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ORIGINAL RESEARCH

Efficacy of Spleen-and-Stomach-Tonifying, Yin-Fire-Purging, and Yang-Raising Decoction Derived from the Trimethylamine N-Oxide Metabolic Pathway of Intestinal Microbiota on Macrovascular Lesions Caused by Type 2 Diabetes Mellitus

Yue Yue[1,*], Han-Bo Cui[2,*], Yue-Jie Chu[2], Gui-Ling Zheng[1]

[1]Department of Endocrinology, The 983 Hospital of the Joint Logistics Force of the Chinese People's Liberation Army, Tianjin, People's Republic of China; [2]Department of Diabetes, Tianjin Academy of Traditional Chinese Medicine Affiliated Hospital, Tianjin, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yue-Jie Chu, Department of Diabetes, Tianjin Academy of Traditional Chinese Medicine Affiliated Hospital, 354 North Road, Hongqiao District, Tianjin, 300120, People's Republic of China, Tel +8613920513352, Email chuyuejie0710@126.com

Objective: We aimed to analyze the mechanisms underlying spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction derived from the trimethylamine N-oxide (TMAO) metabolic pathway of intestinal microbiota in the treatment of macrovascular lesions caused by type 2 diabetes mellitus (T2DM).

Methods: Hartley-guinea pigs were randomly divided into 3 groups—the blank, model, and intervention groups. The T2DM combined with atherosclerosis guinea pig models were established in the model and intervention groups. After successful modeling, spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction were administered intragastrically to the intervention group, while the same volume of normal saline was administered via gavage to the blank and model groups. After 6 weeks of continuous gavage, guinea pigs were sacrificed in all groups, the colon contents were obtained, and the diversity and structural differences of intestinal microbiota were analyzed via bioinformatics. Serum was collected to detect differences in lipids, TMAO, oxidative stress, and inflammation markers between groups.

Results: Compared to the blank group, the species diversity of the intestinal microbiota in the model and intervention groups was significantly reduced. Based on the results of Analysis of Similarities and Multiple Response Permutation Procedure, the microbiota structure of the intervention group was closer to that of the blank group. After modeling, the blood lipid levels of guinea pigs increased significantly, and drug intervention significantly reduced the levels of TC, TG, and LDL-C ($P < 0.05$). TMAO expression was significantly increased after modeling ($P < 0.05$), while drug intervention reduced TMAO expression ($P < 0.05$). Compared to the model group, drug intervention significantly increased the concentrations of SOD while decreasing the concentrations of MDA, ICAM-1, VCAM-1, IL-6, and hs-CRP.

Conclusion: Spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction can reduce the risk of macrovascular lesions in T2DM, and its mechanism may be associated with its ability to regulate the TMAO metabolic pathway of intestinal microbiota.

Keywords: intestinal microbiota, macrovascular lesions, spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction, trimethylamine N-oxide, type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by high blood sugar levels. The latest data from the International Diabetes Federation indicates that approximately 415 million adults aged between 20–79 experienced DM in 2015, and it is anticipated that the number of patients affected with DM will reach 552 million by 2030, with the prevalence rising from 8.8% to 10.4%.[1,2] Some studies suggest that patients diagnosed with DM (mostly type-2 diabetes

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mellitus (T2DM)) are more likely to develop coronary, peripheral, and carotid artery complications than the general population.[3,4]

The intestinal microbiota is the largest micro-ecosystem in the human body; it participates in the body's material and energy metabolism processes and has substantial effects on the normal activities of the human body.[5,6] Recent studies suggest that the onset and progression of DM may be associated with dysbiosis.[7] Some studies suggest that dysbiosis in patients diagnosed with T2DM lead to elevated levels of trimethylamine N-oxide (TMAO).[8,9] TMAO can cause inflammation of the adipose tissue, disrupt insulin signaling pathways, and result in insulin resistance, thereby promoting the onset and progression of T2DM.[10]

TMAO has negative effects on the heart and blood vessels. Tang et al demonstrated that TMAO levels in patients diagnosed with T2DM were significantly higher than in the general population,[11] thereby increasing the risk of cardiovascular adverse events and death in patients with T2DM. In these patients, elevated TMAO concentrations are considered an independent risk factor for atherosclerosis.[12] Furthermore, regulating intestinal microbiota and decreasing TMAO synthesis can reduce the risk of atherosclerosis and prevent or improve T2DM macrovascular lesions.

Spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction is a clinical classic method for treating spleen deficiency and internal hyperactivity of yin fire syndrome caused by stomach damage due to diet, spleen damage due to fatigue, and fire-pathogen invasion.[13] Its therapeutic principles align with those of clearing heat and moisturizing dryness, nourishing yin, and producing body fluid of DM. Several studies suggest that spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction has positive clinical effects in the treatment of T2DM.[14,15] Long et al conducted research on the effect of spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction on blood lipids in the treatment of patients diagnosed with T2DM and discovered that the method not only has a significant hypoglycemic effect but can also regulate lipid metabolism disorders in patients, which may have a protective effect against macrovascular lesions caused by T2DM.[16]

Other studies have shown that components of spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction have the pharmacological effect of regulating intestinal microbiota, but it is unknown whether this recipe can reduce the incidence and development of macrovascular lesions in T2DM by regulating intestinal microbiota. Therefore, using the TMAO metabolic pathway of intestinal microbiota as a starting point, we investigated the effects and mechanism of spleen-and-stomach-tonifying, yin-fire-purging and yang-raising decoction in macrovascular lesions caused by T2DM, as well as to provide new ideas for the intervention and treatment of macrovascular lesions caused by T2DM.

Materials and Methods

Preparation of Intervention Drugs

The drug compositions of spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction ("Spleen and stomach theory") are as follows: astragalus 20 g, codonopsis 15 g, radix bupleuri 20 g, rattletop 12 g, fried cangshu 15 g, notopterygium root 15 g, *Scutellaria baicalensis* 10 g, *Coptis chinensis* 10 g, gypsum 15 g, and licorice 15 g. These medications were purchased by the Affiliated Hospital of Tianjin Institute of Traditional Chinese Medicine. All medications were decocted to obtain a juice, which was concentrated to 100 mL (equivalent to 1.47 g of crude drug/mL) in a water bath at 65 °C and stored in the refrigerator at 4 °C. The above listed dosage is the daily dose for adults, while the equivalent dose for guinea pigs is 7.74 mL/kg based on the body surface area of the animal.

Experimental Methods

Preparation of Animal Models

Male Hartley-guinea pigs were randomly divided into the blank group and model group. Guinea pigs in the model group were fed with a high-fat diet (1% cholesterol, 0.3% sodium cholate, 10% lard, 88.7% regular feed) to induce hyperlipidemia and atherosclerosis.[17] After 2 weeks of high-fat diet, DM modeling was performed on the model group by injecting intraperitoneally with 35 mg/kg streptozotocin (STZ). After 1 week of DM modeling, fasting blood glucose (FBG) was measured in the model group, samples with FBG higher than 9.0 mmol/L were qualified; if the blood glucose of the sample did not meet the standard, this sample was excluded. In the 5th week, three guinea pigs in the model group

were randomly selected and anesthetized by intraperitoneal injection of 2% pentobarbital, sacrificed in the supine position, and the aortic arch of the guinea pig was removed, hematoxylin and eosin (H&E) staining was performed, and the histomorphological changes were observed—the vascular wall of the guinea pig was observed, the lining membrane was rough and irregularly thickened, and the endothelial cells were shed, which was regarded as successful modeling. After successful modeling, a portion of guinea pigs in the model group were randomly selected as intervention group for drug treatment. Finally, there were three groups of guinea pigs in this study, namely the blank group (n=6), model group (n=7), and intervention group (n=9).

Drug Administration

The spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction was administered via gavage into guinea pigs to treat atherosclerosis, after successfully modeling T2DM and atherosclerosis. The spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction was administered intragastrically to the intervention group, whereas the same volume of normal saline was administered via gavage to the blank and model groups once a day for six weeks.

Specimen Sampling

Collection of Blood Samples

Samples were collected after six weeks of continuous gavage. Guinea pigs were fasted for 8 hours and were then anesthetized intraperitoneally with 2% pentobarbital sodium (0.2 mL/100 g). The animals were placed in the supine position and sacrificed; blood was drawn from the inferior vena cava and detected by a biochemical analyzer. A total of 2 mL of serum was obtained from each experimental guinea pig, and stored in a −80 °C refrigerator for preparation.

Pathological Specimen Collection

After collecting blood, the guinea pig was placed in a supine position and the chest cavity was fully exposed. The aortic arch was removed, washed with normal saline, dried with filter paper, and then fixed and stored with 8% neutral formaldehyde solution.

Fecal Sample Collection

After the animals were sacrificed, the abdominal cavity was incised with a scalpel, and the intestine and colon were removed to collect fecal samples. The sample was immediately sent to the laboratory for freezing at −70 °C.

DNA Extraction

Using a centrifugal adsorbent column that can extract DNA and a unique inhibitor adsorption tablet, InhibitEX, genomic DNA was extracted from fecal samples in conjunction with a buffer system. DNA extraction was conducted one month after sample collection. The extracted DNA was stored at −20 °C.

Observation Indicators and Detection Methods

H&E Staining

Slides containing aortic arch tissue were successively dewaxed with xylene and dehydrated with a gradient of ethanol. They were then stained with hematoxylin staining solution for five minutes, placed in a 1% hydrochloric acid alcohol differentiation solution for 3–5 seconds, and rinsed with running water. They were then immersed in an eosin staining solution containing 0.5% eosin for 1 minute, dehydrated with ethanol, and placed in xylene until the slides were transparent. These slides were then sealed and examined using light microscopy.

16S Bioinformatics Analysis of Feces

Tianjin Meiji Biotechnology Co., Ltd. was tasked with sequencing the extracted DNA using the 16S rDNA amplicon, and the between-group differences of the various microbial communities were determined using professional statistical analysis and data processing.

Detection of Basic Biochemical Indicators

A fully automatic biochemical analyzer was used to detect TC, TG, LDL-C, and HDL-C levels in guinea pigs.

Enzyme-Linked Immunosorbent Assay (ELISA) Detection

The stored inferior vena cava serum of the guinea pigs was withdrawn, and the levels of TMAO, MDA, SOD, ICAM-1, VCAM-1, IL-6, and hs-CRP in the guinea pig serum were determined in accordance with the instructions of the corresponding ELISA kit.

Statistical Methods

SPSS25.0 software was used for data statistics and analysis, and the measurement data are expressed as mean ± standard deviation ($\bar{x}\pm$ s). One-way ANOVA was utilized to compare data across multiple groups, while the LSD test was utilized for pairwise comparisons. Bioinformatics was used to analyze intestinal microbiota structural differences: species annotation and microbiota abundance analysis was performed by operational taxonomic unit (OUT) clustering; Alpha diversity analysis was used to analyze the species composition of intestinal microbiota in each group; Multiple Response Permutation Procedure (MRPP) and Analysis of Similarities (ANOSIM) were used for significance test of differences in microbial community structure between groups; and MetaStat and LEfSe were used to analyze the different symbiotic relationships between the groups. $P < 0.05$ indicates statistical differences.

Experiment Results

Microscopic Observation of H&E Stained Slides

The vascular wall of the aortic arch of guinea pigs in the blank group was clearly layered, and the intima was smooth and complete. In the model group, the vascular wall of the aortic arch was unclear, the intima was rough and thickened, the endothelial cells were detached, and lipid deposition and smooth muscle cell hyperplasia were observed in the subendothelial layer. The arrangement of smooth muscle cells in the middle membrane was disordered. In the intervention group, the intima of the aortic arch in guinea pigs was slightly thickened, scattered lipid deposition was observed in the subendothelial layer, smooth muscle cell proliferation was not evident, and the smooth muscle cells in the middle membrane were arranged neatly (Figure 1).

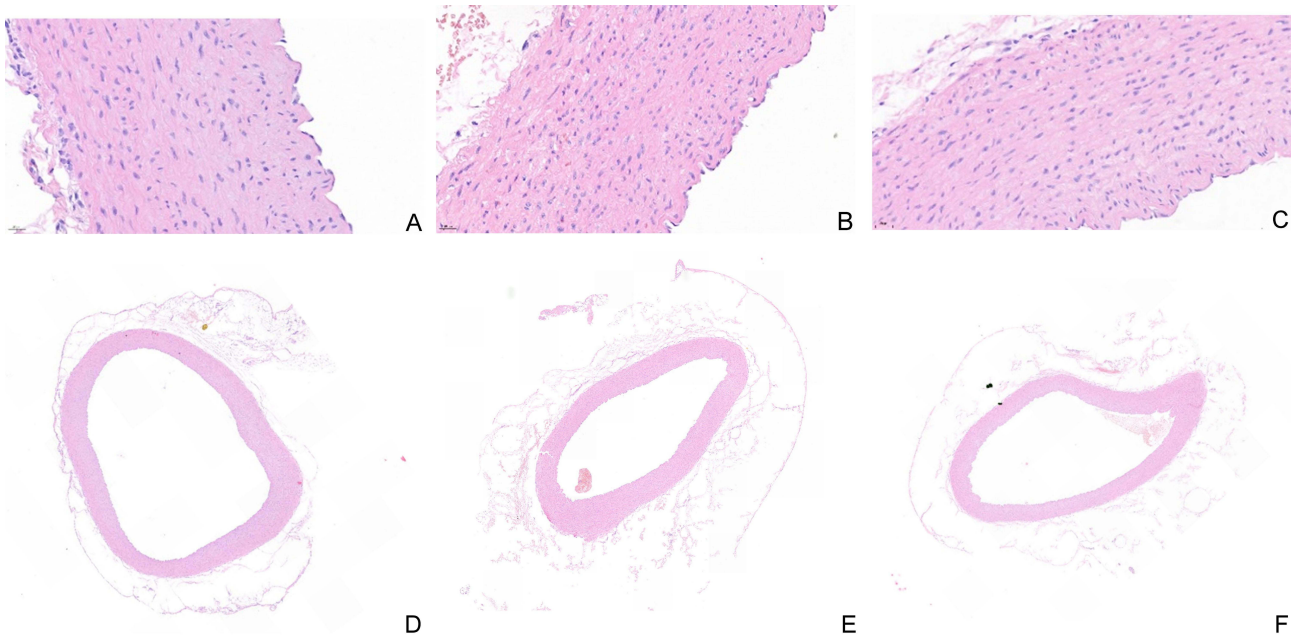


Figure 1 Guinea pig aortic arch was examined using H&E-stained microscope slides in each group. (**A**) Blank group (400x magnification). (**B**) Model group (400x magnification). (**C**) Intervention group (400x magnification). (**D**) Blank group (20x magnification). (**E**) Model group (20x magnification). (**F**) Intervention group (20x magnification).

Intestinal Microbiota Analysis

OTU Analysis

The Illumina Nova platform was used for sequencing and 4807 OTUs were identified. The resulting sequences were then annotated by the Silva138 library. There were 1714 common OTUs in the three experimental groups, with 380 shared by the blank and model groups, 450 shared by the blank and intervention groups, and 709 shared by the model and intervention groups. There were 607 specific OTUs in the blank group, 351 in the model group, and 562 in the intervention group (Figure 2A).

Based on the results of species annotation, the top 10 microbiota at each level of abundance in each sample were counted, and a histogram of relative abundance of species was created at the phylum level. Bacteroidota, Firmicutes, Proteobacteria, Actinobacteriota, unidentified_Bacteria, Verrucomicrobiota, Acidobacteriota, Euryarchaeota, Cyanobacteria, and Chloroflexi were the most prevalent microbiota (Figure 2B).

Difference Analysis of Alpha Diversity Index

Alpha between-group differences analysis allows us to compare the abundance and diversity of intestinal microbiota in each group of guinea pigs. Figure 3A and B demonstrate that the abundance of species in the blank group was significantly higher than in the model group and the intervention group, while there was no significant difference between the model group and the intervention group.

In this study, the observed_species and Shannon indicators were used to reflect the species abundance. As depicted in Figure 4A and B, the indicator values of the blank group were significantly higher than those of the model group and the intervention group, indicating that the blank group had the highest species abundance.

Community Structure Differences Tests Between Groups

The results of ANOSIM revealed that after gavage intervention of spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction, the intestinal microbiota structure of guinea pigs in the intervention group changed significantly compared with the model group ($P = 0.021$) (Table 1).

MRPP analysis indicated that there were significant differences in the intestinal microbiota community structure between the model group and the blank group, the intervention group and the blank group, and the intervention group and the model group. However, the difference between the intervention group and the blank group was smaller than the difference between the model group and the blank group, indicating that the intestinal microbiota structure of guinea pigs with T2DM and atherosclerosis differed from that of normal guinea pigs. Also, the intestinal microbiota structure of guinea pigs in the intervention group was altered following gavage administration of spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction—the structure of intestinal microbiota was distinct from the model group and the blank group, but more similar to that of the guinea pigs in the blank group (Table 1).

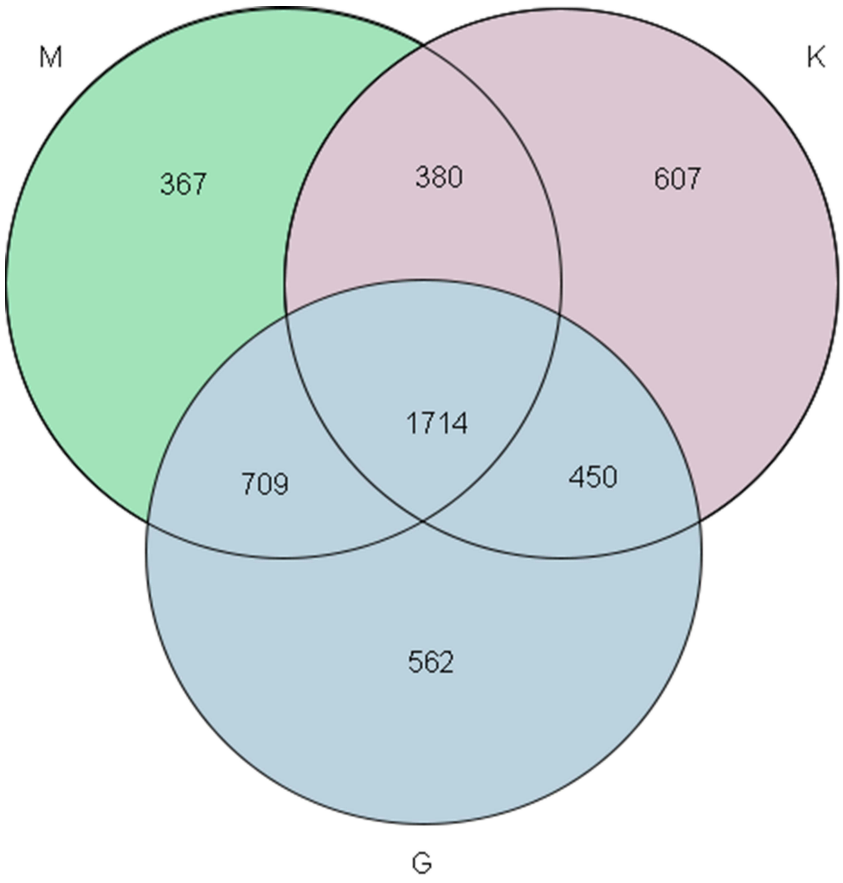
Analysis of Different Species Between Groups

For species with substantial differences, a corresponding heatmap was generated. As shown in Figure 5, compared to the model group, the abundance of *Colidextribacter, Papilibacter*, and *Desulfovibrio* in the intervention group decreased significantly ($P < 0.05$), whereas the abundance of *Faecalibacterium* and *Ruminococcus* increased significantly ($P < 0.05$), but there was no significant difference compared to the blank group ($P > 0.05$).

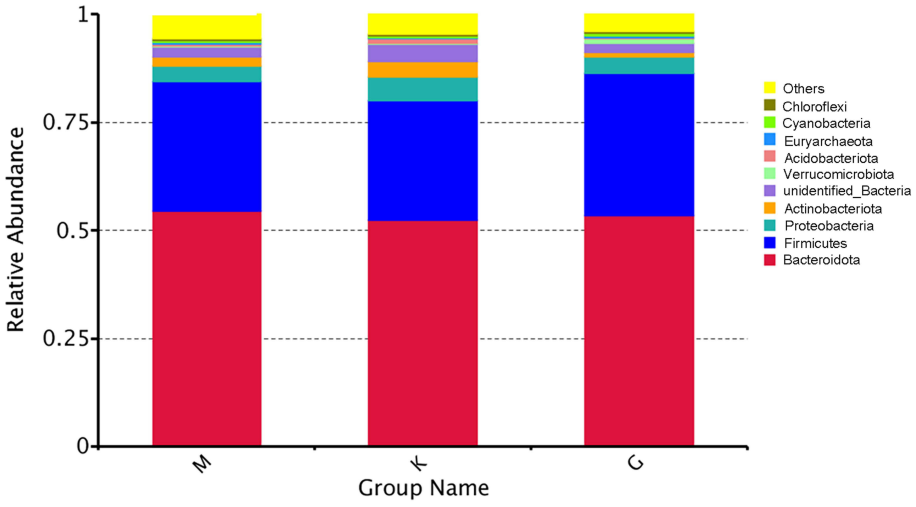
Analysis of Serological Indicators

Comparison of the Four Items in the Blood Lipids Test in Guinea Pigs in Each Group

Compared to the blank group, the levels of TC, TG, and LDL-C in the guinea pigs in the model group were significantly elevated ($P < 0.05$). Compared to the model group, the TC, TG, and LDL-C levels were significantly decreased in the intervention group ($P < 0.05$), while the levels of HDL-C were significantly increased ($P < 0.05$) (Table 2).



A



B

Figure 2 (**A**) Number of common and specific OTUs in the intestinal microbiota of Guinea pigs in each group (unit: pcs). (**B**) Histogram of species relative abundance of the intestinal microbiota of Guinea pigs in each group (phylum level).

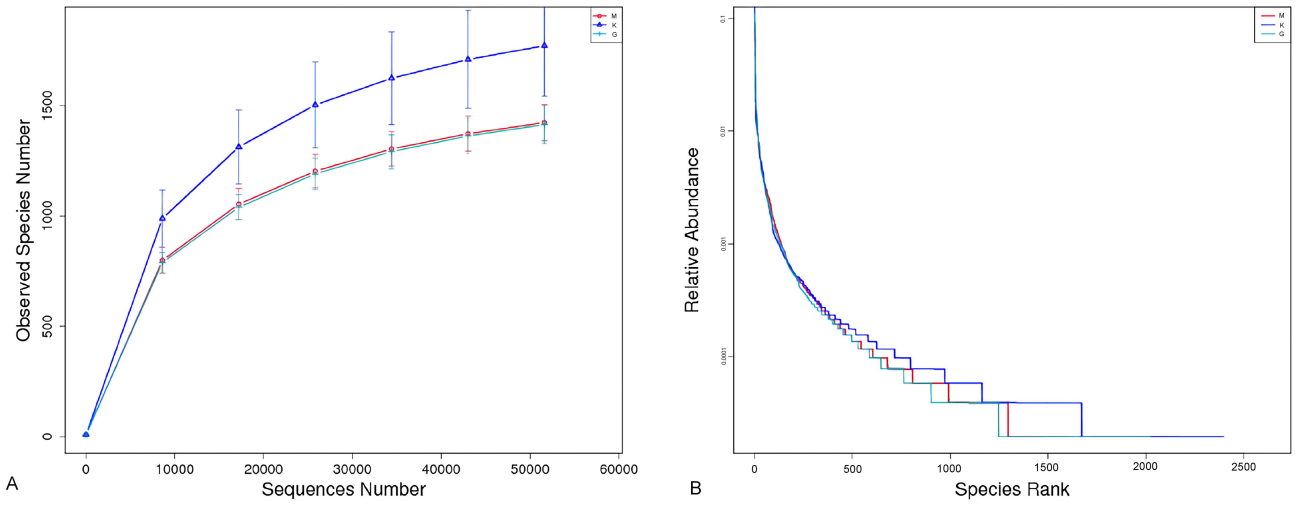


Figure 3 (**A**) Species diversity dilution curve. (**B**) Species diversity hierarchy clustering curve.

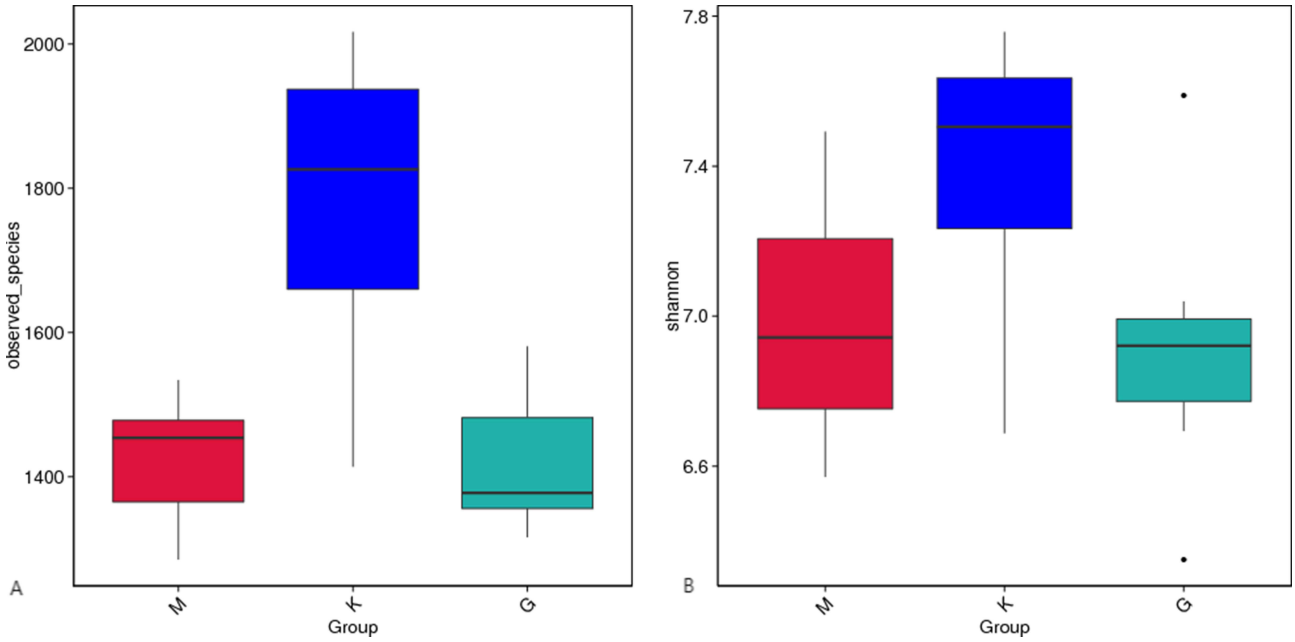


Figure 4 (**A**) Box plot of between-group differences of observed_species indicator. (**B**) Box plot of between-group differences of Shannon indicator.

Comparison of Serum TMAO Levels in Guinea Pigs in Each Group

Compared with the blank group, the TMAO level in the model group was significantly increased ($P < 0.05$). Compared to the model group, the TMAO level was significantly reduced in the intervention group ($P < 0.05$) (Table 3).

Comparison of Oxidative Stress Indicators SOD and MDA in Guinea Pigs in Each Group

Compared with the blank group, the SOD concentration in the model group and the intervention group decreased significantly ($P < 0.05$). Compared to the model group, the SOD concentration in the intervention group increased significantly ($P < 0.05$). Compared to the blank group, the MDA concentration in the model group increased significantly ($P < 0.05$), whereas there was no significant difference in the concentration of MDA in the intervention group ($P > 0.05$). Compared to the model group, the MDA concentration in the intervention group decreased significantly ($P < 0.05$) (Table 4).

Table 1 Between-Group Difference Analysis Using ANOSIM and MRPP

Between-Group Comparison	Anosim		MRPP			
	R-value	P-value	A	Observe Delta	Expect Delta	Significance
Model group and blank group	0.1032	0.077	0.04338	0.4185	0.4375	0.005
Intervention group and blank group	0.2886	0.056	0.04847	0.3823	0.4016	0.008
Intervention group and model group	0.1399	0.021	0.01489	0.4186	0.425	0.029

Notes: The smaller the Observe Delta value, the smaller the difference within the group. The greater the Expect Delta value, the greater the group difference. A value greater than 0 indicates that the difference between groups is larger than the difference within the group, while a value less than 0 indicates that the difference within the group is larger than the difference between groups.

Comparison of Serum Inflammatory Markers ICAM-1, VCAM-1, IL-6, and hs-CRP Concentrations in Guinea Pigs in Each Group

Compared to the blank group, the concentrations of ICAM-1, VCAM-1, IL-6, and hs-CRP in the model group increased significantly ($P < 0.05$). Compared to the model group, the concentrations of ICAM-1, VCAM, IL-6, and hs-CRP in the intervention group decreased significantly ($P < 0.05$) (Table 5).

Discussion

The results of some studies suggest that approximately 80% of patients diagnosed with T2DM die from cardiovascular disease. Current research on macrovascular lesions caused by T2DM focuses mostly on two goals: identifying novel targets for therapy and improving the treatment regimen. The onset of T2DM is inextricably linked to the intestinal microbiota.[18,19] The composition of the intestinal microbiota of patients diagnosed with T2DM differs from that of healthy people, as do the microorganisms and metabolites within the intestines. In this study, the results of 16S rDNA sequencing revealed that the diversity and abundance of intestinal microbiota in the model group changed significantly, which was attributed to the alteration of the internal environment following high-fat feeding and intraperitoneal injection of STZ, which disrupted the diversity and stability of the intestinal microecosystem.

Intestinal dysbiosis reduces the integrity of the intestinal barrier, inhibits nutrient absorption, causes chronic low-grade inflammation, inhibits lipid metabolism, and accelerates oxidative stress, resulting in the formation and enlargement of arterial plaque. Thus, among the macrovascular consequences of T2DM, intestinal dysbiosis plays a prominent role. In this study, we found that the oxidative stress indicator SOD decreased significantly in the model group, while

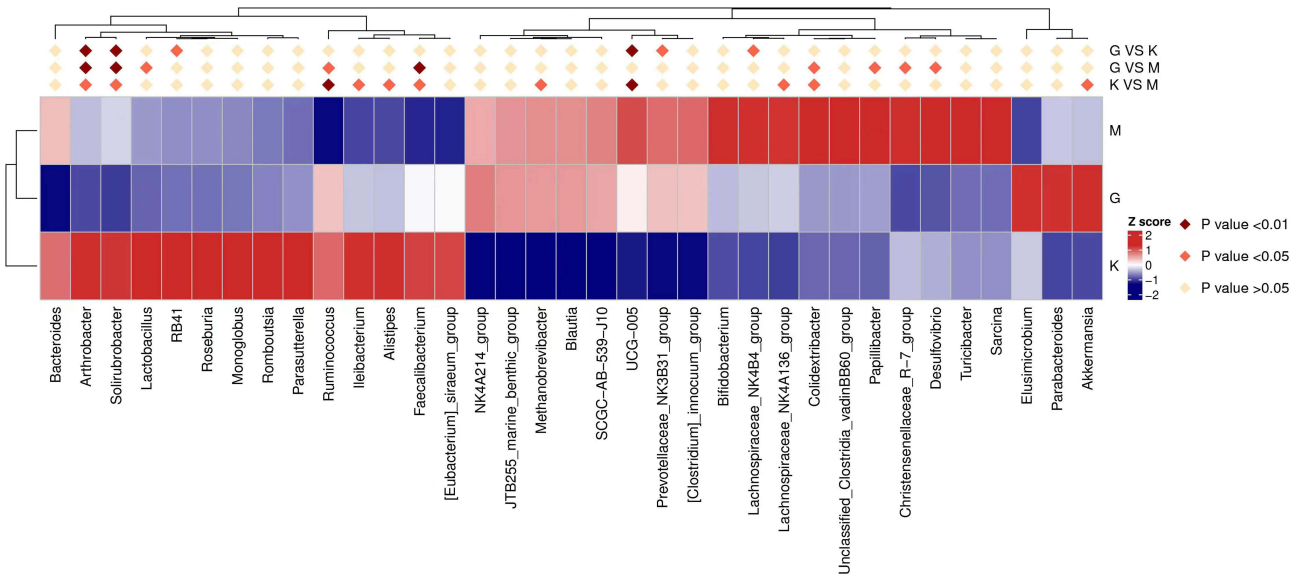


Figure 5 Heatmap figure of species significance differences between groups.

Table 2 Comparison of Four Components of the Blood Lipids Test in the Guinea Pigs of Each Group ($\bar{x}\pm$ s, mmol/L)

Group	n/Cases	TC	TG	HDL-C	LDL-C
Blank group	6	9.12±1.71	0.45±0.08	2.05±0.37	2.03±0.58
Model group	7	13.22±2.97[a]	0.92±0.29[a]	1.06±0.22[a]	3.91±1.05[a]
Intervention group	9	9.90±3.69[b]	0.57±0.15[b]	1.53±0.41[a,b]	2.77±0.62[a,b]

Notes: [a]Indicates that the data of this group were compared with those of the blank group $P < 0.05$; [b]Indicates that the data of this group were compared to those of the model group $P < 0.05$.

Table 3 Comparison of Serum TMAO Concentrations in Guinea Pigs in Each Group ($\bar{x}\pm$ s, ng/mL)

Group	n/Cases	TMAO
Blank group	6	30.61±5.22
Model group	7	40.17±3.04[a]
Intervention group	9	31.68±4.76[b]

Notes: [a]Indicates that the data of this group were compared with those of the blank group $P < 0.05$; [b]Indicates that the data of this group were compared to those of the model group $P < 0.05$.

Table 4 Comparison of the Oxidative Stress Indicators SOD and MDA in the Guinea Pigs in Each Group ($\bar{x}\pm$ s)

Group	n/Cases	SOD (U/mL)	MDA (nmol/mL)
Blank group	6	31.73±2.43	3.63±0.61
Model group	7	18.62±3.98[a]	5.82±1.91[a]
Intervention group	9	23.20±4.51[a,b]	4.17±1.13[b]

Notes: [a]Indicates that the data of this group were compared with those of the blank group $P < 0.05$; [b]Indicates that the data of this group were compared to those of the model group $P < 0.05$.

Table 5 Comparison of Serum Inflammatory Markers in Guinea Pigs in Each Group

Group	n/Cases	ICAM-1 (ng/mL)	VCAM-1 (ng/mL)	IL-6 (ng/L)	hs-CRP (mg/L)
Blank group	6	1.13±0.18	1.04±0.39	37.19±6.73	5.18±0.49
Model group	7	1.50±0.35[a]	1.42±0.21[a]	74.26±9.54[a]	7.38±0.82[a]
Intervention group	9	1.22±0.14[b]	1.06±0.10[b]	46.36±8.14[a,b]	5.84±0.61[a,b]

Notes: [a]Indicates that the data of this group were compared with those of the blank group $P < 0.05$; [b]Indicates that the data of this group were compared to those of the model group $P < 0.05$.

MDA increased significantly. The decrease in SOD activity would result in an accumulation of oxygen radical metabolites in the body, and the body's ability to eliminate oxygen free radicals would decrease. The increase in MDA content indirectly reflected that the cells in the body were attacked by free radicals, and the decrease in SOD and increase in MDA indicated that the intestinal mucosa of guinea pigs in the model group was damaged after their blood glucose increased. Serum concentrations of ICAM-1, VCAM-1, IL-6, and hs-CRP increased significantly in model group, indicating that an inflammatory response occurred in the intestinal mucosal injury, which led to the upregulated expression of adhesion factors.

The serum TMAO concentration of guinea pigs in the model group was significantly higher than that in the blank group, primarily as a result of dysbiosis and the significant increase in the level of TMAO. Intestinal microbiota produce TMAO by fermenting choline and carnitine from food.[20] Some researchers have conducted prospective studies to investigate the relationship between TMAO levels and atherosclerosis, and have confirmed that when systemic TMAO levels increase, both atherosclerotic load and myocardial damage is aggravated.[21] The mechanism by which TMAO induces atherosclerosis may involve multiple pathways: it can upregulate atherosclerosis-associated macrophage scavenger receptor expression, resulting in elevated pro-inflammatory cytokine levels,[22,23] and it can reduce the bioavailability of nitric oxide by promoting oxidative stress, resulting in endothelial dysfunction.[24] The results of this study revealed that while the TMAO level of guinea pigs in the model group increased, the levels of TC, TG, LDL-C, MDA, ICAM-1, VCAM, IL-6, and hs-CRP increased significantly, whereas the levels of HDL-C and SOD decreased significantly, indicating that the increase in TMAO level caused lipid metabolism disorders, promoted oxidative stress, and strengthened inflammatory response, thereby contributing to the development of atherosclerosis. This is consistent with the findings of Mohammad et al.[25]

Macrovascular lesions causes by diabetes belong to the variation of consumptive thirst in traditional Chinese medicine (TCM), of which the main pathogeneses as per TCM theory are body fluid impairment and "Yin fire injures fluids and consumes blood". Spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction is a classic Chinese medicine recipe that uses the methods of strengthening the spleen and invigorating qi, raising yang, and purging yin fire to replenish the spleen and stomach qi deficiency, increase the clear yang, and decrease the turbid yin. It can not only control the consumptive thirst symptom, but also relieve thirst and vessel impediment. This study demonstrated that the spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction can significantly improve the intestinal microbiota structure of guinea pigs and regulate dysbiosis. Previous studies have demonstrated that similar Chinese medicine recipes for strengthening the spleen, removing dampness, invigorating qi, and harmonizing the stomach can inhibit harmful intestinal bacteria, promote the growth of beneficial bacteria, and regulate intestinal microbiota.[26] For example, spleen-stomach-invigorating and qi-replenishing decoction can regulate intestinal microbiota;[27] Sijunzi decoction can significantly increase the diversity of intestinal microbiota and the content of beneficial bacteria in the treatment of spleen deficiency;[28] and Cangshu compound recipe can regulate intestinal microbiota structure and improve the intestinal environment.[29] The regulating effects of recipes that strengthen the spleen, eliminate dampness, and strengthen qi on intestinal microbiota provide novel approaches for treating macrovascular lesions caused by T2DM. We found that spleen-and-stomach-tonifying, yin-fire-purging and yang-raising decoction can significantly increase the abundance of *Faecalibacterium* and *Ruminococcus* in guinea pigs in the intervention group, significantly decrease the TMAO level of guinea pigs in the intervention group, significantly decrease the levels of TC, TG, LDL-C, MDA, ICAM-1, VCAM-1, IL-6 and hs-CRP, and significantly increase the levels of HDL-C and SOD, indicating that spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction can regulate intestinal microbiota, reduce TMAO levels, regulate blood lipids, improve antioxidant capacity, improve inflammatory response, reduce the risk of atherosclerosis, and delay the development of macrovascular lesions caused by T2DM. Some studies suggest that beneficial bacteria such as *Faecalibacterium* and *Ruminococcus* are significantly negatively correlated with TMAO and macrovascular diseases, and their increase can inhibit the production of TMA and thus reduce TMAO levels, thereby preventing the occurrence of atherosclerosis,[30] which is consistent with our findings.

The pharmacological study of the single Chinese herb of spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction revealed that it can regulate intestinal microbiota, promote intestinal metabolism, exert anti-inflammatory effects, and enhance immunity. The study conducted by Yin et al demonstrated that astragalus can increase the diversity of intestinal microbiota in mice, restore intestinal microbiota homeostasis, and reduce the expression of inflammatory factors in the intestinal tissues of aging mice.[31] A study conducted by Lv et al suggests that Cangshu-baishu has the effects of regulating intestinal microbiota, repairing gastrointestinal mucosal damage, and enhancing gastrointestinal motility.[32] The study by Huang et al demonstrated that baked licorice can increase the diversity of intestinal microbiota in rats with a spleen deficiency.[33] Baicalin is the primary component of *S. baicalensis*, and Liu et al found that baicalin inhibits the number of harmful *Desulfovibrio* bacteria in the intestines of mice, thereby playing an anti-inflammatory role.[34] Mu et al discovered that *C. chinensis* can improve dysbiosis in mice, enhance inflammatory response, and play a role in the prevention and treatment of T2DM.[35]

It is evident that the Chinese herbal compound and most single Chinese herbs of the spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction can regulate intestinal microbiota, inhibit intestinal pathogenic bacteria, and promote the growth of probiotics.

Conclusion

Gavage intervention of the spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction can significantly improve the intestinal microbiota structure of guinea pigs, significantly reduce the serum TMAO level of guinea pigs, regulate blood lipid levels, enhance antioxidant capacity, and improve inflammatory response. Therefore, the spleen-and-stomach-tonifying, yin-fire-purging, and yang-raising decoction can reduce the risk of macrovascular lesions caused by T2DM, and its mechanism may involve its capacity to regulate the TMAO metabolic pathway of intestinal microbiota.

Abbreviations

ADA, American Diabetes Association; DM, Diabetes mellitus; FBG, Fasting blood glucose; FMO3, Flavin containing monooxygenase 3; FMOx, Flavin containing monooxygenase x; HDL-C, High density lipoprotein cholesterol; HE, Hematoxylin-eosin; hs-CRP, Hypersensitive C-reactive protein; hs-cTnT, Hypersensitive cardiac troponin-T; ICAM-1, Intercellular cell adhesion molecule-1; IL-1β, Interleukin-1β; IL-6, Interleukin-6; LDL-C, Low density lipoprotein cholesterol; MDA, Malondialdehyde; OTUs, Operational Taxonomic Units; SOD, Superoxide Dismutase; STZ, Streptozotocin; T2DM, Type 2 Diabetes mellitus; TC, Total cholesterol; TG, Triglyceride; TMA, Trimethylamine; TMAO, Trimethylamine oxide; TNF-α, Tumor Necrosis Factor-α; VCAM-1, Vascular cell adhesion molecule-1.

Ethics Approval

All experiments were evaluated and approved by the Ethics Committee of Tianjin Academy of Traditional Chinese Medicine Affiliated hospital and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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Disclosure

The authors report no conflicts of interest in this work.

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