necrosis factor), and iNOS (inducible nitric oxide synthase) [28]. Many of these molecules are implicated in the production of acute inflammation in gout flares [29]. The oxidative stress caused by iNOS can affect synoviocyte survival by controlling mitochondrial functionality [30] and inhibits chondrocyte proteoglycan synthesis through

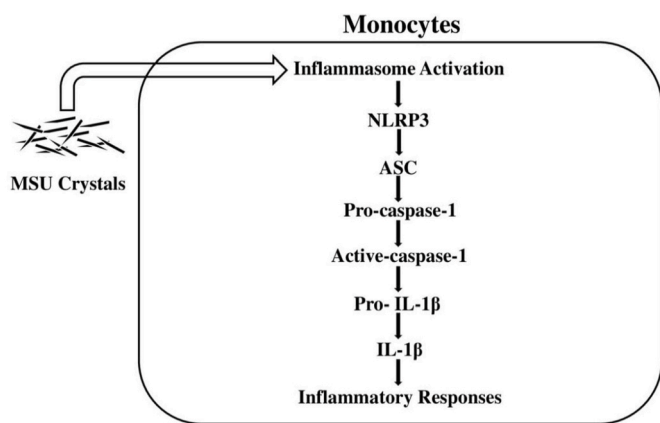


Fig. 3. NLRP3 Inflammasome activation and Pathway of inflammatory response in monocyte (ASC: Apoptosis-associated speck-like protein, IL-1 β: Interleukin 1 β, MSU: Monosodium urate crystals, NLRP3: Nod-like receptor protein 3).

PGE2(Prostaglandin E2) inhibition of chondrocyte apoptosis [31–33]. Suppression of these pro-inflammatory mediators has been shown to reduce the intensity of gouty arthritis inflammation [10].

Monosodium Urate Crystals are made by dissolving the uric acid in 800 mL H<sub>2</sub>O with 9 mL 0.5 N(Normality) NaOH(sodium hydroxide) and adjusting the pH to 8.9 at 60 °C. Crystals are washed and dried after cooling overnight at a very low temperature. Crystals that resemble needles are collected and suspended in sterile saline [34]. The mice, rats, or rabbits are divided into five main groups at random (Control, Colchicine, and test at three doses). Colchicine or test compound is given to each group once a day for five days. One hour after the final administration, the animals are given normal saline to the control group and suspension of 50 µl MSU crystals (25 mg/mL) to the other four groups intra-articularly under anesthesia at the medial side of the right ankle joint [35].

The inflammation is measured by using a vernier caliper. Vernier scale used to measure the thickness of the paw at various times interval for three days. The animals are killed by cervical decapitation after 72 h. The blood of each animal is taken to separate the serum. The liver and spleen are dissected to make a 10% homogenate. The homogenization process includes the use of ice-cold 0.01 M(molar) Tris– HCL(Hydrochloric Acid) buffer and pH 7.4. Lysosomal enzymes, lipid peroxidation, antioxidant status, and the inflammatory mediator tumor necrosis factor are all measured in tissue homogenates from the spleen, liver, and serum [35].

### 2.3. Animal Model of Acute Gouty Arthritis with hyperuricemia

The main therapeutic approach against gouty arthritis is hyperuricemia control and anti-inflammatory therapy. The two primary categories of drugs that are useful in gout include uric acid-lowering drugs and anti-inflammatory drugs. For evaluating the effect of formulation concurrently on parameters, hyperuricemia and inflammation are necessary. The drawback of potassium oxonate-induced hyperuricemia is, we will measure only uric acid level but the assessment of Inflammation parameters is difficult. By combining both models we can assess the gouty arthritis parameter more efficiently in the animal [36].

An experimental animal model of hyperuricemia is induced using potassium oxonate after a 7-day acclimatization duration. In brief, 70 rats were given orally potassium oxonate 1.5 g/kg/day (Gram per Kilogram per Day) dissolved in distilled water once daily for 21 days. The dosing amount is 1 mL/100 g (Milliliter/100 g) body weight. Also, a standard control group of ten rats is used (administration of distilled water only). At weeks 1, 2, and 3, blood samples are taken from the eyelids of model rats, and the levels of uric acid in the serum are assessed. The model is successfully induced when blood uric acid levels were >110 mol/L (moles per liter) at week three. Animals with high or low uric acid levels in their blood are excluded from the study, and 50 rats are chosen for the following experiment. Model treatment, standard control, colchicine, and Test high dose, Test medium dose, and Test low dose rats are randomly divided into six groups (n = 10) after successfully establishing hyperuricemia.

To further optimize the model of acute gouty arthritis with hyperuricemia, all rats are given 2.5% isoflurane and 50 µl (microliter) MSU crystals 25 mg/mL (milligrams per milliliter) or frequent saline injections into the medial side of the right tibiotarsal joint (ankle). The standard for drug injection is the contralateral bulging of the joint capsule. Each group received a Test or colchicine intragastrically once daily for 7 days after receiving MSU. The normal control group and the model control group are assessed using PBS(phosphate buffer solution) [37].

### 2.4. Diet induced hyperuricemia

Gout has also been suggested that it's a distinct risk factor for obesity, hypertriglyceridemia, insulin resistance, and cardiovascular

disease [38]. Uric acid may be endogenous, resulting from de novo synthesis and nucleic acid dissolution, or exogenous, resulting from a high-purine diet.

Dietary purine contributes to almost one-third of the daily purine load [39]. High purine diet includes animal meats (beef, pork, lamb, organ meats, and meat extracts), seafood (fish fillets, salmon, crabs, lobster, clams, and so on), and plants (yeast extracts, peas, beans, lentils, asparagus, and mushrooms). There is strong evidence that the prolonged intake of a high purine diet contributes to the incidence of acute hyperuricemia in diabetics. However, a high-purine diet alone does not entirely justify why diabetic patients have a higher incidence of hyperuricemia/gout than healthy people [40].

The mice are fed with a normal chow diet and are divided into the following groups: a control group, a hyperuricemic model group, and treatment groups, each group with eight mice. Each group is housed in its cage. For eight weeks, the animals in the hyperuricemia model group, and treatment groups are gavaged with 200 µl (microliter) of high purine solution every day. The purine solution contains hypoxanthine 200 mg/kg/day (Milligram per Kilogram per Day) yeast extract 30 mg/kg/day, and potassium oxalate 200 mg/kg/day. During the 8 weeks of dietary treatment, each animal's body weight is reported regularly. Fecal samples are obtained from each mouse at the end of the study and deposited at 80 °C. Following that, the mice are given isoflurane anesthesia, blood samples are collected from the eyelids, and serum is isolated by centrifugation for 15 min at 4 °C and 3000 rpm, and then stored at 80 °C for biochemical studies. The mice are sacrificed through cervical dislocation after sample processing, and their visceral organs are excised, weighed, and preserved at 80 °C before further study [41].

## 3. Evaluation parameters for in-vivo models

### 1) Assessment of swelling ratio:

The swelling ratio would be used to assess the inflammation before and after treatment of animals. Ankle circumference of all rats would be measure before and after at 0, 12, 24, and 48 h of MSU injection by vernier caliper. The swelling ratio (%) would be calculated by using the formula: Swelling ratio (%) = (Ct – C0)/C0, where Ct is the circumference at various times and C0 is the circumference at 0 h [23].

### 2) Inflammation Index and Dysfunction Index:

Both indexes would be used to evaluate macroscopic scoring of the ankle joint. Data would be recorded before and after MSU injection at 12, 24, 48, 72, and 96 h intervals. Two independent observers can evaluate the rat's inflammation and dysfunction scores [23].

To score inflammation, the following inclusion methodology would be used:

- Grade 0 (0 points): The ankle joint is normal with no signs of inflammation.
- Grade 1 (2 points): Skin erythema, moderate swelling, and significant bony marks are present in the joints.
- Grade 2 (4 points): Joints are red and swollen, bony marks have disappeared, and swelling is restricted to the joints.
- Grade 3 (6 points): Swelling outside the joint, more intense inflammatory response, impaired foot ability, and foot often lifted off the ground.

To score dysfunction, the following inclusion methodology would be used:

- Grade 0 (0 points): Normal gait with both feet grounded uniformly.
- Grade 1 (2 points): Toes are not unfolded, and the foot is slightly limp.



- Grade 2 (4 points): Toes are on the ground, and the foot is bent and visibly limping.
- Grade 3 (6 points): A three-legged gait with the foot lifted off the ground.

### 3) Proinflammatory Cytokines:

The animals would be sacrificed using the euthanasia method after the experimental period ended on the 8th day. Irrigation of the joints (articular cavities) would be done with 0.5 mL normal saline and after irrigation centrifugation of the specimen would be done for 15 min at 3000 rpm/min. ELISA (enzyme-linked immunosorbent assay) kits would be used to measure the levels of proinflammatory cytokines such as TNF- $\alpha$ , IL-1, and IL-6 in serum and joint fluid, according to the instructions provided by the manufacturer [29].

### 4) Uric acid measurement in serum plasma:

Blood samples would be taken from the eyelids of rats on the 8th day after treatment. The samples would be kept at room temperature for 30 min before being centrifuged for 15 min at 3000 rpm/min. The phosphotungstic acid procedure [30] would be used to evaluate the concentration of uric acid in the serum using a test kit, according to the instructions provided by the manufacturer.

### 5) Xanthine oxidase activity of liver:

- Liver sample preparation:

Rat livers would be removed as soon as blood was collected, cleaned in 0.9% cold saline, and stored at 80 °C until further use. Enzyme extraction would be carried out as previously noted. Liver homogenates would be homogenized in 5 mL of 80 mM sodium phosphate buffer (pH 7.4) and centrifuged at 3000 rpm for 10 min at 4 °C. After systematically removing the lipid layer, the supernatant would be centrifuged at 10,000 rpm for 60 min at 4 °C. Enzyme assays would be performed on the final supernatant [26].

- Procedure:

XO activity would be measured spectrophotometrically by tracking the formation of uric acid from xanthine. To prevent uric acid oxidation to allantoin, the reaction mixtures contained 50 mM (Millimolar) phosphate buffer (pH 7.4), 50 ml liver homogenate, and 1 mM potassium allantoxanate in a final volume of 1.65 ml. The reaction would be started by adding 350 ml of 250 mM xanthine after a 15-min pre-incubation at 37 °C. After 0 and 30 min, the reaction would be terminated by adding 0.15 ml of 0.6 M HCl to the reaction medium. After that, the solutions would be centrifuged for 5 min at 3000 rpm. UV/VIS spectrophotometer would be used to separate the supernatant and for measurement of the sample absorbance at 295 nm (Nanometer).

The Bradford (1976) technique would be used to quantify protein concentration using spectrophotometry and BSA (Bovine serum albumin) as a standard. XO activity would be measured in nanomoles of uric acid formed per milligram of protein per minute [42].

### 6) Assessment of Renal function:

The renal function would be evaluated with the help of two parameters (i.e., blood urea and creatinine) before and after treatment. The collected blood would be centrifuged for 5 min at 4000 rpm at 4 °C and plasma would be aspirated out for the analysis of urea and creatinine. The plasma concentrations of urea and creatinine would be determined using commercially available biochemical kits by GLDH (glutamate dehydrogenase) kinetic method and alkaline picrate method respectively, according to the manufacturer's instructions [23].

### 7) Antioxidant enzyme activities:

#### a. Catalase biochemical assay:

The kidneys would be homogenized individually in 0.05 M Tris HCl buffer solution (pH 7.0) at a tissue concentration of 50 mg/ml for a biochemical assay of catalase activity. The activity of catalase in the kidney would be measured using the H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide) method. At 550 nm, six more readings would be taken at 30-s intervals [32].

#### b. SOD (Superoxide Dismutase) biochemical assay:

The kidneys were homogenized in ice-cold 100 mM Tris-cocodylate buffer and centrifuged at 10,000 rpm for 20 min at 4 °C to obtain a tissue concentration of 50 mg/ml. The supernatant's SOD activity was determined using the auto-oxidation of the pyragallol method. The enzyme activity that inhibited the auto-oxidation of pyragallol by 50% was characterized as one unit of SOD [33].

#### c. GSH (Glutathione) Biochemical Assay:

To achieve a concentration of 50 mg/ml, the kidneys were homogenized in 0.01 M phosphate buffer (pH 7.2). The GSH content of the kidney was calculated using the DTNB (5,5'-dithiobis-(2-nitrobenzoic acid) method [10].

### 8) Oxidative Stress Marker

#### Malondialdehyde (MDA) Biochemical assay:

The kidneys would be homogenized separately in 0.1 M of ice-cold phosphate buffer at a tissue concentration of 50 mg/ml (pH 7.4). The MDA level would be calculated using the H<sub>2</sub>O<sub>2</sub> method [43], as previously discussed. The extinction coefficient of 1.56 × 10<sup>5</sup> M/cm would be used to quantify the amount of MDA present in the sample, which would be expressed as nM/mg of tissue or nM/ml of plasma.

### 9) Histological analysis of kidney and paw:

The kidneys of rats would be maintained in 4% formaldehyde for 24 h at room temperature. Each kidney would be cut into 4  $\mu$ m thick paraffin sections, stained with hematoxylin and eosin, and examined under a light microscope at a magnification of 200 × .

The paw area of MSU-induced gouty arthritis mice would be isolated and decalcified in a 10% formaldehyde solution, then embedded in paraffin and sectioned at 5 m (meter), followed by hematoxylin and eosin staining and examination under a light microscope at a magnification of 200 × (23).

## 4. Conclusion

In this article, we have reviewed the four established animal models of gouty arthritis, especially the mechanisms, characteristics, and procedures of rodent models. In vivo animal models, each with its own set of advantages and limitations, have been discussed for the study of gouty arthritis. Various drugs can generate animal models of gouty arthritis, and different methods of administration of the same drugs may lead to different outcomes, such as through gavages, intraperitoneal and intraarticular injections. Choosing an appropriate model system best suited for the study undertaken can reveal the pathogenesis of gouty arthritis and drugs targeting molecular events at different levels of the complication can be designed. All in all, therefore, we conclude that the Acute Gouty Arthritis with Hyperuricemia and Diet-induced hyperuricemia animal models are the best of the available four models.

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