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Faecalibacterium prausnitzii (ATCC 27766) has preventive and therapeutic effects on chronic unpredictable mild stress-induced depression-like and anxiety-like behavior in rats

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ABSTRACT

The realization that the microbiota-gut-brain axis plays a critical role in health and disease,including neuropsychiatric disorders, is rapidly advancing . An abundance of preclinical studies have shown that psychobiotics acting via the brain-gut-axis can affect brain development, function and behavior. Here we tested whether potential psychobiotics *Faecalibacterium prausnitzii* (ATCC 27766) has anxiolytic and antidepressant-like effects and reverse the impact of chronic unpredictable mild stress (CUMS) in rats. The experiment was divided into two phases, the first stage was CUMS procedure period and the second stage was convalescence period. SD male rats were administered *Faecalibacterium prausnitzii* for 4 weeks prior to testing during each period. Behavior, growth status, SCFAs produced, plasma cytokine, endocrinology and bone mineral density (BMD) were assessed. Our findings indicate that the administration of *F. prausnitzii* had preventive and therapeutic effects on CUMS-induced depression-like and anxiety-like behavior. In addition, *F. prausnitzii* administration could significantly prevent the reduction of the whole-body, femur and tibia BMD during the recovery phase. Moreover, the growth status of rats fed the *F. prausnitzii* was better than the rats by CUMS. And *F. prausnitzii* administration led to higher levels of SCFAs in the cecum and higher levels of cytokines interleukin-10 (IL-10) in the plasma, prevented the effects on corticosterone, C-reaction protein and cytokines interleukin-6 (IL-6) release induced by CUMS, changes that were associated with the effects seen on behavior. These results provide further evidence that gut microflora play a role in anxiety and depression. Subject to the confirmation of these results, probiotics might offer a useful novel therapeutic approach to neuropathological disorders and/or as adjunct therapies in psychiatric disorders and support the recent broadening of the definition of psychobiotic. Finally, this study supports *F. prausnitzii* has significant potential as a psychobiotic.

1. Introduction

The intestine and the brain are intimately connected via the brain-gut axis, which involves bidirectional communication via neural, endocrine and immune pathways (Mayer et al., 2014; Steenbergen et al., 2015). In recent years, increasing evidence suggests that the microbiota-gut-brain axis plays a key role in regulating brain functions, particularly emotional processing and behavior(Bharwani et al., 2016; Dinan and Cryan, 2012; Rackers et al., 2018).An abundance of preclinical studies has shown that probiotics acting via the brain-gut-axis can affect brain development, function and behavior (Bharwani et al., 2016). It has previously been shown that the prebiotic sialyllactose is able to diminish stress-induced alterations in colonic mucosa-associated microbiota community structure, anxiety-like behavior, and immature neuron cell numbers irrespective of immune or endocrine functionality in mice/rats(Savignac et al., 2013; Tarr et al., 2015). This has prompted a growing interest in the possibility of targeting the gut microbiota to beneficially impact human brain function and

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behavior. Drug therapy in addition to some psychotherapy models may help relieve anxiety, these may be associated with consuming certain foods, for this reason, improving the quality of the diet can be help improve mood and feel comfortable. So, recent scientific evidence indicates that a diet with low nutritional value is associated with an increased risk of anxiety disorder, so maybe linked to an increased risk of developing an anxiety disorder (Begaa and Messaoudi, 2018; Messaoudi and Begaa, 2018).

Psychobiotics have been defined as bacteria that ingested in adequate amounts to produce a positive mental health benefit (Dinan et al., 2013).Considering the potential impact of putative psychobiotics upon central nervous system processes, especially stress, mood, anxiety and cognition (Dinan et al., 2015), the prospect of targeting the gut microbiota as a potential modifiable risk factor for stress-related disorders is appealing. Preclinical research has indicated that chronic probiotic administration can reduce anxiety-like and depressivelike behavior, and can normalise associated physiological outputs such as corticosterone and noradrenaline in plasma, brain-derived neurotrophic factor (BDNF) in the brain and immune function (Janik et al., 2016; Kelly et al., 2017; Rudzki et al., 2019). There is a growing appreciation of the need to translate this promising preclinical work to the clinic while at the same time recognising the challenges inherent in this process (Kelly et al., 2016). The intestinal microbial balance may alter the regulation of inflammatory responses and in so doing may be involved in the modulation of mood and behavior(Forsythe et al., 2012; Foster and McVey Neufeld, 2013). More recently, *Faecalibacterium prausnitzii*, based on a case study in a closed experimental human life support system(Hao et al., 2018), had the highest abundance and showed a significant positive correlation with mood. The moreover, a negative correlation was observed between *Faecalibacterium* and the severity of depressive symptoms, and the relative abundance of *Faecalibacterium* was significantly increased after patients responded to antidepressant therapy (Jiang et al., 2015) *Faecalibacterium* is a genus of bacteria. Its sole known species, *Faecalibacterium prausnitzii* represents around 5% from the total fecal microbiota in healthy adults (Hold et al., 2003). Furthermore, the levels of *F. prausnitzii* have been found to be decreased in patients suffering from intestinal and metabolic disorders such as inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), colorectal cancer (CRC), obesity, celiac disease (Balamurugan et al., 2010; Furet et al., 2010; Rajilićstojanović et al., 2011) and depressive disorder (SP et al., 2005). *F. prausnitzii* exhibits anti-inflammatory effects on cellular and TNBS colitis models, partly due to secreted metabolites able to block NF-kappaB activation and IL-8 production.(Sokol et al., 2008). *F. prausnitzii* produces butyrate and other short-chain fatty acids through the fermentation of dietary fiber (Louis and Flint, 2010). Butyrate along with other fermentation-derived SCFAs (e.g. acetate, propionate) and the structurally related ketone bodies (e.g. acetoacetate and d-β-hydroxybutyrate) show the interesting effects in various diseases, including obesity, diabetes, inflammatory (bowel) diseases, and colorectal cancer as well as neurological disorders (Stilling et al., 2016b). Depression is associated with the presence of biomarkers of inflammation such as elevated interleukin (IL)-6, tumor necrosis factor alpha, and the acute phase protein, C reactive protein (O'Brien et al., 2004). Similar elevated biomarkers of inflammation have been seen in anxiety states and are known to occur as a result of stress. Decreased bone mineral density (BMD) has been reported in patients suffering from major depressive disorder. And excessive cortisol production results in decreased BMD, inflammatory mediators that might have a role in the biology of major depression and a host of other factors may also be involved in somatic consequences of depression such as osteoporosis (Licinio and Wong, 1999). The *F. prausnitzii* may alter the regulation of inflammatory responses and in so doing may be involved in the modulation of mood, behavior and BMD (Forsythe et al., 2012; Foster and McVey Neufeld, 2013). Regarding *F. prausnitzii*, although little is known about its safety, there is a clear potential of this species as a next-generation probiotic (Martin et al., 2017).

However, there is almost no research on *F. prausnitzii* and depression. Therefore, this study used depression model rats to explore the effect and mechanism of *F. prausnitzii* on depression. Models of depression are conducted in order to put animals without depression as closely as possible to the clinical situation. Many different models of depression are used in the research conducted at present, including learned helplessness, forced swim test, or social defeat stress (PORSOLT et al., 1977; Yan et al., 2010). More recently, the chronic unpredictable mild stress (CUMS) model is the most frequently used and considered one of the most perfect models of depression (Gambarana et al., 2001; Yan et al., 2010). In this model, rats or mice are exposed chronically to a constant bombardment of unpredictable micro-stressors (i.e., restriction, inversion of the light-darkness cycle, deprivation of water or food, wet litter, etc.), resulting in the development of a plethora of behavioural changes, including a behavioural correlate of the clinical core symptom of depression and anxiety. However, it can be restored to normal levels by chronic treatment with antidepressant drugs (Willner, 2016).In the current study, the experiment was divided into two phases, the first stage was CUMS procedure period and the second stage was convalescence period. We took these two stages to investigate whether administration of *F. prausnitzii* has preventive and therapeutic effects on CUMS-induced depression-like and anxiety-like behavior in parallel with associated changes in growth status, SCFAs produced, cytokine, endocrinology and bone mineral density in rats.

2. Material and methods

2.1. Animals

Sixty male Sprague-Dawley (SD) rats obtained from Charles River Labs (Beijing) were chosen for this study. At the beginning of the study, the weight of each rat is 230–250 g. All rats received standard laboratory diet (Vital River Laboratory Animal Technology Co. Ltd, Beijing, China) and tap water *ad libitum* under a 12 h light–dark cycle (lights on 0730–1930) and a constant temperature of 21–22 ℃ and humidity of 55 ± 5%. All animals were group-housed in Macrolon cages (37 cm long, 26 cm wide, 17 cm high) and were allowed to adapt to the environment for 2 weeks prior to any experiment. The experimental procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the experiments were approved by the University Animal Use Committee.

2.2. Experimental protocols

2.2.1. Treatments and sacrifice

The experiment was divided into two phases, the first phase was CUMS procedure period (further described in details, from the third week to the sixth week) and the second phase was a convalescence period (from the eighth week to the eleventh week).After 2 weeks of adaptive feeding, the rats were randomly divided into 3 groups during the CUMS procedure period. They are unhandled control group (Free-1, $n = 20$); CUMS group (Cums, $n = 30$) and CUMS + *F. prausnitzii* group (Cums + Fp, $n = 10$). After the end of the first phase, the rest rats were divided into 3 groups during the convalescence period. The rest rats of unhandled control group were named Free -2 ($n = 10$); the rest rats of Cums group were divided into two different treatment groups groups, they are non-treated group (Control, $n = 10$) and *F. prausnitzii*-treated group(Fp, $n = 10$). They were fed with standard laboratory diet *ad libitum* (Vital River Laboratory Animal Technology Co. Ltd, Beijing, China). Rats in each group were weighed every week. Food intake was measured before each supplementary. One week of food intake was defined as the total weight of seven days of all supplementary food minus the weight of the remnant food. Two or three rats of from the same experimental group were housed per cage. And the rats from the same group were re-distributed in per cage every week after the weight of the rats and the amount of food consumed were measured. The

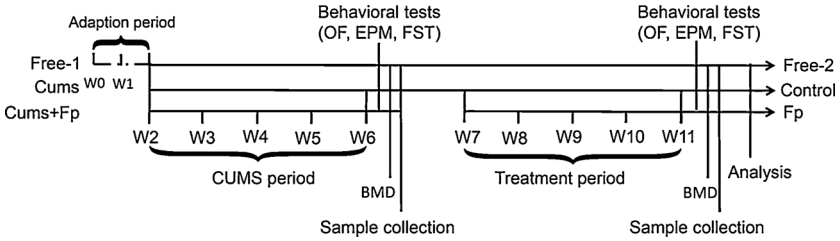

Fig. 1. Animal treatment and experimental procedure. Where: W0-W2, animal adaptation time; Animals were divided into three groups (Free group; Cums group; Cums + Fp group) on the last day of the second week; W2-W6, CUMS procedure period of Cums group and Cums + Fp group; the rest rats of unhandled control group was named Free -2, the rest animals of Cums group were divided into two groups (Control group; Fp group) on the last day of the 7th week; W7-W11, Recovery phase; CUMS, chronic unpredictable mild stress; OF, Open field test; EPM, Elevated plus maze; FST, Forced swim test; BMD, Bone mineral density.

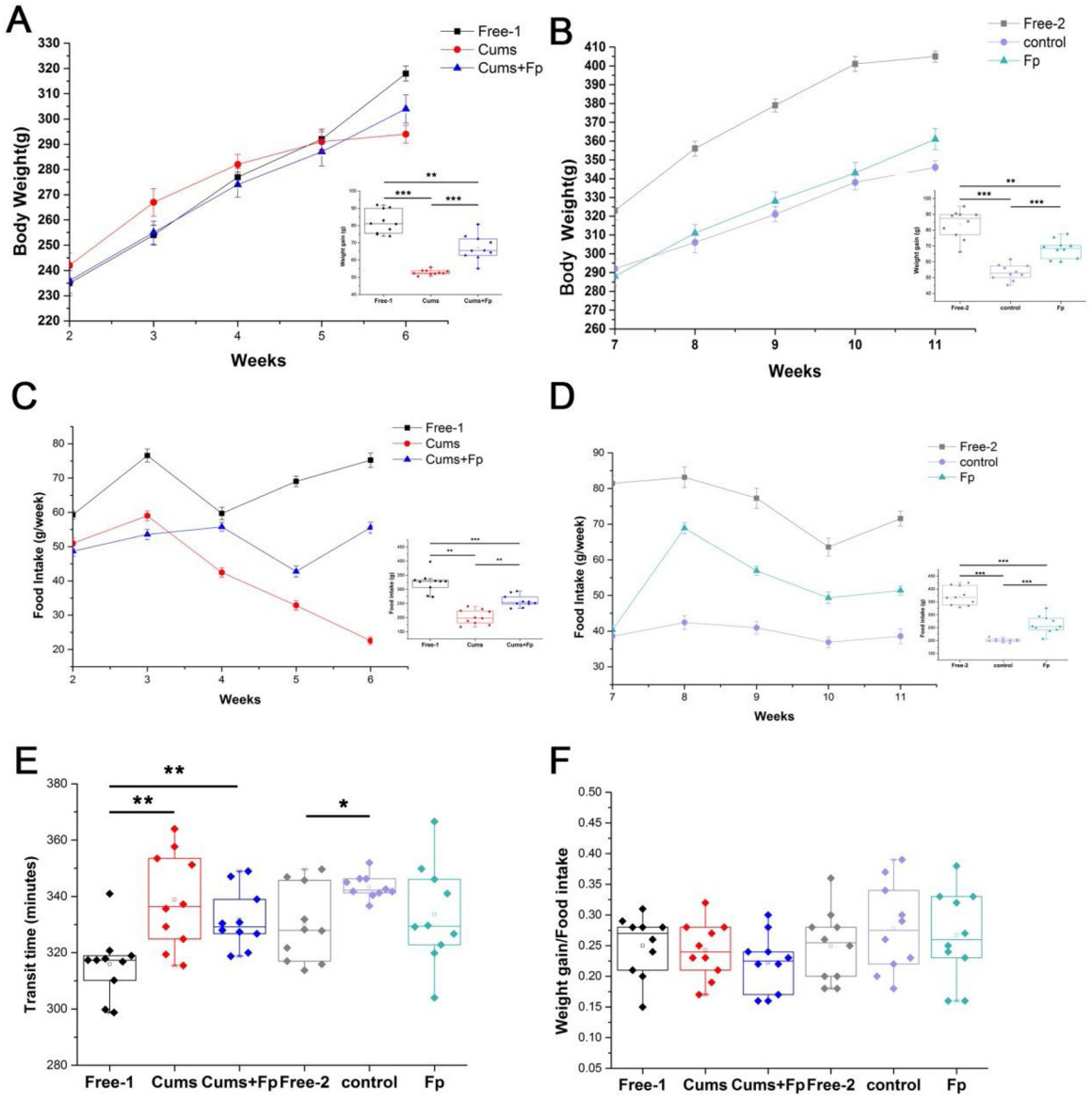

Fig. 2. Body weight, weight gain, food intake, intestinal transit time and food efficiency of rats in different group during the experiment. Temporal evolution of body weights weekly and total weight gain of each phase from 2^{nd}-6^{th} weeks,[A, $F(2,27) = 63.1$] and from 7^{th}-11^{th} weeks [B, $F(2,27) = 49.2$]; Evolution of food intake weekly and total food intake of each phase from 2^{nd}-6^{th} weeks [C, $F(2,27) = 48.6$] and from 7^{th}-11^{th} weeks [D, $F(2,27) = 91.2$]; Intestinal transit time of the different group of rats [E, first phase, $F(2,27) = 7.7$; second phase, $F(2,27) = 2.9$]; Food efficiency(weight gain / food intake) of the different group of rats [F, first phase, $F(2,27) = 0.9$; second phase, $F(2,27) = 0.4$].Data are shown as averages and error bars represent SD in Figs. 2A and 2B; All values in all other figures were presented as single data points superimposed to boxplots (n = 10 in each group)。 $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. One-way ANOVA with Duncan's test during each phase.

amount of food consumed was averaged between the two or three rats. Food efficiency was defined as weight gain divided by food intake. Toward the end of the each phase, the animals underwent a series of behavioral testing including Open field test (OF), Elevated plus maze (EPM) and Forced swim test (FST). In addition, bone mineral density (BMD) was measured before the rats were killed. The details of the experimental procedure are shown in Fig. 1.

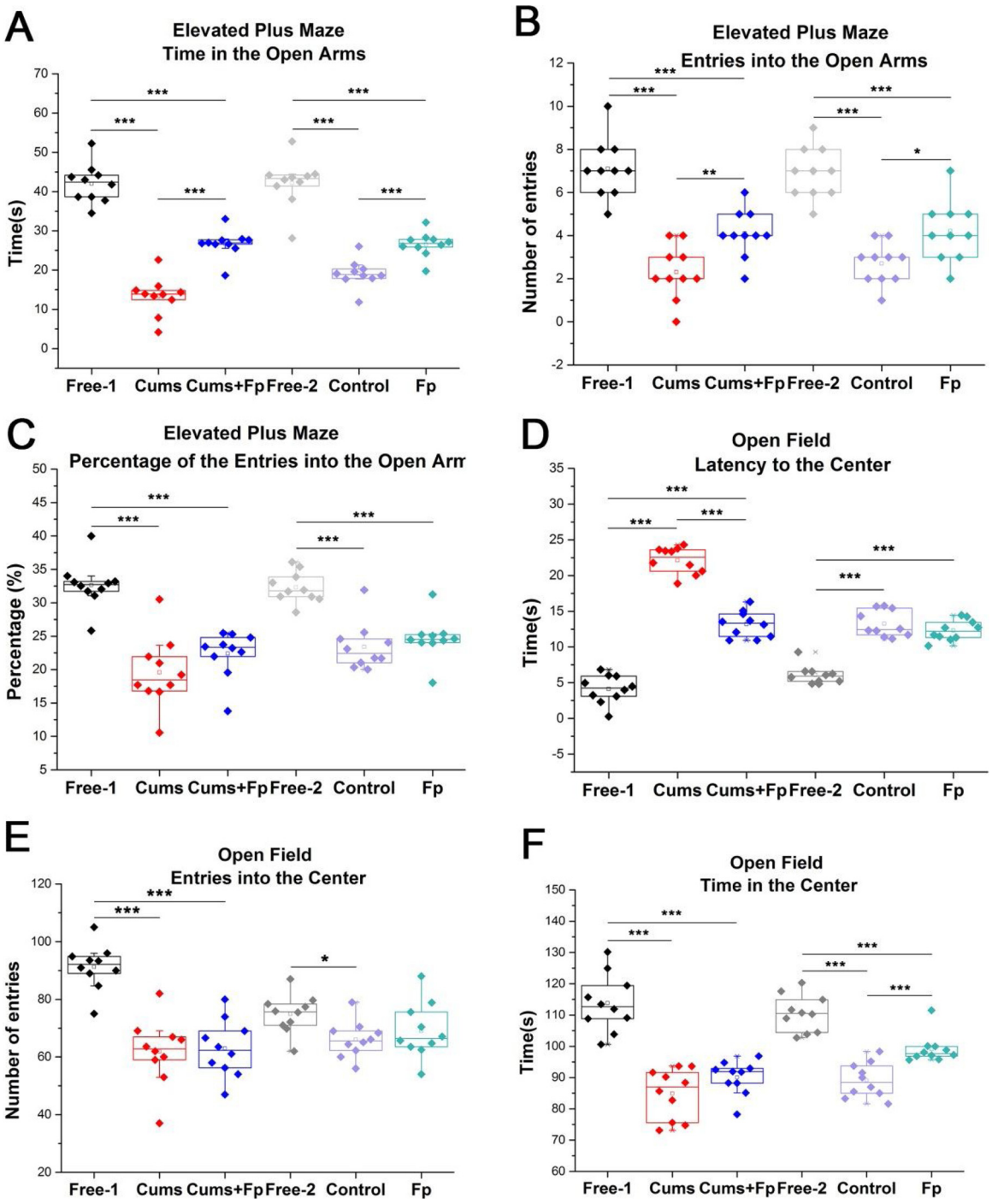

Fig. 3. Anxiety-like behaviors in the elevated-plus maze test (A–C) and open field test (D–F). Time spent in open arms [A, first phase, $F(2,27) = 37.9$; second phase, $F(2,27) = 31.7$], the numbers [B, E, first phase, $F(2,27) = 102.2$; second phase, $F(2,27) = 69.2$] and percentage [C, first phase, $F(2,27) = 27.5$; second phase, $F(2,27) = 25.2$] of the entries into open arms in the elevate plus maze test; Latency to the center [D, first phase, $F(2,27) = 229.4$; second phase, $F(2,27) = 64.2$] the numbers of entries into the center [E, first phase, $F(2,27) = 28.4$; second phase, $F(2,27) = 3.5$] and time in the center of the open field test [F, first phase, $F(2,27) = 40.5$; second phase, $F(2,27) = 39.9$] The values were presented as single data points superimposed to boxplots; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. One-way ANOVA with Duncan's test during each phase.

2.2.2. CUMS procedure

The CUMS protocol was performed as described previously (Biala et al., 2017; Willner et al., 1992; Wu et al., 2007) with minor modifications. In brief, rats were subjected to different kinds of mild stressors, which varied from day to day to make the stress procedure unpredictable. These stressors were randomly scheduled over a 1-week period and repeated throughout the 4 weeks experiment for 2 h daily. There were a total of seven stressors: (1) 1 h warm swim at 31 ℃; (2) 5 min cold swim at 8–10 ℃, after which they were toweled dry; (3) 5 min hot stress in oven at 42 ℃; (4) 1 min tail pinch; (5) lights on overnight; (6) damp sawdust overnight ; (7) 1 h shaker stress (160 r.p.m.). Non-stressed rats were left undisturbed in their home cages. Twenty-four hours after the end of the CUMS protocol, all animals were exposed to one of the behavioral paradigms described below.

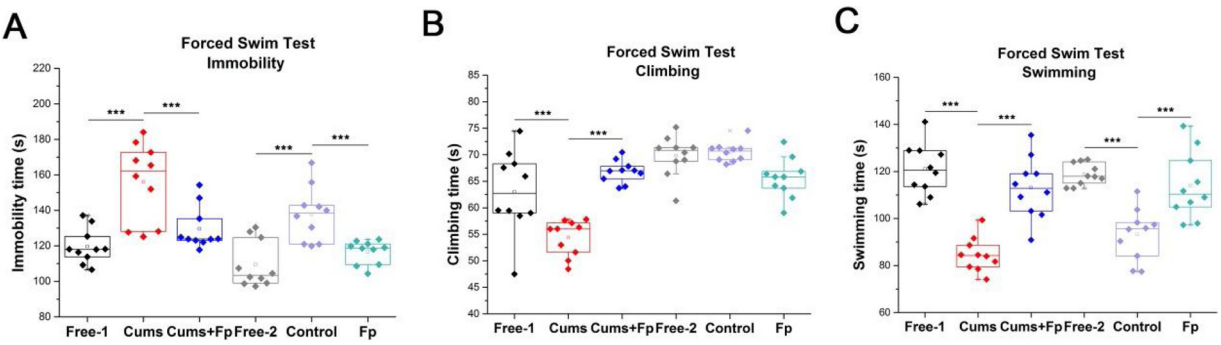

Fig. 4. Depressive-like behaviors in the forced swim test. Immobility time [A, first phase, $F(2,27) = 14.6$; second phase, $F(2,27) = 14.3$] Climbing time [B, first phase, $F(2,27) = 16$; second phase, $F(2,27) = 1.8$] and swimming time [C, first phase, $F(2,27) = 32.9$; second phase, $F(2,27) = 16.3$]. The values were presented as single data points superimposed to boxplots; *p < 0.05, **p < 0.01, ***p < 0.001. One-way ANOVA with Duncan's test during each phase.

Table 1
BMD of whole-body, femur and tibia in six groups after 4-weeks experimental period.

Group	Whole-body BMD (g/cm^2)	Femur BMD (g/cm^2)	Tibia BMD (g/cm^2)
Free-1	0.1653 ± 0.0033	0.3398 ± 0.0135	0.2245 ± 0.00238
Cums	0.1600 ± 0.0037	0.3345 ± 0.0265	0.2258 ± 0.0017
Cums + Fp	0.1643 ± 0.0015	0.3515 ± 0.0294	0.2303 ± 0.0082
Free-2	0.1860 ± 0.0064	0.3720 ± 0.0186	0.2708 ± 0.0060
Control	0.1775 ± 0.0037*	0.3010 ± 0.0860*	0.2583 ± 0.0113*
Fp	0.1890 ± 0.0038	0.3878 ± 0.0128	0.2763 ± 0.0099

Data is expressed as mean ± SD for 8–10 rats per group. Differences between the groups were evaluated with One-way ANOVA with Duncan's test during each phase.
* P < 0.05 vs Free-2 or Fp Group.

2.2.3. *Faecalibacterium prausnitzii administration*

F. prausnitzii (ATCC 27766, Manassas, VA, United States) was cultured anaerobically at 37 °C in LYHBHI medium [main component of brain-heart infusion medium (37 g/L, BD, Franklin Lakes, NJ, United States), yeast extract (5 g/L, Oxoid, Basingstoke, United Kingdom), cellobiose (1 g/L, Sigma, St. Louis, MO, United States), maltose (1 g/L, Amresco, Solon, OH, United States), hemin (5 mg/L, Sigma), and cysteine (0.5 g/L, Sigma)]. Bacterial suspensions were centrifuged at 850 × *g* for 15 min at 20 °C and washed twice with sterile phosphate buffered saline (PBS) to a concentration of 1×10^9 *F. prausnitzii* per mL as determined by a Vitek colorimeter (bioMérieux). This dose was selected based on previous studies (Huang et al., 2016). Rats were fed at the same time each day by oral gavaged with 200 µL of PBS (Free-1 and Cums group from the third week to the sixth week; Free-2 and Control group from the eighth week to the eleventh week), or with 200 µL of resuspended *F. prausnitzii*, 1×10^9 CFU (Cums + Fp group from the third week to the sixth week; Fp group from the eighth week to the eleventh week) daily.

2.3. *Behavioral tests*

Toward the end of each phase, ten rats each group (if the rats in the group are equal to 10, then all were used for testing) were randomly selected for depression-and anxiety-like behavioral tests. Behavioral testing were performed during one week at the end of the each phase in the following order: (1) elevated-plus maze, (2) open field test, and (3) forced swim test. Each testing was performed between 8–12 o'clock within the two days of connection and the rats were allowed to habituate in the test room for 30 min prior to the tests.

2.3.1. *Elevated plus maze test*

The set up was made of a grey plastic cross-shaped maze 1 m elevated from the floor, comprising two open (fearful) and two closed (safe) arms (50 × 5 × 15 cm walls or 1 cm no wall). Experiments occurred under red light (˜5 lx). Rats were individually placed into the center of the maze facing an open arm (to avoid direct entrance into a closed one) and were allowed 5-min free exploration. Experiments were videotaped using a ceiling camera for further parameters analysis using Ethovision software (3.1 version, Noldus, TrackSys, Nottingham, UK). The percentage of time spent, distance moved and the number of entries in each arm were measured, for anxiety behavior and locomotor activity, respectively (entrance in an arm was defined as all four paws inside the arm).

2.3.2. *Open field test*

To assess the response to a novel stressful environment and locomotor activity, rats were placed into open arena (40 × 32 × 23 cm, L × w × h) with ˜60 lx lighting and allowed to explore for 10-mins. Experiments were videotaped using a ceiling camera for further parameter analysis using Ethovision software (3.1 version, Noldus, TrackSys, Nottingham, UK). The distance travelled and the latency to enter a virtual central zone (defined at 50% away from the edges) was scored.

2.3.3. *Forced swim test*

The FST is a behavioural test used in rodents to assess antidepressant-like behavior and was carried out as previously described (Slattery and Cryan, 2012). All experimental animals were first habituate to the testing room 30 min prior testing. A pre-swim (15 min) was conducted first, 24 h prior to the test swim. On test day, all animals were introduced again to the Plexiglas cylinder (46 cm tall x 21 cm in diameter) filled with water (24 °C) to a depth of 30 cm. Test sessions (5 min) were recorded by video camera positioned directly above the cylinder. Animals were removed from their home cage and placed into the tank. After 5 min, the animal was removed from the tank, dried and replaced back in its home cage. The tank was then emptied and fresh water replaced into the tank between animals. Analysis of behavior was conducted by an experienced experimented for the test 5 min. The parameters of interest were the length of time immobile, swimming and climbing. Climbing was defined by the rat presenting its forepaws along the edge of the cylinder in an upwards movement. Any horizontal movement was classified as swimming. Finally, immobility was defined as no additional movement required for the animal to maintain its head above water.

2.4. *Rat intestinal transit time determination*

Animals were single-housed (with a thin layer of bedding) with food and water ad libitum. Three hours after being single-housed, animals were given 200 ul of 6% carmine red in 0.5% methylcellulose (in PBS) given by oral gavage. After the gavage, the cages were inspected every 10 min, and the appearance of the first red fecal pellet was recorded.

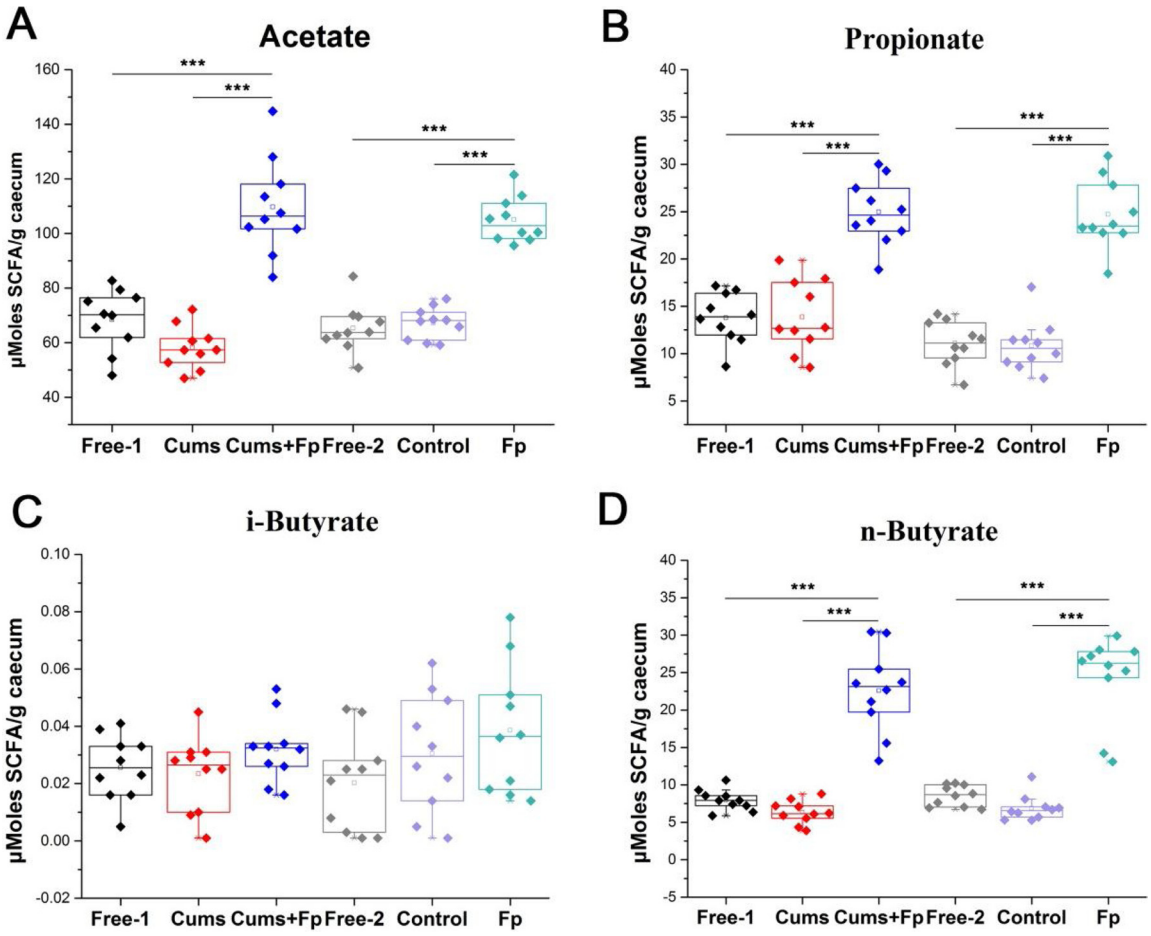

Fig. 5. Short-chain fatty acid (SCFA) concentrations in cecum. *F. prausnitzii* administration significantly increased acetate [A, first phase, F(2,27) = 45.4; second phase, F(2,27) = 84.8], Propionate [B, F(2,27) = 37.8; second phase, F(2,27) = 73.4] and n-butyrate levels [D, F(2,27) = 68.2; second phase, F(2,27) = 71.4]) in the cecum ($p < 0.001$) (Fig. 4A), but it did not alter iso-butyrate levels [C, F(2,27) = 1.38; second phase, F(2,27) = 2.08] in the cecum ($p > 0.05$). The values were presented as single data points superimposed to boxplots; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. One-way ANOVA with Duncan's test during each phase.

After the test, animals were group housed in their home cages.

2.5. Bone mineral density (BMD) measured by dual-energy X-ray absorptiometry (DXA)

On week 6, 11 of the experiment, whole-body, femur and right tibias of the rats were scanned by DXA (Discovery QDR, USA) using small animals' regional high resolution scanning mode after anesthesia (1% pentobarbital sodium: 6 mg/100 g body weight).

2.6. Short chain fatty acids concentration analysis from cecum content

For the determination of SCFAs, the caecum contents, were mixed with ultrapure water at 10 ml/g, followed by centrifugation at 15,000 *g* at 4 °C for 15 min to get supernatant. The supernatant were then filtered with 0.22 µm PVDF membranes (Pall Life Sciences). SCFAs from the filtrates were analyzed using high performance liquid chromatography (HPLC, LC-20AT, Shimadzu), which was equipped with a column (Aminex HPX-87H column, Biorad) at a column temperature of 30 °C and UV detector (The detection wavelength was 215 nm, Shimadzu, Japan) for 60 min. 5 mM H_2SO_4 was used as the eluent at a flow rate of 0.6 ml/min. A standard curve was built with different concentrations of a standard mix containing acetate, propionate, iso-butyrate and n-butyrate (Sigma). Peaks were integrated by using the LabSolutions LC Workstation Ver.5 Single LC software. All SCFA data are expressed as µmol/g.

2.7. Rat Corticosterone, C-reaction protein (CRP) and cytokine determination

Rats were sacrificed by decapitation 24 h after the end of the FST test and immediately trunk blood was collected in EDTA-coated tubes and centrifuged at 3500 × *g* for 15 min. Plasma supernatant was collected and stored on dry ice. All samples were frozen at −80 °C for later analysis. Corticosterone, CRP, IL-6 and IL-10 were determined using commercially available Rat Corticosterone, CRP, IL-6 and IL-10 ELISA Kit (Rigor, Beijing, China) according to the manufacturers' instructions, especially.

2.8. Statistical analysis

All statistical analyses were performed using SPSS IBM version 21 (SPSS Inc., Chicago, IL, USA). All data were presented as mean ± SD. Differences between the groups were evaluated with One-way ANOVA with a Duncan's test. P-values < 0.05 were considered statistically significant.

3. Results

3.1. Growth status, food intake and intestinal transit time

During CUMS procedure period, the weight gain and food intake of rats in the Cums group ($p < 0.01$) and Cums + Fp ($p < 0.01$) significantly decreased compared to the Free-1 group, and the weight gain

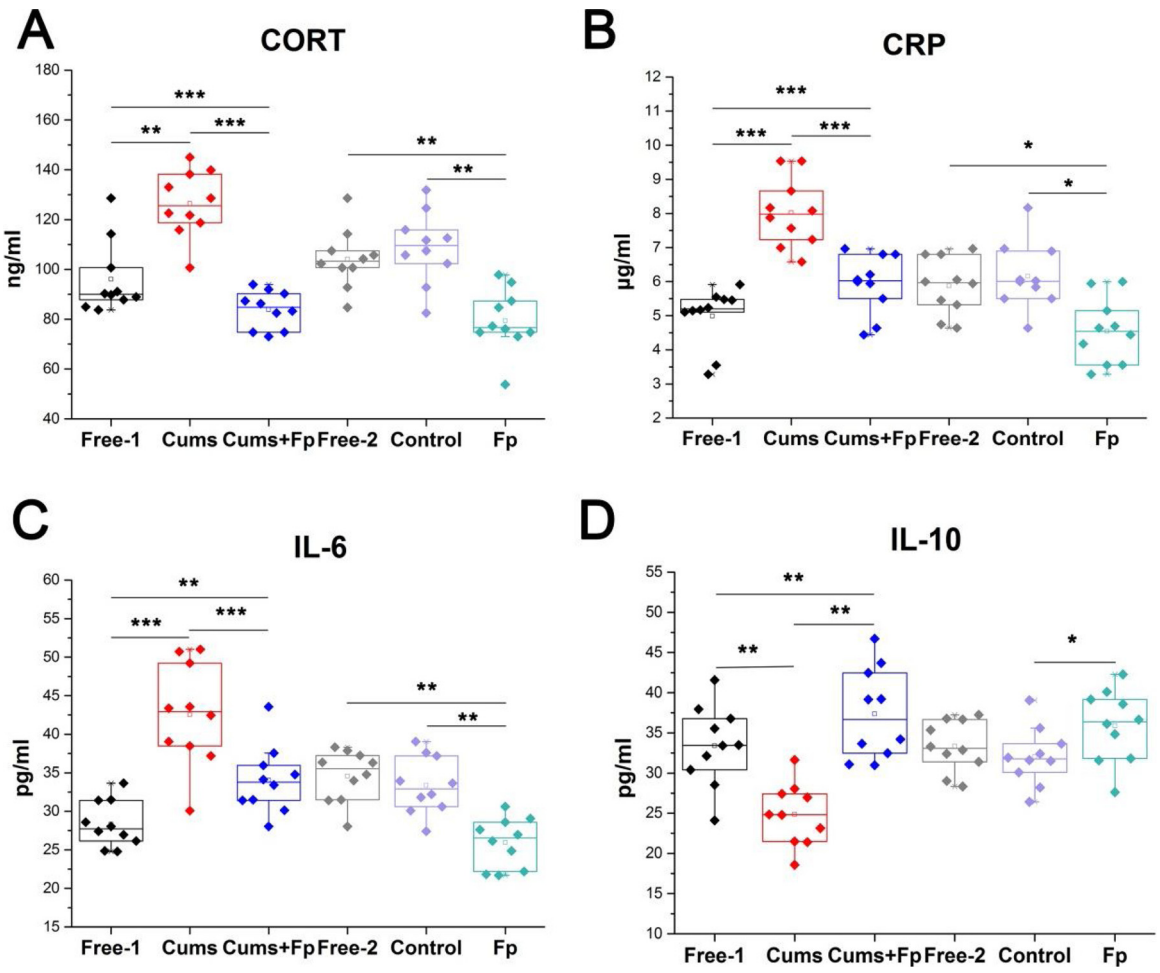

Fig. 6. Rat CORT (A), CRP (B) and cytokine IL-6 (C) and IL-10 (D) levels in blood plasma. The values were presented as single data points superimposed to boxplots. [A, first phase, $F(2,27) = 32.6$, second phase, $F(2,27) = 14.8$; B, first phase, $F(2,27) = 8.8$, second phase, $F(2,27) = 28.9$; C, first phase, $F(2,27) = 21.2$, second phase, $F(2,27) = 18.6$; D, first phase, $F(2,27) = 17.2$; second phase, $F(2,27) = 2.6$]. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. One-way ANOVA with Duncan's test during each phase.

and food intake of rats from the Cums + Fp group significantly increased compared to the Cums group ($p < 0.001$) (Fig. 2A and C). Rats in the Cums group ($p = 0.002$) and Cums + Fp ($p = 0.005$) demonstrated a significant increase in intestinal transit time (Fig. 2E) during CUMS phase. During the recovery phase, although the weight gain and food intake of rats in the Fp group was significantly lower than the Free-2 group ($P < 0.01$), it significantly increased compared to the control group ($p < 0.001$) after 4 weeks of *F. prausnitzii* administration (Fig. 2B).However, there were no significant differences in weight gain/Food intake ($p = 0.30$) between the different groups during the each phase.

3.2. Depression and anxiety like behaviors

3.2.1. Results of EPM & OF

Free gourp rats were significantly more active than CUMS rats in the open field and elevate plus maze test. During CUMS procedure period, rats in the Cums and Cums + Fp group significantly decreased the time spent in open arms ($P < 0.001$), the numbers ($P < 0.001$) and percentage of the entries into open arms ($P < 0.001$)in the elevate plus maze test compared to the Free-1 group. And rats in the control and Fp group also significantly significantly decreased the time spent in open arms ($P < 0.001$), the numbers ($P < 0.001$) and percentage of the entries into open arms ($P < 0.001$) in the elevated plus maze test ($P < 0.05$) compared to the Free-2 group during the recovery phase (Fig. 3A–C). About *F. prausnitzii* administration, Cums + Fp group significantly increased the time spent in the open arms ($P < 0.001$) and the numbers of the entries into open arms ($P < 0.01$), but had no significant difference on percentage of the entries into open arms ($P = 0.31$) in the elevate plus maze test compared to the Cums group during CUMS procedure period. During the recovery phase, Fp group significantly increased the time spent in open arms ($P < 0.001$) and the numbers of the entries into open arms ($P < 0.01$), but had no significant difference on percentage of the entries into open arms ($P = 0.53$)in the elevate plus maze test compared to the control group ($P > 0.05$) (Fig. 3A–C).

During CUMS procedure period, rats in the Cums and Cums + Fp group significantly increased latency to the center zone ($P < 0.001$), and significantly decreased time in the center of the open field test ($P < 0.01$) and the numbers of entries into the center of the open field test ($P < 0.01$) compared to the Free-1 group. And rats in the control and Fp group also significantly increased latency to the center zone ($P < 0.001$), and significantly decreased time in the center ($P < 0.001$) and the numbers of entries into the center of the open field test ($P < 0.001$) compared to the Free-2 group during the recovery phase (Fig. 3D–F). About *F. prausnitzii* administration, Cums + Fp group significantly decreased latency to the center zone compared to the Cums group ($P < 0.001$), but had no significant difference on time in the center ($P = 0.26$) or entries into the center ($P = 0.33$) of the open field test during CUMS procedure period (Fig. 3D–F). During the recovery phase, Fp group significantly increased time in the center of the open field test compared to the control group ($P < 0.001$), but there

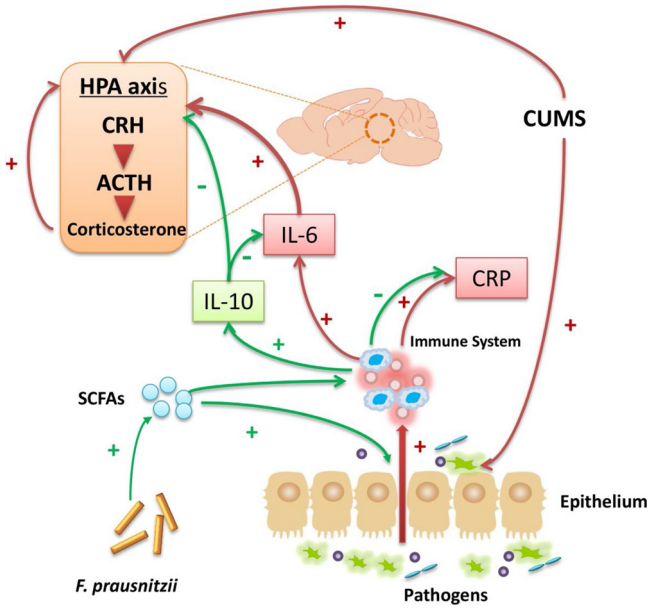

Fig. 7. The administration of *F. prausnitzii* has anxiolytic and antidepressant-like effects and reverses the impact of CUMS in rats (This is a hypothesis and not a summary of the findings of the described study). CUMS results in activation of the hypothalamic-pituitaryadrenal axis by bringing about the release of corticotropin releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and Cortisol/corticosterone, together with altered gut barrier function. The latter may result in the passage of lipopolysaccharide and other molecules (such as pathogens) into the bloodstream with development of a proinflammatory phenotype, characterized by high interleukin-6 (IL-6) and C-reaction protein (CRP) levels. Potential psychobiotics *F. prausnitzii* increased SCFAs such as acetate, propionate and n-butyrate levels, which play a major role in gut physiology and they have pleiotropic effects in intestinal cell life cycle and numerous beneficial effects for health through improving gut barrier function protection against pathogen invasion and modulation of immune system such as they can elevate the anti-inflammatory cytokine interleukin 10 (IL-10), decrease proinflammatory cytokines such as IL-6, and suppress hypothalamic-pituitary-adrenal (HPA) axis activity. This shows that the intestinal microbial balance may alter the regulation of inflammatory responses and in so doing may be involved in the modulation of mood and behavior.

was no effect of *F. prausnitzii* administration (Fp group) on entries into the center of the open field test ($P = 0.42$) or on latency to the center zone compared to the control group ($P = 0.31$). However, *F. prausnitzii* administration increased a tendency to make more entries into the center of the open field test and make the Fp group had no significant difference with a Free-2 group ($p = 0.56$) (Fig. 3E).

3.2.2. Results of FST

During CUMS procedure period, rats in the Cums group significantly increased immobility time ($P < 0.001$), and significantly decreased climbing ($P < 0.001$) and swimming time ($P < 0.001$) in the depressive-like behaviors forced swimming test compared to the Free-1 group (Fig. 4). About *F. prausnitzii* administration, rats in the Cums + Fp group significantly decreased immobility time ($P < 0.001$), and significantly increased climbing ($P < 0.001$) and swimming time ($P < 0.001$) in the forced swimming test compared to the Cums group. During the recovery phase, rats in the Fp group significantly increased swimming time ($P < 0.001$) and decreased immobility time ($P < 0.001$) compared to the control group. However, rats in the Fp group had no significant difference on climbing time compared to the control group ($P > 0.05$) (Fig. 4).

3.3. Bone mineral density

The rats BMD measured are shown in Table 1. During CUMS procedure period, the groups had no significant difference ($P > 0.05$) on whole-body, femur and tibia BMD. *F. prausnitzii* administration also had no effect on the BMD during CUMS procedure period ($P > 0.05$, Cums + Fp group vs Cums group). However, the bone mineral density of the control group induced by CUMS was significant lower ($P < 0.05$) than Free-2 and Fp group during the recovery phase. This showed *F. prausnitzii* administration could significantly prevent the reduction of the whole-body, femur and tibia BMD ($P < 0.05$, Fp group vs Control group) during the recovery phase.

3.4. Short-chain fatty acids

F. prausnitzii administration had a significant effect on cecum SCFAs production, as shown in Fig. 5. *F. prausnitzii* administration significantly increased acetate, Propionate and n-butyrate levels in the cecum ($p < 0.001$) (Fig. 5A, B, D), but it did not alter iso-butyrate levels in the cecum ($p > 0.05$) (Fig. 5C)

3.5. Plasma corticosterone, CRP and cytokine levels

One-way ANOVA with Duncan's test during each phase. CORT, Corticosterone; CRP, C-reaction protein; IL-6, cytokines interleukin-6; IL-10, cytokines interleukin-10.

During the CUMS procedure period, the corticosterone (CORT), CRP, IL-6 levels were significantly higher ($P < 0.001$) and IL-10 level was significantly lower ($P < 0.001$) in the Cums group compared to the Free-1 (Fig. 6). About *F. prausnitzii* administration, rats in the Cums + Fp group significantly decreased the CORT ($P < 0.001$), CRP ($P < 0.001$), and IL-6 levels ($P < 0.001$) and increased the IL-10 ($P < 0.01$) compared to the Cums group, Fp group also significantly decreased the CORT ($P < 0.01$), CRP($P = 0.02$), and IL-6 levels ($P < 0.01$) and increased the IL-10 ($P = 0.03$) compared to the control group (Fig. 6).

4. Disscusion

Mouse and human studies provide tantalizing suggestions that the psychobiotics, such as *Bifidobacterium infantis*, *Bifidobacterium longum* NCC3001, *Lactobacillus helveticus* strain NS8 (Desbonnet et al., 2010; Pinto-Sanchez et al., 2017) etc., may play an active role in driving depressive-like or anxiolytic-like behaviors, suggesting potential new avenues for therapeutic development. *F. prausnitzii* is the most abundant bacterium in the human intestinal microbiota of healthy adults, representing more than 5% of the total bacterial population. Over the past five years, an increasing number of studies have clearly described the changes in the abundance of *F. prausnitzii* have been linked to dysbiosis in several human disorders including depression. (Jiang et al., 2015; Miquel et al., 2013; Munukka et al., 2017). *F. prausnitzii* was also considered a next-generation probiotic (Martin et al., 2017).In this study, we report that potential psychobiotics *F. prausnitzii* was able to significantly prevent and treat depressive-like or anxiolytic-like behaviors caused by CUMS. Moreover, It could markedly modify behavior and hypothalamic–pituitary–adrenal (HPA) axis activity relevant to depression and anxiety in rats. In addition, we report that the growth status of rats fed the *F. prausnitzii* were better than the rats by CUMS. And *F. prausnitzii* administration led to higher levels of SCFAs in the cecum, and higher levels of cytokines interleukin-10 (IL-10) in the plasma, prevented the effects on corticosterone, C-reaction protein and cytokines interleukin-6 (IL-6) release induced by CUMS, changes that were associated with the effects seen on behavior.

In this study, behavioral tests showed that following CUMS procedure, rats displayed great anxiety-like behaviors as indicated by rats in the Cums group were significantly less active than free rats in the open field and elevate plus maze test, and rats displayed great depressive-like behaviors as indicated by rats in the Cums group were significantly increased immobility time and significantly decreased climbing and

swimming time in the forced swim test compared to the Free-1 group. *F. prausnitzii* administration had marked effects on preventing and reducing CUMS-induced anxiety-like behaviors by rats in the Cums + Fp and Fp groups were more active than Cums and control group in the open field and elevate plus maze test, especially (Fig. 3). And *F. prausnitzii* administration could prevent CUMS-induced depressive-like behaviors by rats in the Cums + Fp group significantly decreased immobility time, significantly increased climbing and swimming time in the forced swim test compared to the Cums group during CUMS protocol period, and Fp group selectively increased swimming time and decreased immobility time in the forced swim test compared to the control group during the recovery phase (Fig. 4).

The weight gain and food intake of rats in the Cums group ($p < 0.01$) and Cums + Fp ($p < 0.01$) significantly decreased and the intestinal transit time of rats significantly increased ($p < 0.05$) compared to the Free-1 group during the CUMS phase, indicating anxiety and depression could change gastro-intestinal function (Gorard et al., 1996). During the recovery phase, the weight gain and food intake of rats in the Fp significantly increased compared to the control group ($p < 0.001$), and there were no significant differences in the intestinal transit time between the Fp and Free-2 group. This indicates that *F. prausnitzii* administration could improve gastro-intestinal function. However, there were no significant differences in weight gain/Food intake ($p = 0.30$) between the different groups during the each phase (Fig. 2). These show the significant differences of body weight gain between different groups was mainly due to the changes of food intake.

Microbial metabolites such as SCFAs can reach the circulation, cross the blood brain barrier (Frost et al., 2014; Vijay and Morris, 2014) and activate specific receptors in relevant brain regions underpinning the neurocircuitry pertinent to the expression of depression and anxiety-related behaviors (Kelly et al., 2016; Wei et al., 2014). Indeed, recently it has been demonstrated that SCFAs are key molecules that modulate microglia maturation, morphology, and function (Erny et al., 2015). It is now well-establish that *F. prausnitzii* is an acetate-, propionate-and butyrate-producer species (Lopezsiles et al., 2017).This preclinical data does show that *F. prausnitzii* administration significantly increased acetate, propionate and n-butyrate levels in the cecum (Fig. 5). Butyrate plays a major role in gut physiology and it has pleiotropic effects in intestinal cell life cycle and numerous beneficial effects for health through protection against pathogen invasion, modulation of immune system and reduction of cancer progression (Macfarlane and Macfarlane, 2011). There is ample evidence that production of butyrate by the gut microbiota strongly influences peripheral immune system function, which will in turn shape the brain's immune milieu (Filiano et al., 2015). In addition, butyrate directly affects serotonin and gut hormone release in the enteric nervous system and thereby stimulates the vagus nerve and elicits endocrine signalling, both impacting on brain function. Host metabolism and immune functions are critically dependent on butyrate as an energy source and potent regulator. This implicates butyrate as a key modifiable mediator of host-microbe crosstalk (Stilling et al., 2016a). Thus, by producing butyrate in the gut, *F. prausnitzii* may impact on physiological functions and homeostasis to maintain mental health (Miquel et al., 2013).

More recently, chronic stress and the gut microbiota can interact through complementary or opposing factors to influence HPA axis. The hypothalamic–pituitary–adrenal (HPA) axis has been suggested as a possible mechanism of action of probiotics (Forsythe et al., 2010). HPA axis activity was assessed by measuring corticosterone. Chronic stress is known to activate the HPA axis leading to the release of corticotropin releasing hormone, adrenocorticotropic hormone, and cortisol/corticosterone (RH and G, 2008). Here, the results showed CUMS procedure significantly increased the corticosterone, suggesting enhanced activity of the HPA axis. And we demonstrate that treatment with *F. prausnitzii* significantly reduced the enhanced level of circulating corticosterone, suggesting normalised activity of the HPA axis (Fig. 6A). C-reactive protein (CRP) levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via C1q (D et al., 1999). In this study, the results showed CUMS procedure significantly increased the CRP, suggesting increased inflammation. And we demonstrate that treatment with *F. prausnitzii* significantly reduced the enhanced level of CRP, suggesting *F. prausnitzii* anti-inflammatory effects (Fig. 6B).The pro-inflammatory cytokines interleukin-6 (IL-6) is potent modulators of corticotropin-releasing hormone (CRH) which produces heightened hypothalamic–pituitary–adrenal axis (HPA) activity characterized by increases in ACTH and cortisol/corticosterone, which is reported elevated in major depression (O'Brien et al., 2004). It has been suggested that antidepressants act in part via generation of perhaps the most potent immunoregulatory cytokine, IL-10, thereby suppressing inflammation and depressive mood (Messaoudi et al., 2011). *F. prausnitzii* has been found to be a strong inducer of regulatory T cells secreting IL-10. (Martin et al., 2017) In this study, the results showed CUMS procedure significantly increased the IL-6, suggesting increased inflammation. And this data did show that *F. prausnitzii* administration significantly increased IL-10 level, and decreased IL-6 induced by CUMS (Fig. 6C–D). Li et al. found that the mixture of three probiotic strains (*(Lactobacillus helveticus R0052, Lactobacillus plantarum R1012, and Bifidobacterium longum R0175)*) could alleviate CUMS-induced anxiety- and depressive-like behaviors in mice (Li et al., 2018). The findings revealed that mice subjected to CUMS exhibited anxiety- and depressive-like behaviors along with increased interferon-γ, tumor necrosis factor-α, and indoleamine 2,3-dioxygenase-1 levels in the hippocampus. Probiotics attenuated CUMS-induced anxiety- and depressive-like behaviors, and reversed the CUMS-induced immune changes in the hippocampus, demonstrating that the inextricable relationship between the gut microbiota, and the immune and nervous systems.

Recent studies showed that chronic psychological stress is a risk factor for osteoporosis by various signaling pathways. Increasing evidence confirms the physiological importance of the central nervous system, especially the hypothalamus, in the regulation of bone metabolism. Both animal and human studies indicate that Chronic stress activates the HPA axis and sympathetic nervous system, suppresses the secretion of gonadal hormone and growth hormone, and increases inflammatory cytokines, eventually leading to bone loss by inhibiting bone formation and stimulating bone resorption (Azuma et al., 2015). *F. prausnitzii* administration, proven to be able to significantly prevent and treat depressive-like or anxiolytic-like behaviors caused by CUMS in this study, we guess could attenuate CUMS-induced neuroendocrine responses and ameliorate CUMS-induced bone loss. In this study, results showed during CUMS protocol period, the groups had no significant difference on whole-body, femur and tibia BMD, maybe the processing time was not long enough. However, the bone mineral density of the control group induced by CUMS was lower than Free and Fp group during the recovery phase. This showed longer depression may have decreased BMD, and *F. prausnitzii* administration could significantly prevent the reduction of the whole-body, femur and tibia BMD ($P < 0.05$, Fp group vs Control group) during the recovery phase.

5. Conclusions and recommendation

In conclusion, findings indicate that consumption of *F. prausnitzii* mitigated anxiety- and depression-like behavior in rats. In addition, *F. prausnitzii* administration could significantly prevent the reduction of the whole-body, femur and tibia BMD during the recovery phase. Moreover, the growth status of rats fed the *F. prausnitzii* was better than the rats by CUMS. And *F. prausnitzii* administration led to higher levels of SCFAs in the cecum and higher levels of cytokines interleukin-10 (IL-10) in the plasma, prevented the effects on corticosterone, C-reaction protein and cytokines interleukin-6 (IL-6) release induced by CUMS,

changes that were associated with the effects seen on behavior (Fig. 7). These results provide further evidence that gut microflora play a role in anxiety and depression, perhaps via the enteric nervous system as well as centrally. Subject to the confirmation of these results, probiotics might offer a useful, novel therapeutic approach to neuropathological disorders and/or as adjunct therapies in psychiatric disorders (Logan and Katzman, 2005) and support the recent broadening of the definition of psychobiotic (Sarkar et al., 2016). Finally, this study supports *F. prausnitzii* has significant potential as a psychobiotic.

Conflict of interest

The authors declare no conflicts of interest.

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