

Blended tea ameliorates T2DM via modulation of gut microflora

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Abstract

Increasing evidences suggest that type 2 diabetes mellitus (T2DM) is closely related to gut microflora dysbiosis, which can be improved by dietary intervention. Four natural plant products, including *Cyclocarya paliurus*, Fu brick tea, *Ampelopsis grossedentata*, and *Lithocarpus litseifolius*, which are all rich in bioactive ingredients, are preliminarily found to be individually effective in alleviating T2DM. However, each of these four products has its own limitations in taste or aroma. In this study, these four natural plant products were blended to form a blended teaproduct for obtaining the better flavor. The blended tea was also expected to have excellent pharmacological activity. Therefore, the ameliorative effect of blended tea on T2DM and underlying mechanisms were studied in this study. The results showed that the blended tea extract effectively attenuated the symptoms of glucose and lipid metabolism-related disorders in T2DM mice fed by high-fat and high-sucrose diet (HFHSD). Furthermore, blended tea extract intervention significantly attenuated gut microbiota dysbiosis, characterized by increased community diversity and community richness. Certain core microbes, including *Ruminococcaceae_UCG-005*, *Butyricimonas*, *Roseburia*, *Oscillibacter*, *[Eubacterium]_nodatum_group*, *Muribaculaceae*, *Prevotellaceae UCG 001*, were also found to be improved by blended tea extract. Collectively, our results demonstrated that the blended tea may ameliorate T2DM through modulation of gut microflora. The blended tea may serve as novel functional drink for the treatment of T2DM and dysbiosis of gut microbiota.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder intricately linked to dysregulation in glucose and lipid metabolism, encompassing dyslipidemia, insulin resistance, pancreatic injury, inflammation, and related symptoms [1]. Furthermore, T2DM is closely associated with an elevated susceptibility to cardiovascular diseases, nonalcoholic fatty liver disease, and certain cancers [2, 3]. Projections indicate that the global prevalence of T2DM is set to exceed 700 million patients by 2045, thereby manifesting as a formidable global public health crisis and a significant economic burden. Consequently, the prevention and management of T2DM assume paramount importance in contemporary societies. Recent investigations have illuminated the role of gut microflora disturbances in precipitating diabetes [4]. Epidemiological evidence indirectly underscores a marked distinction between the gut microflora composition of healthy individuals and those afflicted with T2DM, with increased *Proteobacteria* and *Bacteroidetes*, as well as decreased *Firmicutes* and the *Firmicutes* to *Bacteroidetes* ratio in the intestines of T2DM patients [4–6]. Notably, genome-wide association studies among T2DM patients revealed a reduction in the quantity of butyrate-producing bacteria in the gut microflora of diabetic individuals [7, 8]. Research findings indicate an inverse correlation between T2DM and genera such as *Bifidobacterium*, *Akkermansia muciniphila*, *Bacteroides*, *Roseburia*, and *Faecalibacterium*, with *A. muciniphila* serving as a marker for glucose tolerance. Conversely, genera like *Ruminococcus*, *Blautia*, *Lactobacillus*, and *Fusobacterium* exhibit a positive correlation with T2DM [9–12]. Therefore, the regulation of gut microflora dysbiosis emerges as a promising avenue for T2DM treatment.

Studies have found that modulating the gut microflora via dietary intervention would be a novel therapeutic approach to ameliorate T2DM [13–16]. *Cyclocarya paliurus*, which belongs to the genus of *Cyclocarya* Iljinskaja, thrives predominantly in Southern China. Its leaves, known for both medicinal and culinary applications, have been utilized as a functional beverage for conditions such as diabetes mellitus, hypertension, and hyperlipidemia [17]. Enriched with polysaccharides, flavonoids, and triterpenoids, *C. paliurus* demonstrates the capacity to alleviate T2DM symptoms and ameliorate metabolic disorders by influencing the relative abundance of *Spiraudella* and *Clostridia* and modulating short-chain fatty acid levels [18–20]. Fu Brick tea (FBT) is a special kind of dark tea with distinctive flavor fermented dominantly only by *Aspergillus cristatus* (also known as *Eurotium cristatum*) [21–23]. Abundant in polysaccharides, polyphenols, and microbial metabolites, FBT is postulated to exert preventive and therapeutic effects on T2DM through the regulation of gut microbiota and intestinal metabolites [24–27]. *Ampelopsis grossedentata*, often referred to as "Vine tea", belongs to the *Vitaceae* plant family, primarily found in mountainous regions of Southern China. It boasts a high content of dihydromyricetin in its immature leaves, with levels exceeding 20% in most dry leaves and reaching up to 38.5% in rarer variants [28, 29]. Previous review publications have already explored the potential health benefits of dihydromyricetin, encompassing its hepatoprotective, antihypertensive, and antioxidant properties, along with its promising role in preventing or treating diabetes mellitus by enhancing glucose-related metabolism [28–33]. Recent research indicates that the composition of gut microflora can be significantly regulated and the richness and diversity of it can be altered by *A. grossedentata* [34, 35]. *Lithocarpus litseifolius*, a species of evergreen plant in the family *Fagaceae*, has a long history of being consumed as a non-sugar sweet beverage in Chinese folk culture. It is abundant in dihydrochalcones, mainly including three natural sweeteners, phlorizin, phloretin, and trilobatin [36]. Dihydrochalcones have been identified for their manifold health benefits, encompassing antioxidant, anti-inflammatory, antimicrobial, cardioprotective, hepatoprotective, antidiabetic, and anticancer effects [17]. Therefore, *L. litseifolius* has attracted wide interest for its excellent hypoglycemic effect and its potential as a hypoglycemic agent [37, 38]. Furthermore, *L. litseifolius* extract can remodel gut microflora structure of ulcerative colitis mice by enhancing the abundance of beneficial bacteria and butyric-acid-producing bacteria while inhibiting pathogenic ones [39]. Furthermore, it markedly ameliorates gut microbiota dysbiosis in obese mice [40].

Overall, although each of the aforementioned natural products has shown preliminary effectiveness individually in alleviating T2DM, they each exhibit certain limitations in terms of taste and aroma. Therefore, to enhance the overall flavor profile, this study introduces the concept of blended tea [41], leveraging the sweetness of *L. litseifolius* and *C. paliurus* to balance the bitterness and staleness flavors of FBT, while incorporating the "bitter first, sweet later" taste of *A. grossedentata* to provide complexity and layers to the blended tea. By adjusting the proportions, consumers can expect a synergistic effect on taste and aroma. Moreover, the concept of "medicine and food homology" was mentioned in the "Huang Di Nei Jing Su Wen", where the constitution of food in traditional Chinese medicine can be categorized through the "Four Properties Theory" (cold, cool, warm, and

heat). Foods or herbs with different properties serve distinct functions and are suitable for different individuals [42]. According to the Four Properties Theory, *A. grossedentata*, *L. litseifolius*, *C. paliurus*, and FBT are classified as cold, cool, cool, and warm, respectively. Their combination can harmonize the medicinal properties of each plant and expand their potential consumer base. Therefore, we designed an orthogonal experiment, and based on sensory evaluation and fundamental physicochemical analysis results, we initially selected the optimal ratio for the blended tea as follows: *C. paliurus*, Fu brick tea, *A. grossedentata*, and *L. litseifolius* in a proportion of 35.71:35.71:19.05:9.53.

Currently, most research on blended tea products predominantly focuses on flavor analysis [43]. In our study, we aimed to investigate the potential efficacy of blended tea in ameliorating gut microflora dysbiosis in T2DM. To achieve this, we used a T2DM mouse model established through a high-fat and high-sugar diet combined with streptozotocin, and conducted interventions with blended tea extract (BTE) through gavage administration to assess its effectiveness in reducing glucose and fat levels while regulating gut microflora dysbiosis. The primary objective of this study is to find sugar substitute beverages that offer both functionality and exceptional taste, providing a valuable reference for the future development of blended functional beverages and contributing to enhancing the quality of life for individuals with diabetes.

2. Materials and methods

2.1. Optimization of Blended Tea Formulation

2.1.1. Orthogonal experimental design

C. paliurus, Fu brick tea, *A. grossedentata* and *L. litseifolius* were obtained from Changsha Dekang Biotechnology Co., Ltd (Changsha, Hunan Province, China). These raw materials were ground and the resulting material was sequentially sieved through screens with 4-hole and 16-hole specifications. Only the material that passed through the 4-hole sieve was collected. To determine the optimal ratios, preliminary single-factor experiments were conducted, defining the range of levels for each raw material's orthogonal experiment. This study employed a 3-level design, following the L9 (3⁴) orthogonal table (Table 1).

Table 1
Orthogonal test scheme. A stands for *Cyclocarya paliurus*; B is Fu brick tea; C is for *Ampelopsis grossedentata*; D stands for *Lithocarpus litseifolius*.

Level	A/g	B/g	C/g	D/g
1	0.75	0.50	0.30	0.10
2	1.00	0.75	0.40	0.20
3	1.25	1.00	0.50	0.30

2.1.2. Sensory evaluation and physicochemical analysis

Since that the raw materials are fragmented tea, this experiment prepared the blended tea in 3g filter bags. The resulting tea infusions were consistently clear and bright. Therefore, sensory evaluation focused on aroma and taste as the evaluation criteria. The sensory evaluation process adhered to the sensory evaluation standard for tea, GB/T 14487 – 2017. For each treatment group, 3g of evaluation samples were proportionally weighed, and a panel of 10 experienced sensory evaluators conducted blind sensory evaluations, with the results being averaged. Results showed that treatment groups 1, 2, and 8 received the highest composite scores. Based on the results of the orthogonal experiment, primary physicochemical analysis was conducted for the treatment groups 1, 2, and 8. The physicochemical analysis results indicated that treatment group 2 exhibited higher levels of functional components such as soluble sugars, polyphenols, and flavonoids. Based on the results of the orthogonal experiment, primary physicochemical analysis was conducted for the treatment groups 1, 2, and 8. The physicochemical analysis results indicated that treatment group 2 exhibited higher levels of functional components such as soluble sugars, polyphenols, and flavonoids (Table 2). Combining sensory evaluations and physicochemical testing outcomes, the formula of treatment group 2 was selected as the experimental formula. This formula consists of a blend of *C. paliurus*, Fu brick tea, *A. grossedentata*, and *L. litseifolius* in a ratio of 35.71:35.71:19.05:9.53. This blended tea exhibits harmonious aroma, rich in tea fragrance, and a well-balanced taste with distinct tea flavor. The blended tea had a soluble sugar content of 5.90% ± 0.24%, a polyphenol content of 5.43% ± 0.36%, and a flavonoid content of 3.37% ± 0.24%.

Table 2

Sensory evaluation score and physicochemical analysis of blended teas. A stands for *Cyclocarya paliurus*; B is Fu brick tea; C is for *Ampelopsis grossedentata*; D stands for *Lithocarpus litseifolius*.

Items	A	B	C	D	Composite Score	Aroma Score	Taste Score	Aroma Description	Taste Description	Extraction rate (%)	Soluble sugar (%)	Flavonoids (%)	Polyphenols (%)
1	1	1	1	1	90.00	91.00	89.00	Sweet aroma with hints of herbs	Moderately harmonious, sweet	24.17 ± 1.63	5.13 ± 0.33	2.0 ± 0.43	4.92 ± 0.27
2	1	2	2	2	95.00	95.00	95.00	Harmonious with tea aroma	Harmonious with tea flavor	24.46 ± 1.10	5.90 ± 0.24	3.37 ± 0.24	5.43 ± 0.36
3	1	3	3	3	88.00	89.00	87.00	Strong tea aroma	Tea flavor, slightly astringent	-	-	-	-
4	2	1	2	3	74.00	75.00	73.00	Herbal notes, slightly sweet	Astringent	-	-	-	-
5	2	2	3	1	74.00	75.00	73.00	Herbal notes, slightly sweet	Astringent	-	-	-	-
6	2	3	1	2	86.00	85.00	87.00	Sweet aroma	Sweet	-	-	-	-
7	3	1	3	2	88.00	90.00	86.00	Sweet aroma with herbal hints	Sweet	-	-	-	-
8	3	2	1	3	92.00	93.00	91.00	Sweet aroma with herbal hints	Moderately harmonious, sweet	24.20 ± 1.08	5.52 ± 0.41	2.70 ± 0.59	4.72 ± 0.11
9	3	3	2	1	82.50	85.00	80.00	Sweet aroma	Tea flavor, slightly astringent	-	-	-	-

2.2. Preparation of the BTE

The blended tea was extracted with distilled water at 100°C twice for 45 min each time, stirring every 10 min, (tea/water = 1 g/10 mL, 1 g/8 mL). The extracting solution was collected after filtration. Then, the extracting solution was concentrated under vacuum, and finally lyophilized for 30 h, affording BTE. BTE was stored in -80°C ultra-low temperature refrigerator.

2.3. Determination of the dosage of BTE

Research has shown that the recommended daily tea consumption for adults is approximately 6–12 g of raw tea leaves [44, 45]. In this study, we calculated the daily intake as 9 g of blended tea for adults, which equates to 150 mg/kg (based on an assumed adult body weight of 60 kg). The low, medium, and high dosage levels of the blended tea are determined by multiplying the recommended adult intake by 5, 10, and 20, respectively. Therefore, the low dose BTE (BTE-L), middle dose BTE (BTE-M), and high dose BTE (BTE-H) groups are as follows: (150 × 5 × 22%) = 165 mg/kg, (150 × 10 × 22%) = 330 mg/kg, and (150 × 20 × 22%) = 660 mg/kg.

2.4. Experimental design

A total of 60 male Kunming mice (3 weeks of age, with a body weight of 13–15 g) were purchased from Hunan Slake Jingda Laboratory Animal Co., Ltd., (Changsha, Hunan Province, China) (Laboratory Animal Production License No.: SCXK(Su)2021-0013) [Approval number: Lenco 2021 (2137)]. The mice were housed under standard laboratory conditions (25°C, 12 h light/dark cycle). Each cage housed 5 mice with ad libitum access to food and water.

All mice were fed with a standardized diet or a HFHSD (HFHSD; 35% fat, 50% carbohydrate) for 4 weeks following 1 week of acclimatization. All mice fed with HFHSD were injected intraperitoneally with streptozotocin (STZ, 120 mg/kg) after 16 hours of fasting. The normal control group (Control; n = 10) were injected with an equal volume of citrate buffer. Fasting blood glucose (FBG) was detected with a glucometer (Johnson, USA) by tail vein sampling 3 days later. The mice with FBG ≥ 11.1 mmol. L⁻¹ and maintained for 3 days were considered to be T2DM mice. Finally, 50 mice that met the above criteria were further randomly divided into the model group (T2DM; n = 10), metformin-positive group (Metformin; n = 10), high dose BTE group (BTE-H; n = 10), middle dose BTE group (BTE-M; n = 10), and low dose BTE group (BTE-L; n = 10). Mice in the control and T2DM groups were supplemented daily with 0.3 mL of distilled water by intragastric gavage. Mice in the metformin control group were given an

administration of with 0.3 mL metformin (200 mg/kg) daily. Mice in the BTE-H, BTE-M, BTE-L groups were given an administration with 0.3 mL of BTE at 660 mg/kg, 330 mg/kg, 165 mg/kg daily respectively. All groups of mice were fed the standardized diet until the end of the study. Body weights and blood glucose were recorded once a week and drinking volume and amount of diet were monitored daily during the treatment. After the completion of BTE gavage intervention, the feces from each group of mice were collected on the day before the mice were sacrificed. The collected feces were placed directly into frozen storage tubes, immediately fixed with liquid nitrogen, and then transferred to an ultra-cold refrigerator at -80°C. After the 4 weeks of treatment, intraperitoneal glucose tolerance tests (IPGTT) were performed following a fast of over 8 h [46]. All mice were anesthetized with 4% chloral hydrate after overnight fasting and then sacrificed. All mice procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Hunan Agricultural University.

2.5. Biochemical analysis

Serum levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) were measured using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu Province, China). The degree of insulin resistance was calculated using the homeostatic model assessment–insulin resistance (HOMA-IR) index.

2.6. Histology and immunostaining (IHC) analyses

Pancreatic tissues were fixed with 4% paraformaldehyde overnight at 4°C. Then they were dehydrated with ethanol and embedded in paraffin. Sections were cut at 4 mm thickness, and the staining of hematoxylin and eosin (H&E) was performed following standard protocol with sections dehydrated, diaphanized, and covered with coverslips.

2.7. 16S ribosomal ribonucleic acid (rRNA) sequencing of the gut microflora

Feces samples were analyzed by 16S rRNA sequencing analysis. Briefly, DNA was extracted from the samples by using a QIAamp Fast DNA stool Mini Kit (Qiagen) according to the manufacturer's protocols. PCR amplification was conducted with barcoded specific bacterial primers targeting the variable region 3–4 (V3–V4) of the 16S rRNA gene: forward primer 5'-ACTCTACGGGAGGCAGCA-3' and reverse primer 5'-GGACTACHVGGGTWTCTAAT-3' [47]. Construction of sequencing libraries and paired-end sequencing were carried out on an Illumina MiSeq platform at Biomarker Technologies Co, Ltd. (Beijing, China) according to standard protocols. Raw sequences were merged and low-quality reads as well as duplicate sequences were removed by FLASH, Trimmomatic, and UCHIME, respectively [48]. The resulting sequences were aligned against the Greengenes database of 16S rRNA gene sequences. Reads were performed using quantitative insights into microbial ecology (QIIME).

2.8. Analysis of data

All data were presented as the mean $\pm$ SD. All statistical analyses involving 16S rRNA sequencing data were performed using Mothur and QIIME. Comparisons among groups were analyzed with one-way analysis of variance (ANOVA) and Duncan's multiple comparison test. $P < 0.05$ was considered to indicate a significant difference.

3. Results

3.1. Antihyperglycemic and hypolipidemic effect of BTE

We validated the effectiveness of the STZ-induced T2DM mouse model in this study. T2DM mice showed significant weight loss, reduced food and water intake (Fig. 1A-C), impaired glucose tolerance, and insulin resistance (Fig. 1E-F) compared with the control group. Histopathological examination further revealed islet atrophy and inflammatory cell infiltration in pancreas of T2DM mice (Fig. 1G). Notably, significant disparities in blood glucose and lipid-related parameters were observed between the metformin-positive control group and the T2DM model group. T2DM mice also exhibited elevated serum levels of TC, TG, and LDL-C, along with reduced levels of HDL-C (Fig. 1G-J). These findings indicate that T2DM disrupts blood lipid homeostasis in mice along with glucometabolic disorder. These results support the reliability and utility of the generated T2DM mouse model for future studies (Fig. 1).

In this study, we investigated the therapeutic potential of BTE in regulating hypoglycemia and lipid hemostasis in T2DM mice. Remarkably, BTE administration resulted in reduced body weight, increased food and water intake, and lowered FBG levels in T2DM mice (Fig. 1A-D). Histology analysis also revealed that the pancreatic structure of the BTE group was relatively healthier and more similar to that of the control group. Additionally, BTE treatment led to improved insulin resistance, impaired glucose tolerance, and dyslipidemia (Fig. 1E-K). Collectively, these outcomes suggest that BTE treatment significantly ameliorates disorders related to glucose and lipid metabolism in T2DM mice.

3.2. Analysis of the gut microflora diversity

The regulatory effect of BTE on T2DM-induced gut microflora dysbiosis was investigated by 16S rRNA sequencing of feces. The Shannon and Simpson indices were used to evaluate community diversities, while the ACE and Chao indices were used to identify the community richness. The results suggested that T2DM induced a lower gut microflora community diversity (Fig. 2A, B) and decreased community richness of the gut microflora (Fig. 2C, D). After BTE treatment, T2DM mice exhibited a significant increase in both gut microflora community diversities and richness. Interestingly, the BTE-L group showed higher microflora diversity, while the BTE-H group exhibited a higher microflora richness (Fig. 2A-D). Further

PCA analysis revealed a clear separation of the T2DM group from the control group, while BTE-treated groups distributed closer to the control group (Fig. 2E), suggesting a similar composition of intestinal flora between BTE-treated T2DM mice and control mice.

3.3. Species distribution of the gut microflora

To depict the species distribution of the mouse gut flora, species histograms of the top 10 abundance of each group at the phylum were drawn. The top 10 intestinal bacteria are: *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Spirochaetes*, *Epsilonbacteraeota*, *Verrucomicrobia*, *Patescibacteria*, *Actinobacteria*, *Deferribacteres*, and *Cyanobacteria* (Fig. 3). The species distribution based on the phylum level is similar to another study [49]. Members of the *Bacteroidetes* and *Firmicutes* represented a substantial amount of the bacterial diversity in healthy mice (Fig. 3A). The increases in the relative abundance of *Actinobacteria*, *Spirochaetes*, *Verrucomicrobia*, *Proteobacteria*, and *Patescibacteria* were observed in the T2DM group compared with the control group ($P < 0.05$). The relative abundance of *Actinobacteria* and *Spirochaetes* were significantly decreased in the BTE group compared with the T2DM group ($P < 0.05$), while the relative abundance of *Verrucomicrobia* was not significantly decreased. Among them, abundance of *Actinobacteria* and *Spirochaetes* was decreased after BTE-L and BTE-H interventions, with BTE-L group showing a more pronounced, but not statistically significant effect (Fig. 3A).

At the family level, the relative abundance of *Muribaculaceae* was significantly reduced and those of *Spirochaetaceae* and *Akkermansiaceae* were significantly increased in the T2DM group compared with the control group ($P < 0.05$), while the gut microflora of mice after BTE intervention was closer to that of the control group. Among them, the metformin-positive control group also functions to improve the species distribution of the gut in T2DM (Fig. 3B). Of note, the improvement effect was significantly better in the BTE group than in the metformin-positive group.

3.4. Analysis of the rank-sum test based at the genus level

To clarify the regulatory effect of the blended tea on the gut microflora genus, microbial diversity analysis was conducted among various treatment groups. This analysis revealed 26 significantly distinct genera among the top 50 genera based on relative abundance, as determined by the Wilcoxon rank sum test. T2DM resulted in significant increases in the genera of *Akkermansia*, accompanied by decreases in the genera of *uncultured_bacterium_f_Muribaculaceae*, *Ruminococcaceae_UCG-005*, *Weissella*, *Butyricimonas*, *GCA-900066575*, *uncultured_bacterium_f_Peptococcaceae*, *Roseburia*, *Intestinimonas*, *Ruminiclostridium*, *Oscillibacter*, *uncultured_bacterium_f_Erysipelotrichaceae*, *Ruminiclostridium_6*, *Angelakisella*, *Rikenellaceae_RC9_gut_grou*, and *[Eubacterium]_nodatum_group*. Interestingly, BTE treatment significantly reversed the T2DM-induced alteration in the abundance of most bacterial genera, such as *uncultured_bacterium_f_Muribaculaceae*, *Ruminococcaceae_UCG-005*, *Weissella*, *Butyricimonas*, *Roseburia*, *GCA-900066575*, *Oscillibacter*, and *[Eubacterium]_nodatum_group* ($P < 0.05$), with the exception of *Ruminiclostridium* and *Angelakisella* (Fig. 4).

3.5. LefSe analysis based on the phylum to genus level

Biomarkers of the gut microflora are biomolecules, metabolites, or other characteristics that can be used to measure or indicate the state of the gut microbiome. By detecting the biomarkers of gut microflora, the health status of gut microflora can be assessed, the diagnosis and treatment of diseases related to gut microflora can be carried out, and the occurrence of diseases can be prevented. Common gut microflora biomarkers include microflora components, microflora metabolites, intestinal mucosal barrier, and immune function, etc. Linear discriminant analysis effect size (LefSe) analysis showed those abundance from phylum to genus between groups, only taxa with an LDA (linear discriminant analysis) significant threshold of 3.5 was shown (Fig. 5). *Oscillibacter*, *Ruminiaceae_Rumliniclostridium*, and *Prevotellaceae_Prevotellaceae_NK3B31_group* were identified as biomarkers of gut microflora in the control group. The cladogram representative of the structure of *Akkermansia*, which belonged to the phylum *Verrucomicrobia*, was identified as a biomarker of gut microflora that associated with the T2DM group. In addition, *Muribaculaceae* and *Prevotellaceae_UCG_001*, both belonging to the *Bacteroidetes* phylum, exhibited potential as gut microflora biomarkers in mice following BTE treatment.

4. Discussion

4.1. BTE regulates glucose and lipid metabolism disorders in T2DM

The basic features of T2DM with disorders of glucose and lipid metabolism include weight loss, excessive drinking and eating, higher FBG levels, lower glucose tolerance, insulin resistance, and dyslipidemia. In our study, FBG levels and AUG were significantly elevated in the T2DM group compared to the control group at different time points of the test, whereas the BTE intervention significantly reversed the upregulation of FBG and AUG, decreased TC, TG, and LDL-C levels, and increased HDL-C levels. As analyzed histopathologically, diabetes has a significant destructive effect on the pancreas, whereas BTE has a favorable protective effect on the pancreas. The above results indicate that BTE can effectively regulate glucose and lipid metabolism disorders in T2DM.

4.2. BTE regulates the gut microflora structure

The gut microflora constitutes a stable and independent ecosystem within the human body, conducting a wide range of metabolic activities that profoundly impact the health of the host. These activities include the digestion of complex carbohydrates, the synthesis of vitamins and amino acids, and the production of metabolites such as short-chain fatty acids and bile acids [50]. The composition of the gut microflora plays a pivotal

role in human health, including regulating the gut immune system, maintaining the gut mucosal barrier, aiding in digestion and absorption, as well as inhibiting the growth of harmful bacteria. Therefore, maintaining a balanced and diverse environment of gut microflora is one of the key measures in maintaining human health. In this study, the HFHSD treatment led to the decrease in both the alpha and beta diversity of the gut microflora, consistent with previous studies [51]. Study indicates that low diversity is a typical characteristic of gut microflora dysbiosis [52]. We found that the HFHSD-induced decrease in the alpha and beta diversity was restored by BTE intervention. Moreover, at the phylum level, *Bacteroidetes* and *Firmicutes* emerge as the two most abundant phyla in the gut. The former is recognized as a key contributor to the intricate homeostasis and overall well-being maintained by the gut microbiota. Previous studies pointed out that various members of *Firmicutes* and *Bacteroidetes* can encode and secrete carbohydrate-active enzymes, thereby facilitating more efficient carbohydrate hydrolysis and utilization [53]. Meanwhile, *Firmicutes* produce more absorbable energy from hydrolyzed carbohydrates than *Bacteroidetes* [54]. Currently, many studies have confirmed a decreased abundance of *Bacteroidetes* and an increased abundance of *Firmicutes* in diabetic mice [55, 56]. This is consistent with our findings. The results of this study showed that BTE intervention reduced the enrichment of *Firmicutes* and *Bacteroidetes* induced by a HFHSD compared with the T2DM group, suggesting that BTE intervention may reduce host energy absorption by altering the composition of the gut microflora. Additionally, a positive correlation between a high-fat diet and *Actinobacteria* abundance has been reported [57]. Moreover, the relative abundance of *Proteobacteria*, which contains a variety of pathogenic microorganisms, was significantly reduced after BTE treatment compared to the model group, suggesting that BTE may have an inhibitory effect on the growth of certain pathogenic bacteria [58].

At the family level, The *Firmicutes* phylum is composed of two widely recognized families: *Lachnospiraceae* and *Ruminococcaceae*, both contributing to the protection of the colonic endothelium by producing butyrate [59]. A decrease in the levels of *Lachnospiraceae* and *Ruminococcaceae* within the intestinal tract can lead to malfunctioning of the intestinal endothelium [60]. Studies showed that the diversity of *Lachnospiraceae* was significantly negatively correlated with FBG [61], which was similar to the results of our study. After BTE intervention, the relative content of them were increased, and it was speculated that BTE could promote the growth of beneficial bacteria. Our research showed that the relative abundance of *Muribaculaceae* and *Akkermansia* in the gut of T2DM patients is altered compared with that of healthy individuals. T2DM patients typically exhibit fewer *Muribaculaceae* and more *Akkermansia* in their gut [62, 63]. *Muribaculaceae* is an anaerobic bacteria and one of the most common members of the gut microflora. It can metabolize polysaccharides such as cellulose and pectin, and produce short-chain fatty acids such as propionic acid and butyric acid, which are beneficial to gut health. In addition, *Muribaculaceae* is involved in maintaining gut mucosal barrier function and immune regulation [64]. *Akkermansia*, a Gram-negative bacterium, is another vital member of the gut microflora. It primarily utilizes mucin as its energy source and contributes to gut health by maintaining the gut mucosal barrier function and participating in anti-inflammatory responses [65]. Since T2DM patients often consume a high-fat, high-sugar, low-fiber diet, it can lead to changes in the gut microflora. *Muribaculaceae* has a weaker ability to utilize dietary fiber, while *Akkermansia* can use mucin as its energy source. Therefore, changes in diet may lead to alterations in the abundance and relative abundance of *Muribaculaceae* and *Akkermansia*. Interactions between gut microflora may also affect the relative abundance of *Muribaculaceae* and *Akkermansia*. For example, some studies have shown that the relative abundance of *Muribaculaceae* and *Akkermansia* in the gut is closely related to the presence and abundance of other microbiota, possibly involving mutual promotion or competition [66]. Therefore, the increase in *Akkermansia* in the T2DM group was accompanied by a decrease in *Muribaculaceae*, suggesting a possible functional dysregulation of the microbiota. The ratio of the two has improved after BTE intervention, predicting that it may be related to the fact that BTE provides substances such as dietary fiber.

Furthermore, whole-genome sequencing analysis revealed that the abundance of *Faecalibacterium*, *Clostridium* and *Alistipes* was abundant in healthy individuals, whereas their abundance was significantly reduced in T2DM patients, leading to insulin resistance and metabolic abnormalities [67]. This is due to their ability to participate in the metabolism of short-chain fatty acids, thereby promoting the production of butyrate in the gut. Studies have found that the dominant family of *Bacteroidales* and *Prevotella* was positively associated with propionate, which can reduce hepatic fatty acid content, increase insulin sensitivity and improve T2DM symptoms [68–72]. Our findings show that BTE intervention attenuated the HFHSD-induced gut microflora dysbiosis and increased certain core microbes that can produce butyrate, such as *Ruminococcaceae_UCG-005*, *Butyricimonas*, *Roseburia*, *Oscillibacter*, *[Eubacterium]_nodatum_group* [38, 73, 74]. Taken together, it is hypothesized that the mechanism by which BTE improves gut microflora dysbiosis in T2DM may be through improving the abundance of gut microflora.

4.3. BTE has potentialities to treat T2DM

As a first-line hypoglycemic medication, metformin was traditionally believed to primarily inhibit hepatic gluconeogenesis, thus improving insulin resistance [75]. However, Duca et al. found that the hypoglycemic effect of metformin was significantly decreased by inhibiting the rat duodenal AMPK, and it was speculated that the main action target of metformin is in the intestine [76]. Bryrup et al. recruited 27 cases of healthy Danish young Danish men for an 18-week non-blinded, one-armed intervention study [77]. During the period, metformin was taken orally and feces of volunteers were collected regularly. For the primary outcome the abundance of five bacterial genera including *Enterobacter*, *Clostridium* and *Dichelix* significantly decreased and the abundance of *Bifidobacteria* and *Escherichia coli* significantly increased during the metformin intervention but returned to baseline levels after treatment cessation. It provides strong support for the gut microflora mediated the antidiabetic effect of metformin. This study also demonstrated the efficacy of metformin in regulating the gut microflora.

Interestingly, the efficacy of BTE in this study showed results similar to those of metformin group. This suggests the possibility of considering blended tea as an adjunctive treatment for diabetics. Future research could explore the combined use of blended tea intervention with hypoglycemic drugs compared to using either hypoglycemic drugs or blended tea in isolation. Moreover, from the results, it can be observed that there is a non-

linear relationship between the BTE dosage groups. This could be attributed to the higher concentration of active ingredients in the blended tea compared to single teas, suggesting that the recommended daily tea consumption for adults used in this study may not be suitable for this particular blended tea. It is also possible that BTE at the same concentration may exert varying effects on different gut microflora. Addressing these concerns will be the focus of our next research steps.

5. Conclusion

In conclusion, our results indicate that BTE attenuates the HFHSD-induced T2DM by regulating glucose and lipid metabolism and modulating the gut microflora. Certain core microbes, including *Ruminococcaceae_UCG-005*, *Butyricimonas*, *Roseburia*, *Oscillibacter*, *[Eubacterium]_nodatum_group*, *Muribaculaceae*, *Prevotellaceae UCG 001*, were also found to be improved by BTE. Our results suggest that BTE can be developed as a beverage with both flavor and function to prevent and ameliorate T2DM and gut microflora dysbiosis in individuals with T2DM. However, there is still work to be done in finding the optimal ratio of blended tea and the optimal dose of BTE, and the mechanisms and channels by which BTE attenuates intestinal diseases need to be further investigated.

Declarations

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Conflict of Interest

There are no conflicts to declare.

Author Contributions

SL1† and XD 2†: conceptualization, investigation, data curation, writing-original draft, writing-review and editing, visualization. These authors contributed equally to this work and share first authorship. JZ and XZ: investigation, data curation. MZ: writing-review and editing, validation. KW and DF: conceptualization, resources, supervision, project administration, funding acquisition. All authors have read and approved the final version of this manuscript.

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Figures

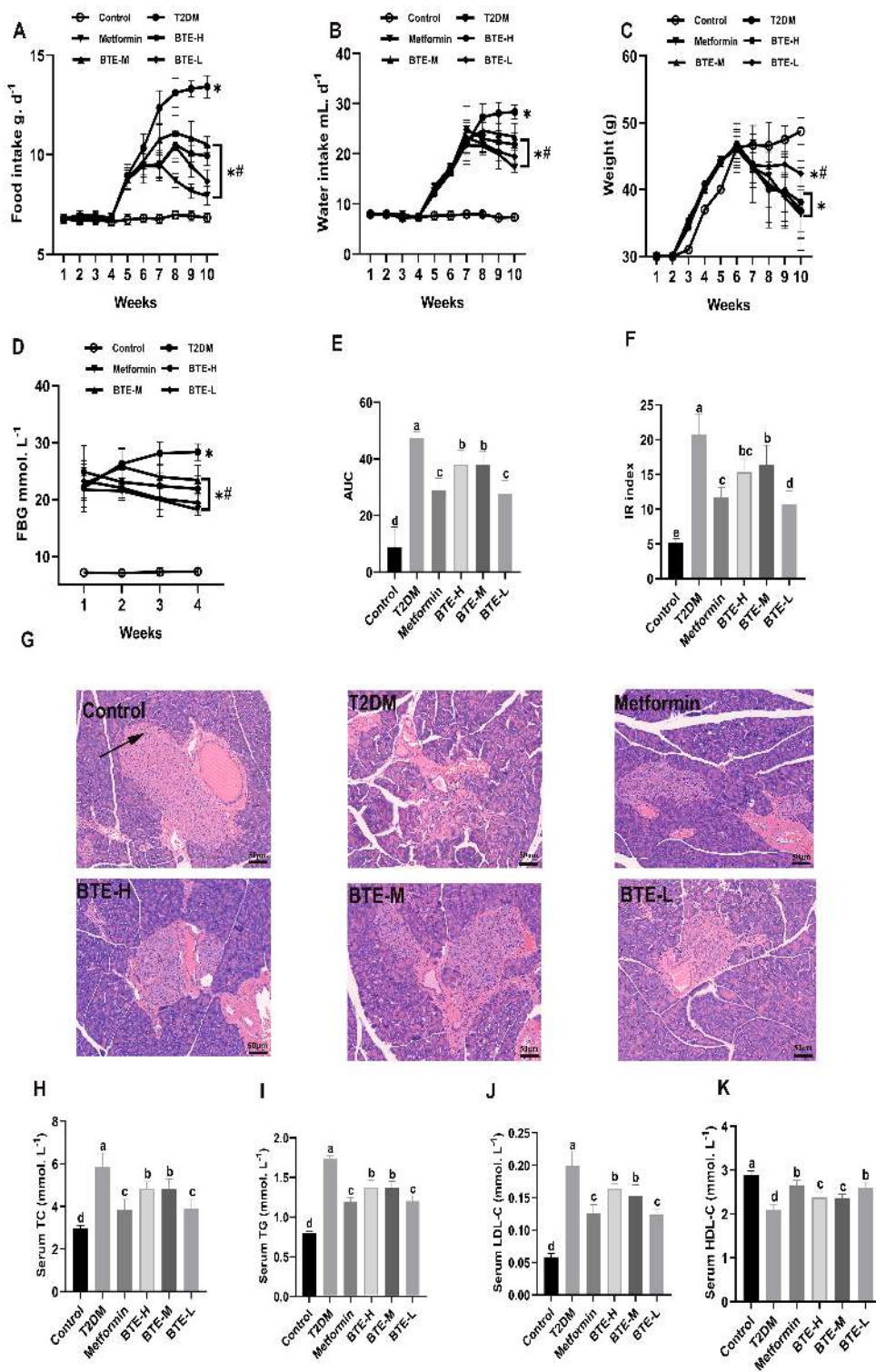


Figure 1

Effects of BTE on disorders of glucose and lipid metabolism in T2DM mice. (A) Weekly food intake per mouse during the experiment; (B) Weekly water intake per mouse during the experiment; (C) Changes in body weight of mice in each group; (D) Changes in FBG in different groups during the treatment; (E) Area under the curve (AUC) of blood glucose. (F) Insulin resistance (IR) index; (G) Histopathological changes of the pancreas (H&E staining, ×200 magnification); (H) Total cholesterol (TC) in serum; (I) Total triglycerides (TG) in serum; (J) Low-density lipoprotein (LDL-C) level in serum; (K) High-density lipoprotein (HDL-C) level in serum. Large black arrows show pancreatic islets. Control: normal Control group; T2DM: type 2 diabetes model group; Metformin: metformin-positive control group; BTE-H: high dose BTE group, BTE-M: middle dose BTE group, BTE-L: low dose BTE group; Significant differences ($p < 0.05$) are denoted by different lowercase letters.

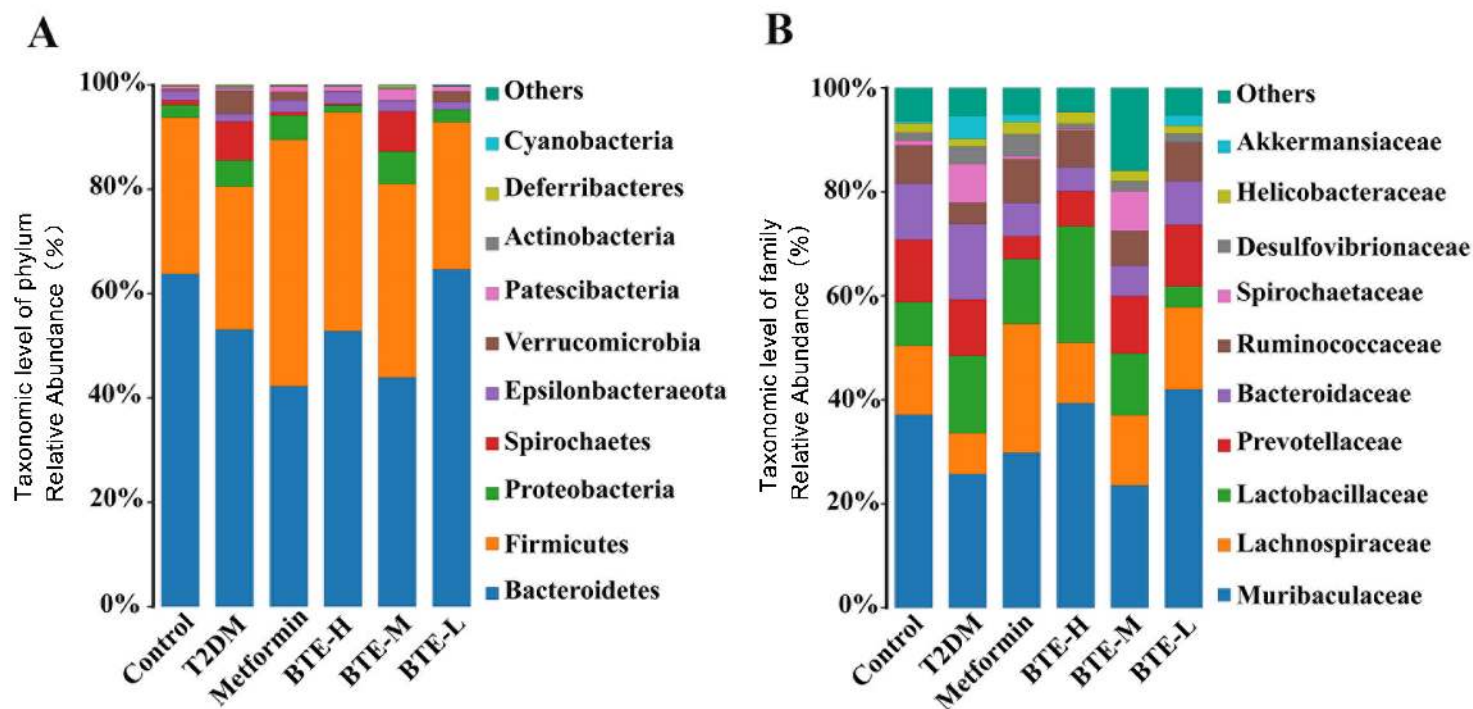


Figure 2

Diversity analysis of intestinal flora in mice. (A) Simpson index; (B) Shannon index; (C) Chao 1 index; (D) ACE index; (E) PCA component analysis 3D diagram; Control: normal Control group; T2DM: type 2 diabetes model group; Metformin: metformin-positive control group; BTE-H: high dose BTE group, BTE-M: middle dose BTE group, BTE-L: low dose BTE group; Significant differences ($p < 0.05$) are denoted by different lowercase letters.

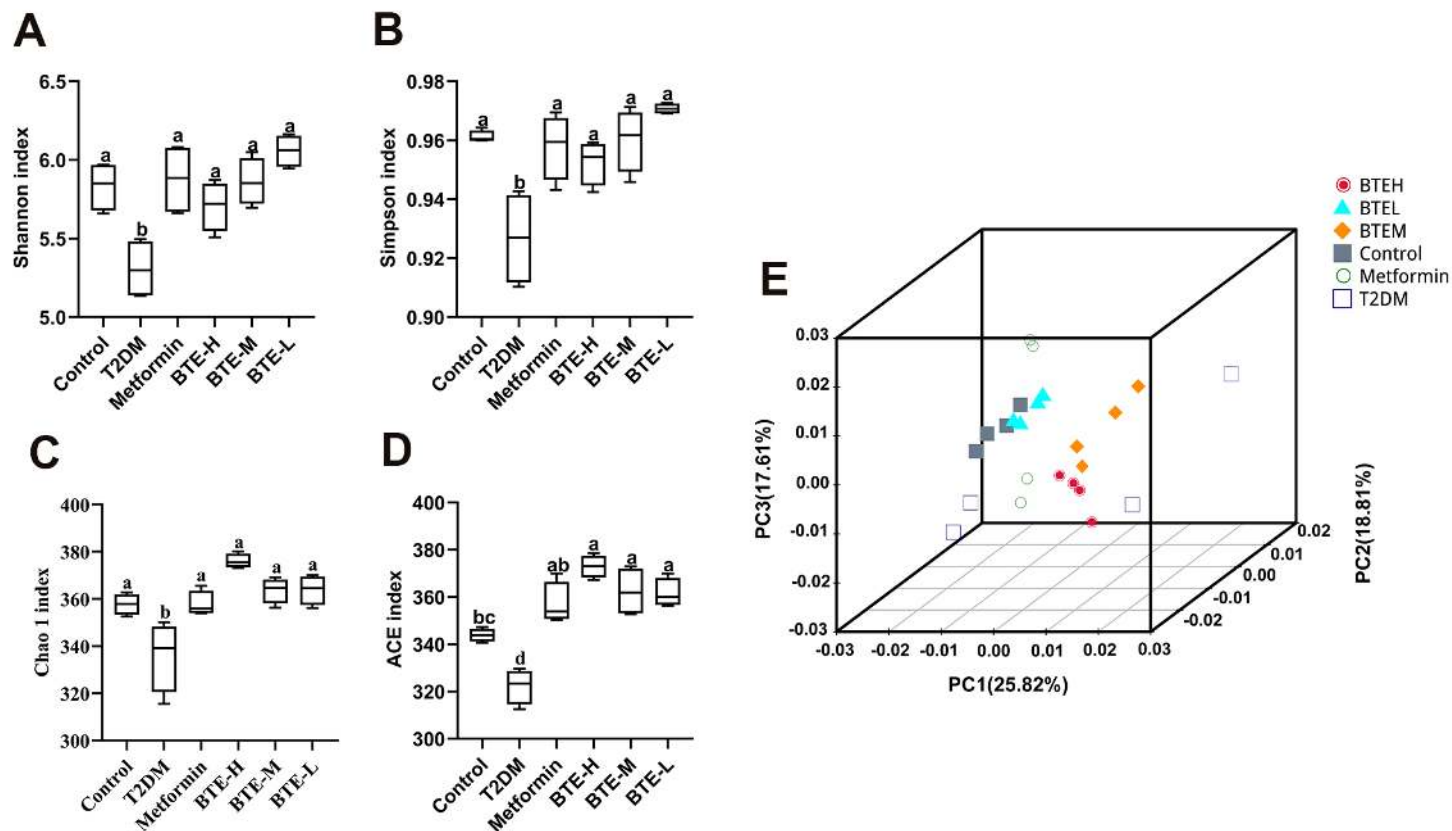


Figure 3

Abundance and structure of the top 10 gut microbiota at the phylum and family levels in different groups. (A) At the phylum level; (B) At familylevel; Control: normal Control group; T2DM: type 2 diabetes model group; Metformin: metformin-positive control group; BTE-H: high dose BTE group, BTE-M: middle dose BTE group, BTE-L: low dose BTE group. Abundance and structure of the top 10 gut microbiota at the phylum and family levels in different groups.

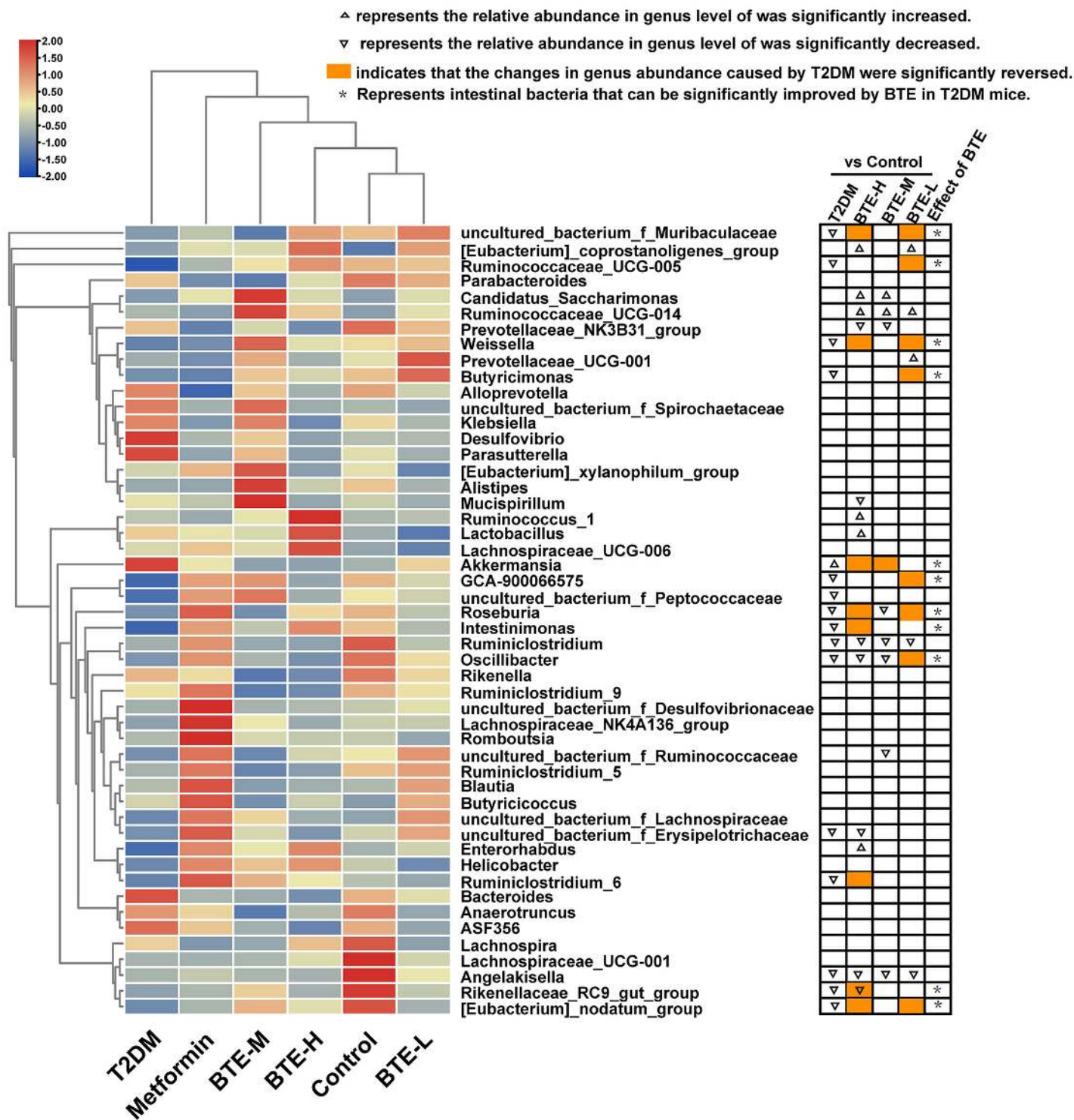


Figure 4
 Rank sum test analysis based on genus classification level. ▲ represents a significant increase in relative abundance compared with the control group. ▼ represents a significant decrease in relative abundance compared with the control group; The orange rectangle represents the improvement effect of different doses of blended tea on species richness change induced by T2DM. * represents the intestinal bacteria of T2DM mice that can be significantly improved by blended tea; The relative abundance of the first 50 genera in each group was included in the heat map. The relative abundance of each genus was represented by box colour, and the abundance similarity of each genus or sample was represented by hierarchical clustering; Control: normal Control group; T2DM: type 2 diabetes model group; Metformin: metformin-positive control group; BTE-H: high dose BTE group, BTE-M: middle dose BTE group, BTE-L: low dose BTE group; P values less than 0.05 were considered statistically significant.

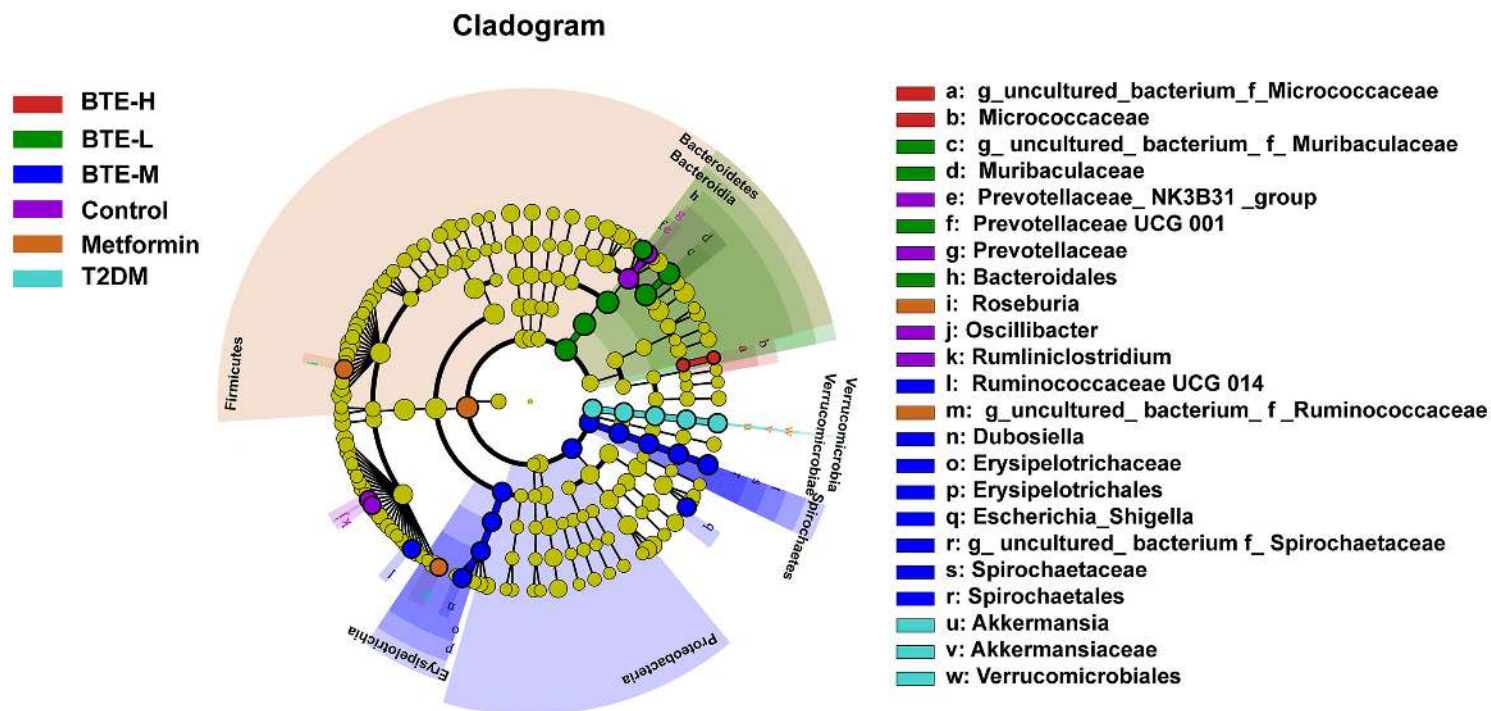


Figure 5

LefSe analysis based on phylum to genus classification level. Cladogram generated from LefSe analysis, representing taxa enriched in control group (purple), T2DM group (aquamarine), metformin-positive group (brown), BTE-H (red), BTE-M (blue), BTE-L group (green). The circles radiated from inside to outside, representing the taxonomic level from the phylum to the genus, respectively. The diameter of each circle represents the relative abundance of the taxon. When full identification was not possible, g_ was used for genus.

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