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Anti-hyperuricemic effects of *Ampelopsis grossedentata* extract and its major component dihydromyricetin via targeted regulation of uric acid metabolism

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ABSTRACT

Hyperuricemia (HUA), a prevalent metabolic disorder, is increasingly recognized as a critical contributor to the onset and progression of various chronic diseases, including cardiovascular and renal conditions. However, the adverse effects and long-term dependence associated with conventional pharmacological therapies highlight the pressing need for safe and effective nutritional interventions. This study systematically investigated the anti-hyperuricemic potential of an ethanol extract of *Ampelopsis grossedentata* (AMP) and its principal flavonoid, dihydromyricetin (DMY), through integrated *in vitro* and *in vivo* approaches. In the mouse model of HUA, AMP (125, 500 mg/kg) xanthine oxidase (XOD) activity and modulation of key urate transporters, including glucose transporter 9, urate transporter 1, and ATP-binding cassette subfamily G member 2. Phytochemical profiling revealed that DMY accounts for 52.97% of the total flavonoids in AMP. Further mechanistic studies demonstrated that DMY (50, 100, 200 mg/kg) exerted dose-dependent inhibition of XOD activity, regulated the expression of urate transporter genes, and protected renal tubular cells from uric acid-induced cytotoxicity. Collectively, AMP displays multi-targeted regulatory effects on uric acid metabolism, with DMY identified as its core bioactive constituent. These findings offer new insights into the molecular mechanisms of natural flavonoids in managing uric acid homeostasis and support the development of *A. grossedentata*-derived functional ingredients as promising nutritional strategies for HUA prevention and therapy.

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1. Introduction

Uric acid (UA) is the final metabolite of purine catabolism in the human body, with approximately 75% excreted via the kidneys to maintain physiological balance. Hyperuricemia (HUA) occurs when UA production exceeds renal excretion capacity or when excretory dysfunction arises^[1]. It is defined as serum UA levels ≥ 7.0 mg/dL in men and ≥ 6.0 mg/dL in women. The development of HUA is influenced not only by dietary factors but also by sex,

age, environmental conditions, and genetic predisposition. In recent years, the global prevalence of HUA has risen markedly, ranging from 2.6% to 36%. According to the National Health and Nutrition Examination Survey (NHANES) in the United States, approximately 21% of adults are affected by HUA^[2]. In Asia, the Korean NHANES reported a prevalence of 11.4% (17.0% in men and 5.9% in women)^[3], while in China, the prevalence increased from 11.1% (2016) to 14.0% (2019), with a significantly higher incidence in men (24.4%) compared to women (3.6%) and a notable trend toward younger individuals^[4]. Epidemiological and clinical evidence has linked HUA to several chronic conditions, including chronic kidney disease, non-alcoholic fatty liver disease, metabolic syndrome, hypertension, insulin resistance, obesity, type 2 diabetes, and cardiovascular and

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cerebrovascular diseases, positioning HUA as an emerging global public health concern^[5].

To understand the pathophysiology of HUA, it is important to first examine the regulation of UA levels, which involve both endogenous and exogenous factors. Approximately 67% of UA originates from endogenous purine metabolism, while 33% is derived from dietary purines. UA is produced primarily through the action of xanthine oxidoreductase (XOR) and xanthine dehydrogenase (XDH), enzymes involved in purine degradation. Increased cellular turnover, elevated purine biosynthesis, and high-purine diets contribute to HUA^[6]. The liver and other tissues rich in xanthine oxidase (XOD) are the main sites of UA production. Approximately 65% of UA is excreted by the kidneys, while the remainder is eliminated through the gastrointestinal tract. Serum UA levels reflect a dynamic balance between production, intestinal and renal excretion, and reabsorption^[7]. Renal excretion is mediated by a coordinated network of urate transporters. Under normal conditions, more than 70% of urate is excreted *via* transporter-mediated renal pathways, while only 5%–10% is directly eliminated in the urine^[8]. Key urate transporters involved in reabsorption include urate transporter 1 (URAT1), glucose transporter 9 (GLUT9), and organic anion transporter 4 (OAT4), while OAT1 and OAT3 function mainly in urate secretion. URAT1 is located on the apical membrane of renal tubular epithelial cells and plays a central role in urate reabsorption, accounting for approximately 90% of filtered urate^[9]. GLUT9, expressed on the basolateral membrane of proximal tubular cells, facilitates basolateral urate transport. Breast cancer resistance protein (BCRP), an ATP-binding cassette transporter located on the apical membrane of renal and intestinal epithelial cells, is critical for urate excretion; mutations or dysfunction of its gene (*ABCG2*) are associated with reduced UA clearance and increased HUA risk^[10]. Impaired renal excretion is considered the predominant pathogenic mechanism of HUA, accounting for approximately 90% of all cases^[11]. Currently, therapeutic agents for HUA are classified into three main types: XOD inhibitors (such as allopurinol (AP), febuxostat, and topiroxostat), uricosuric agents (such as probenecid and benzbromarone), and uricase-based agents (such as tranilast and uricase). However, long-term use of these drugs may lead to adverse effects such as renal burden, hepatotoxicity, and allergic reactions^[12]. As a result, there is growing interest in identifying safer and more effective natural interventions.

Ampelopsis grossedentata Hand.-Mazz., a traditional herbal infusion made from its dried leaves and stems, has gained attention for its promising health benefits and functional food potential^[13]. This plant is rich in flavonoids, including dihydromyricetin (DMY), myricetin, myricitrin, quercetin, quercitrin, resveratrol, polydatin, kaempferol, vitexin, taxifolin, and naringenin^[14]. Among these, DMY is the predominant compound. DMY has been reported to exhibit potent antioxidant, anti-inflammatory, antihypertensive, and hepatoprotective effects, and shows potential in preventing and managing metabolic disorders such as non-alcoholic fatty liver disease, diabetes, atherosclerosis, and osteoporosis^[15]. *In vitro* and cellular studies have shown that *A. grossedentata* extract significantly inhibits the activities of adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP), and XOD^[16]. However, the active constituents, effective dosages, safety profile, and the potential effects on liver and kidney function remain unclear, necessitating further investigation. Despite the promising bioactivities of *A. grossedentata*, its *in vivo* efficacy and underlying mechanisms against HUA remain insufficiently characterized,

particularly regarding renal urate handling, regulation of urate transporters, and dose-dependent therapeutic potential.

This study utilized vine tea from Zhangjiajie, Hunan Province, to systematically evaluate the anti-hyperuricemic effects of the ethanol extract of *A. grossedentata* (AMP) and its major flavonoid, DMY. Using both *in vitro* and *in vivo* models, we explored their modulatory effects on UA biosynthesis and excretion pathways. These findings aim to elucidate the urate-lowering mechanism of AMP and identify its key bioactive constituents, providing scientific evidence and practical support for the development of functional foods and dietary interventions for HUA management.

2. Materials and methods

2.1 Main materials and reagents

A. grossedentata, a medicinal plant native to Zhangjiajie, Hunan Province, China, was selected as the raw material for this study. Specifically, the tender tips of the plant were used. The following reference standards were all purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China), with the stated purities determined by HPLC: apigenin ($\geq 98\%$), epicatechin ($\geq 98\%$), epigallocatechin gallate (EGCG, $\geq 98\%$), hesperidin ($\geq 98\%$), hesperetin ($\geq 98\%$), diosmin ($\geq 95\%$), DMY ($\geq 98\%$), taxifolin ($\geq 98\%$), isoquercitrin ($\geq 98\%$), quercetin ($\geq 98\%$), acacetin ($\geq 98\%$), hyperoside ($\geq 98\%$), rutin ($\geq 98\%$), luteolin ($\geq 98\%$), apigenin ($\geq 98\%$), kaempferol ($\geq 98\%$), myricetin ($\geq 98\%$), myricitrin ($\geq 98\%$), isorhamnetin ($\geq 98\%$), naringenin ($\geq 98\%$), procyanidin B2 ($\geq 98\%$), astragalin ($\geq 98\%$), catechin ($\geq 98\%$), XOD ($\geq 90\%$), hypoxanthine ($\geq 99\%$), xanthine ($\geq 98\%$), and AP ($\geq 98\%$). UA ($\geq 99\%$) was purchased from Sigma-Aldrich (St. Louis, USA).

2.2 Preparation of AMP

The plant samples were ground into a fine powder and passed through a 20-mesh sieve to remove coarse particles. The powder was then extracted with 70% ethanol at a material-to-solvent ratio of 3:7, assisted by ultrasonic extraction for 15 min at room temperature. The supernatant was collected, and the residue was re-extracted 3 additional times using the same solvent volume. All supernatants were combined and subjected to vacuum filtration. The filtrate was vacuum-concentrated at temperatures below 40 °C until complete removal of ethanol, followed by freeze-drying at a cold trap temperature of −60 °C to obtain a yellowish-green powder, designated as AMP, with an extraction yield of 31.2%. The final powder was stored in airtight, light-protected containers at −20 °C until use.

2.3 Liquid chromatography-mass spectrometry (LC-MS) analysis

To prepare the AMP solution, 100 mg of AMP powder was suspended in 1 mL of 80% methanol in a 5 mL centrifuge tube, vortexed for 1 min, and ground using a tissue homogenizer. The sample was then sonicated at 4 °C for 30 min and left standing for 60 min. After centrifugation at 12 000 r/min for 10 min, the supernatant was filtered through a 0.22 μ m organic microporous membrane. For standard preparation, 24 flavonoid standards were each dissolved in 80% methanol to 5 mg/L to form stock solutions, which were mixed and filtered (0.22 μ m) before analysis.

Chromatographic separation was performed on a Waters Acquity UPLC HSS T₃ column (2.1 mm × 100 mm, 1.8 μm) at 42 °C, with a flow rate of 0.3 mL/min and an injection volume of 2 μL. Mobile phase A was 0.1% formic acid in water, and mobile phase B was acetonitrile. Mass spectrometry was carried out using LC-MS/MS in multiple reaction monitoring (MRM) mode under electrospray ionization (ESI) in both positive and negative ion modes: capillary voltage was set at 4.0 kV (positive) and 3.5 kV (negative), with an atomizer temperature of 320 °C, gas flow rate of 9 L/min, and nebulizer pressure of 40 psi. Flavonoid-specific MS parameters are listed in Table S1. The linearity of each analyte was evaluated by constructing calibration curves using at least 6 concentration levels. The calibration curves were generated by plotting peak area (y) against standard concentration (x), and the resulting regression equations and correlation coefficients (R^2) are summarized in Table S2. All analytes showed good linearity within the tested concentration ranges, with R^2 greater than 0.999, indicating high reliability of the quantification method.

2.4 Cell culture and treatment

HK-2 cells were cultured in DMEM medium containing 10% fetal bovine serum (FBS) at 37 °C in a humidified incubator with 5% CO₂. Cells were seeded into 6-well plates at a density of 2×10^6 cells/mL. AMP was dissolved in DMSO and filtered through a 0.22 μm sterile membrane for subsequent use. The AMP treatment groups included: control group (no treatment), model group (3 mmol/L UA), low-dose AMP group (3 mmol/L UA + 40 μg/mL AMP), and high-dose AMP group (3 mmol/L UA + 80 μg/mL AMP). The DMY treatment groups were similarly divided: control, model, low-dose (3 mmol/L UA + 80 μmol/L DMY), and high-dose (3 mmol/L UA + 160 μmol/L DMY). All treatments were incubated for 48 h before samples were harvested for RNA extraction and reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis.

2.5 Cell viability assay

Cell viability was assessed using the CCK-8 assay (purchased from Beijing Solarbio Science & Technology Co., Ltd., China). HK-2 cells were treated with various concentrations of AMP (0, 1, 20, 40, 60, 80, 90, 100, 110, and 140 μg/mL) and DMY (0, 1, 10, 50, 100, 150, 175, 200, and 225 μg/mL). After 24 h incubation, 10 μL of CCK-8 reagent was added to each well and incubated for 1 h. After incubation, the absorbance of each well was read at 490 nm using a microplate reader to assess cell viability.

2.6 XOD inhibition assay

AMP and DMY solutions of varying concentrations were mixed with XOD (0.05 U/mL) and incubated at 37 °C for 30 min. The reaction was initiated by adding 150 μL of xanthine solution (0.48 mmol/L). AP was used as a positive control. Absorbance at 290 nm (A) was recorded every 30 s for 20 cycles. The inhibition rate was calculated as follows:

$$\text{Inhibition rate (\%)} = \frac{(\text{d}A/\text{d}t)_{\text{blank}} - (\text{d}A/\text{d}t)_{\text{sample}}}{(\text{d}A/\text{d}t)_{\text{blank}}}$$

Where, $(\text{d}A/\text{d}t)_{\text{blank}}$ is the initial rate of the control group (without inhibitor, containing the same volume of solvent), and $(\text{d}A/\text{d}t)_{\text{sample}}$ is the initial rate of the sample group.

2.7 Animals and experimental design

All animal protocols were approved by the Animal Ethics Committee of China Agricultural University and adhered to the Guidelines for the Care and Use of Laboratory Animals (AW81114202-5-04). SPF-grade Kunming male mice (4 weeks old) were purchased from Beijing Keao Xieli Feed Co., Ltd. (China). Mice were housed under standard conditions ((22 ± 2) °C, 12 h light/dark cycle) with free access to food and water. Mice were purchased from SBF Biotechnology Co., Ltd.

AMP intervention: 50 mice were randomized into 5 groups ($n = 10$ per group): normal control (CON), HUA model (MOD), low-dose AMP (LAMP, 125 mg/kg), high-dose AMP (HAMP, 500 mg/kg), and AP (10 mg/kg). DMY intervention: 72 mice were randomized into 6 groups ($n = 12$ per group): CON, MOD, low-dose DMY (LDMY, 50 mg/kg), medium-dose DMY (MDMY, 100 mg/kg), high-dose DMY (HDMY, 200 mg/kg), and AP (10 mg/kg). All treatments were administered by oral gavage in saline solution (0.1 mL/10 g body weight) daily at 10:30 a.m. for 21 consecutive days.

2.8 Sample collection

Following 12 h of fasting, mice were euthanized *via* cervical dislocation. Liver, kidneys, and spleen were excised, weighed, and processed. The left kidney was fixed in 4% paraformaldehyde for histopathological evaluation, and the right kidney was snap-frozen in liquid nitrogen. Blood samples were allowed to clot for 2 h at room temperature, centrifuged at 12 000 r/min for 15 min, and the serum was collected and stored at -80 °C for further analysis.

2.9 RNA extraction and quantification

For cells: culture medium was discarded, and each well was lysed with 1 mL TRIzol reagent on ice for 5 min. For tissues: 100 mg of frozen tissue was homogenized in 1 mL pre-chilled TRIzol using zirconium oxide beads. Samples were lysed overnight at 4 °C. Chloroform (200 μL) was added, and samples were centrifuged at 12 000 r/min for 15 min. The aqueous phase was transferred to new tubes, mixed with an equal volume of isopropanol, and incubated for 10 min. After centrifugation, RNA pellets were washed with 75% ethanol, air-dried, and dissolved in 20 μL DEPC water. RNA concentration and purity were determined by measuring OD_{260 nm} and OD_{280 nm} using a microplate reader.

2.10 RT-qPCR analysis

Reverse transcription was performed using a commercial kit from Beijing Polyme Biotechnology Co., Ltd. (China), following the manufacturer's instructions. qPCR was conducted using kits from Beijing Polyme Biotechnology Co., Ltd. (China). Reactions were prepared on ice and run on a qPCR platform using β -actin as the reference gene. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.11 Histological analysis

Kidney tissue fixed in 4% paraformaldehyde for 24 h was dehydrated through graded ethanol, cleared in xylene, embedded in paraffin, and sectioned at 5 μm thickness. Sections were mounted on slides, dried at 45 °C, stained with hematoxylin for 10 min, washed with water, rinsed with 70% and 90% ethanol, and stained with eosin for 2 min. After dehydration and clearing, slides were evaluated under a light microscope. The ileum and liver sections were processed in the same way as above. We have performed blinded histopathological scoring of liver and kidney sections by 2 independent evaluators using established criteria. Histological scores were assigned by 2 independent, blinded pathologists based on semi-quantitative criteria adapted from previous studies. The average of the 2 scores was used for analysis. Detailed scoring criteria are provided in Tables S3 and S4.

2.12 Serum biochemical analysis

Commercial kits (Nanjing Jiancheng Bioengineering Institute, China) were used to measure serum UA, creatinine, urea nitrogen (UN), and XOD activity, following the manufacturer’s protocols^[17]. Absorbance was measured using a microplate reader, and concentrations were calculated using standard curves.

2.13 Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism. Differences among groups were evaluated by One-way ANOVA, and a *P*-value < 0.05 was considered statistically significant. Figures and charts were generated using Excel 2019 and GraphPad Prism 8.0.2.

3. Results

3.1 Identification of flavonoid components in AMP

The preparation procedure for the AMP is shown in Fig. 1A. Based on the analytical methods described by Luo et al.^[18] and Zhang et al.^[19], with appropriate modifications, the flavonoid profile of AMP was systematically analyzed. Quantification was performed using calibration curves generated from the peak areas and corresponding concentrations of 24 flavonoid standards (Table S2).

A total of 23 flavonoid compounds were identified in AMP using LC-MS/MS. The total flavonoid content of the AMP extract was (111 337.3 ± 24 352.53) μg/g (8.70%–13.57%, *m/m*). These included apigenin, catechin, epicatechin, EGCG, hesperidin, hesperetin, diosmin, DMY, taxifolin, isoquercitrin, quercetin, acacetin, hyperoside, rutin, luteolin, apigenin, kaempferol, myricetin, myricitrin, isorhamnetin, naringenin, procyanidin B2, astragalín, and kaempferol. Detailed quantitative data are presented in Table 1. Among these compounds, DMY, taxifolin, EGCG, myricitrin, and myricetin were the most abundant. Notably, DMY and taxifolin were present at concentrations of (58 849.27 ± 11 908.50) and (47 548.8 ± 10 608.80) μg/g, respectively, accounting for 52.97% and 42.67% of the total flavonoid content. Based on the extract yield, the estimated total flavonoid content in *A. grossedentata* was (35.69 ± 7.80) g/100 g of raw material, with DMY contributing (18.86 ± 3.82) g/100 g.

Table 1
Content and percentage of flavonoid monomers in *A. grossedentata* extract.

Compound	Content in AMP (μg/g)	Proportion of total flavonoids (%)	Content in <i>A. grossedentata</i> (g/100 g)
DMY	58 849.27 ± 11 908.50	52.97 ± 0.86	18.86 ± 3.82
Taxifolin	47 548.80 ± 10 608.80	42.67 ± 0.56	15.24 ± 3.40
EGCG	2 499.08 ± 1 187.06	2.17 ± 0.54	0.80 ± 0.38
Myricitrin	993.94 ± 105.80	0.91 ± 0.10	0.32 ± 0.03
Myricetin	945.56 ± 360.73	0.83 ± 0.13	0.30 ± 0.12
Catechin	152.62 ± 16.48	0.14 ± 0.04	0.049 ± 0.005
Isoquercitrin	149.14 ± 120.93	0.12 ± 0.07	0.048 ± 0.039
Hyperoside	80.96 ± 17.23	0.076 ± 0.029	0.026 ± 0.006
Quercetin	49.05 ± 7.68	0.044 ± 0.003	0.016 ± 0.002
Diosmin	37.42 ± 17.01	0.033 ± 0.007	0.012 ± 0.005
Naringenin	24.03 ± 1.17	0.022 ± 0.004	0.008 ± 0.000
Epicatechin	3.12 ± 0.36	0.003 ± 0.000	0.001 ± 0.000
Luteolin	1.69 ± 0.08	0.002 ± 0.000	0.001 ± 0.000
Astragalín	0.97 ± 0.07	0.001 ± 0.000	< 0.001
Kaempferol	0.57 ± 0.26	0.001 ± 0.000	< 0.001
Apigenin	0.35 ± 0.11	< 0.001	< 0.001
Isorhamnetin	0.27 ± 0.02	< 0.001	< 0.001
Hesperetin	0.23 ± 0.16	< 0.001	< 0.001
Acacetin	0.12 ± 0.07	< 0.001	< 0.001
Quercetin	0.03 ± 0.02	< 0.001	< 0.001
Apigenin	0.03 ± 0.00	< 0.001	< 0.001
Hesperidin	ns	ns	ns
Rutin	ns	ns	ns
Procyanidin B2	ns	ns	ns
Total flavonoids	111 337.30 ± 24 352.53	100	35.69 ± 7.80

Note: “ns” indicates not detected.

3.2 AMP alleviates HUA via XOD inhibition and transporter regulation in HK-2 cells

XOD activity directly influences UA levels and serves as a critical therapeutic target in HUA. *In vitro* assays demonstrated that AMP significantly inhibited XOD activity in a dose-dependent manner, with a half-maximal inhibitory concentration (IC₅₀) of approximately 870 μg/mL (*P* < 0.05). In comparison, AP, a commonly used XOD inhibitor, exhibited a markedly lower IC₅₀ of approximately 65 μmol/L (Figs. 1B, C).

To further assess the safety profile and the maximal non-toxic concentration of AMP in HK-2 cells, we treated cells with a range of AMP concentrations (0, 1, 20, 40, 60, 80, 90, 100, 110, and 140 μg/mL) for 48 h and evaluated cell viability using the CCK-8 assay. As shown in Fig. 1D, AMP concentrations up to 40 μg/mL exhibited a slight but non-significant promotion of cell growth. Notably, 60 μg/mL AMP significantly enhanced cell proliferation (*P* < 0.05). However, at concentrations ≥ 110 μg/mL, cell viability declined significantly (*P* < 0.001), indicating evident cytotoxicity. Based on these findings, 80 μg/mL was selected as the optimal concentration for subsequent *in vitro* experiments to ensure both efficacy and cellular safety. A cellular model of UA-induced renal injury was established by treating HK-2 cells with 3 mmol/L UA.

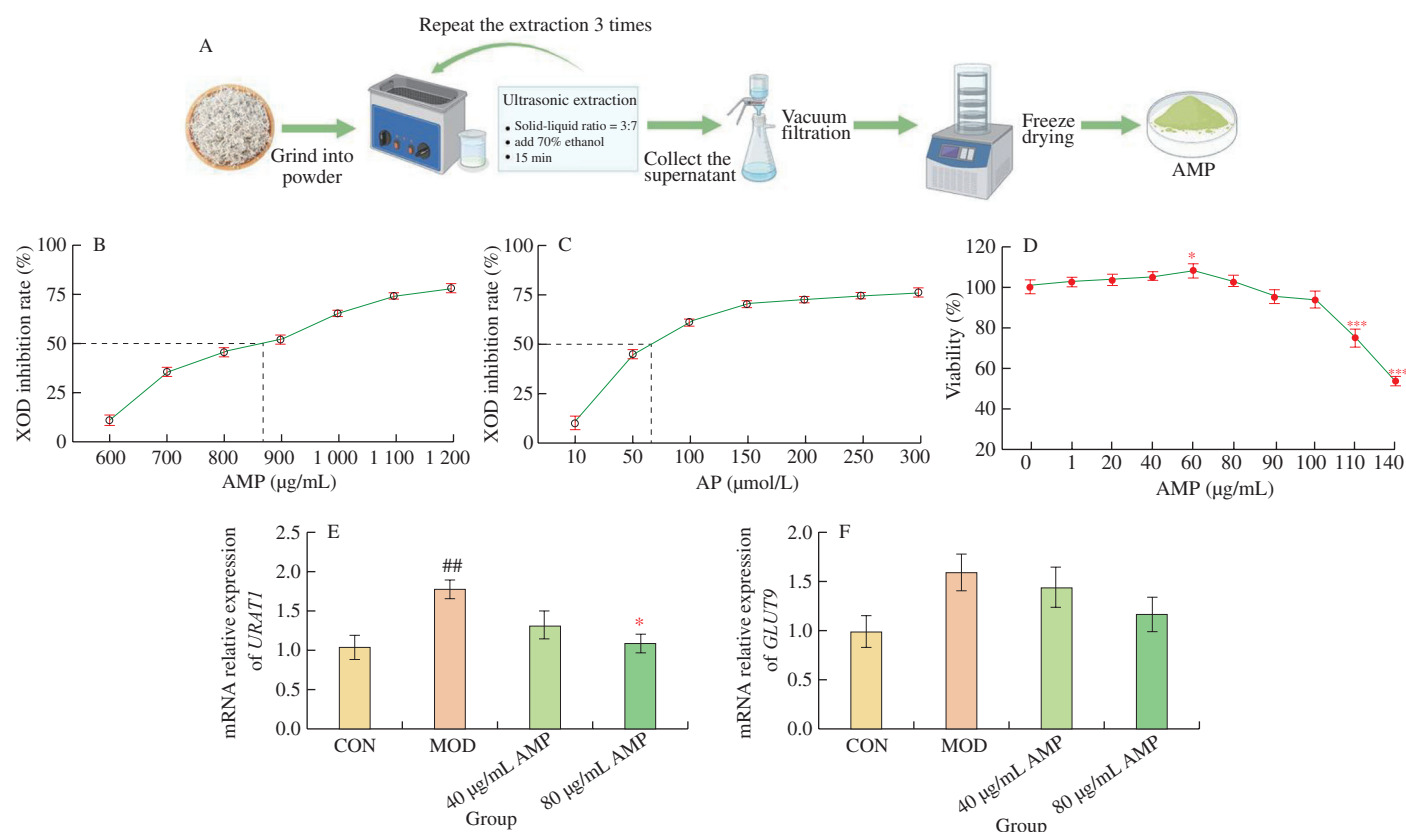


Fig. 1 Effects of AMP on XOD activity and UA metabolism in HK-2 cells. (A) Schematic diagram of AMP extraction procedure. (B-C) Inhibitory curves of (B) AMP and (C) AP against XOD *in vitro*. (D) Effect of AMP on the viability of HK-2 cells. (E-F) Effects of AMP on the mRNA expression levels of urate transporters in HK-2 cells, (E) *URAT1* and (F) *GLUT9*. ## $P < 0.01$ vs. CON; * $P < 0.05$ *** $P < 0.001$ vs. MOD.

In the model group, *URAT1* expression was significantly upregulated ($P < 0.05$), while *GLUT9* also showed an upward trend, indicating enhanced intracellular UA reabsorption. Treatment with 80 μg/mL AMP significantly downregulated *URAT1* expression ($P < 0.05$), suggesting that AMP reversed UA-induced overexpression of this transporter (Figs. 1E, F). Collectively, these results indicate that AMP exerts a dose-dependent inhibitory effect on XOD activity *in vitro* and significantly reduces *URAT1* expression in HK-2 cells at non-toxic concentrations, thereby potentially alleviating HUA-induced renal damage.

3.3 AMP reduces serum UA, creatinine, and UN levels in HUA mice

AMP was administered at varying concentrations to HUA mice, as illustrated in Fig. 2A. The serum UA levels in the HUA model group (MOD group) were significantly higher than those in the normal control group (CON group, $P < 0.001$), confirming the successful establishment of the HUA model. Treatment with AMP at 125 mg/kg (LAMP), 500 mg/kg (HAMP), and AP groups resulted in significant reductions in serum UA levels compared to the MOD group ($P < 0.001$), with a clear dose-dependent effect (Fig. 2B). Furthermore, the serum creatinine levels in the MOD group were markedly elevated compared to the CON group ($P < 0.001$) (Fig. 2C), suggesting kidney impairment in the HUA state. After AMP treatment, particularly in the HAMP group, creatinine levels were significantly reduced ($P < 0.05$) (Fig. 2C), indicating a protective effect of AMP on kidney function. Additionally, UN levels in the

MOD group were significantly higher than in the CON group ($P < 0.001$), while LAMP and HAMP treatments led to significant decreases in UN levels ($P < 0.05$) (Fig. 2D). Among all treatment groups, the AP group exhibited the lowest levels of both creatinine and UN ($P < 0.001$).

3.4 AMP alleviates organ injury and histopathological alterations in HUA mice

No significant differences in food intake were observed among groups, indicating that AMP intervention did not affect dietary consumption (Fig. 2E). Liver coefficients showed no significant changes, suggesting no apparent hepatic toxicity during AMP administration (Fig. 2F). Compared with the CON group, the MOD group exhibited a significantly increased kidney coefficient ($P < 0.01$), which was markedly reduced by AMP treatment ($P < 0.05$), indicating improved renal morphology (Fig. 2G). Similarly, the spleen coefficient was elevated in MOD mice ($P < 0.01$) but significantly decreased following LAMP and HAMP treatments ($P < 0.001$) (Fig. 2H). Histological analysis further demonstrated the protective effects of AMP. The CON group showed normal renal architecture, whereas MOD mice exhibited tubular epithelial cell necrosis, dilation, and vacuolar degeneration. AP treatment partially alleviated these changes, while AMP, particularly HAMP, markedly restored renal structure (Fig. 2I). Liver histology revealed disrupted architecture in MOD mice, whereas AMP treatment improved hepatic organization and reduced pathological alterations, with HAMP showing morphology comparable to the CON group (Fig. 2J).

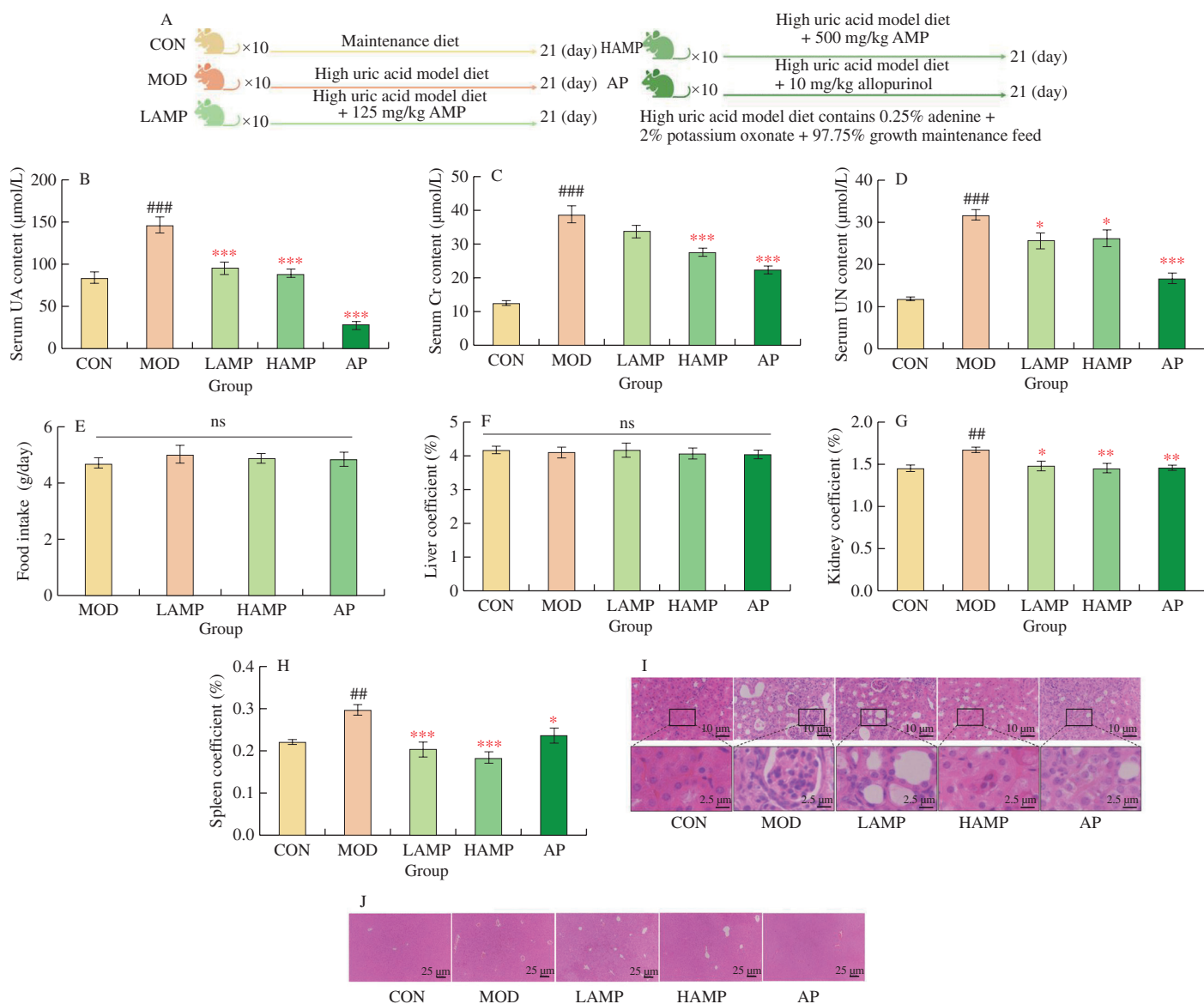


Fig. 2 Ameliorative effects of AMP on hyperuricemic mice. (A) Schematic of the AMP intervention in the hyperuricemia mouse model. (B-E) Effects of AMP on serum levels of (B) UA, (C) creatinine, and (D) UN; (E) food intake in mice. (F-H) Organ indices of (F) liver, (G) kidney, and (H) spleen. (I-J) Representative H&E-stained histological sections of the (I) kidney and (J) liver. ## $P < 0.01$, ### $P < 0.001$ vs. CON; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. MOD. ns, not significant ($P > 0.05$).

3.5 AMP suppresses UA synthesis via inhibition of XOD activity and expression in serum, liver, and intestine

To evaluate the effects of AMP on XOD activity and gene expression, XOD activity in the serum (Fig. 3A) and mRNA expression levels in the liver and small intestine (Figs. 3B and C) were measured in HUA mice treated with different doses of AMP. Compared to CON group, serum XOD activity was significantly elevated in MOD group ($P < 0.001$), indicating enhanced UA synthesis in the hyperuricemic state. Both low-dose and high-dose AMP treatments significantly reduced serum XOD activity in a dose-dependent manner, with the HAMP group showing a more pronounced reduction ($P < 0.001$), comparable to that of the AP group.

Similarly, XOD mRNA expression levels in the liver and intestine were significantly upregulated in the MOD group compared to the CON group (liver: $P < 0.01$; intestine: $P < 0.05$), further indicating

increased UA biosynthesis under hyperuricemic conditions. AMP treatment led to a dose-dependent downregulation of XOD gene expression in both tissues. Notably, XOD expression in the HAMP group was suppressed to a level comparable with the AP group ($P < 0.01$), confirming the strong inhibitory effect of AMP. These findings demonstrate that AMP effectively reduces UA synthesis by inhibiting serum XOD activity and suppressing XOD gene expression in the liver and intestine, with efficacy comparable to that of AP.

3.6 AMP regulates urate transporter gene expression to modulate UA reabsorption and excretion in HUA mice

To explore whether AMP mediates its UA-lowering effects through regulation of UA transport, the expression levels of key urate transporter genes were evaluated in the kidney and intestine of HUA mice.

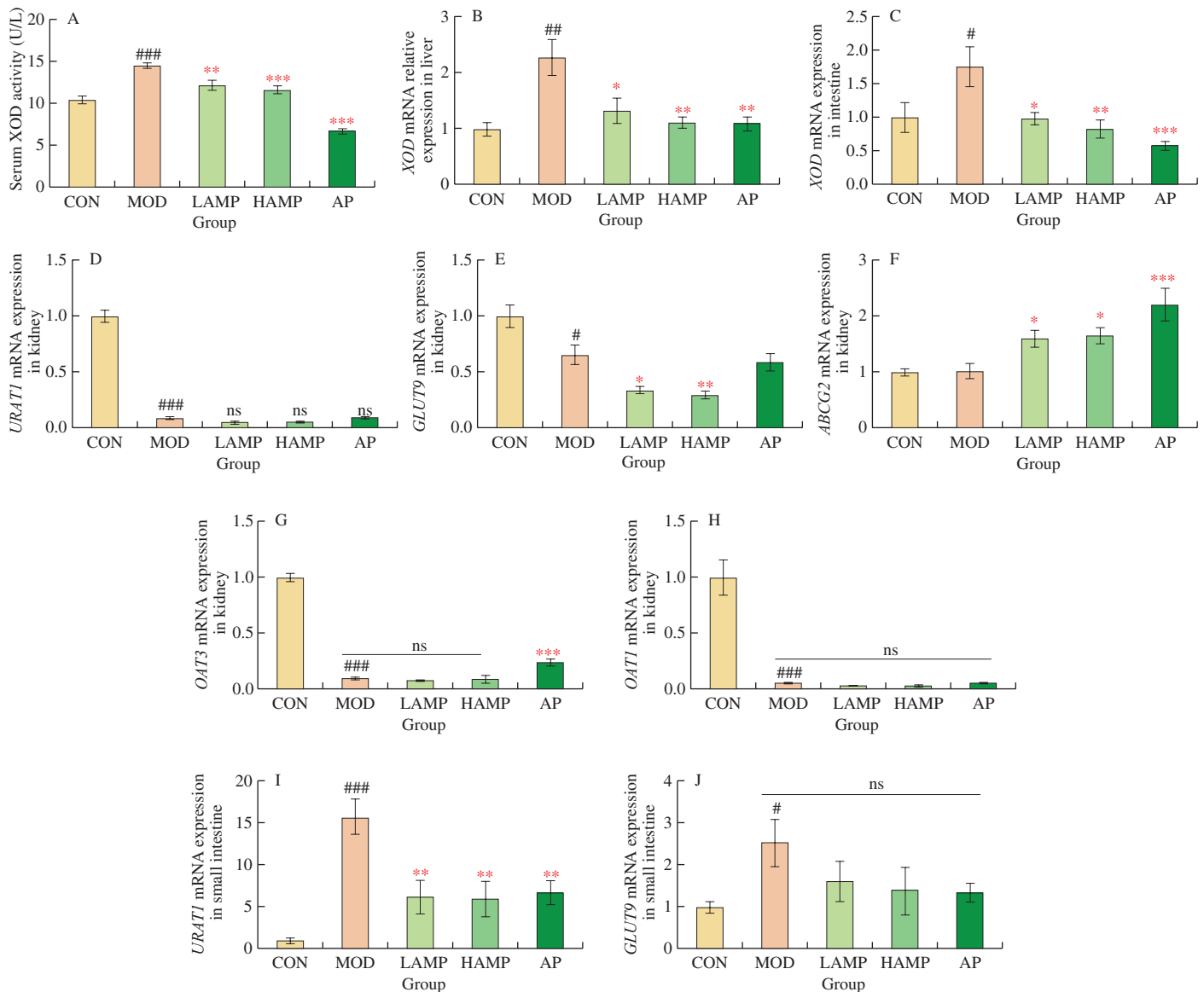


Fig. 3 Effects of AMP on XOD activity, *XOD* gene expression, and urate transporter gene expression in hyperuricemic mice. (A–C) AMP-induced changes in (A) XOD activity in serum, (B) liver, and (C) intestine. (D–H) Effects of AMP on renal expression of urate transporter genes (D) *URAT1*, (E) *GLUT9*, (F) *ABCG2*, (G) *OAT3*, and (H) *OAT1*. (I–J) Effects of AMP on intestinal expression of urate transporter genes (I) *URAT1* and (J) *GLUT9*. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. CON; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. MOD. ns, not significant ($P > 0.05$).

In the kidney, expression levels of *URAT1* and *GLUT9* were significantly elevated in the MOD group compared to the CON group (*URAT1*: $P < 0.01$; *GLUT9*: $P < 0.05$), suggesting enhanced renal UA reabsorption. AMP treatment did not significantly alter *URAT1* expression; however, it significantly decreased *GLUT9* expression ($P < 0.05$), indicating that AMP may inhibit UA reabsorption by downregulating *GLUT9* (Figs. 3D and E). For urate excretion-related genes, no significant difference in *ABCG2* expression was observed between the MOD and CON groups. Nonetheless, *ABCG2* expression was significantly upregulated following AMP treatment ($P < 0.05$), suggesting a promotive effect on UA excretion (Fig. 3F). In contrast, *OAT1* and *OAT3* expression levels were markedly reduced in the MOD group and were not significantly restored by AMP treatment. A significant upregulation of *OAT3* was observed only in the AP group (Figs. 3G and H).

In the intestine, *XOD* gene expression was significantly increased in the MOD group ($P < 0.05$), indicating elevated local UA production. Additionally, *URAT1* expression was markedly upregulated ($P < 0.001$), implying increased intestinal reabsorption of UA. AMP and AP treatments significantly downregulated both *XOD* and *URAT1* expression in a dose-dependent manner, suggesting that AMP can reduce intestinal UA synthesis and reabsorption by suppressing the expression of these genes (Fig. 3I). Although *GLUT9* expression was significantly elevated in the MOD group compared to the CON group ($P < 0.05$), AMP and AP treatments showed only a downward trend without statistical significance (Fig. 3J). Collectively, these findings indicate that AMP exerts its UA-lowering effects through multiple mechanisms: by downregulating renal *GLUT9* and upregulating *ABCG2* to reduce UA reabsorption and enhance excretion, and by suppressing *XOD* and *URAT1* expression in the intestine to inhibit UA production and reabsorption. These coordinated actions contribute to the overall reduction of serum UA levels in HUA mice.

3.7 DMY inhibits XOD activity and modulates UA transporters in HK-2 cells

DMY, a major flavonoid compound derived from *A. grossedentata*, accounts for more than 50% of the total flavonoid content in the extract^[20]. In this study, the DMY content was determined to be (18.86 ± 3.82) g/100 g of dry weight. DMY is recognized for its antioxidant, anti-inflammatory, and potential antitumor activities, along with its hypoglycemic and hepatoprotective effects^[21]. To clarify the role of DMY in the anti-hyperuricemic activity of the AMP extract, we investigated its effects on XOD activity and UA metabolism in HK-2 cells, using concentrations that reflect its levels in AMP. DMY significantly inhibited XOD activity in a dose-dependent manner ($P < 0.05$), with an IC_{50} of approximately 1 800 $\mu\text{mol/L}$ ($576.45 \mu\text{g/mL}$), which was notably higher than that of the positive control AP ($IC_{50} = 70 \mu\text{mol/L}$) (Figs. 4A, B). In HK-2 cells, DMY at concentrations of 50–150 $\mu\text{mol/L}$ markedly enhanced cell viability, indicating a pro-proliferative effect. However, when the concentration reached 175 $\mu\text{mol/L}$ or higher, a noticeable decline in viability was observed, and at 200 $\mu\text{mol/L}$, DMY exerted clear cytotoxic effects (Fig. 4C). Based on these findings, 160 $\mu\text{mol/L}$ was selected as the optimal concentration for subsequent experiments to ensure data accuracy and reliability.

In UA-stimulated HK-2 cells, DMY significantly modulated the expression of UA transport-related genes. Both 80 and 160 $\mu\text{mol/L}$ DMY elevated the expression of *URAT1* and *GLUT9* compared to the model group, but only 160 $\mu\text{mol/L}$ DMY significantly reversed the UA-induced upregulation of *URAT1* ($P < 0.05$), while the effect of 80 $\mu\text{mol/L}$ DMY was not statistically significant (Figs. 4D, E). These findings suggest that DMY, as a principal active flavonoid in AMP, inhibits XOD activity in a concentration-dependent manner and significantly reverses UA-induced *URAT1* overexpression at 160 $\mu\text{mol/L}$, indicating its key regulatory role in AMP's anti-hyperuricemic effect.

3.8 DMY attenuates HUA and renal dysfunction through dose-dependent effects

To further evaluate the effects of DMY *in vivo*, HUA mice were treated with different DMY doses (Fig. 5A). Following successful model establishment, serum UA levels in the MOD group were significantly elevated compared to the CON group ($P < 0.001$), confirming effective induction of HUA. Treatment with low (LDMY, 50 mg/kg), medium (MDMY, 100 mg/kg), and high (HDMY, 200 mg/kg) doses of DMY significantly reduced serum UA levels in a dose-dependent manner ($P < 0.001$, Fig. 5B). Notably, UA levels in the MDMY and HDMY groups were comparable to those in the CON group, underscoring DMY's potent hypouricemic effect. The positive control group treated with AP (10 mg/kg) showed the most pronounced UA-lowering activity, consistent with its well-established role as an XOD inhibitor.

DMY administration also significantly decreased serum creatinine and UN levels in a dose-dependent fashion. In the HDMY group, levels of UA, creatinine, and UN were not significantly different from those in the AP group (Figs. 5C, D), indicating that high-dose DMY confers renal protective effects comparable to those of AP in HUA mice.

3.9 Effects of DMY on serum XOD activity and gene expression in HUA mice

Regarding serum XOD activity, the MOD group exhibited a significantly higher level compared to the CON group ($P < 0.001$), indicating increased UA production in HUA mice, which is consistent with the elevated serum UA levels observed (Fig. 5E). After intervention with DMY, serum XOD activity decreased in a dose-dependent manner, with both the MDMY and HDMY groups showing significant inhibition ($P < 0.05$), suggesting that DMY effectively reduces UA production by inhibiting XOD activity. In

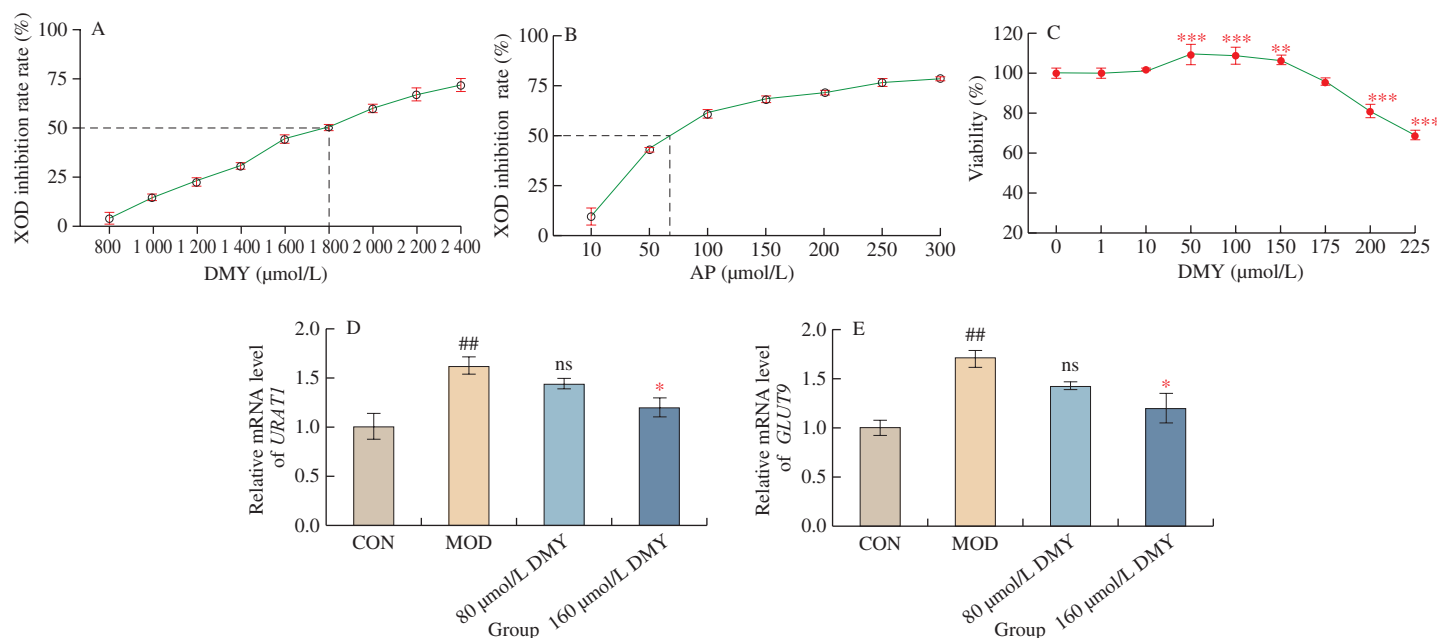


Fig. 4 Effects of DMY on XOD activity and UA metabolism in HK-2 cells. (A-B) Inhibitory curves of (A) DMY and (B) AP against XOD *in vitro*. (C) Effect of DMY on the viability of HK-2 cells. (D-E) Effects of DMY on mRNA expression levels of urate transporters in HK-2 cells, (D) *URAT1* and (E) *GLUT9*. ## $P < 0.01$ vs. CON; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. MOD. ns, not significant ($P > 0.05$).

terms of *XOD* gene expression, the MOD group exhibited markedly upregulated *XOD* expression in both liver and small intestine compared to the CON group ($P < 0.001$ and $P < 0.01$, respectively). DMY and AP treatments significantly downregulated *XOD* gene expression. Notably, the reduction in the LDMY and HDMY groups was comparable to that observed in the AP group ($P < 0.01$), indicating that DMY may suppress UA production by inhibiting *XOD* expression in hepatic and intestinal tissues (Figs. 5F and G).

3.10 DMY regulates urate transporter expression in kidney and intestine to facilitate UA excretion

In the kidneys, *URAT1* expression was significantly decreased in the MOD group compared to the CON group; however, no significant differences in *URAT1* expression were observed between the MOD group and any of the DMY-treated or AP groups, suggesting that DMY does not exert its UA-lowering effect via regulation of renal *URAT1* expression (Fig. 5H). In contrast,

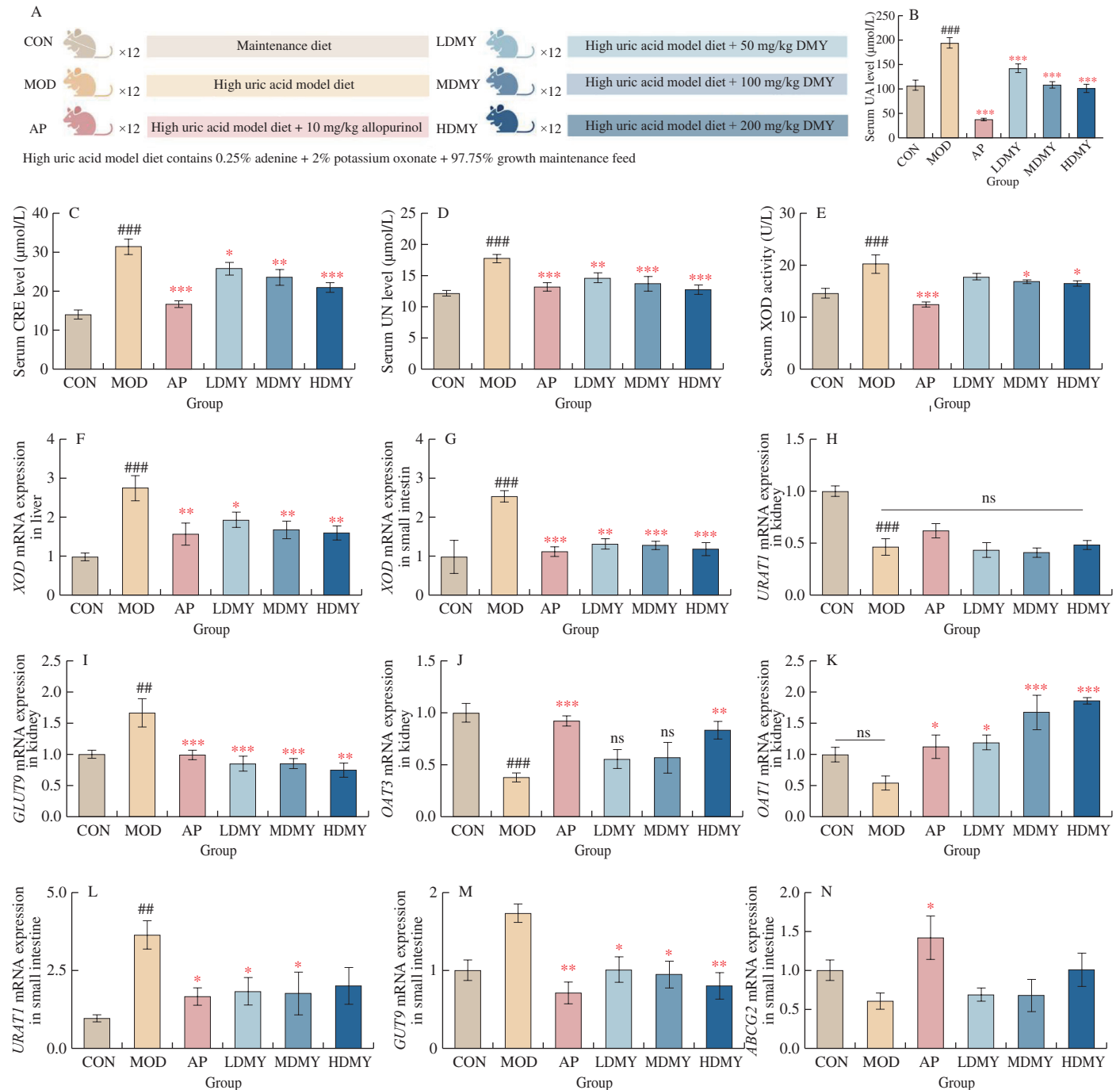


Fig. 5 Ameliorative effects of DMY on hyperuricemic mice. (A) Schematic diagram of DMY intervention in HUA mouse model. (B-D) Effects of DMY on (B) serum UA, (C) creatinine, and (D) UN levels in HUA mice. (E) Effect of DMY on serum XOD activity in HUA mice. (F-G) Effect of DMY on *XOD* mRNA expression levels in the (F) liver and (G) small intestine of HUA mice. (H-K) Effect of DMY on renal mRNA expression of urate transporters in HUA mice, (H) *URAT1*, (I) *GLUT9*, (J) *OAT3*, and (K) *OAT1*. (L-N) Effect of DMY on intestinal mRNA expression of urate transporters in HUA mice, (L) *URAT1*, (M) *GLUT9*, and (N) *ABCG2*. ** $P < 0.01$, *** $P < 0.001$ vs. CON; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. MOD. ns, not significant ($P > 0.05$).

GLUT9 expression was significantly elevated in the MOD group ($P < 0.01$) and significantly decreased following DMY treatment at all doses ($P < 0.001$). The reduction in *GLUT9* expression in all 3 DMY groups exceeded that observed in the AP group, indicating that DMY effectively suppresses renal UA reabsorption (Fig. 5I). *OAT3* and *OAT1* expressions were both downregulated in the MOD group compared to the CON group, with the reduction in *OAT3* reaching statistical significance ($P < 0.001$), suggesting impaired UA excretion (Figs. 5J and K). Compared with the MOD group, significant upregulation of *OAT3* expression was observed only in the HDMY and AP groups, indicating a potential enhancement of UA excretion. All 3 DMY doses, as well as AP, significantly increased *OAT1* expression, with DMY exhibiting a dose-dependent effect, suggesting that DMY promotes renal UA excretion *via* upregulation of *OAT1*.

In the small intestine, *URAT1* gene expression was significantly increased in the MOD group ($P < 0.01$), but this elevation was significantly reversed by all 3 doses of DMY and AP treatment. Although *GLUT9* expression also showed an upward trend in the MOD group, its expression was significantly reduced after DMY and AP intervention, with the HDMY group showing an effect comparable to that of AP. Additionally, DMY treatment did not enhance the expression of *ABCG2* in the intestine (Figs. 5L-N).

3.11 DMY exerts nephroprotective, hepatoprotective, and intestinal barrier-restoring effects in HUA mice

There were no significant differences in body weight among the treatment groups (Fig. 6A). The liver coefficient showed no significant changes across all experimental groups, indicating that DMY did not cause apparent hepatic injury during the treatment period (Fig. 6B). Compared with the CON group, the kidney coefficient in the MOD group was significantly increased ($P < 0.05$), suggesting that the pathological condition may lead to renal hypertrophy (Fig. 6C). However, DMY treatment resulted in a

dose-dependent reduction in kidney coefficient relative to the MOD group, with the most pronounced effect observed in the HDMY group ($P < 0.01$), indicating the potential nephroprotective effect of DMY. Similarly, the spleen coefficient was significantly elevated in the MOD group compared to the CON group ($P < 0.01$), whereas DMY administration at low, medium, and high doses significantly reduced the spleen coefficient in HUA mice ($P < 0.05$) (Fig. 6D). These findings suggest that DMY exerts beneficial effects on the kidney and spleen indices of HUA mice, particularly at higher doses, while having no adverse impact on liver weight.

Histological analysis further confirmed these findings. Mice in the MOD group exhibited apparent pathological changes in the kidneys, including tubular epithelial cell necrosis, dilation, and vacuolation. AP treatment partially alleviated the vacuolation, while DMY intervention improved renal histopathology in a dose-dependent manner. Notably, the HDMY group exhibited renal structures most similar to those of the CON group, indicating superior protective effects. In the liver, HUA mice showed disrupted lobular architecture and structural damage, indicating hepatic impairment. DMY treatment improved hepatocyte arrangement and reduced inflammation, with the HDMY group again showing liver morphology most comparable to the control group (Fig. 6E).

As a major organ involved in UA excretion, the integrity of the intestinal barrier plays a critical role in maintaining UA homeostasis. In the CON group, ileal histology appeared normal, with well-aligned villi and no signs of edema or congestion. In contrast, the MOD group displayed severe pathological changes, including shortened and thickened villi, extensive mucosal damage, and inflammatory cell infiltration. DMY treatment markedly alleviated these pathological alterations, as evidenced by the restoration of mucosal epithelial integrity and regular villus arrangement, with no visible inflammatory infiltration. These results suggest that DMY supplementation helps restore intestinal barrier function in HUA mice, which may facilitate UA excretion and contribute to maintaining systemic UA homeostasis.

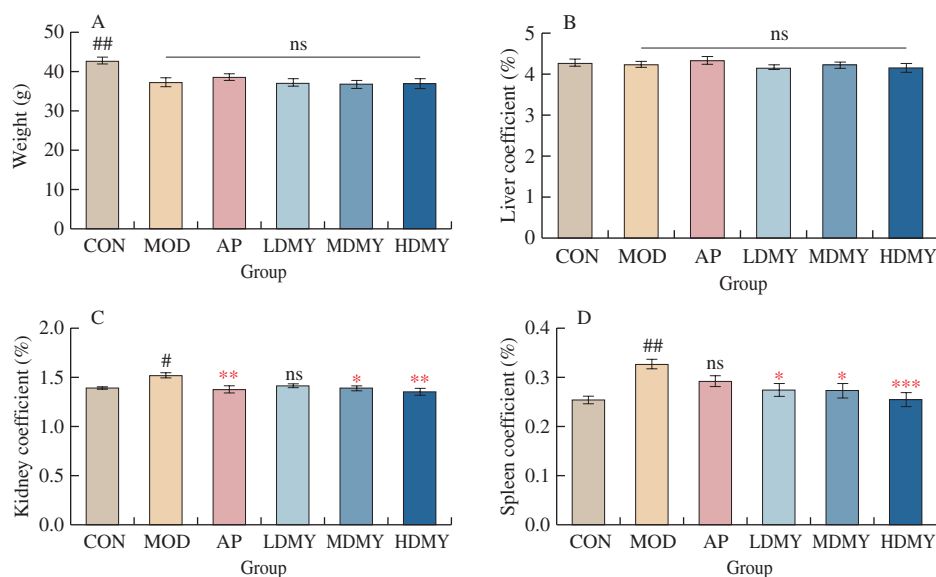


Fig. 6 Effects of DMY on the kidney, liver, and ileum of hyperuricemic mice. (A) Body weight of mice. (B-D) Organ index of (B) liver, (C) kidney, and (D) spleen. (E) Representative H&E-stained histological sections of the liver, kidney, and ileum. [#] $P < 0.05$, ^{##} $P < 0.01$ vs. CON; $P < 0.05$, $P < 0.01$, $P < 0.001$ vs. MOD. ns, not significant ($P > 0.05$).

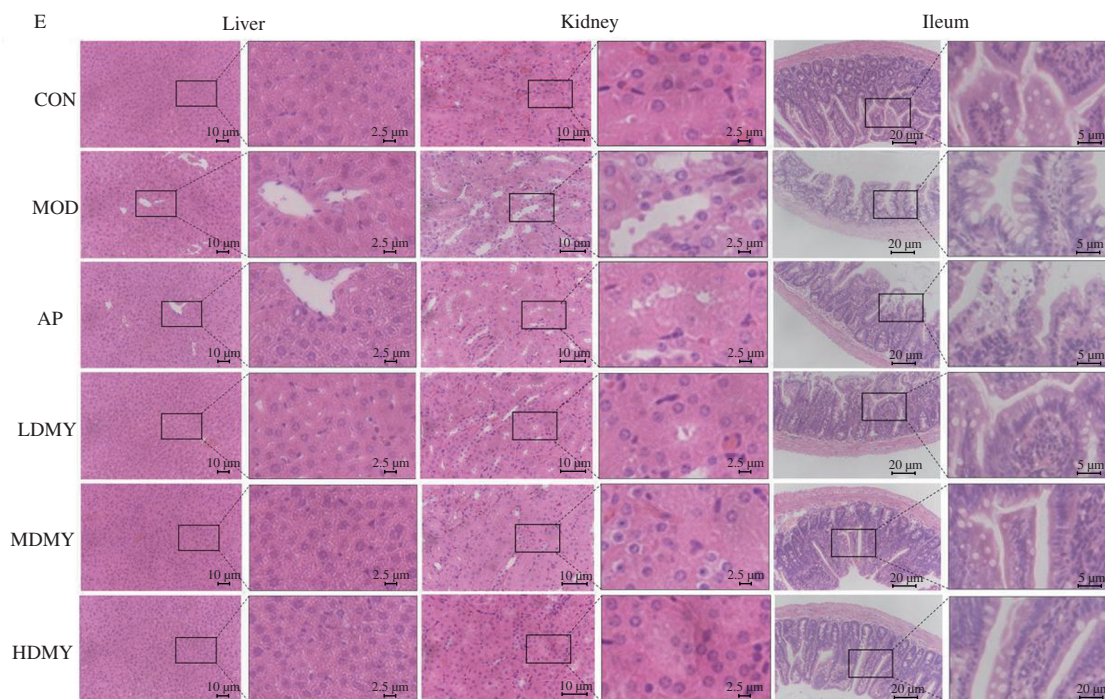


Fig. 6 (Continued)

4. Discussion

In China, the prevalence of HUA among adults is steadily increasing and showing a trend toward younger populations, making it the second most common metabolic disorder after diabetes. HUA, characterized by abnormally elevated serum UA levels, is a hallmark of metabolic dysfunction and is associated with various health issues, including gout, nephropathy, and cardiovascular diseases. Therefore, identifying safe and effective strategies to regulate UA levels is of critical importance.

A. grossedentata, a dual-purpose edible and medicinal plant, contains a complex mixture of bioactive compounds, particularly flavonoids^[22]. Recent studies have demonstrated that both water and ethanol extracts of *A. grossedentata* possess significant anti-HUA effects *in vitro* and *in vivo* models. However, current research lacks a comprehensive evaluation that integrates the origin of raw materials, extraction processes, compositional analysis, and the *in vivo* and *in vitro* effects into a unified framework. In this study, *A. grossedentata* harvested from Zhangjiajie, Hunan Province, China, was selected as the raw material, and its ethanol extract was obtained *via* ultrasonic-assisted extraction, followed by rotary evaporation and freeze-drying. The extraction yield was 31.2%. LC-MS analysis revealed that the flavonoid content in the extract reached $(111\,337.3 \pm 24\,352.53) \mu\text{g/g}$, corresponding to $(35.69 \pm 7.80) \text{ g/100 g}$ dry weight of AMP. Notably, DMY was the predominant flavonoid, accounting for $(18.86 \pm 3.82) \text{ g/100 g}$, or $(52.97 \pm 0.86)\%$ of the total flavonoid content. Compared to the DMY yield of 16.21% reported by Huang et al.^[23], our study achieved a higher extraction efficiency. Additionally, the DMY content obtained here aligns with findings from samples collected in Lichuan $((18.63 \pm 1.04) \text{ g/100 g})$ ^[24]. Nevertheless, our extraction method is more economical and practical. The extraction technology used in this study not only ensured high efficiency but also laid a solid foundation for the further development and application of

AMP-derived bioactive components. Differences in extraction yields across studies may be attributed to variations in raw material origin and extraction conditions, including time, temperature, and solvent-to-solid ratio, underscoring the need for standardization of raw material sourcing and processing methods in future product development.

Although UA-lowering medications targeting XOD exist, their use is often limited by adverse effects and increased cardiovascular risks. This highlights the necessity of developing safe and effective food-derived XOD inhibitors from natural sources. In this study, the AMP extract exhibited an IC_{50} value of $870 \mu\text{g/mL}$ against XOD, which is significantly lower than that of *Rosa roxbunghii* extract ($\text{IC}_{50} = 12\,720 \mu\text{g/mL}$)^[25]. Although the IC_{50} value is higher than that of other herbal extracts, such as *Chaenomeles speciosa* ($334.2 \mu\text{g/mL}$)^[26], *Artemisia nilagirica* $((144.23 \pm 2.04) \mu\text{g/mL})$ ^[27], the discrepancies may stem from differences in the types, abundances, and bioactivities of constituent. These findings highlight the importance of further research aimed at optimizing extraction processes, enriching active fractions, and conducting systematic comparative evaluations across functional food plants with dual medicinal and dietary uses.

Establishing a reliable HUA animal model is a prerequisite for evaluating therapeutic effects of natural compounds. Common modeling approaches include increasing purine intake, inhibiting UA excretion, suppressing UA metabolism, and genetic modification^[28]. Potassium oxonate, a uricase inhibitor, is widely used to induce HUA rapidly. However, due to its short action duration, it is often administered in combination with UA precursors such as adenine, hypoxanthine, fructose, or yeast extract to simulate the chronic pathological process more accurately^[29–30]. For instance, Wen et al.^[31] reported that co-administration of potassium oxonate and adenine stabilized serum UA levels from day 7 in the modeling process. To mimic the chronic HUA condition caused by a high-purine diet, we adopted a modified modeling protocol using 0.25% adenine and

2% potassium oxonate in feed. The model's success was confirmed by significantly elevated serum levels of UA, creatinine, and UN compared to the control group.

Subsequently, AMP intervention was applied. Consistent with studies on *Sanghuangporus*^[32] and AMP extracts, our results showed that AMP dose-dependently reduced the kidney index and significantly decreased the spleen index to levels comparable with controls. Key biochemical markers revealed that AMP significantly and dose-dependently decreased serum UA, creatinine, UN, and XOD activity. Hematoxylin and eosin (H&E) staining further confirmed the attenuation of renal and hepatic lesions. These effects may be attributed to reduced XOD expression in the liver and intestine, as well as modulation of UA transporters in the kidney, including downregulation of *GLUT9* and upregulation of *ABCG2*. The minimal effective dose of AMP was determined to be 125 mg/kg, equivalent to an intake of approximately 710 mg AMP or 2.28 g AMP raw material for a 70 kg adult. These findings support the potential of AMP as a natural agent to reduce serum UA and protect renal function, likely by inhibiting XOD-mediated UA production.

Due to the complex composition of AMP, it remains challenging to pinpoint its precise mechanism of action. DMY, which constitutes over 50% of total flavonoids in AMP^[20], was quantified at (18.86 ± 3.82) g/100 g in this study. DMY is recognized for its antioxidant, antitumor, antimicrobial, and anti-inflammatory properties, as well as its protective effects against cardiovascular and renal disorders^[33]. Although Li et al.^[34] used molecular docking to demonstrate that DMY forms stable interactions with *URAT1*, *GLUT9*, *ABCG2*, and *XOD* via van der Waals forces and hydrogen bonds, these findings remain limited to *in vitro* observations, lacking *in vivo* validation. *In vitro*, DMY exhibited an IC_{50} of 1 800 μ mol/L against XOD, equivalent to 576.45 μ g/mL. Achieving this concentration would require approximately 1 090 mg of AMP to deliver the necessary DMY content. Low-dose DMY (50 mg/kg) significantly reduced serum UA, creatinine, and UN levels in HUA mice, while medium (100 mg/kg) and high doses (200 mg/kg) significantly inhibited XOD activity. Histopathological analysis showed that DMY alleviated tubular dilation and vacuolar degeneration in the kidneys, and restored intestinal barrier integrity. At low doses, DMY downregulated *URAT1* and *GLUT9* in the small intestine, while high doses promoted *ABCG2* expression. The minimum effective dose of DMY was identified as 50 mg/kg, equivalent to an intake of 283.5 mg DMY or 1.56 g AMP for a 70 kg adult. This is significantly lower than the minimum effective dose of AMP (2.28 g), offering important insights for future dose optimization and clinical application.

Beyond its urate-lowering properties, AMP and its primary flavonoid, DMY, exhibit notable antioxidant and anti-inflammatory activities that may contribute to renal protection under hyperuricemic conditions. AMP, rich in total polyphenols and bioactive compounds, has demonstrated strong antioxidant capacity *in vitro* and *in vivo*. Studies have shown that AMP scavenges free radicals (ABTS, DPPH) in a dose-dependent manner, suppresses menadione-induced cell death in zebrafish, and inhibits ROS production in human leukocytes^[13,35-36]. DMY has similarly been shown to enhance systemic antioxidant status in animal models by increasing antioxidant enzyme levels and reducing oxidative stress markers, partly through activation of the Nrf2/HO-1 signaling pathway^[37-38]. AMP and DMY also display potent anti-inflammatory effects.

In previous research, XOD was reported to localize to both the cytoplasmic and mitochondrial compartments and to act upstream of the PI3K/AKT signaling pathway^[39-40]. In LPS-induced macrophage models, AMP significantly reduces the production of pro-inflammatory mediators such as NO, tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6)^[36,41]. In models of renal injury, DMY mitigates cisplatin-induced nephrotoxicity by reducing serum levels of renal function markers, including blood UN and creatinine, enhancing the activity of antioxidant enzyme systems, and attenuating inflammatory responses through inhibition of both the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome and the nuclear factor kappa-B (NF- κ B) signaling pathway. These actions collectively protect the kidney from oxidative damage^[42]. These findings highlight the considerable anti-inflammatory potential of DMY, mediated by its ability to inhibit the activation of the NLRP3 inflammasome and NF- κ B pathway, as well as to downregulate the expression of multiple pro-inflammatory cytokines. These findings suggest that the antioxidant and anti-inflammatory actions of AMP and DMY may synergistically support renal health, thereby contributing indirectly to the amelioration of HUA-related kidney damage.

Further research on AMP should emphasize the terroir and processing technologies that influence the composition and stability of functional ingredients. Strict quality control standards are essential, particularly with regard to quantifying key bioactive compounds such as DMY. Such standards would ensure the consistency and safety of AMP-based products, thereby improving their reliability and efficacy in practical applications.

5. Conclusion

This study systematically elucidated the bioactivity and underlying mechanisms of AMP and its principal flavonoid component, DMY, in the intervention of HUA. Using an *in vitro* UA-induced injury model and a high-purine diet-induced mouse model, AMP was found to significantly reduce serum levels of UA, creatinine, and UN levels, alleviate renal pathological damage, and improve UA metabolism through a multifaceted mechanism involving the inhibition of XOD activity and expression, as well as the regulation of key urate transporters such as *URAT1*, *GLUT9*, and *ABCG2*. Phytochemical analysis revealed that DMY is the predominant flavonoid in AMP, accounting for 52.97% of the total flavonoid content. Further investigations confirmed that DMY exerts significant XOD-inhibitory and renoprotective effects at both cellular and animal levels. Its urate-lowering mechanism involves multi-target regulation of UA production, transport, and excretion. Specifically, DMY suppresses XOD expression in the liver and intestine to reduce UA synthesis; concurrently, it downregulates *URAT1* and *GLUT9* expression in the kidney and intestine to inhibit UA reabsorption, while upregulating *OAT3* and *ABCG2* expression to enhance UA excretion. In conclusion, DMY is identified as the key active compound responsible for the anti-hyperuricemic effects of AMP. This study not only reveals the molecular basis by which DMY modulates purine metabolism and maintains UA homeostasis but also provides theoretical support and practical guidance for the development of natural urate-lowering functional ingredients.

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Conflicts of interest

The authors declare no conflict of interest.

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