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Original Research Article

Micromeria biflora in alleviating hindgut mucosal injury in type 1 diabetic rats

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Abstract

Objective: This study investigated the alleviating effect of *Micromeria biflora* on hindgut mucosal damage in rats with type 1 diabetes.

Materials and Methods: 7-week-old male Sprague-Dawley rats were randomly divided into a control group (NC, n=15), a diabetic rat group (DC, n=15), and a *Micromeria biflora* treatment group (MbT, n=15, 300 mg/kg/day for 21 days by gavage). Changes in prostaglandin E2 (PGE2) concentration, cyclooxygenase-2 (COX-2) activity, and gene and protein expression of related signaling pathway components in the caecal mucosa were detected using enzyme-linked immunosorbent assay (ELISA), PCR, and Western blot, respectively.

Results: Compared with the DC group, *M. biflora* treatment significantly increased mRNA expression levels of CCND2 (1.4-fold, $p<0.05$) and MKI67 (1.9-fold, $p<0.05$), and increased protein expression of CCND2 (1.7-fold, $p<0.05$) and Sox9 (1.6-fold, $p<0.05$). It elevated β-defensin protein levels (40% increase, $p<0.05$) and interleukin-10 (IL-10) content (60% increase, $p<0.05$), and enhanced COX-2 activity (35% increase, $p<0.05$) as compared to the DC group. Treatment also increased local PGE2 content (75% increase, $p<0.05$) and membrane prostaglandin receptor 4 (EP4) levels (2.3-fold, $p<0.05$) as compared to the DC group. Furthermore, it upregulated epidermal growth factor receptor (EGFR) mRNA expression (1.5-fold, $p<0.01$) and increased protein abundances of phosphorylated CREB (pCREB) (2.0-fold, $p<0.05$) and phosphorylated AKT (pAKT) (1.8-fold, $p<0.05$) as compared to the DC group. No significant change in phosphorylated ERK (pERK) was observed.

Conclusion: *M. biflora* ameliorates diabetic hindgut injury by specifically activating the PGE2/EP4 signaling axis, evidenced by increased COX-2 activity (35%), PGE2 synthesis (75%), and EP4 receptor levels. This activation drives downstream phosphorylation of CREB and AKT, ultimately promoting epithelial proliferation and enhancing mucosal barrier function.

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Introduction

Diabetes mellitus (DM) represents a significant global health burden, frequently causing autonomic nervous system dysfunction (Kume et al. 2014; Luo et al. 2016). This dysfunction often leads to numerous complications, including those affecting the gastrointestinal (GI) tract (Careyva and Stello 2016; Horvath et al. 2014). GI symptoms in diabetics, arising from the foregut to the mid and hindgut, are predominantly linked to dysmotility (Careyva and Stello 2016; Krishnan et al. 2013). Given that diabetes is a systemic disorder (Brownlee 2001; Kume et al. 2014; Yan 2014), it is notable that up to 50% of patients experience disabling GI symptoms (Horvath et al. 2014), with up to 12% exhibiting symptoms consistent with specific GI dysfunctions (Careyva and Stello 2016; Horvath et al. 2014). A previous study indicates that increased luminal acidity in the hindgut can damage the mucosal epithelium and promote cell apoptosis (Tao et al. 2015). Apoptosis is often accompanied by reduced cell proliferation (Fernandez et al. 2015), and an imbalance in epithelial proliferation contributes to mucosal injury in models like colitis in mice (Araki et al. 2010). However, a critical question remains: does a decline in cell proliferation contribute to the severe mucosal damage observed in the hindgut under hyperglycemic conditions?

Given the established role of prostaglandin signaling in promoting epithelial proliferation and mucosal repair (Rodriguez-Lagunas et al. 2010; Roulis et al. 2014), interventions targeting this pathway represent a rational therapeutic strategy for diabetes-associated gut injury.

Natural products rich in bioactive compounds, particularly polyphenols found in medicinal herbs, have shown promise in modulating inflammatory and proliferative pathways. A previous study indicated that prostaglandin E2 (PGE2) as a bioactive lipid substance could regulate cell proliferation (Ansari et al. 2008), which involves distinct cyclooxygenase (COX) activities through a two-step process (Goren et al. 2017). COX as a key enzyme controlled the process of catalyzing arachidonic acid conversion to PGE2, which required presence of COX-1 and COX-2 (Chen et al. 2017; Goren et al. 2017). Selective inhibition of COX-2 activity could induce apoptosis and inhibit cell proliferation (Sirianni et al. 2009). The biological actions of PGE2 exerts by binding to specific PGE2 receptors (EP) , such as EP1, EP2, EP3 and EP4 (Hata and Breyer 2004). Subsequently, activation of the PGE2/EP receptor pathway, particularly downstream signals like cAMP response element binding protein (CREB), promotes compensatory epithelial proliferation and intestinal homeostasis (Rodriguez-Lagunas et al. 2010; Roulis et al. 2014). Furthermore,, proteinase-activated receptor-2 inducing COX-2 expression could increase PGE2 production and transactivation epidermal growth factor receptors in intestinal epithelial cells (Roulis et al. 2014), stimulating intestinal epithelial cell proliferation through ERK 1/2 and PI3K-AKT signaling pathways (Waseem et al. 2014). This highlights the central role of the PGE2-EP axis in mucosal maintenance and repair.

Micromeria biflora (*M. biflora*) is a common member of the family Lamiaceae, and as an important medicinal herb, it is a rich source of diverse classes of phytochemicals notably polyphenols including Rosmarinus acid and Flavonoids, compounds previously implicated in modulating COX-2/PGE2 pathways and epithelial function in other models (Liu 2024; Scheckel 2008). Also, *M. biflora* is commonly identified as white leaf savory and qurniyya. Its leaves are aromatic, and they can be used in the preparation of decoctions traditionally employed in some regions for GI ailments. Previous research reported that aromatic herbs are rich in polyphenols, and dietary supplementation of these compounds has been shown to produce broad health effects in the human body (Arenas 2017; Godos 2017; Lafay

2008; Saibabu et al. 2015), including potential benefits for GI integrity and inflammation modulation. And dietary supplementation of these compounds has been shown to produce broad health effects in the human body, including potential benefits for GI integrity and inflammation modulation.

For better understanding *M. biflora* function, and based on its phytochemical profile and traditional use suggestive of gut-protective potential, this study specifically aimed to investigate the potential of *M. biflora* to modulate the cell proliferation, PGE2 synthesis, and its downstream signaling pathways (CREB, ERK, and PI3K-AKT) in the hindgut mucosa of type 1 diabetic rats, addressing the identified gap concerning hyperglycemia-induced mucosal damage mechanisms.

Materials and Methods

Ethics statement, and experimental design and sampling

M. biflora plant materials were collected from Guiyang, Guizhou, China. A specimen of the fresh obtained plant was recognized and the marked specimen No. CHGY/Bot2644 was stored at the departmental herbarium. Complete *M. biflora* was extracted by water extraction process. The ratio of medicine to liquid was 1:15, each extraction lasted for 2 hr, and a total of 3 extractions were carried out. The filtrates were combined, concentrated, and then sprayed dry to obtain the extract of ginger-flavored grass, which was set aside for later use. The extraction rate was 23.61%. The extracted samples were dissolved in water and stored for later use.

All animal experimental procedures were approved by Guizhou University of Traditional Chinese Medicine Animal Care and Use Committee and the law of laboratory animals of the Guizhou province Zoological Society (Ethics Review Number: 2023128). The seven-week-old male Sprague-Dawley rats were obtained from SLAC Laboratory Animal Company (Shanghai, China). In brief, one week before the start of this experiment, rats were acclimatized to the laboratory conditions of temperature (22±2°C) and maintained on standard food pellets and tap water. After laboratory conditions adaptation, a total of 50 rats were randomly divided into two initial groups: 15 rats served as the control group (NC), while the remaining 35 rats received a single intraperitoneal injection of streptozotocin (STZ, 65 mg/kg) to establish the type 1 diabetes mellitus (T1DM) model. The control group simultaneously received an equal volume of citrate buffer solution (0.1 mol/L, pH 4.5). Diabetes was confirmed 72, 96, and 120 hr post-injection by fasting plasma glucose (FPG) levels ≥16.7 mmol/L accompanied by polydipsia, polyuria, and polyphagia. During the modeling process, 2 rats died and 3 rats were injured and excluded from the study, resulting in 30 successfully modeled diabetic rats. After successful modeling, diabetic rats were further randomized into two subgroups: the diabetic control group (DC, n=15) and the *M. biflora*-treated diabetic group (MbT, n=15, 300 mg/kg body weight by gavage once a day) treated for 21 days (Khazaei 2025). Upon experiment completion, hindgut mucosal tissues were immediately harvested, and the epithelial layer was carefully separated from the muscular layer by blunt dissection. The isolated epithelial tissues were washed four times with ice-cold phosphate-buffered saline (PBS) to remove adherent fat and blood residues. Tissues were then snap-frozen in liquid nitrogen and stored at -80°C for subsequent analysis.

RNA extraction, cDNA synthesis and qPCR

Total RNA was extracted from frozen hindgut samples by TRIzol reagent (TaKaRa, Dalian, China) according to the manufacturer's instructions. The concentration and quality of the RNA were measured based on the absorbance at 260

nm in a Nano-1000 Spectrophotometer (Eppendorf-Biotech, Hamburg, Germany).

The mRNA expression was measured by qRT-PCR according to our previous study (Xie et al. 2015) and performed with MyiQ2 Real-time PCR system (Bio-Rad, Hercules, USA). The expression of β-actin mRNA was chosen to be the reference gene because it is not affected by the experimental factors. All PCR primers were synthesized by the Sangon Biotech (Shanghai, China), and the primer sequences are listed in Table 1. The use of a single reference gene (β-actin) without formal stability validation is a limitation, though its selection was based on widespread application in diabetic models and internal consistency checks. Future studies will implement multi-gene normalization protocols.

Table 1. The primers sequence and parameters

Primers	GenBank number	Primers sequence (5'→3')	Orientation
β-actin	NM_031144.3	CACCATGTACCCAGGCATTG	Forward
		ACAGTCCGCCTAGAAGCATT	Reverse
CCND2	NM_022267.1	CATTGAGCACATCCTTCGCA	Forward
		TGCATTCACTTCCTCGTCCT	Reverse
MKI67	BC058559.1	GAACCTGACTGTGCCCTTTC	Forward
		CAGAACTGCCCTCTCACTCT	Reverse
EGFR	AF275367.1	CCAAGCCCTACAGACTCCAA	Forward
		ACGGCAGCTCCCATTTCTAT	Reverse

Protein extraction, SDS-PAGE and western blot analysis

Protein was extracted from frozen hindgut samples using basic lysis buffer and added the protease inhibitor cocktail (Roche Applied Science) and protein Extraction Kit (Sigma-Aldrich Co. LLC, Beijing, China) (Deml et al. 2015), according to the manufacturer's instructions. The protein concentration was measured with a Pierce BCA Protein Assay Kit (Pierce, Rockford, IL, USA). The protein samples of SDS-PAGE experiments was pretreatment and the step of SDS-PAGE was performed to separate proteins according to the previous study description (Xie et al. 2017; Yan et al. 1997). The protein antibody of β-Actin was used as a loading control. The protein expression of Sox9, CCND2 and EP4 and the Protein phosphorylation of CREB, AKT and ERK were determined. Protein abundance is expressed as the fold change relative to the mean value of the control group. The effective concentration of primary antibodies was used according to the manufacturer's instructions (Abcam). Finally, the blot was washed and detected by enhanced chemiluminescence (ECL) using the LumiGlo substrate (Pierce, USA) and Clarity Western ECL Substrate (BioRad, USA).

Determination of COX-2 activity and β-defensin, IL-10 (IL-10), and PGE2 content

COX-2 activity and the content of β-defensin, IL-10 and PGE2 in hindgut mucosa tissues were measured by enzyme-linked immunosorbent assay kits according to the manufacturer's instructions. The kits were purchased from Shanghai Langdun Biotechnology Co. Ltd (Shanghai, China).

Statistical analysis

The results were analyzed by LSD model of ANOVA (SPSS-20.0, Chicago, IL, USA). Data are expressed as Means±SE and statistical significance was considered when $p<0.05$.

Results

Proliferation-related genes and proteins expression in hindgut mucosa

As shown in Figure 1, compared with NC rats, the DC group exhibited significantly decreased mRNA expression levels of *CCND2* and *MKI67* in the caecal and colonic mucosa ($p<0.01$). Additionally, the protein expression levels of CCND2 and Sox9 in the caecal mucosa were significantly downregulated in the DC group ($p<0.05$) as compared to the NC group. Compared with the DC group, *M. biflora* treatment significantly increased mRNA expression levels of *CCND2* (1.4-fold, $p<0.05$) and *MKI67* (1.9-fold, $p<0.05$), and increased protein expression of CCND2 (1.7-fold, $p<0.05$) and Sox9 (1.6-fold, $p<0.05$).

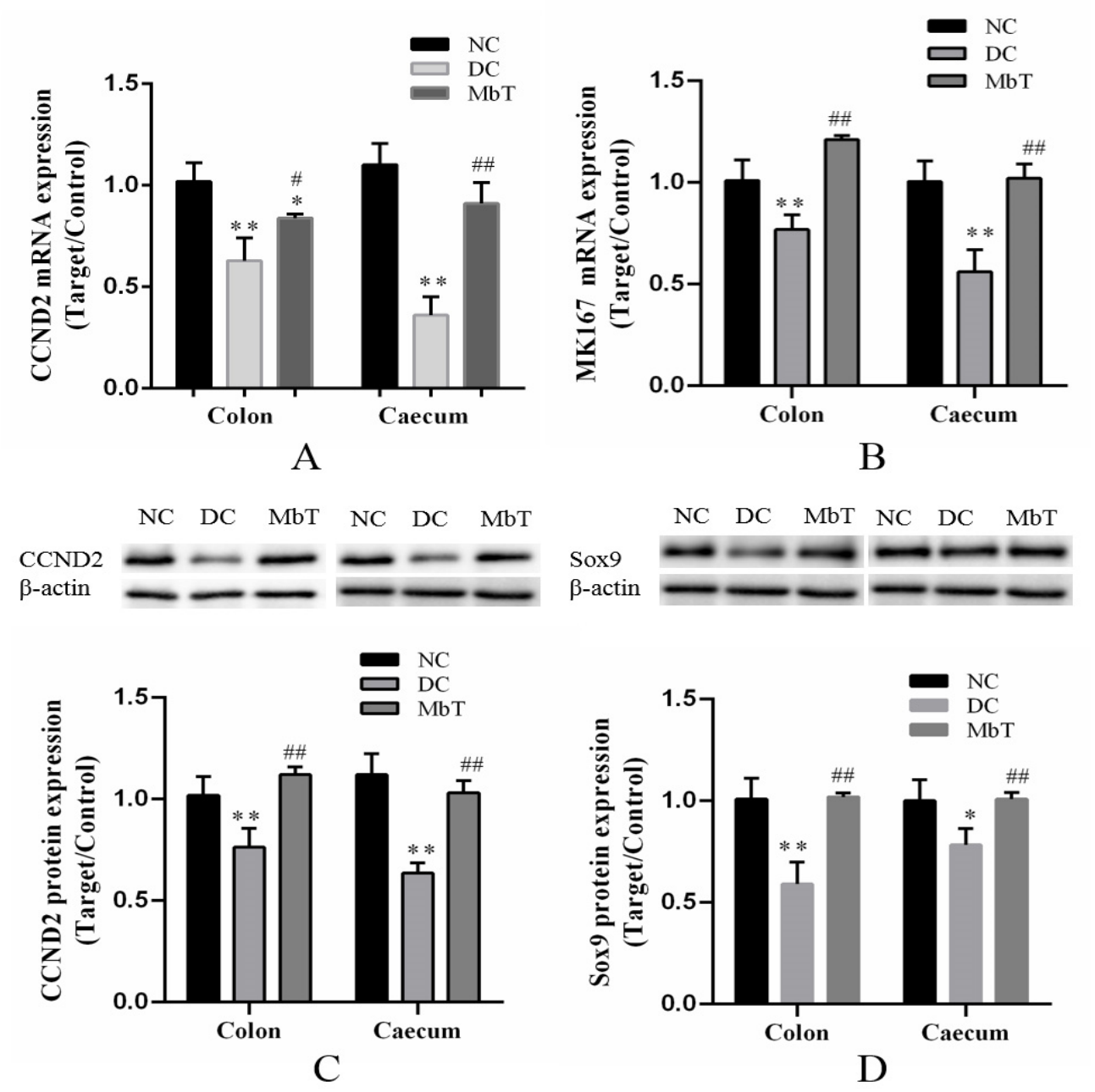

Figure 1. Changes in proliferation-related gene and protein expression in the hindgut mucosa: (A) CCND2 mRNA, (B) MKI67 mRNA; (C) CCND2 protein, and (D) Sox9 protein. Data are presented as Means ± SE. *$p<0.05$ and **$p<0.01$ vs. NC group; #$p<0.05$ and ##$p<0.01$ vs. DC group.

Contents of β-defensin protein and IL-10 and COX-2 activity in hindgut mucosa

As shown in Figure 2, compared with the NC group, the DC group exhibited significantly decreased IL-10 levels in the colon mucosa (Figure 2B) ($p<0.05$), while no significant difference in β-defensin levels was observed between the two groups ($p>0.05$). In the caecal mucosa, both β-defensin (Figure 2A) and IL-10 (Figure 2B) levels were significantly lower in the DC group than in the NC group ($p<0.05$ and $p<0.01$, respectively). Additionally, caecal mucosal COX-2 activity was significantly reduced in the DC group ($p<0.05$), whereas no significant change in colonic mucosal COX-2 activity was detected ($p>0.05$, Figure 2C) as compared to the DC group. Following treatment with *M. biflora*, it elevated β-defensin protein levels (40% increase, $p<0.05$) and IL-10 content (60% increase, $p<0.05$), and enhanced COX-2 activity (35% increase, $p<0.05$).

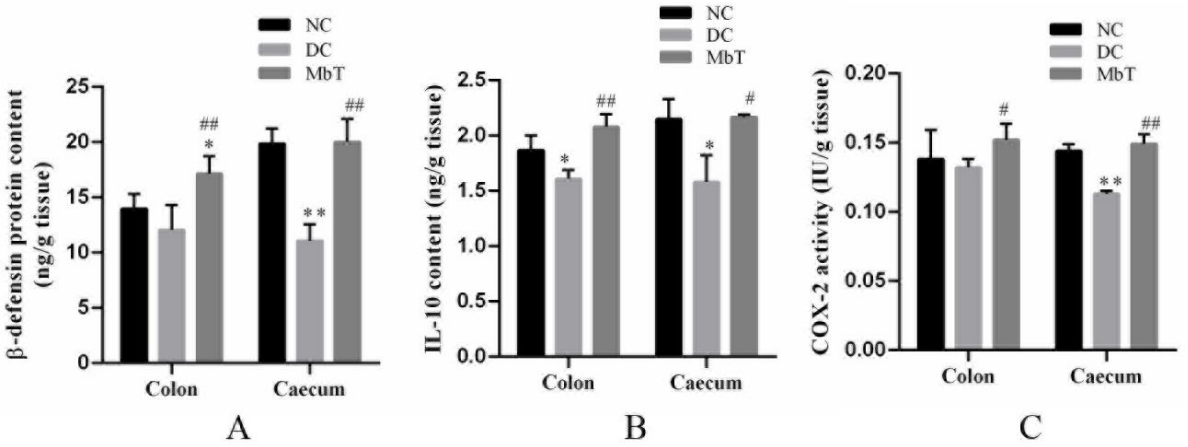

Figure 2. Levels of β-defensin (A), IL-10 (B), and cyclooxygenase-2 (COX-2) activity (C) in the hindgut mucosa. Data are presented as Means ± SE. * $p<0.05$ and ** $p<0.01$ vs. NC group; # $p<0.05$ and ## $p<0.01$ vs. DC group.

Effect of *M. biflora* on PGE2 concentration and PGE2 signaling pathway

As shown in Figure 3, compared with the NC group, the DC group exhibited significantly decreased PGE2 concentrations in both the colonic and caecal mucosa ($p<0.01$, Figure 3A). Consistent with PGE2, EP4 protein expression was also significantly downregulated ($p<0.01$, Figure 3B). Following treatment with *M. biflora*, both local PGE2 content (75% increase, $p<0.05$) and membrane prostaglandin receptor 4 (EP4) levels (2.3-fold, $p<0.05$) were significantly higher in the treated groups compared to the DC group ($p<0.01$).

In the PGE2 signaling pathway, compared with the NC group, the DC group showed significantly downregulated EGFR mRNA expression in both the caecal and colonic mucosa (1.5-fold, $p<0.01$, Figure 3C), along with significantly reduced total CREB and phosphorylated CREB protein levels in the caecal mucosa ($p<0.01$, Figure 3D). After treatment, except for the colonic mucosal phosphorylated CREB protein levels, *M. biflora* treatment group displayed significantly elevated indices compared to the DC group (2.0-fold, $p<0.05$ and $p<0.01$). Furthermore, the DC group exhibited significantly lower AKT protein phosphorylation levels in the caecal mucosa compared to the NC group ($p<0.01$, Figure 3E). However, *M. biflora* treatment significantly increased AKT phosphorylation levels in the caecal mucosa compared to the DC group (1.8-fold, $p<0.05$), while no significant changes in ERK phosphorylation levels were observed in either the caecal or colonic mucosa ($p>0.05$, Figure 3F).

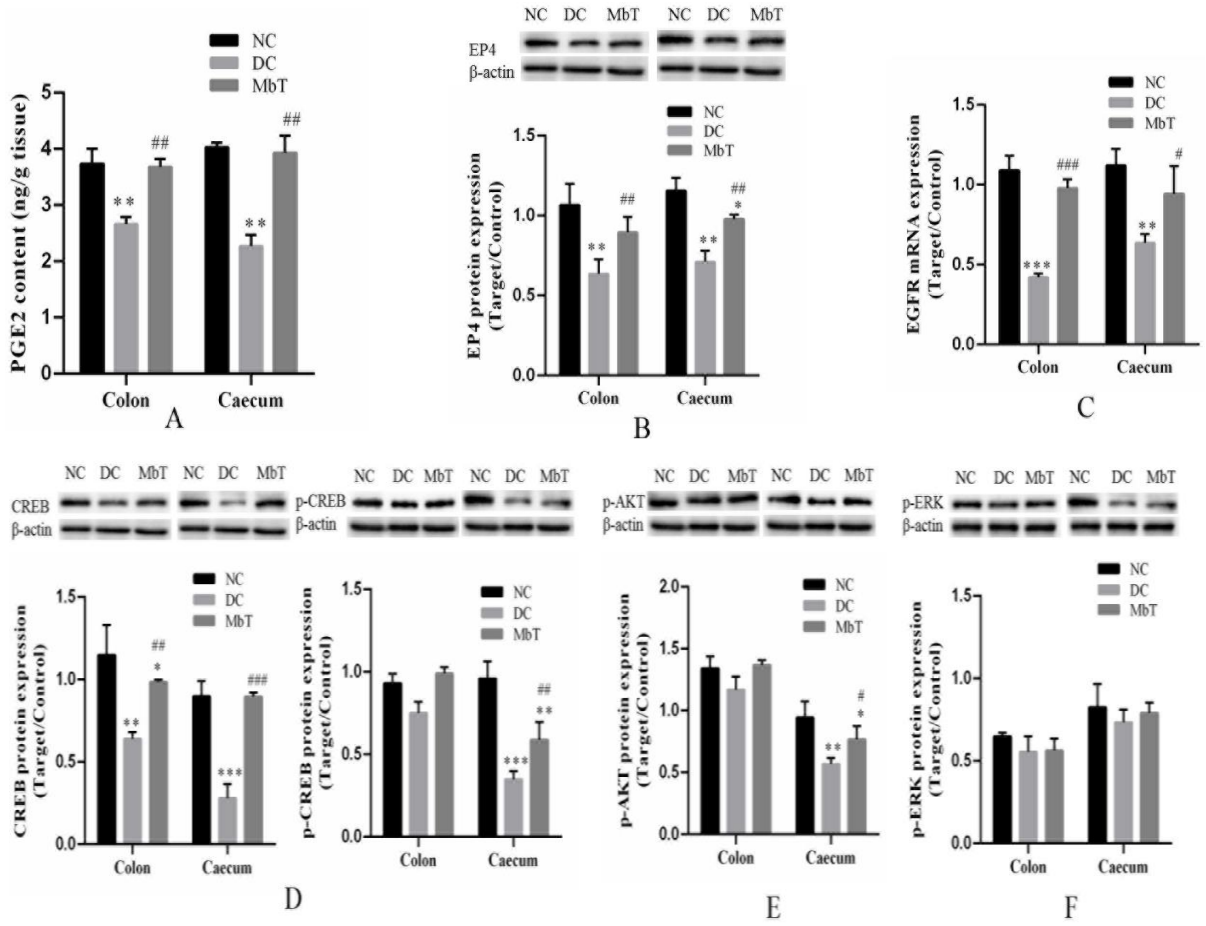

Figure 3. Prostaglandin E2 (PGE2) signaling pathway: (A) PGE2 concentration; (B) EP4 protein expression; (C) EGFR mRNA expression; (D) CREB and phosphorylated CREB protein expression; (E) phosphorylated AKT protein expression; and (F) phosphorylated ERK protein expression. Data are presented as Means ± SE. * $p<0.05$ and ** $p<0.01$ vs. NC group; # $p<0.05$ and ## $p<0.01$ vs. DC group.

Discussion

Gastrointestinal symptoms, including enteropathy or colonic dysfunction such as constipation, diarrhea, and fecal incontinence, are frequently observed in diabetic patients and are particularly distressing for many (Maisey 2016). However, diabetes-induced mucosal damage is complex and primarily associated with chronic hyperglycemia and gastrointestinal autonomic neuropathy during the course of diabetes (Boland et al. 2013; Careyva and Stello 2016; Krishnan et al. 2013; Maisey 2016).

Previous results have shown that high-grain diets may increase hindgut acidity, potentially damaging the hindgut mucosal epithelium (Tao et al. 2015). Epithelial growth is inhibited in diabetes due to reduced cell proliferation, which correlates with decreased cyclin expression (Zhang et al. 2017).

In the present study, the upregulation of *CCND2* and *MKI67* by *M. biflora* suggests direct stimulation of epithelial cell cycle progression. This represents a critical mechanism counteracting the diabetes-associated suppression of proliferation previously reported (Zhang et al. 2017). These findings suggest the potential therapeutic value of *M. biflora* for repairing mucosal epithelial damage in the hindgut of diabetic rats. *M. biflora* is a medicinal herb characterized by modulating cytokine levels including increased anti-inflammatory cytokines (e.g. IL-10) and decreased pro-inflammatory cytokines (e.g. IL-1 and IL-4) and mediators (β-defensin) (Alhumaydhi et al. 2021; Jan 2019; Rauf 2020; Uddin et al. 2016). Our results revealed that *M. biflora* treatment

significantly elevated IL-10 and β-defensin protein levels in the mucosal tissue, suggesting enhanced defensive capabilities in diabetic rats. Furthermore, *M. biflora* treatment markedly improved mucosal cell proliferation and epithelial defense markers in the colonic mucosa of diabetic rats. These differences indicate that *M. biflora* can repair diabetes-induced epithelial barrier damage, potentially through mechanisms involving PGE2 synthesis and activation of its downstream signaling pathways.

Recent data indicated that in Tpl2 D/D mice, PGE2 administration suppresses the COX-2 pathway during dexamethasone sodium-induced inflammatory bowel disease injury, thereby restoring epithelial cell proliferation and structure (Roulis et al. 2014). Furthermore, endogenous PGE2 exerts proliferative and anti-apoptotic effects on epithelial cells in GI injury (Stenson 2007). Similar findings demonstrate that COX-2-derived PGE2 binding to EP4 activates protein kinase A (PKA) and CREB to regulate cell proliferation (Ansari et al. 2008; Sirianni et al. 2009). Upregulated COX-2 protein levels and activity are critical factors in hyperglycemia- or diabetes-associated endothelial dysfunction (Roulis et al. 2014). Earlier studies suggested that the phosphatidylinositol 3-kinase (PI3K)/Akt signaling and transcriptional factors NF-κB and CREB are involved in these processes (Shanmugam et al. 2004). In the present study, we observed that *M. biflora* treatment significantly increased COX-2 enzymatic activity, PGE2 content, and EP4 protein expression in the hindgut mucosa of type 1 diabetic rats. This is consistent with the activation of the PGE2-EP4 signaling axis. These findings provided evidence for the role of COX-2 and PGE2 signaling in regulating epithelial structure and function. Additionally, elevated phosphorylated CREB levels in the hindgut mucosa post-treatment clearly indicate enhanced local PGE2 synthesis, consistent with activation of the PGE2-EP4-PKA pathway in diabetic rats compared to the model group. This result aligns with previous studies in HT-29 and Caco-2 cells, demonstrating that cell proliferation is mediated by CREB phosphorylation and the PGE2-EP4-PKA pathway (Engstrom et al. 2014; Kisslov et al. 2012). Concurrently, increased local PGE2 rapidly induces epidermal growth factor receptor (EGFR) expression (Lu et al. 2014; Oshima et al. 2011), subsequently activating the PI3K-AKT and MAPK pathways (Ansari et al. 2008). In the present study, *M. biflora*-treated diabetic rats exhibited significantly upregulated *EGFR* mRNA expression and increased phosphorylated AKT levels in the caecal mucosa, while phosphorylated ERK1/2 (pERK1/2) levels remained unchanged. Notably, *M. biflora* markedly elevated PGE2 content and EP4 receptor protein expression in the colonic mucosa of diabetic rats. These findings suggest that *M. biflora* extract enhances PGE2/EP4 expression in diabetic rats and modulates colonic mucosal cell repair. While our data point towards the involvement of the PGE2-EP4-PKA-CREB and PI3K/AKT pathways in the beneficial effects of *M. biflora*, it is important to consider that other signaling pathways not investigated here might also contribute to or modulate the observed improvements in mucosal repair and anti-inflammatory responses in diabetes-induced mucosal damage. For instance, pathways involving other prostaglandin receptors (e.g. EP2), Wnt/β-catenin signaling crucial for epithelial homeostasis, or additional growth factor signaling cascades could play compensatory or synergistic roles. Furthermore, the unchanged pERK1/2 levels suggest that the MAPK/ERK pathway might not be a primary mediator of *M. biflora* proliferative effects in this specific context, highlighting the potential specificity of the pathways identified. This selectivity is mechanistically significant, unlike broad-spectrum proliferative agents (e.g. growth factors) that non-specifically activate both PI3K-AKT and MAPK

cascades (Ansari et al. 2008; Waseem et al. 2014), *M. biflora* appears to preferentially target the CREB/AKT branch downstream of EP4. Such targeted activation may reduce off-target effects and enhance therapeutic precision for diabetic mucosal repair, a novel finding underscoring the unique mechanistic profile of this botanical intervention.

In conclusion, *M. biflora* demonstrated mechanisms for ameliorating intestinal complications in type 1 diabetic rats (Figure 4). On one hand, it effectively repairs hindgut mucosal epithelial cells by upregulating CCND2 and MKI67 mRNA and protein expression levels, thereby counteracting the diabetes-induced inhibition of epithelial cell proliferation-a critical step in diabetes-induced mucosal damage. On the other hand, its anti-inflammatory properties are equally noteworthy. *M. biflora* extract not only modulates cytokine balance by increasing anti-inflammatory cytokines IL-10 and the defense peptide β-defensin, thereby enhancing mucosal defense capacity, but also promoted colonic mucosal cell repair and elevated epithelial defense marker protein levels, contributing to barrier restoration. Crucially, this study elucidated *M. biflora*'s impact on the COX-2 and PGE2 signaling pathway. Post-treatment, diabetic rats exhibited significantly elevated COX-2 enzymatic activity, PGE2 content, EP4 protein expression, and phosphorylated CREB levels in the hindgut mucosa, indicating enhanced local PGE2 synthesis and the possible activation of the PGE2-EP4-PKA pathway. These findings align with prior cell-based studies, confirming *M. biflora* role in mediating cell proliferation. Additionally, *M. biflora* upregulated EGFR mRNA expression and increased AKT phosphorylation in the caecal mucosa, further supporting its regulatory effects on cell growth and colonic mucosal repair. Collectively, these results highlight *M. biflora* dual mechanisms in repairing intestinal damage and modulating inflammatory responses, providing a scientific basis for its therapeutic application in diabetes-induced mucosal damage.

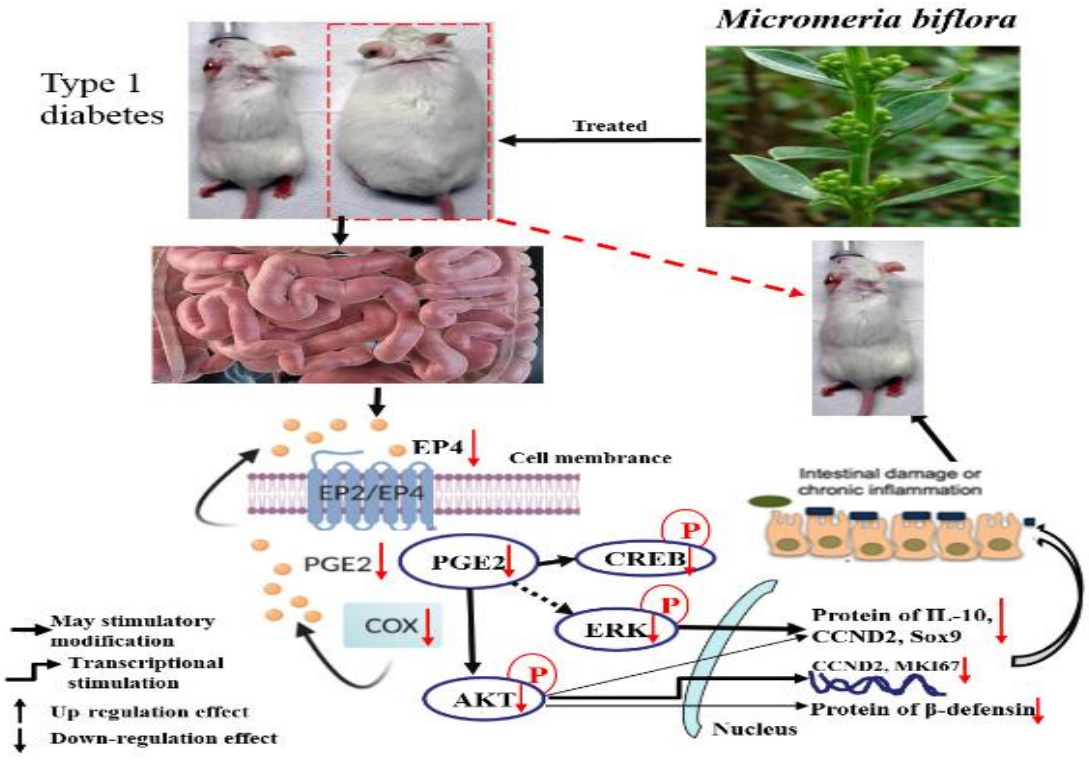

Figure 4. M. biflora has demonstrated the mechanisms in treating intestinal complications in type 1 diabetic rats.

It is important to acknowledge the limitations of this study. First, while the STZ-induced type 1 diabetic rat model recapitulates key features of hyperglycemia and complications, it may not fully represent the long-term progression of diabetes-induced mucosal damage observed in humans, limiting direct clinical extrapolation. Second, although we demonstrate the beneficial effects of *M. biflora* and its association with activation of the PGE2/EP4 signaling axis (evidenced by increased COX-2 activity, PGE2 synthesis, and EP4 levels), the specific bioactive compounds within the extract responsible for these effects remain unidentified. While existing literature attributes the bioactivity of *Lamiaceae* herbs like *M. biflora* to polyphenols such as rosmarinic acid and flavonoids (Liu 2024; Scheckel 2008), direct phytochemical profiling of our extract was not performed here. Third, histological validation of mucosal injury and subsequent repair was not performed. While the significant changes in molecular markers (e.g. upregulated CCND2, MKI67, Sox9, β-defensin, IL-10; activated PGE2/EP4/pCREB/pAKT signaling) strongly support our conclusions regarding enhanced proliferation and barrier function, direct morphological assessment would provide crucial complementary evidence. Future work should integrate detailed phytochemical characterization to identify the active principles, alongside comprehensive histopathological analysis of the hindgut mucosa, to strengthen the mechanistic understanding and translational relevance of *M. biflora* for treating diabetic enteropathy.

To date, the current study has yielded substantial findings, yet certain aspects require further elucidation. For instance, the precise relationship between enhanced PGE2 synthesis and EP4 protein expression in the colonic mucosa and *M. biflora* regulation of apoptosis requires more direct and conclusive evidence. Future research should focus on dissecting the specific molecular mechanisms underlying *M. biflora* therapeutic effects, optimizing its extraction protocols to improve efficacy, and conducting clinical trials to evaluate its potential in alleviating GI symptoms in diabetic patients. These steps will be critical to lay a more solid foundation for translating *M. biflora* from bench research to clinical applications.

Acknowledgment

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Conflicts of interest

The authors have declared that no competing interests exist.

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Ethical Considerations

All animal experimental procedures were approved by Guizhou University of Traditional Chinese Medicine Animal Care and Use Committee and the law of laboratory animals of the Guizhou province Zoological Society (Ethics Review Number: 2023128).

Code of Ethics

All animal experimental,design and implementation of this study strictly followed the requirements of the "Good Clinical Practice for Drugs" (NMPA, of China 2020)

Authors' Contributions

Jing Zhang contributed to experimental design, drafted the manuscript and finalized the manuscript. Ye Wang, Lihong Chen, Zhengsheng Li, Liang Yang, Yinan Yang, Jie Xu, Jun Zhan performed the experiment and analyzed the data. All authors have agreed to be accountable for all aspects of the work and approved the final version of the manuscript.

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