Potential role of tea drinking in preventing hyperuricemia in rats: biochemical and molecular evidence

Hui Li (LHCA@fudan.edu.cn)
Fudan University https://orcid.org/0000-0002-7642-215X
Siyao Sang
Fudan University School of Life Sciences
Lufei Wang
Fudan University School of Life Sciences
Taotao Liang
Henan Cancer Hospital
Mingjie Su
Fudan University School of Life Sciences

Research Article

Keywords: tea, hyperuricemia, uric acid, kidney injury, inflammation

Posted Date: March 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1460341/v1

Potential role of tea drinking in preventing hyperuricemia in rats: biochemical and molecular evidence

Siyao Sang[1], Lufei Wang[2,5], Taotao Liang[3], Mingjie Su[2,4], Hui Li[2,4,5*]

1. State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai 200438, China.

2. MOE Key Laboratory of Contemporary Anthropology, Human Phenome Institute, Fudan University, Shanghai 200438, China.

3. Department of Hematology, Affiliated Tumor Hospital of Zhengzhou University, Henan Cancer Hospital, Zhengzhou, 450008, China.

4. Human Phenome Institute, Fudan University, Shanghai 200438, China.

5. Shanxi Academy of Advanced Research and Innovation, Fudan-Datong Institute of Chinese Origin, Datong 037006, China.

*Corresponding author: Hui Li. E-mail: LHCA@fudan.edu.cn. (H.L.) Tel: +86-02131246787; Fax: +86-02131246787; Address: 2005 Songhu Road, Bio-E-601, Shanghai, 200438, China.

Full list of author information is available at the end of the article.

Abstract

Background: Lifestyle and diet play a significant role in hyperuricemia. Accumulating evidence indicates that tea consumption is associated with hyperuricemia and the risk of gout. However, diverse compounds in different types of tea make it quite difficult to find out the relevant molecular mechanism. Here, we compared the effects of six types of tea on hyperuricemia induced by potassium oxonate (PO) and hypoxanthine in rats and investigated possible underlying mechanisms.

Methods: Rats were randomly assigned into ten groups: control group, hyperuricemia model group, benzbromarone positive control group, traditional Chinese Medicine *Simiao San* positive control group, green tea, yellow tea, black tea, white tea, red tea, and cyan tea treatment groups. After 21 days, uric acid (UA), xanthine oxidase (XOD), blood urea nitrogen (BUN), and creatinine (CRE) were assessed. By enzyme-linked immunosorbent assay, serum levels of interleukin-1β. By hematoxylin-eosin staining and immunohistochemistry, liver and kidney injury were measured.

Results: The levels of UA, CRE, and BUN in the treatment group were decreased to extent degrees. There was a significant reduction of UA, CRE, BUN levels for yellow tea compared to the positive control drugs. Yellow tea suppressed the XOD activity to lessen hepatic and kidney injury. Network pharmacology and untargeted metabolomics indicated that ten yellow tea bioactive ingredients and 35 targets were responsible for preventing hyperuricemia mediated by 94 signaling pathways, such as IL-1β and TNF.

Conclusion: These findings indicate that green tea cannot reduce the serum uric acid level of hyperuricemic rats. Yellow tea can significantly improve hyperuricemia by

regulating inflammatory response, autophagy, and apoptosis. This study provides a potential candidate for the treatment of hyperuricemia and a basis for selecting tea for patients with hyperuricemia.

Keywords: tea; hyperuricemia; uric acid; kidney injury; inflammation

Background

Hyperuricemia and gout is a chronic disease of urate crystal deposition, accompanied by elevated serum uric acid concentration and monosodium urate precipitation in joints and other tissues[1]. Disorders of purine metabolism cause hyperuricemia and its prevalence are affected by multiple factors such as heredity, gender, age, lifestyle, dietary habits, drug treatment, and economic development[2, 3]. Recent epidemiological studies show that hyperuricemia may be involved in hypertension, diabetes, atherosclerosis, chronic kidney disease, atrial fibrillation (AF), and the occurrence of cardiovascular events[4-6]. The prevalence of hyperuricemia was 20.2% in men and 20.0% in women[7]. The prevalence of hyperuricemia increased with age, reaching its highest at 27.8% in people aged 80 years and older. With the change of people's dietary structure, the prevalence of hyperuricemia is increasing year by year. Over the past 5-10 years, much progress has been made in the pathophysiology and genetics of hyperuricemia and gout, but the quality of care remains a major challenge in gout management[8].

Uric acid is a metabolite of hypoxanthine and xanthine oxidized by xanthine oxidase (XOD) in the liver[9, 10]. It is filtered by the glomerulus, then reabsorbed by the renal

tubules, and finally excreted from the body through the bladder. The excessive production of uric acid in the purine metabolism in the liver or the reduction of the filtration effect of the kidney on it can lead to the deposition of urate crystals and hypoxanthine[4]. Hyperuricemia can induce diabetes, hypertension, myocardial infarction, and other diseases. There are many feasible drugs for treating gout. The first-line urate-lowering medications for chronic gout are xanthine oxidase inhibitors-allopurinol and febuxostat[11]. Colchicine is used both during acute episodes and chronic maintenance therapy[12]. Benzbromarone and probenecid treat hyperuricemia and gout by inhibiting renal reabsorption of uric acid[13]. The therapeutic effect of these drugs is not ideal and easy to cause damage to other organs, are resistant to long-term use, and have high medical costs. Given the potential impact of gout on cardiovascular and metabolic diseases, there is an urgent need to find safe and effective intervention treatments.

Tea, the leaf of *Camellia sinensis*, is the second most consumed beverage in the world after water and may be associated with hyperuricemia and gout risk. There are thousands of tea productions in the world. In 1979, Chinese tea scientists analyzed the productive procedures and chemical components of the teas and successfully divided them into six categories: green tea, cyan tea, red tea (commonly known as black tea), white tea, black tea (commonly known as dark tea), and yellow tea[14]. Over the past several decades, teas and their metabolites have been widely investigated for their wide-ranging beneficial health effects in preventing and alleviating resistance of metabolic disorders, cancer, oxidation, and inflammation. Research on the effects of tea on

hyperuricemia has focused on red and green tea[15, 16]. Some researchers have conflicting conclusions about the same tea. A recent study has shown that daily drinkers of green tea exhibited a dose-dependent and statistically significant four-fold increase with hyperuricemia[17]. Still, an earlier study has shown that green tea drinking did not lead to a statistically significant trend with serum uric acid[18]. Therefore, we explored the effect of tea drinking on hyperuricemia, especially in the liver and kidney. Then, we used Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) and network pharmacology system to identify and analyze tea's effective active ingredients and potential targets and explore their possible mechanisms of action.

Methods

Chemicals and Reagents

Hypoxanthine and Monosodium urate (MSU) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium oxonate (PO) was purchased from Adamas Reagent Co. Ltd. (Shanghai, China). Assay kits for serum UA, BUN, CRE, and XOD were purchased from Nanjing Jian Cheng Bioengineering Institute (Nanjing, China). Enzyme-linked immune sorbent assay (ELISA) kits of IL-1β were purchased from DAKEWE (Beijing, China). All antibodies were obtained from Abcam (Cambridge, UK).

Turquoise Pearls (green tea), Golden Bricks (yellow tea), Golden Fungi (black tea), Red Garment (cyan tea), Old Eyebrows (white tea), and Red Grape (red tea) were purchased from Bud-Chem Tea Co. Ltd. (Jiangkou, Guizhou, China).

Tea preparation

Added 3 g of green tea, cyan tea, and red tea leaves to 10 mL of water at 80℃, 95℃, and 99℃, respectively, and soaked for 2-5 minutes. Boiled 3 g of white tea, yellow tea, and black tea in 20 mL of water and simmered for 15-20 minutes, respectively. The detailed protocols for tea preparation have been described previously [19].

Animal study

Male Sprague-Dawley (SD) rats (body weight 200 ± 20 g) were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Certificate No.: 20170005059225). The rats were maintained in specific pathogen-free conditions, a 12 h light/dark cycle at 20-22 °C and 45 ± 5%humidity. All rats were randomly assigned into ten groups: (a) control group (con, n=10), (b) hyperuricemia and gout model group (Veh, n=10), (c) benzbromarone treatment group (ben, n=10, 4mg/kg/day), (d) traditional Chinese Medicine (TCM) *Simiao San* treatment Group (SMS, n=10, 480 mg/kg/day), (e) green tea treatment group (G, n=10,10 mL/kg/day), (f) yellow tea treatment group (Y, n=10, 10 mL/kg/day), (g) black tea treatment group (B, n=10, 10 mL/kg/day), (h) white tea treatment group (W, n=10, 10 mL/kg/day), (i) red tea treatment group (R, n=10, 10 mL/kg/day), (j) cyan tea treatment group (C, n=10, 10mL/kg/day). Except for the con group, rats in each group were intraperitoneally injected with PO (300 mg/kg): hypoxanthine (300 mg/kg) suspension once a day. The rats in the Con group were treated with PBS at the same schedule. The (c)-(j) group was given intragastric administration once a day, and the Con and Veh groups were treated with water. On day 21, the rats were killed after anesthetization, and blood samples were collected by

cardiac puncture. The kidney and liver were surgically harvested and fixed in 4% paraformaldehyde.

Histological analyses

For histological evaluation, the right kidneys and livers were fixed with 4% paraformaldehyde and embedded in paraffin. The kidneys and livers were cut into 3-μm sections and stained with hematoxylin and eosin. Next, the slices were photographed under a light microscope and analyzed by a pathologist blinded to the animal groups and drug treatments.

Immunohistochemistry (IHC)

The expression of NLRP3 in the kidney was measured by IHC. For antigen retrieval, 4-μm-thick paraffin sections were placed in citrate buffer and microwaved with medium heat for 8 min and heat preservation for 8 min, followed by moderate-low heat for 7 min. After blocking 3% bovine serum albumin, sections were incubated with anti-NLRP3 antibodies. Next, they were mixed with the secondary antibody and incubated. Finally, the sections were stained with diaminobenzidine and counterstained with hematoxylin stain solution. Notably, positive staining presented a brown-yellow color under a microscope (Eclipse Ci-L, Nikon, Tokyo, Japan).

Enzyme-linked immunosorbent assay (ELISA)

We assayed serum contents of IL-1β with ELISA kits. The ELISA assay has been described previously[20].

XOD, BUN, CRE and UA activity assay

The blood samples were kept at 4 ℃ for four hours to clot and then centrifuged at 2500

g for 10 min to obtain the serum supernatant. Then, the supernatants were used to determine XOD, BUN, CRE and UA activity using the assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's guidelines.

LC-MS-based untargeted metabolomics analysis

Tea infusion was analyzed with an LC-MS/MS system, a Waters 2D ultra-performance liquid chromatography (Waters, USA) coupled with a high-resolution mass spectrometer Q Exactive HF (Thermo Fisher Scientific, USA). Chromatographic separation was performed using the Hypersil GOLDaQ column (100 × 2.1mm, 19μm, Thermo Fisher Scientific, USA). The column temperature and detection wavelength were set at 40 ℃, and the injection volume was set to 5μL. The mobile phase consisted of 0.1% formic acid in 100% water (A) and 0.1% formic acid in 100% acetonitrile (B) at a flow rate of 5μL/s. The linear gradient elution was as follows: 0-2min, 5% B; 2-22min, increase to 5-95%; 22-27min, hold at 95%; 27-30min, decrease to 5%.

The raw data for MS1 and MS2 was acquired from Q exactive mass spectrometer (Thermo Fisher Scientific, USA). The parameters of electrospray source under the negative ionization were as follows: spray voltage, 3.2 kV (ESI−); capillary temperature, 320 °C; Aux gas heater temperature, 350 °C; m/z range, 150-1500 m/z. The parameters of the MS1 scan were as follows: resolution (70,000), automatic gain control (AGC) target (1.0×10^6), maximum injection time (100 ms). For the data-dependent in MS2, the following parameters were used: resolution (35,000), AGC target (2.0×10^5), maximum injection time (50 ms). The stepped normalized collision energy (NCE) values were set at 20, 40, and60 eV. Each sample was performed in six repetitions.

The raw data was processed by Compound Discoverer 3.1 (Thermo Fisher Scientific, USA), including the peak extraction, alignment, and quantification. Compounds identification was verified by authentic standards and MS/MS fragmentations. Meanwhile, PubMed and HMDB also were used to identify the compounds.

Gene set of the identified compounds in yellow tea and hyperuricemia

The identified compounds were filtered using ADMET properties. ADME parameters were acquired from the TCMSP (https://tcmsp-e.com/), used to evaluate the chemicals' absorption, distribution, metabolism, and excretion. The yellow tea compounds were filtered based on the criteria of oral bioavailability (OB) ≥ 30% and drug-likeness ≥ 0.18. After initial filtering, the gene set of the bioactive compounds was collected from the TCMSP database, HERB database (http://herb.ac.cn/).

Genes of treatment and prevention for hyperuricemia were collected from four databases: Genecards (https://www.genecards.org/), DisGeNet (https://www.disgenet.org/). Gene sets obtained from taking intersection between YT compound-related genes, and anti-hyperuricemia-related genes were used for further analysis.

Construction of PPI network and Compound-targets network

The STRING database was used to construct the protein-protein interaction network for the overlapping targets between hyperuricemia and yellow tea. The species “Homo sapiens” was selected. Meanwhile, the interaction network of the yellow tea compounds and the overlapping targets was established using the Cytoscape 3.9.0 software. Crucial targets were identified using molecular complex detection (MCODE) (a plugin in

Cytoscape).

Enrichment analysis of GO and KEGG pathway

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was used to unveil the potential molecular mechanism and the critical signaling pathways. The "ClusterProfile" package performed enrichment analysis for GO and KEGG pathways in R software version 3.4.0.

Statistical analyses.

The values are expressed as mean ± s.e.m. The unpaired t-test (GraphPad Prism 9 Software) was used for statistical analysis. The data points were not excluded. The researchers involved in this study were not blinded during sample collection or data analysis. Select the sample size based on the preliminary results to ensure sufficient power. P-values < 0.05 were considered significant.

Results

Effect of tea on the UA, CRE, BUN level of rats with hyperuricemia

UA, an essential indicator of hyperuricemia, excessive production or insufficient excretion can cause kidney damage. As shown in Fig. 1a, there was no difference in UA levels between the green tea treatment group and the Veh group. In contrast, the other types of tea treatment groups could markedly reduce the serum UA levels in rats with hyperuricemia. The yellow tea and black tea treatment groups' UA levels were remarkably lower than the Veh group (P<0.0001).

The level of CRE in the serum shows the filtration function of the glomerulus. As can

be observed in Fig. 1b, all the types of tea can decrease the level of CRE to restore the filtration function of the glomerulus, which were similar with positive control groups (Ben group and SMS group).

BUN is also an important indicator of kidney injury. As can be observed in Fig. 1c, the BUN level of the Veh group was significantly higher than that of the Con group. Compared with the Veh group, the TCM *Simiao San* can reduce the BUN level in the serum of hyperuricemic rats. The effect of white tea is similar to *Simiao San* (P<0.001). Compared with the Veh group, red tea and cyan tea can slightly relieve BUN levels in the serum of hyperuricemic rats (P<0.005). In contrast, the green tea group and Ben groups' BUN levels had no considerable reduction. However, the BUN levels were significantly reduced in the yellow tea group.

Effect of tea on the liver of rats with hyperuricemia

XOD is a critical enzyme that promotes the production of uric acid. As shown in Fig. 2a, the serum XOD level in the Veh group were significantly higher than those in the Con group. Except for the black tea group, the XOD levels of all intervention groups decreased. It is worth noting that yellow tea and white tea significantly reduce XOD levels, and their effects are similar to the SMS group. Liver histological changes of Congroup and experimental mice were depicted in Fig. 2b. Veh group exhibited several characterized histologic alterations, including inflammatory cell infiltration (yellow arrow) under the capsule in many tissues, inflammatory damage (black arrow) around the local vein, and parenchyma. Some liver cells die and disappear, and fibroblasts proliferate. The inflammatory cell infiltration and hepatocellular disease were

alleviated in the tea treatment and positive control groups. Yellow tea group and black tea group treatment significantly alleviated pathological lesions induced by PO and hypoxanthine.

Effect of tea on histopathology of rat kidney induced by PO and hypoxanthine

The changes in the organizational structure of the kidney were observed as an apparent pathological characteristic of hyperuricemia. Kidney histological changes of rats are shown in Fig. 3. The renal tissues in control rats showed a normal morphology without evidence of inflammation. The kidney in rats treated by PO and hypoxanthine exhibited several characterized histologic alterations, including conspicuous dilation of renal tubules (black arrow), swelling and proximal tubule necrosis. Compared with the Veh group, tubulointerstitial and glomerular lesions were facilitated in the tea treatment groups and the positive control group, and with regulated proximal tubule cells and the cytoplasm were relatively straightforward. Yellow tea treatment alleviated PO and hypoxanthine-induced pathological lesions patently.

The candidate ingredients of yellow tea and its targets

The above results indicate that yellow tea can reduce biochemical indicators and histopathological changes in hyperuricemia rats. Therefore, we analyzed and identified the LC-MS system's active ingredients in yellow tea infusion. Based on the OB and DL parameters, ten active compounds of yellow tea were retained (Table 1). Nine flavonoids mainly included two flavones, four flavonols, and three flavan-3-ols. By searching the HERB database and TCMID database, we obtained 730 potential targets of 10 yellow tea compounds after removing the repetitive targets.

Construction of the PPI Network of the Active Ingredients of yellow tea and Hyperuricemia

Hyperuricemia-related targets were searched and retrieved from DisGeNET and GeneCards, including 196, 773 targets, respectively. We finally retained 127 overlapping targets by taking the intersection (Fig. 4a). Thirty-five overlapping targets between yellow tea compounds and targets related to hyperuricemia were obtained (Fig. 4b). Compounds-targets network of 10 compounds from yellow tea and 35 targets was also constructed (Fig. 4c). These common targets were imputed into STRING to construct a PPI network, and the network was visualized using Cytoscape 3.9.0. The PPI network consisted of 41 nodes with 295 edges (Fig. 4d). The core targets of yellow tea for the treatment of hyperuricemia were detected by MCODE consist of NLRP3 inflammasome (NLRP3), peroxisome proliferators-activated receptor-α (PPARA), IL-1β (IL1B), Interleukin-6 (IL6), tumor necrosis factor (TNF), peroxisome proliferative activated receptor gamma (PPARG), C-C motif chemokine ligand 2 (CCL2), Vascular Endothelial Growth Factor A (VEGFA), signal transducer and activator of transcription (STAT3) (Supplementary Table 1).

GO analysis and KEGG pathway enrichment analysis of common targets

To investigate the functions of these genes, we performed the GO annotation and KEGG pathway enrichment analysis. GO enrichment analysis was used to identify the potential biological processes (BP), molecular functions (MF), and cellular components (CC) of the 35 genes of yellow tea against hyperuricemia. According to the criteria of adjusted p-value < 0.05 and q value < 0.05, we generated the 1482 GO terms, including 1414

BP-related, 15 CC-related, and 52 MF-related GO terms. The top 10 GO terms for BP, CC, MF are shown in Fig.5a. Enrichment was observed in the BP: the regulation of inflammatory response, epithelial cell proliferation, response to oxidative stress, response to reactive oxygen species, response to tumor necrosis factor, response to lipopolysaccharide, regulation of lipid localization, endothelial cell proliferation, and lipid storage. Enrichment was observed in the MF: cytokine receptor binding, receptor-ligand activity, signaling receptor activator activity, cytokine activity, peptide binding, repressing transcription factor binding, lipoprotein particle binding, protein-lipid complex binding, and RNA polymerase II transcription factor activity. KEGG enrichment analysis was employed the potential cellular signaling pathways. 94 KEGG pathways were significantly enriched to reveal the possible molecular mechanism of yellow tea to prevent hyperuricemia. The top 20 KEGG pathways are illustrated in Fig. 5b. Among those enriched, the signaling pathways associated with hyperuricemia include TNF, IL-17, lipid and atherosclerosis, and the AGE-RAGE signaling pathway, suggesting that yellow tea may play a role in treating hyperuricemia mainly through the regulation of the above signaling pathways.

Teas alleviated the inflammatory response in hyperuricemic rats

The above experimental results indicate that yellow tea may reduce hyperuricemia in rats by inhibiting inflammation. Therefore, we detected the levels of pro-inflammatory factors IL-1β in serum and NLRP3 expression in the kidney. As shown in Fig. 6a, black tea, cyan tea, and red tea had no significant effect on the level of IL-1β in the serum of hyperuricemic rats. White tea and green tea can be slightly reduced, while yellow tea

304 can significantly reduce the level of IL-1β in the serum of rats with hyperuricemia. We
305 found that all six kinds of tea could down-regulate the expression of NLRP3 protein in
306 the kidneys of hyperuricemic rats, especially yellow and white tea (Fig. 6b and Fig. 6c).
307 These data suggest that yellow tea may alleviate hyperuricemia in rats by inhibiting
308 NLRP3 inflammasome activation.
309

310 ## Discussion

311 As we all know, the diet has a stimulating effect on hyperuricemia and even gout. Our
312 study investigated the impact of different types of tea on hyperuricemia in rats. We
313 evaluated the biochemical indicators of uric acid and explored the effects of different
314 teas on the liver and kidney of rats with hyperuricemia. Our study is the first time to
315 compare the impact of various teas on hyperuricemia in rats. Here, we show that
316 drinking green tea cannot reduce the uric acid level in the serum of rats with
317 hyperuricemia, which is consistent with the point raised in the previous study by
318 Zhang[16]. Other types of tea can reduce the serum uric acid level of hyperuricemic
319 rats to varying degrees. However, it was inconsistent with a previous cross-sectional
320 study showing that frequent green tea consumption did not increase the risk of
321 hyperuricemia compared with a 'no intake' reference group[21]. More interestingly, we
322 found that black and yellow tea can significantly reduce the levels of various
323 biochemical indicators (UA, CRE, BUN, XOD) of hyperuricemia. In contrast, yellow
324 tea can reduce the biochemical indicators while reversing liver and kidney damage. Our
325 data shows that the natural compounds in yellow tea are likely to be good candidates

for dietary supplements or drugs.

A variety of active ingredients in tea have been confirmed to have anti-cancer, anti-obesity and reversing steatohepatitis effects. We identified and analyzed the candidate components of yellow tea and obtained 35 targets related to hyperuricemia based on LC-MS/MS. Our research also examined the identified possible targets and participating signal pathways by GO analysis and KEGG pathway enrichment analysis. The active ingredients in yellow tea participate in many cell signaling pathways by binding to different proteins, such as TNF, atherosclerosis, and the AGE-RAGE signaling pathway. Our experiments show that green tea and white tea can slightly reduce the pro-inflammatory cytokines IL-1β of hyperuricemia rats; however, yellow tea can significantly reduce the level. Epigallocatechin gallate (EGCG) attenuates microglial inflammation by inhibiting inflammasome activation[22]. Procyanidin B1 inhibited apoptosis of oocytes and cumulus cells by reducing oxidative stress[23]. These ingredients may exert the anti-inflammatory and antioxidant effects of tea. We did not distinguish the specific actual impact of every compound because of the lack of standards for quantitative analysis of active compounds. Nevertheless, our results may provide more details for studying the mechanism of hyperuricemia and the efficacy of tea in the prevention or possible treatment of hyperuricemia and provide a reference for patients with hyperuricemia to drink tea.

Conclusions

Collectively, to our knowledge, this is the first report that compares the effects of six

types of tea on hyperuricemia and found that green tea cannot reduce the serum uric acid level of hyperuricemic rats. Compared with other teas, yellow tea can significantly improve hyperuricemia, which may be by regulating inflammatory response, autophagy, and apoptosis. Therefore, this study provides a potential candidate for the treatment of hyperuricemia and a basis for selecting tea for patients with hyperuricemia.

Abbreviation

PO, Potassium Oxonate; UA, Uric Acid; XOD, Xanthine Oxidase; BUN, Blood Urea Nitrogen; CRE, Creatinine; ELISA, enzyme-linked immunosorbent assay;IL-1β, interleukin-1β; LC-MS/MS, Liquid Chromatography Tandem Mass Spectrometry; MSU, Monosodium Urate; TCM, Traditional Chinese Medicine; IHC, Immunohistochemistry; NLRP3, NOD-like Receptor Protein 3; ADMET, Absorption, Distribution, Metabolism, Excretion, and Toxicity; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; MCODE, Molecular Complex Detection; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction; STRING, Search Tool for Recurring Instances of Neighboring Genes; BP, biological processes; MF, molecular functions; CC, cellular components; EGCG, Epigallocatechin gallate

Acknowledgements

Not applicable.

Author's contributions

Siyao Sang: Methodology, Data curation, Writing-Original draft preparation. Lufei Wang: Conducted experiments and data analysis. Taotao Liang: Participated in research design and revised manuscript. Mingjie Su: conducted experiments. Hui Li: Conceptualization, Supervision, Project administration, Funding acquisition. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work, ensuring integrity and accuracy.

Fundings

This work was supported by B&R Joint Laboratory of Eurasian Anthropology (18490750300), Shanghai Ziranerran Chinese Medicine Development Foundation (201501).

Availability of data and materials

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

Declarations

Ethics approval and consent to participate

All experiments were carried out in accordance with the guidelines of the Ethics Committee of Fudan University and approved by the Research Ethics Committee of Fudan University School of Life Sciences, China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai 200438, China.

[2]MOE Key Laboratory of Contemporary Anthropology, Human Phenome Institute, Fudan University, Shanghai 200438, China.

[3]Department of Hematology, Affiliated Cancer Hospital of Zhengzhou University, Henan Cancer Hospital, Zhengzhou, 450008, China.

[4]Human Phenome Institute, Fudan University, Shanghai 200438, China.

[5]Shanxi Academy of Advanced Research and Innovation, Fudan-Datong Institute of Chinese Origin, Datong 037006, China.

References

1. Dalbeth, N., et al., *Gout.* Lancet, 2021. **397**(10287): p. 1843-1855.

2. Taylor, W.J., et al., *Study for Updated Gout Classification Criteria: Identification of Features to Classify Gout.* Arthritis Care Res (Hoboken), 2015. **67**(9): p. 1304-1315.

3. Mallat, S.G., et al., *Hyperuricemia, Hypertension, and Chronic Kidney Disease: an Emerging Association.* Curr Hypertens Rep, 2016. **18**(10): p. 74.

4. Johnson, R.J., et al., *Hyperuricemia, Acute and Chronic Kidney Disease, Hypertension, and*

Cardiovascular Disease: Report of a Scientific Workshop Organized by the National Kidney Foundation. Am J Kidney Dis, 2018. **71**(6): p. 851-865.

5. Mortada, I., *Hyperuricemia, Type 2 Diabetes Mellitus, and Hypertension: an Emerging Association.* Curr Hypertens Rep, 2017. **19**(9): p. 69.

6. Wang, D., et al., *Increased Susceptibility of Atrial Fibrillation Induced by Hyperuricemia in Rats: Mechanisms and Implications.* Cardiovasc Toxicol, 2021. **21**(3): p. 192-205.

7. Borghi, C., et al., *Expert consensus for the diagnosis and treatment of patient with hyperuricemia and high cardiovascular risk: 2021 update.* Cardiol J, 2021. **28**(1): p. 1-14.

8. Stamp, L.K., *Major unanswered questions in the clinical gout field.* Curr Opin Rheumatol, 2017. **29**(2): p. 171-177.

9. de Oliveira, E.P. and R.C. Burini, *High plasma uric acid concentration: causes and consequences.* Diabetol Metab Syndr, 2012. **4**: p. 12.

10. Xu, C., et al., *Xanthine oxidase in non-alcoholic fatty liver disease and hyperuricemia: One stone hits two birds.* J Hepatol, 2015. **62**(6): p. 1412-9.

11. Baidurin, S., et al., *[Errors in the Diagnostics and Treatment of Patients with Gout and the Algorithm of Therapeutic Tactics in Different Periods of the Disease (Clinical Case)].* Georgian Med News, 2020(303): p. 103-108.

12. Vargas-Santos, A.B. and T. Neogi, *Management of Gout and Hyperuricemia in CKD.* Am J Kidney Dis, 2017. **70**(3): p. 422-439.

13. Cicero, A.F.G., et al., *Therapeutic Strategies for the Treatment of Chronic Hyperuricemia: An Evidence-Based Update.* Medicina (Kaunas), 2021. **57**(1).

14. Brody, H., *Tea.* Nature, 2019. **566**(7742): p. S1.

15. Towiwat, P. and Z.G. Li, *The association of vitamin C, alcohol, coffee, tea, milk and yogurt with uric acid and gout.* Int J Rheum Dis, 2015. **18**(5): p. 495-501.

16. Zhang, Y., et al., *Is tea consumption associated with the serum uric acid level, hyperuricemia or the risk of gout? A systematic review and meta-analysis.* BMC Musculoskelet Disord, 2017. **18**(1): p. 95.

17. Teng, G.G., et al., *Serum urate levels and consumption of common beverages and alcohol among Chinese in Singapore.* Arthritis Care Res (Hoboken), 2013. **65**(9): p. 1432-40.

18. Kiyohara, C., et al., *Inverse association between coffee drinking and serum uric acid concentrations in middle-aged Japanese males.* Br J Nutr, 1999. **82**(2): p. 125-30.

19. Jin, W., et al., *Infrared imageries of human body activated by teas indicate the existence of meridian system.* 2020.

20. Lequin, R.M., *Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA).* Clin Chem, 2005. **51**(12): p. 2415-8.

21. Lee, J.S., et al., *Impact of Coffee/Green Tea/Soft Drink Consumption on the Risk of Hyperuricemia: A Cross-Sectional Study.* Int J Environ Res Public Health, 2021. **18**(14).

22. Zhong, X., et al., *Epigallocatechin-3-Gallate Attenuates Microglial Inflammation and Neurotoxicity by Suppressing the Activation of Canonical and Noncanonical Inflammasome via TLR4/NF-kappaB Pathway.* Mol Nutr Food Res, 2019. **63**(21): p. e1801230.

23. Zhu, J. and C. Du, *Could grape-based food supplements prevent the development of chronic kidney disease?* Crit Rev Food Sci Nutr, 2020. **60**(18): p. 3054-3062.

Figure Legends

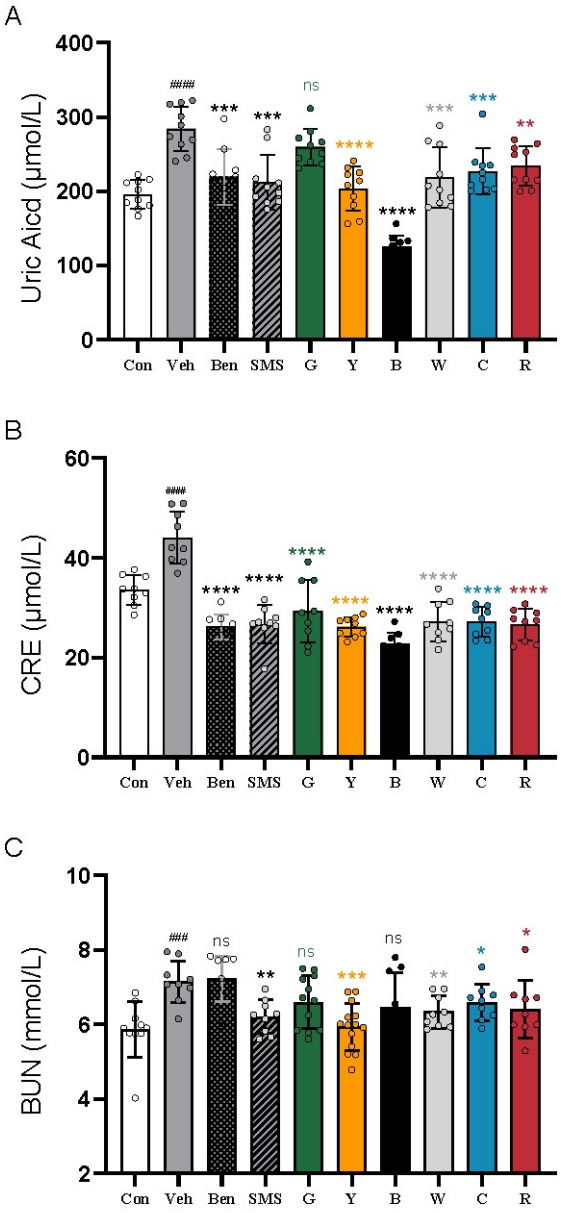


Fig. 1. Effects of tea on UA, CRE, BUN level in PO- and hypoxanthine-induced hyperuricemia in rats. Data were given as Mean ± SD (n=10). Ben, benzbromarone (4mg/kg/day) treatment group; SMS, traditional Chinese Medicine *Simiao San* (480 mg/kg/day) treatment group; G, green tea (10 mL/kg/day) treatment group; Y, yellow tea (10 mL/kg/day) treatment group; B, black tea (10 mL/kg/day) treatment group; W, white tea (10 mL/kg/day) treatment group; C, cyan tea (10 mL/kg/day) treatment group;

R, red tea (10 mL/kg/day) treatment group; # represents significant difference as compared with Con group, ###p<0.001; *represents significant difference as compared with vehicle group, *p<0.05, **p<0.01, ***p<0.001, **** p<0.0001.

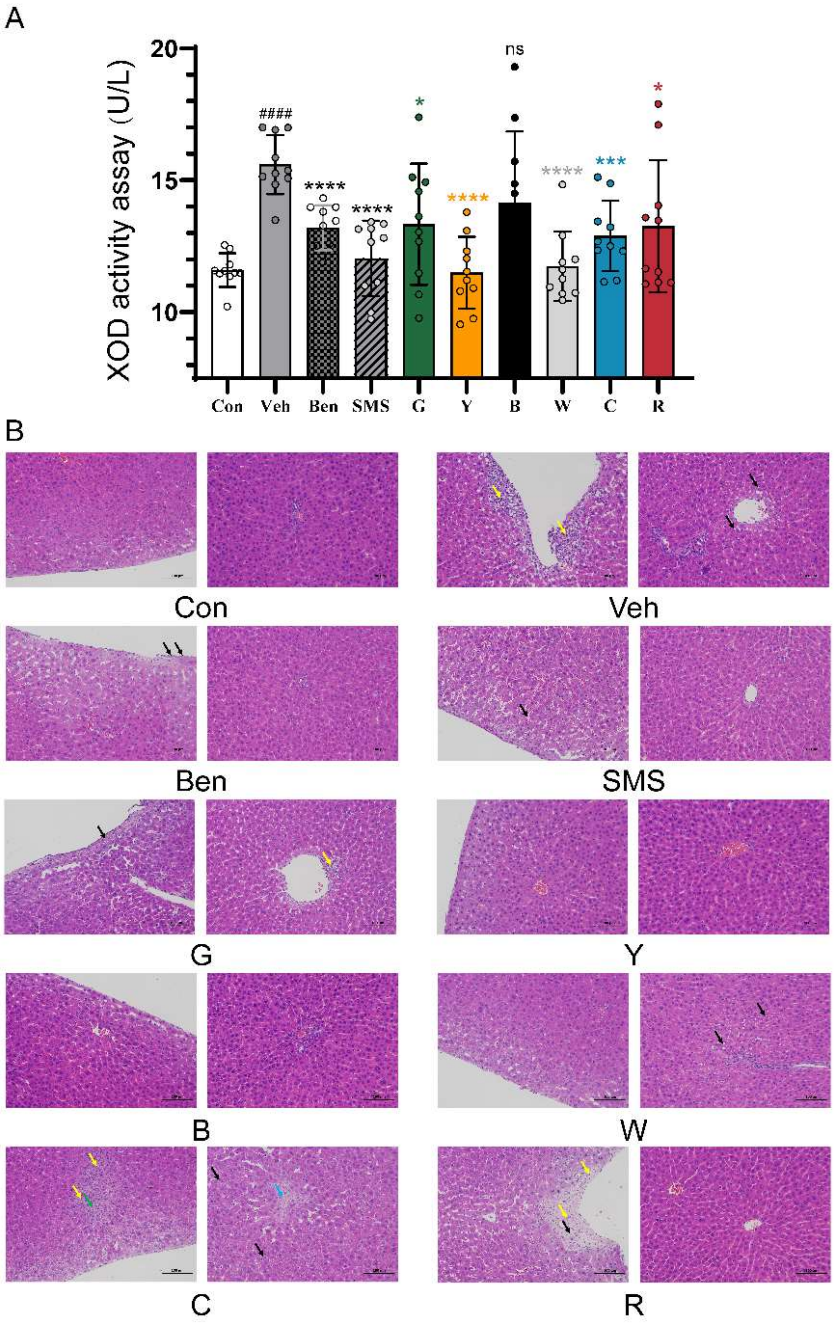


Fig. 2. Effect of tea on the liver of rats with hyperuricemia. (A). The effects of teas on hepatic XOD activity in PO- and hypoxanthine-induced hyperuricemia in rats. Data

were given as Mean ± SD (n=10). (B). Hematoxylin and eosin (H&E)-stained in liver tissues. Scale bars, 100 μm. #represents significant difference as compared with Con group, ###p<0.001; *represents significant difference as compared with vehicle group, *p<0.05, **p<0.01.

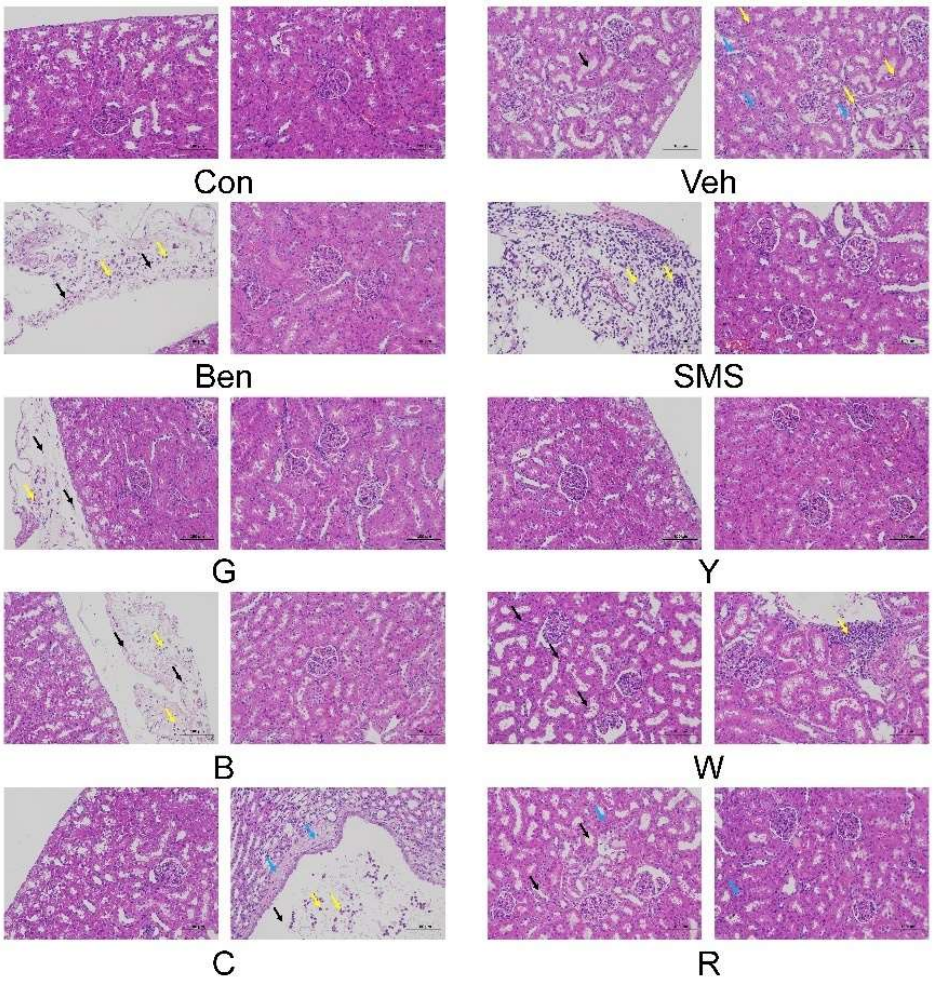


Fig. 3. Effect of tea on histopathology of rat kidney induced by PO and hypoxanthine. The right kidney of rats was fixed, embedded in paraffin, sectioned, and stained by H&E. The inflammatory cells infiltration in renal tubular, as well as tubular dilation and vacuole in renal tubular epithelial cells of these animals were evaluated and semi-scored under light microscopy (n=3). Scale bars, 100 μm.

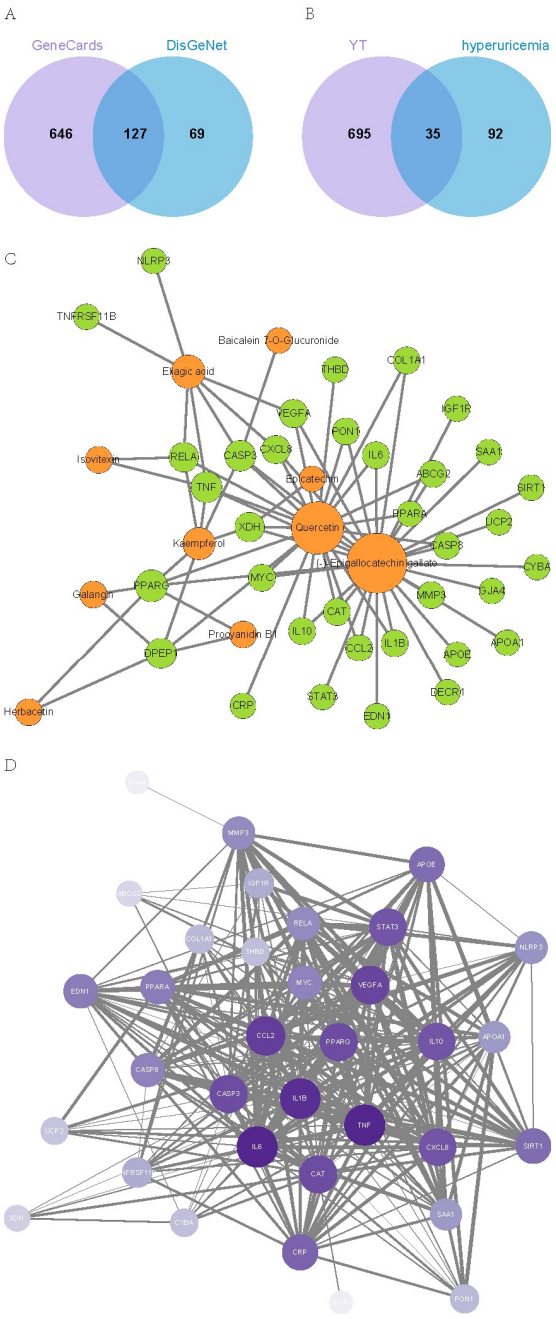


Fig. 4. Screening of active ingredients related to hyperuricemia in yellow tea. (A). Intersection targets related to hyperuricemia from GeneCards and DisGeNet databases. (B). The overlapping targets for YT bioactive compounds and targets related to hyperuricemia. (C). The active ingredient-target network for yellow tea. The orange node represents ingredient nodes, the green node represents predicted targets, and the connecting lines represent the interaction between the ingredients and the targets. The

node size represents the corresponding degree value.D). PPI network of the common targets of active ingredients of yellow tea and hyperuricemia.

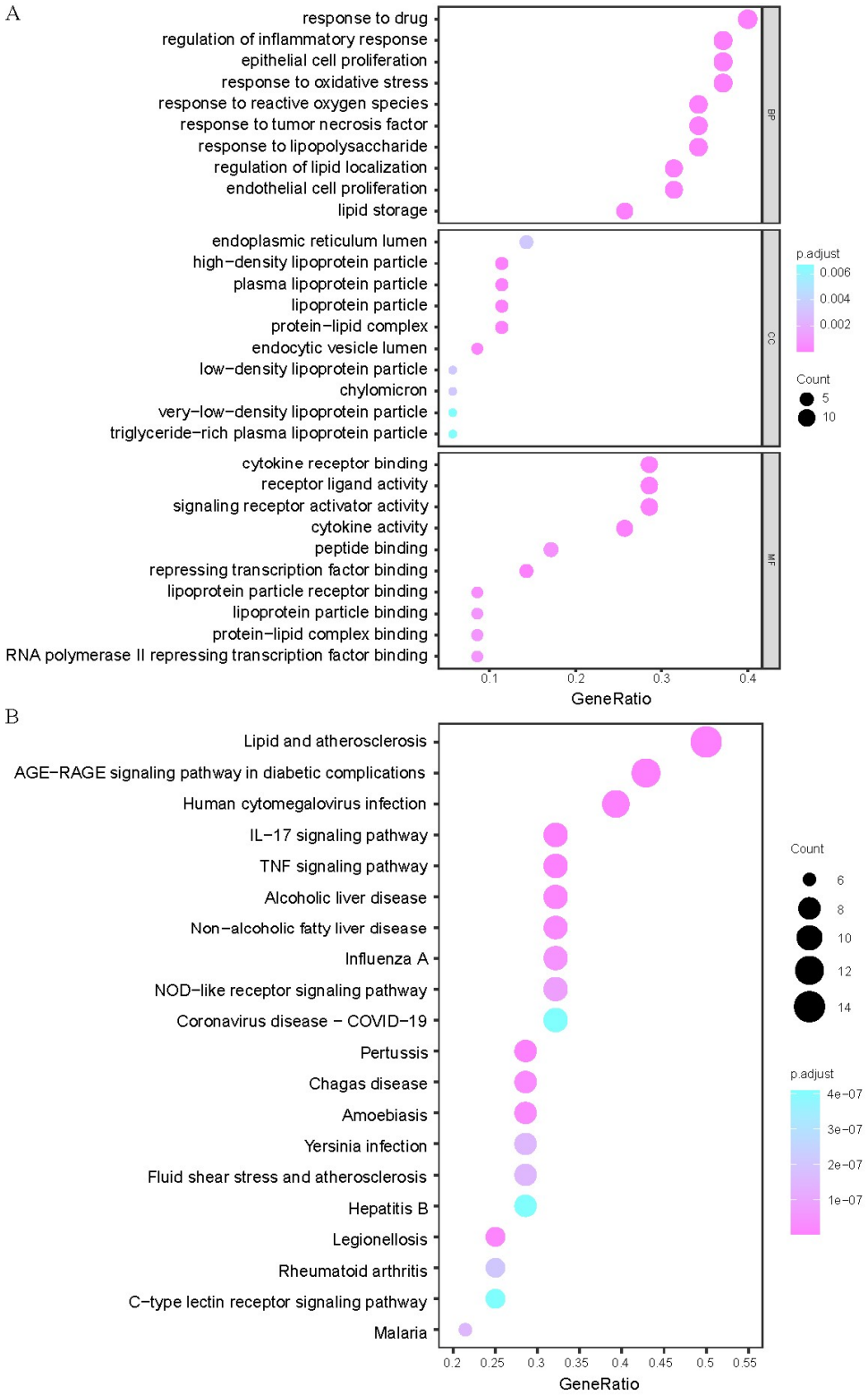


Fig. 5. GO analysis and KEGG pathway enrichment analysis of common targets.

(A). Bubble diagram of GO enrichment of common targets. (B). KEGG pathway analysis of common targets. Gene ratio refers to the percentage of enriched targets to all targets, and the counts refer to the number of enriched targets.

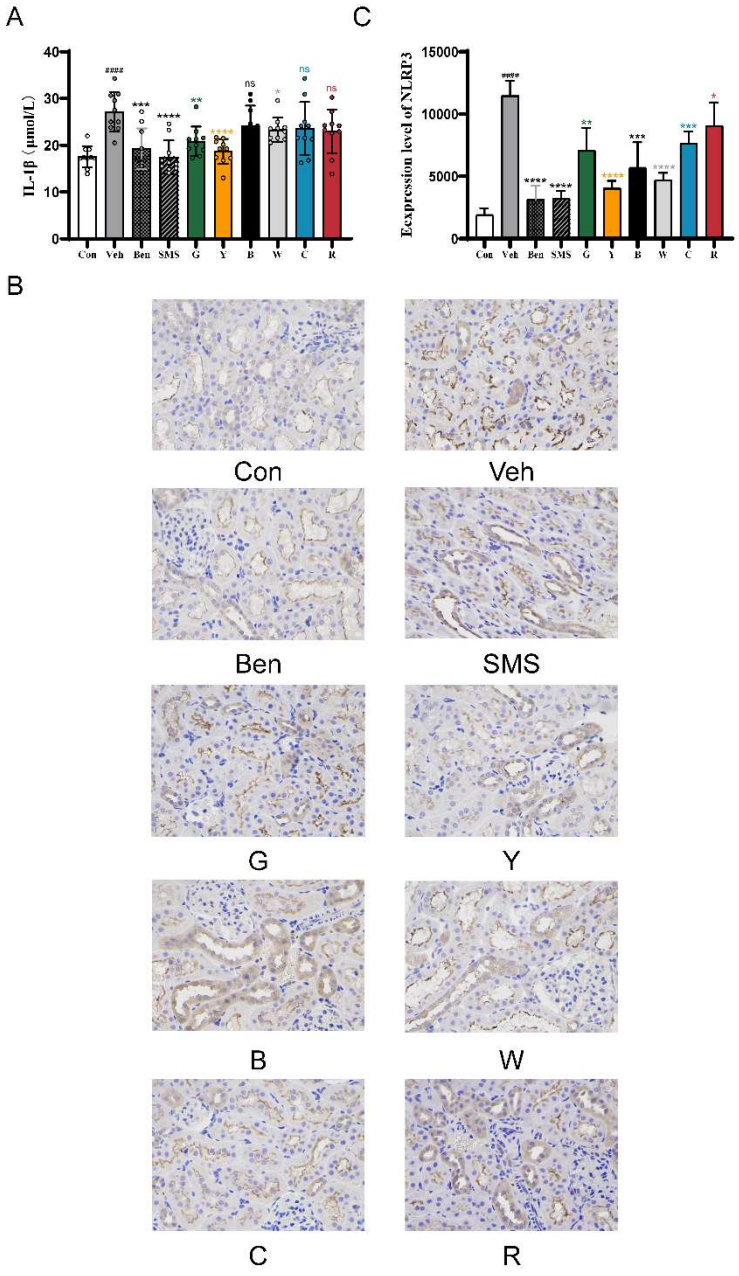
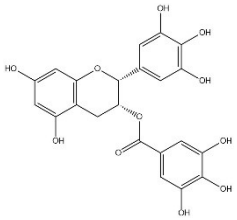
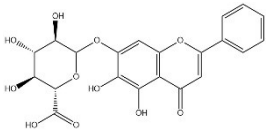
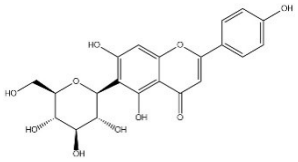
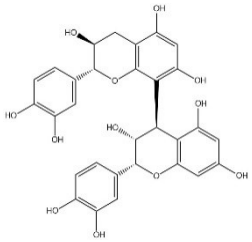
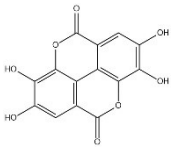
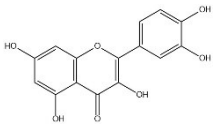
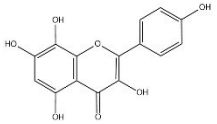
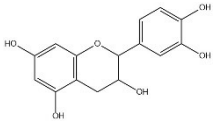
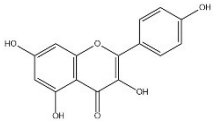
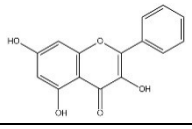


Fig. 6. Teas alleviated the inflammatory response in hyperuricemia rats. (A). Serum was measured by the IL-1β ELISA kit. Data are shown as means ± sem (n = 10 rats). (B). Immunohistochemical of NLRP3 was acquired in the kidney tissue. Scale bars, 20 μm. (C). Statistics of areal density of NLRP3 in the kidney.

503 **Table 1. The candidate ingredients of yellow tea and its targets.**

Compound ID	Compound name	Chemical structure	OB (%)	DL
5.301_458.08396	(-)-Epigallocatechin gallate		55.09	0.77
5.705_446.08371	Baicalein 7-O-Glucuronide		40.12	0.75
6.548_432.10466	Isovitexin		31.29	0.72
4.712_578.14173	Procyanidin B1		67.87	0.66
6.352_302.00551	Ellagic acid		43.06	0.43
8.341_302.04179	Quercetin		46.43	0.28
7.662_302.04178	Herbacetin		36.07	0.27
5.149_290.07823	Epicatechin		48.96	0.24
9.424_286.04692	Kaempferol		41.88	0.24
7.739_270.05195	Galangin		45.55	0.21

504

505 **Supplementary Materials**

506 **Supplementary Table 1.** The core targets of yellow tea for the treatment of

507 hyperuricemia.

MCODE:: Clusters	MCODE:: Node Status	MCODE:: Score	name	selected	shared name
Cluster 0	Seed	14	NLRP3	TRUE	NLRP3
Cluster 0	Clustered	14	PPARA	TRUE	PPARA
Cluster 0	Clustered	13.88333333	EDN1	TRUE	EDN1
Cluster 0	Clustered	13.81578947	CASP3	TRUE	CASP3
Cluster 0	Clustered	13.81578947	CCL2	TRUE	CCL2
Cluster 0	Clustered	13.81578947	CXCL8	TRUE	CXCL8
Cluster 0	Clustered	13.81578947	IL10	TRUE	IL10
Cluster 0	Clustered	13.81578947	IL1B	TRUE	IL1B
Cluster 0	Clustered	13.81578947	IL6	TRUE	IL6
Cluster 0	Clustered	13.81578947	SIRT1	TRUE	SIRT1
Cluster 0	Clustered	13.81578947	STAT3	TRUE	STAT3
Cluster 0	Clustered	13.81578947	TNF	TRUE	TNF
Cluster 0	Clustered	13.81578947	VEGFA	TRUE	VEGFA
Cluster 0	Clustered	13.76666667	CASP8	TRUE	CASP8
Cluster 0	Clustered	13.69117647	APOE	TRUE	APOE
Cluster 0	Clustered	13.69117647	CRP	TRUE	CRP
Cluster 0	Clustered	13.0994152	CAT	TRUE	CAT
Cluster 0	Clustered	13.01754386	PPARG	TRUE	PPARG
Cluster 0	Clustered	12.87619048	RELA	TRUE	RELA
Cluster 0	Clustered	12.675	MYC	TRUE	MYC
Cluster 0	Clustered	12	SAA1	TRUE	SAA1
Cluster 0	Clustered	11.86813187	MMP3	TRUE	MMP3

508