

Research Article

Laxative Effect of Polysaccharides From *Dendrobium officinale* on Slow Transit Constipation: Regulation Effect of Intestinal Microbiota and 5-HT System

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Background: *Dendrobium officinale* (DOF), a highly valued traditional Chinese medicinal herb and functional food, boasts a millennia-long history of medicinal use. Our previous research has demonstrated its efficacy in alleviating acute constipation, primarily attributed to its polysaccharide (DOP). Nevertheless, the therapeutic potential of DOP in addressing slow transit constipation (STC) and its underlying mechanisms remain unexplored. This research seeks to elucidate the protective effects and underlying mechanistic pathways of DOP in loperamide-induced STC.

Methods: KM mice were administered loperamide to establish the STC model, followed by DOP intervention. After treatment, gastrointestinal motility, fecal output, and key gastrointestinal regulatory hormones were all detected. The 5-hydroxytryptamine (5-HT) pathway was further analyzed by analyzing enterochromaffin cells (ECs), serotonin transport (SERT), and 5-HT receptors (5-HTRs). Additionally, 16S rDNA sequencing was performed to evaluate gut microbiota composition. Changes in levels of short-chain fatty acids (SCFAs) and their receptor were also quantified.

Results: DOP significantly ameliorated defecation dysfunction in STC mice, enhancing gastrointestinal motility and increasing 24-h fecal output. Additionally, DOP treatment restored the colonic levels of key gastrointestinal hormones, especially in 5-HT concentration. This effect may be attributed to the expansion of ECs and suppression of SERT expression. Moreover, DOP selectively upregulated 5-HT₄R expression without affecting 5-HT₃R. Beyond neurotransmitter regulation, DOP modulated gut microbiota composition by increasing microbial diversity and altering the community structure, particularly through shifts in the Bacteroidetes/Firmicutes ratio. Furthermore, DOP elevated SCFA levels and activated their downstream receptor G Protein-Coupled Receptor 43 (GPR43), which may contribute to the observed 5-HT system modulation.

Conclusions: DOP demonstrates significant efficacy in ameliorating constipation symptoms in STC mice through the dual modulation of the 5-HT system and gut microbiota composition. These results furnish robust scientific support, substantiating the therapeutic potential of DOP in the daily health care or management of STC.

Keywords: 5-HT; *Dendrobium officinale* polysaccharides; intestinal microbiota; SCFAs; slow transit constipation

1. Introduction

Slow transit constipation (STC), a multifactorial gastrointestinal (GI) condition, originates from dysfunctional intestinal motility, neuropathic dysfunction, or microbial dysbiosis [1, 2]. Clinically, it presents with infrequent bowel movements, straining during defecation, and abdominal distension, significantly compromising patients' quality of life [3]. Therapeutic strategies focus on symptom alleviation and motility restoration. Current pharmacologic interventions predominantly rely on laxatives (such as lactulose) and prokinetics (such as mosapride and cisapride) [4]. However, these conventional treatments often yield suboptimal efficacy and carry risks of adverse reactions [5], which underscored the demand for safer and more effective alternatives.

An imbalanced intestinal microbiota has been identified as a potential contributor to STC pathogenesis [6]. Probiotic supplementation presents a viable therapeutic approach through normalizing microbial balance, modulating luminal osmolarity, and optimizing fecal water content, which in turn enhances intestinal peristalsis and facilitates bowel movements [7]. Additionally, probiotics exert regulatory effects on gut function through the synthesis of short-chain fatty acids (SCFAs) [8]. Consequently, the targeted regulation of the gut microbiome represents a viable therapeutic approach on controlling and ameliorating STC manifestation. 5-Hydroxytryptamine (5-HT) acts as a vital neurotransmitter and paracrine signaling factor [9]. 5-HT predominantly localized in the digestive tract and exerts its regulatory effects through seven subtypes [10]. Among them, 5-HT₃R and 5-HT₄R exert critical effects on intestinal motility. Dysregulation of these receptors' secretion and signaling has been implicated in various GI pathologies [11]. Notably, diminished 5-HT₄R expression directly impairs intestinal peristalsis, representing a key pathophysiological mechanism underlying STC [12]. Growing evidence suggests that dysregulation in neuroendocrine regulation, notably involving 5-HT, substance P (SP), and vasoactive intestinal peptide (VIP), represents a core pathological hallmark of STC [13–15]. Consequently, therapeutic strategies targeting 5-HT signaling pathways and receptor expression may offer promising interventions for STC management.

Dendrobium officinale (DOF), a famous Chinese herbal medicine with a long-standing history of therapeutic application [16, 17], contains valuable bioactive components including polysaccharides, alkaloids, dietary fiber, and essential vitamins. This functional food is commonly prepared as soups or fresh juices, offering both GI benefits and immune-enhancing properties [18, 19]. Among its active constituents, DOF polysaccharides (DOP) demonstrate significant pharmacological potential, exhibiting anti-inflammatory [20], immunoregulation [18, 21], anticollitis [22], and antitumor effects [23]. Furthermore, research has demonstrated that DOP promotes SCFAs' generation while regulating the ratio of Firmicutes to Bacteroidetes [24]. Our prior findings revealed DOP's constipation-alleviating effects through improved colonic motility, increased fecal hydration, and gut hormone regulation [25], suggesting

potential laxative properties in STC models. However, DOP's therapeutic mechanisms concerning 5-HT deficiency and microbiota dysbiosis in STC remain unexplored. This study therefore investigates DOP's regulatory effects on 5-HT signaling and microbial homeostasis in STC pathogenesis.

2. Materials and Methods

2.1. Chemicals and Reagents. DOF was taxonomically confirmed by Prof. Zhenbiao Zhang, a principal investigator at the Guangdong Academy of Agricultural Sciences (Guangzhou, China). India ink, loperamide, mosapride, and lactulose were the products of Guangzhou JiaYan Biological Technology Co., Ltd. (Guangzhou, China). ELISA kits designed for the detection of SP, VIP, and 5-HT were purchased from the Beijing Chenglin Biotechnology Technology Co., Ltd. (Beijing, China). Other chemicals and solvents were analytical reagents. Primary antibodies including Chromogranin A (CGA), SERT, G Protein-Coupled Receptor 43 (GPR43), 5-HT₃R, and 5-HT₄R and were purchased from Proteintech Group, Inc. (Wuhan, China).

2.2. Extraction, Purity Evaluation, and Molecular Weight Characterization of DOP. DOP were extracted and purified following our previously optimized protocol. Subsequently, purity level and molecular weight characteristics of the purified DOP were quantified via an HPLC/HPGPC system. All analytical methods were developed in accordance with standardized protocols reported in the prior literature [25].

2.3. Animal. Male KM mice (22–25 g, 7–8 weeks) were obtained from ZhuHai Bestest Biotechnology Co., Ltd. (Zhuhai, China) and subsequently maintained under specific pathogen-free conditions, with temperature controlled at $22 \pm 2^\circ\text{C}$, relative humidity maintained at 60%–70%, and a 12-h light–dark cycle [27]. All experimental protocols accorded to the National Institutes of Health guidelines (Publication No. 8023) and the Institutional Animal Care and Use Committee of the Tea Research Institute, Guangdong Academy of Agricultural Sciences (Certificate Number Agricultural Science No. 019).

2.4. Experimental Design. Eighty-four mice were randomly divided into seven groups, with 12 mice in each group: intact, vehicle, mosapride, lactulose, and three DOP dosage groups. The entire experimental period spanned 14 days. Mice in the intact control group received daily oral gavage with normal saline (0.1 mL/10 g/day) from Day 1 to Day 14, whereas all other groups were orally administered loperamide (5 mg/kg/day) during the same timeframe to induce constipation. Additionally, all groups received their respective treatments concurrently from Days 1 to 14: intact control, oral gavage with saline at 0.1 mL/10 g/day; vehicle control, oral gavage with saline at 0.1 mL/10 g/day; mosapride-treated, oral gavage with mosapride at 3 mg/kg/day; lactulose-treated, oral gavage with lactulose at 0.25 mL/

day; low-dose DOP, oral gavage with DOP at 12.5 mg/kg/day; medium-dose DOP, oral gavage with DOP at 25 mg/kg/day; and high-dose DOP, oral gavage with DOP at 50 mg/kg/day. The dosages of DOP were established based on our preliminary experimental data.

2.5. Measurement of GI Transit Ratio and Fecal Weight.

On Day 14 post-treatment, GI motility was evaluated in six randomly selected mice per group using an India ink tracer method. Following a 30-min fasting period, animals received 5% India ink suspension (0.1 mL/10 g) via oral gavage. After precisely 30 min of tracer administration, mice were humanely euthanized by cervical dislocation following deep anesthesia with an intraperitoneal injection of sodium pentobarbital (150 mg/kg). Death was confirmed by the cessation of vital signs. After that, the entire intestinal tract (pylorus to anus) was immediately excised and carefully straightened on a chilled surface. Two critical measurements were obtained: total intestinal length and India ink migration distance. The GI motility was calculated as

$$\text{GI transit rate (\%)} = \frac{\text{Migration distance of India ink}}{\text{Entire intestinal length}} * 100\%. \quad (1)$$

Following GI transit assessment, the remaining six mice per group were individually housed in metabolic cages for 24-h fecal collection. Total wet fecal weight was measured as a primary outcome parameter for evaluating intestinal motility. After the observation period, mice were humanely euthanized, and the colonic tissue was immediately dissected, frozen in liquid nitrogen, and subsequently preserved at -80°C for later biochemical assays, molecular investigations, and histopathological examinations.

2.6. Measurement of GI Hormones. In the present study, the quantitative assessment of 5-HT, VIP, and SP expression levels was conducted in colorectal tissue using commercially available the ELISA kits. Briefly, the colon tissue underwent initial rinsing with ice-cold saline to eliminate the remaining blood component, followed by homogenization in PBS. The resulting tissue lysates were centrifuged (10,000 rpm, 10 min, 4°C), followed by careful collection of the supernatant fraction. The protein concentrations of supernatant fraction were first quantified by the bicinchoninic acid (BCA) assay kit (supplied by Solarbio, China). To standardize sample inputs for ELISA, all samples were adjusted to equivalent protein concentrations based on the BCA results. Subsequently, ELISA detection was conducted strictly in accordance with the manufacturer's instructions (Shanghai KALANG, China). Briefly, the standardized supernatant fraction sample was added to precoated and blocked microplate wells, followed by incubation to facilitate target analyte binding to the immobilized capture reagents. Unbound components were then removed through sequential washing steps. An enzyme-labeled detection antibody was introduced, and the plate was incubated to form a "capture-analyte-detection" complex. After repeated washing to eliminate the unbound detection

antibody, a substrate specific to the enzyme was added to generate a quantifiable signal (colorimetric or fluorescent). The signal intensity was measured using a microplate reader, and analyte concentrations in the original samples were determined by comparing the obtained signals to a standard curve generated with known analyte concentrations.

2.7. Histological Analysis. Colonic tissues underwent fixation with 4% paraformaldehyde, followed by paraffin embedding, then sectioning at a thickness of 5 μm , and hematoxylin-eosin (H&E) staining using a standard protocol with minor adjustments. The H&E-stained colon sections were scanned using Digital Pathology Slide Scanner (JF-KF-400, Konfoong Bioinformation Tech. Co., Ltd, China), and pathological changes in colorectal tissues were captured via KFSlideOS software at 10 $\times$ and 20 $\times$ magnification.

2.8. Measurement of Enterochromaffin Cells (ECs), SERT, and GPR43 Expression. ECs, serotonin transporter (SERT), and Free Fatty Acid Receptor 4 (GPR43) expression were assessed via immunohistochemistry (IHC). Colonic tissues were resected and then immersed in 4% paraformaldehyde at 25°C for 24 h prior to paraffin embedding. After embedding, tissue sections were processed through xylene for dewaxing, followed by antigen retrieval using sodium citrate buffer (0.01 M, pH 6.0) under microwave irradiation, and finally incubated with 5% bovine serum albumin (BSA) in phosphate-buffered saline for 1 h. Primary antibodies against CGA (Proteintech, 10529-1-AP, 1:50), SERT (Proteintech, 19559-1-AP, 1:100), and GPR43 (Proteintech, 19952-1-AP, 1:100) were applied overnight at 4°C . Following washing steps, horseradish peroxidase (HRP)-labeled secondary antibodies underwent incubation at ambient temperature for 1 h. Immunoreactivity was visualized using diaminobenzidine (DAB), followed by hematoxylin counterstaining. Subsequently, the slides were subjected to dehydration, clearing, and mounting procedures. Microscopic images were acquired by a Nikon E100 microscope (Nikon Corporation, Tokyo, Japan) and quantified with ImageJ software (National Institutes of Health, USA).

2.9. Measurement of 5-HT Receptors (5-HTRs). Cryopreserved colonic samples were subjected to homogenization in radioimmunoprecipitation assay (RIPA) lysis buffer. The protein concentration levels were quantified via the BCA assay kit, adhering to the manufacturer's recommended protocol. The samples were subjected to boiling with a loading buffer over a 6-min period, followed by separation via SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrophoretic transfer to Millipore membranes (0.25 μm pore size). Subsequently, the membranes were blocking at room temperature for 1 h using either nonfat dry milk (5%) or BSA (5%) prepared in TBST. Following TBST washing, the membranes were incubated overnight at 4°C with respective primary antibodies. The primary antibodies employed included 5-HT₃R (Proteintech, 1:1000) and 5-HT₄R (Proteintech, 1:1000). Following three rounds of TBST washing, the membranes were

incubated with secondary antibodies (Proteintech, 1:5000 dilution) in TBST at 25°C for 1 h. Subsequently, the membranes were subjected to three additional 5-min washes with TBST, after which protein bands were visualized through chemiluminescent detection using an ECL substrate. Band density analysis was performed via ImageJ software. GAPDH were served as the loading control.

2.10. Measurement of SCFAs in Feces. Fecal samples were subjected to preparatory processing for SCFA analysis via spiking with 100 μ L of aqueous phosphoric acid solution (20%) and 500 μ L of 4-methyl valeric acid (50 μ g/mL) serving as an internal standard. The mixture was homogenized followed by centrifugation to facilitate supernatant collection. Calibration standards were formulated to comprise acetic (ethanoic), propionic, butyric, valeric, isobutyric, and isovaleric acids, which were prepared alongside the samples for quantitative analysis. GC-MS analysis was performed utilizing an Agilent 5977BGC/MSD system equipped with an Agilent DB-FFAP capillary column (dimensions: 30 m $\times$ 250 μ m $\times$ 0.25 μ m). A 1- μ L aliquot of the supernatant was injected at a 10:1 split ratio, with helium acting as the carrier gas flowing at 1.0 mL/min. The injector temperature was set to 90°C, while the column temperature was maintained isothermally at 240°C during the entire analytical run. Data processing was performed using Agilent MSD ChemStation software. SCFA concentrations were quantified by constructing standard curves via the external standard method.

2.11. 16S rDNA Gene Sequencing and Intestinal Microbiota. Fecal specimens were aliquoted into cryogenic tubes and frozen at -80°C to enable the subsequent microbiota profiling. Genomic DNA was isolated from these collected fecal samples using a commercial Stool DNA Kit, with procedures strictly according to the manufacturer's recommended protocols. The concentration and purity of the purified DNA were determined via optical density measurements at the A260/A280 ratio, measured using a spectrophotometer. DNA samples that met predefined quality criteria were subsequently utilized for the construction of a gene library. Following this, the qualified library was sequenced on the Illumina MiSeq Sequencing Platform (Illumina, San Diego, USA). Additionally, bioinformatics analysis was performed on the sequencing data. Using USEARCH (Version 7.0.1090), tags displaying $\geq 97\%$ sequence similarity were clustered into operational taxonomic units (OTUs) and the relative abundances of these OTUs were analyzed from the phylum to genus levels. Leveraging the relative abundances of known OTUs within sequenced data, anticipated values of individual α indices during tag extraction were computed. Subsequently, rarefaction curves were generated using observed values and their paired expected α index values. The linear discriminant analysis effect size (LEfSe) methodology was subsequently employed to identify pivotal microbial taxa.

2.12. Statistical Analysis. Statistical analysis was performed using SPSS 23.0 software (SPSS Inc., Chicago, USA), with numerical results expressed as mean $\pm$ standard deviation

(SD). The normality of the data distribution was assessed at the first time. For data that violated the normality assumption, nonparametric tests were employed. For data satisfying the normality criterion, pairwise comparisons were conducted via one-way analysis of variance (one-way ANOVA) followed by the least significant difference (LSD) posthoc test. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Molecular Weight and Purity of DOP. As shown in Figure 1, HPLC/HPGPC analysis demonstrated that the molecular weight of DOP was determined to be 8218 Da, with a purity of 97.82%.

3.2. DOP Mitigated the Defecation Status of STC Mice. The GI transit ratio in the vehicle group exhibited a marked decrease relative to the intact group (Figure 2(a)), confirming successful induction of the STC model by loperamide. Both positive control drugs (mosapride and lactulose) effectively increased the GI transit ratio. Notably, high-dose DOP treatment ($p < 0.01$) demonstrated the most pronounced effect on improving GI motility. As shown in Figure 2(b), STC mice exhibited significantly decreased fecal weight compared to intact controls ($p < 0.01$). Administration of DOP and mosapride all significantly increased fecal weight (all $p < 0.01$). These findings demonstrate that DOP administration potentially enhances defecation and ameliorates the constipation-related phenotype in STC model mice.

3.3. DOP Alleviated Colonic Injury. H&E-stained colon sections from the intact group are presented in Figure 3(a). The colon tissue structure is normal, the mucosal crypts are closely arranged (orange arrows), the goblet cells are abundant (green arrows), and the epithelial cells (black arrows) are not denatured and exfoliated. However, In the vehicle group (Figure 3(b)), the colonic structure exhibited significant disruption, characterized by markedly reduced or rarely observed crypts, sparse goblet cells, epithelial cell shedding, and exposure of the lamina propria. Treated with mosapride, lactulose, and DOP (shown in Figures 3(c), 3(d), and 3(e)), colonic injury was significantly ameliorated. The colonic mucosal cells in these groups remained intact, accompanied by abundant goblet cells and tightly arranged crypts. Pathological findings revealed that DOP significantly improved the pathological changes in mice with chronic constipation.

3.4. DOP Regulated the GI Hormones. GI hormones including VIP, SP, and 5-HT play critical roles in STC pathophysiology. Measurement of these hormones provides valuable insights into DOP's regulatory mechanisms in STC. As shown in Figures 4(a) and 4(b), levels of both SP and VIP were significantly reduced in the vehicle group relative to the intact control group ($p < 0.01$). Notably, DOP treatment (25 and 50 mg/kg) effectively restored these hormone levels (both $p < 0.05$), with the higher dose showing particularly pronounced effects. Figure 4(c) illustrates that DOP

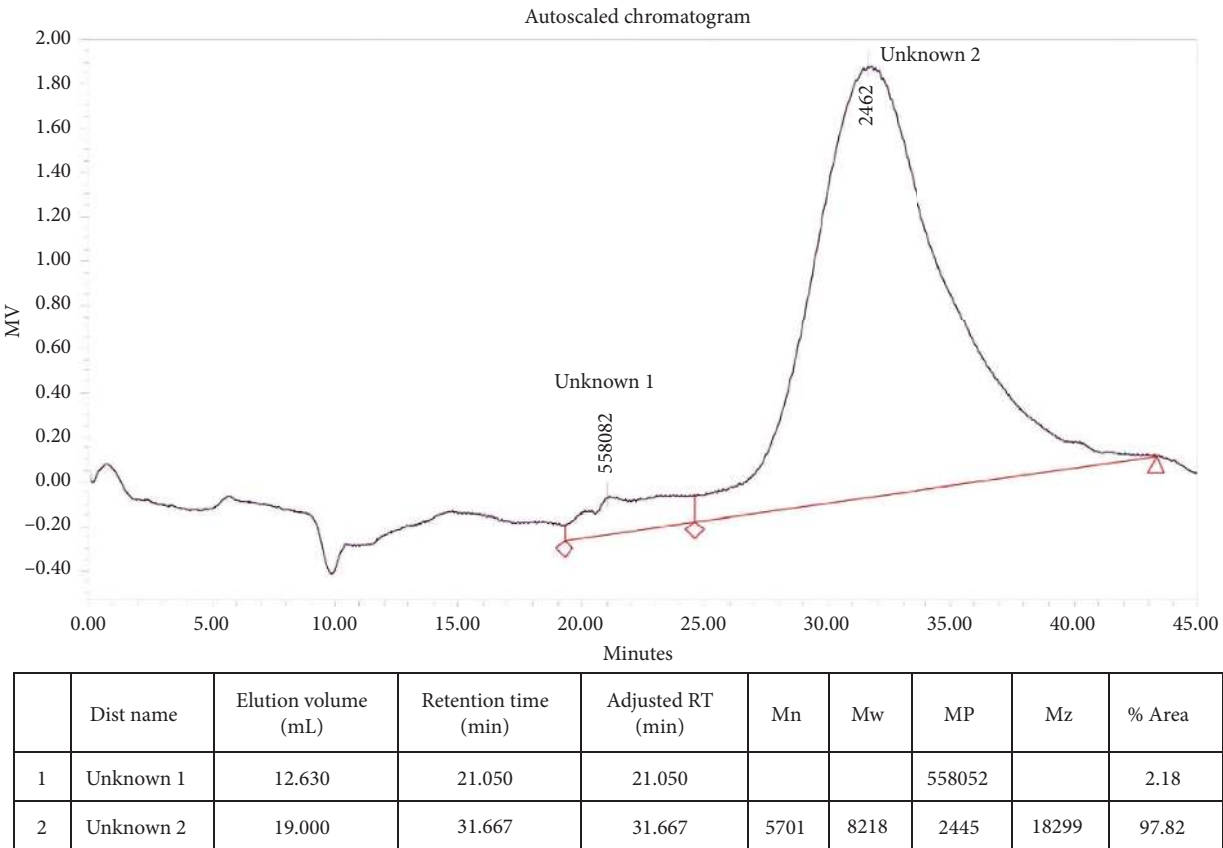


FIGURE 1: HPLC/HPGPC analysis results of DOP.

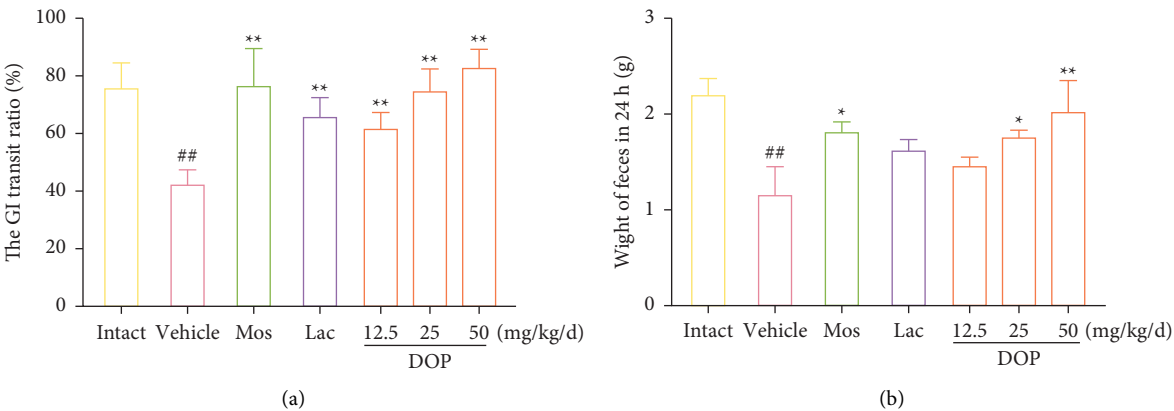


FIGURE 2: DOP mitigated the defecation status of STC mice. (a) GI transit ratio. (b) Weight of feces in 24 h. Data are expressed as mean $\pm$ SD ($n = 6$). ## $p < 0.01$ vs. intact group; * $p < 0.05$, ** $p < 0.01$ vs. vehicle group.

administration induced a significant elevation in 5-HT levels in STC mice versus the vehicle group ($p < 0.01$). These findings collectively indicate that DOP exerts a modulatory effect on key GI hormones involved in STC regulation.

3.5. DOP Upregulated the 5-HT System. To comprehensively assess the effects of DOP on the 5-HT system, we examined both its biosynthesis/inactivation pathways and receptor-mediated activation. Specifically, we assessed ECs counts (a marker of 5-HT biosynthesis) and the expression of SERT, while 5-HTR

expression (5-HT₃R and 5-HT₄R) reflected receptor-mediated activation. Figure 5 illustrates that DOP administration significantly elevated the count of ECs, suggesting enhanced 5-HT biosynthesis. Concurrently, DOP markedly reduced SERT expression (Figure 6), which would be expected to decrease 5-HT reuptake. These combined effects resulted in elevated 5-HT levels (Figure 2(c)). Regarding receptor expression (Figure 7), DOP selectively enhanced 5-HT₄R expression while having no significant effect on 5-HT₃R, indicating a specific modulation of 5-HT receptor signaling pathways.

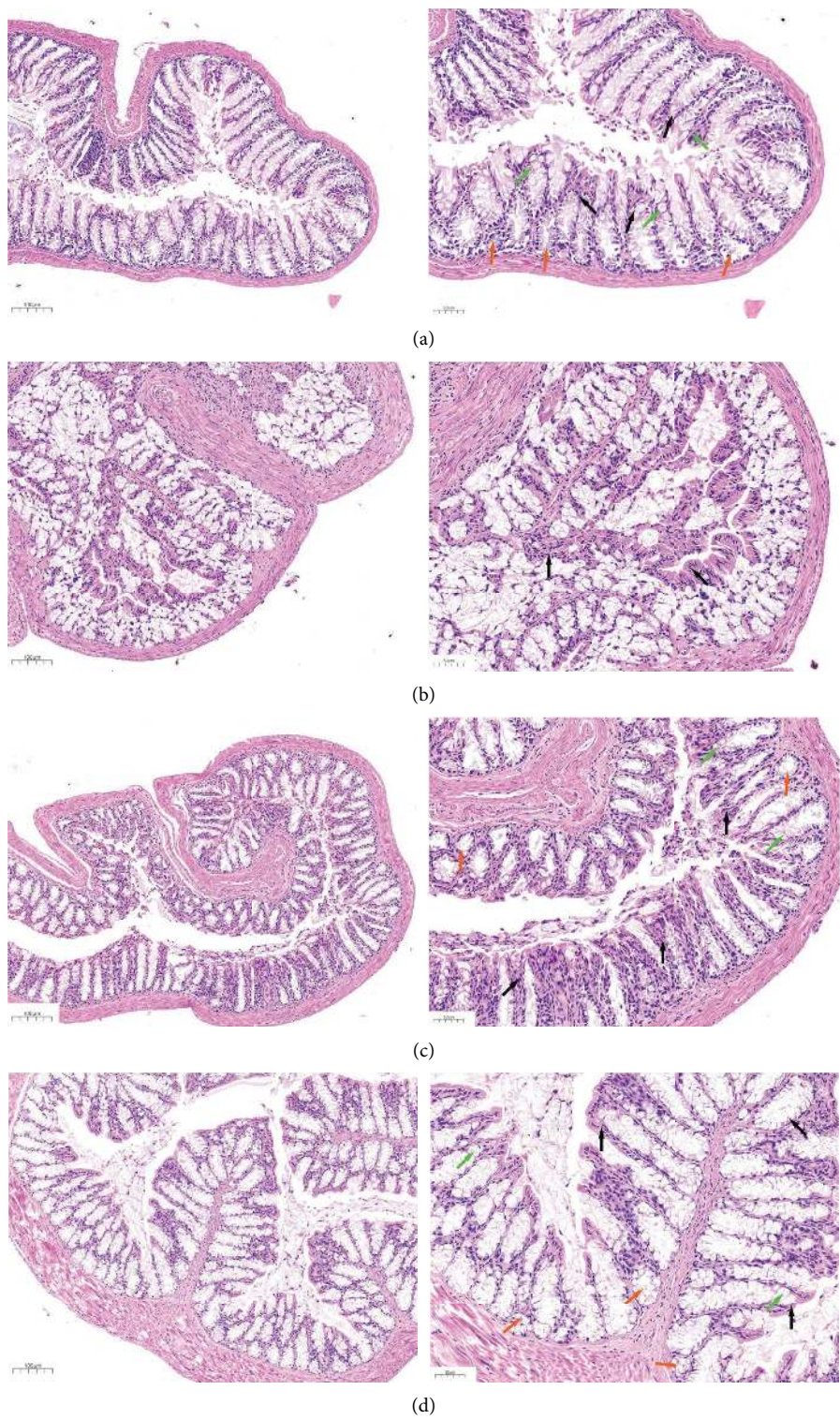


FIGURE 3: Continued.

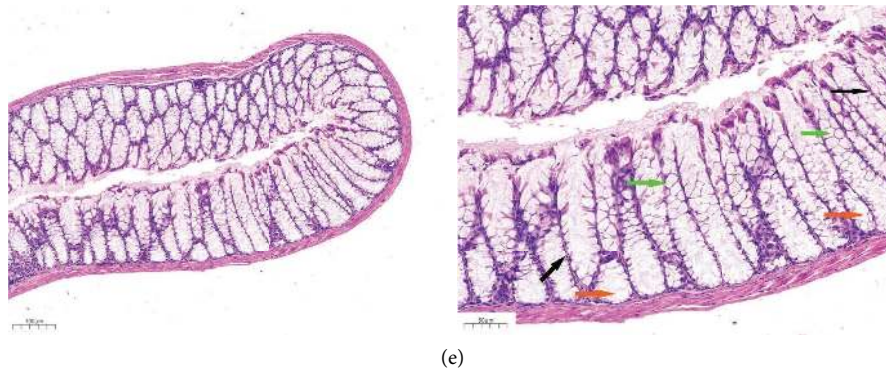


FIGURE 3: H&E staining results of mice colon from (a) intact, (b) vehicle, (c) mos, (d) lac, and (e) DOP. The black arrows indicate the epithelial cells, the green arrows signify the goblet cells, and orange arrows signify the mucosal crypts.

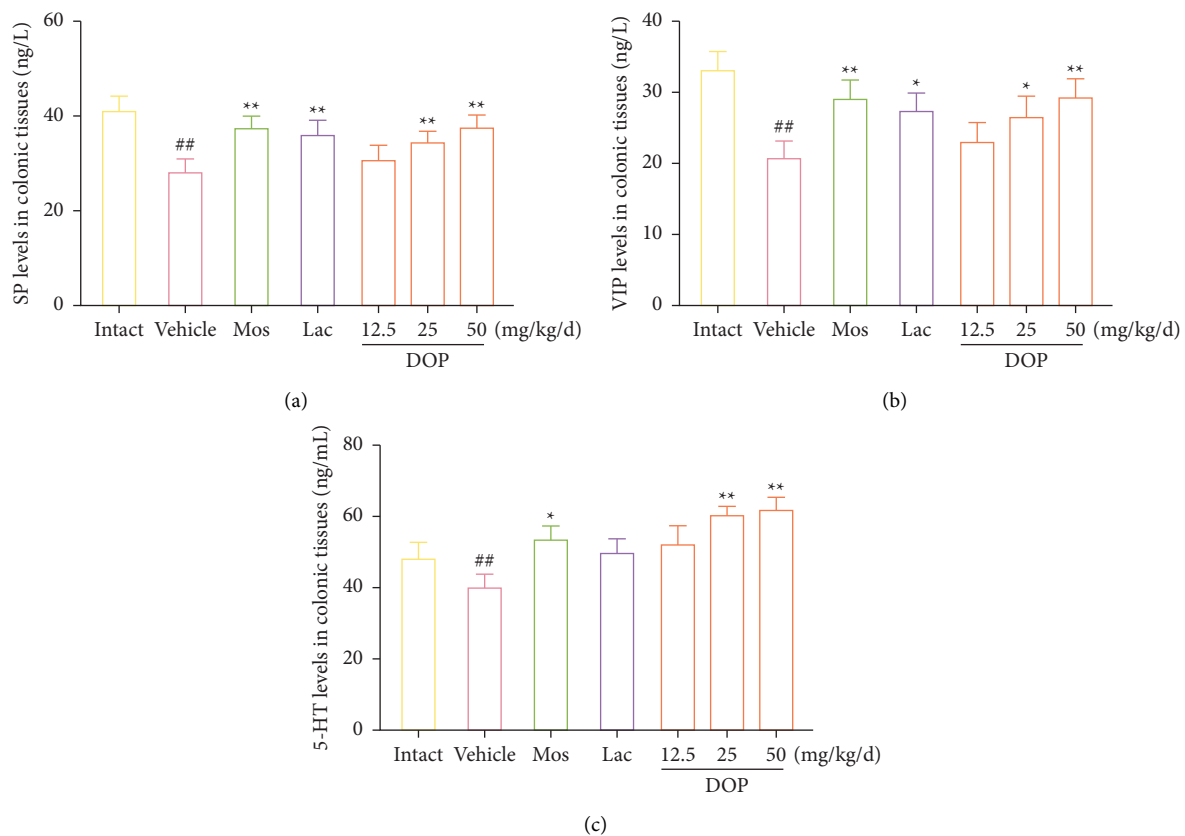


FIGURE 4: DOP regulated the gastrointestinal hormones. The level of (a) SP, (b) VIP, and (c) 5-HT as measured by ELISA kits. Data are expressed as mean $\pm$ SD ($n = 6$). ^{##} $p < 0.01$ vs. intact group; ^{*} $p < 0.05$, ^{**} $p < 0.01$ vs. vehicle group.

3.6. DOP Regulated the Intestinal Microbiota. Dysbiosis of the gut microbiota has been significantly correlated with the pathophysiology of STC [28]. To explore this relationship, We characterized the composition of fecal microbial community via 16S rRNA amplicon sequencing. The results consistently demonstrated that DOP administration effectively modulated the microbial profile in STC mice.

As illustrated in Figures 8(a) and 8(b), DOP treatment markedly elevated the alpha-diversity of STC mice, as evidenced by significant increases in the Simpson and Shannon indices ($p < 0.05$). Similarly, Figures 8(c) and 8(d) reveal that

DOP altered the beta-diversity relative to the vehicle group, as demonstrated by principal coordinate analysis (PCoA).

To profile the gut microbial community composition, OTUs were analyzed, and their abundance profiles were analyzed across taxonomic ranks, including phylum, class, order, family, genus, and species (data are presented at the phylum and genus levels). The CircosChord diagram (Figure 9(a)) demonstrated that the vehicle group displayed an elevated relative abundance of Firmicutes alongside a reduced abundance of Bacteroidetes at the phylum level when compared with the intact group, aligning with prior

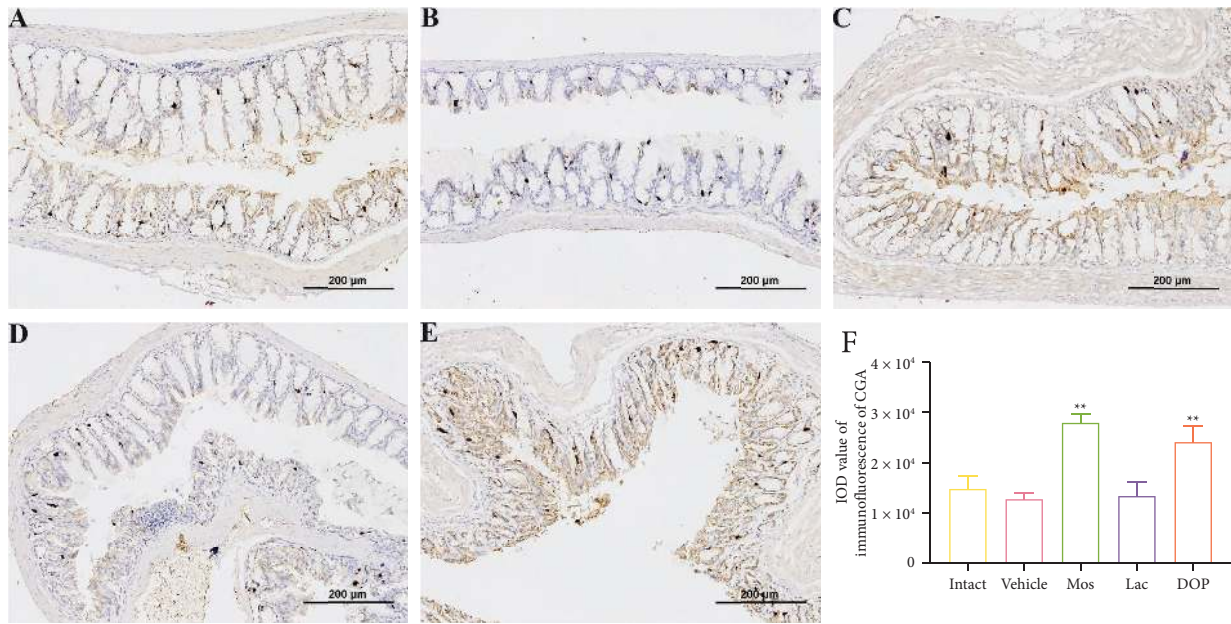


FIGURE 5: DOP upregulated the biosynthesis of 5-HT in colons. The ECs' immunohistochemistry staining of colonic tissue from (A) intact, (B) vehicle, (C) mos, (D) lac, and (E) DOP, and quantified by (F) the IDO value. Data are expressed as mean $\pm$ SD ($n = 3$). ** $p < 0.01$ vs. vehicle group.

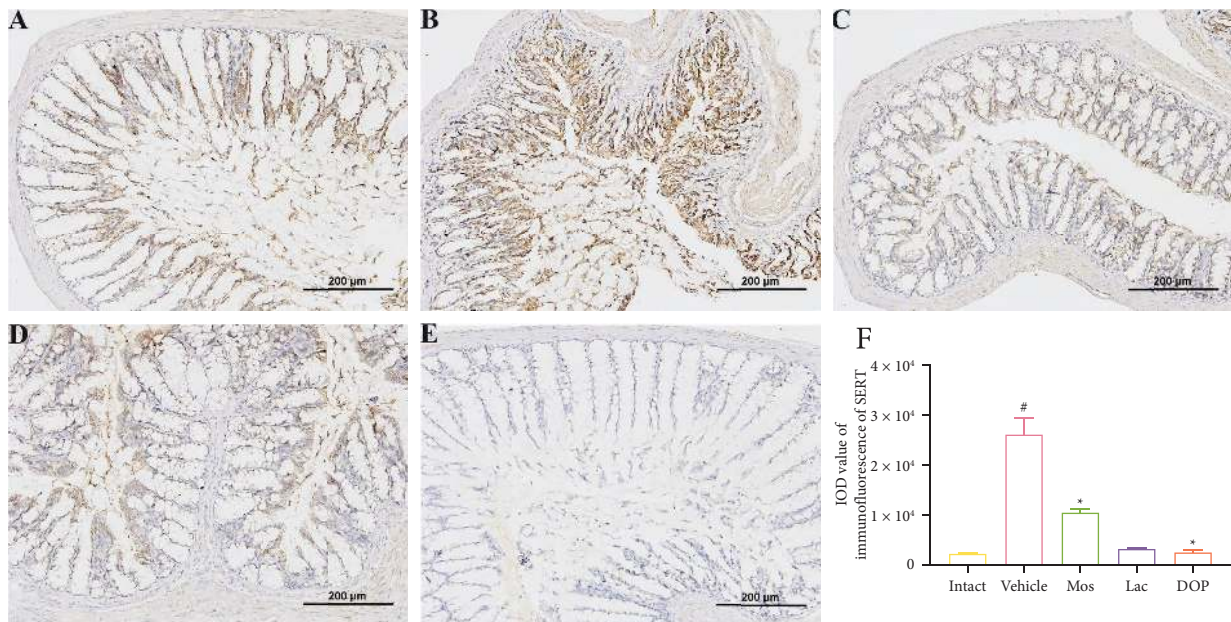


FIGURE 6: DOP inhibited the inactivation of 5-HT in colons. The SERT immunohistochemistry staining of colonic tissue from (A) intact, (B) vehicle, (C) mos, (D) lac, and (E) DOP, and quantified by (F) IDO value. Data are expressed as mean $\pm$ SD ($n = 3$). # $p < 0.05$ vs. intact group; * $p < 0.05$ vs. vehicle group.

research findings [29]. Treatment with DOP significantly increased the proportion of Bacteroides at the phylum level. Additionally, the vehicle group exhibited decreased relative abundances of Bacteroides and Lactobacillus at the genus level, effects that were reversed by DOP (Figure 9(b)). Notably, both Bacteroidetes and Lactobacillus are functionally associated with SCFA biosynthesis.

3.7. DOP Enhanced SCFA Levels and Activation in STC Mice. Metabolites derived from the intestinal microbiota, notably SCFAs, exert a pivotal role in modulating GI motility. Perturbations in the community composition and diversity of the intestinal microbiota can substantially influence SCFA biosynthesis, resulting in dynamic changes in systemic SCFA levels. Given their essential contributions to host health,

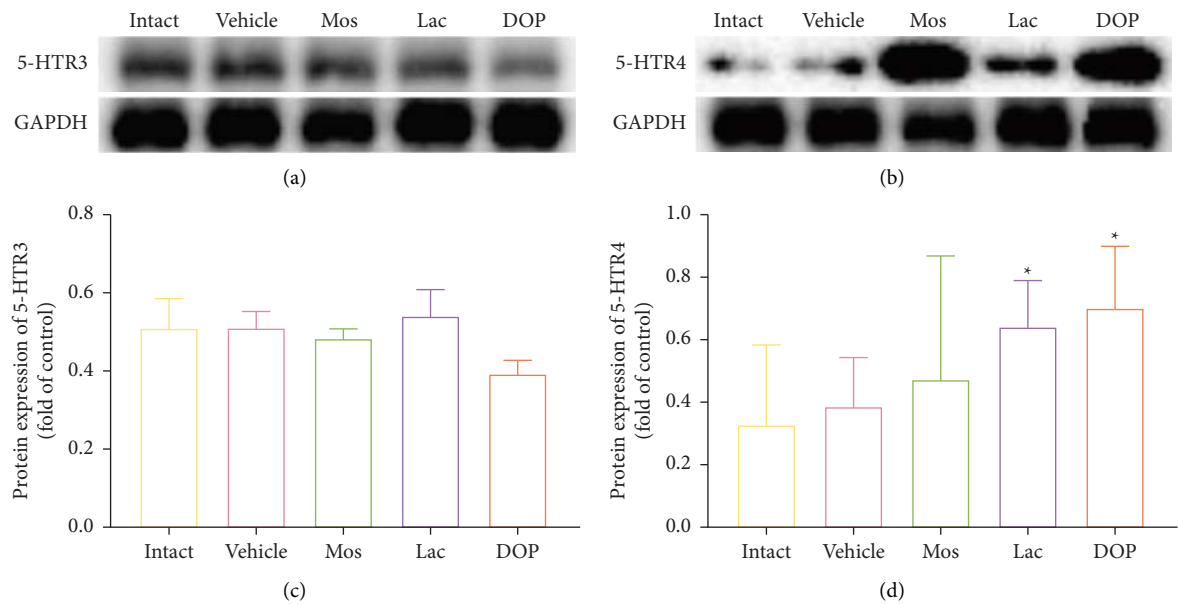


FIGURE 7: DOP influenced 5-HTR expression in colons. (a, b) Representative lanes of 5-HTR₃ and 5-HTR₄, (c, d) protein expressions of 5-HTR₃ and 5-HTR₄. Data are expressed as mean $\pm$ SD ($n = 3$). * $p < 0.05$ vs. vehicle group.

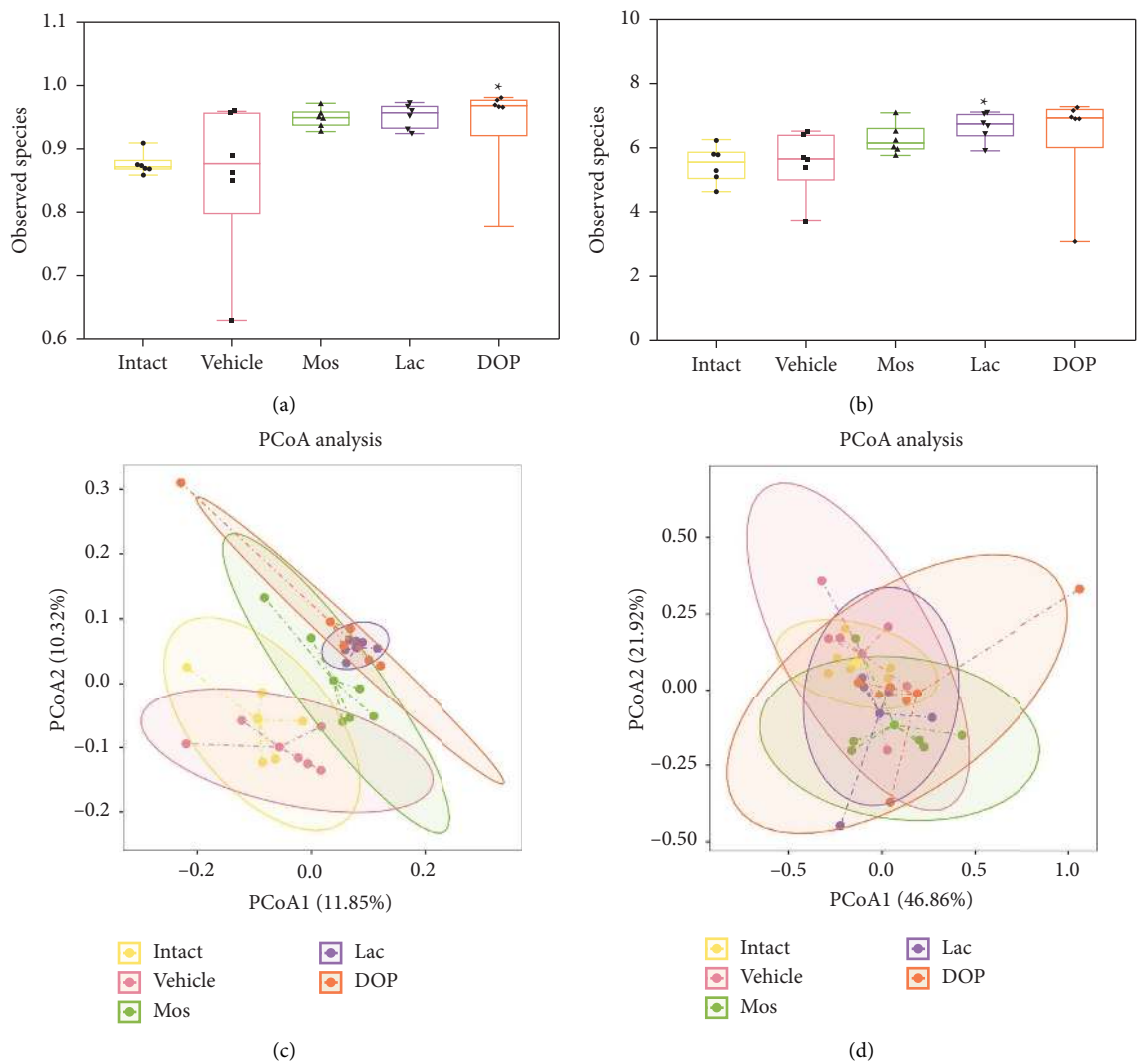


FIGURE 8: DOP modulated the composition of gut microbial communities in STC mice. The alpha-diversity was assessed via (a) Simpson's index and (b) Shannon indices. Beta-diversity was investigated using (c) unweighted UniFrac distance and (d) weighted UniFrac distance. * $p < 0.05$ vs. vehicle group.

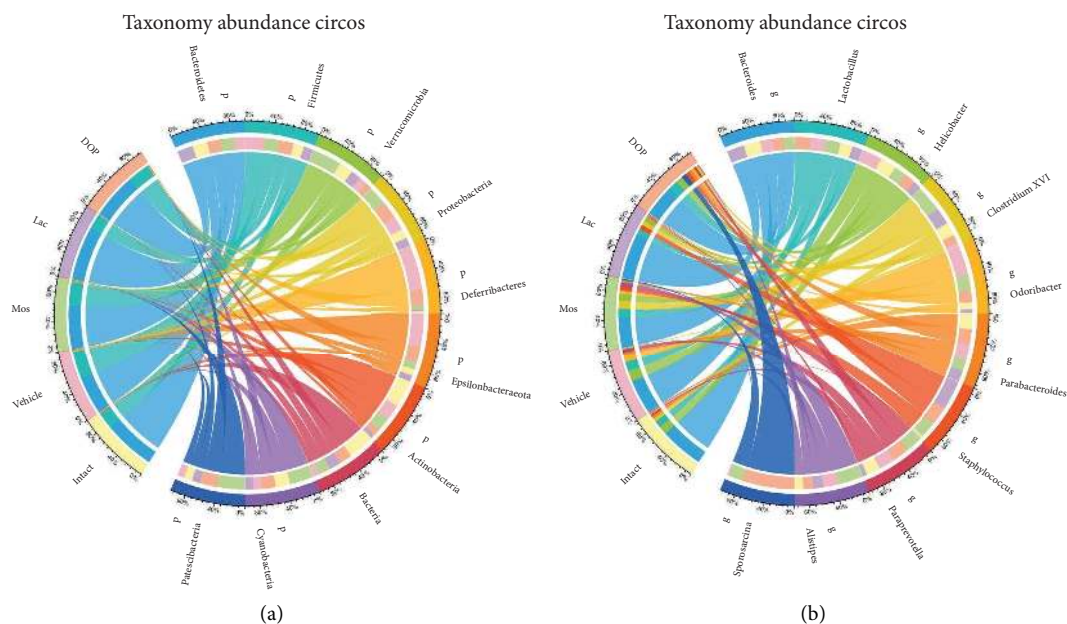


FIGURE 9: DOP regulated the structure of intestinal microbiota in STC mice. Relative abundance of intestinal microbial at (a) phylum level and (b) genus level.

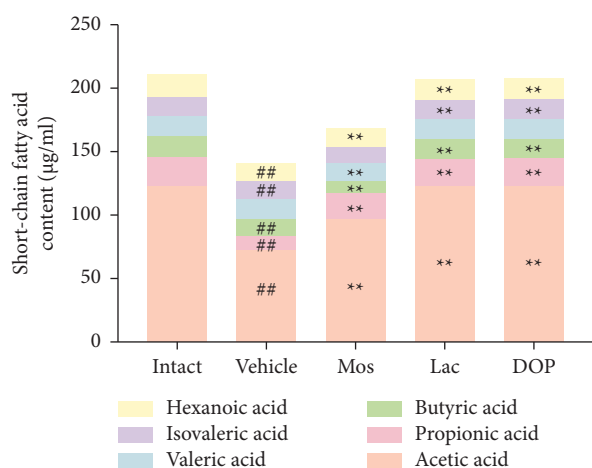


FIGURE 10: DOP increased rebalanced SCFA levels in STC mice. Quantification of SCFAs (ethanoic acid, propanoic acid, valeric acid, butyric acid, and caproic acid) in colonic tissues was detected by GC-MS. # $p < 0.01$ vs. intact group; ** $p < 0.01$ vs. vehicle group.

maintaining an optimal endogenous SCFA balance is crucial for physiological homeostasis.

As shown in Figure 10, administration with loperamide for 14 days induced a marked reduction in fecal SCFA levels in the vehicle group ($p < 0.01$). In contrast, DOP intervention markedly increased fecal concentrations of SCFAs, with notable elevations specifically observed in hexanoic acid, butyric acid, propionic acid, and acetic acid ($p < 0.01$). Furthermore, as demonstrated in Figure 11, DOP markedly upregulated the expression of GPR43, a key SCFA receptor. This suggests that DOP not only enhances SCFA production but also activates their signaling pathways, ultimately modulating the 5-HT system.

4. Discussion

STC is a prevalent health issue that significantly impairs the quality of life. Herbal medicines have received extensive acceptance in clinical practice owing to their strong patient compliance and favorable safety profiles. DOF, a widely recognized medicinal-edible plant species, has been traditionally used to treat GI disorders for centuries [30]. Our previous studies demonstrated that ultrafine DOF powder exerts a potent laxative effect in loperamide-induced STC mice [26]. Notably, DOF is rich in polysaccharides [31], which are known to modulate intestinal microbiota, potentially contributing to its therapeutic effects. For instance, polysaccharides from *Nostoc sphaeroides* have been shown to alleviate constipation by increasing fecal *Bifidobacterium* levels and improving stool consistency [32]. In this study, we purified DOP according to our previous way. DOP was characterized by a molecular weight of 8218 Da and a purity of 97.82%, which confirmed the stability and reliability of the previously established extraction and purification methods. Although the therapeutic potential of DOP in STC remains unexplored, we hypothesized that its polysaccharide composition may confer beneficial effects on gut motility. In this investigation, we conducted a systematic assessment of DOP's therapeutic efficacy against STC and explored the underlying mechanisms involved.

STC is clinically characterized by hard stools, difficulty defecating, irregular intestinal water absorption, and dysregulated GI hormones [14]. Common etiological factors of STC including colonic motility dysfunction, neurologic abnormalities, and a low-fiber diet [33]. Diminished intestinal propulsion has been repeatedly documented in patients with STC [34, 35]. Macroscopically, GI motility and defecation status serve as key diagnostic criteria for STC.

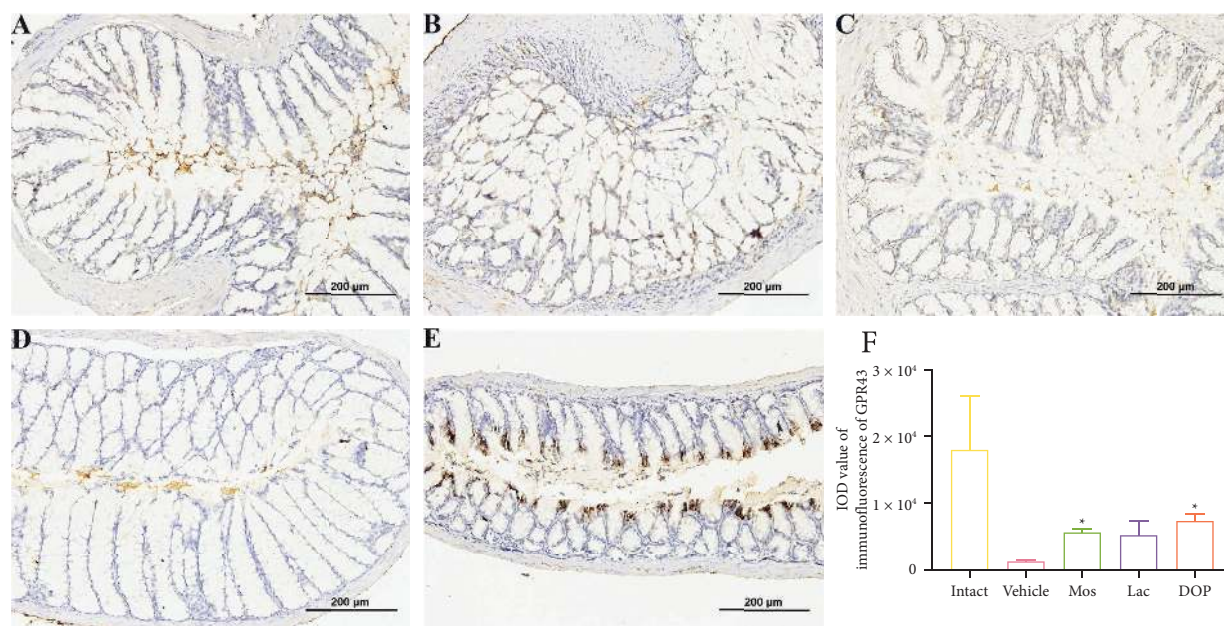


FIGURE 11: DOP enhanced the activation of SCFAs in colons. The GPR43 immunohistochemistry staining of colonic tissue from (A) intact, (B) vehicle, (C) mos, (D) lac and (E) DOP, and quantified by (F) the IDO value. Data are expressed as mean $\pm$ SD ($n = 3$). * $p < 0.05$ vs. vehicle group.

Peristalsis, the primary mechanism driving GI content movement, is typically assessed by measuring the distance traveled by ink in the small intestine following administration in constipation studies. Notably, DOP significantly enhances the GI transit ratio and fecal weight within 24 h, which effectively improves defecation function. Additionally, DOP significantly ameliorated the histopathological damage in mice with chronic constipation, indicating that it exerts a certain protective effect on the intestinal tract, which aligns with previous reports [36]. STC is also associated with significant disturbances in GI hormones, including SP, VIP, and 5-HT [37]. Many research studies indicate that enteric neurotransmitter imbalance is the primary pathological mechanism underlying STC [38]. Therapeutic strategies targeting these imbalanced hormones may offer promising approaches for STC management. Under physiological conditions, VIP stimulates peristalsis and promotes intestinal water–electrolyte secretion [39]. In our study, compared to the vehicle group, DOP treatment increased SP and VIP levels in STC mice, suggesting that DOP modulates GI hormone levels by stimulating the GI tract. This hormonal regulation may contribute to improved GI peristaltic function.

The enteric nervous system (ENS) regulates GI motility primarily through 5-HT mediated signaling in intestinal neurons, which controls the release of neurotransmitters such as SP and VIP to modulate intestinal smooth muscle activity [40]. Reduced 5-HT levels are associated with decreased stool frequency, altered stool consistency, and STC symptoms [41]. In this study, we observed abnormal 5-HT levels in loperamide-induced STC mice, which were effectively normalized by DOP treatment (Figure 4). This correction may contribute to the restoration of intestinal fluid

metabolism and GI function. 5-HT regulates GI motility and secretion by acting on 5-HT receptors, with 5-HT₃R and 5-HT₄R being particularly relevant to GI motility. Dysregulation of these receptors can disrupt secretion and transport, leading to GI disorders [42]. Compared to the intact group, the colonic expression of 5-HT₄R was markedly reduced in the vehicle group, suggesting that STC pathogenesis may involve impaired 5-HT receptor signaling. 5-HT₄R enhances intestinal smooth muscle contraction and secretion, playing a critical role in STC development [43]. Clinically, prucalopride, a high-affinity 5-HT₄R agonist, significantly improves the bowel frequency and alleviates STC symptoms [44]. In line with these results, our research confirmed increased 5-HT₄R protein levels in the DOP-treated group (Figure 7), suggesting that DOP may ameliorate STC by modulating 5-HT neurotransmitter levels, thereby restoring 5-HT₄R expression in colonic tissue and improving disease outcomes.

Alterations in intestinal microbiota are closely associated with STC [28]. Recent studies have increasingly reported intestinal microbiota dysbiosis in STC patients [45, 46]. Therapeutic strategies such as probiotics or fecal microbiota transplantation (FMT) can correct microbial imbalance, restore normal bacterial composition, and regulate intestinal motility, thereby effectively alleviating STC symptoms [2, 47]. These findings underscore the critical role of intestinal microbiota dysbiosis as a core pathological mechanism in STC. In this investigation, we utilized high-throughput 16S rRNA sequencing to assess alterations in the fecal microbiota composition of mice subjected to DOP treatment. Alpha- and beta-diversity analyses revealed that STC mice exhibited reduced microbial diversity compared to healthy controls, which was partially restored by DOP

treatment (Figures 8(a) and 8(b)). Notably, DOP effectively corrected the relative imbalance between Firmicutes and Bacteroides, promoting a microbiota structure in STC mice that more closely resembled that of healthy controls (Figure 9). These results demonstrate DOP's ability to rebalance the damaged intestinal microbiota in STC mice. Intestinal microbiota serves as a rich source of metabolites that influence STC, and it is well established that microbial metabolism, particularly SCFA production, plays a key role in host physiology [48]. SCFAs regulate intestinal fluid secretion and enhance GI motility, making them promising therapeutic candidates for STC [49]. In accordance with these observations, our research revealed that fecal levels of SCFAs were markedly decreased in STC mice, which displayed a significant increase following DOP administration (Figure 10), highlighting DOP's modulatory effects on microbial metabolism.

SCFAs primarily composed of acetate, propionate, and butyrate exert an essential role in intestinal physiological processes by lowering luminal pH and serving as key metabolites of gut microbiota [50]. Importantly, SCFAs enhance 5-HT synthesis, which in turn promoting intestinal motility. Consistent with this mechanism, our study demonstrated that DOP treatment increased 5-HT levels in STC mice by activating the GPR43 receptor (Figures 4 and 11). This activation led to elevated EC numbers and reduced SERT expression (Figures 5 and 6), further supporting the role of SCFAs in modulating 5-HT homeostasis. Our findings highlight the critical link between intestinal microbiota, SCFA production, and DOP's therapeutic effects in STC. Specifically, DOP altered the relative abundance of Bacteroides and Lactobacillus (Figure 9), two SCFA-producing bacterial genera that contribute to 5-HT synthesis and release. This microbial modulation likely explains the observed improvements in defecation, as increased 5-HT levels subsequently activate 5-HT₄R, ultimately alleviating STC symptoms [51].

5. Conclusions

DOP demonstrated significant laxative effects in loperamide-induced STC mice, exerting its therapeutic action through multiple mechanisms: (1) enhancing GI motility, (2) modulating 5-HT and 5-HT₄R expression, and (3) improving intestinal microecology. Notably, DOP treatment markedly increased the abundance of SCFAs-producing microbiota, which may contribute to 5-HT system activation—a pathway warranting further investigation. Collectively, these findings provide robust scientific evidence supporting DOP as a promising therapeutic agent for STC.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Dandan Luo: conceptualization, methodology, investigation, writing—original draft, and funding acquisition; Wen Li: resource and formal analysis; Chen Zhang: data curation and validation; Suiqin Ni: investigation and writing—review and editing; Zhenbiao Zhang: conceptualization, methodology, and writing—review and editing.

Zhenbiao Zhang and Suiqin Ni contributed equally to this work.

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