Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2023**; 28(9): 228
https://doi.org/10.31083/j.fbl2809228

Original Research

Honey Mushroom, *Armillaria mellea* (Agaricomycetes) and Its Fermentation Products Target Regulation of OAT1/OAT3 Proteins to Reduce Hyperuricemia in Mice

Zheng-Long Li[1], Shu-Min Wang[1,*], Huan Wang[1,2,*]

[1]Institute of College of Pharmacy, Changchun University of Traditional Chinese Medicine, 130117 Changchun, Jilin, China
[2]Ginseng Research Institute, Changchun University of Traditional Chinese Medicine, 130117 Changchun, Jilin, China
*Correspondence: wangsm@ccucm.edu.cn (Shu-Min Wang); wanghuanmyco@163.com (Huan Wang)

Academic Editor: Marcello Iriti
Submitted: 12 December 2022 Revised: 28 February 2023 Accepted: 14 March 2023 Published: 27 September 2023

Abstract

Background: Disorders of purine metabolism are the main cause of hyperuricemia. Current drugs for the treatment of hyperuricemia usually cause a degree of cardiovascular damage. **Methods**: This study aimed to investigate the therapeutic effects of *Armillaria mellea* fruiting body (AFB), *Armillaria* rhizomorph (AR) and *Armillaria mellea* fermentation product (after rhizomorphs removal) (AFP) on hyperuricemic mice. The hyperuricemia mouse model was established by oral administration of potassium oxonate 0.9 g·kg^{-1} and hypoxanthine 0.5 g·kg^{-1} for two weeks. Starting from the third week, the intragastric administration of the intervention drug group was as follows: Allopurinol 0.013 g·kg^{-1}, AFB (3.9 and 7.8 g·kg^{-1}), AR (3.9 and 7.8 g·kg^{-1}), AFP (1.95 and 3.9 g·kg^{-1}) once daily for 14 days. **Results**: Results showed that AFB, AR, and AFP reduced the contents of serum uric acid, serum creatinine, and blood urea nitrogen in hyperuricemic mice and the mechanism of action might be through up-regulation of the expression levels of organic anion transporter 1/organic anion transporter 3 proteins in kidney tissue. AR and AFP both exhibited better uric acid-lowering effects than AFB, which may be due to the higher purine content of AFB. **Conclusions**: *Armillaria mellea* and its fermentation products can treat hyperuricemia by up-regulating OAT1 protein and OAT3 protein, reducing uric acid content in mice.

Keywords: medicinal mushrooms; *Armillaria mellea*; serum uric acid; transporter protein

1. Introduction

Disorders of purine metabolism cause hyperuricemia, a disease of recent concern [1]. Nationally, representative National Health and Nutrition Examination Survey data showed the prevalence of hyperuricemia in the United States was 20.2% for men and 20.0% for women in 2015–2016 [2]. A survey showed the co-prevalence of hyperuricemia was 13.3% on the Chinese mainland during 2000–2014 [3]. Hyperuricemia is associated with cardiovascular disease [4], kidney damage [5], diabetes [6,7], hypertension [8], dyslipidemia [9], rheumatism, and other diseases [10].

Uric acid is a product of the purine metabolic pathway. Uric acid is deposited in the form of sodium salts in joints, soft tissues, cartilage, and kidneys, resulting in elevated uric acid levels in the body that gradually evolves into hyperuricemia and other diseases when the human purine metabolism is disordered and serum uric acid (SUA) levels rise sharply. Research has shown that uric acid in the renal interstitium enters tubular epithelial cells in a retro-electrochemical gradient through organic anion transporter 1 (OAT1) and organic anion transporter 3 (OAT3) in the basement membrane of epithelial cells, while intracellular ketoglutaric acid is excreted into the renal interstitium, thereby promoting the reabsorption of uric acid and reducing uric acid levels [11,12]. Current therapeutic drugs and natural alternatives for hyperuricemia have a SUA-lowering effect, but their long-term use has adverse effects on the cardiovascular system and other diseases [13]. Thus, it is necessary to develop drugs that have fewer side effects during the stabilization and effective reduction of uric acid. A number of natural alternatives help treat hyperuricemia (Table 1) [14–17]. Honey mushroom, *Armillaria mellea* (Vahl.) P. Kummer (Physalacriaceae, Agaricomycetes) is widely distributed in tropical and temperate forests and is a representative edible and medicinal fungi in China [18,19]. Modern pharmacological studies have demonstrated that *Armillaria mellea* ameliorates insulin resistance and diabetes-induced kidney damage [20,21], reduces Lipopolysaccharides (LPS) stimulated glial cell inflammation in BV-2 mice [22], is an antioxidant [23,24], anticancer [25,26], relieves depression [27,28], improves sleep [29], and other effects, but a high consumption leads to gastric upset [30]. Evidence suggests that aqueous extracts of *Armillaria mellea* fruiting body (AFB) have a degree of uric acid-lowering properties [31].

Therefore, this study targets the OAT1/OAT3 membrane channel protein to explore the protective effects of *Armillaria mellea* and its fermentation products on mice with hyperuricemia induced by oral potassium oxonate (PO) and hypoxanthine (HX). HPLC was simultaneously employed to determine the purine content of AFB, *Armil-*

Table 1. Natural substitutes and treatment methods.

Natural alternatives	Therapeutic method
Inonotus obliquus (Chaga)	Triterpenoid acid from *Inonotus obliquus* reduces SUA level by inhibiting XOD activity
G. applanatum	*Ganoderma applanatum* reduces SUA by up-regulating OAT1 protein and down-regulating GLUT9 protein expression
Agrocybe aegerita	*Agrocybe aegerita* inhibits XOD activity, increases OAT1 protein expression and reduces uric acid content
Caffeic acid phenethyl ester	Caffeic acid phenethyl ester in dietary plants reduces uric acid by inhibiting XOD and up-regulating OAT3 protein expression
Anthocyanins	Anthocyanins reduces uric acid by regulating the expression of renal urate transport proteins OAT1 and OAT3

SUA, serum uric acid; XOD, xanthine oxidase; OAT1, organic anion transporter 1; OAT3, organic anion transporter 3; G. applanatum, Ganoderma applanatum; GLUT9, glucose transporter 9.

laria mellea rhizomorph (AR), and *Armillaria mellea* fermentation product (AFP).

2. Materials and Methods

2.1 Materials and Reagents

Potassium oxonate (PO, 98%) was obtained from Macleans Biotechnology Corporation (Shanghai, China), HX (98.5%) was provided by Yuanye Biotechnology Co., Ltd. (Shanghai, China), and allopurinol (99.5%) supplied by Hefei Jiulian Pharmaceutical Co., Ltd (Anhui, China). SUA, serum creatinine (SCr), blood urea nitrogen (BUN) kits were provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Mouse anti-OAT1 and OAT3 were purchased from Affinity (San Francisco, CA, USA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) from Cell Signaling Technology Inc. (Beverly, MA, USA). All other reagents used in the experiment were analytically pure. Phenylmethanesulfonyl fluoride (PMSF) and radio immunoprecipitation assay were obtained from Beyotime Biotechnology (Shanghai, China). *Armillaria mellea* strains were high-quality *Armillaria mellea* (Vahl) P. Kumm. provided by Xingtianjian Pharmaceutical Group Company (Benxi, China).

2.2 Preparation of Sample

Preparation of *Armillaria mellea* strains. The liquid shaker flask culture medium was transferred from the *Armillaria mellea* slant strain, placed in a constant temperature shaking incubator, 28 °C, 150 r/min culture for 15 days and subcultured 1–2 times for seven days each time to obtain stable liquid culture strains. The above liquid culture medium (Glucose 2%, Yeast powder 1%, KH_2PO_4 0.15%, $MgSO_4$ 0.075%, VB 10.01%) was incubated as a seed medium in 1 L culture flasks with 5% of the inoculum amount. The fermentation product (rhizomorphs and fermentation broth) was obtained after 40 days of incubation at constant temperature and away from light.

Considering the convenience of oral administration and avoiding secondary injury to mice caused by intraperitoneal injection, oral administration was chosen. Refer to the usage and dosage of *Armillaria mellea* in the Encyclopedia of Chinese Medicinal Materials, take 30–60 g of decoction or grind it orally, such that 60 g is the high dose and 30 g is the low dose. The dose for mice was converted according to a body weight of 70 kg and its equivalent dose ratio was 0.0026 (converted according to the body surface area). AFB: soaked 78.0 g fruiting body in water for 30 min and decocted twice for 60 min each time, combine the two filtrates and concentrate to 100 mL. AR: Filter the rhizomorphs, dry at 65 °C at a constant temperature, pulverized, soaked 78.0 g rhizomorphs in water for 30 min and decocted twice for 60 min each time, combine the two filtrates and concentrate to 100 mL. AFP: the fermentation product (after rhizomorphs removal) was dried at a constant temperature of 105 °C, pulverized, and 39.0 g of the fermentation product powder was taken and dissolved in 100 mL of normal saline.

Preparation of purine reference substance: Precisely weigh an amount of HX, xanthine and adenine reference substance, add water, and dissolve in a 10 mL volumetric flask to prepare HX (0.526 mg·mL^{-1}), xanthine (0.732 mg·mL^{-1}) and adenine (0.382 mg·mL^{-1}) reference solution (if HX is insoluble in water add a trace amount of 0.1 moL·L^{-1} NaOH). Preparation of purine test sample: Weigh 1.0 g of fruiting body and rhizomorphs, respectively, add 10 mL of distilled water, ultrasonically extract for 90 min (80 kW), centrifuge at 5000 rpm for 10 minutes, take the supernatant and dilute to 10 mL.

2.3 Chromatographic Conditions

Waters XSelect HSS T3 column (4.6 mm × 250 mm, 5 µm); Mobile phase: acetonitrile (A)-water (B), gradient elution (0–15 min, 99% B; 15–25 min, 99% B-99% B; 25–30 min, 99% B-90% B; 30–37 min, 90% B-5% B; 37–38 min, 5% B-5% B; 38–48 min, 5% B-99% B). Flow rate: 1.0 mL·min^{-1}; column temperature: 35 °C; injection volume: 10 µL. The detection wavelength of three purine components was 254 nm; the theoretical plate number should not be less than 1000 calculated according to the peaks of HX, xanthine and adenine.

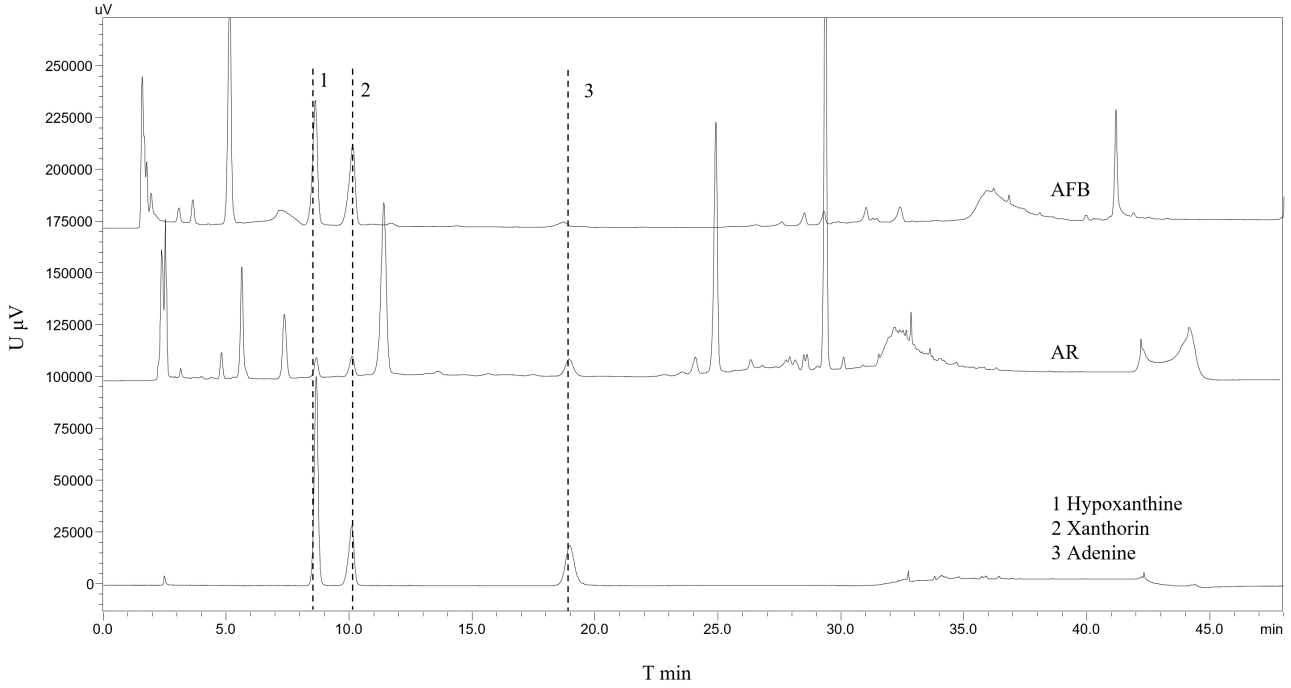

Fig. 1. Liquid Chromatogram of Samples and Standards. 1: hypoxanthine; 2: xanthine; 3: adenine.

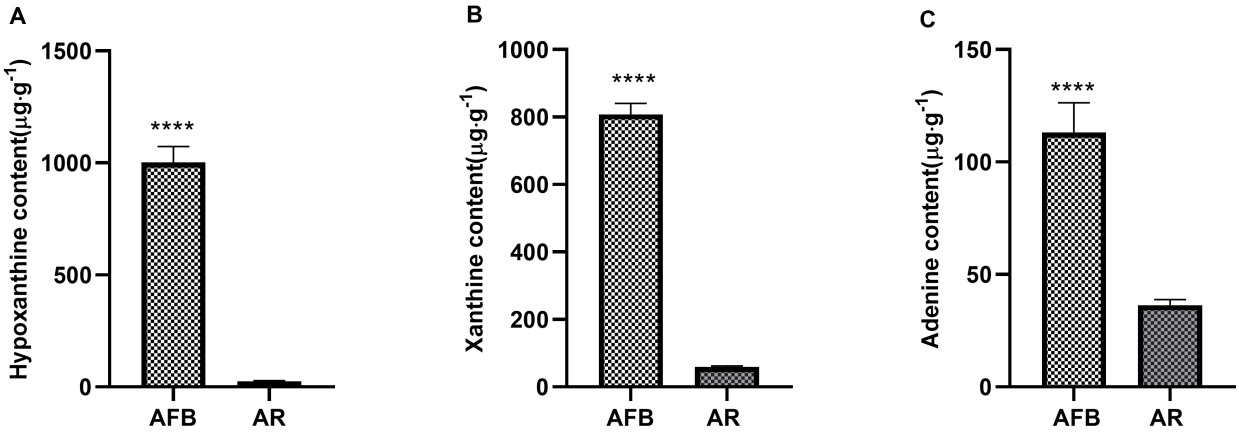

Fig. 2. Comparative data of AFB and AR in hypoxanthine, xanthine, and adenine content. A: hypoxanthine content; B: xanthine content; C: adenine content. Data expressed as mean ± SD (n = 6). $^{****}p < 0.0001$, versus the AR group. AFB, *Armillaria mellea* fruiting body; AR, *Armillaria rhizomorph*.

2.4 Animals and Experimental Groups

90 healthy male C57 mice (SPF, weighing 18 ± 2 g) were obtained from Liaoning Changsheng Biotechnology Co., Ltd., animal experiments were conducted by the Animal Experiment Center of Changchun University of Traditional Chinese Medicine. The experimental animals freely ate and drank water (air temperature 23 ± 1 °C, relative humidity 55 ± 5%, 12 h light-dark cycle). The experimental program was approved by the Experimental Animal Ethics Committee of Changchun University of Traditional Chinese Medicine and strictly complied with the regulations on the use of experimental animals and the provisions of the China Animal Welfare Law.

The 90 mice were divided into nine each groups containing 10 mice. After one week of adaptation an animal model of hyperuricemia were established in the mice by intragastric administration of PO (0.9 $g \cdot kg^{-1}$) and HX (0.5 $g \cdot kg^{-1}$) for two weeks. The specific drug administration regimens were as follows. (a) Control group: Intragastric administration of an equal volume of normal saline. (b) Model group: Intragastric administration of an equal volume of normal saline. (c) Allopurinol group: Intragastric administration of 0.013 $g \cdot kg^{-1}$ allopurinol. (d) AFB (3.9 $g \cdot kg^{-1}$) group: Intragastric administration of 3.9 $g \cdot kg^{-1}$ AFB. (e) AFB (7.8 $g \cdot kg^{-1}$) group: Intragastric administration of 7.8 $g \cdot kg^{-1}$ AFB. (f) AR (3.9 $g \cdot kg^{-1}$) group: In-

tragastric administration of 3.9 g·kg^{-1} AR. (g) AR (7.8 g·kg^{-1}) group: Intragastric administration of 7.8 g·kg^{-1} AR. (h) AFP (1.95 g·kg^{-1}) group: Intragastric administration of 1.95 g·kg^{-1} AFP. (i) AFP (3.9 g·kg^{-1}) group: Intragastric administration of 3.9 g·kg^{-1} AFP. The foregoing groups were administered daily for two weeks.

2.5 Determination of Pharmacological Parameters

The serum obtained from mouse whole blood was centrifuged at 3000 rpm for 10 min at 4 °C. SUA, SCr, and BUN in mice serum were determined by commercially available kits.

2.6 Histopathological Examination

Stomach tissue was fixed with 4% paraformaldehyde solution and embedded in paraffin, cut at a thickness of 5 µm, and stained with hematoxylin and eosin (H&E). Histopathological changes of the stomach were then observed by light microscope.

2.7 Western Blot Analysis of Kidney Tissue

Following addition of 500 µL precooled protein lysate (containing PMSF) to 100 mg of kidney tissue, samples were homogenized and incubated on ice for 30 min. The protein concentration of the tissue supernatant was determined by Bicinchoninic Acid Assay (BCA) after centrifugation at 3500 rpm for 10 minutes at 4 °C. Electrophoresis was performed with equal amounts of total protein for sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE), then transferred to nitrocellulose membranes. Non-specific binding was prevented by incubating with 5% skimmed milk powder at room temperature for two hours. The blot was incubated with primary antibody (anti-OAT1, anti-OAT3, and anti-GAPDH) overnight at 4 °C, then incubated with the secondary antibody plus fluorescent markers for two hours at room temperature. A chemiluminescent detection system was used to detect bands and optical density analysis with ImageJ software (ImageJ 1.48v Jave1.6.0-20(64-bit), National Institutes of Health, USA) was used to measure band intensity. The ratio of OAT1 and OAT3 to the corresponding control GAPDH was calculated for each sample.

2.8 Immunohistochemical Analysis of Kidney Tissue

Part of the gastric tissue was fixed with 4% paraformaldehyde solution, embedded in paraffin, and sectioned into 5 µm slices. Phosphate buffered saline with Tween (PBST) washed 3 times, 3% hydrogen peroxide room temperature was closed for 20 min, PBST washed 3 times, 5% bovine serum albumin was closed at room temperature for 1 hour, primary antibody incubation overnight at 4 °C. Samples were incubated with PBST and horse radish peroxidase-labeled secondary antibody at room temperature for 45 min, PBS washed three times, the diaminobenzidine was color developed, stained under the microscope, and washed with deionized water. Hematoxyfolk was counterstained, dehydrated after transparency, and sealed with neutral gum.

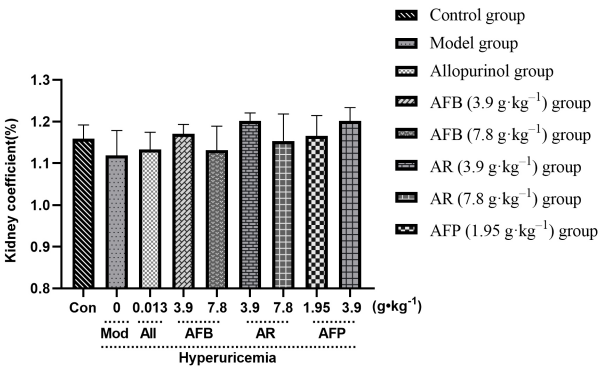

Fig. 3. Effect of *Armillaria mellea* and its fermentation products on the kidney organ coefficient of mice. Data expressed as mean ± SD (n = 6). Con, control group; Mod, model group; All, allopurinol group; AFP, *Armillaria mellea* fermentation product.

2.9 Statistical Analysis

Data were processed with the aid of Graph Pad Prism 8.3 (Graph Pad Software Inc., San Diego, CA, USA), the difference between groups was analyzed by one-way ANOVA, and purine content was analyzed by paired t-test. Experimental data were expressed as mean ± SEM, and $p < 0.05$ suggested significant statistical difference.

3. Results

3.1 Purine Content

The chromatogram of sample and control products are given (Fig. 1) and show that HX, xanthine, and adenine in both AFB and AR exhibit a good chromatographic peak shape and that AFB contains more purine components. The content of three purines was too low and did not reach the detection limit in AFP. Analytic results show that the density of HX, xanthine, and adenine in AFB were 1001.87, 807.87, and 113.12 µg·g^{-1}, respectively, and that the sum of the three common purines was 2 mg·g^{-1} (Fig. 2). The contents of HX, xanthine, and adenine in AR were 25.81, 59.09, 36.34 µg·g^{-1}, respectively, with the sum of the three common purines 120 µg·g^{-1}.

3.2 Kidney Coefficient

There was no significant difference in the kidney coefficient (bilateral kidney weight (mg)/body weight (g), Fig. 3) indicating that *Armillaria mellea* and their fermentation products had no effect on the kidney.

3.3 Histopathological Section

Histopathological results showed (Fig. 4) that the surface envelope of kidney tissue in the control group was

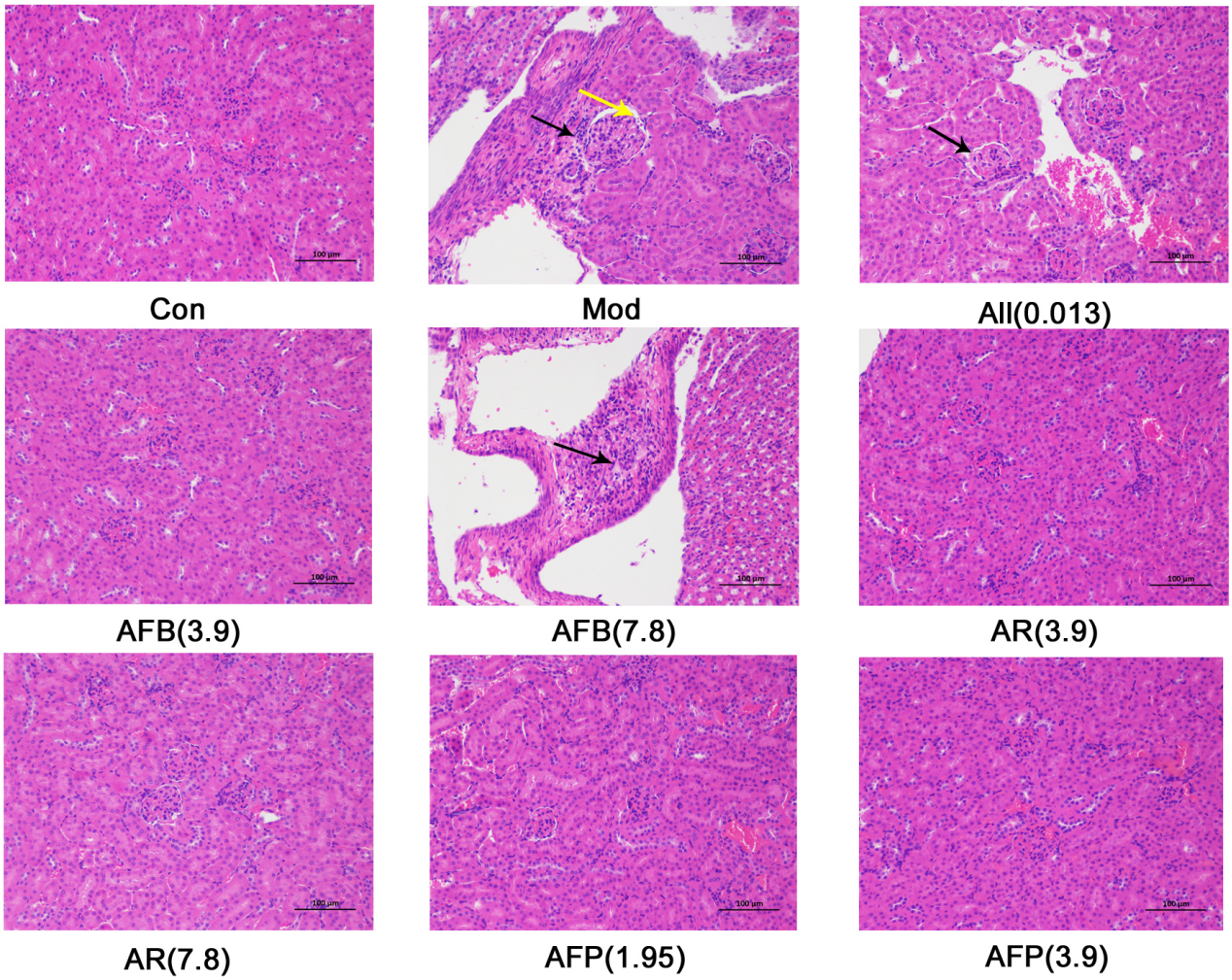

Fig. 4. Effect of *Armillaria mellea* and its fermentation products on kidney tissue sections in mice kidney. Histopathological changes are shown following H&E staining (magnification, ×200).

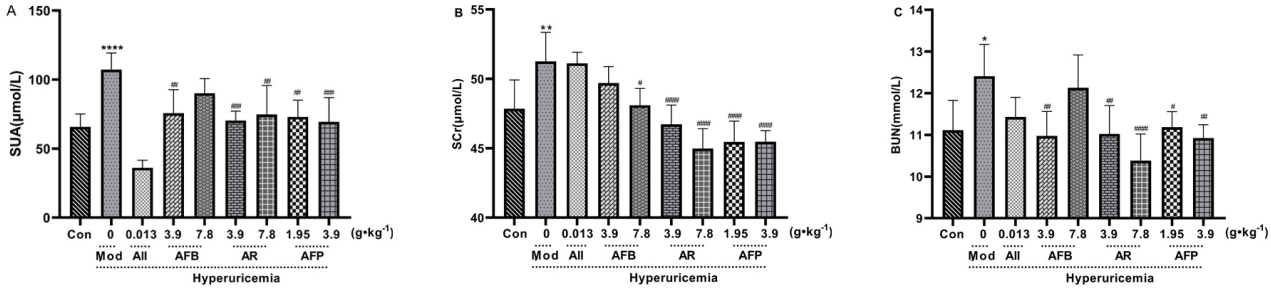

Fig. 5. Effect of *Armillaria mellea* and its fermentation products on SUA, BUN, and SCr. A: SUN content; B: SCr content; C: BUN content. Data are expressed as mean ± SD ($n = 6$). $^{\#\#\#\#}p < 0.0001$, $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ versus the model group. $^{****}p < 0.0001$, $^{**}p < 0.01$, $^{*}p < 0.05$ versus the control group. SUA, serum uric acid; SCr, serum creatinine; BUN, blood urea nitrogen.

composed of dense connective tissue with uniform thickness. No obvious abnormalities were found in the kidney parenchyma, neither was there significant hyperplasia of the kidney interstitium, nor obvious inflammatory changes. Compared with the control group, punctate infiltration of lymphocytes (black arrow) and eosinophilic substances (yellow arrow) were seen in the kidney capsule in the model group. After administration, exception for a small amount of eosinophils in the AFB (7.8 g·kg^{-1}) and allopurinol groups, there were no obvious lesions in the renal tissue sections of any other group.

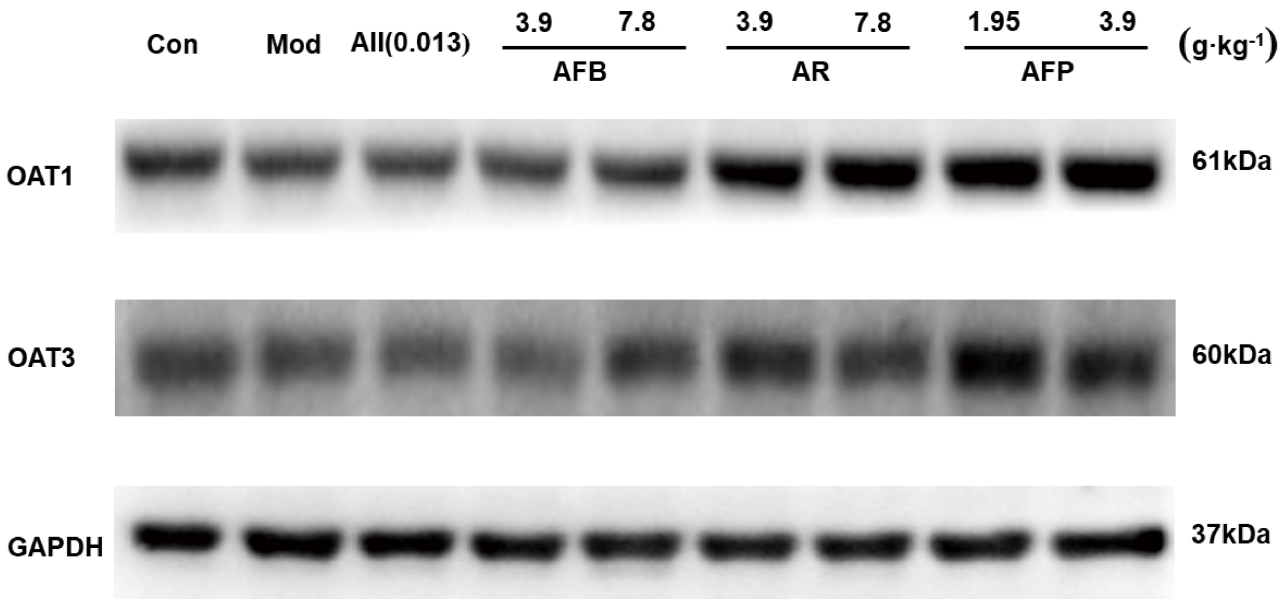

Fig. 6. OAT1/OAT3 protein expression results.

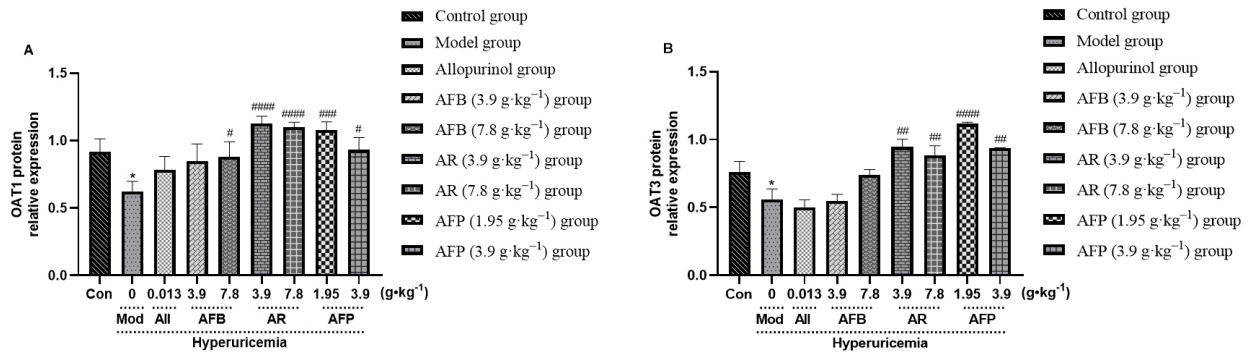

Fig. 7. *Armillaria mellea* and its fermentation products increase the activation of OAT1 and OAT3 in kidney tissue. A: OAT1 protein content; B: OAT3 protein content. Data expressed as mean ± SD (n = 6). $^{\#\#\#\#}p < 0.0001$, $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ versus the model group. $^{*}p < 0.05$ versus the control group.

3.4 Biochemical Index Detection

Compared with the control group, the serum levels of SUA, SCr, and BUN in the model group were significantly improved ($p < 0.05$, $p < 0.01$ or $p < 0.0001$, Fig. 5). The allopurinol group had only a significantly reducing effect on SUA ($p < 0.0001$, Fig. 5). The AFB (3.9 g·kg^{-1}) group had a reduced effect on SUA and BUN ($p < 0.01$, Fig. 5A,C), while the AFB (7.8 g·kg^{-1}) group had a reducing effect on SCr ($p < 0.05$, Fig. 5B) when compared with the model group. The AR (3.9 and 7.8 g·kg^{-1}) groups and the AFP (1.95 and 3.9 g·kg^{-1}) groups significantly decreased the levels of serum SUA, SCr and BUN ($p < 0.05$, $p < 0.01$, $p < 0.001$ or $p <$0.0001, Fig. 5). The findings showed that AR and AFP had a certain protective effect on kidney function.

3.5 OAT1 and OAT3 Protein Content Detection

The OAT1/PAT3 protein results are shown in Fig. 6. The expression level of OAT1 and OAT3 proteins in the kidney tissue of the model group decreased significantly compared with the control group ($p < 0.05$, Fig. 7A,B). Compared with the model group, the allopurinol group had no significant effect on the protein content of OAT1 and OAT3. The AFB (7.8 g·kg^{-1}) group had a significant increase in effect on OAT1 ($p < 0.05$, Fig. 7A). Both AR (3.9 and 7.8 g·kg^{-1}) and AFP (1.95 and 3.9 g·kg^{-1}) groups significantly increased the protein content of OAT1 and OAT3 ($p < 0.05$, $p < 0.01$ or $p < 0.0001$, Fig. 7).

3.6 OAT1 Immunohistochemical Results

OAT1 protein levels were significantly reduced in the hyperuricemia model group ($p < 0.001$, Figs. 8, 9) when

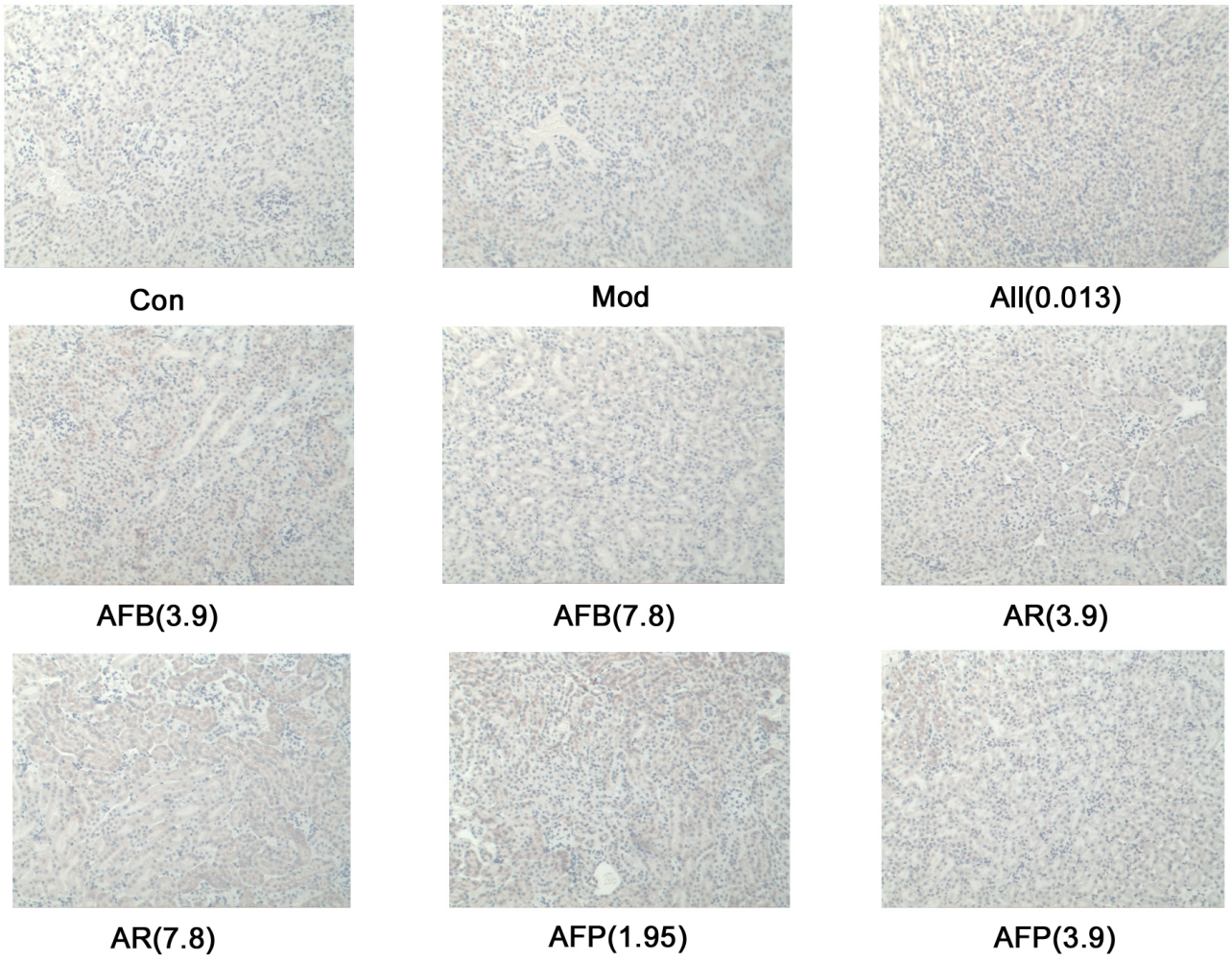

Fig. 8. OAT1 Immunohistochemical results.

compared to the control group. AFB (3.9 g·kg^{-1}) group significantly increased OAT1 protein content, the AR (3.9 and 7.8 g·kg^{-1}) and AFP (1.95 and 3.9 g·kg^{-1}) groups significantly increased OAT1 protein content in kidney tissue compared with the model group ($p < 0.0001$, Figs. 8, 9). The experimental results were found to be identical to Western blot results.

4. Discussion

There are two major types of hyperuricemia mouse model: genetically induced and environmentally induced. PO and HX are commonly used to establish hyperuricemia models to study hyperuricemia-related diseases. The definition of uric acid content for hyperuricemia is not the same in humans and mice, and urate concentrations in mice have not been specified. Following the experiment, the serum content of mice in the model group could reach the urate concentration standard of some mouse models [32], even in the case of reversible hyperuricemia [33]. This showed that the serum urate content of mice after modeling is greater than the serum urate content of mice after the experiment. In this experiment the model was used to study the uric acid-lowering effect of *Armillaria mellea* and its fermentation products. Results showed that AFB (3.9 g·kg^{-1}), AR (3.9 and 7.8 g·kg^{-1}) and AFP (1.95 and 3.9 g·kg^{-1}) enhanced uric acid excretion and decreased SUA *in vivo*, suggesting a significant uric acid-lowering effect. AR (3.9 g·kg^{-1}) had the greatest uric acid-lowering effect.

PO has traditionally been thought to seriously impair kidney function. SCr and BUN levels are commonly used indicators to assess kidney health. In this experiment, the model group increased the concentration of Scr, BUN, and damaged kidney tissue. The AR (3.9 and 7.8 g·kg^{-1}) and AFP (1.95 and 3.9 g·kg^{-1}) groups may reduce the infiltration of punctate lymphocytes and eosinophils in kidney tissue by reducing the contents of SCr and BUN, thus reducing kidney tissue damage. Compared with the AFB group, the AR and AFP groups have more comprehensive therapeutic effects, as the contents of SCr and BUN were simultaneously reduced.

Uric acid homeostasis primarily depends on an equilibrium between liver production, secretion, renal reabsorp-

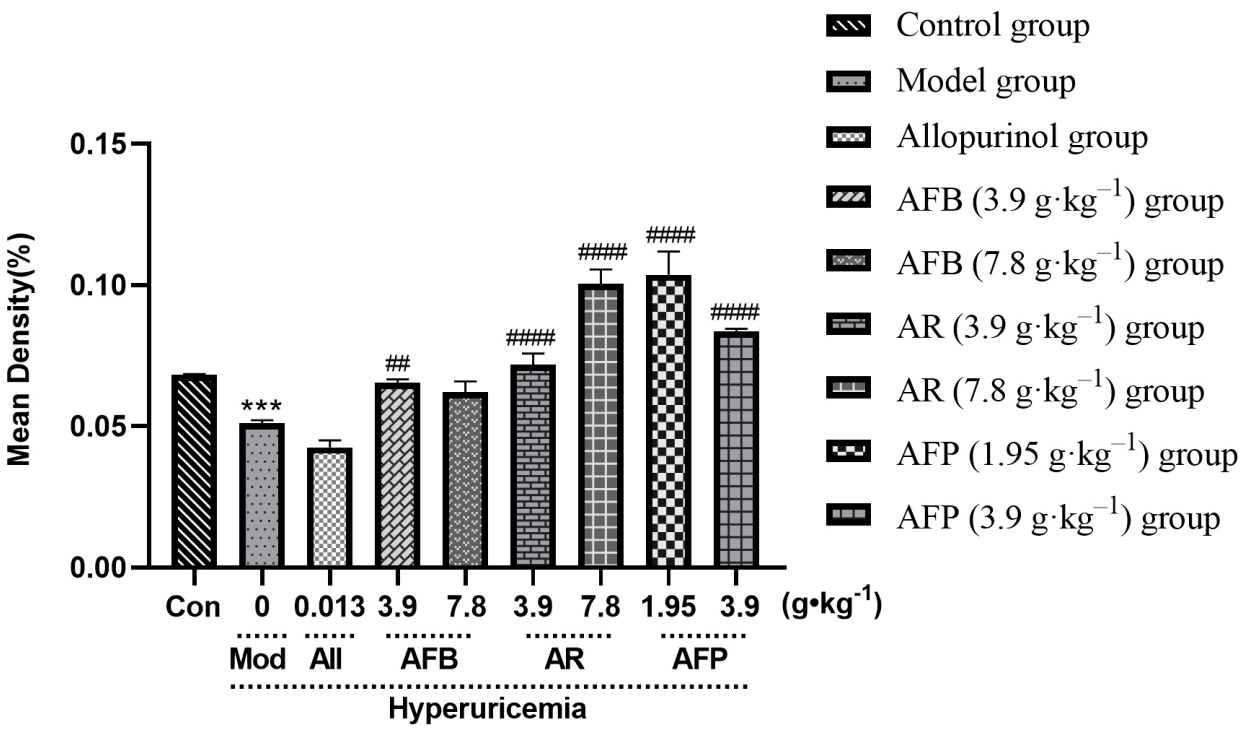

Fig. 9. Immunohistochemical analysis of *Armillaria mellea* and its fermentation products on mice OAT1 protein. Data expressed as mean ± SD (n = 3). $^{\#\#\#\#}p < 0.0001$, $^{\#\#}p < 0.01$ versus the model group. $^{***}p < 0.001$ versus the control group.

tion, and intestinal excretion, among them, the kidney reabsorbs about 90% of the filtered urate, so increased reabsorption is extremely important for urination drugs [34,35]. OAT1 was identified as a molecular transporter regulating the secretion of uric acid in the first step, which drives other OATs (OAT2, OAT3, OAT4) that together constitute an amphiphilic solute transporter family (Slc22a) within the major facilitator superfamily [36]. OAT3 is not only the most expressed protein in the kidney, but also has the same function of exchanging substrates as OAT1, which has great research significance. In terms of uric acid transport, the kidneys of humans are similar to those of mice. OAT1 and OAT3 are basolateral urate transporters encoded by SLC22A6 and SLC22A8, respectively, that are involved in urate secretion and urate conversion in the blood, which is transported to proximal tubule cells. This study showed that AR (3.9 and 7.8 g·kg^{-1}) and AFP (1.95 and 3.9 g·kg^{-1}) may promote uric acid metabolism and reduce SUA in mice by up-regulating OAT1/OAT3 protein expression. Based on the results of OAT1 protein immunohistochemistry, the AR (3.9 g·kg^{-1}) group showed a better ability to enhance the level of OAT1 protein.

Yong's [31] experimental results showed that both alcoholic and aqueous extracts of AFB reduced the uric acid levels of hyperuricemic mice by reducing xanthine oxidase activity, while both up-regulating the expression level of OAT1 and down-regulating the expression of GLUT9 in kidney tissue and CNT2 in the gastrointestinal tract. The current study confirms that AMW reduces uric acid levels and shows that AFB also reduces uric acid levels in hyperuricemic mice by increasing the expression of OAT1 protein in kidney tissue, but that the activity of AFB is far less than that of AR and AFP. Increased HX content leads to increased SUA. When combined with the purine content experiment results, it was revealed that AFB contained much more purine than AR and AFP. Therefore, the down-regulation effect of uric acid on hyperuricemia mice is weaker than AR and AFP. Additionally, it shows that the fermentation products AR and AFP of *Armillaria mellea* are more effective and more meaningful in treating hyperuricemia. *Armillaria mellea* and is often used for research into the state of fruiting bodies [37]. Nevertheless, in this study, it was found that the fermentation products of *Armillaria mellea* have better effect in treating hyperuricemia, which suggests that the fermentation products of *Armillaria mellea* also have research significance.

5. Conclusions

Armillaria mellea and its fermentation products can treat hyperuricemia by up-regulating OAT1 protein and OAT3 protein, reducing the content of uric acid, creatinine and urea nitrogen in mice, and the fermentation products have better therapeutic effect.

Abbreviations

AFB, *Armillaria mellea* fruiting body; AFP, *Armillaria mellea* fermentation product (after rhizomorphs removal); AR, *Armillaria mellea* rhizomorphs; BUN, blood urea nitrogen; GLUT9, glucose transporter 9; H&E, hematoxylin-eosin staining; HPLC, High Performance Liquid Chromatography; HX, hypoxanthine; IHC, Immunohistochemistry; OAT1, organic anion transporter 1; OAT3, organic anion transporter 3; PO, potassium oxonate; SCr, serum creatinine; SUA, serum uric acid; WB, Western blot.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

ZL and SW conceived the study, designed the experiments, supervised all the research, analyzed the data, and revised the manuscript. HW and ZL completed the experiments and analyzed the data. ZL wrote the manuscript. ZL carried out the experiments. All authors have read and agreed to the published version of the manuscript.

Ethics Approval and Consent to Participate

All procedures and experimental methods were approved by the Animal Ethics Committee of Changchun University of Traditional Chinese Medicine (Certificate No.: 2021196).

Acknowledgment

De Hu helped the writing of the manuscript.

Funding

This research was partially supported by the Traditional Chinese Medicine Science and Technology Project of Jilin Province (2021006), the Jilin Province Science and Technology Development Plan Project Technology Innovation Guidance-Medical and Health Industry Development Special (YDZJ202301ZYTS509), and the "Xinglin Scholars Project" Young Scientist Program of Changchun University of Traditional Chinese Medicine (QNKXJ2-2021ZR03).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Lima WG, Martins-Santos MES, Chaves VE. Uric acid as a modulator of glucose and lipid metabolism. Biochimie. 2015; 116: 17–23.

[2] Chen-Xu M, Yokose C, Rai SK, Pillinger MH, Choi HK. Contemporary Prevalence of Gout and Hyperuricemia in the United States and Decadal Trends: The National Health and Nutrition Examination Survey, 2007-2016. Arthritis & Rheumatology. 2019; 71: 991–999.

[3] Liu R, Han C, Wu D, Xia X, Gu J, Guan H, *et al*. Prevalence of Hyperuricemia and Gout in Mainland China from 2000 to 2014: A Systematic Review and Meta-Analysis. BioMed Research International. 2015; 2015: 762820.

[4] Zhang S, Wang Y, Cheng J, Huangfu N, Zhao R, Xu Z, *et al*. Hyperuricemia and Cardiovascular Disease. Current Pharmaceutical Design. 2019; 25: 700–709.

[5] Kostka-Jeziorny K, Widecka K, Tykarski A. Study of epidemiological aspects of hyperuricemia in Poland. Cardiology Journal. 2019; 26: 241–252.

[6] Song SH, Oh TR, Choi HS, Kim CS, Ryu DR, Kim SG, *et al*. Hyperuricemia is a risk factor for the progression to end-stage renal disease in minimal change disease. Kidney Research and Clinical Practice. 2021; 40: 411–418.

[7] Wang H, Zhang H, Sun L, Guo W. Roles of hyperuricemia in metabolic syndrome and cardiac-kidney-vascular system diseases. American Journal of Translational Research. 2018; 10: 2749–2763.

[8] Lee TH, Chen JJ, Wu CY, Yang CW, Yang HY. Hyperuricemia and Progression of Chronic Kidney Disease: A Review from Physiology and Pathogenesis to the Role of Urate-Lowering Therapy. Diagnostics. 2021; 11: 1674.

[9] Tatsumi Y, Asayama K, Morimoto A, Satoh M, Sonoda N, Miyamatsu N, *et al*. Hyperuricemia predicts the risk for developing hypertension independent of alcohol drinking status in men and women: the Saku study. Hypertension Research. 2020; 43: 442–449.

[10] Stewart DJ, Langlois V, Noone D. Hyperuricemia and Hypertension: Links and Risks. Integrated Blood Pressure Control. 2019; 12: 43–62.

[11] McHugh J. Hyperuricaemia or gout in patients with RA. Nature Reviews. Rheumatology. 2019; 15: 384.

[12] Mandal AK, Mount DB. The molecular physiology of uric acid homeostasis. Annual Review of Physiology. 2015; 77: 323–345.

[13] Luo LS, Wang Y, Dai LJ, He FX, Zhang JL, Zhou Q. Triterpenoid acids from medicinal mushroom Inonotus obliquus (Chaga) alleviate hyperuricemia and inflammation in hyperuricemic mice: Possible inhibitory effects on xanthine oxidase activity. Journal of Food Biochemistry. 2022; 46: e13932.

[14] Yong T, Chen S, Xie Y, Chen D, Su J, Shuai O, *et al*. Hypouricemic Effects of *Ganoderma applanatum* in Hyperuricemia Mice through OAT1 and GLUT9. Frontiers in Pharmacology. 2018; 8: 996.

[15] Yong T, Chen S, Xie Y, Shuai O, Li X, Chen D, *et al*. Hypouricemic Effects of Extracts From *Agrocybe aegerita* on Hyperuricemia Mice and Virtual Prediction of Bioactives by Molecular Docking. Frontiers in Pharmacology. 2018; 9: 498.

[16] Yong T, Liang D, Chen S, Xiao C, Gao X, Wu Q, *et al*. Caffeic acid phenethyl ester alleviated hypouricemia in hyperuricemic mice through inhibiting XOD and up-regulating OAT3. Phytomedicine. 2022; 103: 154256.

[17] Qian X, Wang X, Luo J, Liu Y, Pang J, Zhang H, *et al*. Hypouricemic and nephroprotective roles of anthocyanins in hyperuricemic mice. Food & Function. 2019; 10: 867–878.

[18] Sipos G, Anderson JB, Nagy LG. Armillaria. Current Biology. 2018; 28: R297–R298.

[19] Qin GF, Zhao J, Korhonen K. A study on intersterility groups of Armillaria in China. Mycologia. 2007; 99: 430–441.

[20] Yang R, Li Y, Cai J, Ji J, Wang Y, Zhang W, *et al*. Polysaccharides from *Armillariella tabescens* mycelia ameliorate insulin resistance in type 2 diabetic mice. Food & Function. 2020; 11: 9675–9685.

[21] Yang R, Li Y, Mehmood S, Yan C, Huang Y, Cai J, *et al*. Polysaccharides from Armillariella tabescens mycelia amelio-

rate renal damage in type 2 diabetic mice. International Journal of Biological Macromolecules. 2020; 162: 1682–1691.

[22] Yang S, Yan J, Yang L, Meng Y, Wang N, He C, *et al*. Alkali-soluble polysaccharides from mushroom fruiting bodies improve insulin resistance. International Journal of Biological Macromolecules. 2019; 126: 466–474.

[23] An S, Lu W, Zhang Y, Yuan Q, Wang D. Pharmacological Basis for Use of *Armillaria mellea* Polysaccharides in Alzheimer's Disease: Antiapoptosis and Antioxidation. Oxidative Medicine and Cellular Longevity. 2017; 2017: 4184562.

[24] Erbiai EH, da Silva LP, Saidi R, Lamrani Z, Esteves da Silva JCG, Maouni A. Chemical Composition, Bioactive Compounds, and Antioxidant Activity of Two Wild Edible Mushrooms *Armillaria mellea* and *Macrolepiota procera* from Two Countries (Morocco and Portugal). Biomolecules. 2021; 11: 575.

[25] Bohnert M, Scherer O, Wiechmann K, König S, Dahse HM, Hoffmeister D, *et al*. Melleolides induce rapid cell death in human primary monocytes and cancer cells. Bioorganic & Medicinal Chemistry. 2014; 22: 3856–3861.

[26] Chi CW, Chen CC, Chen YJ. Therapeutic and radiosensitizing effects of armillaridin on human esophageal cancer cells. Evidence-Based Complementary and Alternative Medicine. 2013; 2013: 459271.

[27] Zhang T, Du Y, Liu X, Sun X, Cai E, Zhu H, *et al*. Study on antidepressant-like effect of protoilludane sesquiterpenoid aromatic esters from *Armillaria Mellea*. Natural Product Research. 2021; 35: 1042–1045.

[28] Lin YE, Wang HL, Lu KH, Huang YJ, Panyod S, Liu WT, *et al*. Water extract of Armillaria mellea (Vahl) P. Kumm. Alleviates the depression-like behaviors in acute- and chronic mild stress-induced rodent models via anti-inflammatory action. Journal of Ethnopharmacology. 2021; 265: 113395.

[29] Li IC, Lin TW, Lee TY, Lo Y, Jiang YM, Kuo YH, *et al*. Oral Administration of *Armillaria mellea* Mycelia Promotes Non-Rapid Eye Movement and Rapid Eye Movement Sleep in Rats. Journal of Fungi. 2021; 7: 371.

[30] Muszyńska B, Sułkowska-Ziaja K, Wołkowska M, Ekiert H. Chemical, pharmacological, and biological characterization of the culinary-medicinal honey mushroom, Armillaria mellea (Vahl) P. Kumm. (Agaricomycetideae): a review. International Journal of Medicinal Mushrooms. 2011; 13: 167–175.

[31] Yong T, Chen S, Xie Y, Chen D, Su J, Shuai O, *et al*. Hypouricemic Effects of Armillaria mellea on Hyperuricemic Mice Regulated through OAT1 and CNT2. The American Journal of Chinese Medicine. 2018; 46: 585–599.

[32] Jakše B, Jakše B, Pajek M, Pajek J. Uric Acid and Plant-Based Nutrition. Nutrients. 2019; 11: 1736.

[33] Dhouibi R, Affes H, Salem MB, Moalla D, Marekchi R, Charfi S, *et al*. Creation of an adequate animal model of hyperuricemia (acute and chronic hyperuricemia); study of its reversibility and its maintenance. Life Sciences. 2021; 268: 118998.

[34] Ishikawa T, Aw W, Kaneko K. Metabolic Interactions of Purine Derivatives with Human ABC Transporter ABCG2: Genetic Testing to Assess Gout Risk. Pharmaceuticals. 2013; 6: 1347–1360.

[35] Bakhiya A, Bahn A, Burckhardt G, Wolff N. Human organic anion transporter 3 (hOAT3) can operate as an exchanger and mediate secretory urate flux. Cellular Physiology and Biochemistry. 2003; 13: 249–256.

[36] Sweet DH, Chan LMS, Walden R, Yang XP, Miller DS, Pritchard JB. Organic anion transporter 3 (Slc22a8) is a dicarboxylate exchanger indirectly coupled to the Na+ gradient. American Journal of Physiology. Renal Physiology. 2003; 284: F763–F769.

[37] Li Z, Wang Y, Jiang B, Li W, Zheng L, Yang X, *et al*. Structure, cytotoxic activity and mechanism of protoilludane sesquiterpene aryl esters from the mycelium of Armillaria mellea. Journal of Ethnopharmacology. 2016; 184: 119–127.