Phytomedicine 83 (2021) 153471

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Total glycosides from stems of *Cistanche tubulosa* alleviate depression-like behaviors: bidirectional interaction of the phytochemicals and gut microbiota

Li Fan, Ying Peng, Jingwen Wang, Ping Ma, Lijuan Zhao, Xiaobo Li *

School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, P. R. China

ARTICLE INFO

Keywords:
Cistanche tubulosa
total glycosides
hydroxytyrosol
depression
gut microbiota

ABSTRACT

Background: As the most frequently used kidney-yang tonifying herb in traditional Chinese medicine, dried succulent stems of *Cistanche tubulosa* (Schenk) Wight (CT) have been shown to be effective in the treatment of depression. However, the antidepressant components and their underlying mechanism remain unclear.

Purpose: To explore the active components of CT against depression, as well as the potential mechanisms.

Study design and methods: Behavioral despair tests were used to assess the antidepressant activities of polysaccharides, oligosaccharides and different glycoside-enriched fractions separated from CT, as well as the typical gut microbiota metabolites including 3-hydroxyphenylpropionic acid (3-HPP) and hydroxytyrosol (HT). Furthermore, the effects of bioactive fractions and metabolites on chronic unpredictable mild stress (CUMS) model were explored with multiple pharmacodynamics and biochemical analyses. Changes in colonic histology and the intestinal barrier were observed by staining and immunohistochemical analysis. Gut microbial features and tryptophan-kynurenine metabolism were explored using 16S rRNA sequencing and western-blotting, respectively.

Results: Total glycosides (TG) dramatically alleviated depression-like behaviors compared to different separated fractions, reflecting in the synergistic effects of phenylethanoid and iridoid glycosides on the hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis, severe neuro- and peripheral inflammation, and deficiencies in 5-hydroxytryptamine (5-HT) and brain-derived neurotrophic factor in the hippocampus. Moreover, TG mitigated low-grade inflammation in the colon and intestinal barrier disruption, and the abundances of several bacterial genera highly correlated with the HPA axis and inflammation in CUMS rats. Consistently, the expression of indoleamine 2, 3-dioxygenase 1 (IDO1) in the colon was significantly reduced after TG administration, accompanied by the suppression of tryptophan-kynurenine metabolism. On the other hand, HT also exerted a marked antidepressant effect by ameliorating HPA axis function, pro-inflammatory cytokine release, and tryptophan-kynurenine metabolism, while it was unable to largely adjust the disordered gut microbiota in the same manner as TG. Surprisingly, superior to fluoxetine, TG and HT could further improve dysfunction of the hypothalamic-pituitary-gonadal axis and abnormal cyclic nucleotide metabolism.

Abbreviations: AB-PAS, Alcian blue-periodic acid-Schiff; ACTH, adrenocorticotropic hormone; BDNF, brain-derived neurotrophic factor; CT, dried succulent stems of *Cistanche tubulosa* (Schenk) Wight; CUMS, chronic unpredictable mild stress; CORT, corticosterone; CRH, corticotrophin-releasing hormone; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CTE, *Cistanche tubulosa* aqueous extract; ELISA, enzyme-linked immunosorbent assay; FLX, fluoxetine; FST, forced swimming test; GnRH, gonadotropin-releasing hormone; 5-HT, 5-hydroxytryptamine; 3-HPP, 3-hydroxyphenylpropionic acid; HPA, hypothalamic-pituitary-adrenal; HT, hydroxytyrosol; HPG, hypothalamic-pituitary-gonadal; H&E, hematoxylin and eosin; IL-1β, interleukin-1beta; IFN-γ, interferon-gamma; IDO1, indoleamine 2,3-dioxygenase 1; Imp, imipramine; Kyn/Trp, kynurenine-to-tryptophan ratio; LEfSe, linear discriminant analysis effect size; OFT, open field test; PCoA, principal coordinates analysis; SSRIs, selective serotonin reuptake inhibitors; SPT, sucrose preference test; TCM, traditional Chinese medicine; TNF-α, tumor necrosis factor-alpha; T, testosterone; TP, total polysaccharides; TO, total oligosaccharides; TG, total glycosides; TG-IrG, total irodoid glycosides; TG-PhG, total phenylethanoid glycosides; TST, tail suspension test; ZO-1, zonula occludens 1.

* Corresponding author at: School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, People's Republic of China.
E-mail address: xbli@sjtu.edu.cn (X. Li).

https://doi.org/10.1016/j.phymed.2021.153471
Received 25 October 2020; Received in revised form 31 December 2020; Accepted 14 January 2021
Available online 22 January 2021

Conclusion: TG are primarily responsible for the antidepressant activity of CT; its effect might be achieved through the bidirectional interaction of the phytochemicals and gut microbiota, and reflect the advantage of CT in the treatment of depression.

Introduction

Depression, is a chronic, recurring and potentially life-threatening mental disorder that affects up to 20% of the population worldwide (Nabavi *et al.*, 2017). At present, although numerous antidepressants have been approved, the side effects are concerning. Notably, long-term treatment with selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, the major pharmacological agent for depression, exacerbated problems such as chronic sexual dysfunction (Bijlsma *et al.*, 2014); this has drawn increasing attention to the discovery of novel antidepressants from natural herbs (Wang *et al.*, 2019). In traditional Chinese medicine (TCM) theory, kidney-yang deficiency results in performance features similar to the clinical symptoms of depression such as feeling down in the dumps, loss of interest and sentimentality (Yang *et al.*, 2020), promoting the thoughts to alleviate depression-like behaviors by tonifying kidney-yang. Coincidentally, many kidney-yang tonifying herbs possess the advantage of improving sexual function (Wu *et al.*, 2015). Such studies make us pay attention to the potential application of Cistanches Herba, which is the most frequently used kidney-yang tonifying herb in TCM and is effective in treating male sexual dysfunction (Wang *et al.*, 2020), in terms of antidepressant developments. Cistanches Herba, officially recorded as the dried succulent stems of *Cistanche tubulosa* (Schrenk) Wight (CT) and *Cistanche deserticola* (Y. C. Ma) in the Chinese Pharmacopoeia (Wang *et al.*, 2017), has been used in China and other East Asian countries since the 15th century to treat conditions such as kidney-yang deficiency, impotence and female infertility. Modern pharmacological studies indicate that Cistanches Herba has multiple bioactivities, such as immunity enhancement, neuroprotection, and antioxidant, antiaging and antifatigue effects (Wang *et al.*, 2017). The neuroprotective properties of Cistanches Herba suggest its therapeutic potential in cognitive-related illnesses such as stroke, depression and Alzheimer's disease (Wang *et al.*, 2020). In our previous study, we found that CT extract alleviated the depression-like behaviors of chronic unpredictable mild stress (CUMS) rats (Li *et al.*, 2018). To date, chemical analysis of CT showed that its main constitutes include phenylethanoid glycosides, iridoid glycosides, polysaccharides and oligosaccharides (Jiang and Tu, 2009), however, the type of compound that plays a dominant role in its antidepressant effect remains unclear.

Numerous studies have confirmed that the physiological action of the gut (especially commensal microbiota) plays an important role in the development of depression and can regulate the neuroendocrine, autonomic nervous system and immune systems (Yang *et al.*, 2020). Moreover, the relationship linking tryptophan metabolism, the gut microbiota, and depression has gradually attracted much attention. Altered gut microbiota can activate the tryptophan-kynurenine pathway, subsequently reducing peripheral and cerebral tryptophan availability, which results in 5-HT deficiency (Agus et al., 2018). Our previous study showed that phenylethanoid glycosides in CT generally exert low bioavailability, and can be quickly metabolized to 3-hydroxyphenylpropionic acid (3-HPP) and hydroxytyrosol (HT) by the gut microbiota (Li *et al.*, 2016). Additionally, CT extract was reported to restore gut microbiota homeostasis in CUMS rats (Li *et al.*, 2018). Based on such research, we inferred that the bidirectional interaction of the phytochemicals and gut microbiota might govern the antidepressant effects of specific bioactive components of CT. Specifically, the compounds might be transformed into absorbable metabolites (such as 3-HPP and HT) in the gastrointestinal tract by gut microbiota, and subsequently exert antidepressant effects. In turn, bioactive component-induced gut microbiota structural alterations can simultaneously play a vital role in the antidepressant activity.

Herein in our study, we used behavioral despair tests to assess the antidepressant activities of polysaccharides, oligosaccharides and diverse glycosides-enriched fractions separated from CT aqueous extract (CTE), and the typical metabolites by gut microbiota (including 3-HPP and HT), which we further confirmed in chronic unpredictable mild stress (CUMS) model with multiple pharmacodynamics and biochemical analyses. Subsequently, we investigated whether its mechanism against depression was relevant to the bidirectional interaction of the phytochemicals and gut microbiota.

Materials and Methods

Materials

All standards used for chemical analysis were purchased from Durst (Sichuan, China). 3-HPP, HT, fluoxetine and imipramine were purchased from Aladdin (Shanghai, China). The BCA protein quantitative kit was purchased from Boster (Wuhan, China). Nanjing Jiancheng (Nanjing, China) provided 5-HT, BDNF, tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), and interferon-gamma (IFN-γ) enzyme-linked immunosorbent assay (ELISA) kits. Corticosterone (CORT), adrenocorticotropic hormone (ACTH), and corticotrophin-releasing hormone (CRH) ELISA kits were purchased from Multi-Sciences (Hangzhou, China). Cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), testosterone (T) and gonadotropin-releasing hormone (GnRH) ELISA kits were purchased from Enzyme-linked Biotechnology (Shanghai, China). Tryptophan and kynurenine ELISA kits were obtained from Small Molecule Antibodies (Bordeaux, France). Primary/secondary antibodies including zonula occludens 1 (ZO-1), indoleamine 2, 3-dioxygenase 1 (IDO1), β-actin, and HRP goat anti-rabbit IgG (H+L) were purchased from Proteintech (Wuhan, China). All other reagents of analytical grade or higher purity were obtained from commercial suppliers.

Preparation of different fractions separated from CTE

CT were collected in November 2015 in Hetian, Xinjiang, China, and authenticated by Prof. Xiaobo Li. The voucher specimen (20151108) was deposited at the School of Pharmacy at Shanghai Jiao Tong University.

Crushed samples were extracted three times with distilled water (1:10, w/v) for 2 h at 80~90 °C to obtain CTE (the yield was 57.1%). CTE was dissolved in distilled water and then slowly stirred with ethanol (1:4, v/v) and placed at 4 °C for 24 h. Then, the precipitate and supernatant were collected by centrifugation at 4000 rpm for 20 min. Total polysaccharides (TP; the yield was 5.7%) were obtained by washing with 80% ethanol, and deproteinization with Sevage reagent three times from the collected precipitate. The remaining supernatant was then chromatographed over a D101 microporous resin column and eluted with distilled water and 40% ethanol. The eluted water and 40% ethanol fractions contained total oligosaccharides (TO; the yield was 35.1%) and total glycosides (TG; the yield was 12.1%), respectively. TG were further eluted with distilled water and 5%, 10%, and 40% ethanol through D101 microporous resin again, and the eluted fractions of 5% and 40% ethanol were total iridoid glycosides (TG-IrG; the yield was 2.7%) and total phenylethanoid glycosides (TG-PhG; the yield was 8.7%), respectively.

Chemical analysis

First, the main chemical constituents of different fractions separated

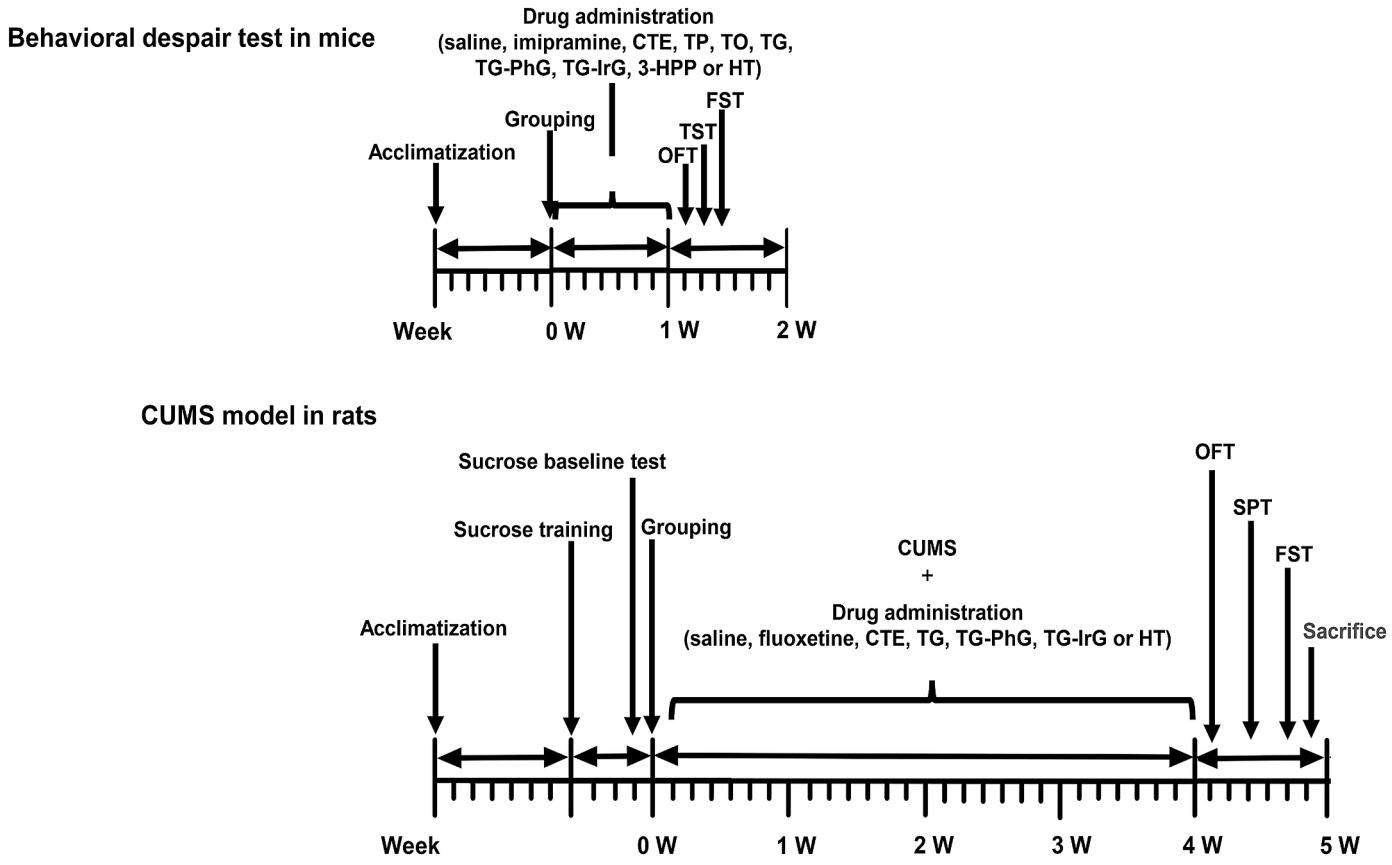

Fig. 1. Flow chart of animal experiments (CUMS: chronic unpredictable mild stress; OFT: open-field test; TST: tail suspension test; FST: forced swimming test; SPT: sucrose preference test).

Table 1
Subjects and the dosage for mice or rats in each group.

Class	Mice			Rats		
	Groups	Subjects	Dosage	Groups	Subjects	Dosage
Control	Con	Aqueous solution	10 mL/kg.d.bw	Con	Aqueous solution	10 mL/kg.d.bw
Model	——			CUMS	Aqueous solution	10 mL/kg.d.bw
Positive	Imp	Imipramine aqueous solution	15 mg/kg.d.bw	CUMS+FLX	Fluoxetine aqueous solution	10 mg/kg.d.bw
Treatment	CTE	CTE aqueous solution	2.60 g/kg.d.bw	CUMS+CTE	CTE aqueous solution	1.80 g/kg.d.bw
	TP	TP aqueous solution	0.26 g/kg.d.bw	——		
	TO	TO aqueous solution	1.60 g/kg.d.bw	——		
	TG	TG aqueous solution	0.55 g/kg.d.bw	CUMS+TG	TG aqueous solution	0.38 g/kg.d.bw
	TG-PhG	TG-PhG aqueous solution	0.40 g/kg.d.bw	CUMS+TG- PhG	TG-PhG aqueous solution	0.28 g/kg.d.bw
	TG-IrG	TG-IrG aqueous solution	0.13 g/kg.d.bw	CUMS+TG-IrG	TG-IrG aqueous solution	0.09 g/kg.d.bw
	3-HPP	3-HPP aqueous solution	0.07 g/kg.d.bw	——		
	HT	HT aqueous solution	0.07 g/kg.d.bw	CUMS+HT	HT aqueous solution	0.05 g/kg.d.bw

from CTE were characterized by UPLC-QTOF-MS. Second, the relative contents of total carbohydrates and glycosides were determined by the UV-Vis method. Third, the major components (echinacoside, verbascoside, isoverbascoside, 8-epiloganic acid and geniposidic acid) were quantified by a validated HPLC method. The detailed parameters and method validation are shown in the Supplementary Materials.

Animal experiments

The specific animal experimental schedules are shown in Fig. 1. All experimental animals were housed and acclimatized for 1 week prior to the experiments in the Laboratory Animal Center of Shanghai Jiao Tong University (Shanghai, China), under controlled room temperatures (25 ± 2 °C; 55 ± 10% relative humidity) with a 12 : 12 h light-dark cycle. This research was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of SJTU. The animal facilities and protocols were approved by the Animal Ethics Committee of SJTU (No. A2019008).

One hundred and fifty male ICR mice (weighing 18–20 g, 6 weeks old) were purchased from Shanghai Slack Biotechnology Company (Shanghai, China) and randomly divided into 10 groups (n = 15/group). The subjects and doses for behavioral despair mice in each group are shown in Table 1. The doses of CTE and its separated fractions were calculated by the following formula: dosage = 4.55 g/kg.d.bw × yield, which was determined according to three human equivalent dose calculations based on body surface area. The doses of the positive drug imipramine and gut microbiota metabolites (3-HPP and HT) were set up according to the previous studies (Gupte *et al.*, 2016; Pablos *et al.*, 2019). After intragastric administration once daily for 7 days, the open field test (OFT), tail suspension test (TST) and forced swimming test (FST) were

Table 2
Contents of echinacoside, verbascoside, isoverbascoside, 8-epiloganic acid and geniposidic acid in each fraction (mean ± SEM, n = 3).

Group	Content (mg/g)				
	geniposidic acid	8-epiloganic acid	echinacoside	verbascoside	isoverbascoside
CTE	7.8 ± 0.3	9.7 ± 0.8	75.0 ± 1.4	16.0 ± 0.2	14.8 ± 0.3
TP	—	—	—	—	—
TO	—	—	—	—	—
TG	31.2 ± 1.0	40.2 ± 0.8	328.1 ± 1.4	69.4 ± 1.8	62.4 ± 0.5
TG-PhG	—	—	393.5 ± 0.8	76.8 ± 0.5	67.0 ± 0.3
TG-IrG	118.9 ± 0.8	146.9 ± 1.5	—	—	—

performed on the 8th, 9th and 10th days, respectively. The specific methods of the behavioral tests are outlined in the Supplementary Materials.

Seventy-two male Sprague-Dawley rats (weighing 160–180 g, 6 weeks old) were purchased from Shanghai Slack Laboratory Animal Company (Shanghai, China). Prior to the formal experiments, all rats were subjected to the sucrose preference test (SPT) after the corresponding training. The rats were divided into 8 groups (n = 9/group) according to their sucrose preference (Supplementary Fig. S1). The subjects and doses for the rats in each group are shown in Table 1. The doses were set up according to the conversion coefficient between mouse and rat based on their effective doses in the behavioral despair mice. And the dose of the positive control drug fluoxetine was consistent with the previous study (Hou *et al.*, 2017). All groups received a series of chronic unpredictable mild stress (CUMS) except for control group. Stressors were applied continuously and randomly for 4 weeks (see Supplementary Table S1 for details). Food and water were freely available to the control rats, which remained undisturbed in another room, except for the behavioral tests. The subjects were intragastrically administered 1 h before the CUMS procedure over the course of 4 weeks. After 28 days of continuous stressors and drug administration, the OFT, SPT and FST were performed on the 29th, 31st and 33rd days, respectively. The specific methods of the behavioral experiments are shown in the Supplementary Materials.

Sample collection and biochemical analysis

After the behavioral tests, the fasting rats were anesthetized with pentobarbital sodium, and whole blood was collected to obtain serum samples. After heart perfusion with saline, the hippocampus, colon and cecal contents were collected and stored in -80 °C. Distal colonic segments were removed and fixed in 4% paraformaldehyde. The levels of CORT, CRH, ACTH, TNF-α, IL-1β, IFN-γ, cAMP, cGMP, T, GnRH, tryptophan, and kynurenine in the serum, 5-HT, BDNF, and TNF-α in the hippocampus, and TNF-α and IFN-γ in the colon were measured according to the instructions provided with the corresponding ELISA kits.

Histology, immunohistochemistry and western-blotting assay

Hematoxylin and eosin (H&E) and Alcian blue-periodic acid-Schiff (AB-PAS) staining were used to observe the pathological changes in colon tissue. In addition, immunohistochemistry assessments of these sections were performed to observe ZO-1 protein expression in colonic epithelial cells (Ding *et al.*, 2020). Details regarding sample preparation and western-blotting assay to determine IDO1 protein expression in the colon and hippocampus are also described in the Supplementary Materials.

Compositional profile analysis of gut microbiota

16S rRNA high-throughput sequencing was performed by MajorBio Co., Ltd. (Shanghai, China), and the detailed method and data processing are shown in the Supplementary Materials.

Statistical analysis

The data are expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used to compare multiple groups by using SPSS 20.0 software. A difference was considered significant at $p < 0.05$. The correlations between gut microbes and certain depression-related physiological factors were analyzed using the Spearman correlation coefficient based on heatmap analysis.

Results

Chemical analysis of different fractions separated from CTE

The main components were characterized using UPLC-QTOF-MS (Supplementary Table S2 and Fig. S2), and total of 21 constituents were identified, including 16 constituents of phenylethanoid glycosides, 2 constituents of phenolic glycosides in CTE, TG and TG-PhG; and 3 constituents of iridoid glycosides in CTE, TG, and TG-IrG. Overall, the relative contents of total carbohydrates in TP and TO were 60.3% and 80.8%, respectively. The relative contents of total glycosides in TG were 87.7%. Correspondingly, phenylethanoid and iridoid glycosides accounted for 86.7% and 53.4% of TG-PhG and TG-IrG, respectively.

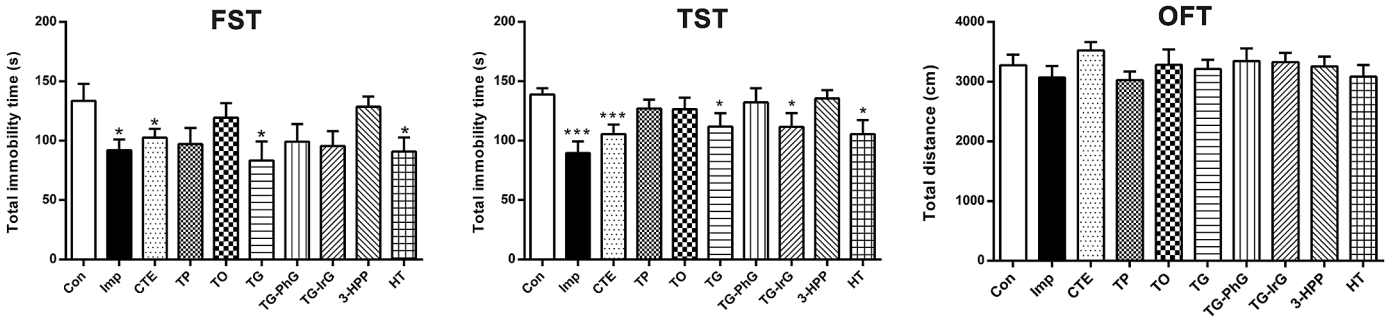

Fig. 2. The effects of different fractions separated from CTE and the typical metabolites by gut microbiota on the FST, TST, OFT in mice. Data represent mean ± SEM (n = 13-15/group). ANOVA analysis, compared with control group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 3
The effects of different glycoside-enriched fractions and HT on the FST, SPT and OFT in CUMS rats.

Group	FST Total immobility times (s)	SPT Sucrose preference (%)	OFT Total distance (cm)	Rearing number
Con	67.1±6.3	86.4±4.0	2458.4±65.6	27.7±1.1
CUMS	133.2±12.2$^{\#\#}$	50.2±6.1$^{\#\#\#}$	1532.8±48.6$^{\#\#\#}$	13.3±0.7$^{\#\#\#}$
CUMS+FLX	76.8±11.3**	81.0±3.9***	1921.5±99.0**	24.3±2.0**
CUMS+CTE	64.9±10.3**	88.7±5.5***	1875.3±118.3*	22.5±1.3**
CUMS+TG	71.5±19.8**	81.3±6.8***	1992.7±95.5**	24.3±2.0*
CUMS+TG-PhG	92.3±14.0*	77.6±6.2**	1863.6±98.7*	19.8±1.8
CUMS+TG-IrG	85.1±15.1**	79.4±6.2**	1947.1±134.8**	17.7±0.9
CUMS+HT	87.3±18.3*	81.5±5.7**	2112.8±82.6**	25.8±2.6*

Data represent mean ± SEM (n=8-9/group). ANOVA analysis, compared with control group, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$; compared with CUMS group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

The contents of echinacoside, verbascoside, isoverbascoside, 8-epiloganic acid and geniposidic acid in each fraction are shown in Table 2, and the HPLC chromatogram and method validation data are listed in Supplementary Table S3 and Fig. S3.

Effects of different fractions separated from CTE and the typical metabolites by gut microbiota on behavioral despair tests in mice

As shown in Fig. 2, compared with control group, mice subjected to 7 days of imipramine (positive control) administration exhibited significant reductions in the immobility times in both FST and TST, and showed no significant effect on the total distance traveled in the OFT. Similar alterations were also observed in CTE, TG and HT groups, indicating that CTE, TG and HT exerted the antidepressant activity and the effects were not related to excitability. Hence, TG and HT were the dominant bioactive fraction separated from CTE and typical metabolite by gut microbiota, respectively. Interestingly, no significant changes in the immobility times in the FST were shown after TG-PhG and TG-IrG administration compared with those of the controls, or in the TST after TG-PhG administration, indicating that the presence of phenylethanoid and iridoid glycosides might be essential in the antidepressant activity of CTE.

Pharmacodynamic effects and biochemical changes of different glycoside-enriched fractions and HT on CUMS rats

As shown in Table 3, rats subjected to 28 days of the CUMS paradigm showed significant reductions ($p < 0.001$) in sucrose preference in the SPT, total distance traveled, and rearing number in the OFT, as well as a significant increase ($p < 0.01$) in total immobility time in the FST compared with the controls, suggesting the induction of depression-like phenotypes in CUMS rats. The depressive performances were reversed in FLX (positive control) group. Similarly, CTE, TG, TG-PhG, TG-IrG and HT treatment resulted in the restoration of sucrose preference to normal levels in CUMS rats, which were 102.7%, 94.1%, 89.8%, 91.9% and 94.3% of the controls, respectively. Consistently in the FST, CTE and TG groups exhibited the most obvious effects, and the immobility times reached 103.3% and 93.4% of those of control group, respectively, while the immobility times of TG-PhG, TG-IrG and HT groups just reached 62.1%, 73.2%, and 69.9% of those of control group. The similar significant increases ($p < 0.05$) were observed in the total distance traveled and rearing number in the OFT after CTE, TG and HT administration, and TG-PhG and TG-IrG treatment trended toward revising the rearing number in CUMS rats. The results indicated that TG was the dominant bioactive antidepressant fraction separated from CTE, and phenylethanoid and iridoid glycosides were indispensable in exerting its antidepressant effect. Moreover, HT was demonstrated to be one of the active metabolites of TG *in vivo*.

Consistently, suffering from CUMS significantly increased the levels of CORT, CRF, ACTH, TNF-α, IL-1β, and IFN-γ in the serum and TNF-α in the hippocampus of rats compared with those of control group ($p < 0.05$), resulting in significant reductions ($p < 0.05$) in the levels of hippocampal 5-HT and BDNF (Fig. 3). Except for a non-significant difference in the level of hippocampal BDNF between FLX and CUMS groups, the above-mentioned alterations in CUMS rats could be significantly reversed ($p < 0.05$) after administration of fluoxetine, CTE, TG and HT, indicating that CTE, TG and HT showed ameliorative effects on the hyperactivation of HPA axis, severe peripheral and neural inflammation, and deficiencies in 5-HT and BDNF. Likewise, TG-IrG and TG-PhG treatment significantly reduced the levels of serum CORT, TNF-α, IFN-γ, and hippocampal TNF-α, and increased the level of hippocampal 5-HT in CUMS rats ($p < 0.05$). Significant decreases ($p < 0.05$) in the levels of serum CRF and ACTH were also observed in TG-IrG group compared with CUMS group. Interestingly, the levels of serum CORT, TNF-α, ACTH and CRF in TG-PhG and TG-IrG groups were significantly higher than those in TG group ($p < 0.05$), and the levels of hippocampal 5-HT and BDNF exhibited the opposite expression pattern ($p < 0.05$). These results showed that the effects of TG-PhG and TG-IrG on the hyperactivity of the HPA axis, peripheral and neural inflammation, and shortage of 5-HT were clearly inferior to TG, indicating that phenylethanoid and iridoid glycosides might exert synergistic effects on the above-mentioned multiple aspects.

TG and HT regulate the HPG axis and cyclic nucleotide metabolism in CUMS rats

The levels of T, GnRH, cAMP, and cGMP and the ratio of cAMP and cGMP (cAMP/cGMP) in the serum subjected to CUMS were measured, and the results are shown in Table 4. The concentrations of T, GnRH, cAMP and cAMP/cGMP in the serum were significantly decreased, and the level of cGMP was significantly increased ($p < 0.05$) in CUMS group compared with the controls, indicating that subjected to CUMS resulted in suppression of the HPG axis and cyclic nucleotide metabolism dysfunction. Compared with CUMS group, there were no significant changes in FLX group. Surprisingly, after TG and HT treatment, significant increases ($p < 0.05$) were exhibited in the levels of serum T, GnRH, cAMP and cAMP/cGMP, while the level of serum cGMP was significantly reduced ($p < 0.05$). These results indicated that TG and HT regulated the HPG axis and cyclic nucleotide metabolism, which was distinctly superior to the positive control fluoxetine.

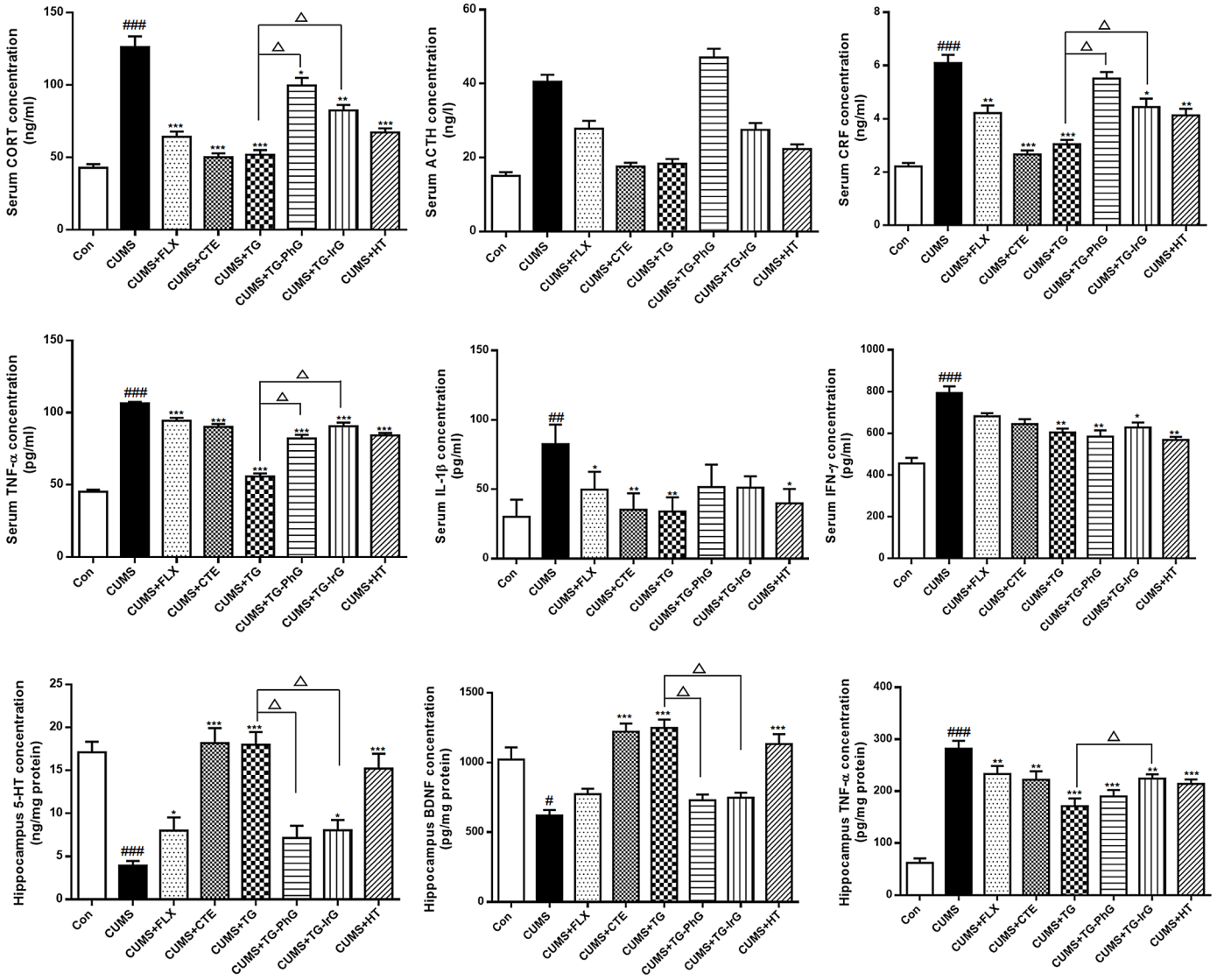

Fig. 3. Effects of different glycoside-enriched fractions and HT on the levels of CORT, ACTH, CRF, TNF-α, IL-1β, IFN-γ in the serum and 5-HT, BDNF, TNF-α in the hippocampus of CUMS rats. Data represent mean ± SEM (n = 8-9/group). ANOVA analysis, compared with control group, $^{\#\#}p$ <0.01, $^{\#\#\#}p$ <0.001; compared with CUMS group, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; compared with TG group, $^{\Delta}p < 0.05$.

Table 4
Effects of TG, HT or fluoxetine on HPG axis-related hormones and nucleotide metabolism-related indexes in the serum of CUMS rats.

Group	GnRH (mIU/mL)	T (pg/mL)	cAMP (nmmol/L)	cGMP (nmmol/L)	cAMP/cGMP
Con	30.3±1.0	108.2±2.0	5.5±0.1	1.6±0.1	3.7±0.3
CUMS	13.0±0.5$^{\#\#\#}$	42.4±3.4$^{\#\#\#}$	2.8±0.1$^{\#\#\#}$	3.9±0.3$^{\#\#\#}$	1.1±0.4$^{\#\#\#}$
CUMS+FLX	13.5±0.9	46.3±3.0	3.5±0.2	3.5±0.3	1.4±0.5
CUMS+TG	15.9±1.0*	68.8±3.7***	4.6±0.2***	2.7±0.2***	2.0±0.4***
CUMS+HT	20.2±1.8***	84.4±4.0***	4.0±0.1***	3.1±0.3***	1.7±0.5***

The values are expressed as mean ± SEM (n = 8-9/group). ANOVA analysis, compared with control group, $^{\#\#\#}P < 0.001$; compared with CUMS group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

TG and HT alleviate low-grade inflammation in the colon and intestinal barrier disruption in CUMS rats

H&E staining was used to assess colonic morphological damage, and no significant differences in the colon were observed among the experimental groups (Fig. 4a). Furthermore, the effects of TG and HT on inflammation in the colon and intestinal barrier disruption were also analyzed (Fig. 4b-d). Compared with those of control group, the number of goblet cells and the thickness of the mucus layer were reduced in CUMS group. Consistently, the protein expression of ZO-1, and the levels of IFN-γ and TNF-α in the colon were significantly decreased ($p < 0.05$) and increased ($p < 0.05$), respectively, suggesting that although no obvious histological damage was induced, subjected to CUMS resulted in low-grade inflammation in the colon and intestinal barrier disruption.

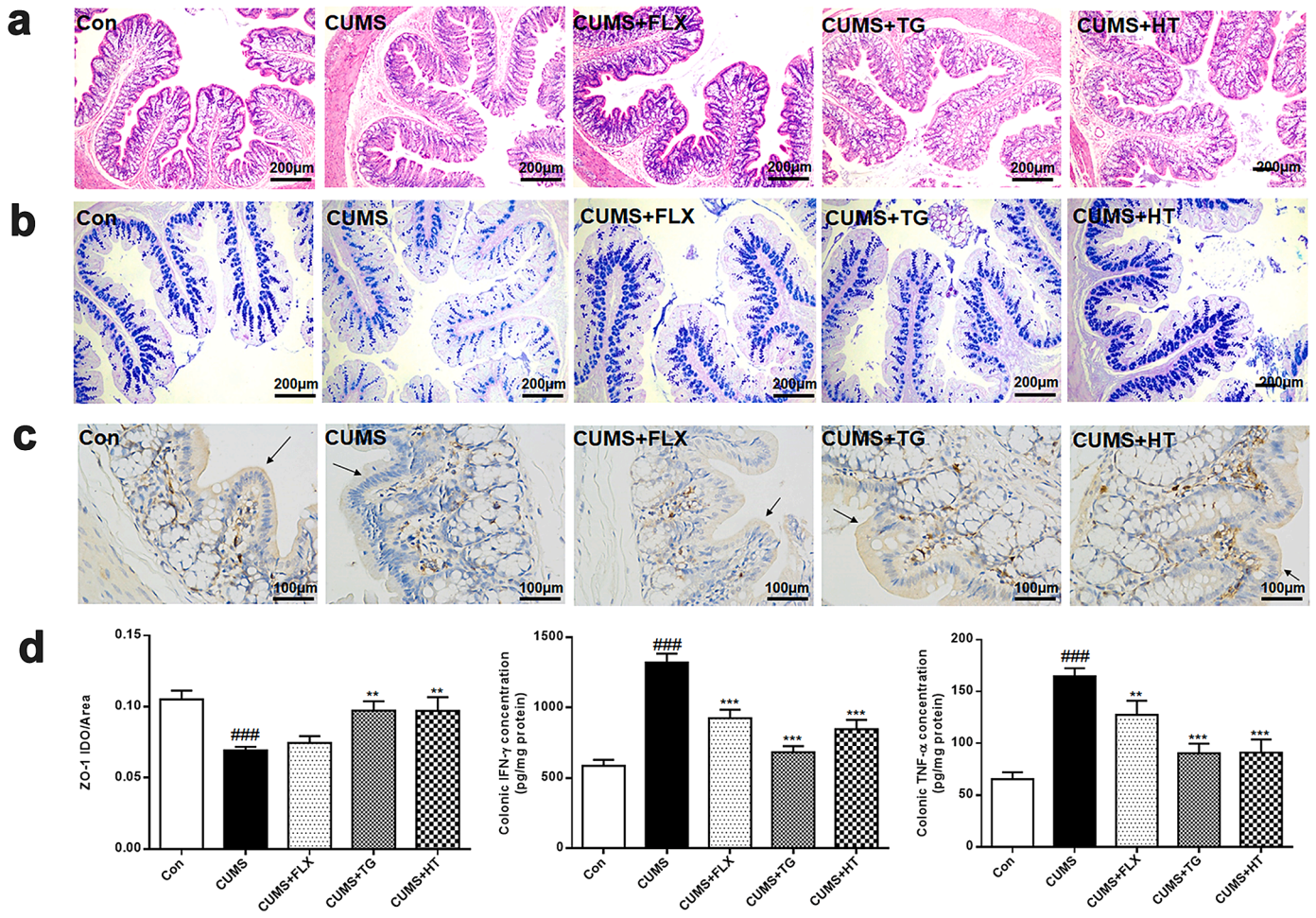

Fig. 4. Effects of TG, HT or fluoxetine on the morphologic changes, ZO-1 protein expression, IFN-γ and TNF-α concentrations in the colon of CUMS rats. (a) Typical H&E staining section; (b) typical AB-PAS staining section; (c) typical immunohistochemistry section of ZO-1; (d) quantification of mean optical density of ZO-1, IFN-γ and TNF-α concentrations. Data represent mean ± SEM (n = 8-9/group). ANOVA analysis, compared with control group, $^{\#\#\#}p < 0.001$; compared with CUMS group, $^{**}p < 0.01$, $^{***}p < 0.001$.

After fluoxetine, TG, and HT treatment, the number of goblet cells and the thickness of the mucus layer were increased, and the levels of colonic IFN-γ and TNF-α were significantly reduced ($p < 0.05$). In addition, TG and HT significantly increased ($p < 0.05$) the protein expression of ZO-1 to a level similar to that of control group. These results showed that TG and HT could alleviate low-grade inflammation in the colon and intestinal barrier disruption in CUMS rats.

Effects of TG and HT on gut microbiota composition

After removing unqualified sequences, a total of 1,874,721 effective reads were obtained. The alpha diversity (Fig. 5a) and beta diversity (Fig. 5b) results showed that although the richness (Chao) and diversity (Shannon) of the gut microbiota in the cecal contents did not differ, the overall structure of the gut microbiota in the principal coordinates analysis (PCoA) plot indicated a striking difference between control and CUMS groups. Moreover, TG and HT treatment significantly increased the bacterial richness of CUMS rats and induced marked differences in the PCoA plot in comparison with those of CUMS rats, showing clear similarities with the controls, especially when TG was assayed.

The taxonomic changes in microbiota further confirmed that TG exerted a dramatic regulatory effect on gut microbiota composition. After TG administration, the abundances of the majority of altered taxa in CUMS rats were reversed to a level similar to those of control group. A significant reduction (14.2%, $p < 0.05$) and enrichment (53.6%, $p < 0.05$) in the abundances of the phylum *Firmicutes* and *Bacteroidetes* were observed in TG group compared with CUMS group (Fig. 5c), respectively. Consistently, at the family level (Supplementary Table S4), TG decreased the abundances of *Ruminococcaceae* (phylum *Firmicutes*) and *Peptococcaceae* (phylum *Firmicutes*) by 33.9% and 82.9%, respectively, compared with CUMS group ($p < 0.05$). Conversely, significant enrichments in the abundances of family *Erysipelotrichaceae* (phylum *Firmicutes*, 78.4%, $p < 0.05$) and *Muribaculaceae* (phylum *Bacteroidetes*, 77.3%, $p < 0.05$) existed in TG group. Consistently at the genus level (Fig. 5d), the abundances of *Anaerotruncus, Harryflintia, Ruminiclostridium_9, unclassified_f_Ruminococcaceae* (members of the family *Ruminococcaceae*), and *Peptococcus* (a member of the family *Peptococcaceae*) were significantly decreased while *norank_f_Erysipelotrichaceae, Allobaculum, Dubosiella* (members of the family *Erysipelotrichaceae*) and *norank_f_Muribaculaceae* (a member of the family *Muribaculaceae*) displayed higher abundances in TG group than those of CUMS group ($p < 0.05$). In addition, TG decreased the abundances of *Tyzzerella_3, Acetatifactor* and *norank_f_Lachnospiraceae* assigned to the family *Lachnospiraceae* ($p < 0.05$), although the abundance of the family *Lachnospiraceae* was similar across all experimental groups. Unlike TG, HT decreased the abundances of partially altered taxa induced by CUMS, including the family *Ruminococcaceae*, 3 genera (*Anaerotruncus, Harryflintia,* and *Ruminiclostridium_9*) assigned to the family *Ruminococcaceae* and 2 genera (*Tyzzerella_3, Acetatifactor*) assigned to the family *Lachnospiraceae*. Additionally, converse variations were observed in the

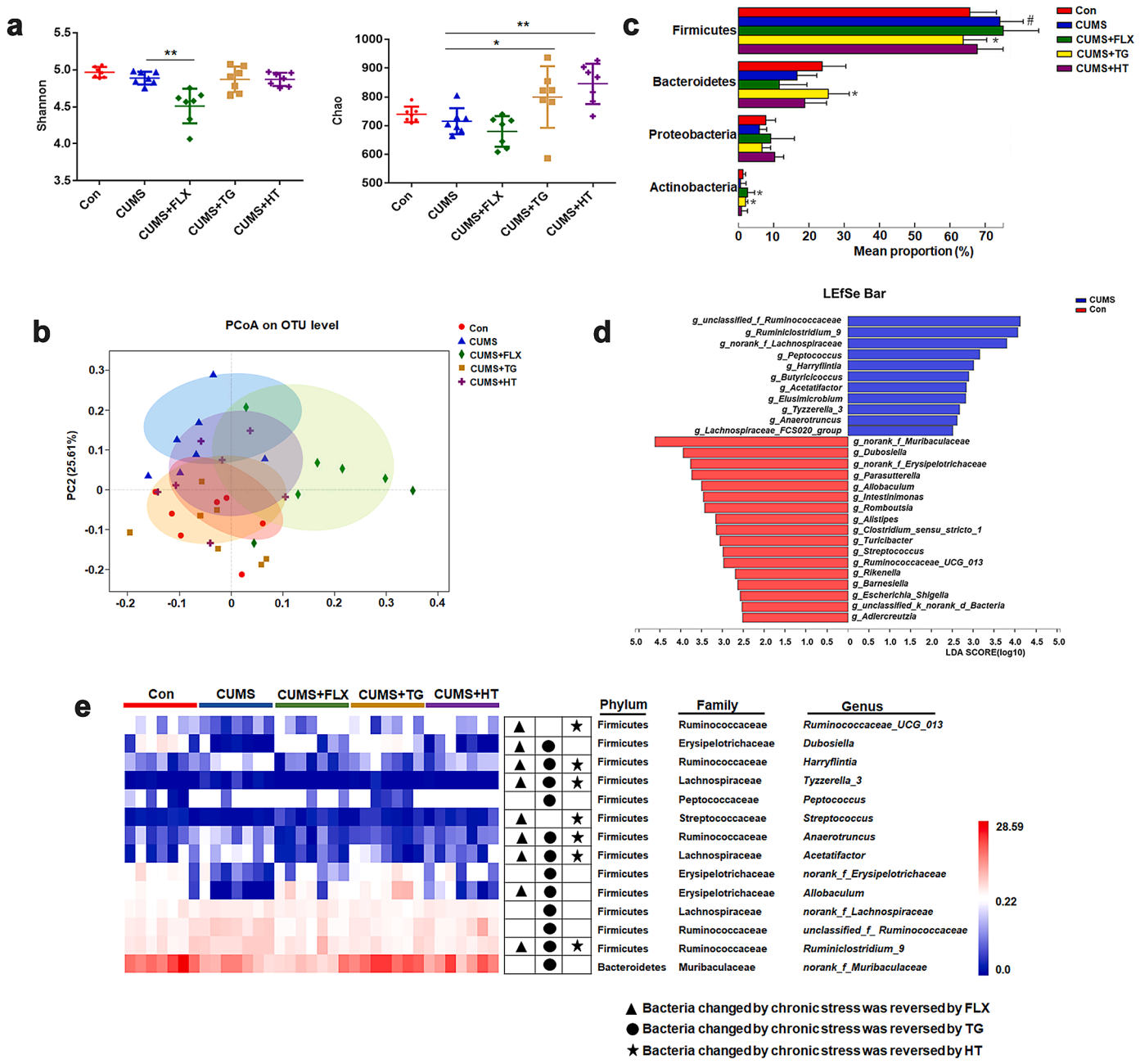

Fig. 5. Analysis of the diversity, composition and differences of gut microbiota in cecal samples. (a) Alpha diversity indexes in each group; (b) PCoA plot of 16S rRNA profiles obtained from cecal samples of rats; (c) relative abundances of selected phylum with significant differences among each group; (d) LEfSe analysis between control and CUMS group at the genus level; (e) heatmap of the relative abundances of selected genera with significant differences among each group. Data represent mean ± SEM (n = 7/group). Kruskal-Wallis *H*-test, compared with control group, $^{\#}p < 0.05$; compared with CUMS group, $^{*}p < 0.05$, $^{**}p < 0.01$.

abundances of *Ruminococcaceae_UCG_013* and *Streptococcus* in HT group. These results indicated that TG and HT exerted clear differences in the regulation of gut microbiota composition, and the effect of TG was superior to that of TG especially in altering the genera assigned to the family *Erysipelotrichaceae, Peptococcaceae,* and *Muribaculaceae*.

Correlation analysis of altered microbial genera affected by TG and HT, HPA axis-related hormones, pro-inflammatory cytokines, 5-HT and BDNF

To elucidate the associations between changes in the gut microbiota composition affected by TG and HT, and corresponding depression related traits, Spearman's correlation analysis was performed. As shown in Fig. 6, the levels of 5-HT and BDNF in the hippocampus were only significantly positively correlated with *norank_f_Muribaculaceae*. Otherwise, clear correlations could be identified between the altered microbial genera and HPA axis-related hormones. In particular, *Dubosiella, norank_f_Muribaculaceae, norank_f_ Erysipelotrichacea* and *Peptococcus* were strongly related to HPA axis function. Serum CORT, CRF and ACTH had negative correlations with *Dubosiella, norank_f_Muribaculaceae* and *norank_f_Erysipelotrichacea*, and positive associations with *Peptococcus*. Furthermore, *Dubosiella* and *norank_f_ Erysipelotrichacea* exhibited negative associations with colonic IFN-γ and hippocampal TNF-α, while similar relationships existed in *Allobaculum, Harryfinita*, and serum and hippocampal TNF-α, as well as in *norank_f_Lachnospiracea*, and serum IFN-γ and TNF-α. In contrast, *Peptococcus* was positively associated with colonic TNF-γ and serum TNF-α. Likewise, *norank_f_Muribaculaceae* was

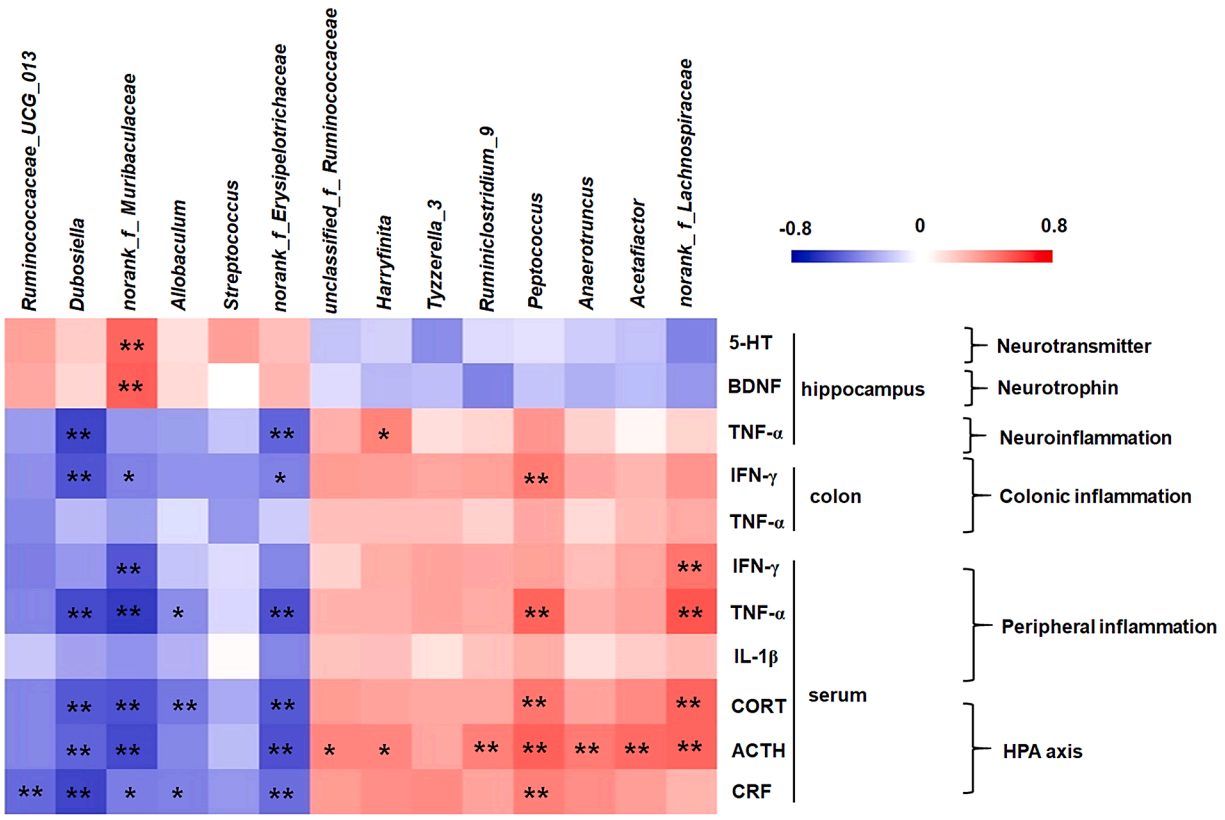

Fig. 6. The Spearman's correlation analysis of altered microbial genera and HPA axis-related hormones in the serum, pro- inflammatory cytokines in the serum, colon and hippocampus, 5-HT and BDNF in the hippocampus. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

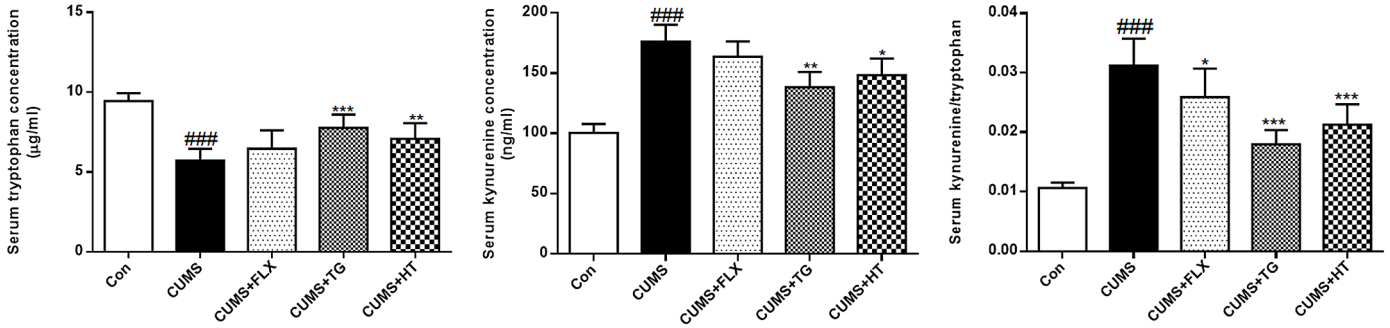

Fig. 7. Effects of TG, HT or fluoxetine on tryptophan, kynurenine concentrations and Kyn/Trp in the serum of CUMS rats. Data represent mean ± SEM (n = 8-9/group). ANOVA analysis, compared with control group, $^{###}p < 0.001$; compared with CUMS group,$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

positively related to serum IFN-γ and TNF-α. These results indicated that the altered microbial genera, especially certain genera belonging to the family *Erysipelotrichaceae, Peptococcaceae* and *Muribaculaceae*, which were specifically affected by TG, showed strong associations with HPA axis-related hormones and pro-inflammatory cytokines simultaneously, demonstrating the critical role of gut microbiota in regulating HPA axis function and inflammation.

TG and HT inhibit tryptophan-kynurenine metabolism in CUMS rats

The effects of TG and HT on the levels of tryptophan and kynurenine in the serum of rats, as well as the expression of IDO1 in the colon and hippocampus, were determined. As shown in Fig. 7 and Fig. 8, compared with that of control group, the level of tryptophan in the serum was significantly reduced in CUMS group, in contrast with the increased level of kynurenine and kynurenine-to-tryptophan ratio (Kyn/Trp) and IDO1 protein expression in the colon and hippocampus. Compared with CUMS group, serum Kyn/Trp in FLX group was significantly reduced ($p < 0.05$). TG and HT treatment resulted in a significant increase in the serum tryptophan level in CUMS rats ($p < 0.05$), and a decrease in the levels of serum kynurenine and Kyn/Trp ($p < 0.05$). In addition, TG significantly inhibited the expression of IDO1 protein in the colon of CUMS rats ($p < 0.05$), while a non-significant difference in the expression of IDO1 protein in the hippocampus was observed between CUMS and TG groups. However, the effect of HT on the expression of IDO1 protein in the colon and hippocampus was contrary to that of TG, indicating that TG and HT inhibited tryptophan-kynurenine metabolism by decreasing the expression of IDO1 in the colon and hippocampus of CUMS rats, respectively.

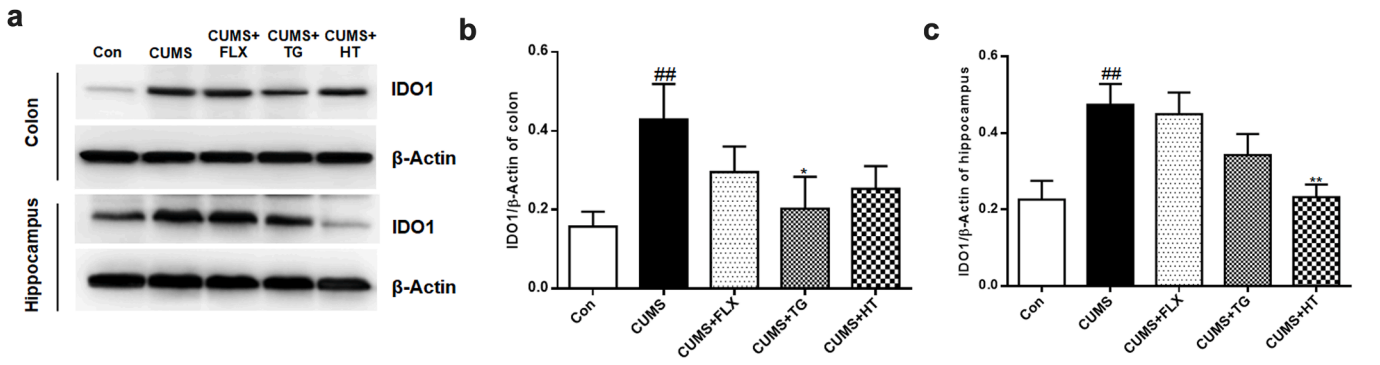

Fig. 8. Effects of TG, HT or fluoxetine on the expression of IDO1 protein in the colon and hippocampus of CUMS rats. (a) Western-blotting analysis, β-Actin was used as an internal reference; (b) histogram of colonic IDO1 protein expression; (c) histogram of hippocampus IDO1 protein expression. The values are expressed as mean ± SEM (n = 6/group). ANOVA analysis, compared with control group, $^{\#\#}p$ <0.01; compared with CUMS group, $^{*}p < 0.05$, $^{**}p < 0.01$.

Discussion

In this study, two different animal models were used to screen for bioactive compounds in CT that alleviate depression-like behaviors for the first time. TP and TO were most likely ineffective ingredients in CTE, while TG exhibited the most potential in the treatment of depression. Previous studies on CT, as well as Cistanche plants, have mostly focused on the bioactivities of phenylethanoid glycosides with the highest contents, and neglected the importance of iridoid glycosides and other less abundant ingredients. Interestingly, our study showed that phenylethanoid and iridoid glycosides showed synergistic effects on the hyperactivity of the HPA axis, severe peripheral and neural inflammation, and deficiencies in hippocampal 5-HT and BDNF, which may be the critical reason for the advantageous antidepressant effect of TG. These results further suggest the multicomponent and multitarget characteristics of herbal medicine, and remind us that the constituents with lower levels, even trace ingredients might also play a key role in pharmacological effects.

Chronic stress induces the hyperactivity of the HPA axis, which promotes the onset of depression, as well as the suppression of the HPG axis *via* decreased GnRH release and/or pituitary sensitivity of GnRH (Kirby *et al.*, 2009). Meanwhile, cAMP and cGMP are important intermediate hubs that relied on by many neurotransmitters and hormones to exert their physiological effects (Siawrys *et al.*, 2002). Recent studies have consistently shown that the occurrence of kidney-yang deficiency is closely related to dysfunction of the hypothalamus-pituitary-target gland axis (Zhang *et al.*, 2019). In kidney-yang deficiency syndrome, marked reductions in the levels of serum cAMP and cAMP/cGMP have also been observed. In this study, we found that TG could inhibit the levels of serum CORT, CRF, and ACTH, and increase serum T and GnRH contents in CUMS rats, indicating the recovery of HPA and HPG axis dysfunction after TG administration. Additionally, TG reversed a significant decrease in the level of serum cAMP/cGMP in rats subjected to CUMS. Thus, the potential underlying mechanism of CT in the treatment of depression and kidney-yang deficiency may be partially similar, and deserves to be explored further. Moreover, the production of sexual desire and behaviors is a complex neural reflex process. Sex hormones are major sources of central sexual excitement, and lacks of sex hormones can cause the less sexual desire or sexual dysfunction (Holloway and Wylie, 2015). In particular, excessive 5-HT inhibits the release of GnRH, which is a dominant reason for the occurrence of chronic sexual dysfunction after long-term treatment with SSRIs (Prasad *et al.*, 2015). In our study, fluoxetine (representative of SSRIs) was ineffective in the HPG axis dysfunction. However, although TG significantly improved the level of 5-HT in the hippocampus, similar alterations in serum T and GnRH levels were also observed, thus ameliorating the suppression of the HPG axis. These results demonstrate the distinct advantage of CT in the treatment of depression, and the intrinsic mechanism responsible for these effects needs to be further studied.

Increasing evidence has shown that the gut is a potential target for the treatment of chronic diseases using natural compounds with low bioavailability (Zhou *et al.*, 2020). Recent research (Wei *et al.*, 2019) has indicated that CUMS treatment induces fecal microbiome alterations and intestinal barrier defects, which facilitate bacterial invasion into the colonic mucosa and exacerbate inflammatory reactions in the colon. Consistent with that report, our results showed that 4 weeks of CUMS exposure induced an extensive depletion of goblet cell number and mucus layer thickness in the rat colon, as well as the onset of intestinal barrier disruption represented by increased gut permeability, which was determined by measuring the ZO-1 protein. In addition, increased expressions of pro-inflammatory factors (such as TNF-α and IFN-γ) were observed in the colon. Accordingly, these findings indicated that although no obvious histological damage was induced, CUMS treatment initiated colonic inflammation and intestinal barrier disruption. Previous studies have reported that CT exerted improved efficacy in preventing DSS-induced colitis in mice (Jia *et al.*, 2014). In consistent, TG was also shown to alleviate low-grade inflammation in the colon and intestinal barrier disruption of CUMS rats, which underscores its potential for clinically treating colonic inflammation-related diseases.

Extensive studies have also demonstrated that the relationship linking commensal gut microbiota, the HPA axis and inflammation is complex and plays a crucial role in the development of depression (Misiak, *et al.*, 2020). Alterations in gut microbiota composition may contribute to the enhanced release of cytokines and the synthesis of small bioactive molecules (Du *et al.*, 2020). Certain cytokines (such as TNF-α) might pass through the blood-brain barrier and are potent activators of the HPA axis (Misiak *et al.*, 2020). In turn, the hyperactivation of the HPA axis can contribute to gut microbiota dysbiosis, chronic inflammation in the colon, and altered gut permeability (Misiak *et al.*, 2020). Consistent with prior studies, TG-medicated modification of the gut microbiota is likely responsible for its ameliorating effects on depression. We observed that TG reversed the abundances of microbial taxa at different levels in rats subjected to CUMS to a level similar to those of the controls. In particular, the main altered genera belonging to the family *Peptococcaceae, Erysipelotrichaceae*, and *Muribaculaceae* were found to be highly correlated with the HPA axis and inflammation. Consistent with our results, family *Peptococcaceae, Erysipelotrichaceae* and *Muribaculaceae* have been reported to play an important role in maintaining mucosal barrier integrity and the occurrence of inflammation (Kankoush, 2015; Borton *et al.*, 2017; Zhang *et al.*, 2020). Therefore, we conjectured that TG could moderate the abundances of some genera assigned to the family *Erysipelotrichacea, Peptococcaceae* and *Muribaculaceae*, subsequently alleviating the low-grade inflammation in the colon, hyperactivity of the HPA axis and disruption of mucosal barrier integrity. Interestingly, in our study, only one genus named *norank_f_Muribaculaceae* was correlated with 5-HT and BDNF in the

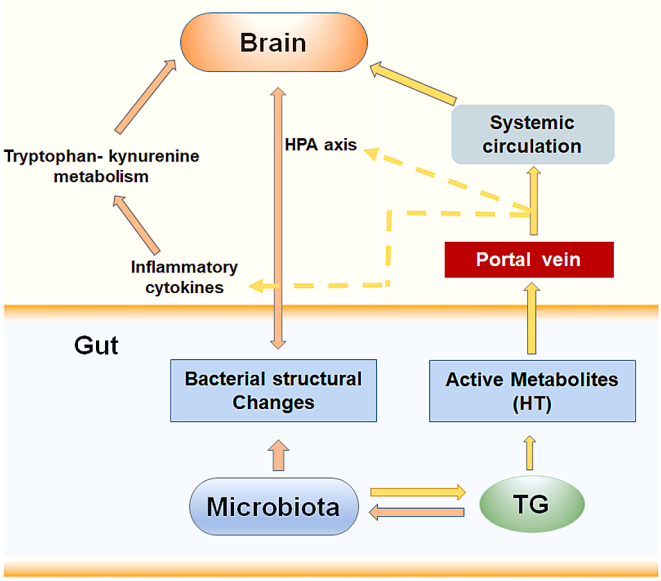

Fig. 9. Schematic diagram of the antidepressant mechanism in the treatment of depression with TG administration.

hippocampus. The specific mechanism of the relationship remains unclear and needs to be scrutinized further. Otherwise, the findings also suggest that it is extremely difficult for the majority of the altered genera caused by TG to directly affect the levels of 5-HT in the hippocampus. To explain how changes in gut microbiota composition influence the level of hippocampal 5-HT after TG treatment, the intricate relationship connecting tryptophan metabolism, the HPA axis, inflammatory cytokines and 5-HT captured our attention. Tryptophan is an essential amino acid for humans and less than 1% is used in protein synthesis, with the majority (over 90%) being converted by IDO1 and tryptophan 2,3-dioxygenase 2 (TDO2) into kynurenines, and approximately 5% being driven by tryptophan hydroxylase down the serotonin pathway (Duan, *et al.* 2018). The level of 5-HT in the brain largely depends on peripheral tryptophan availability, given that tryptophan is transported into the brain *via* neutral amino acid transporters (Duan *et al.*, 2018). IDO1, the rate-limiting enzyme in the first step of the kynurenine pathway, is primarily induced by IFN-γ, and other pro-inflammatory cytokines, such as IL-1β and TNF-α (Kennedy *et al.*, 2017), which were shown to be associated with gut microbiota in our study. As reported, more tryptophan is metabolized through the kynurenine pathway, thereby competitively reducing the conversion of tryptophan to 5-HT, and linking to the decreased 5-HT commonly found in depression (O'Mahony *et al.*, 2015). Thus, lower tryptophan levels and higher Kyn/Trp are common and coincide with an increased risk of depressive mood. After TG administration, the overexpression of IDO1 in the colon was inhibited, resulting in increased serum tryptophan levels and a reduction in serum Kyn/Trp, which revealed the potential linkage between gut microbiota and hippocampus 5-HT level affected by TG. Meanwhile, BDNF, a neurotrophin family member, is essential for cell differentiation, neuronal survival, synapse formation, and neuroplasticity processes in depression (Greenberg *et al.*, 2009). Recent studies have shown that BDNF has found to be closely associated with intestinal mucosal barrier function and gut microbiota (Maqsood and Stone, 2016). As well, the changes in gut microbiota have reported to possess the potential to increase the levels of BDNF expression and thus influence the development of depressive-like behaviors (Du *et al.*, 2020). However, similar to 5-HT variation, it was extremely impossible for most altered genera caused by TG in our study to directly affect the levels of BDNF in the hippocampus, and the intrinsic molecular connection and mechanism need further examination.

In contrast, HT, a gut microbiota metabolite of TG, also exhibited an antidepressant effect in the present study, which is consistent with its advantaged neuroprotective activity (Hu *et al.*, 2014). Of concern, HT could also moderate pro-inflammatory cytokines, HPA and HPG axis-related hormones, and hippocampal 5-HT concentrations to a level similar to that of the controls in CUMS rats, while most of the altered microbial genera affected by HT had weak correlations with the above physiological indexes, suggesting that the gut microbiota might not be the target of the antidepressant activity of HT. Furthermore, HT suppressed the overexpression of IDO1 in the hippocampus but not the colon, accompanied by the increased serum tryptophan levels. The occurrence of the apparent differences between TG and HT may be due to the excellent absorption of HT into the bloodstream and capacity for HT to cross the blood-brain barrier (Robles-Almazan *et al.*, 2018), which also indicates that the strong metabolic capacity of the gut microbiota is especially important in the antidepressant activity of TG, in addition to its physiological functions. Hence, we have preliminarily proven that the bidirectional interaction of the phytochemicals and gut microbiota plays a critical role in the treatment of depression with TG administration (as shown in Fig. 9).

Conclusion

In summary, TG was mainly responsible for the antidepressant activity of CT, reflecting in the synergistic effects of phenylethanoid and iridoid glycosides on the hyperactivation of the HPA axis, severe peripheral and neural inflammation, and deficiencies in 5-HT and BDNF in the hippocampus. Furthermore, the potential molecular mechanism of the antidepressant effect of TG was achieved through the bidirectional interaction of the phytochemicals and gut microbiota. In addition, amelioration of the suppressed HPG axis and cyclic nucleotide abnormality by TG and HT, which were not regulated by treatment with SSRIs represented by fluoxetine, indicated the distinct advantage of CT in the treatment of depression. Such modulations represent a promising strategy for the treatment of depression using CT and similar traditional herbal medicines.

Author contributions

X.L., Y.P., and L.F. designed the experiments and analyzed data. L.F. and J.W. performed the experiment and analyzed data. L.F. and X.L. drafted the manuscript. P.M. contributed to the animal experiment and sample testing, and L.Z. contributed to the analysis of gut microbiota composition. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by grants from National Key Research and Development Project (2017YFC1702400).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2021.153471.

References

Agus, A., Planchais, J., Sokol, H., 2018. Gut microbiota regulation of tryptophan metabolism in health and disease. Cell Host Microbe 23, 716–724.

Bijlsma, E.Y., Chan, J.S.W., Olivier, B., Veening, J.G., Millan, M.J., Waldinger, M.D., Oosting, R.S., 2014. Sexual side effects of serotonergic antidepressants: mediated by

inhibition of serotonin on central dopamine release? Pharmacol Biochem Behav 121, 88–101.
Borton, M.A., Sabag-Daigle, A., Wu, J.K., Solden, L.M., O'Banion, B.S., Daly, R.A., Wolfe, R.A., Gonzalez, J.F., Wysocki, V.H., Ahmer, B.M.M., Wrighton, K.C., 2017. Chemical and pathogen-induced inflammation disrupt the murine intestinal microbiome. Microbiome 5, 47.
Duan, K.M., Ma, J.H., Wang, S.Y., Huang, Z.D., Zhou, Y.Y., Yu, H.Y., 2018. The role of tryptophan metabolism in postpartum depression. Metab Brain Dis 33, 647–660.
Ding, F.M., Wu, J.J., Liu, C.Y., Bian, Q.L., Qiu, W.Q., Ma, Q.Y., Li, X.J., Long, M., Zou, X. J., Zou, X.J., Chen, J.X., 2020. Effect of Xiaoyaosan on colon morphology and intestinal permeability in rats with chronic unpredictable mild stress. Front Pharmacol 11, 1069.
Du, Y., Gao, X.R., Peng, L., Ge, J.F., 2020. Crosstalk between the microbiota-gut-brain axis and depression. Heliyon 6, e04097.
Greenberg, M., Xu, B.J., Lu, B., Hempstead, B.L., 2009. New insights in the biology of BDNF synthesis and release: implications in CNS function. J Neurosci 29, 12764–12767.
Gupte, P.A., Dawane, J., Wele, A.A., 2016. Experimental evaluation of Hiṅgvādi Ghṛta in behavioral despair using animal models. Anc Sci Life 36, 84–89.
Holloway, V., Wylie, K., 2015. Sex drive and sexual desire. Curr Opin Psychiatry 28, 424–429.
Hu, T., He, X.W., Jiang, J.G., Xu, X.L., 2014. Hydroxytyrosol and its potential therapeutic effects. J Agric Food Chem 62, 1449–1455.
Hou, T.H., Li, X.B., Peng, C.S., 2017. Borneol enhances the antidepressant effects of asiaticoside by promoting its distribution into the brain. Neurosci Lett 646, 56–61.
Jiang, Y., Tu, P.F., 2009. Analysis of chemical constituents in Cistanche species. J Chromatogr A 1216, 1970–1979.
Jia, Y.M., Guan, Q.N., Jiang, Y., Salh, B., Guo, Y.H., Tu, P.F., Du, C.G., 2014. Amelioration of dextran sulphate sodium-induced colitis in mice by echinacoside-enriched extract of Cistanche tubulosa. Phytother Res 28, 110–119.
Kirby, E.D., Geraghty, A.C., Ubuka, T., Bentley, G.E., Kaufer, D., 2009. Stress increases putative gonadotropin inhibitory hormone and decreases luteinizing hormone in male rats. Proc Natl Acad Sci U S A 106, 11324–11329.
Kaakoush, N.O., 2015. Insights into the role of Erysipelotrichaceae in the human host. Front Cell Infect Microbiol 5, 84.
Kennedy, P.J., Cryan, J.F., Dinan, T.G., Clarke, G., 2017. Kynurenine pathway metabolism and the microbiota-gut-brain axis. Neuropharmacology 112, 399–412.
Li, Y., Zhou, G.S., Peng, Y., Tu, P.F., Li, X.B., 2016. Screening and identification of three typical phenylethanoid glycosides metabolites from Cistanches Herba by human intestinal bacteria using UPLC/Q-TOF-MS. J Pharm Biomed Anal 118, 167–176.
Li, Y., Peng, Y., Ma, P., Yang, H.L., Xiong, H.Y., Wang, M.Y., Peng, C.S., Tu, P.F., Li, X.B., 2018. Antidepressant-like effects of *Cistanche tubulosa* extract on chronic unpredictable stress rats through restoration of gut microbiota homeostasis. Front Pharmacol 9, 967.
Maqsood, R., Stone, T.W., 2016. The gut-brain axis, BDNF, NMDA and CNS disorders. Neurochem Res 41, 2819–2835.
Misiak, B., Łoniewski, I., Marlicz, W., Frydecka, D., Szulc, A., Rudzki, L., Samochowiec, J., 2020. The HPA axis dysregulation in severe mental illness: Can we shift the blame to gut microbiota? Prog Neuropsychopharmacol Biol Psychiatry 102, 109951.
Nabavi, S.M., Daglia, M., Braidy, N., Nabavi, S.F., 2017. Natural products, micronutrients, and nutraceuticals for the treatment of depression: A short review. Nutr Neurosci 20, 180–194.
O'Mahony, S.M., Clarke, G., Borre, Y.E., Dinan, T.G., Cryan, J.F., 2015. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. Behav Brain Res 277, 32–48.
Prasad, P., Ogawa, S., Parhar, I.S., 2015. Serotonin reuptake inhibitor citalopram inhibits GnRH synthesis and spermatogenesis in the male zebrafish. Biol Reprod 93, 102.
Pablos, R.M., Espinosa-Oliva, A.M., Hornedo-Ortega, R., Cano, M., Arguelles, S., 2019. Hydroxytyrosol protects from aging process via AMPK and autophagy; a review of its effects on cancer, metabolic syndrome, osteoporosis, immune-mediated and neurodegenerative diseases. Pharmacol Res 143, 58–72.
Robles-Almazan, M., Pulido-Moran, M., Moreno-Fernandez, J., Ramirez-Tortosa, C., Rodriguez-Garcia, C., Quiles, J.L., Ramirez-Tortosa, M., 2018. Hydroxytyrosol: Bioavailability, toxicity, and clinical applications. Food Res Int 105, 654–667.
Siawrys, G., Bogacka, I., Okrasa, S., Kaminski, T., Przala, J., 2002. The effect of stimulators and blockers of adrenergic receptors on LH secretion and cyclic nucleotide (cAMP and cGMP) production by porcine pituitary cells in vitro. Anim Reprod Sci 69, 73–89.
Wu, Z.Q., Chen, D.L., Lin, F.H., Lin, L., Shuai, O., Wang, J.Y., Qi, L.K., Zhang, P., 2015. Effect of bajijiasu isolated from *Morinda officinalis* F. C. how on sexual function in male mice and its antioxidant protection of human sperm. J Ethnopharmacol 164, 283–292.
Wang, N.Q., Ji, S.Z., Zhang, H., Mei, S.S., Qiao, L.M., Jin, X.L., 2017. Herba Cistanches: Anti-aging. Aging Dis 8, 740–759.
Wang, Y.S., Shen, C.Y., Jiang, J.G., 2019. Antidepressant active ingredients from herbs and nutraceuticals used in TCM: pharmacological mechanisms and prospects for drug discovery. Pharmacol Res 150, 104520.
Wei, L.N., Li, Y., Tang, W.J., Sun, Q., Chen, L.X., Wang, X., Liu, Q.Y., Yu, S.Q., Yu, S.Y., Liu, C.Y., Ma, X.L., 2019. Chronic unpredictable mild stress in rats induces colonic inflammation. Front Physiol 10, 1228.
Wang, Q.X., Dong, J.T., Lu, W.J., He, H., Sun, X.Q., Zhang, K., Song, Q.Q., Jiang, Y., Wang, Y., Li, J., Tu, P.F., 2020. Phenylethanol glycosides from *Cistanche tubulosa* improved reproductive dysfunction by regulating testicular steroids through CYP450-3β-HSD pathway. J Ethnopharmacol 251, 112500.
Wang, F.J., Li, R.Y., Tu, P.F., Chen, J.P., Zeng, K.W., Jiang, Y., 2020. Total glycosides of *Cistanche deserticola* promote neurological function recovery by inducing neurovascular regeneration via Nrf-2/Keap-1 pathway in MCAO/R rats. Front Pharmacol 11, 236.
Yang, M., Luo, C.H., Zhu, Y.Q., Liu, Y.C., An, Y.J., Iqbal, J, Wang, Z.Z., Ma, X.M., 2020. 7,8-dihydroxy-4-methylcoumarin reverses depression model-induced depression-like behaviors and alteration of dendritic spines in the mood circuits. Psychoneuroendocrinology 119, 104767.
Yang, Z.L., Li, J.J., Gui, X.H., Shi, X.Q., Bao, Z.W., Han, H.J., Li, M.D., 2020. Updated review of research on the gut microbiota and their relation to depression in animals and human beings. Mol Psychiatry.
Zhang, J.Y., Hong, C.L., Chen, H.S., Zhou, X.J., Zhang, Y.J., Efferth, T., Yang, Y.X., Li, C. Y., 2019. Target identification of active constituents of *Shen Qi Wan* to treat kidney yang deficiency using computational target fishing and network pharmacology. Front Pharmacol 10, 650.
Zhou, N., Gu, X.Y., Zhuang, T.X., Xu, Y., Yang, L., Zhou, M.M., 2020. Gut microbiota: A pivotal hub for polyphenols as antidepressants. J Agric Food Chem 68, 6007–6020.
Zhang, Y.W., Chen, L.Y., Hu, M.J., Kim, J.J., Lin, R.B., Xu, J.L., Fan, L.N., Qi, Y.D., Wang, L., Liu, W.L., Deng, Y.Y., Si, J.M., Chen, S.J., 2020. Dietary type 2 resistant starch improves systemic inflammation and intestinal permeability By modulating microbiota and metabolites in aged mice on high-fat diet. Aging (Albany NY) 12, 9173–9187.