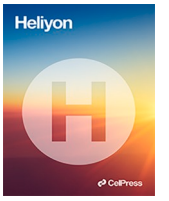
Heliyon 10 (2024) e30379

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

The therapeutic effects of traditional Chinese medicine on insulin resistance in obese mice by modulating intestinal functions

Lirong Ma [a,1], Yongquan Bai [c,1], Jun Liu [a,1], Kaimin Gong [a], Qirui He [a], Jintao Zhao [a], Yina Suo [a], Wenwen Wang [c], Guo Chen [b,c,*], Zifan Lu [b,c,**]

[a] Yinchuan Traditional Chinese Medicine Hospital, 750001, Ningxia, China
[b] Translational Medicine Center of Shaanxi Provincial People's Hospital, Xi'an, 710068, China
[c] Department of Bio-pharmacy, Fourth Military Medical University, Xi'an, China

ARTICLE INFO

Keywords:
Obesity
Chinese medicine
Lipid metabolism
Inflammation
Intestinal flora
Artemisia capillaris
Astragalus propinquus
Phellodendron amurense
Salvia miltiorrhiza
Poria cocos
Anemarrhena asphodeloides

ABSTRACT

Introduction: Obesity, mainly caused by excessive accumulation of visceral fat, excessive fat metabolism will cause hormone secretion imbalance and inflammation and other diseases. is extremely detrimental to human health. Although many treatments are available for obesity, most treatments fail to exert a radical effect or are associated with several side effects. Traditional Chinese medicine (TCM) for regulating the intestinal flora, lipid content and inflammation is considered effective. Based on previous studies, *Artemisia capillaris*, *Astragalus propinquus*, *Phellodendron amurense*, *Salvia miltiorrhiza*, *Poria cocos*, and *Anemarrhena asphodeloides* were selected to prepare an innovative herbal formula.

Methods: TCM was characterized by UHPLC-Q-Orbitrap-MS. The anti-inflammatory and lipid-lowering effects of the TCM formula prepared were evaluated in a high-fat diet-fed obese mouse model. The effects of the TCM formula on the intestinal flora were also investigated.

Results: Weights and insulin resistance, as well as inflammation, decreased in the mice after treatment. At the same time, lipid metabolism increased after the mice were gavaged with the TCM formula for 2 weeks. The intestinal motility of the drug administration group was enhanced, with partial restoration of the intestinal flora.

Conclusion: In summary, our innovative Chinese herbal formula significantly reduced weight, reduced intestinal inflammation, improved intestinal motility, and improved lipid metabolism in obese mice. Furthermore, the innovative formula effectively prevented relevant obesity-induced metastatic diseases in the mice.

1. Introduction

The global prevalence of obesity has been sustainably increasing over the past 40 years, from less than 1 % in 1975 to 6%–8% in 2016 [1].Obesity has become an epidemic, with more than 2 billion people globally being overweight or obese. The World Health Organization has estimated that approximately 300 million people will be obese by 2035. Therefore, developing drugs to treat this

* Corresponding author. Translational Medicine Center of Shaanxi Provincial People's Hospital, Xi'an, 710068, China.
** Corresponding author. Translational Medicine Center of Shaanxi Provincial People's Hospital, Xi'an, 710068, China.
E-mail addresses: fmmu.cg@163.com (G. Chen), luzfliuq@fmmu.edu.cn (Z. Lu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e30379
Received 16 August 2023; Received in revised form 24 April 2024; Accepted 24 April 2024
Available online 6 May 2024

life-threatening pandemic is very important [2]. Obesity refers to excessive fat accumulation relative to height, characterized by increased mass of adipose tissue and adipose tissue dysfunction, leading to systemic lipid extravasation and low-grade inflammation, which may ultimately lead to other diseases, such as type 2 diabetes (T2DM) and cardiovascular disease [3]. The intestinal flora structure differs significantly between obese patients and healthy people, possibly causing changes in the intestinal peristaltic function and intestinal barrier function [4]. Traditional treatments for obesity, weight loss, and/or improvement in disordered blood glucose control mainly involve weight loss surgery and drugs. However, surgery is often highly invasive, irreversible in most cases, and associated with risks. Most importantly, surgery is unrealistic as an overall strategy for tackling the global "diabetes" pandemic. Moreover, when most weight loss drugs are used as single hormone therapy, their efficacy and safety are unsatisfactory [5]. Therefore, new and effective treatments with fewer side effects are urgently required to reduce obesity and improve blood glucose control.

Appetite inhibition is a very effective method of treating obesity [6]. Intestinal hormones secreted by intestinal endocrine cells, such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY), regulate appetite. Rodents treated with combinations of these gut-derived peptides exhibited a reduction in food intake and body weight [7]. Both CCK and GLP-1 are satiety signal hormones that activate the CCK receptor (CCK1R) and GLP-1 receptor (GLP-1R) of the intestinal vagus nerve to stimulate that nerve and inhibit appetite. In addition, CCK may travel directly through the blood circulation to the brain's center and bind to CCK1R in the nucleus tractus solitarii (NTS) and six hypothalamic areas, thereby inhibiting eating [8,9]. PYY may activate proopiomelanocortin and inhibit neuropeptide Y neurons through the hormone pathway, triggering a sense of satiety and thus reducing food intake [10]. Along with the upregulation of saturated signal hormones, the downregulation of food-promoting signals is the direction worth taking to reduce obesity. Gastrin is the only orexin with peripheral activity. It reaches the brain through blood circulation and enters the hypothalamus through the vagus nerve and nucleus of the solitary tract, affecting the center of energy regulation and inducing an increase in food intake [11,12]. Increased food intake leads to obesity, which may further result in insulin resistance in the target tissue [13]. Insulin resistance is the pathological state in which the glucose uptake-promoting efficiency of insulin is decreased. Insulin resistance occurs for various reasons. Skeletal muscle, being the main glucose uptake tissue, and fat play crucial roles in insulin resistance [14]. In the presence of glucose, insulin stimulates protein kinase B (AKT) phosphorylation, which leads to glucose uptake. During insulin resistance, AKT phosphorylation is inhibited, and glucose uptake by each target tissue is significantly weakened. Consequently, the blood glucose concentration is maintained at a high level [15,16].

The gut is a vital organ in humans. In addition to its digestion–absorption capacity, this organ functions as a barrier protecting the body [17]. Numerous bacteria are present in the human intestinal tract. These bacteria form a microbial line of defense, namely the microbial barrier, by competing with pathogenic bacteria for colonization sites and nutrients or secreting bacteriostatic substances to inhibit the growth of pathogenic bacteria. In addition to their barrier function, the normal flora plays a major role in lipid metabolism. Regulating the intestinal flora is a new therapeutic strategy for controlling metabolic syndromes such as obesity, diabetes, and non-alcoholic fat disease [18]. The intestinal mucosal barrier is the most critical structure in the intestine and serves as a mechanical barrier, and the most crucial structural basis for the function of this barrier is the tight junction between intestinal mucosal epithelial cells. Tight junctions of intestinal mucosal epithelial cells are mainly regulated by claudin, occludin, zonula occludens (ZO-1), and other factors [19]. When a high-fat diet induces obesity, the expression of occludin and ZO-1 proteins is inhibited, and the intestinal barrier function is impaired [20]. Being the largest mucosa-associated lymphoid tissue in the whole body, the intestinal tract has a strong immune function. The intestinal tissue has numerous immune cells, which offer good protection to the intestinal tract [21]. However, long-term consumption of a high-fat diet results in inflammation of the distal small intestine and colon. In such cases, the expression of pro-inflammatory factors, such as IL-6 and TNF-α, increases, whereas that of anti-inflammatory factors, such as IL-10 and IL-7α, decreases. These inflammatory reactions are not conducive to intestinal barrier stability [22]. In addition to intestinal inflammation, obesity also induces systemic chronic low-grade inflammation, which is mainly manifested as increased blood concentration of pro-inflammatory factors and decreased blood concentration of anti-inflammatory factors [23]. Meanwhile, obesity causes oxidative stress in the intestinal tract and decreases the expression of genes encoding antioxidant enzymes such as SOD, thereby resulting in intestinal damage [24].

Along with the intestinal barrier function, the intestinal motor function is also impaired in obese mice [25]. 5-Hydroxytryptamine (5-HT) is a crucial component of the brain–gut axis regulation pathway and plays a critical role in maintaining normal gastrointestinal function [26]. In the body, 90 % of 5-HT is synthesized, secreted, and activated by enterochromaffin cells in the gut. Tryptophan hydroxylase (TPH) acts on tryptophan to produce 5-hydroxytryptophan, which is then transformed to 5-HT under the action of 5-hydroxytryptophan decarboxylase. 5-HT mainly binds to the receptors 5-HT1, 5-HT2, 5-HT3, 5-HT4, and 5-HT7 in the intestinal tract to regulate the gastrointestinal motor function. The 5-HT2 receptor mainly exists in peripheral tissues such as the digestive tract. When 5-HT stimulates the aforementioned receptor, the smooth muscle contraction of the gastrointestinal tract occurs, thereby promoting intestinal movement [27].

Because obesity is a chronic systemic metabolic disease, achieving the desired therapeutic effect from a single phenotypic therapy in obese patients is difficult [5]. Traditional Chinese medicine (TCM) exhibits a good effect in the treatment of metabolic diseases [28]. Many Chinese medicine monomers, such as *Poria cocos* and *Salvia miltiorrhiza* polysaccharides, have also been found to regulate the intestinal flora in animal models. These monomers improve hyperglycemia and hyperlipidemia by regulating intestinal microorganisms [29,30]. The *Cortex phellodendri* extract significantly improved the intestinal flora and increased the serum GLP-1 level of mice [31]. In addition, light body powder, seaweed light body soup, lotus leaf ash prescription, and other TCMs have a good effect on weight loss [28]. However, most effects of these formulas are mainly directed toward clearing heat and dehumidifying, removing stagnation, and stopping diarrhea. Currently, herbal formulas with good therapeutic effects on metabolic dysfunction caused by obesity, intestinal peristalsis, and systemic inflammation are lacking. In the ancient book "Medical Jin Jian" of TCM, a medicine called Zhibai Dihuang Pill was mainly used to treat Yin deficiency. Modern pharmacological studies have also reported its pharmacological effects. Zhibai

Dihuang Pill has been reported to reduce blood glucose levels and antitumor effects [32]. However, there are no relevant articles on how this drug exerts its hypoglycemic effect at this stage, and there are even fewer articles on how to discuss the relevant mechanism from the perspective of intestinal flora.,However, too many Chinese herbs are present in this prescription, and no further research has been conducted in this regard. In this study, we selected *Artemisia* hairy stem, *Astragalus*, *Phellodendron*, *S. miltiorrhiza*, *Poria cocos*, *Anemarrhena*, and other TCMs to investigate their impact on obesity. Early reports have suggested that these herbs can curb obesity. But whether these drugs can be equally effective in combination is the main aim of our research at this stage. Early drug use has certain limitations, so the dosage needs to be fumbled. Through numerous experiments, we determined the drug dose. This formula was an improvement and innovation of the traditional formula and was expected to exert a therapeutic effect on insulin resistance in obese individuals through the intestinal tract. We here investigated the new formula through numerous pre-experiments. We used high-fat diet-fed mice as the obesity model. The mouse model was successfully established through the statistical analysis of mouse weight monitoring, adipose histopathological examination, glucose tolerance results, and insulin tolerance results. The mice in the experimental group were treated intragastrically with the prepared formula. Through 16S sequencing of microbial flora, serological detection, and related protein detection of intestinal samples, we confirmed that the prepared TCM formula alleviates obesity by regulating lipid metabolism and influencing the intestinal microenvironment flora of mice.

2. Methods and materials

Primary anti-P-Akt and AKT antibodies were purchased from Signalway Antibody (USA). ZO-1 and occludin (02902) antibodies were purchased from ProteinTech (USA). The biotinylated goat secondary antibody (G23303) was purchased from Servicebio (Wuhan, China). Beta-actin (#8457) was purchased from Cell Signaling Technology (Cambridge, MA, USA). The ELISA kits for mouse GLP-1 and 5-HT were purchased from cloud-clone (Wuhan, China). The ELISA kits for mouse IL-6 and IL-10 were purchased from Dakewe Biotech Co. The CFX96 real-time PCR instrument was purchased from Bio-Rad (USA). The Powerlab 8/35 data acquisition and analysis system was from AD Instruments (Australia).

3. Extraction of herbal components

Dried *A. capillaris*, *Astragalus propinquus*, *P. amurense*, *S. miltiorrhiza*, *Poria cocos*, and *Anemarrhena asphodeloides* (Shengsheng TCM Yinpian, Shaanxi) were extracted individually under reflux by using deionized water for 1 h. The extraction was repeated. The extracts were filtered, room temperature centrifuged 3000 rpm/min 3 min to remove undissolved particles, and freeze-dried into powder. All the extracts were mixed in a ratio of 1:3, dissolved in normal saline, and stored at 4 °C.

4. Characterization of TCM by ultra-high performance liquid chromatography-Q

4.1. Exactive-Orbitrap-MS (UHPLC-Q-Orbitrap-MS)

The TCM were analyzed by a high-resolution UHPLC-Q-Orbitrap/MS according to the following conditions. Chromatographic separation was carried out at 35 °C on AQ-C18 (2.1 × 150 mm, 1.8 μm Welch), Gradient elution was performed using mobile phase A (0.1 % formic acid) and mobile phase B (methanol) at a flow rate of 0.30 mL/min. The injection volume and flow rate were 5 μL and 0.3 mL/min, respectively.

Q-Orbitrap-MS identification was carried out in positive ion mode (ESI+) with a scan range of *m/z* 100.0–1500.0. The other parameters are as follows: sheath gas flow rate 40 L/min, auxiliary gas flow rate 15 L/min, spray voltage 3.2 kV, ion transfer tube temperature 300 °C, The mass spectrometry mode was Full Mass-ddMS2 with the resolution of 70000, and the operation and acquisition of data were monitored by the Xcalibur workstation (Thermo Fisher Scientific, Germany).

4.2. Animals and animal treatment

Male C57 mice (age: 4–6 weeks) were purchased from the Experimental Animal Center of the Fourth Military Medical University. The mice were housed in cages under controlled temperature (24 °C ± 2 °C) and 12 h light/12 h dark cycles with free access to water and chow. All animal procedures were approved by the Laboratory Animal Center and Animal Care and Welfare Ethics Committee of the Fourth Military Medical University.

The mice were randomized into the blank control group, the high-fat control group, the high-fat drug group (drug administered: 26, 13, and 6.5 g/kg/day, respectively) and the Metformin positive drug group (n = 10). The blank control group and the high-fat control group were fed a normal diet and a high-fat diet, respectively (Research Diets, USA). After 8 weeks, 6.5, 13, and 26 g/kg herbal extracts were given to the high-fat drug group daily through gavage,250 mg/kg the metformin were given positive drug group(MET),while the high-fat control group was administered normal saline. Dietary patterns remained the same during that time. During the preliminary experiments, no significant difference was observed between the high-dose and middle-dose groups, whereas the therapeutic effect of the low-dose group was relatively weak. Therefore, after comprehensive consideration, the middle-dose group (13 g/kg) was selected for subsequent experiments.

After 12 weeks of dietary treatments, the mice were euthanized through cervical dislocation. Orbital blood was collected from the euthanized mice, and plasma was obtained through centrifugation at 3000 rpm/min for 10 min at room temperature. The intestine was dissected, measured, and divided into different portions (large intestine, small intestine, and colon). The liver and fat were also

harvested. The tissues obtained were rinsed with protease inhibitor-containing PBS, frozen in liquid nitrogen, and stored at −80 °C until further analysis.

4.3. Small intestine motor function experiment

This experiment investigated the improvement effect of the drug on the small intestine motor ability of the mice. The small intestine contractility under the same pressure and different drugs was measured through Power Lab polysomnography. Acetylcholine (ACH, Sigma, USA) excites the gut, whereas atropine (Sigma, USA) inhibits this effect. Based on the ability of the gut to respond to ACH and atropine, we evaluated the gut motor function. The small intestine was cut into several pieces and placed in a good physiological salt buffer. The intestinal segment with the best spontaneous peristalsis was selected and fixed on the pressure sensor. Then, 0.5 g pressure was applied, and the intestine was allowed to adapt to the buffer for some time. Atropine (10^{-3} mM) was added to the buffer. The maximum contraction of the mouse intestine was recorded and the buffer was replaced. When intestinal movements became stable, 10^{-4}mM ACH was added to the buffer. The waveform was allowed to stabilize, and the maximum diastolic value of the mouse intestine was determined. The old buffer was replaced with a new one to start with the next group of experiments.

4.4. Fecal microbiome transplantation

To eliminate as much bacteria as possible from in the gut of the SPF mice, penicillin (Sigma-Aldrich, 2000 U/mL) and streptomycin (Sigma-Aldrich, 2 mg/mL), which were typical spectrum antibiotics, dissolved in sterile distilled water in the final concentrations. The mixed solution was administered to the mice (500 μL/mouse) through oral gavage that lasted for 3 days. Feces were collected from the formula-treated mice fed a high-fat diet and stored at −80 °C until use. The feces were dissolved in 1.5 mL PBS, homogenized, and filtered through a 100-μm nylon mesh strainer. The filtered contents were resuspended with PBS in a total volume of 2 mL. Then, 200 μL of the suspension was administered to obese mice through oral gavage.

4.5. Glucose tolerance tests in mice

The mice that had to be treated were fasted the day before and could not stop drinking water. After 16 h of fasting, each mouse in each group was marked and removed from the cage. The fasting blood glucose level was measured using a glucometer (Sigma, USA). The measured value was considered the blood glucose level at 0 min. These steps were performed as carefully as possible to prevent excessive fright among the mice. The mice were left undisturbed for 30 min, and their weights were determined. Glucose was injected intraperitoneally at 0.01 mL/g, and the timing was started. Blood samples were collected at 15, 30, 60, 90, and 120 min to detect blood glucose levels. Feed was added to each cage at the end of the experiment. The experimental results were analyzed using the software.

4.6. Insulin tolerance tests in mice

The mice were fasted for 4 h, and each mouse in each group was marked and removed from the cage. Then, the fasting blood glucose levels of the mice were measured using the glucometer. The measured value was considered as the blood glucose level at 0 min.

Table 1
Detailed information of identified compounds by LC-MS.

No	ID	RT(min)	*m/z*([M+H]+)	Match COS	Match Name	Verification method	Classification
1	102	22.141	439.35684	99.9	Oleanolic acid	Compound database	triterpenes
2	319	19.697	295.13248	99.8	Tanshinone IIA	Compound database	anthraquinones
3	357	14.715	447.09238	99.8	Baicalin	Compound database	flavonoid
4	382	11.938	195.06525	99.8	Ferulic acid	Compound database	Organic acids
5	456	15.943	269.08047	99.7	Formononetin	Compound database	aglycone
6	609	7.712	353.08774	99.7	Chlorogenic acid	Compound database	Organic acids
7	625	9.314	153.01817	99.7	Gentisic acid	Compound database	phenol
8	657	10.018	159.06512	99.7	Pimelic acid	Compound database	Organic acids
9	786	23.059	271.05972	99.6	Baicalein	Compound database	flavonoid
10	1525	10.645	151.03889	99.2	Vanillin	Compound database	Others
11	1299	13.769	257.0809	99.3	Isoliquiritigenin	Compound database	flavone
12	2185	12.463	303.04999	98.1	Quercetin	Compound database	flavonol
13	2624	12.214	433.11307	97.6	Vitexin	Compound database	Flavone glucoside
14	2874	8.248	353.08728	96.9	Neochlorogenic acid	Compound database	triterpenes
15	2895	1.67	193.07082	96.8	D-(−)-Quinic acid	Compound database	Organic acids
16	742	5.774	129.01808	99.6	Citraconic acid	Compound database	Organic acids
17	967	13.488	187.09659	99.5	Azelaic acid	Compound database	Organic acids
18	3459	19.755	515.11951	94.8	4,5-Dicaffeoylquinic acid	Compound database	Organic acids
19	3260	13.255	271.06009	95.1	Emodin	Compound database	anthraquinone
20	3651	14.404	471.20108	94.1	Limonin	Compound database	triterpenoid
21	4008	7.266	159.09172	92.4	Indole	Compound database	benzpyrole
22	4363	1.44	365.10532	90.8	D-(+)-Maltose	Compound database	Others

The mice were undisturbed for 30 min, and their weights were determined. The mice were intraperitoneally injected with insulin at 0.01 mL/g. The timing was started. The blood glucose level of each mouse was measured at 15, 30, 45, and 60 min.

4.7. Quantitative PCR

For quantitative PCR, mouse tissues were separately homogenized, and RNA was extracted using the Trizol reagent (Tiangen Biotech, China) according to the manufacturer's protocol. Briefly, 1 mL Trizol reagent was added to 40 mg colonic tissues of each sample. To fully homogenize the sample, the colon tissue was homogenized in a mechanical homogenizer until no large tissue mass was left. The lithium chloride method was used to remove all polysaccharides. cDNA was synthesized using Reverse Transcriptase kits (Takara, Japan). Real-time PCR was performed using SYBR Green (Vazyme, China). Data were acquired using the ABI fluorescence quantitative PCR apparatus (7500, USA) and analyzed using the comparative Ct method. Table 1 lists the primers used. Target gene transcription of each sample was normalized to the respective levels of the β-actin gene.

4.8. Western blot analysis

A 1-cm section of the colon tissue was immediately frozen in liquid nitrogen, stored at −80 °C, and homogenized in cell extraction buffer (Thermo-Fisher Scientific, NSW, Australia) supplemented with 1 mM phenylmethanesulfonyl fluoride (Sigma, USA) and protease inhibitor cocktail (Sigma, USA). The total protein was obtained and quantified using the BCA method. The isolated proteins were separated through SDS-PAGE, transferred onto NC membranes, blocked with 5 % skim milk in Tris buffer saline-Tween 20 (TBST), and washed three times for 5 min each time. Then the membranes were incubated overnight at 4 °C with respective antibodies, washed with TBST three times for 15 min, and again incubated with HRP-conjugated secondary antibodies (1:10000) for 1 h at room temperature. The membranes were finally washed in TBST, and proteins were detected using the ECL chemiluminescence detection system (MIniChemi 610, China).

4.9. 16S rRNA gene amplicon sequencing

The bacterial 16S rRNA gene V4–V5 region was PCR amplified using the forward primer 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and the reverse primer 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The reaction mixtures for PCR contained 5 μL of Q5 reaction buffer (5 ×), 5 μL of Q5 High-Fidelity GC buffer (5 ×), 0.25 μL of Q5 High-Fidelity DNA Polymerase (5 U/μL), 2 μL (2.5 mM) of dNTPs, 1 μL (10 μM) of each primer, 2 μL of DNA

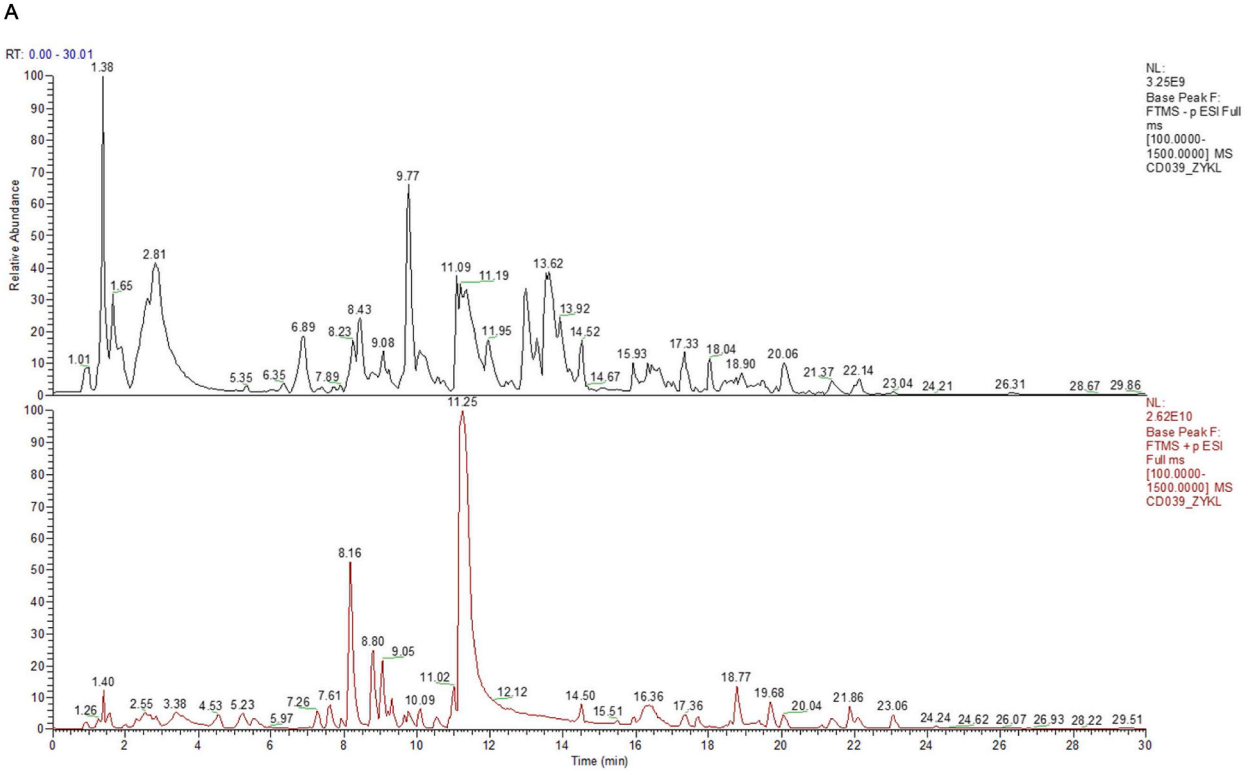


Fig. 1. The high-resolution extracted ion chromatogramsof TCM

template, and 8.75 μL of ddH_2O. Thermal cycling consisted of an initial denaturation at 98 °C for 2 min, followed by 25 cycles of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension of 5 min at 72 °C. PCR amplicons were purified using Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN, USA) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). Following the individual quantification, the amplicons were pooled in equal amounts. Paired-end 2 × 300 bp sequencing was performed using the Illlumina MiSeq platform with MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China)

4.10. Statistical analysis

Statistical and significance analyses were performed using one-way ANOVA. Statistically analyzed data were expressed as means ± SEM. Post-mortem testing was performed using GraphPad Prism software version 6.0 (GraphPad Software, USA). P values < 0.05 were considered statistically significant.

5. Results

1. Preparation and component analysis of TCM

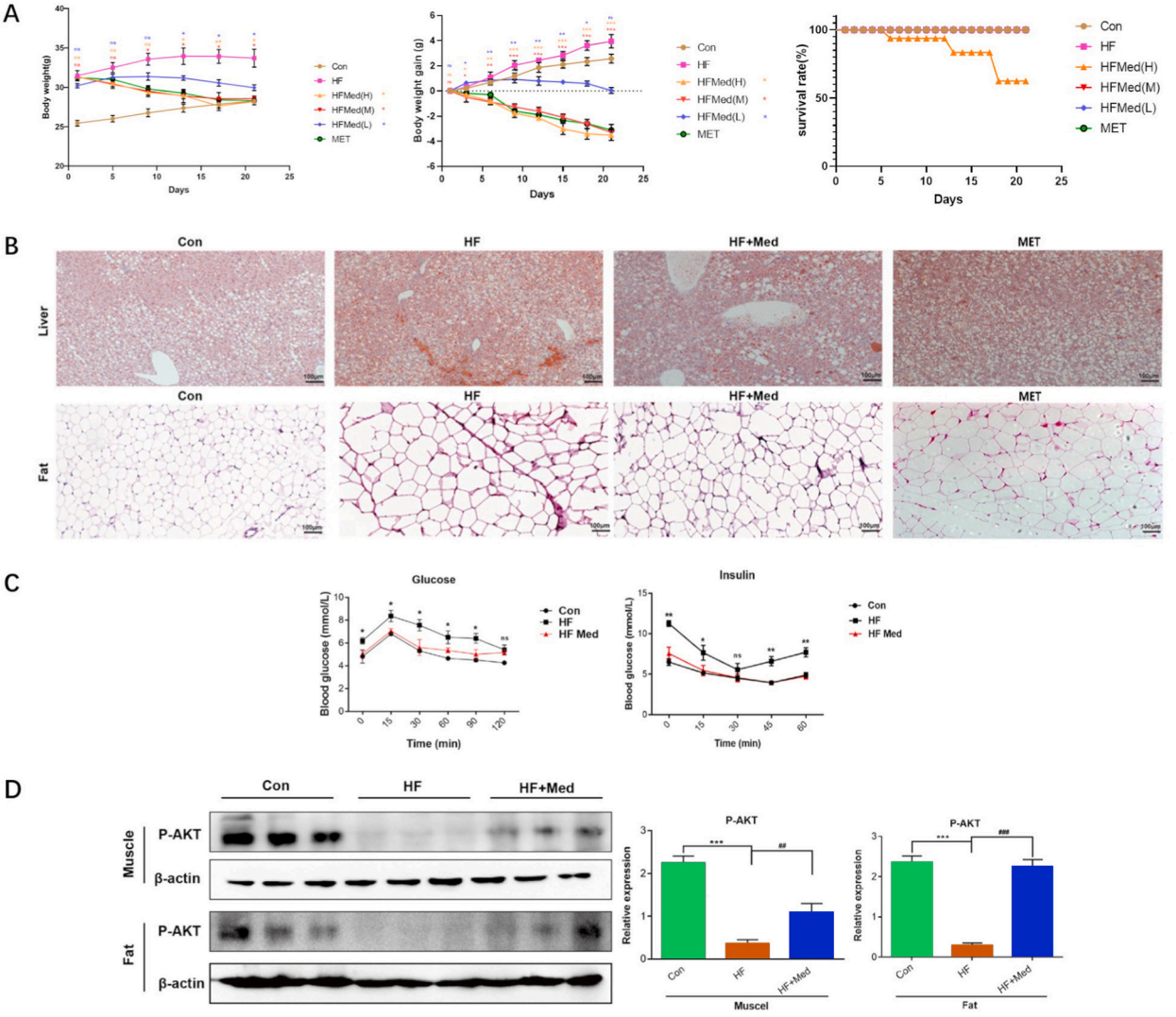


Fig. 2. TCM formula would relieve the obesity symptoms of mice. (A) The change in mice weight and weight increment in each group. (B) Mice liver and fat oil red staining. (C) Glucose tolerance tests (GTT) and insulin tolerance tests (ITT). (D) Western blotting to detect P-AKT of muscle and the fat protein levels in mice. The results are presented as the means ± SEM of 8 animals/treatment. The values with different superscripts were significantly different (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns > 0.05, one-way ANOVA test).

The composition of **TCM** was determined using UHPLC-Q-Orbitrap- MS in the positive and negative ion modes. The total ion current (TIC) chromatogram is shown in Fig. 1.Comparison with standard material and chemical information in the mass spectrometry library mz Cloud revealed that the active ingredients of **TCM** include Organic acids, flavonoids, triterpenoid, phenolic compounds, and others (supplementary S1-3).

2. TCM formula relieved obesity symptoms in mice

After 21 days of continuous treatment, the weights of the high-fat control group increased, whereas those of the high-fat administration group decreased significantly, (Fig. 2A). The liver lipid droplets in the administration group also decreased significantly compared with those in the control group, and the fat cells decreased (Fig. 2B). Insulin and glucose tolerance tests revealed that the Chinese herbal formula improved the high-fat diet-induced insulin resistance (Fig. 2C). Immunoblotting revealed that (Fig. 2D) *p*-Akt expression in the muscle and fat of the high-fat drug group was significantly higher than that in the muscle and fat of the high-fat drug control group ($p < 0.05$).

3. TCM reduced fat content by affecting the secretion of the gastrointestinal endocrine peptide

The formula improved the fat phenotype of the mice. Therefore, the levels of endocrine peptides were quantified. GLP-1 levels (Fig. 3A) in the small intestine and colon were higher in the drug group than in the high-fat control group. However, ghrelin levels did not differ significantly between the small intestine partial administration group and the high-fat control group. By contrast, ghrelin levels were lower in the colonic partial administration group than in the high-fat control group (Fig. 3B). Considering that appetite also affects obesity in mice, we detected PYY secretion in the small intestine and colon of the mice (Fig. 3C). PYY expression in the small intestine and colon was higher in the treated group than in the high-fat control group. CCK expression (Fig. 3D) in the small intestine and colon was consistent with PYY expression. The transcriptional levels of the endocrine peptides, except ghrelin, in the small intestine and colon were statistically significant among the groups. Finally, the serum expression level of GLP-1 was detected in the mice (Fig. 3E). Serum GLP-1 levels were higher in the treated group than in the high-fat control group ($p < 0.05$). The drug thus exerted a certain effect on endocrine peptide secretion in mice. The serum GLP-1 levels were higher in the administration group than in the high-

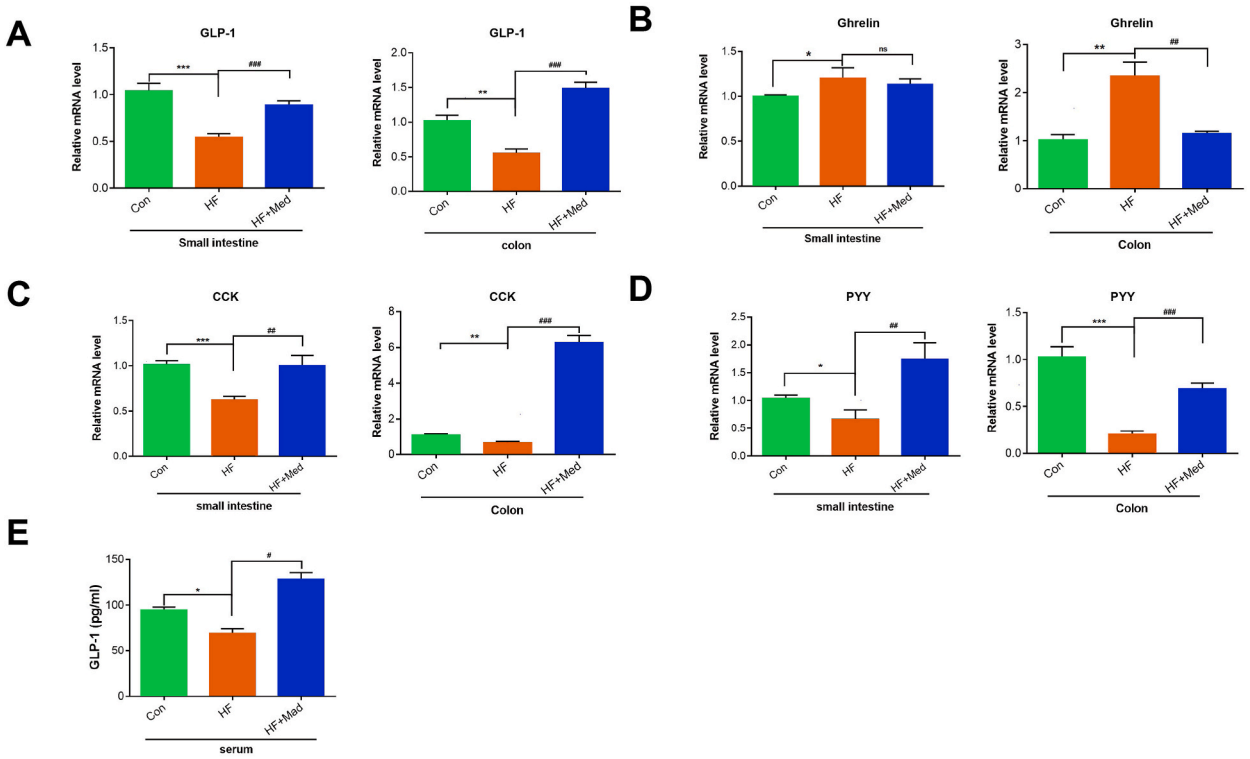


Fig. 3. Effect of Chinese medicine prescription on the secretion of intestinal endocrine peptide. The expression of GLP-1. (A) RT-PCR to detect the GLP-1 mRNA expression of the small intestine and colon of mice in each group. (B) RT-PCR to detect the ghrelin mRNA expression of the small intestine and colon of mice in each group. (C) RT-PCR to detect the CCK mRNA expression of the small intestine and colon of mice in each group. (D) RT-PCR to detect the PYY mRNA expression of the small intestine and colon of mice in each group. € ELISA method to detect the mice serum concentrations of GLP-1. The results are expressed as the means ± SEM (n = 8). Values with different superscripts were significantly different (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns > 0.05, one-way ANOVA test).

fat control group (Fig. 3e) ($p < 0.05$).

4. TCM inhibits intestinal inflammation and enhances the intestinal barrier function of mice

The inflammatory factor (IL-6) levels in the small intestine and colon were lower in the treated group than in the high-fat control group (Fig. 4A). Levels of the anti-inflammatory factor (IL-10) and antioxidant enzymes (SOD) in the small intestine and colon were higher in the drug group than in the high-fat control group (Fig. 4B and C). The results revealed that the TCM prescription played a certain role in inhibiting high-fat diet-induced intestinal inflammation. Obesity affects the intestinal barrier function of mice. However, after drug treatment, the intestinal barrier function recovered to a certain extent in the obese mice compared with the high-fat control mice (Fig. 4D). A high-fat environment increased inflammation in the mice, but after intragastric administration, serum IL-6 levels decreased, whereas serum IL-10 levels increased to varying degrees in the mice (Fig. 4E).

5. The TCM prescription enhanced the intestinal motor function of mice

5-HT secreted by the intestinal ECs was related to intestinal motility and inflammation. We thus detected the expression of the 5-HT ligand in the small intestine and colon of the mice. The high-fat environment decreased 5-HT2c secretion in the intestines of the mice. However, the 5-HT2c levels in the intestine were partially restored after the formula was administered (Fig. 5A). Serum 5-HT expression in the mice was consistent with the results of the ligand (Fig. 5B). As a rate-limiting enzyme involved in the 5-HT synthesis, tph secretion in the intestine was also detected. The results revealed that the high-fat environment could increase tph1 secretion and decrease tph2 secretion in the mouse intestine. This state was restored after administration (Fig. 5C and D). We then used the mouse small intestine to detect the intestinal motor function. The intestinal spontaneous peristalsis was weaker in the obese mice than in the drug-treated mice. After drug stimulation in vitro, the intestinal contractile and diastolic abilities of the drug-treated group were

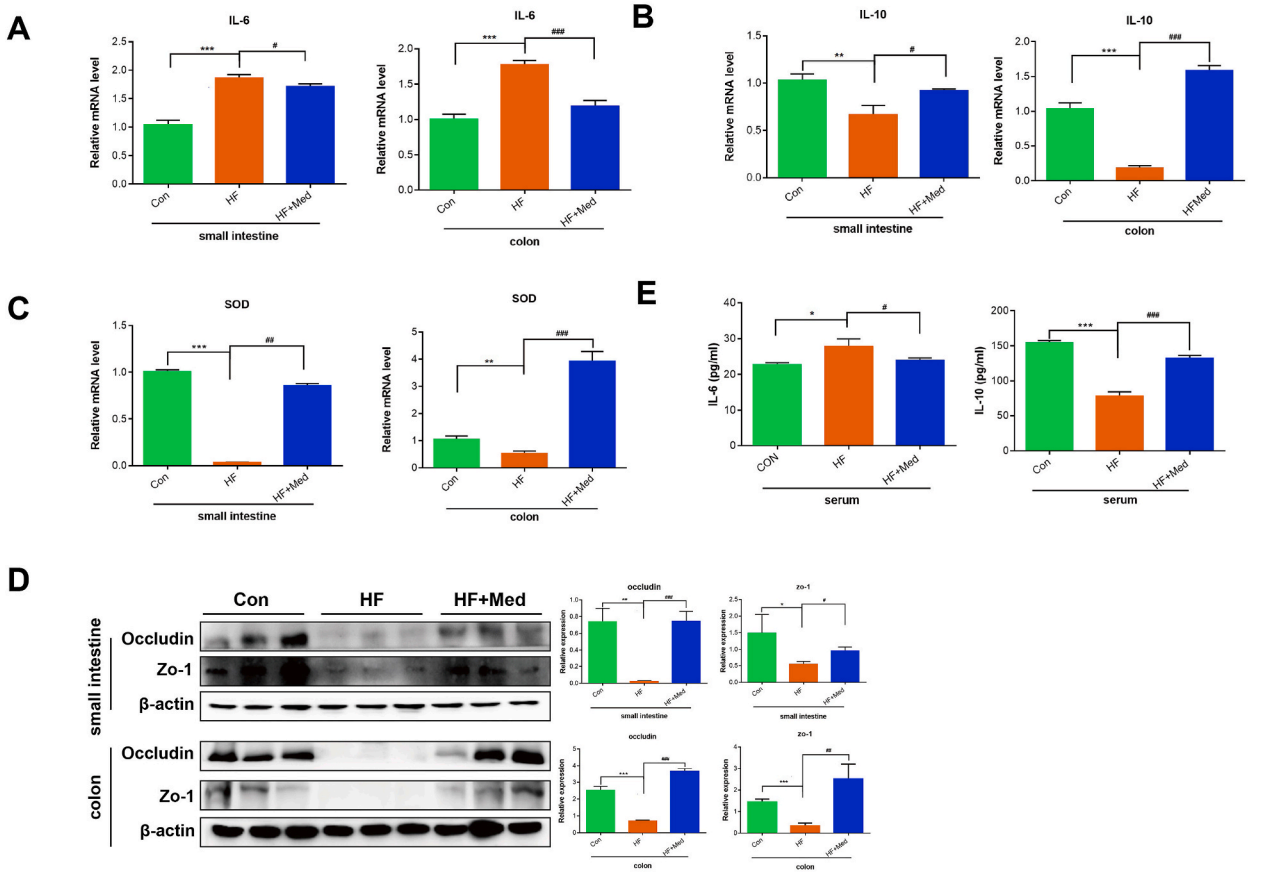


Fig. 4. Evaluation of the inflammation and intestinal barrier functions in mice. (A) RT-PCR to detect the IL-6 mRNA expression of the small intestine and colon of mice in each group. (B) RT-PCR to detect the IL-10 mRNA expression of the small intestine and colon of mice in each group. (C) RT-PCR to detect the SOD mRNA expression of the small intestine and colon of mice in each group. (D) Western blotting was used to detect and quantify the protein levels of occluding and zo-1 in the small intestine and colon of mice in each group. (E) ELISA method to detect different groups of mice serum IL-6 and IL-10. The results are expressed as the means ± SEM ($n = 8$). Values with different superscripts were significantly different (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns > 0.05, one-way ANOVA test).

stronger than those of the high-fat control group (Fig. 5E).

6. The TCM prescription affects the intestinal flora distribution and lipid metabolism in mice.

Early detection of the intestinal flora in groups of mice revealed that the high-fat environment changed the intestinal flora in the mice. The drug altered the microbial environment to that of normal mice in mice (Fig. 6A). Cluster analysis revealed an increase in the proportion of Desulfovibrionaceae in obese mice. This bacterium is associated with obesity [33], whereas the TCM formula inhibited its proliferation. The TCM formula also promoted rumen bacteria breeding in the obese mice so as to reduce the constipation rate in the mice [34] (Fig. 6B). The body weight of the obese mice in the flora recovery group was partially lower than that of the mice in the control group (Fig. 6C). Intestinal inflammation decreased to some extent, whereas the levels of anti-inflammatory factors increased to varying degrees. The expression of the intestinal tight junction protein was also partially upregulated in the obese mice (Fig. 6D). Compared with the control mice, intestinal GLP-1 expression in the obese mice was also upregulated to a certain extent (Fig. 6E).

6. Discussion

The global prevalence of obesity has been sustainably increasing over the past 40 years, from less than 1 % in 1975 to 6%–8% in 2016 [1].Many factors can cause obesity, such as genetic factors, environmental factors, abnormal endocrine regulation, inflammation, and changes in the intestinal flora [6]. Our study mainly discussed the effect of a TCM formula on insulin resistance in obese mice. This TCM prescription was an innovation of the traditional Chinese prescription "Zhibai Dihuang Tang." We here combined the characteristic drugs in the original prescription to observe the overall effect of the drug on mice.

A. capillaris, a member of the Asteraceae plant family, grows wild in Asia. Numerous studies have reported the antisteatotic, antioxidative, anti-inflammatory, choleretic, antiviral, antifibrotic, and antitumor functions of *A. capillaris*. *Astragalus propinquus* and *P. amurense* are widely used in TCM for treating viral and bacterial infections, inflammation, as well as cancer in in vitro experiments

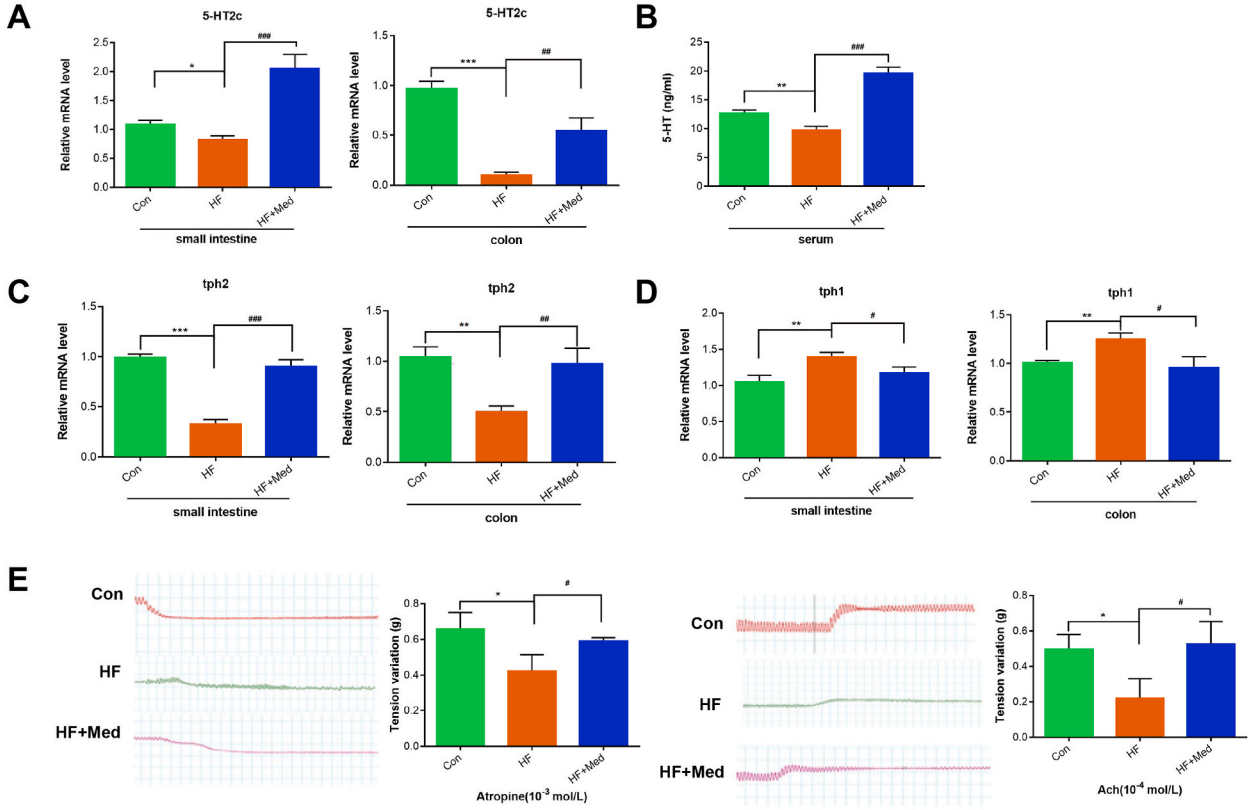


Fig. 5. Traditional Chinese medicine prescription would enhance the intestinal motor function of mice. (A) RT-PCR to detect the 5-TH2c mRNA expression of the small intestine and colon of mice in each group. (B) ELISA method was performed to detect the different groups of mice serum 5-TH levels. (C) RT-PCR to detect the thp-1 mRNA expression of the small intestine and colon of mice in each group. (D) RT-PCR to detect the thp-2 mRNA expression of the small intestine and colon of mice in each group. (E) The diastolic and contractile values of the small intestine of mice in each group were determined by Power Lab polysomnograph after Atrop treatment of 10-4 mm Ach and 10-3 mm under 0.5 g pressure. The results are expressed as the means ± SEM (n = 8). Values with different superscripts were significantly different (*p < 0.05, **p < 0.01, ***p < 0.001, ns > 0.05, one-way ANOVA test).

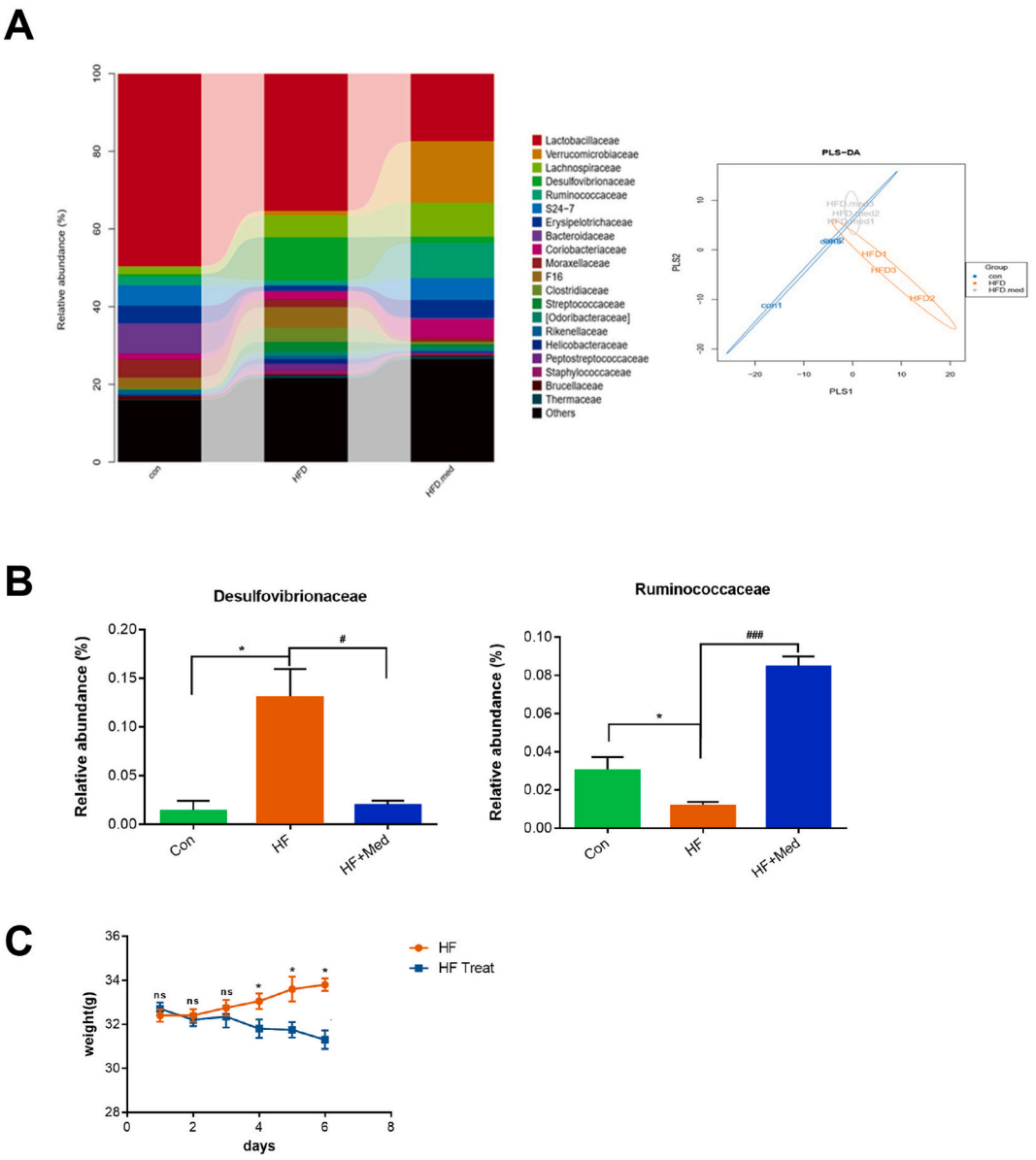


Fig. 6. Effects of drugs on the intestinal flora of mice. (A) In lavage for 21 days after the mice feces were collected for 16s RNA sequencing. (B) The statistical data of desulfovibrionaceae bacteria and rumen bacteria in the mice intestines. (C) High-fat feeding mice were treated with dung obtained from the TCM group to flora transplants and the weights were recorded for 7 days. (D) RT-PCR to detect the IL-6, IL-10, and occludin mRNA expression of the small intestine and colon of mice after treatment with flora transplants. (E) RT-PCR to detect the GLP-1 mRNA expression of the small intestine and colon of mice after treatment with flora transplant. The results are expressed as the means ± SEM (n = 8). Values with different superscripts were significantly different (*p < 0.05, **p < 0.01, ***p < 0.001, ns > 0.05, one-way ANOVA test).

[31,35,36]. In vivo experiments have revealed that *P. cocos* and its derivatives have anticancer, anti-inflammatory, antioxidative, antiviral, and other beneficial biological activities. *Anemarrhena* possesses anticancer, antinervous disorder, anti-inflammatory, anticoagulation, and other pharmacological activities [29,30]. Clinical trials have shown that *S. miltiorrhiza* can improve redox homeostasis and inhibit cell apoptosis and inflammatory responses [37]. In the previous experiment, the TCM prescriptions were found to exert an impact on the phenotype of the obese mice and had a certain impact on their intestinal tracts. Therefore, we conducted our study from this viewpoint. First, we established three doses, namely 6.5, 13, and 26 g/kg/day. However, after intragastric administration, the phenotypic effects of low doses on the experimental mice were weaker than those of the other doses. Meanwhile, there was little difference in weight loss between high-dose and medium-dose mice, but high-dose drugs had a certain impact on the survival rate of mice, and we suspected that high-dose drugs might have some toxicity. Finally, we selected the medium dose (13 g/kg/day) for our

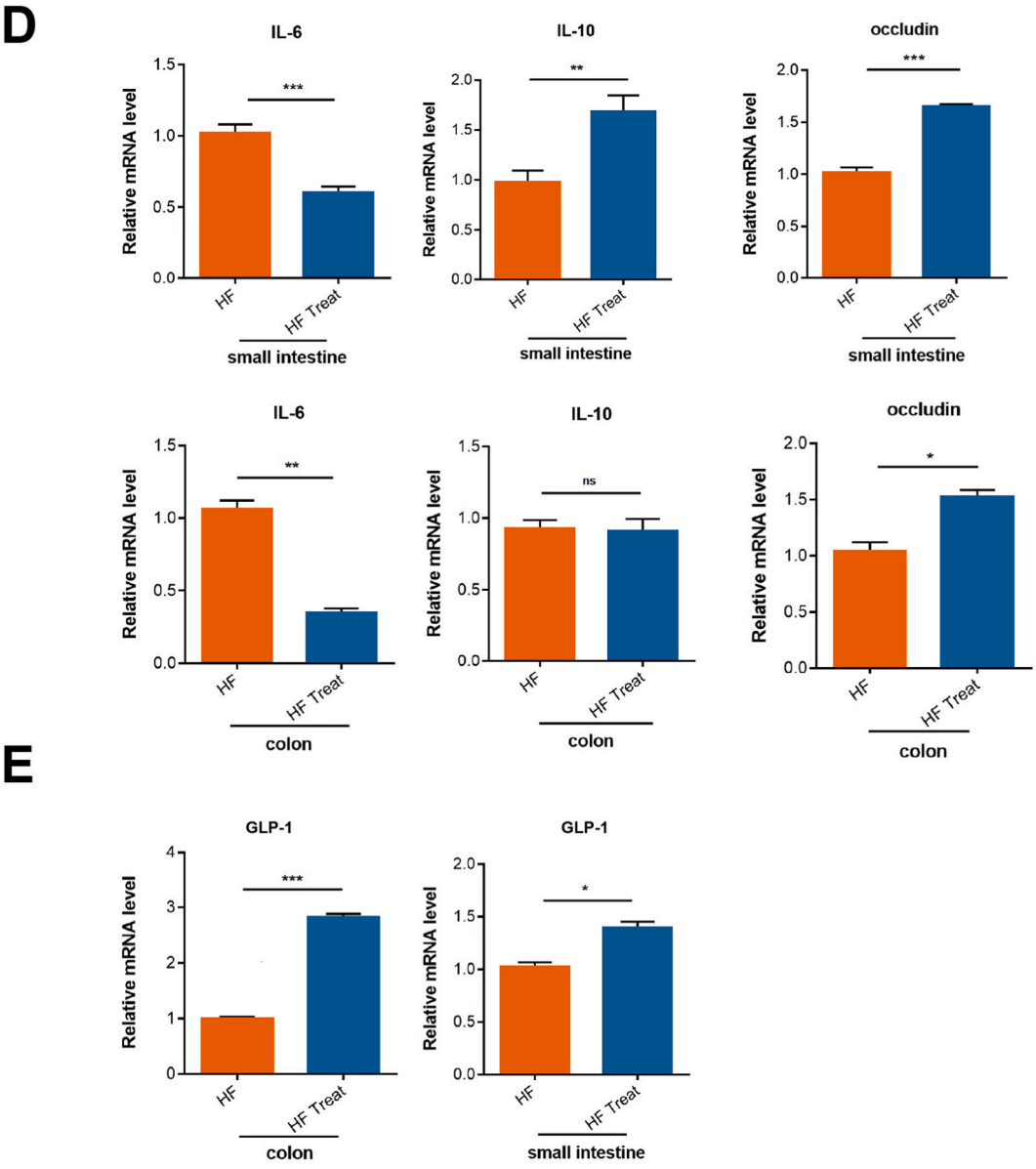


Fig. 6. (*continued*).

study (Fig. 2A). The gut is the main body organ responsible for energy intake. It communicates with the brain through hormones and neural pathways. With food intake, intestines secrete various peptides. Among them, PYY, CCK, and GLP-1 feedback to the NTS by stimulating the vagus nerve [7–9]. Herefore, the inflammatory response of mice can be observed through the feedback of intestinal peptides on energy metabolism and fat in mice.Obesity can cause intestinal barrier damage. At present, the treatment of obesity and its related diseases mostly focuses on drugs, and some scholars pay attention to intestinal flora. However, no relevant research has been conducted on the relationship between intestinal motor function and diseases. Some data show that obesity can slow down the transmission rate of stool, and many obese patients are also plagued by constipation. Therefore, our data may serve as a direction to provide new ideas for the treatment of diseases such as obesity. The TCM formula could reverse this damage. In this study, we also tested the active ingredients of the drugs, thus help in better understanding the potential mechanisms responsible for the clinical efficacy and safety of TCM.Many studies have shown that changes in the body affect intestinal flora distribution. Intestinal flora redistribution affected the distribution of the intestinal flora in the mice in turn. Therefore, we detected the intestinal flora distribution in mice at a later stage. The TCM formula affected the intestinal flora distribution in the mice. We then determined whether changes in the microflora and intestinal flora of the TCM prescription-fed mice could alleviate some of the damage caused by the high-fat environment. Our results revealed that the microflora and intestinal flora of the TCM prescription-fed mice recovered and had a certain effect on microflora redistribution in the hyperlipidemic mice. They also decreased the expression of inflammatory factors and

affected the phenotype of the mice. However, the overall effect was weaker in the control group than in the TCM-treated group. This may be due to the short time of bacteria transplantation. This experiment had a limitation. The sample size of 16s sequencing of mice was not very large, and we cannot therefore count the flora more accurately. In the future experiment, the clinical application of the TCM prescription and intestinal flora will be investigated in detail to determine the clinical therapeutic effect of the prescription and the application of the enriched dominant microflora as a microbial preparation for treating intestinal diseases.

7. Conclusion

This Chinese herbal formula has been illustrated to improve insulin resistance, reduce inflammation, improve intestinal motor function, and change the intestinal flora distribution in obese mice through the intestinal tract. This study provides a basis for clinical research on the treatment of obesity-induced constipation or insulin resistance.

Ethics approval and consent to participate

Every experiment in this article was performed in accordance with the protocols approved by the Fourth Military Medical University Committee on Animal Care. The ethic approval number is: IACUC-20191206.

Consent for publication

All authors gave final approval of version to be published.

Availability of data and materials

The [DATA TYPE] data used to support the findings of this study are available from the corresponding author upon request.

Funding

This study was funded by the grants from the Key R&D Program of Ningxia Province, the Medical Enhancement Program, Incubation fund of Shaanxi Provincial People's Hospital, Ningxia Hui Autonomous Region key research and development project and Ningxia Yinchuan City science and technology innovation leading talent training (No.2021BEG03037, 2020SWAQ06, 2023YJY-15, 2022BEG02035 and 2022LJRC13).

CRediT authorship contribution statement

Lirong Ma: Writing – review & editing, Data curation. **Yongquan Bai:** Formal analysis, Conceptualization. **Jun Liu:** Formal analysis, Data curation. **Kaimin Gong:** Software, Methodology. **Qirui He:** Methodology, Formal analysis. **Jintao Zhao:** Data curation. **Yina Suo:** Supervision. **Wenwen Wang:** Project administration, Methodology, Formal analysis. **Guo Chen:** Supervision, Software, Conceptualization. **Zifan Lu:** Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

I would like to express my gratitude to all those who have helped me during the writing of this thesis. I gratefully acknowledge the help of my supervisor Professor Z.F.L and G.C. I do appreciate her patience, encouragement, and professional instructions during my thesis writing. Thanks for the platform support provided by the Bio-pharmaceutical Teaching and Research Office of The Air Force Military Medical University, China.

Acronym

TCM	traditional Chinese medicine
T2DM	type 2 diabetes
CCK	cholecystokinin
GLP-1	glucagon-like peptide-1
PYY	peptide YY
POMC	proopiomelanocortin
MET	metformin
NPY	neuropeptide Y

TNS	nucleus of solitary tract
AKT	protein kinase B
p-AKT	phosphorylation-AKT
ZO	zonula occludens
IL-6	interleukin-6
IL-10	interleukin-10
IL-7α	interleukin-7α
SOD	Superoxide Dismutase
5-HT	5-hydroxytryptamine
TPH	tryptophan hydroxylase
ACH	acetylcholine
GTT	glucose tolerance tests
ITT	insulin tolerance tests
HE	hematoxylin-eosin staining
NTS	nucleus solitone

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30379.

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