



The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item	Recommendation	Section/line number, or reason for not reporting
Study design	1 For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	A: Groups: control, model, benzbromarone, PUE low-/medium-/high-dose; second in-vivo experiment: control, model, benzbromarone, 70%-EtOH fraction low/high dose, 95%-EtOH fraction low/high-dose. Blank control and model control included. Control groups included. See MATERIALS AND METHODS. B: Experimental unit: individual male Kunming mouse. See MATERIALS AND METHODS — Animals.
Sample size	2 a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done.	A: HUA mice: PUE dose-response in-vivo experiment: n=10 per group, total 60 mice. Fraction efficacy evaluation experiment: n=10 per group, total 70 mice. See MATERIALS AND METHODS — Experiments of sample on HUA mice. B: No <i>a priori</i> sample-size power calculation was performed. n=10 animals per group was selected referencing previously published hyperuricemia mouse models, balancing statistical power and animal 3R-reduction principles.
Inclusion and exclusion criteria	3 a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established <i>a priori</i>. If no criteria were set, state this explicitly.	A: Random allocation was performed. Animals were assigned using random-number table. B: Animals from different groups were distributed

		b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of <i>n</i> in each experimental group.	across multiple cages; sample-collection order was randomised to reduce cage-position and time-dependent confounding factors. C: For each group, <i>n</i>=10 in HUA experiment. Exact <i>n</i> values are reported in each table and figure legend.
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	A: Yes, Outcome assessment (biochemical assay, renal histopathological scoring) was performed with encoded sample identifiers; assessors were blinded to group allocation. Blinding during animal administration / handling was not feasible due to physical differences of test articles. Data analysis was performed on coded datasets to reduce observer bias. B: No specific strategy to minimise confounders (e.g., order of treatments/measurements, cage location) was described in the manuscript.
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Blinding was not explicitly described in the manuscript. It is not stated who was aware of group allocation during allocation, conduct of experiment, outcome assessment, or data analysis.
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).	A: In-vivo outcome measures: serum uric acid, urinary uric acid, CRE, BUN, XOD activity, inflammatory cytokines (IL-6, TNF-α), renal URAT1/OAT1 mRNA and protein, renal histopathology. In-vitro RAW264.7 outcomes: NO, SOD, MDA, pro-inflammatory cytokine mRNA, transporter-gene expression. See Materials & Methods and Results.

		b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	B: serum uric acid level and renal histopathological injury score; remaining indices were designated secondary outcome measures.
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	A: Statistical methods: Two-tailed independent-samples t-test for two-group comparison; one-way ANOVA with post-hoc LSD (homogeneous variance) / Dunnett's T3 (heterogeneous variance). Software: SPSS Statistics 20.0 (IBM). See MATERIALS AND METHODS — Statistical analysis. B: Normality was tested by Shapiro-Wilk test; homogeneity of variance was tested by Levene's test prior to ANOVA. Effect sizes and confidence intervals were not calculated and reported, acknowledged as study limitation.
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	A: Animals: Male Kunming mice, SPF grade, 6-8 weeks old, body weight 20-24 g. See MATERIALS AND METHODS — Animals. B: Animals obtained from licensed commercial vendor (Henan Skbes Biotechnology Co., Ltd.), SPF health status; no genetic modification, no prior experimental manipulations before this study.
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	A: Procedures described in MATERIALS AND METHODS: Hyperuricemia mouse model: hypoxanthine i.g. + potassium oxonate i.p.; drug oral administration; biochemistry, qRT-PCR, western-blot, H&E histopathology; MSU-stimulated RAW264.7 macrophage model for in-vitro assessment. B: Treatment given once

		daily for 14 days; 7-day pre-experiment acclimatisation period. See MATERIALS AND METHODS. C: All animal experiments performed at Wannan Medical University. 7-day environmental acclimatisation before modelling. D: Rationale: this mouse HUA model is widely accepted to evaluate anti-hyperuricemic substances from herbal medicine; refer to Introduction section. See INTRODUCTION and MATERIALS AND METHODS.
Results	10	A: Results are presented as mean $\pm$ SD for each experimental group. See all tables (Table 1–3) and figures (Fig. 1–5) in RESULTS. B: Effect sizes with confidence intervals were not reported in the manuscript.

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Section/line Recommendation number, or reason for not reporting
Abstract	11	See Abstract
	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	
Background	12	INTRODUCTION (Paragraphs 1-2) — includes scientific background, rationale, and explains use of HUA mice models with relevance to human gout.
	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	
Objectives	13	INTRODUCTION (last paragraph, starting with "The present investigation was architected around four integrated objectives...")
	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	
Ethical statement	14	Ethics statement (Approval No. WNMC-AWE-2023476)
	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	

	15		
Housing and husbandry		Provide details of housing and husbandry conditions, including any environmental enrichment.	MATERIALS AND METHODS — Animals: Temperature $22 \pm 2^{\circ}\text{C}$, relative humidity $50 \pm 5\%$, 12 h light-dark cycle, standard rodent chow and water ad libitum; no special environmental enrichment applied.
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	a. No analgesics were administered for this non-surgical hyperuricemia model. b. No adverse events reported. c. Humane endpoints not predefined; animals monitored daily.
Interpretation/scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	a. DISCUSSION (Paragraphs 1-4) — interprets results via transporter and cytokine mechanisms. b. DISCUSSION (final part) — notes limitations and need for further trials.
Generalisability/translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	DISCUSSION (last paragraph) — discusses translational potential for human gout.
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	No protocol was prepared or registered before the study.
Data access	20	Provide a statement describing if and where study data are available.	Data availability statement
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	a. No conflict of interest declared. b. Funding sources listed in Acknowledgements; funders had no role in design, analysis, or reporting.