Hepatic antioxidant and gut ecological modulation properties of long-term intake of tea (*Camellia sinensis* L.) flower extract *in vivo*

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RESEARCH ARTICLE

Abstract

Tea (*Camellia sinensis* L.) flower extract (TFE) is a new type of tea beverage. The aim of this study was to explore the possible function after intake of TFE for a fixed period. In the study, 200 mg/kg body weight (BW)/day (d) of TFE was given to mice for 14 weeks. The results showed that the levels of hepatic superoxide dismutase and reduced glutathione were increased but the formation of malondialdehyde was reduced, compared to the normal control (NC) group. Meanwhile, administration of TFE contributed to the prior number of colonic goblet cells (1,505 ± 124 vs. 1,162 ± 112, per mm^2) and enhancement of colonic messenger RNA expression of mucin 2 and Claudin5. Additionally, TFE intervention modulated the composition and metabolic pathways of gut microbiota with an important role in dietary metabolism. Representatively, the relative abundance of genera *Bacteroides*, *Prevotella*, and *Lachnospiracea_incertae_sedis*, and the levels of short-chain fatty acids (SCFAs) and immunoglobulin A were increased. Taken together, long-term intake of TFE could promote hepatic antioxidant and modulate gut ecological status. These results could provide a reference for the development of TFE as a functional beverage.

Keywords: Camellia sinensis L.; tea flower; antioxidation; gut microbiota; short-chain fatty acids; immunoglobulin A

Introduction

Tea, with a unique taste and flavor, is one of the most popular beverages worldwide. Tea is traditionally produced from the leaves of tea plants, *Camellia sinensis* (L.) O. Kuntze or *Camellia sinensis* var. assamica (Mast.) Kitamura, Theaceae (Zhang *et al.*, 2019). What makes tea popular, besides its good taste, is that it possesses many beneficial health properties. Tea contains a variety of functional substances, including polyphenols, amino acids, caffeine, vitamins, and carbohydrates, although their content varies with variety and origin (Zhang *et al.*, 2019). Numerous studies have extensively reported the beneficial health properties of tea leaves and its bioactive substances. It was found that short- and/or long-term consumption of tea or isolated substances was significantly associated with a reduced risk of certain diseases, including stroke (Wang *et al.*, 2021), pneumonia (Mhatre *et al.*, 2021), hypertension (Kokaze *et al.*, 2012), osteoporosis (de Amorim *et al.*, 2018), cognitive impairment (Kakutani *et al.*, 2019), depression (Teng *et al.*, 2017), and psychological distress (Hozawa *et al.*, 2009). Although tea flowers have long been considered a waste, the discovery that the flowers of *C. sinensis* have

a similar composition as that of its leaves, and may have the same valuable properties (Chen *et al.*, 2018; 2020b), has received more attention in the past two decades.

Many insightful and useful discoveries have been made in physiological genetics, isolation, identification, and evaluation of functional substances of tea flowers (Chen *et al.*, 2020a), given that tea flowers perform diverse biological activities, including catechin- and polysaccharide-derived antioxidant, polysaccharide-derived antidiabetic and immunomodulatory, saponin-derived antiallergic, antiobesity, antihyperlipidemic, and antihyperglycemic properties (Chen *et al.*, 2018, 2020a, 2020b;). The safety, usage, and dosage of tea flower extract (TFE) or its bioactive components have also been confirmed. TFE exhibited very low acute (moderate lethal dose [LD_{50}] > 12.0 g lyophilized powder/kg·body weight [BW]) and subchronic (no observed adverse effect level of 4.0 g/kg·BW/day [d]) toxicity in animals (Li *et al.*, 2011). It is understood that the International Institute of Tea Flowers and the International Research and Development Center of Tea Flowers have been established in Japan and China, respectively. In 2013, tea flowers have been recognized as a new food source by the Minister of Health of China (Chen *et al.*, 2018). Hence, a wide range of applications of tea flowers are to be explored.

Recently, a variety of health/functional foods and beverages made from tea flowers have been developed in China and Japan (Chen *et al.*, 2018). Our previous work (Chen *et al.*, 2020b) discovered that TFE includes 34.02 ± 1.42% carbohydrates, 11.57 ± 0.14% phenolic compounds, 27.72 ± 3.07% crude proteins, and 2.81 ± 0.00% saponins, which contributed to the improvement of intestinal barrier, dysbacteriosis, immunoreactions, and hepatic injury in cyclophosphamide-induced immunosuppressed mice at a dosage of 200 mg/kg·BW/d for 10 days. However, there is little perception of the effect of TFE consumption for a relatively prolonged period. Therefore, in this study, we aimed to investigate the effects of long-term consumption (14 weeks) of TFE on the host's health and provided support for the functional applications of TFE. An effective dose of 200 mg/kg BW/d was used as explored previously (Chen *et al.*, 2020b).

Materials and Methods

Materials and chemicals

Dried flowers of the tea plant (Longjing 43) were provided by the Department of Tea Science, Zhejiang University (Hangzhou, China). Twenty male BALB/c mice (6-week old) were purchased from the Nanjing Qinglong Mountain Breeding Farm (Nanjing, China, SCXK Jiangsu). MiniBEST Universal RNA extraction kit and reverse transcription kit were purchased from TaKaRa Bio. Inc. (Dalian, China). SYBR® Green Fast Mix was purchased from Qtsingke Biotechnology (Beijing, China). Primer sequences for the targeted genes of mice, including glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), mucin 2 (*MUC2*), and *Claudin5* were purchased from Sangon Biotechnology (Shanghai, China). Commercial assay kits for measuring lactate, malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Fast DNA SPIN kit for feces was purchased from MP Biomedicals (Solon, USA). Commercial enzyme-linked immunosorbent serologic assay (ELISA) kit for immunoglobulin A (IgA) measurement was purchased from Multi Sciences (Lianke) Biotech Co. Ltd. (Hangzhou, China). All other chemical reagents used were of analytical grade.

Preparation of TFE

TFE was prepared according to the published literature (Chen *et al.*, 2020b). Briefly, 100 g of dried tea flowers were crushed to powder and extracted with 2,500 mL of hot water at 95°C for 1 h. The infusion was centrifuged (4,000 rpm, 20 min) and the supernatant was collected and lyophilized to obtain TFE. Thus, TFE mainly consisted of 34.02 ± 1.42% carbohydrates, 11.57 ± 0.14% phenolic compounds, 27.72 ± 3.07% crude proteins, and 2.81 ± 0.00% saponins.

Animals experimental design

Mice (6-week old, weighing 19.7 ± 0.7 g) were allowed to acclimate for 7 days, and then randomly divided into two groups, the normal control (NC) and the TFE group. Mice in the TFE group were fed *ad libitum* a chow diet plus intragastric administration of 200 mg/kg BW/d of TFE daily. Mice in the NC group was fed the same chow diet with a pseudo-gastric gavage of an equal amount of water. All mice were caged under a standardized room condition (21 ± 2°C, 14-h light/10-h dark cycle). Body weight was collected once a week for each mouse. After 14 weeks, all mice were euthanized and their liver, colon, cecal contents, and feces were collected. All these procedures were complied with the Yangzhou University Animal Ethics Committee and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Hepatic oxidative status evaluation

Tissue from the same location of each liver was taken to make a homogenate on a Precellys tissue homogenizer

equipped with a Cryolys condensing system (Bertin, France) prior to analysis (Chen *et al.*, 2020b). The levels of hepatic GSH, MDA, and SOD were assessed using commercial assay kits by following the manufacturer's operation manual.

Histomorphology observation

A part of fresh colonic tissue at the same position was picked, fixed in 10% formalin, and embedded in paraffin. A 5-mm thick of each slice was cut on a microtome and routinely stained with hematoxylin and eosin (H&E) (Ding *et al.*, 2021). Each pathological specimen was observed under a light microscope and photographed.

RNA extraction and quantification of gene expression

The total RNA of each colonic tissue was extracted using a Universal RNA Extraction kit. Evaluation of integrity and concentration of RNA was carried out on a Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). Each qualified RNA was converted into complementary DNA (cDNA) as a replication template for quantitative reverse-transcription polymerase chain reaction (qRT-PCR). The qRT-PCR was performed using a SYBR Green Fast Mix protocol and analyzed on a Roche LightCycler® 480 device (Roche, USA). The relative expression of *MUC2* and *Claudin5* was calculated bythe $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) and normalized to the *GAPDH* gene. The sequences of PCR primer are shown in Table 1.

DNA extraction and fecal microbiota analysis

Microbial DNA of mice was extracted from their fresh feces and quantified using a Nanodrop 2000 spectrophotometer. All qualified DNA was used to construct a library. The microbial 16S recombinant DNA (rDNA) sequencing was analyzed on an Illumina MiSeq platform (Illumina, USA) with an amplification procedure of V4 region (Primer F = Illumina adapter sequence 1+GTGCCAGCMGCCGCGGTAA, Primer R = Illumina adapter sequence 2+GGACTACHVGGGTWTCTAAT). The bioinformatics analysis was conducted with sequencing data. The off-board data were filtered to eliminate low-quality reads, and the remaining high-quality clean data were spliced into tags. The tags were clustered into operational taxonomic units (OTUs) at 97% similarity and compared with the database to obtain taxonomic ranks. Then, alpha diversity, beta diversity, and screening of different species were performed based on OTUs and taxonomic ranks.

Table 1. Target genes and primers sequence.

Gene	Sequence (5′-3′)
Mucin 2 (*MUC2*)	F: TTCTATGAGGCCTGTGTGCA
	R: CTTGGATGGGGAAGGAAGGT
Claudin5	F: TTCTTCTATGCGCAGTTGGC
	R: TTGGTGCCTACTTCACCGAT
GAPDH	F: GGACTTACAGAGGTCCGCTT
	R: CTATAGGGCCTGGGTCAGTG

Content of short-chain fatty acids (SCFAs) of IgA

SCFAs, including acetate, propionate, and butyrate, were analyzed using gas chromatography (GC) with 2-ethylbutyric acid as an internal standard (Tian *et al.*, 2016). Cecal contents or feces, 200 mg, were dissolved into 1 mL of distilled water and mixed with 1 mL of 0.3-mg/mL 2-ethylbutyric acid (prepared in 0.2 M HCl). After centrifugation at 30,000× *g* for 5 min, 200 μL of supernatant was mixed with 50 μL of 0.15 M oxalic acid, and the mixture was centrifuged again after 30 min. The supernatant was collected and passed through a 0.22-μm filter membrane for analysis, which was performed on an Agilent 6890N GC Station. Each sample, 1 μL, was injected onto an HP-INNOWAX column (30 m × 0.25 mm × 0.25 μm; Agilent, USA). The flow rate of nitrogen as a carrier gas was 19.0 mL/min. Air, hydrogen, and nitrogen were used as a makeup gas with flow rates of 260, 30, and 30 mL/min, respectively. The initial temperature of the oven was 100°C, which was maintained for 1 min, then increased to 160°C at a rate of 5°C/min and held for 4 min. Lactate and IgA were analyzed using the commercial kit according to described instructions.

Statistical analysis

Data were demonstrated as mean ± standard deviation (SD). Statistical analysis between two groups was performed using IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY, USA) based on an independent two-tailed Student's *t*-test, or R package (V 3.4.1). *P*-value (or corrected) < 0.05 was considered statistically significant between groups.

Results and Discussion

Body weight and food intake

The effect of intervention of dietary components varies in different organismal states. TFE was proved to have a role in body weight gain and food intake in

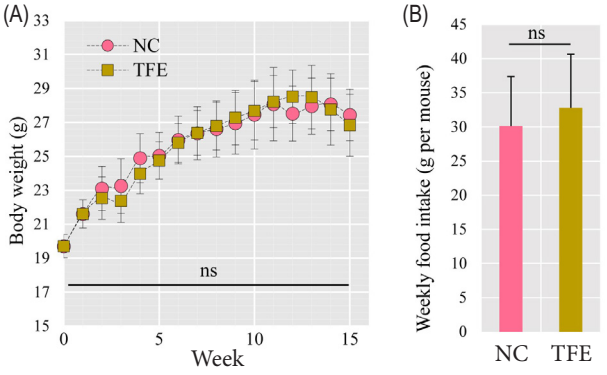

Figure 1. Effects of TFE on body weight and food intake. (A) Body weight, and (B) food intake. Values are reported as mean ± SD (n = 10 in the NC group, n = 9 in the TFE group). ns: no significant difference ($P > 0.05$), using the independent sample t-test. NC: normal control; TFE: tea flower extract.

immunosuppressed mice (Chen *et al.*, 2020b). However, there was no significant difference in body weight gain and weekly food intake between the NC group and TFE group during 14 weeks of administration ($P > 0.05$) (Figure 1). Similarly, *Ganoderma lucidum* extract reduced the body weight in obese mice but not in normal mice (Chang *et al.*, 2015). *Lycium barbarum* polysaccharide contributed to increasing body weight and food intake in immunosuppressed mice but had no significant effect in case of mice on normal diet (Ding *et al.*, 2021).

Notably, long-term consumption of extract of tea (leaves) (for more than 10 years) leads to a decrease in the percentage of total body fat (Wu *et al.*, 2003), which is mainly due to rich polyphenol content, especially epigallocatechin gallate (EGCG) (Li *et al.*, 2020). It is possible that low content of catechins in the extract of tea flowers, compared to that of leaves, had no significant effect of TFE on body weight (Chen *et al.*, 2018; 2020a).

In a previous toxicological study, the mean body weight, food consumption, and total feed efficiency of rats fed with TFE at 1.0–4.0 g/kg·BW for 13 weeks were also not significantly different from those of the control group (Li *et al.*, 2011).

TFE improves oxidative stress capacity

Given that the green tea extract has performed well in hepatoprotection, one of the important ways is to improve the oxidative stress capacity of the liver (El-Bakry *et al.*, 2017). However, there are hepatotoxic effects of excessive consumption of green tea and its extracts, which are positively correlated with total catechin and EGCG content (Hu *et al.*, 2018).

In an evaluated subacute toxicity trial in Sprague-Dawley rats, chronic administration (for 13 weeks) of TFE at a dose of 1.0, 2.0, or 4.0 g/kg·BW/d did not cause any significant changes in serum biochemical parameters, including alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) (Li *et al.*, 2011), which could be related to its lower catechin and EGCG content. Notably, our previous study discovered that TFE had an ameliorative effect on the oxidative damage of the liver caused by cyclophosphamide (Chen *et al.*, 2020b). However, the effect of consumption of TFE on the oxidative stress function of the liver in a healthy state is currently unknown. Therefore, the present work evaluated the effect on hepatic oxidative stress after 14 weeks of intake of TFE. As shown in Table 2, the level of hepatic SOD was a significant difference between the NC (107.81 ± 2.70 U/per gram of protein [mgprot]) group and the TFE (116.99 ± 3.80 U/mgprot) group. GSH level of the Liver was increased by nearly 1.5-fold after TFE intervention, compared to mice in the normal diet group (3.19 ± 0.32 vs. 2.06 ± 0.30 μmol/gprot). Meanwhile, hepatic MDA level was significantly lower in mice after TFE intervention than that in the NC group (0.87 ± 0.08 vs. 1.26 ± 0.23 mmol/gprot). Antioxidant defense systems, including antioxidant enzymes (such as SOD) and antioxidant molecules (such as GSH), in the body can effectively neutralize oxidative damage and regulate oxidation (Baez-Duarte *et al.*, 2016). MDA, a product of free radical damage to polyunsaturated membrane lipids, is a marker of oxidative stress (Dusak *et al.*, 2017). Long-term consumption of TFE can improve hepatic oxidative stress capacity by promoting the level of SOD and GSH, and by resisting the secretion of MDA, which is consistent with our previous observations in cyclophosphamide mice (Chen *et al.*, 2020b).

TFE improves intestinal mucosal barrier

The intestine is both digestive and immune organ. The gut-associated lymphoid tissue and goblet cells form the bulk of intestinal immune system (Hachimura *et al.*, 2018). Large intestinal epithelium mucosal surface is

Table 2. The antioxidant capacity of the liver and IgA in cecal contents in two groups.

Items	NC	TFE	P-value
SOD (U/mgprot)	107.81 ± 2.70	116.99 ± 3.80	<0.001
GSH (μmol/gprot)	2.06 ± 0.30	3.19 ± 0.32	<0.001
MDA (mmol/gprot)	1.26 ± 0.23	0.87 ± 0.08	<0.001
IgA (ng/mL)	9.91 ± 2.68	22.13 ± 7.62	0.004

Data are expressed as mean ± SD (n = 9–10) and determined by independent sample t-test. GSH: reduced glutathione; IgA: immunoglobulin A; MDA: malondialdehyde; NC: normal control; SOD: superoxide dismutase; TFE: tea flower extract.

essential for maintaining the stability of intestinal environment. It serves as both physiological barrier and focal point for immune defense and communication between bacteria and immune cells, thus determining the risk of intestinal diseases (Allaire *et al.*, 2018). As shown in Figure 2A, histological observation of the colon (scale bar 50 μm) indicated that the mice fed with TFE showed a closer arrangement and greater number of colonic goblet cells (1505 ± 124 per mm^2 vs. 1162 ± 112 per mm^2) (Figure 2B), compared with the NC group.

Likewise, the qRT-PCR analysis revealed that the colonic mRNA expression of *MUC2* and *Claudin5* in the TFE group was higher than that in the NC group (Figure 2C; $P < 0.05$). Normal epithelial cell transport relies on the function and regulation of mucosal tight junction proteins, such as MUC2 and Claudin5. MUC2 is produced by goblet cells to form a protective mucus blanket covering intestinal epithelium as the first line of defense for innate host defense (Tawiah *et al.*, 2018).

The present work determined that long-term consumption of TFE could enhance mucosal intestinal barrier by promoting the number of goblet cells and the expression of MUC2 and Claudin5.

Similarly, TFE was discovered to repair intestinal barrier and contribute to the recovery of body's immune function in cyclophosphamide-treated mice (Chen *et al.*, 2020b).

TFE modulates the composition and metabolic pathways of gut microbiota

Gut microbiota plays an important role in mammalian health. Diet is a crucial factor concerned with gut microbiota and their metabolic pathways, which also influence host's health and disease.

Emerging evidences have revealed that dietary choices and food components can target modulate the composition of gut microbiota and thus regulate intestinal microecology and its functions (Rinninella *et al.*, 2019). In the present work, 16S rDNA high-throughput sequencing technology was used to analyze the composition of gut

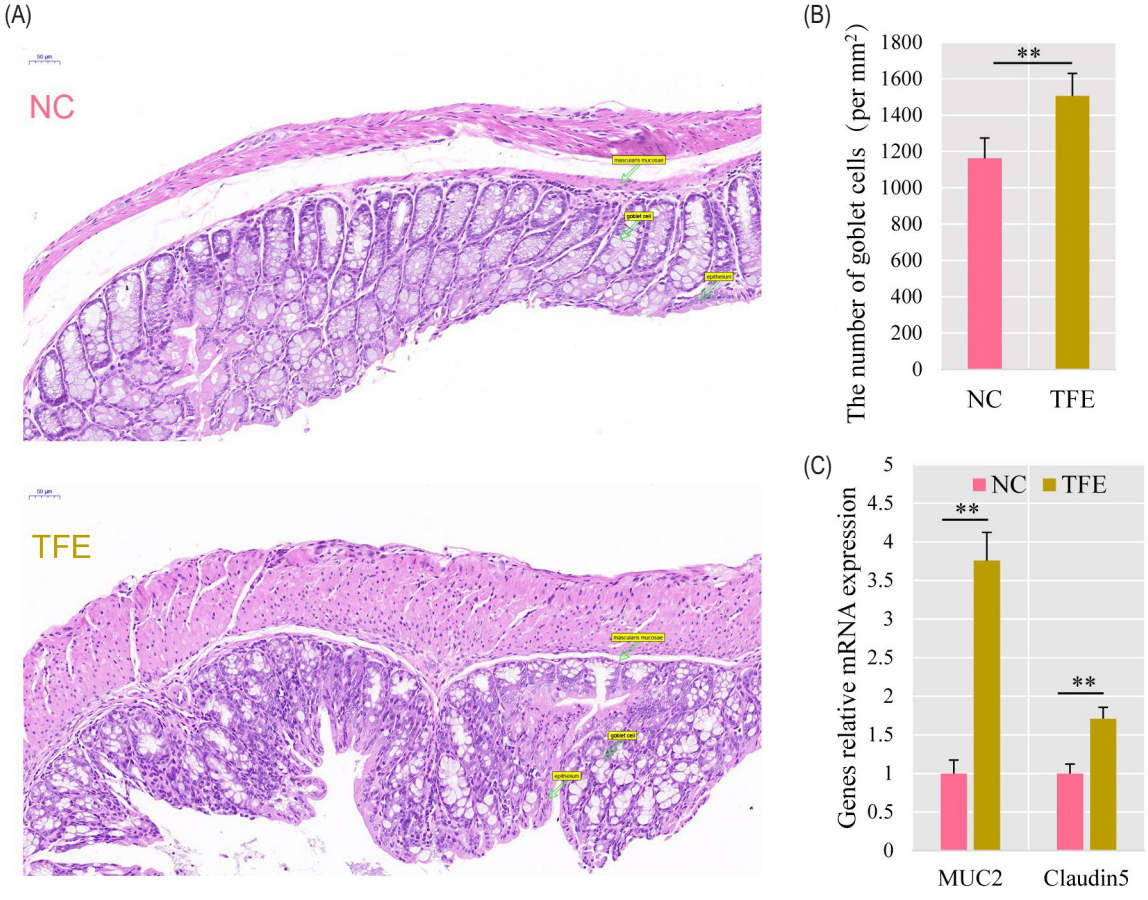

Figure 2. Effect of TFE on intestinal structure. (A) Representative histological observation of hematoxylin and eosin-stained colonic tissues (scale bar 50 mm). (B) qRT-PCR analysis of colonic MUC2 and Claudin5 mRNA expressions. (C) Number of goblet cells in colonic tissue. **$P < 0.01$, using the independent sample *t*-test. NC: normal control; TFE: tea flower extract.

microbiota, and more than 50,000 high-quality bacterial gene reads were acquired per sample.

Principal component analysis (PCA) based on the number of OTUs showed that there was an obvious variation in the gut microbial community between TFE and NC groups (Figure 3A). The hierarchical clustering of Bray–Curtis distances demonstrated the similarity of microbial composition among samples (Figure 3B). A high similarity of gut microbial composition within the NC group (>0.5) was established. Similarity between the NC group and TFE group was less than 0.5. To further understand detailed changes in gut microbiota, linear discriminant analysis (LDA) of bacteria was performed at the genus level (Figure 3C).

In all, 13 genera were altered after consumption of TFE, including three increased (*Bacteroides*, *Prevotella*, and *Lachnospiracea_incertae_sedis*) and 10 decreased (*Clostridium_sensu_stricto*, *Alistipes*, *Barnesiella*, *Saccharibacteria_genera_incertae_sedis*, *Staphylococcus*, *Streptococcus*, *Marvinbryantia*, *Anaeroplasma*, *Murimonas*, and *Parasutterella*) genera, compared with the NC group. The relative abundance of 13 genera in two groups based on LDA is displayed as a heat map in Figure 3D. Here, *Bacteroides* and *Prevotella* represent the predominant genera of Bacteroidetes phyla. It has been shown that *Bacteroides* and *Prevotella* are two species contributing to glycan degradation and are influenced by various components of food. Fecal communities clustered into enterotypes that are closely related to a long-term diet, especially abundance of *Bacteroides* formed by protein and animal fat versus the dominant genus *Prevotella* formed by carbohydrates (Wu *et al.*, 2011). It is related to 34.02 ± 1.42% carbohydrates and 27.72 ± 3.07% crude proteins of TFE. It was found that a higher dietary intake of fruits, vegetables, legumes, and whole grains increased the abundance of *Lachnospiracea_incertae_sedis* (Breuninger *et al.*, 2021), which may be a function of some phenolics (Xie *et al.*, 2017) and carbohydrates (Li *et al.*, 2021).

Among microorganisms reduced after TFE intervention, one study identified *Barnesiella* and *Marvinbryantia* as

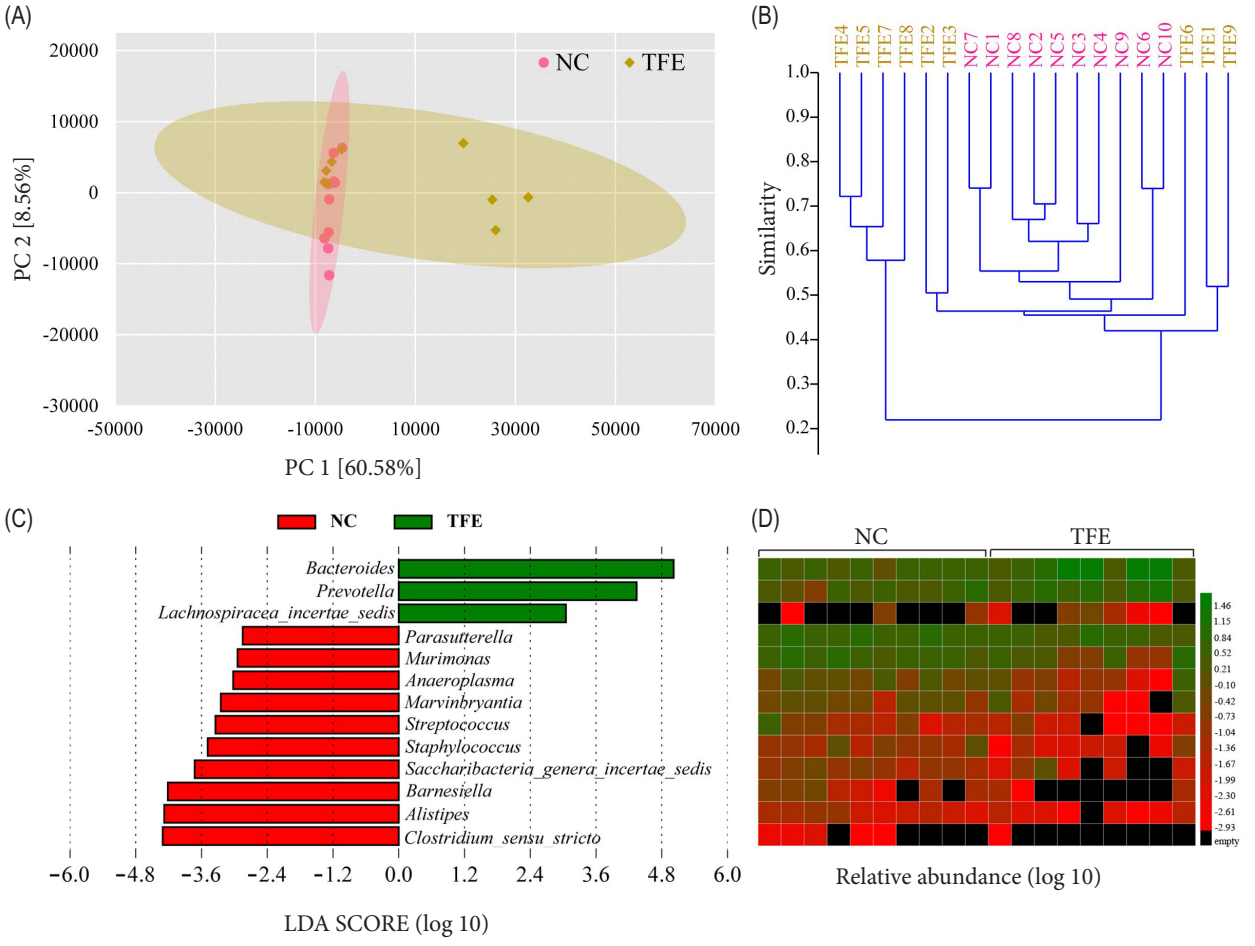

Figure 3. Effects of TFE on gut microbiota composition. (A) Principal component analysis (PCA). (B) Hierarchical clustering on Bray–Curtis distances. (C) Linear discriminant analysis (LDA) of bacteria at the genus level. (D) Heat map shows the relative abundance of 13 genera in two groups based on LDA. NC: normal control; TFE: tea flower extract.

primary contributors to colitis-associated colorectal cancer (Hu *et al.*, 2016). *Alistipes* is a genus of Bacteroidetes phylum, which is highly relevant in dysbiosis and diseases, such as liver fibrosis, colitis, cancer immunotherapy, and cardiovascular disease (Parker *et al.*, 2020). One previous study found that the proportion of *Clostridium_sensu_stricto* was decreased with reduced protein concentration (Fan *et al.*, 2017). However, it is biased to talk about gut microbial changes without specific conditions, because the dynamic balance between gut microbes and hosts maintains gut homeostasis and organismal health.

To gain insight into potential differential functional metabolic pathways between TFE and NC groups, the contribution of various differential species to the understanding of biological pathways was assessed using PICRUSt (bioinformatics software package) analysis based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Langille *et al.*, 2013). Consequently, 50 different functional pathways were identified between TFE and NC groups (Figure 4). Among these, 22 pathways, mainly focused on carbohydrate metabolism (such as pentose and glucuronate interconversions, starch and sucrose metabolism, etc.), glyoxylate and dicarboxylate metabolism, phenylpropanoid biosynthesis, and biosynthesis of siderophore group nonribosomal peptides, were increased in the TFE group, compared to the NC group ($P < 0.05$).

As reported, *Bacteroides, Prevotella*, and *Lachnospiracea_incertae_sedis* are closely related to carbohydrate metabolism (Li *et al.*, 2021); it is because TFE contains 34.02 ± 1.42% carbohydrates. The glyoxylate and dicarboxylate metabolism pathway is associated with high glucose intake (Ouyang *et al.*, 2020). Phenylpropanoid biosynthesis pathway has been found to be associated with the antioxidant and whitening activities of ginseng (Jin and Hyun, 2020).

Siderophores are a structurally diverse group of important natural products that play a key role in the acquisition of iron by most microorganisms (Swayambhu *et al.*, 2021). The mechanism of siderophore formation usually involves the activity of nonribosomal peptide synthetases, while nonribosomal peptide synthesis pathway produces representative metabolites, such as antibiotics, antitumor compounds, and immunosuppressants, that are essential for the host's health (Oide and Turgeon, 2020).

Likewise, 28 metabolic pathways were significantly reduced in the TFE group, such as nucleotide excision repair, limonene and pinene degradation and glycerophospholipid metabolism pathways ($P < 0.05$), in comparison to the NC group. The associations between long-term TFE intervention diet, microbiota, and their metabolic pathways identified in this analysis not only extend the current knowledge of long-term TFE intervention diet–microbiota–health relationships but also suggest that certain microbiota could be modulated through TFE diet interventions and thus have the potential to influence organismal health. However, the microbes and associations observed in this work need to be replicated in the future studies.

TFE promotes the production of SCFAs and IgA

In addition to directly influencing the composition of gut microbiota, the diet also affects host homeostasis and biological processes through the production of metabolites of nutrients fermented by microorganisms, especially SCFAs. SCFAs, which primarily include acetate, propionate, and butyrate, are key players in symbiotic relationships as messengers facilitating cross-talk between the host and gut microbiota. They are mainly produced in the cecum and ascending colon, where they regulate intestinal microenvironments, influence nutrient bioavailability, and promote normal functioning (Overby and Ferguson, 2021).

SCFAs are known to promote intestinal epithelial cell proliferation, maintain intestinal barrier function and intestinal homeostasis, and improve immune tolerance (Cani, 2019). The concentrations of SCFAs in cecal content and feces are presented in Figures 5A and 5C, respectively. Compared with the NC group, intake of TFE significantly increased the concentrations of lactate, butyrate, and total SCFAs in both cecal content and feces ($P < 0.05$).

SCFAs in the gut are mainly derived from selective fermentation of microbial accessible carbohydrates or probiotics by symbiotic bacteria. Much progress has been made in the identification of SCFA-producing bacteria. *Bacteroides, Prevotella*, and *Lachnospiracea* are reported to contribute to lactate and/or butyrate production (Morrison and Preston, 2016).

Lactate is easily produced by intestinal lactic acid bacteria, *Bifidobacteria*, and other anaerobes. *Lactobacillus*, the most typical lactate-producing bacteria, did not change significantly in the present study. Besides, differences in lactate content may be due to the body's own glycolysis and oxidative metabolism (Brooks, 2018). *Lachnospiraceae* is an abundant butyrate-producing group that converts lactate to butyrate (Duncan *et al.*, 2004). The levels of acetate and propionate in cecal content and feces did not show significant difference between the two groups ($P > 0.05$). Except for lactate, amount of each SCFA in cecal contents was higher than that in feces, which established that SCFAs could

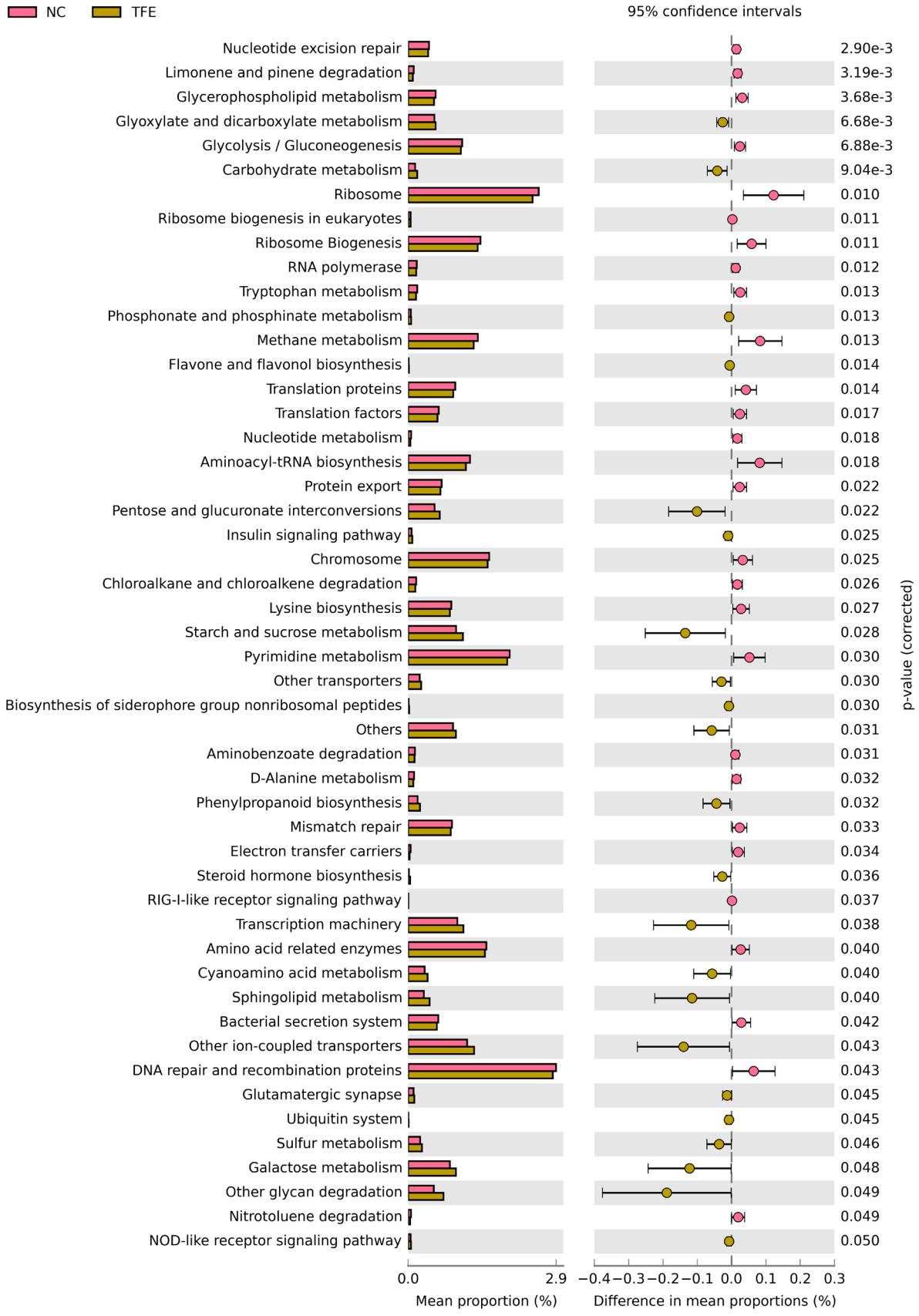

Figure 4. Effects of TFE on predicted microbial community functions by PICRUSt bioinformatics software. All KEGG pathways are shown with $P < 0.05$. NC: normal control; TFE: tea flower extract.

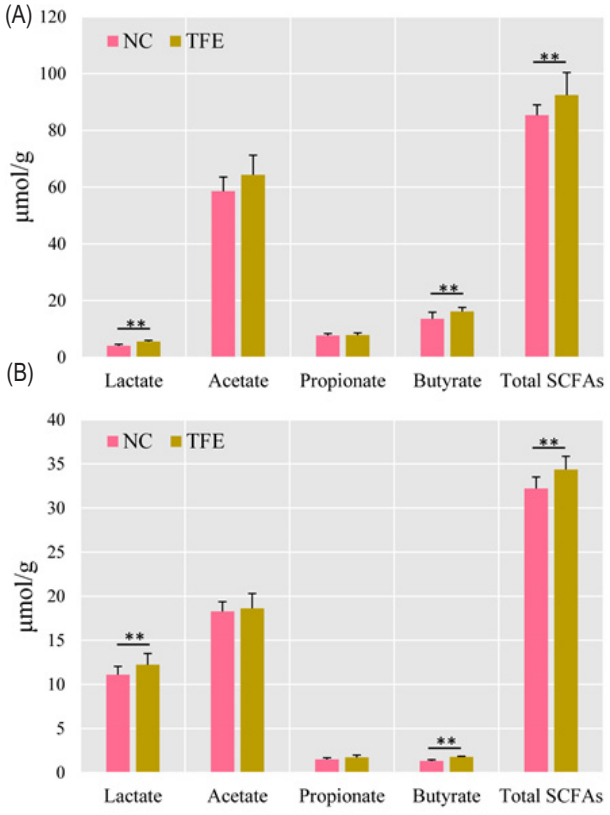

Figure 5. Effects of TFE on the production of SCFAs in (A) cecal content, and (B) feces. **$P < 0.01$, using independent sample *t*-test. NC: normal control; SCFAs: short-chain fatty acids; TFE: tea flower extract.

have been absorbed and metabolized by the organism in colonic segment.

Butyrate is the main source of energy for the epithelial cells of colonic mucosa (Salvi and Cowles, 2021). Butyrate is proved to promote mucus production through histone deacetylase inhibition and G protein-coupled receptor signaling, which epigenetically regulates the expression of MUC2 in goblet cells (Gaudier *et al.*, 2004). Long-term intake of TFE significantly increased butyrate levels in cecum content and feces, which is consistent with our previously reported results (Chen *et al.*, 2020b).

In addition, mice administrated with TFE showed notable improvement in IgA concentration in cecal content (22.13 ± 7.62 ng/mg versus 9.91 ± 2.68 ng/mL; $P < 0.05$) (Table 2). IgA is an immunoglobulin produced in the gut and plays an important role in the establishment of intestinal barrier function and adaptive immune system (Pabst and Slack, 2020). IgA induced by both food antigens and gut microbiota is one of the most important indicators to evaluate intestinal immune status (Singh *et al.*, 2017). It was found that symbiotic bacterial-derived butyrate promotes T-cell-independent IgA responses in the colon mediated by G protein-coupled receptor 41 (GPR41/FFA3) and GPR109a/HCA2, thus contributing to the maintenance of intestinal immune homeostasis (Isobe *et al.*, 2020). In this study, we found that TFE intervention could increase IgA and butyrate production to promote adaptive immunity, thus having a role in intestinal health.

Conclusion

Long-term consumption of TFE at a dose of 200 mg/kg·B-W/d exhibited a health-promoting effect by improving hepatic oxidative stress capacity by increased SOD and GSH levels and reduced MDA level. It also improved gut ecological environment by improving the number colonic goblet cells and colonic mRNA expression of MUC2 and Claudin5, enrichment of genera *Bacteroides*, *Prevotella*, and *Lachnospiracea_incertae_sedis* as well as promotion of the level of SCFAs and IgA. Understanding the possible effects of TFE on the host's health is expected to provide new ideas for the exploitation of tea flowers and to open new therapeutic avenues for the prevention and treatment of liver oxidative damage and intestinal microenvironmental disorders. However, the mechanisms by which TFE exerts its effects are being explored.

Acknowledgments

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Conflict of Interest

The authors have declared no conflict of interest.

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