 doi:10.20944/preprints202211.0088.v1

Article

Supplementation of *Lactobacillus Plantarum* (TCI227) Prevented Potassium Oxonate Induced Hyperuricemia in Rats

Chih-Yu Chien [1,#], Yu-Jou Chien [1,#], Yung-Hao Lin [2], Yung-Hsiang Lin [3], Shu-Ting Chan [3], Wei Chun Hu [3], Han-Fang Wu [3], Chi-Fu Chiang [3] and Chin-Lin Hsu [1,4,*]

[1] Department of Nutrition, Chung Shan Medical University, Taichung 40201; chihyu0903@gmail.com (C.-Y.C.); aa910149@gmail.com (Y.-J.C.); clhsu@csmu.edu.tw (C.-L.H.)
[2] Baiyuete Limited Company, Shanghai, China; inos@tci-bio.com (Y.-H. L.)
[3] Research & Design Center, TCI CO., Ltd., Taipei, Taiwan.; vincent@tci-bio.com (Y.-H. L.); rebecca.chan@tci-bio.com (S.-T. C.); hubert.hu@tci-bio.com (W.-C. H.); sindy.wu@tci-bio.com (H.-F. W.); jimmy.chiang@tci-bio.com (C.-F. C.)
[4] Department of Nutrition, Chung Shan Medical University Hospital, Taichung 40201; clhsu@csmu.edu.tw (C.-L.H.)
* Correspondence: clhsu@csmu.edu.tw
# These authors contributed equally to this work

Abstract: Hyperuricemia (HC) was one of the important risk factors for gout, arteriosclerosis, and cardiovascular disease. Animal studies have shown that *Lactobacillus plantarum* can improve the microbiota, immune regulation, and inhibit the uric acid production. However, it was not clear whether *L. plantarum* can improve HC and intestinal microbiota. We used potassium oxonate (PO) to induce HC in male SD rats, and then treated L. plantarum TCI227 in a dose dependent (HC+LD, HC+MD, HC+HD) for 4 weeks, and examined weight organs, biochemical examination of blood and urine, and analyzed the intestinal microbiota in feces by 16s rDNA sequence analysis. In this study, TCI227 improved body weight, decreased creatinine, serum uric acid, and increased urine uric acid compared to the HC group. Furthermore, TCI227 increased short-chain fatty acids (SCFAs). In fecal microbiota (family), TCI227 increased the level of *Lactobacillaceae*, and then decreased the level of *Deferribacteres* and *Prevotellaceae* compared to the HC group. Finally, in fecal microbiota (genus), TCI227 decreased the level of *Prevotella*, and then increased the level of *Lactobacillus* and *Ruminococcus* compared to the HC group. This study suggested that TCI227 can improve HC and change the compositions of the intestinal microbiota in PO induced male HC SD rats.

Keywords: hyperuricemia; *Lactobacillus plantarum*; microbiota; uric acid

1. Introduction

Uric acid (UA) was the final product of nucleic acid metabolism in the body [1]. Purine in the body was metabolized by the liver to form uric acid, and finally the kidneys excrete uric acid from the body[2]. If too much UA was produced in the body or was poorly excreted by the kidneys, it formed crystals and deposited in the body, leading to hyperuricemia (HC), which was a chronic disease[3]. Risk factors include obesity, the consumption of too much fructose, meat, and seafood[4]. HC patients were often accompanied by chronic diseases such as gout, diabetes, heart disease, and kidney disease[5]. In addition to adjusting diet and weight control, the most direct way of treatment was drug intervention[6], but it had side effects such as allergy, nephrotoxicity, liver toxicity, nausea and vomiting, and most patients with HC were unable to achieve long-term control due to high purine diets and poor treatment efficacy[7]. Therefore, in recent years, natural-derived dietary supplements have often been used as a preventive and adjuvant treatment for HC.

UA was secreted into the intestines and was rapidly metabolized by intestinal bacteria[8]. Studies had shown that the intestinal microbiota of gout patients was significantly

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 November 2022 doi:10.20944/preprints202211.0088.v1

different compared to normouricemic subjects[8], suggesting that interaction between the microbiota and intestinal UA metabolism and excretion could potentially modulate serum uric acid levels. Studies also had shown that changing the structure and amount of the intestinal microbiota affect the metabolic rate of UA, thus reducing the UA content in the blood[9]. Furthermore, the transport protein of UA was found to be secreted by various indigenous microbes in the human gut[10]. Probiotic supplements have anti-uric acid, anti-inflammatory, immune regulation, and gastrointestinal barrier functions[11]. Therefore, the development of probiotic treatment strategies to reduce UA concentrations would be useful[11, 12].

Probiotics were live microbial dietary supplements that exert beneficial effects on host health due to their potential effectiveness for the prevention and treatment of immune diseases[13]. Lactobacillus was the most common probiotic in the human body, it was a kind of lactic acid bacteria. L. plantarum had a variety of potential beneficial effects on human health[14], such as improvement of the microbiota, immune regulation, suppression of fat accumulation, and reduction in serum triglycerides and cholesterol. Furthermore, L. plantarum treatment significantly reversed UA levels induced by the high-fat diet (HFD) and reduced the size of the adipocytes[12, 14]. However, it was still unclear whether L. plantarum can improve the HC and intestinal microbiota.

Therefore, this study used potassium oxonate (PO), a selectively competitive uricase inhibitor, to induce HC in rats[15], and then evaluated the evaluation of *L.plantarum* TCI227 to improve the HC and intestinal microbiota.

2. Materials and Methods

2.1. Animals and ethics statement

A total of forty-eight adult male SD rats (6-weeks-old) were ordered for this study. All animals were kept in steel rodent cages, and the animal room temperature is controlled at 22±2°C, the humidity is controlled at 60-80%, and with a 12 light/dark cycle (light period:07:00-19:00, dark period:19:00-07:00). The animal use protocol listed below has been reviewed and approved by the Chung Shan Medical University / Institutional Animal Care and Use Committee (IACUC approval no: 2413). All animals were fed regular formula feed and distilled water until 8-weeks-old, then the test sample started the intervention.

2.2. The design of animal experiments

Forty-eight Wistar, SD (Sprague-Dawley) rats were randomly assigned to 6 groups of 8 each. The control group was injected intraperitoneally with vehicle, while the others were injected intraperitoneally with potassium oxonate suspension (Sigma-Aldrich, Schelldorf, Germany): 250 mg/kg for hyperuricemia induction every day for 4 consecutive weeks, and test samples were administered during the hyperuricemia induction phenotype. The six groups were divided as: (a) normal control group (Control, C); (b) hyperuricemia control group (Hyperuricemia control, HC) (c) Low dose (10^8 CFU/kg rat/day) of TCI227 oral gavage treatment of hyperuricemia induced group (low dose, LD) [HC+LD] (d) Medium dose (10^9 CFU/kg rat/day) of TCI227 oral gavage treatment of hyperuricemia induced group (medium dose, MD) [HC+MD] (e) High dose (10^{10} CFU/kg rat/day) of TCI227 oral gavage treatment of hyperuricemia induced group (high dose, HD) [HC+HD]. (f) allopurinol (10 mg/kg rat/day) treatment of the hyperuricemia induced group [HC+AP]. From day 0 to day 28, the rats in groups (b)-(f) were treated once daily with PO, and groups (c)-(f) were treated with oral gavage of different doses of TCI227 and allopurinol. The total animals were sacrificed on day 28. On day 21, stools were collected from animals in RNA stabilizer reagent (Cat. No. MLBRS0500, Taichung City, Taiwan) until sacrificed (day 28). 8-12 hours of fasting blood samples were collected to measure additional serum biochemical parameters from the tail veins prior to sacrificed. Meanwhile, organ tissues, including heart, lung, liver, spleen, kidney, and adipose tissues (perirenal fat, epididymal fat, subcutaneous fat, mesenteric fat and brown adipose tissue) and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 November 2022 doi:10.20944/preprints202211.0088.v1

muscle tissues (gastrocnemius and soleus) are removed, using PBS wash and record the weight. Then freeze it with liquid nitrogen, and store it at -80 ˚C.

2.3. Chemicals

Potassium oxonate (PO), allopurinol, and oxonic acid (potassium salt) were purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany). The powder of *L. plantarum* (TCI227) was prepared by TCI Co. Ltd. Uric acid assay kit was purchased from Abcam (Cambridge, UK). Sodium chloride (NaCl) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Formalin (HCHO) was purchased from Choneye Co., Ltd. (New Taipei City, Taiwan).

2.4. Serum biochemical parameters analysis

The blood was collected in a serum separator tube (BD Vacutainer, Plymouth, UK), centrifuged at 3000 ×g for 15 minutes, then serum supernatant was transferred to the tube and stored at -80 ˚C for analysis. The level of serum aspartate transaminase (AST), alanine transaminase (ALT), creatinine, blood urea nitrogen (BUN), triglycerides, total cholesterol, sodium ion (Na^+), potassium ion concentration (K^+) and chloride ion (Cl^-) is analyzed with a fully automatic biochemical immunoassay analyzer (C501, Roche, Basel, Switzerland).

2.5. The uric acid content of serum and urine analysis

Before the chemical intervention, blood sample was collected as control at week 0. Four weeks after intervention, the blood sample was also collected for analysis. The blood was centrifuged at 3000 ×g for 15 minutes and serum was taken out for analysis. Before the chemical intervention (week 0), the second and fourth weeks of the intervention, the rats were placed in a metabolic cage for 24 hours, and their urine was collected in a centrifuge tube. Subsequently, the urine was centrifuged at 3000 ×g for 10 min at 4˚C and the supernatant was collected. The levels of uric acid in serum and urine were measured with a commercially available analysis kit (Abcam, Cambridge, UK).

2.6. Analysis of short-chain fatty acids composition in feces

The weight of 0.5 g of rat feces was added to 5 mL of deionized water, homogenize for 2 minutes, centrifuge at 7000 rpm for 5 minutes at 25°C, and filter the supernatant. Take 1 mL of supernatant, add internal standard isocaproic acid and 100 μL 50% (v/v) sulfuric acid solution and 1 mL of ether, homogenize for 2 minutes, centrifuge at 4000 rpm for 5 minutes at 25°C, and take the supernatant. Then it was further analyzed by gas chromatography (6890, Agilent Technologies, Santa Clara, USA) for the content of acetic acid, propionic acid, butyric acid and valeric acid in the stool.

2.7. Stool DNA purification and use next-generation sequencing for intestinal microbiota analysis

Fecal nucleic acid extraction was purified using the QIAamp Fast DNA Stool Mini Kit to extract nucleic acid from bacteria in feces. Fecal samples were removed from the preservation solution at 13200 rpm for 10 min, and the feces were shaken and dissolved by adding InhibitEX buffer, and subsequent reagents were added in sequence. The supernatant was then collected and washed by transfer into the QIAmp spin column. Finally, preheated elution buffer was used for the elution of the DNA. And used NanoDrop 2000 for concentration determination. Then used next-generation sequence for further microbiota identification. The illumina 16S Metagenomic Sequencing Library Preparation Manual Protocol was performed for intestinal microbiota analysis. The V3 and V4 gene fragments of bacterial 16S rRNA were amplified to develop the amplicon libraries and further sequenced paired ends by the Illumina MiSeq. The MiSeq reagent kit, V3 (600 cycles) was used and sequencing quality Q30 ≥ 80%, single sample ≥ 100000 reads.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 November 2022 doi:10.20944/preprints202211.0088.v1

2.8. Statistical analyses

The experimental data were analyzed using statistical product and service solutions (SPSS) version 22.0 of statistical software. The variance analysis was performed using the Student's *t*-test with unpaired individuals, and the experimental data were presented as the mean ± SEM, and p-value < 0.05 was considered statistically significant.

3. Results

3.1. TCI227 can improve body weight and diet in PO-induced HC rats

To evaluate whether TCI227 improved HC and intestinal microflora, we used potassium oxonate (PO) (250 mg/kg, intraperitoneal injection) to induce HC in SD rats, and then treated TCI227 in a dose-dependent manner (LD, MD, and HD) by oral administration for 4 weeks. Allopurinol can be used as an anti-hyperuricemia drug [16]. The detailed process was showed in the Supplementary figure 1. We examined body weight and diet in PO-induced HC rats. Table 1 showed that there were no significant differences in the initial body weight (week 0). However, the final weight (week 4) of the HC group was significantly decreased than that of the control group. Furthermore, the weight change in the HC group was significantly decreased than in the control group, and in the HC+MD and HC+HD groups was significantly increased than in the HC group. The food intake in the HC group was significantly decreased than the control group, and in the HC+LD, HC+MD, and HC+HD groups were significantly increased than in the HC group. Water intake in the HC group was significantly decreased than in the control group, and in the HC+HD and HC+AP groups were significantly increased than in the HC group. We also examined organ weight, adipose tissue, and muscle tissue weight in PO-induced HC rats.

Table 1. Effects of TCI227 on the initial body weight, final body weight, weight change, food intake, and water intake in potassium oxonate (PO) induced hyperuricemia SD rats.

Groups	Control	HC	HC+LD	HC+MD	HC+HD	HC+AP
Initial body weight (g)	280.20±4.60	292.94±4.58	294.65±6.58	293.30±3.36	286.70±6.95	287.20±2.72
Final body weight (g)	399.83±12.75	365.44±7.75#	374.65±4.79	388.24±6.54	376.64±8.87	374.04±6.90
Weight change (g)	119.63±8.71	72.50±3.78#	80.00±8.25	93.94±6.89*	89.94±3.75*	86.84±6.29
Food intake (g/rat/day)	26.94±0.25	22.68±0.35#	24.40±0.37*	24.97±0.32*	24.45±0.41*	23.35±0.32
Water intake (mL/rat/day)	45.02±0.84	42.53±1.11#	44.80±1.13	42.11±1.37	49.78±0.91*	46.85±1.67*

The reported values are the mean ± SEM (n=8). #Mean values were significantly different with control group (p<0.05). *Mean values were significantly different with HC group (p<0.05). HC, hyperuricemia control. LD, low dose (10^8 CFU/kg rat). MD, medium dose (10^9 CFU/kg rat). HD, high dose (10^{10} CFU/kg rat). AP, allopurinol. Weight change (g) = final body weight (g) − initial body weight (g).

Table 2 showed that the weight of liver and spleen, the HC group was significantly increased than the control group in week 4, and the weight of perirenal adipose tissue, the HC group was significantly decreased than the control group in week 4. No obvious changes in other organs and tissues. These results indicated that treatment with TCI227 can improve body weight and diet in PO-induced HC rats.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 November 2022 doi:10.20944/preprints202211.0088.v1

Table 2. Effects of TCI227 on the weights of organs, adipose tissues, and muscle tissues in PO induced hyperuricemia SD rats.

Weight of organs/tissues (mg/g rat)	Control	HC	HC+LD	HC+MD	HC+HD	HC+AP
Liver	33.62±0.62	38.51±0.57#	38.29±0.73	38.79±0.87	37.08±0.82	37.45±0.74
Heart	3.53±0.06	3.66±0.12	3.58±0.09	3.48±0.10	3.49±0.14	3.38±0.05
Lung	3.72±0.11	3.94±0.09	3.82±0.13	3.83±0.10	4.04±0.07	4.07±0.15
Spleen	2.36±0.33	5.66±0.36#	6.52±0.29	5.78±0.37	5.27±0.40	5.82±0.34
Kidney	8.43±0.35	8.51±0.17	8.45±0.23	9.00±0.17	8.49±0.31	8.13±0.12
Epididymal adipose tissue	8.89±0.59	7.08±0.67	8.51±0.69	6.97±0.53	7.83±0.57	6.98±1.12
Perirenal adipose tissue	11.05±1.07	8.00±0.89#	8.87±1.20	7.31±0.56	8.02±0.93	7.67±0.58
Mesenteric adipose tissue	6.00±0.78	8.55±1.36	10.12±1.00	10.31±2.03	9.05±1.15	8.47±0.68
Subcutaneous adipose tissue	13.28±1.04	11.48±1.19	12.74±1.17	11.50±0.67	12.75±0.69	9.59±0.73
Brown adipose tissue	0.64±0.07	0.53±0.04	0.45±0.08	0.61±0.10	0.49±0.06	0.60±0.05
Gastrocnemius muscle	7.87±1.00	8.50±0.95	9.14±0.94	8.71±0.94	9.11±1.01	9.65±0.87
Soleus muscle	0.92±0.05	0.86±0.03	0.84±0.03	0.83±0.04	0.90±0.03	0.91±0.03

The reported values are the mean ± SEM (n=8). #Mean values were significantly different with control group (p<0.05). *Mean values were significantly different with HC group (p<0.05).

3.2. TCI227 improved hyperuricemia and did not affect liver and kidney function

Next, we examined blood and urine in PO-induced HC rats in week 4. Table 3 showed that there were no statistical differences between the groups in glucose, total cholesterol, AST, ALT, BUN, and potassium. Triglyceride and sodium ion in the HC group decreased significantly compared to the control group, and triglyceride in the HC+AP group decreased significantly than in the HC group. The creatinine in the HC+MD group decreased significantly compared to the HC group, and the chloride ion in the HC group increased significantly compared to the control group.

Table 3. Effects of TCI227 on the serum biochemical parameters in PO induced hyperuricemia SD rats.

Groups	Control	HC	HC+LD	HC+MD	HC+HD	HC+AP
Glucose (mg/dL)	97.25±3.52	97.88±4.49	89.13±1.90	97.38±6.73	90.13±3.04	91.13±3.85
Total cholesterol (mg/dL)	44.88±2.07	46.00±3.39	44.75±3.79	48.88±2.70	48.38±2.99	40.63±2.91
Triglyceride (mg/dL)	83.75±10.43	50.88±4.31#	45.00±4.52	43.75±5.06	42.38±3.77	37.50±2.07*
AST (U/L)	75.50±1.99	77.00±5.26	84.38±15.82	69.63±4.79	77.75±5.11	73.63±3.96
ALT (U/L)	38.88±2.42	30.13±3.32	36.50±2.08	31.50±3.11	37.50±5.61	30.50±3.10
BUN (mg/dL)	14.38±0.46	14.13±0.67	14.38±0.95	13.25±0.65	14.75±1.03	16.50±1.57
Creatinine (mg/dL)	0.35±0.02	0.34±0.01	0.33±0.02	0.28±0.01*	0.31±0.01	0.34±0.02
Na^+ (mmol/L)	145.25±0.59	141.75±0.62#	142.50±0.35	141.25±0.56	141.38±0.78	144.00±0.65
K^+ (mmol/L)	6.36±0.15	6.64±0.19	6.84±0.19	6.84±0.13	6.75±0.33	6.48±0.16
Cl^- (mmol/L)	96.76±0.49	99.05±0.96#	99.48±0.36	98.49±0.49	98.70±0.68	100.15±0.35

The reported values are the mean ± SEM (n=8). #Mean values were significantly different with control group (p<0.05). *Mean values were significantly different with HC group (p<0.05).

In addition, Table 4 showed that serum uric acid in the HC group was increased in week 4, while the HC+LD, HC+MD, and HC+AP groups decreased significantly compared to the HC group. The urine uric acid in the HC group was decreased than in the HC group in week 2, 4, and the HC+LD and HC+MD groups were significantly increased

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 November 2022 doi:10.20944/preprints202211.0088.v1

than in the HC group in week 4 and week 2,4 respectively. These results suggested that the administration of low (10^8 CFU/kg/rat) and medium (10^9 CFU/kg/rat) doses of TCI227 can decrease serum uric acid and increase urine uric acid, and TCI227 did not influence liver and kidney function.

Table 4. Effects of TCI227 on the serum uric acid and urine uric acid in PO induced hyperuricemia SD rats.

Groups	Control	HC	HC+LD	HC+MD	HC+HD	HC+AP
Serum uric acid (mg/dL)						
Week 0	4.05±0.60	4.41±0.60	4.11±0.87	4.04±0.98	4.08±0.60	4.27±0.79
Week 4	4.83±0.25	6.52±0.32#	4.96±0.63*	5.16±0.54*	5.22±0.89	1.12±0.38*
Urine uric acid (mg/dL)						
Week 0	7.41±1.07	7.68±1.16	6.55±0.19	7.70±0.99	7.86±1.25	7.64±0.90
Week 2	6.66±0.63	3.14±0.79#	4.48±0.89	5.21±1.01*	5.59±0.98	3.23±0.57
Week 4	6.39±0.50	3.91±0.60#	6.08±0.61*	5.41±0.25*	5.15±0.25	4.71±0.59

The reported values are the mean ± SEM (n=8). #Mean values were significantly different with control group (p<0.05). *Mean values were significantly different with HC group (p<0.05).

3.3. TCI227 increased SCFAs and diversity of intestinal microbiota

Studies had shown that intestinal bacteria can use dietary fiber to ferment into short chain fatty acids (SCFAs), such as acetic acid, propionic acid, butyric acid, and valeric acid[17]. SCFAs can provide energy for intestine cells, maintain intestinal mucosa integrity, inhibit harmful bacteria, and promote the growth of beneficial bacteria[18]. Next, we examined fecal SCFAs in PO-induced HC rats. Table 5 showed that acetic acid, butyric acid, and valeric acid in the HC group was significantly decreased compared to the control group. SCFAs in the HC+LD, HC+MD, and HC+HD groups were increased more than in the HC group. Then, we examined the diversity of the intestinal microbiota, and the Shannon's diversity index was used to estimate the level of community diversity[19].

Table 5. Effects of TCI227 on the fecal short-chain fatty acids (SCFAs) in PO induced hyperuricemia SD rats.

Groups	Control	HC	HC+LD	HC+MD	HC+HD	HC+AP
Acetic acid (μmol/g)	8.22±0.61	5.21±0.18#	7.17±0.82	5.56±0.60	5.34±0.21	4.67±0.43
Propionic acid (μmol/g)	2.62±0.35	1.79±0.15	2.30±0.27	1.97±0.25	1.97±0.08	1.84±0.19
Butyric acid (μmol/g)	8.60±1.55	4.14±0.46#	5.41±0.64	5.68±1.87	4.34±0.42	4.69±1.22
Valeric acid (μmol/g)	0.77±0.07	0.50±0.04#	0.50±0.05	0.55±0.07	0.51±0.02	0.46±0.04

The reported values are the mean ± SEM (n=8). #Mean values were significantly different with control group (p<0.05). *Mean values were significantly different with HC group (p<0.05).

Figure 1 showed that Shannon's diversity index in the HC group decreased slightly compared to the control group, and in the HC+LD and HC+MD groups increased slightly compared to the HC group. And through the principal coordinate analysis (Figure 2), we found a significant difference between the HC group and the control group in microbiota composition, and also found significant difference between the HC+LD, HC+MD, and HC+HD groups and the HC group. These results showed that HC reduced SCFAs and the diversity of intestinal microbiota, and TCI227 can recover this phenomenon, and regulate the composition of the microbiota.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 November 2022 doi:10.20944/preprints202211.0088.v1

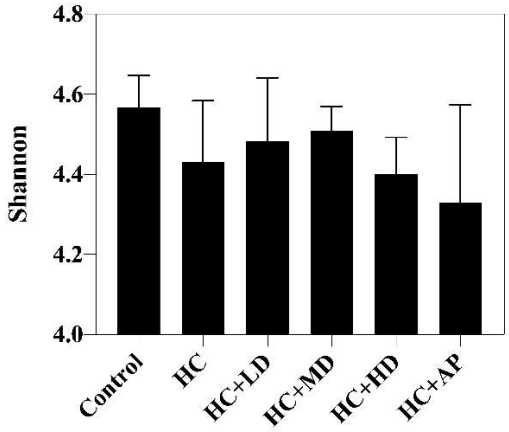


Figure 1. Alpha-diversity indexes of richness of fecal microbiota compositions in rats with PO induced hyperuricemia and fed TCI227. Shannon show species richness and evenness. The reported values are the mean ± SEM (n=3). The significant difference between all the groups was not detected.

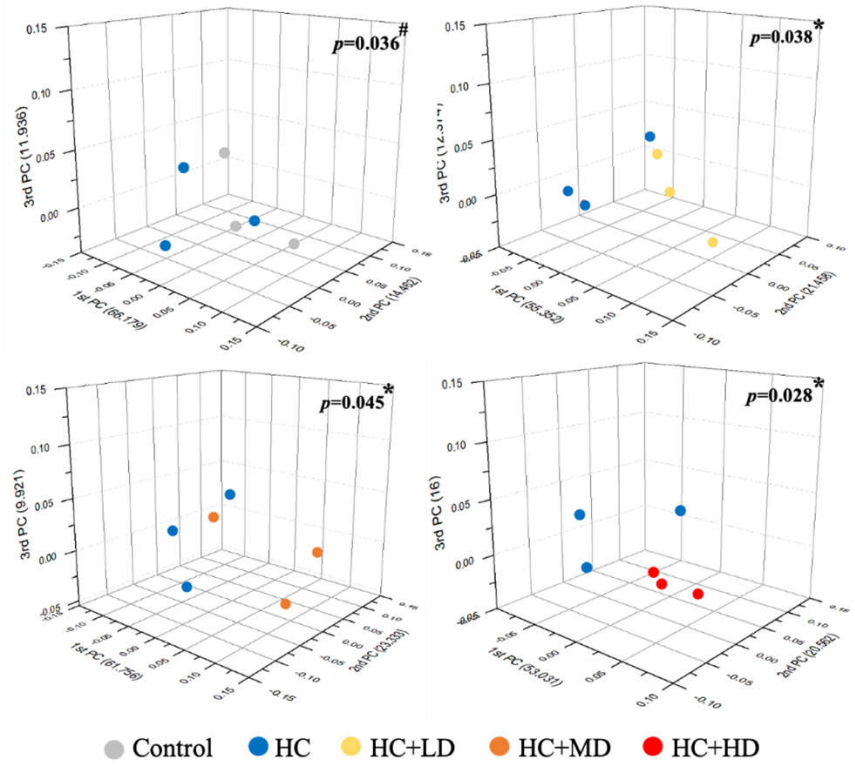


Figure 2. Effect of TCI227 on Principal coordinate analysis (PCoA) index level of fecal microbiota in rats with PO induced hyperuricemia. Based on the unweighted Jensen-Shannon distance measure of all samples based on OTU-level relative abundance profiles. Results are the means for n=3. #Mean values were significantly different with control group (p<0.05). *Mean values were significantly different with HC group (p<0.05). [Jensen-shannon, genus, exclude unclassified OTU (Reads)].

3.4. TCI227 changed the composition of the intestinal microbiota

Next, we want to explore whether TCI227 can change the composition of intestinal microbiota, using 16S microbiota ribosomal DNA sequencing. The eight most abundant fecal microbiota in phylum of the CTL, HC, HC+LD, HC+MD, HC+HD, and HC+AP groups were shown (Figure 3). The most abundant microbiota (phylum) were *Firmicutes* and *Bacteroridetes.* When the ratio of *Firmicutes*/*Bacteroidetes* or the number of *Deferribacteres* was increased, it can promote obesity or gout in mice[20, 21]. Figure 4 showed that the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 November 2022 doi:10.20944/preprints202211.0088.v1

relative abundance of *Deferribacteres* in the HC group was significantly increased than in the control group, while the HC+MD and HC+HD group can significantly decrease the relative abundance of *Deferribacteres* than in the HC group.

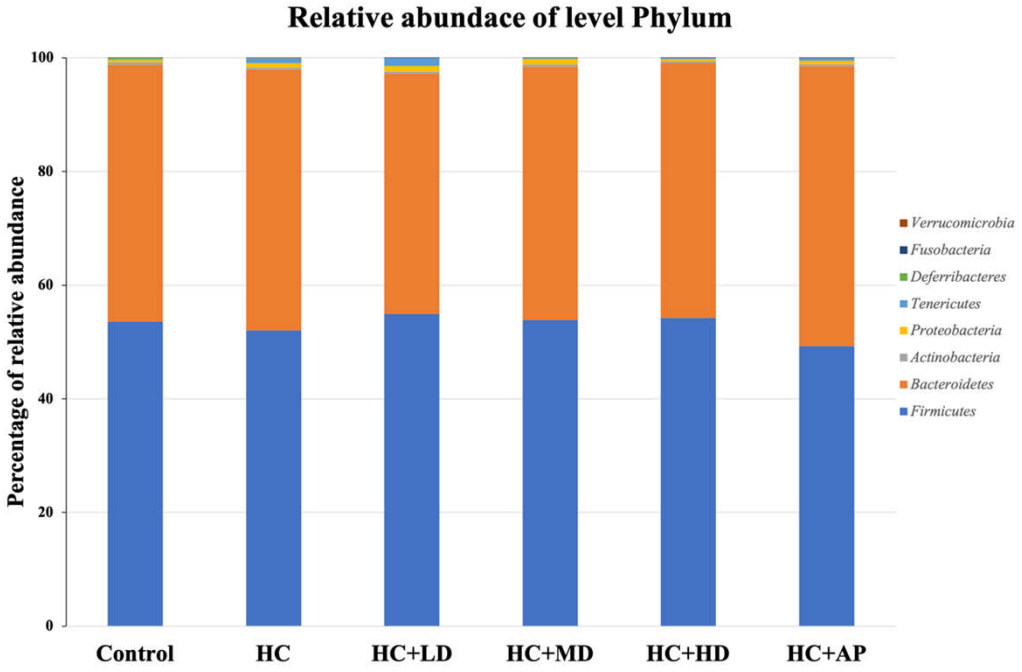


Figure 3. Effect of TCI227 on fecal microbiota phylum level compositions in PO induced hyperuricemia rats. The reported values are the mean (n=3).

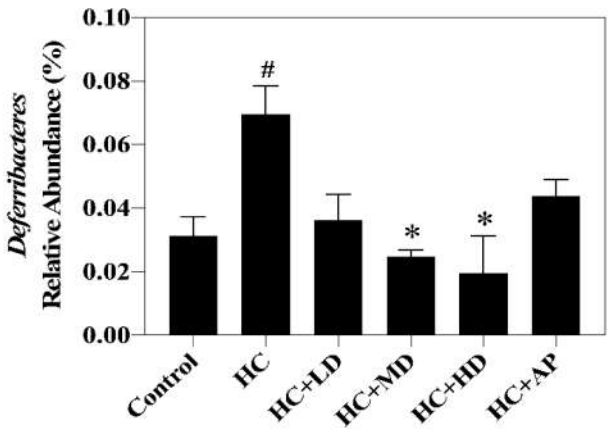


Figure 4. Effect of TCI227 on relative abundance of *Deferribacteres* in feces. The reported values are the mean ± SEM (n=3). #Mean values were significantly different with control group (p<0.05). *Mean values were significantly different with HC group (p<0.05).

In addition, the top 30 most abundant fecal microbiota family of the CTL, HC, HC+LD, HC+MD, HC+HD, and HC+AP groups were also shown (Figure 5). The most abundant microbiota (family) were *Muribaculaceaec* and *Ruminococcaceae*. Figure 6 showed that the relative abundance of *Prevotellaceae* of the HC group was significantly increased compared to the control group, and the HC+MD group can significantly decrease compared to the HC group. In addition, the relative abundance of *Lactobacillaceae* in the HC group decreased compared to the control group, and the HC+HD group can significantly increase. The relative abundance of *Deferribacteraceae* in the HC group increased compared to the HC group, and the HC+MD and HC+HD group can significantly decrease.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 November 2022 doi:10.20944/preprints202211.0088.v1

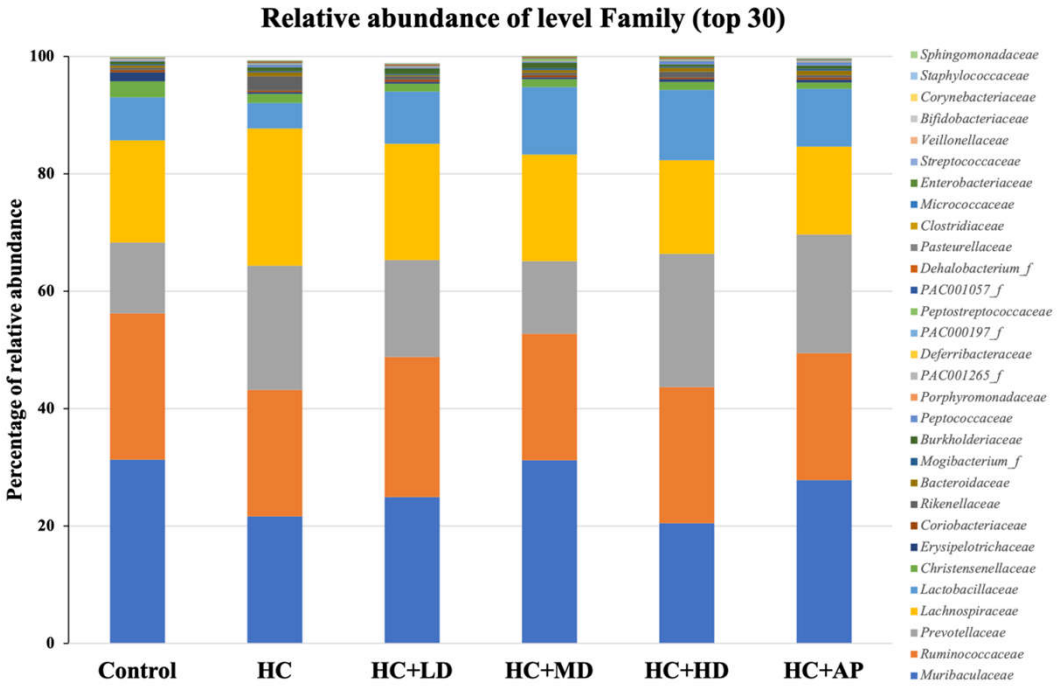


Figure 5. Effect of TCI227 on fecal microbiota family level compositions in PO induced hyperuricemia rats. The reported values are the mean (n=3).

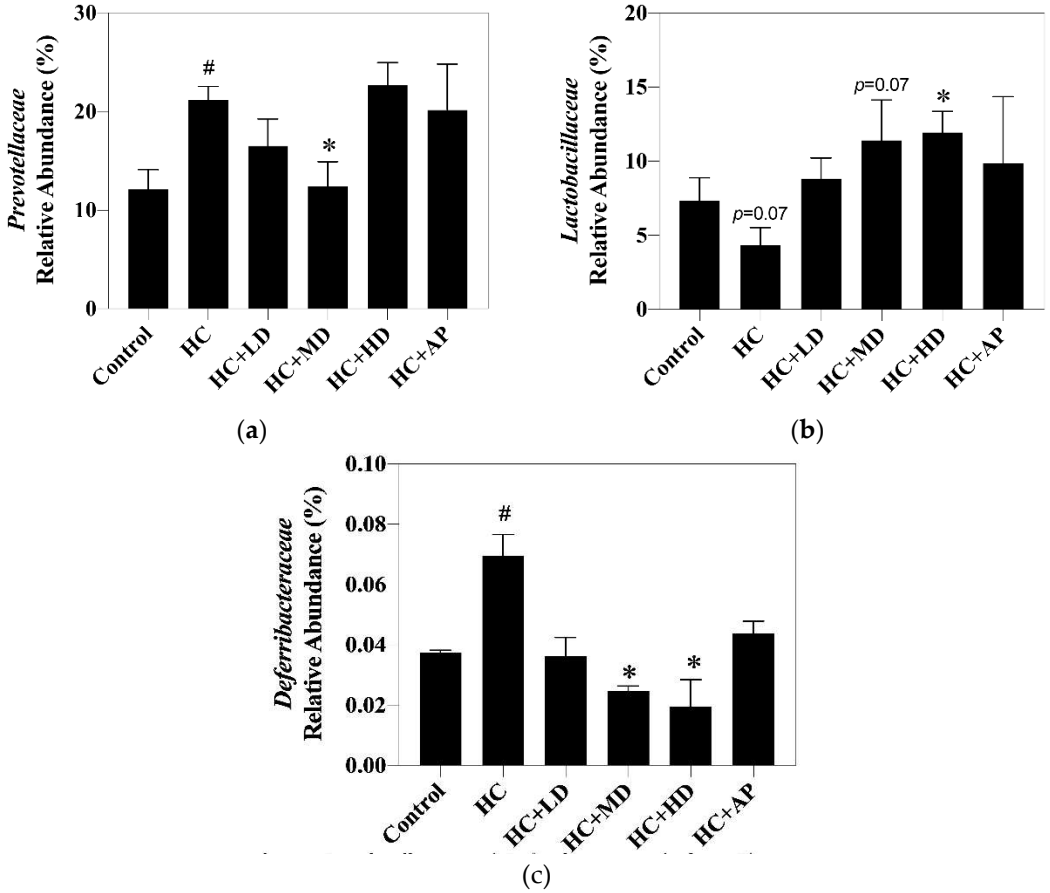


Figure 6. Effect of TCI227 on relative abundance of *Prevotellaceae* (A), *Lactobacillaceae* (B), and *Deferribacteraceae* (C) in feces. The reported values are the mean ± SEM (n=3). #Mean values were significantly different with control group (p<0.05). *Mean values were significantly different with HC group (p<0.05).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 November 2022 doi:10.20944/preprints202211.0088.v1

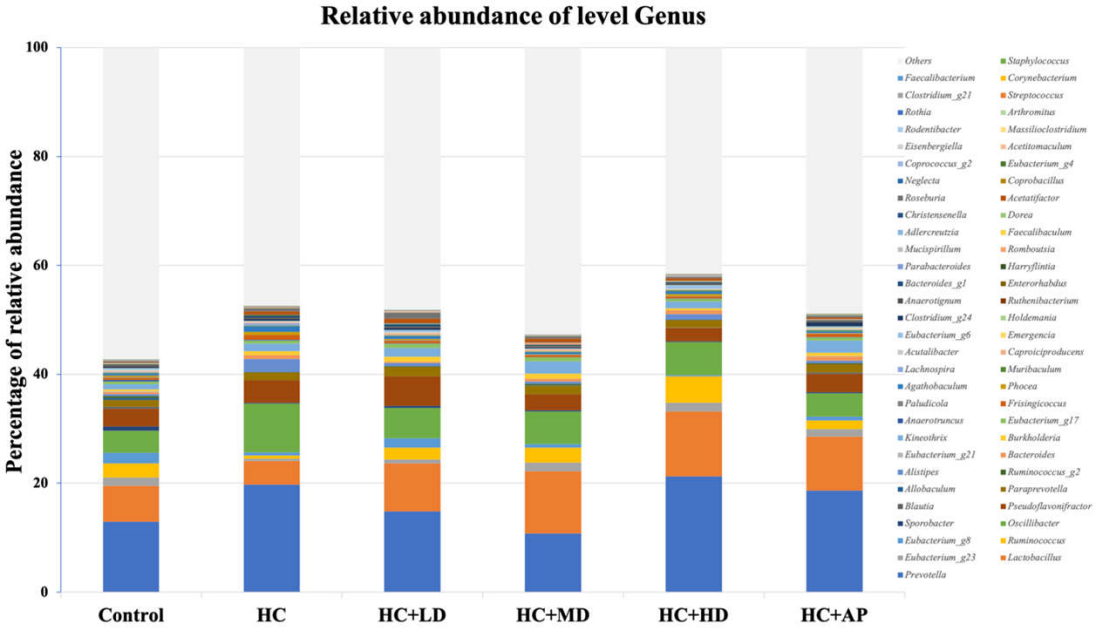


Figure 7. Effect of TCI227 on fecal microbiota genus level compositions in PO induced hyperuricemia rats. The reported values are the mean (n=3).

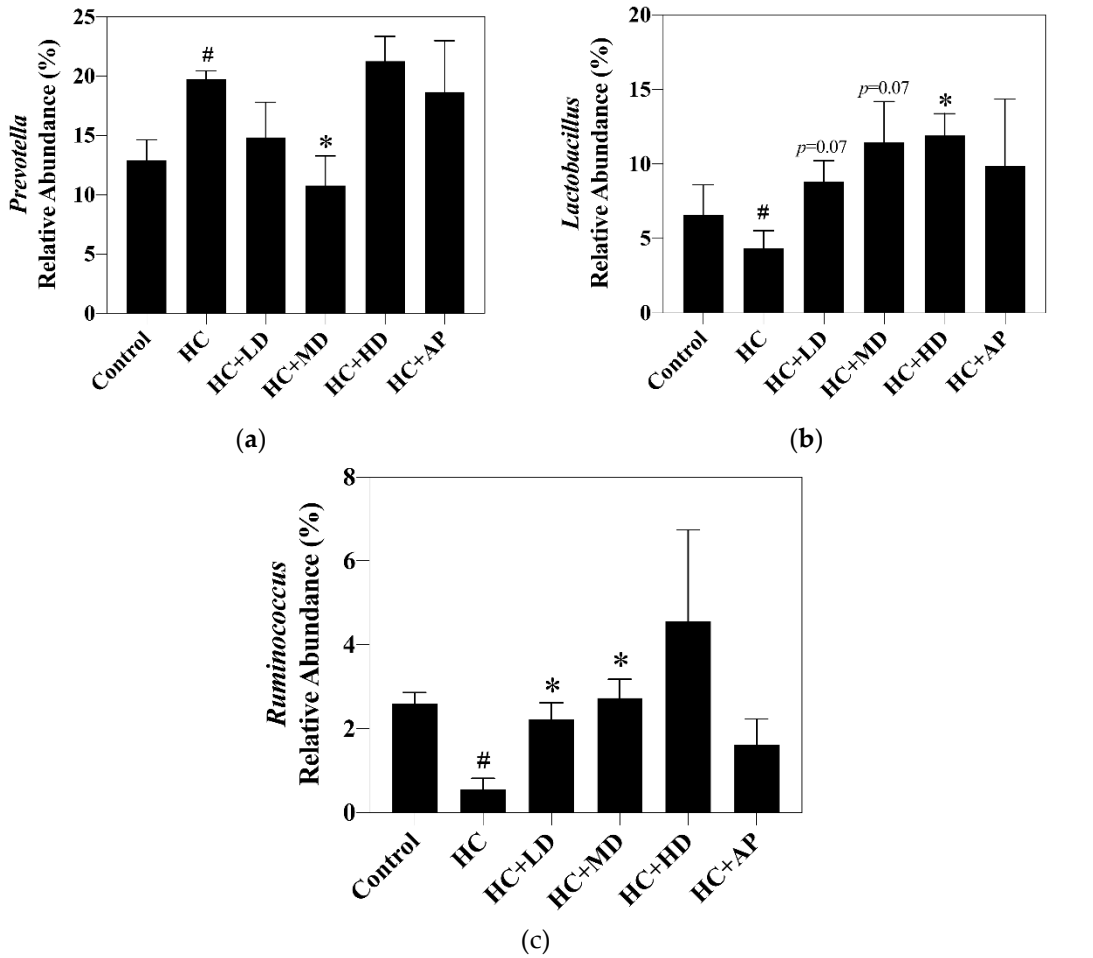


Figure 8. Effect of TCI227 on relative abundance of *Prevotella* (A), *Lactobacillus* (B), and *Ruminococcus* (C) in feces. The reported values are the mean ± SEM (n=3). #Mean values were significantly different with control group (p<0.05). *Mean values were significantly different with HC group (p<0.05).

 doi:10.20944/preprints202211.0088.v1

The major HC relative abundant fecal microbiota (genus) were *Prevotella*, *Oscillibacter*, and *Ruminococcus* (Figure 7). Figure 8 showed the relative abundance of *Prevotella* in the HC group increased significantly compared to the control group, and the HC+MD group could significantly decrease its abundance. The relative abundance of *Lactobacillus* in the HC group decreased in comparison to the control group, and the HC+HD group could significantly increase its abundance. Moreover, the relative abundance of *Ruminococcus* in the HC group was significantly decreased compared to the control group, and the HC+LD and HC+MD group can significantly increase its abundance. The above results indicated that TCI227 can change the composition of the intestinal microbiota.

4. Discussion

Most mammals contain uricase, which can metabolize uric acid as allantoin. However, several primates, including humans have lost the function of uricase enzyme activity. It's been reported that uricase gene display two stop codons and act as a pseudogene in human [22, 23]. Without the metabolic ability of uric acids, elevated uric acid levels cause accumulation of urate crystals and lead to gout and gouty arthritis. In addition, hyperuricemia is associated with other metabolic diseases, including hyperlipidemia, hypertension, cardiovascular diseases, and diabetes[24]. Until now, anti-hyperuricemia drugs have been identified, including the xanthine oxidase (XOD) inhibitors (allopurinol and febuxostat) and uricosuric agent (benzbromarone and probenecid) in clinical practice. Unfortunately, side effects limit the use of these drugs for hyperuricemia treatment[25, 26]. Therefore, in this study, the uricase inhibitor, which is the model of potassium oxonate treated rats[27], was performed with the probiotics TCI227 intervention to investigate a new improved strategy of hyperuricemia.

The previous study showed that the body weight of the PO-induced hyperuricemia mouse model was significantly lower than that of the control rats[28], which is similar to the Table 1 of this study. More precisely, not only body weight, but also the results of food intake and water consumption records are still within the physiological range of normal rats[29, 30]. In the organ weight result of Table 2, the liver and spleen of PO-induced rats were significantly heavier than the control group ($p < 0.05$), and the weight of the perirenal fat tissue of PO-induced rats was significantly lighter than the control group ($p < 0.05$). We speculated on two reasons for the increase in organ weight. First, the results of the histological section revealed some necrosis in peritoneal fat and an inflammation phenomenon after intraperitoneal injection of PO (data not shown), which is probably associated with the formation of urate and leads to foreign body, necrosis, and chronic granulomatous lesions. Second, the literature suggested that when an organ develops disease, and then the abundance of macrophages also increases[31]. Hence, after PO conducted the hyperuricemia phenotype of rats, the increase of macrophages of organs will result in an increase in organ weight.

Regarding to the toxicity test, the serum biochemical parameter analysis was performed as shown in Table 3. The concentrations of serum triglycerides, creatinine, sodium ion, and chloride ion in the hyperuricemia group significantly different compared to the control group ($p < 0.05$). All of these chemical parameters are within the physiological range of normal rats in other literatures[32, 33]. In brief, the TCI227 intervention did not cause liver injury, renal dysfunction, or electrolyte dysregulation. This evidence suggested that the TCI227 can be used as a safe oral health food to ameliorate hyperuricemia.

According to Table 4, the low and medium doses of the TCI227 intervention can significantly reduce the serum uric acid concentration of hyperuricemia rats ($p < 0.05$). Previous literature reports also suggested that oral gavage probiotics *Lactobacillus* (1.5×10^9 CFU/mL/day) from kimchi or ursolic acid (5 and 10 mg/kg) can improve blood uric acid concentration in PO-induced hyperuricemia rats[34, 35]. Again, studies have shown that in PO-induced hyperuricemia mice, giving Prunus mume fruit extract (140 mg/kg) for 1 week can increase urine uric acid excretion of mice[36]. These evidences suggested that

 doi:10.20944/preprints202211.0088.v1

supplemented with natural dietary supplements has the effect of ameliorating hyperuricemia.

Recently, many chronic diseases (eg. obesity, diabetes, and non-alcoholic fatty liver disease) have been associated with unbalanced dysregulation of intestinal flora-mediated inflammation[37]. A previous study indicated that the hyperuricemia rats with a high level of long-term urate concentration in the blood caused increased production of reactive oxygen species, and lead to activated NF-κB or other response of the inflammation pathway. Subsequently, it promotes the expression of the inflammation factors IL-1β and TNF-α expression, causing immune dysfunction and leaky gut syndrome. Then the exaggerated immune response and intestinal barrier dysfunction, which triggers hyperuricemia progression[34, 38].

The decrease in gut microbial diversity is always accompanied by an abnormal decreased in bacterial flora decrement and is associated with most human diseases[39]. Some studies suggested that supplement intake has the effect of elevated microbial diversity. Figure 1 showed that the PO-induced hyperuricemia rats reduced the abundance and α-diversity of the gut microbiota using Shannon's diversity index analysis ($p > 0.05$), after medium and low doses of TCI227 intervention, it can slightly increase the Shannon's diversity index and improve the decreased in microbiota diversity decrement ($p > 0.05$). Although this result was not significant, the trend was very similar to previous PO-induced hyperuricemia Kunming mice microbiota analysis. In that study, after the *Lactobacillus fermentum* JL-3 intervention, inflammatory markers and indicators of oxidative stress (IL-1β, MDA, CRE, and blood urea nitrogen) related to hyperuricemia improved and increased microbial diversity. Notably, another study has shown that the allopurinol intervention did not significantly improve the diversity of the gut flora ($p > 0.05$) in the high-fat diet containing 10% yeast extract induced hyperuricemia SD rats, which is very similar to our result in Figure 1[40].

In the analysis of the composition of microbiota phylum, Figure 4 suggested that the abundance of *Deferribacteres* was increased in PO-induced hyperuricemia rats fecal. Follow previous publications, the abundance of *Deferribacteres* is positively correlated with the concentration of IL-6 in plasma[41]. The pro-inflammatory factor IL-6 plays a crucial role in chronic inflammation diseases. Furthermore, past research has shown that in PO-induced hyperuricemia rats (250 mg/kg, rats) could be increased the IL-6 and other inflammation indicators[42]. In this study, the PO-induced hyperuricemia groups had a higher abundance of *Deferribacteres* ($p < 0.05$); The medium and high doses of the TCI227 intervention significantly reversed the abundance of *Deferribacteres* ($p < 0.05$). *Deferribacteres* was correlated with immune response, which is positive associated with the levels of IL-6, IL-2, and TNF-α in serum. In the mouse model with sepsis treated with *Lactobacillus rhamnosus GG* (2×10^9 CFU/mL) was improved intestinal permeability and microbiota dysbiosis. In that study, the abundance of *Deferribacteraceae* was reduced after *Lactobacillus rhamnosus GG*[43]. Then, in microbiota family composition analysis, the result was similar to other group findings[44]. The intestinal dysbiosis phenomenon was observed in hyperuricemia-induced rats. The abundance of *Prevotellaceae* was increased in Figure 6, which is significantly similar to the TCI227 intervention in this study ($p < 0.05$). Furthermore, the abundance of *Lactobacillaceae* was reduced, which corresponds to ICR mouse oral gavage PO by 250 mg/kg from another group[45]. In the analysis of the composition of microbiota genus, the abundance of *Prevotella* associated with chronic inflammation significantly increased in hyperuricemia rats ($p < 0.05$). The intervention of TCI227 can significantly reverse the abundance of *Prevotella* ($p < 0.05$) in Figure 7. According to a previous study[44], the abundance of *Lactobacillaceae* and *Rumminococcus* was decreased in hyperuricemia mice. Another study also suggested that the abundance of *Rumminococcus* decreased in mice inactivated with uricase[46]. Our result suggested that the abundance of *Lactobacillaceae* and *Rumminococcus* in hyperuricemia rats was lower than in control ($p < 0.05$), and this phenomenon was reversed by the TCI227 intervention. *Lactobacillaceae* and *Rumminococcus* can produce short-chain fatty acids (SCFAs), which can ameliorate kidney function.

 doi:10.20944/preprints202211.0088.v1

Hence, the increase in the abundance of *Lactobacillaceae* and *Rumminococcus* was facilitated in uric acid metabolism, and the intake of the TCI227 may improve hyperuricemia.

A previous study suggested that the intestinal microbiota can participate in purine and uric acid metabolism. On the other hand, SCFAs not only provide an energy resource for enterocytes, but also facilitated mucus secretion[47]. These bacterial fermentation products may play an important role in mucoprotection on gut health by supporting the integrity of the mucosal barrier integrity and prevented the systemic inflammatory response. In hyperuricemia rats, less SCFAs production bacterial abundance was observed, including *Alistipes*, *Lactobacillus*, and *Ruminococcus*, resulted in low SCFAs concentration mediated intestinal barrier dysfunction and increased intestinal permeability. In Table 5, the acetic acid, butyric acid, and valeric acid in the HC group was significantly lower than in the control group. After the TCI227 intervention, these SCFAs content were significantly increased ($p < 0.05$). No significant increase in SCFAs by the AP co-treatment group may be derived from the differently regulated mechanism between AP and TCI227 in the amelioration of hyperuricemia.

5. Conclusions

Supplementation of the TCI227 (low and medium dose, 10^8 and 10^9 CFU/kg rat) could be ameliorated the concentration of uric acid in serum, and excretion of uric acid in urea in PO-induced hyperuricemia rats. Besides, administration of the TCI227 not only increased the abundance of intestinal microbiota but also increased the concentrations of acetic acid, butyric acid, and valeric acid in PO-induced hyperuricemia rats. All these studies demonstrated that administration of the TCI227 improved hyperuricemia rats induced by potassium oxonate (PO).

Supplementary Materials: Table S1: The schematic of probiotics prevented hyperuricemia progression model.

Author Contributions: C.-L.H., Y.-H. L., and Y.-H. L. designed the experiments. C.-Y.C., Y.-J.C., S.-T. C., W.-C. H., H.-F. W., and C.-F. C. carried out the laboratory experiments. C.-L.H., C.-Y.C., Y.-J.C., S.-T. C., W.-C. H., H.-F. W., C.-F. C. analyzed the data, interpreted the results, prepared the figures and wrote the manuscript. C.-L.H., Y.-J.C., Y.-H. L., and Y.-H. L. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was sponsored by a grant from the TCI CO., Ltd.

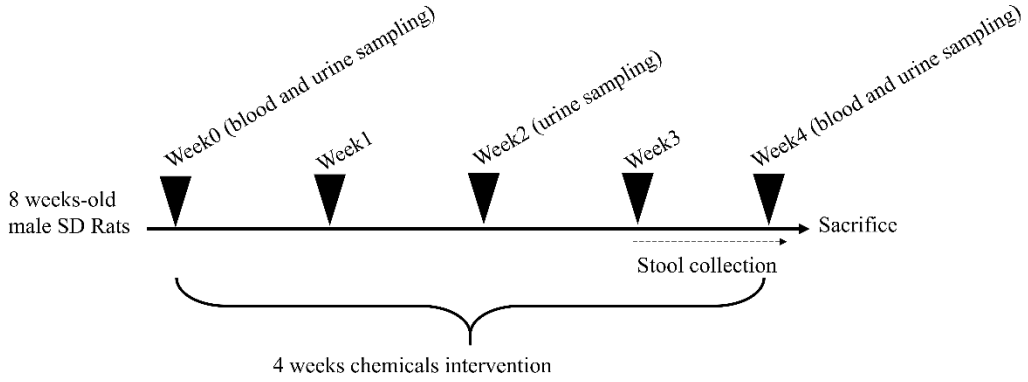
Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of the Chung Shan Medical University / Institutional Animal Care and Use Committee (IACUC approval no: 2413 and date of approval).

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets analyzed or generated during this study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A



(a) Control, C
(b) Hyperuricemia, HC
(c) hyperuricemia + Low dose TCI227 (Low dose, LD)
(d) hyperuricemia + Medium dose TCI227 (Medium dose, MD)
(e) hyperuricemia + High dose TCI227 (High dose, MD)
(f) hyperuricemia + allopurinol (HC+AP)

Supplement Table 1. The schematic of probiotics prevented hyperuricemia progression model.

References

1. Akram, M.; Asghar, M.N.; Saleem Khan, M.; Shahid, S.; Abdur Rahman, H.M.; Nadeem, I. Development and validation of an economical uric acid-Fe(3+)/Fe(2+)-ferrozine-based colorimetric assay to estimate uric acid level of pure and biological samples. *Biosci Biotechnol Biochem* **2020**, *84*, 1967-1974, doi:10.1080/09168451.2020.1781593.
2. Trinchieri, A.; Montanari, E. Biochemical and dietary factors of uric acid stone formation. *Urolithiasis* **2018**, *46*, 167-172, doi:10.1007/s00240-017-0965-2.
3. Mandal, A.K.; Mount, D.B. The molecular physiology of uric acid homeostasis. *Annu Rev Physiol* **2015**, *77*, 323-345, doi:10.1146/annurev-physiol-021113-170343.
4. Bardin, T.; Richette, P. Definition of hyperuricemia and gouty conditions. *Curr Opin Rheumatol* **2014**, *26*, 186-191, doi:10.1097/BOR.0000000000000028.
5. Torralba, K.D.; De Jesus, E.; Rachabattula, S. The interplay between diet, urate transporters and the risk for gout and hyperuricemia: current and future directions. *Int J Rheum Dis* **2012**, *15*, 499-506, doi:10.1111/1756-185X.12010.
6. Ben Salem, C.; Slim, R.; Fathallah, N.; Hmouda, H. Drug-induced hyperuricaemia and gout. *Rheumatology (Oxford)* **2017**, *56*, 679-688, doi:10.1093/rheumatology/kew293.
7. Kubota, M. Hyperuricemia in Children and Adolescents: Present Knowledge and Future Directions. *J Nutr Metab* **2019**, *2019*, 3480718, doi:10.1155/2019/3480718.
8. Guo, Z.; Zhang, J.; Wang, Z.; Ang, K.Y.; Huang, S.; Hou, Q.; Su, X.; Qiao, J.; Zheng, Y.; Wang, L., et al. Intestinal Microbiota Distinguish Gout Patients from Healthy Humans. *Sci Rep* **2016**, *6*, 20602, doi:10.1038/srep20602.
9. Wong, J.; Piceno, Y.M.; DeSantis, T.Z.; Pahl, M.; Andersen, G.L.; Vaziri, N.D. Expansion of urease- and uricase-containing, indole- and p-cresol-forming and contraction of short-chain fatty acid-producing intestinal microbiota in ESRD. *Am J Nephrol* **2014**, *39*, 230-237, doi:10.1159/000360010.
10. Hosomi, A.; Nakanishi, T.; Fujita, T.; Tamai, I. Extra-renal elimination of uric acid via intestinal efflux transporter BCRP/ABCG2. *PLoS One* **2012**, *7*, e30456, doi:10.1371/journal.pone.0030456.
11. Andres, M.; Sivera, F.; Falzon, L.; Buchbinder, R.; Carmona, L. Dietary supplements for chronic gout. *Cochrane Database Syst Rev* **2014**, CD010156, doi:10.1002/14651858.CD010156.pub2.
12. Hsu, C.L.; Hou, Y.H.; Wang, C.S.; Lin, S.W.; Jhou, B.Y.; Chen, C.C.; Chen, Y.L. Antiobesity and Uric Acid-Lowering Effect of Lactobacillus plantarum GKM3 in High-Fat-Diet-Induced Obese Rats. *J Am Coll Nutr* **2019**, *38*, 623-632, doi:10.1080/07315724.2019.1571454.
13. Kim, S.K.; Guevarra, R.B.; Kim, Y.T.; Kwon, J.; Kim, H.; Cho, J.H.; Kim, H.B.; Lee, J.H. Role of Probiotics in Human Gut Microbiome-Associated Diseases. *J Microbiol Biotechnol* **2019**, *29*, 1335-1340, doi:10.4014/jmb.1906.06064.
14. Xiao, Y.; Zhang, C.; Zeng, X.; Yuan, Z. Microecological treatment of hyperuricemia using Lactobacillus from pickles. *BMC Microbiol* **2020**, *20*, 195, doi:10.1186/s12866-020-01874-9.
15. Hongyan, L.; Suling, W.; Weina, Z.; Yajie, Z.; Jie, R. Antihyperuricemic effect of liquiritigenin in potassium oxonate-induced hyperuricemic rats. *Biomed Pharmacother* **2016**, *84*, 1930-1936, doi:10.1016/j.biopha.2016.11.009.

16. White, W.B.; Saag, K.G.; Becker, M.A.; Borer, J.S.; Gorelick, P.B.; Whelton, A.; Hunt, B.; Castillo, M.; Gunawardhana, L.; Investigators, C. Cardiovascular Safety of Febuxostat or Allopurinol in Patients with Gout. *N Engl J Med* **2018**, *378*, 1200-1210, doi:10.1056/NEJMoa1710895.
17. Morrison, D.J.; Preston, T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* **2016**, *7*, 189-200, doi:10.1080/19490976.2015.1134082.
18. Li, L.; Ma, L.; Fu, P. Gut microbiota-derived short-chain fatty acids and kidney diseases. *Drug Des Devel Ther* **2017**, *11*, 3531-3542, doi:10.2147/DDDT.S150825.
19. Jiang, H.; Ling, Z.; Zhang, Y.; Mao, H.; Ma, Z.; Yin, Y.; Wang, W.; Tang, W.; Tan, Z.; Shi, J., et al. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav Immun* **2015**, *48*, 186-194, doi:10.1016/j.bbi.2015.03.016.
20. Indiani, C.; Rizzardi, K.F.; Castelo, P.M.; Ferraz, L.F.C.; Darrieux, M.; Parisotto, T.M. Childhood Obesity and Firmicutes/Bacteroidetes Ratio in the Gut Microbiota: A Systematic Review. *Child Obes* **2018**, *14*, 501-509, doi:10.1089/chi.2018.0040.
21. Zhong, Y.; Nyman, M.; Fak, F. Modulation of gut microbiota in rats fed high-fat diets by processing whole-grain barley to barley malt. *Mol Nutr Food Res* **2015**, *59*, 2066-2076, doi:10.1002/mnfr.201500187.
22. Chang, B.S. Ancient insights into uric acid metabolism in primates. *Proceedings of the National Academy of Sciences of the United States of America* **2014**, *111*, 3657-3658, doi:10.1073/pnas.1401037111.
23. Kratzer, J.T.; Lanaspa, M.A.; Murphy, M.N.; Cicerchi, C.; Graves, C.L.; Tipton, P.A.; Ortlund, E.A.; Johnson, R.J.; Gaucher, E.A. Evolutionary history and metabolic insights of ancient mammalian uricases. *Proceedings of the National Academy of Sciences of the United States of America* **2014**, *111*, 3763-3768, doi:10.1073/pnas.1320393111.
24. Dalbeth, N.; So, A. Hyperuricaemia and gout: state of the art and future perspectives. *Annals of the Rheumatic Diseases* **2010**, *69*, 1738, doi:10.1136/ard.2010.136218.
25. Pacher, P.; Nivorozhkin, A.; Szabó, C. Therapeutic Effects of Xanthine Oxidase Inhibitors: Renaissance Half a Century after the Discovery of Allopurinol. *Pharmacological Reviews* **2006**, *58*, 87, doi:10.1124/pr.58.1.6.
26. Hao, S.; Zhang, C.; Song, H. Natural Products Improving Hyperuricemia with Hepatorenal Dual Effects. *Evidence-based complementary and alternative medicine : eCAM* **2016**, *2016*, 7390504, doi:10.1155/2016/7390504.
27. Sarvaiya, V.N.; Sadariya, K.A.; Pancha, P.G.; Thaker, A.M.; Patel, A.C.; Prajapati, A.S. Evaluation of antigout activity of Phyllanthus emblica fruit extracts on potassium oxonate-induced gout rat model. *Vet World* **2015**, *8*, 1230-1236, doi:10.14202/vetworld.2015.1230-1236.
28. Shi, H.; Liang, X.S.; Huang, L.W.; Luo, Z.G.; Tan, L. [The optimization and assessment of the method for inducing hyperuricemia in rats]. *Zhongguo ying yong sheng li xue za zhi = Zhongguo yingyong shenglixue zazhi = Chinese journal of applied physiology* **2020**, *36*, 223-227, doi:10.12047/j.cjap.5933.2020.049.
29. Piao, Y.; Liu, Y.; Xie, X. Change trends of organ weight background data in sprague dawley rats at different ages. *Journal of toxicologic pathology* **2013**, *26*, 29-34, doi:10.1293/tox.26.29.
30. Křížova, E.; Imek, V.S.; Abelenda, M.; Puerta, M. Food intake and body weight in rats with daily food-availability restrictions. *Physiology & Behavior* **1996**, *60*, 791-794, doi:https://doi.org/10.1016/0031-9384(96)00161-8.
31. Sica, A.; Erreni, M.; Allavena, P.; Porta, C. Macrophage polarization in pathology. *Cellular and molecular life sciences : CMLS* **2015**, *72*, 4111-4126, doi:10.1007/s00018-015-1995-y.
32. Lee, J.M.; Lee, M.A.; Do, H.N.; Song, Y.I.; Bae, R.J.; Lee, H.Y.; Park, S.H.; Kang, J.S.; Kang, J.K. Historical control data from 13-week repeated toxicity studies in Crj:CD (SD) rats. *Laboratory animal research* **2012**, *28*, 115-121, doi:10.5625/lar.2012.28.2.115.
33. Abubakar, S.; Sule, M.S.J.B.J.o.P.; Sciences, A. Effect of oral administration of aqueous extract of cassia occidentalis l. seeds on serum electrolytes concentration in rats. **2010**, *3*, 183-187.
34. Lv, Q.; Xu, D.; Zhang, X.; Yang, X.; Zhao, P.; Cui, X.; Liu, X.; Yang, W.; Yang, G.; Xing, S. Association of Hyperuricemia With Immune Disorders and Intestinal Barrier Dysfunction. *Frontiers in physiology* **2020**, *11*, 524236, doi:10.3389/fphys.2020.524236.
35. Abu-Gharbieh, E.; Shehab, N.G.; Almasri, I.M.; Bustanji, Y. Antihyperuricemic and xanthine oxidase inhibitory activities of Tribulus arabicus and its isolated compound, ursolic acid: In vitro and in vivo investigation and docking simulations. *PLoS One* **2018**, *13*, e0202572, doi:10.1371/journal.pone.0202572.
36. Yi, L.-T.; Li, J.; Su, D.-X.; Dong, J.-F.; Li, C.-F. Hypouricemic effect of the methanol extract from Prunus mume fruit in mice. *Pharm Biol* **2012**, *50*, 1423-1427, doi:10.3109/13880209.2012.683115.
37. Zmora, N.; Bashiardes, S.; Levy, M.; Elinav, E. The Role of the Immune System in Metabolic Health and Disease. *Cell metabolism* **2017**, *25*, 506-521, doi:10.1016/j.cmet.2017.02.006.
38. Ma, C.; Yang, X.; Lv, Q.; Yan, Z.; Chen, Z.; Xu, D.; Liu, X.; Yang, W.; Xing, S. Soluble uric acid induces inflammation via TLR4/NLRP3 pathway in intestinal epithelial cells. *Iranian journal of basic medical sciences* **2020**, *23*, 744-750, doi:10.22038/ijbms.2020.44948.10482.
39. Mosca, A.; Leclerc, M.; Hugot, J.P. Gut Microbiota Diversity and Human Diseases: Should We Reintroduce Key Predators in Our Ecosystem? *Frontiers in microbiology* **2016**, *7*, 455, doi:10.3389/fmicb.2016.00455.
40. Yu, Y.; Liu, Q.; Li, H.; Wen, C.; He, Z. Alterations of the Gut Microbiome Associated With the Treatment of Hyperuricaemia in Male Rats. *Frontiers in microbiology* **2018**, *9*, 2233-2233, doi:10.3389/fmicb.2018.02233.
41. Li, K.; Zhang, L.; Xue, J.; Yang, X.; Dong, X.; Sha, L.; Lei, H.; Zhang, X.; Zhu, L.; Wang, Z., et al. Dietary inulin alleviates diverse stages of type 2 diabetes mellitus via anti-inflammation and modulating gut microbiota in db/db mice. *Food & function* **2019**, *10*, 1915-1927, doi:10.1039/c8fo02265h.

42. Chen, R.J.; Chen, M.H.; Chen, Y.L.; Hsiao, C.M.; Chen, H.M.; Chen, S.J.; Wu, M.D.; Yech, Y.J.; Yuan, G.F.; Wang, Y.J. Evaluating the urate-lowering effects of different microbial fermented extracts in hyperuricemic models accompanied with a safety study. *Journal of food and drug analysis* **2017**, *25*, 597-606, doi:10.1016/j.jfda.2016.07.003.
43. Chen, L.; Li, H.; Li, J.; Chen, Y.; Yang, Y. Lactobacillus rhamnosus GG treatment improves intestinal permeability and modulates microbiota dysbiosis in an experimental model of sepsis. *International journal of molecular medicine* **2019**, *43*, 1139-1148, doi:10.3892/ijmm.2019.4050.
44. Xu, D.; Lv, Q.; Wang, X.; Cui, X.; Zhao, P.; Yang, X.; Liu, X.; Yang, W.; Yang, G.; Wang, G., et al. Hyperuricemia is associated with impaired intestinal permeability in mice. *American journal of physiology. Gastrointestinal and liver physiology* **2019**, *317*, G484-g492, doi:10.1152/ajpgi.00151.2019.
45. Han, J.; Wang, X.; Tang, S.; Lu, C.; Wan, H.; Zhou, J.; Li, Y.; Ming, T.; Wang, Z.J.; Su, X. Protective effects of tuna meat oligopeptides (TMOP) supplementation on hyperuricemia and associated renal inflammation mediated by gut microbiota. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2020**, *34*, 5061-5076, doi:10.1096/fj.201902597RR.
46. Guo, Y.; Yu, Y.; Li, H.; Ding, X.; Li, X.; Jing, X.; Chen, J.; Liu, G.; Lin, Y.; Jiang, C., et al. Inulin supplementation ameliorates hyperuricemia and modulates gut microbiota in Uox-knockout mice. *Eur J Nutr* **2021**, *60*, 2217-2230, doi:10.1007/s00394-020-02414-x.
47. Willemsen, L.E.M.; Koetsier, M.A.; van Deventer, S.J.H.; van Tol, E.A.F. Short chain fatty acids stimulate epithelial mucin 2 expression through differential effects on prostaglandin E(1) and E(2) production by intestinal myofibroblasts. *Gut* **2003**, *52*, 1442-1447, doi:10.1136/gut.52.10.1442.