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Partially Hydrolyzed Guar Gum Modulates Gut Microbiota, Regulates the Levels of Neurotransmitters, and Prevents CUMS-Induced Depressive-Like Behavior in Mice

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Scope: Depression is the leading cause of disability around the world; however, most antidepressants have drug tolerance and serious side effects. In this study, it is explored whether partially hydrolyzed guar gum (PHGG) is a safe food that exhibits protection in a mouse model of depression.

Methods and Results: PHGG is orally administered to mice with depression induced by chronic unpredictable mild stress (CUMS) in two animal experiments (prevention trial and intervention trial) to characterize the potentially protective effect of PHGG. The results in the prevention trial show that PHGG significantly inhibits the loss of body weight, and prevents CUMS-induced depressive-like behavior in mice. The beneficial effects may be associated with PHGG modulating the gut microbiota structure and then increasing the levels of short-chain fatty acids in mice feces and the levels of 5-hydroxytryptamine and dopamine in serum, striatum, and hippocampus. Besides, PHGG in the intervention trial is less effective than that in the prevention trial, but it may have a synergistic effect on improving depression with fluoxetine.

Conclusions: This study suggests that moderate daily intake of PHGG can contribute to relieving depressive-like behavior.

1. Introduction

Depression, as a chronic mental illness associated with heredity, psychology, biology, and environment, is the leading cause of disability around the world.[1] A report from the World Health Organization[2] shows that there were about 322 million people with depression in the world, and it is expected to be the largest contributor to disease burden by 2030. Nowadays, the development of most antidepressants used in the clinic, such as fluoxetine, paroxetine, and sertraline, is mainly based on the monoamine hypothesis, which was first put forward more than 50 years ago.[3] These drugs play an antidepressant role mainly by increasing the concentrations of 5-hydroxytryptamine (5-HT), norepinephrine, and dopamine (DA) in the synaptic space.[3] However, most of them are prone to having drug tolerance and serious side effects, such as nausea, insomnia, and sexual dysfunction.[4] Therefore, it is very necessary to find an effective and relatively safer way to treat or improve depression.

In recent years, increasing evidence demonstrated that mental illness was closely associated with the change of gut microbiota composition.[5,6] Zheng et al.[7] and Li et al.[8] transplanted the fecal bacteria respectively from depressed patients and depressed mice into the sterile mice, leading to depressive-like behavior in the mice. At the same time, some studies indicated that the depressive-like behavior was reversed in depressed mice by giving probiotics, such as *Clostridium butyricum* and *Lactobacillus kefiranofaciens* ZW3.[9,10] Based on the strong evidence, a theory regarding the microbiota-gut-brain axis is proposed for stating that there may be an important relationship between the change of gut microbiota and depression. Furthermore, some studies found that patients with depression have lower levels of probiotics in the intestine,[11] which results in a low concentration of short-chain fatty acids (SCFA).[12] And one study points out that SCFA can increase the concentration of 5-HT,[13] which may contribute to improving depression. Thus, it may be a potentially effective way to prevent or treat depression by modulating the gut microbiota using prebiotics.

Partially hydrolyzed guar gum (PHGG) as the hydrolysis product of guar gum is considered to be a functional food[14] without toxic side effects, because it has some beneficial health functions, such as decreasing symptoms in constipation-predominant and diarrhea-predominant forms of irritable bowel syndrome,[15] lowering the rate of diarrhea occurrence in patients with the total as well as supplemental enteral nutrition,[16] and promoting gut health.[17] PHGG could not be digested and absorbed in

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mammals, and is easy to reach the large intestine, and is fermented by colonic bacteria, which can support the growth of probiotics[18,19] and result in a large number of SCFA productions[20] in the intestine. Therefore, it is supposed that PHGG supplementation may prevent or treat depression by promoting the growth of intestinal probiotics, increasing the concentration of SCFA, and then regulating the concentration of 5-HT in the brain, which is associated with depression.

In this study, prevention and intervention trials were carried out to investigate the antidepressant effects of PHGG in mice. Chronic unpredictable mild stress (CUMS) was used to establish the mice model of depression, and CUMS-induced depressive-like behavior was evaluated by sucrose preference test (SPT), forced swimming test (FST), and open field test (OFT). Next, we analyzed the changes of intestinal flora by 16S sequencing and then determined the concentration of SCFA and neurotransmitters in mice.

2. Experimental Section

2.1. Chemicals

PHGG was obtained from Taiyo Kagaku Co., Ltd. (Tokyo, Japan). Fluoxetine and standards including lactic acid, acetic acid, propionic acid, butyric acid, isovaleric acid, valeric acid, 5-HT, and DA were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China) and Sigma-Aldrich (St. Louis, MO, USA). Chromatography grade methanol and acetonitrile were provided by Merck (Darmstadt, Germany), and other reagents used were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Characterization of PHGG

Viscosity was analyzed using a viscometer (Brookfield, WI,USA) with spindle no. 61 and no. 64 at 100 rpm and 25 °C temperatures. Nuclear magnetic resonance spectra of PHGG were recorded on a Bruker 400 MHz spectrometer using D_2O as the solvent. The average molecular weight of PHGG was calculated by the Mark–Houwink–Sakurada equation ($[\eta] = KM^{\alpha}$). The intrinsic viscosity was measured as described[21] using an Ubbelohde viscometer and the K and α were corrected by dextran with different molecular weights.

2.3. Animal Treatments

All experimental protocols employed herein were approved by the Committee on the Care of Laboratory Animal Resources, College of Biological Science and Engineering, Fuzhou University (2019-SG-007). Male SPF C57BL/6 mice (age 5 weeks, 18–20 g) were purchased from Shanghai Ling Cheong Biotechnology Co., Ltd. (Shanghai, China). Mice were kept in a room with a controlled temperature of 23 ± 1 °C and humidity of 50 ± 1%, under a reversed 12 h light/dark cycle (lights on 08:00–20:00 h) with ad libitum access to food and water, except during the experiments. Animals were first acclimated to the lab for 1 week before the start of the experiments.

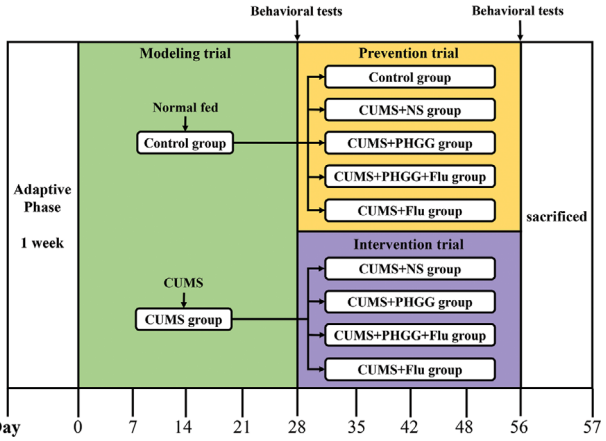

Figure 1. Schematic diagram of the prevention and intervention trial, respectively. For the prevention trial, mice were first fed normally for 28 days, then divided into five groups and subjected to different trials for another 28 days. For the intervention trial, mice were first treated with CUMS for 28 days, then divided into four groups and subjected to different trials (CUMS+NS: normal saline; CUMS+Flu: 1 mg kg^{-1} bw^{-1} fluoxetine; CUMS+PHGG: 600 mg kg^{-1} bw^{-1} PHGG; CUMS+PHGG+Flu: 600 mg kg^{-1} bw^{-1} PHGG and 0.5 mg kg^{-1} bw^{-1} fluoxetine).

As shown in **Figure 1**, 60 mice were randomly divided into two groups for mouse modeling ($n = 30$ per group). One was given CUMS procedure as the CUMS group, and the other was feed normally as the control group. After 28 days, both the CUMS group and the control group were subjected to a series of behavioral tests: SPT, OFT, and FST. Then the mice in the control group were used for the prevention trial, while the mice in CUMS group were used for the intervention trial. After that, mice were confronted with behavioral tests and then were dissected to collect serum, striatum, and hippocampus, which were immediately frozen and stored at −80 °C for the measurement of neurotransmitters concentrations. The body weight and sucrose preference were measured every week, and mice feces were collected every week and stored at −80 °C in the experimental process.

2.4. Dosage Information

In the prevention trial, the mice were housed in five groups: Control group: mice were fed normally for 28 days. CUMS+NS group: mice were given normal saline (NS) orally with CUMS for 28 days. CUMS+PHGG group: mice were given PHGG (600 mg $kgbw^{-1}$ day^{-1}) orally with CUMS for 28 days. CUMS+PHGG+Flu group: mice were given PHGG (600 mg $kgbw^{-1}$ day^{-1}) and fluoxetine (0.5 mg $kgbw^{-1}$ day^{-1}) orally with CUMS for 28 days. CUMS+Flu group: mice were given fluoxetine (1.0 mg $kgbw^{-1}$ day^{-1}) orally with CUMS for 28 days.

In the intervention trial, the mice were housed in four groups: CUMS+NS group: mice were given NS orally with CUMS for 28 days. CUMS+PHGG group: mice were given PHGG (600 mg $kgbw^{-1}$ day^{-1}) orally with CUMS for 28 days. CUMS+PHGG+Flu group: mice were given PHGG (600 mg $kgbw^{-1}$ day^{-1}) and fluoxetine (0.5 mg $kgbw^{-1}$ day^{-1}) orally with CUMS for 28 days. CUMS+Flu group: mice were given fluoxetine (1.0 mg $kgbw^{-1}$ day^{-1}) orally with CUMS for 28 days.

The dose of PHGG (600 mg kg^{-1}) was based on the best dose obtained from the previous human in vitro fermentation trial (data not shown) and the dose used in other research,[19] and then converted into the equivalent dose in mouse based on the body surface area.[22]

2.5. CUMS Procedure

The CUMS procedure was performed as previously described[23,24] with some modifications. The mice were exposed to different kinds of stressors several times a day for 4 weeks in a chronic, inevitable, and unpredictable way. The stressors included confinement in a tube for 4 h, cage tilting (45 °C) for 11 h, tail nipping for 2 min, swimming in ice water (4 °C) or hot water (47 °C) for 5 min, damp sawdust for 5 h, removing the sawdust for 5 h, deprivation of food and water for 24 h, exposure to 85 dB noise for 4 h, and inversion of the light/dark cycle. The stressor sequence was changed every week to make the stress procedure unpredictable.

2.6. Behavioral Tests

SPT, FST, and OFT were used to evaluate the change of interest, behavioral despair, the level of spontaneous motor activities, and investigative behavior. The whole process of all behavioral experiments was monitored by two trained observers blinded to the study group assignment.

The SPT was performed as a description of the previous report.[25,26] Briefly, 2 days before the test, the mice were adapted to sugar water by giving two bottles of 1% sucrose water at the same time in each cage. After 24 h (8:00 to the second day at 8:00), two bottles of 1% sucrose water were changed to a bottle of 1% sucrose water and a bottle of pure water. The position of two bottles was exchanged every half an hour for 3 h before the test. Twenty-four hours later, the consumption of sucrose water and pure water was measured. The sucrose preference = sucrose consumption/(sucrose consumption + water consumption) × 100%.

The FST was performed as previously described.[27] In brief, mice were individually placed in a beaker (30 cm in height × 15 cm in diameter) filled with 15 cm ultrapure water (23 ± 2 °C). After 2 min adaptation period, mice were forced to swim for 4 min, and the immobility time-related behaviors were recorded. Immobility time was defined as the time when mice float on the surface of the water with slight movement or when the body was perpendicular to the surface with only the nose out of the water.

The testing apparatus of OFT was a box (45 × 45 × 40 cm) which was divided into 25 equal squares at the bottom and was placed in a quiet and suitable lighting environment. The day before the test mice were put into the box for 15 min to adapt to the environment. In the test, the mice were placed in the center of the apparatus followed by 2 min of adaptation, and behaviors were recorded for the next 4 min using recording equipment. The number of squares crossed, the number of rearing, and time in the center (the time mice stayed in the central square) were counted to reflect the free activities, exploration ability, and anxiety level of mice, respectively.

2.7. Fecal SCFA Detection

Fecal SCFA was measured using high-performance liquid chromatography (HPLC) according to a previously described method[28] with some modifications. Mice feces were dissolved with 1.8×10^{-3} M sulfuric acid at 10 μL mg^{-1}. After 2 h of ultrasonic treatment, the mixed liquid was centrifuged for 10 min at 15 000 rpm at 4 °C, then supernatants were syringe filtered with 0.22×10^{-6} M filters (Corning). The concentrations of SCFA were measured by HPLC (Thermo, MA, USA) with Column Acclaim TM Organic Acid C18 (5 μm, 250 × 4.0 mm) (SN: 002227) at a column temperature of 30 °C and wavelength of 210 nm. Samples (20 μL) were injected into the HPLC, the autosampler was set at a temperature of 6 °C.

2.8. DNA Extraction from Fecal Samples and 16S rRNA Sequencing

Total DNA was extracted using the DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocols. DNA samples were used for the analysis of fecal microbiota using Illumina HiSeq technology at Biomarker Technologies Co., Ltd. (Beijing, China). The V3–V4 region of the bacteria 16S ribosomal RNA genes was amplified by PCR using primers designed according to the conserved region with an adapter at the end. Amplification products were purified, quantified, and homogenized to form a sequencing library, which was sequenced by Illumina HiSeq 2500 after passing the quality inspection. The original image data files obtained by high-throughput sequencing (such as Illumina HiSeq platform) were transformed into the sequenced reads by base calling analysis. Sequenced reads in each sample were merged into longer raw tags according to their overlap using Flash v1.2.7 software. Raw tags were quality-filtered using Trimmomatic v0.33 software to obtain clean tags, and then chimeras were removed using UCHIME v4.2 software to get effective tags. The effective tags were clustered to generate operational taxonomic units (OTUs) at the 97% similarity level, using UCLUST in QIIME (version 1.8.0) software. Microbial community composition in each sample at a different level was calculated according to corresponding species classification information of each OTU, which was obtained by comparing the representative sequences of OTUs with the microbial database. The a-diversity indexes including ACE index, Chao1 index, Simpson, and Shannon indexes were evaluated using Mothur (version v.1.30) software.

2.9. Measurement of DA and 5-HT in Brain Tissues and Serum

The detection of DA and 5-HT was performed according to the procedure described previously.[29] The samples of hippocampus and striatum were minced, suspended in the chilled homogenizing buffer, and then were homogenized using a 10 mL Dounce type homogenizer. After putting on ice for 30 min, samples were centrifuged at 15 000 r min^{-1} for 15 min at 4 °C. The collected supernatants were mixed with the same volume of perchloric acid solution (5%). The mixed solutions were centrifuged at 10 000 g for 15 min at 4 °C. Finally, 20 μL aliquots of the filtered

supernatants were used for the analysis. The levels of DA and 5-HT were measured by HPLC (Hitachi, Tokyo, Japan) coupled with FLD (Hitachi, Tokyo, Japan) and Column Hypersil GOLD C18 (5 μm, 250 × 4.6 mm) (SN: 10512640) with emission wavelength at 330 nm, excitation wavelength at 280 nm, and isocratic elution at the current speed of 1.0 mL min^{-1}.

2.10. Real-Time RT-PCR

Total RNA was isolated from mouse intestinal tissue using TRIzol reagent (Invitrogen, USA) following the manufacturer's instructions. cDNA was synthesized by reverse transcription kit (TransGen Biotech, China). Real-time PCR was performed using SYBR Green qPCR Supermix (TransGen Biotech, China). Primers (Table S1, Supporting Information) were used to screen the mRNA expression of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH). The internal control was GAPDH.

2.11. Statistical Analysis

SPSS statistical package (SPSS, Chicago, IL, USA) was used to perform statistical analysis. Levene's test was used to assess the equality of variances of data. If the data showed the equality of variances, Student's *t*-test was applied for comparisons between averages of two samples; if the data did not show the equality of variances, the data were transformed to restore equal variances. The minimal level of significance chosen was $p < 0.05$.

3. Results

3.1. CUMS Increased Depressive-Like Behaviors and Decreased Body Weight in Mice

As shown in **Figure 2**A, the body weight in the CUMS-induced mice was significantly lower than that of the control at 28 days. Regarding sucrose preference (Figure 2B), the mice from CUMS group showed a dramatic reduction in relative sucrose intake at 28 days, and a significant difference in the control and CUMS groups was observed from the start of 14 days (Figure S1, Supporting Information). Figure 2C–F showed that the CUMS-induced had a significant increase of immobility time and decrease of time in center, the number of squares crossed and rearing when compared with the control.

3.2. PHGG Affected Body Weight and Behaviors in Depressed Mice

In both prevention trial and intervention trial (**Figure 3**A–J), daily intake of PHGG (M_w = ~33 kDa, Figure S2, Supporting Information), Flu, and PHGG+Flu for 28 days significantly reduced the CUMS-induced weight loss, the increase of immobility time (no effect of PHGG and Flu in the intervention trial), and the decrease of sucrose consumption, time in center, and number of

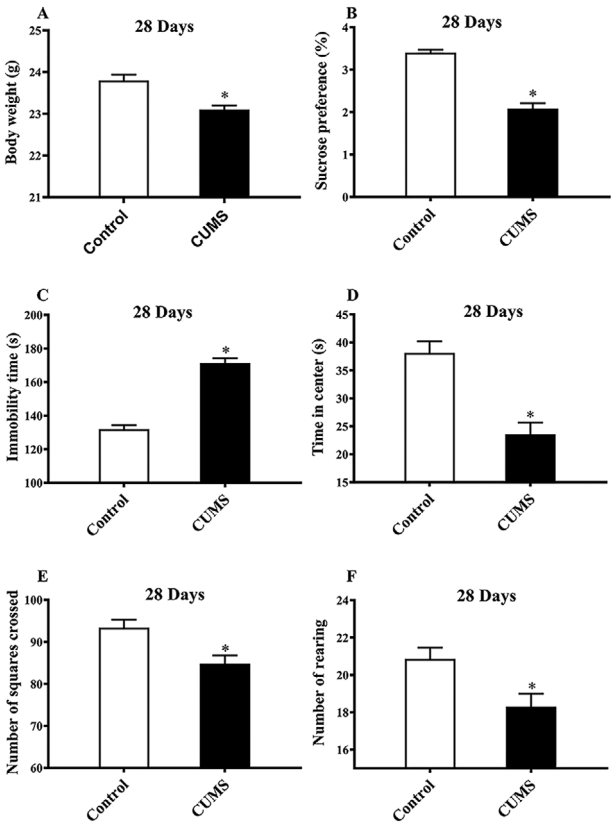

Figure 2. Changes of body weight and depressive-like behavior in mice treated with CUMS for 4 weeks. A) Body weight changes. B) Sucrose preference test. C) Forced swimming test. D) Time in the center in the open field test. E) Crossing number in the open field test. F) Rearing number in the open field test. *$p < 0.05$, based on the Student's *t*-test, and CUMS group was significantly different from the controls. Data are shown as mean ± SD. The number of mice in each group is 30.

squares crossed in mice. As to the number of rearing, significant improvements were observed in the prevention trial (Figure 3K), but not in the intervention trial (Figure 3L). The results indicated that PHGG could prevent weight loss and depressive-like behaviors caused by CUMS in mice. The observed protective effects of PHGG in mice, such as improvements in weight loss and sucrose preference, firstly appeared at 49 days in the prevention trial (Figures S3C and S4E, Supporting Information) and at 42 days in the intervention trial (Figures S3B and S4D, Supporting Information).

3.3. PHGG Affected the SCFA Concentration in Depressed Mice

As shown in Table S2, Supporting Information, CUMS-induced mice had significantly lower concentrations of lactic acid (at 7, 14, 21, and 28 days), acetic acid (at 28 days), and valeric acid (at 21 and 28 days) in feces than those of the control, but no significant differences were found in propionic acid, butyric acid, and isovaleric acid before 28 days. However, as mice got older, CUMS-induced decreases of propionic acid, butyric acid, and isovaleric acid concentrations were found at 7, 14, and 21 days, respectively (**Table 1**).

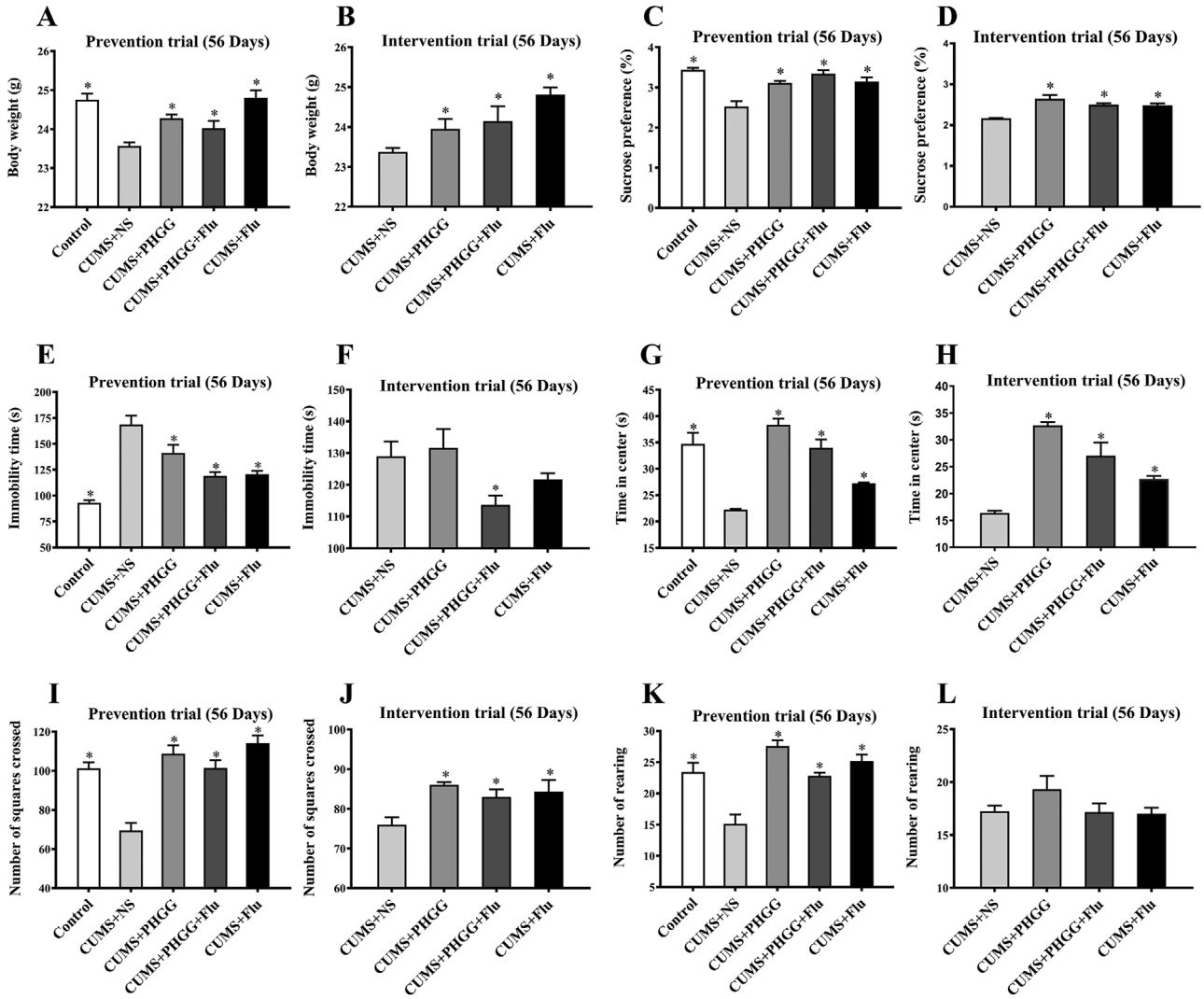

Figure 3. Effects of daily intake of PHGG, Flu, and a mixture of PHGG and Flu on the body weight and depressive-like behavior in mice subjected to CUMS. A,B) Bodyweight changes. C,D) Sucrose preference test. E,F) Forced swimming test. G,H) Time in the center in the open field test. I,J) Crossing number in the open field test. K,L) Rearing number in the open field test. $*p < 0.05$, based on the Student's *t*-test, and significantly different from the CUMS+NS groups. Data are shown as mean ± SD. The number of mice in each group in the prevention trial is six; the number of mice in each group in the intervention trial is seven.

Effects of PHGG, Flu, and the mixture of PHGG and Flu on the SCFA concentration in mice feces in the prevention trial and intervention trial were summarized in **Tables** 1 and **2**, respectively. In the prevention trial, daily intake of PHGG, Flu, and PHGG+Flu significantly increased the concentrations of lactic acid, acetic acid, propionic acid, and valeric acid when compared with the CUMS+NS group. Moreover, PHGG and PHGG+Flu might also prominently elevate the concentrations of butyric acid and isovaleric acid, but not in the Flu group. On the other hand, after Flu or PHGG+Flu intervention, all SCFA concentrations were improved significantly when compared with CUMS+NS group in an intervention trial. However, PHGG intervention had a significant increase in only propionic acid. Moreover, the concentration of lactic acid was increased by Flu or PHGG+Flu intervention at 49 days, but less effect at 56 days.

3.4. PHGG Modulated Gut Microbiota in Depressed Mice

Species richness, reflected by ACE and Chao 1 indexes, was lower in samples from CUMS+NS group than that of the control in the prevention trial, and the change was significantly inhibited by daily intake of PHGG and PHGG+Flu (**Figure 4**A,C). Species diversity, expressed as Simpson and Shannon indexes, showed no significant difference between the CUMS+NS group and the control in the prevention trial (Figure 4E,G). However, the Simpson index was markedly decreased and the Shannon index was increased by daily intake of PHGG and PHGG+Flu in the prevention trial. Similar results were observed in species richness and species diversity in the intervention trial (Figure 4B,D,F,H). Additionally, daily intake of Flu had no significant effects on ACE, Chao 1, Simpson, and Shannon indexes.

Table 1. Effects of daily intake of PHGG, Flu, and a mixture of PHGG and Flu on the SCFA concentrations of mice feces in the prevention trial.

Day	Group	Lactic acid [mmoL g^{-1}]	Acetic acid [mmoL g^{-1}]	Propionic acid [mmoL g^{-1}]	Butyric acid [μmoL g^{-1}]	Isovaleric acid [μmoL g^{-1}]	Valeric acid [μmoL g^{-1}]
35	Control	0.81 ± 0.08	0.54 ± 0.05	0.43 ± 0.07*	1.09 ± 0.05	36.72 ± 6.67	4.23 ± 0.42*
	CUMS+NS	0.80 ± 0.12	0.53 ± 0.05	0.33 ± 0.09	1.01 ± 0.11	31.41 ± 8.13	3.37 ± 0.58
	CUMS+PHGG	0.86 ± 0.05	0.50 ± 0.07	0.43 ± 0.04*	1.05 ± 0.19	34.72 ± 2.51	3.75 ± 1.05
	CUMS+PHGG+Flu	0.83 ± 0.08	0.55 ± 0.05	0.40 ± 0.06*	1.02 ± 0.03	32.56 ± 7.83	3.39 ± 1.30
	CUMS+Flu	0.84 ± 0.09	0.57 ± 0.05	0.40 ± 0.04*	1.04 ± 0.32	32.87 ± 3.50	3.81 ± 0.85
42	Control	0.57 ± 0.13*	0.38 ± 0.09*	0.41 ± 0.09*	1.01 ± 0.05*	32.78 ± 1.61	3.89 ± 0.63*
	CUMS+NS	0.36 ± 0.06	0.24 ± 0.05	0.31 ± 0.04	0.78 ± 0.05	29.63 ± 7.32	2.70 ± 0.20
	CUMS+PHGG	0.44 ± 0.09*	0.27 ± 0.11	0.41 ± 0.02*	0.94 ± 0.10*	30.02 ± 2.10	2.74 ± 0.43
	CUMS+PHGG+Flu	0.57 ± 0.08*	0.28 ± 0.15	0.39 ± 0.08*	0.86 ± 0.23	32.96 ± 2.96	2.63 ± 0.31
	CUMS+Flu	0.52 ± 0.04*	0.29 ± 0.06	0.43 ± 0.06*	0.71 ± 0.14	33.03 ± 4.62	3.06 ± 1.06
49	Control	0.48 ± 0.12*	0.29 ± 0.05*	0.40 ± 0.03*	1.01 ± 0.06*	29.98 ± 3.84*	3.95 ± 0.14*
	CUMS+NS	0.23 ± 0.05	0.19 ± 0.02	0.32 ± 0.03	0.71 ± 0.07	22.61 ± 5.45	2.34 ± 0.24
	CUMS+PHGG	0.32 ± 0.14*	0.30 ± 0.06*	0.41 ± 0.06*	0.87 ± 0.19*	25.07 ± 7.66	3.01 ± 0.22*
	CUMS+PHGG+Flu	0.41 ± 0.09*	0.27 ± 0.09*	0.43 ± 0.04*	0.84 ± 0.12*	23.36 ± 2.95	3.64 ± 0.57*
	CUMS+Flu	0.55 ± 0.06*	0.27 ± 0.07*	0.41 ± 0.02*	0.77 ± 0.13	25.64 ± 7.87	3.18 ± 0.71*
56	Control	0.36 ± 0.09*	0.20 ± 0.03*	0.41 ± 0.04*	0.98 ± 0.08*	26.83 ± 2.97*	3.90 ± 0.74*
	CUMS+NS	0.13 ± 0.03	0.10 ± 0.02	0.19 ± 0.03	0.57 ± 0.03	19.27 ± 2.04	1.68 ± 0.27
	CUMS+PHGG	0.28 ± 0.08*	0.18 ± 0.06*	0.45 ± 0.08*	0.74 ± 0.14*	24.62 ± 5.24*	2.74 ± 0.14*
	CUMS+PHGG+Flu	0.22 ± 0.04*	0.19 ± 0.08*	0.43 ± 0.05*	0.70 ± 0.11*	25.02 ± 5.71*	2.61 ± 0.66*
	CUMS+Flu	0.20 ± 0.04*	0.21 ± 0.08*	0.39 ± 0.08*	0.68 ± 0.20	21.01 ± 7.98	2.49 ± 0.86*

*$p < 0.05$, based on the Student's *t*-test, and significantly different from the CUMS+NS groups. Data are shown as mean ± SD. The number of mice in each group is six.

Table 2. Effects of daily intake of PHGG, Flu, and a mixture of PHGG and Flu on the SCFA concentrations of mice feces in the intervention trial.

Day	Group	Lactic acid [mmoL g^{-1}]	Acetic acid [mmoL g^{-1}]	Propionic acid [mmoL g^{-1}]	Butyric acid [μmoL g^{-1}]	Isovaleric acid [μmoL g^{-1}]	Valeric acid [μmoL g^{-1}]
35	CUMS+NS	0.62 ± 0.07	0.42 ± 0.07	0.43 ± 0.07	0.92 ± 0.11	31.20 ± 6.10	3.29 ± 0.73
	CUMS+PHGG	0.70 ± 0.10	0.43 ± 0.07	0.47 ± 0.09	0.85 ± 0.06	31.65 ± 7.51	3.75 ± 0.29
	CUMS+PHGG+Flu	0.64 ± 0.10	0.43 ± 0.09	0.49 ± 0.03	0.90 ± 0.32	35.59 ± 10.88	3.46 ± 0.73
	CUMS+Flu	0.70 ± 0.08	0.48 ± 0.04	0.50 ± 0.08	0.89 ± 0.17	38.68 ± 9.42	3.27 ± 0.41
42	CUMS+NS	0.53 ± 0.06	0.44 ± 0.10	0.38 ± 0.03	0.81 ± 0.18	23.69 ± 3.52	2.35 ± 0.21
	CUMS+PHGG	0.55 ± 0.08	0.44 ± 0.11	0.46 ± 0.04*	0.80 ± 0.29	25.32 ± 5.28	2.39 ± 0.31
	CUMS+PHGG+Flu	0.64 ± 0.02*	0.40 ± 0.04	0.50 ± 0.03*	0.77 ± 0.24	24.09 ± 8.28	2.50 ± 0.41
	CUMS+Flu	0.59 ± 0.08	0.42 ± 0.14	0.44 ± 0.03*	0.79 ± 0.16	27.78 ± 9.73	2.62 ± 0.56
49	CUMS+NS	0.42 ± 0.04	0.33 ± 0.08	0.30 ± 0.03	0.47 ± 0.06	20.88 ± 1.35	2.68 ± 0.12
	CUMS+PHGG	0.45 ± 0.09	0.38 ± 0.11	0.40 ± 0.05*	0.51 ± 0.08	21.71 ± 5.52	2.74 ± 0.18
	CUMS+PHGG+Flu	0.55 ± 0.12*	0.40 ± 0.13	0.43 ± 0.03*	0.47 ± 0.11	24.52 ± 3.62*	2.87 ± 0.42
	CUMS+Flu	0.51 ± 0.08*	0.37 ± 0.12	0.39 ± 0.05*	0.58 ± 0.09*	27.66 ± 5.08*	2.74 ± 0.15
56	CUMS+NS	0.39 ± 0.07	0.18 ± 0.03	0.29 ± 0.02	0.28 ± 0.05	20.81 ± 2.01	2.16 ± 0.26
	CUMS+PHGG	0.42 ± 0.06	0.20 ± 0.04	0.36 ± 0.06*	0.35 ± 0.04	21.01 ± 2.72	2.42 ± 0.17
	CUMS+PHGG+Flu	0.38 ± 0.07	0.26 ± 0.04*	0.42 ± 0.03*	0.44 ± 0.15*	24.32 ± 1.88*	2.58 ± 0.30*
	CUMS+Flu	0.42 ± 0.09	0.23 ± 0.04*	0.36 ± 0.04*	0.47 ± 0.06*	24.13 ± 3.08*	2.66 ± 0.50*

*$p < 0.05$, based on the Student's *t*-test, and significantly different from the CUMS+NS groups. Data are shown as mean ± SD. The number of mice in each group is seven.

The gut microbiota composition in mice shifted at different taxonomic levels. At the phylum level, the relative abundance of *Firmicutes* was significantly lower, and the relative abundance of *Bacteroidetes* was higher with no significant difference in samples from CUMS+NS group than that of the controls in the prevention trial (**Figure 5**A,C). However, the CUMS-induced decrease in the relative abundance of *Firmicutes* was markedly reversed by daily intake of PHGG and PHGG+Flu. Similar effects were found in PHGG and PHGG+Flu in the intervention trial (Figure 5B). The relative abundance of *Bacteroidetes* was significantly

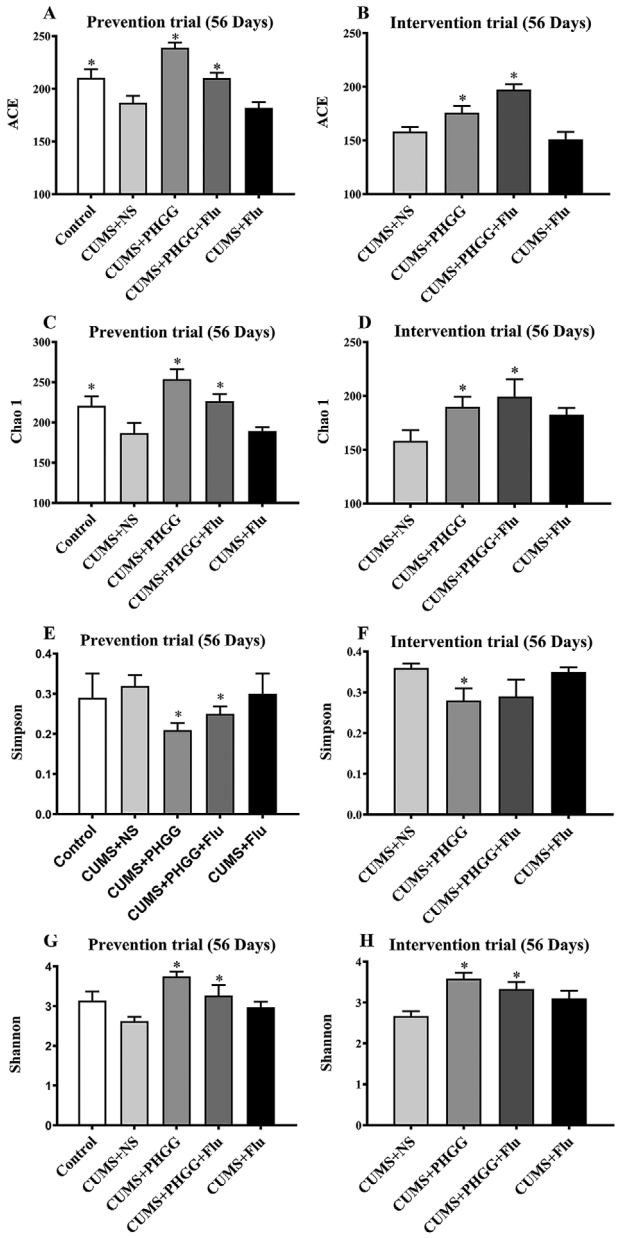

Figure 4. Effects of daily intake of PHGG, Flu, and a mixture of PHGG and Flu on the ACE, Chao 1, Simpson, and Shannon indexes in mice feces. A–D) The analysis of species richness based on ACE and Chao 1 indexes. E–H) The analysis of species diversity based on Simpson and Shannon indexes. *$p < 0.05$, based on the Student's *t*-test, and significantly different from the CUMS+NS groups. Data are shown as mean ± SD. The number of mice in each group in the prevention trial is six; the number of mice in each group in the intervention trial is seven.

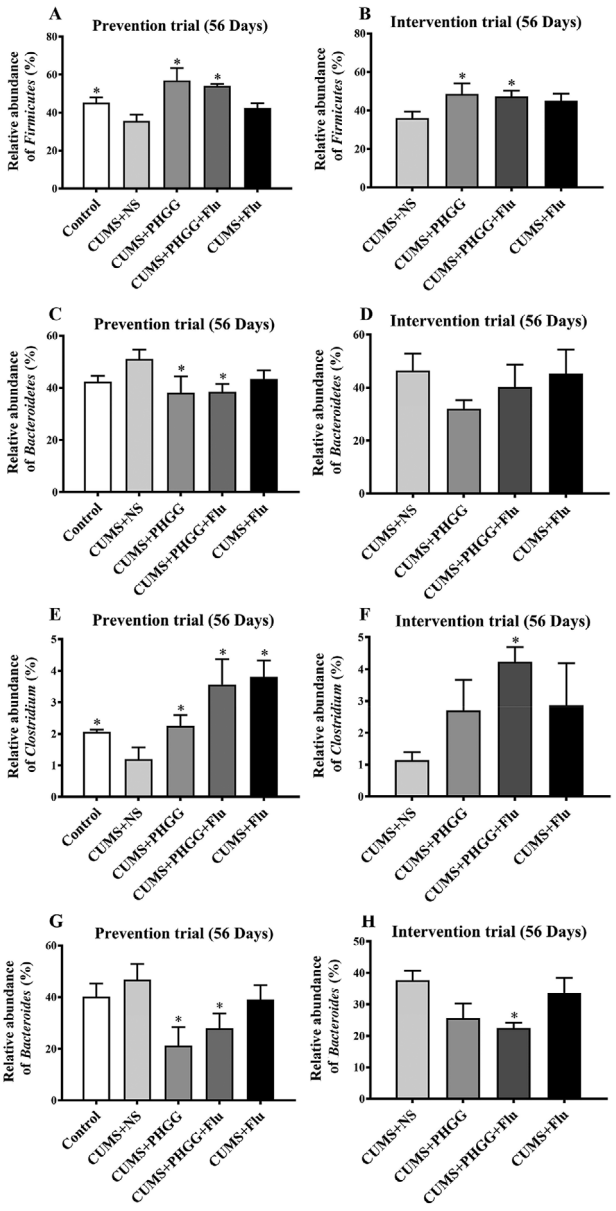

Figure 5. "Effects of daily intake of PHGG, Flu, and a mixture of PHGG and Flu on the gut microbiota at the phylum and genus levels. A–D) The relative abundances of *Firmicutes* and *Bacteroidetes* at the phylum level. E–H) The relative abundances of *Clostridium* and *Bacteroides* at the genus level. *$p < 0.05$, based on the Student's *t*-test, and significantly different from the CUMS+NS groups. Data are shown as mean ± SD. The number of mice in each group in the prevention trial is six; the number of mice in each group in the intervention trial is seven.

increased in CUMS+PHGG and CUMS+PHGG+Flu groups, compared with that of CUMS+NS group in the prevention trial (Figure 5C), and no significant difference was found among the four groups in the intervention trial (Figure 5D). The composition of gut microbiota of fecal samples of each group based on genus level was exhibited in Figure S5. At the genus level, the relative abundance of *Clostridium* was significantly lower in samples from CUMS+NS group than that of the control, but it could be elevated significantly by daily intake of PHGG, Flu, and PHGG+Flu in the prevention trial (Figure 5E). A similar change was observed in CUMS+PHGG+Flu group in the intervention trial (Figure 5F). No significant difference was found in the relative abundance of *Bacteroides* between CUMS+NS group and the control group, but PHGG and PHGG+Flu exerted a significant inhibitory effect in the prevention trial (Figure 5G), and PHGG+Flu worked in the intervention trial (Figure 5H).

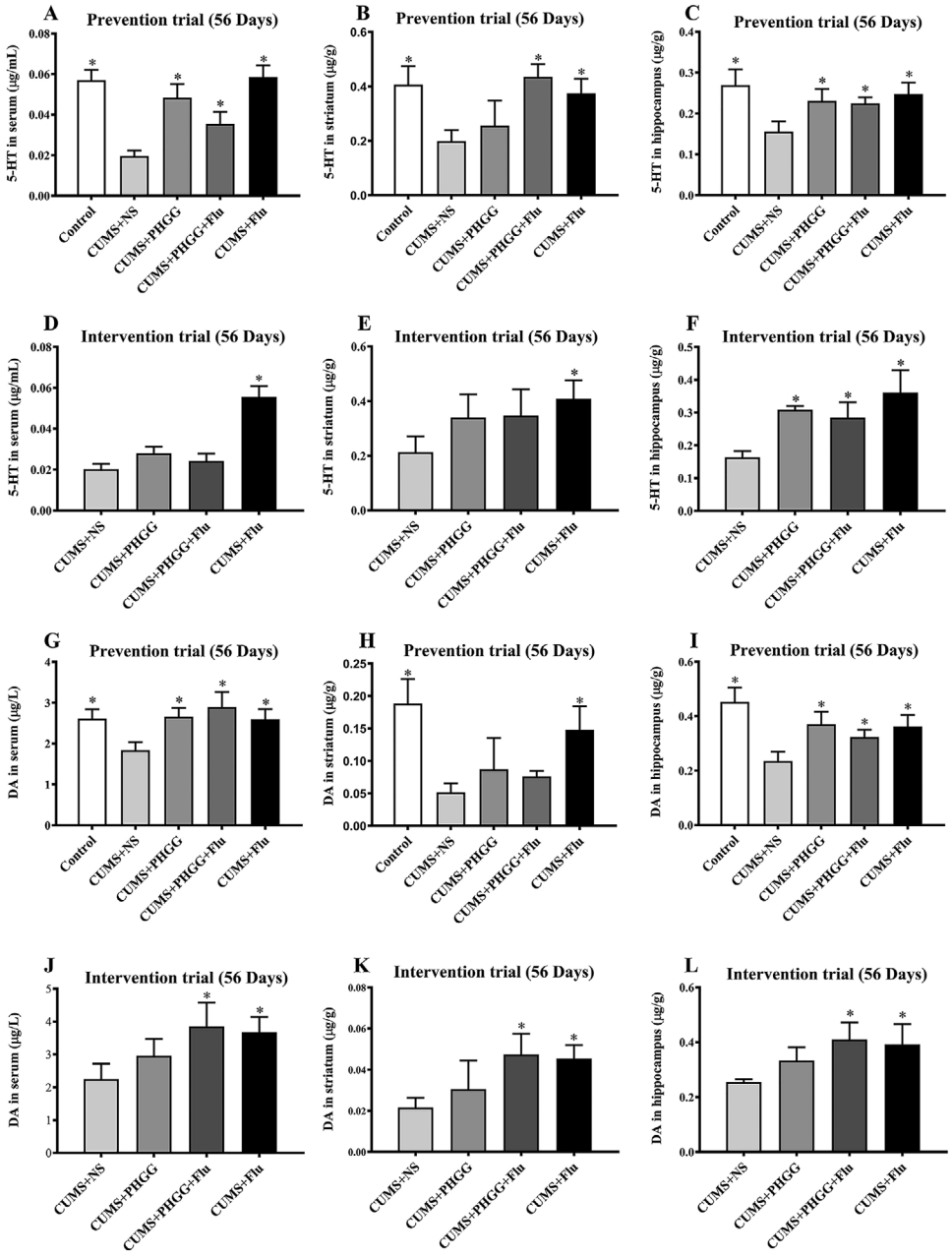

Figure 6. Effects of daily intake of PHGG, Flu, and a mixture of PHGG and Flu on 5-HT and DA levels in serum and brain tissues from mice. A,D,G,J) 5-HT and DA levels were determined in serum. B,E,H,K) 5-HT and DA levels were determined in the striatum. C,F,I,L) 5-HT and DA levels were determined in the hippocampus. $*p < 0.05$, based on the Student's *t*-test, and significantly different from the CUMS+NS groups. Data are shown as mean ± SD. The number of mice in each group in the prevention trial is six; the number of mice in each group in the intervention trial is seven.

3.5. PHGG Affected Neurotransmitter Concentrations in Depressed Mice

Twenty-eight days after CUMS intervention, the concentrations of 5-HT and DA in serum, striatum, and hippocampus were decreased significantly compared with those of the controls in the prevention trial (**Figure 6**A,B,C,G,H,I). Daily intake of PHGG, Flu, and PHGG+Flu significantly prevented the decrease of 5-HT and DA concentrations caused by CUMS in serum and hippocampus from mice in prevention trials (Figure 6A,C,G,I). Moreover, the 5-HT concentration in the striatum was significantly increased after administration with Flu and PHGG+Flu (Figure 6B), while the DA concentration in the striatum was increased just by oral administration of Flu (Figure 6H). In the intervention trial, PHGG and PHGG+Flu intervention significantly increased the concentration of 5-HT in the hippocampus, and Flu intervention markedly increased the concentrations of 5-HT in serum, striatum, and hippocampus (Figure 6D,E,F). The DA concentration was significantly higher than that of CUMS+NS group in the intervention trial by daily

intake of PHGG+Flu and Flu (Figure 6J,K,L). Furthermore, the CUMS+PHGG+Flu group had mice with higher DA concentration than the CUMS+Flu group in serum, striatum, and hippocampus in the intervention trial.

4. Discussion

The gut microbiota and their metabolites are receiving extensive attention owing to their ability to modulate emotional behavior and neurological processes.[30–33] In this study, we found that daily intake of PHGG could effectively ameliorate the depressive-like behavior measured by the SPT, FST, and OFT in CUMS-induced depression mice, and the data further demonstrated that PHGG supplementation improved the gut microbiota richness, and increased the levels of SCFAs in feces and 5-HT and DA concentrations in serum and brain tissues.

CUMS, which is well-known as the most predictive mouse model of depression, is widely used to mimics depression-related behaviors in humans.[34] Combined with the previous reports,[35,36] our results of the weights and behavioral experiments might confirm the successful establishment of a mouse model of depression. To verify whether PHGG could affect the depressive behaviors in mice, in the following prevention trial and intervention trial, the PHGG was as the experimental group, fluoxetine as a positive control group, and the data indicated that improvement effects of PHGG on the body weight and behaviors of depressed mice were consistent with those of fluoxetine[37] in the prevention trial, suggesting that PHGG played a protective role in CUMS-induced depression mice. However, PHGG did not have an ideal effect on the immobility time and the rearing number in the intervention trial. One speculation is that the time of model construction is too long, resulting in mice with a state of treatment-resistant depression. And some studies demonstrated that it is difficult to achieve the desired efficacy of a single antidepressant in the treatment of intractable depression.[38,39]

The previous study reported that the composition of gut microbiota in patients with depression was different from that of healthy humans,[40] and animal experiments also confirmed that different bacterial compositions in the gut could affect the behaviors in mice.[7,8] In the present study, the results showed that PHGG could significantly improve the species richness and diversity of gut microbiota in depression model mice. Moreover, at the phylum level, the decreased relative abundances of *Firmicutes* and increased relative abundances of *Bacteroidetes* in the depressed mice were inhibited by PHGG in the prevention trial. It was supported by the findings of Liu et al.[41] that patients with depression had lower levels of *Firmicutes* and higher levels of *Bacteroidetes*. And the regulatory effect of PHGG on gut microbiota could contribute to preventing the CUMS-induced intestinal barrier damage, possibly due to that the changed levels of *Firmicutes* were proved to be associated with the intestinal barrier functions.[42] Additionally, PHGG significantly attenuated the decreased levels of *Clostridium* and increased levels of *Bacteroides*, the genus of bacteria, in the prevention trial. The *Clostridium* clusters XIVa, IV, and XVIII isolated from human fecal samples have also been documented for the ability to accumulate Treg cells in the colon and thereby suppress inflammation.[43] *Bacteroides* can affect the intestinal immune system, such as *Bacteroides fragilis* had been shown to correlate with regulatory T cells for immune tolerance and maintenance of intestinal homeostasis.[44,45] However, fluoxetine rarely changed the composition of gut microbiota except for the genus *Clostridium*, but it was possible to help to modulate gut microbiota using the combination of PHGG and fluoxetine, including both the prevention trial and intervention trial.

SCFAs, as metabolites of microbial fermentation in the intestine, play a key role in the gut microbiota-brain axis.[46] Previous studies showed that CUMS significantly decreased the concentration of SCFA,[47,48] and similar results were also observed in this study, including the decreases of lactic acid, acetic acid, valeric acid, propionic acid, butyric acid, isovaleric acid at different time stage of constructing depression model. Some evidence shows that SCFAs might influence the brain by crossing the blood-brain barrier and modulating neurotransmission. Lactate played an antidepressant role by activating protein kinase C and upregulating the expression of TH (the enzyme involved in the biosynthesis of DA) and TPH (the rate-limiting enzyme for 5-HT synthesis) in ovariectomized rats.[49] Butyrate and propionate could induce TH gene transcription[50] and promote host 5-HT biosynthesis.[51] One study found that acetate and butyrate promoted TPH1 transcription in a human-derived enterochromaffin cell model.[13] Valeric acid has a structure similar to γ-aminobutyric acid (a neurotransmitter related to depression), which may contribute to the regulation of depression. Moreover, isovaleric acid also was found to be associated with human depression.[47] PHGG as the fermentation substrate of gut microbiota, significantly attenuated the decrease of SCFA concentrations in depressed mice in the prevention trial but had fewer effects in the intervention trial. So, it suggests that PHGG has a better preventive effect. Moreover, we found that fluoxetine might inhibit the CUMS-induced reduction of SCFA concentrations in two trials. One of the mechanisms supported by our data is that fluoxetine induces minor but significant changes in the relative abundance of *Clostridium*, which has been reported to be the producer of SCFA.[52] However, the relationship between bacterial taxa and SCFA is complex. More studies are needed to prove this issue. Likely, the restoration of SCFA concentrations by fluoxetine may partly explain its robust antidepressant actions. Furthermore, PHGG had a supportive role in the intervention trial, based on the data showing that the depressed mice were administered half-dose fluoxetine combined with PHGG had the same effect as full-dose fluoxetine in modifying the SCFA concentrations.

The gene expression levels of TH and TPH in the colon of mice have been determined and shown in Figure S12, Supporting Information. The TH mRNA levels varied differently in the prevention and intervention trials. In the prevention trial, the TH mRNA levels in CUMS+NS group were significantly increased, compared with that in the control group. Some studies suggest CUMS can induce colonic inflammation in rats,[53] which results in an increased expression level of TH in the colon of mice and patients.[54,55] After PHGG treatment, the TH mRNA levels decreased probably due to PHGG improving the intestinal flora to alleviate colonic inflammation. It should be noted that fluoxetine, which has been perceived as contributing to the high expression of TH,[56] increased all the TH mRNA levels in all the related groups. On the other hand, there was no significant difference in the TPH mRNA levels among these groups. Recent research has shown that TH expression levels induced by CUMS exist an

enormous difference in different organizations.[57] More effort is needed to address the link between depression and colonic inflammation induced by CUMS.

Besides, it should be noted that SCFA concentrations had a significant decrease with an increase in age, which reflected that age was an important factor for changes in the SCFA concentrations. Previous studies showed that the concentration of SCFA in feces decreased with age in mice[58] and humans.[59] It might also result in covering up the actual effects of PHGG/fluoxetine on the SCFA concentrations in CUMS-induced mice and normal mice. For example, in the intervention trial, we found that PHGG+fluoxetine and fluoxetine had significant increases in the lactic acid level in depressed mice at 49 days, but not at 56 days. Thus, further studies are needed to elucidate the roles of age, treatment factors such as PHGG, PHGG+fluoxetine, fluoxetine, and CUMS in depression and SCFAs.

SCFAs were reported to have effects on regulating the synthesis and release of 5-HT.[51,60] The monoamine transmitters such as DA, 5-HT, have great importance in regulating stress-induced behavior, mood, and emotion,[61] and the decreased levels of DA and 5-HT could increase the depression risk.[62] In this study, CUMS resulting in the apparent decreases of DA and 5-HT in serum and brain tissues were relieved by fluoxetine as an antidepressant agent, mainly due to that fluoxetine significantly increased the levels of DA and 5-HT. Excitedly, PHGG as a safe food supplement improves the neurotransmitter disorder in depressed mice of the preventive trial. PHGG had fewer effects of increasing the levels of monoamine transmitters in the intervention trial, but it could still help to enhance the antidepressant effect of fluoxetine in depressed mice. Yano et al.[51] found that gut microbiota and its metabolites played a key role in regulating 5-HT concentration in the enterochromaffin cell, and Fukumoto et al.[60] found that intracolonic injection of SCFA significantly increased the 5-HT concentration in the colon of rats in vitro. The relationship between the content of 5-HT in serum, striatum, and hippocampus and the content of SCFA in mice feces in the prevention and intervention trials is shown in Figures S6–11, Supporting Information. The content of SCFA and 5-HT in CUMS+NS group was at a low level in both prevention and intervention trials, while the content of SCFA and 5-HT in most of the mice was increased after intragastric administration of PHGG or fluoxetine. Taken combined with our data mentioned above, the evidence indicates that PHGG may increase the concentrations of SCFAs by regulating gut microbiota, then promote the synthesis and release of 5-HT and DA, and finally ease depression.

In summary, PHGG supplementation prevents and improves body weight loss and depressive-like behavior in depressed mice induced by CUMS, and this may be associated with the regulation of gut microbial composition, increases of SCFA concentrations, and neurotransmitter concentrations. We believe this study casts a new light on the mechanism of PHGG's antidepressant effect and suggests that PHGG has a considerable potential in developing tonics for assisting prevent depression.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Y.C. and M.W. contributed equally to this work. Y.W. and Z.W. designed the study and reviewed the manuscript. Y.C. and M.W. wrote the manuscript and performed experiments and analyzed the data. Y.Z., T.G., and Y.Z. performed experiments and analyzed the data. F.Y. and D.H. discussed the results and commented on the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

depression, gut microbiota, neurotransmitter, partially hydrolyzed guar gum, short-chain fatty acids

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