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(-)-Epicatechin targets gut microbiota-barrier crosstalk to ameliorate colitis-induced cognitive defects

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ABSTRACT: Inflammatory bowel disease is closely linked to neuroinflammation and cognitive dysfunction via the gut-brain axis, yet strategies targeting this interplay remain limited. This study investigated the therapeutic potential of (-)-epicatechin, a bioactive flavonoid, in alleviating colitis-associated brain functional impairments and explored its underlying mechanisms. We demonstrated that (-)-epicatechin treatment significantly mitigated colitis symptoms, restored colon morphology, and improved intestinal barrier integrity in dextran sulfate sodium (DSS)-induced colitis mice. Furthermore, (-)-epicatechin normalized brain oxidative stress and neuroinflammation and suppressed the glial cell activation. These improvements in the neuroimmune environment contributed to reduced neuronal apoptosis and maintenance of synaptic integrity, which was accompanied by enhanced cognitive performance. Gut microbiota profiling revealed reversed DSS-induced dysbiosis by (-)-epicatechin, as reflected by the altered alpha diversity indices and relative abundance of pathogenic and beneficial taxa. Notably, the increased abundance of barrier-disrupting genera, such as *Escherichia*, *Shigella* and *Mucispirillum* were strongly correlated with the weakened intestinal barriers and brain functions, which were reversed by (-)-epicatechin treatment. These findings suggest that (-)-epicatechin attenuates colitis-associated brain dysfunction by restoring gut microbial balance, reinforcing intestinal barrier integrity, and dampening systemic and neuroinflammation. This study highlights the pivotal role of the microbiota-gut-brain axis in mediating the beneficial effects of (-)-epicatechin, positioning it as a promising prebiotic for managing neurological complications associated with IBD.

Keywords: (-)-Epicatechin; Colitis; Gut microbiota; Inflammation; Neurological disorder

1. Introduction

Inflammatory bowel disease (IBD), a chronic inflammatory condition of the gastrointestinal tract, is increasingly associated with systemic complications, including neurological impairments such as neuroinflammation, cognitive decline, and disruption of the brain barrier [1, 2]. The gut-brain axis is a bidirectional communication network linking intestinal homeostasis to central nervous system (CNS) function, which plays an important role in mediating these effects [3]. Notably, gut microbial dysbiosis and compromised intestinal barrier integrity in colitis have been shown to exacerbate systemic inflammation,

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which can in turn promote neuroinflammatory responses and brain pathology [4, 5]. Emerging evidence suggests that prebiotic interventions, such as dietary fibers or polyphenols that modulate gut microbial ecology, can strengthen intestinal barrier function, reduce systemic inflammation, and subsequently alleviate neuroinflammation, highlighting their therapeutic potential for improving both intestinal and neurological health [6, 7].

(-)-Epicatechin, a prominent flavonoid abundant in foods such as green tea and cocoa, exhibits potent anti-inflammatory, antioxidant, and microbiota-modulating properties [8, 9]. Preclinical studies reveal that (-)-epicatechin ameliorates intestinal inflammation by restoring gut barrier function and suppressing pro-inflammatory pathways [10, 11]. It is noteworthy that epicatechin from highland barley grain is able to modulate gut microbiota and attenuate intestinal barrier dysfunction [8]. This improvement may stem from microbial regulation and potential synergistic effects. For instance, intervention with epigallocatechin-3-gallate, a green tea polyphenol, significantly enriched beneficial short-chain fatty acid (SCFA)-producing bacteria such as *Akkermansia*, resulting in elevated levels of protective SCFAs like butyrate and subsequent attenuation of intestinal inflammation and injury [12]. Similarly, (-)-epicatechin specifically suppressed potentially harmful intestinal bacteria while promoting the growth of beneficial microbes, including SCFA-producing bacteria such as *Eubacterium* [13]. Nevertheless, evidence regarding the precise microbiota-mediated mechanisms and synergistic contributions to barrier protection remains limited.

On the other hand, the neuroprotective potential of (-)-epicatechin, particularly in mitigating neuroinflammation and preserving synaptic plasticity, has been documented [14, 15]. These effects are associated with improvements in anxiety [16], depression [17], learning and memory [18, 19]. The neuroprotective role of (-)-epicatechin may be mediated through gut microbiota regulation, characterized by an increase in beneficial bacteria and a reduction in potential pathogens, thereby reducing systemic and neuroinflammation [14]. For instance, (-)-epicatechin supplementation in obese mice alleviated dysbiosis by restoring the abundance of *Lactobacillus* and *Enterobacter* and preventing hippocampal inflammation, suggesting a microbiota-driven pathway for neuroprotection [20–22]. Nevertheless, it remains unclear whether (-)-epicatechin can ameliorate colitis-induced brain dysfunction through restoration of intestinal damage and gut microbiota remodeling.

In this study, we investigated the protective effects of (-)-epicatechin on dextran sulfate sodium (DSS)-induced colitis in mice, focusing on its capacity to attenuate associated brain dysfunction via alteration of gut microbiota. Using a multidimensional approach, we evaluated colitis severity, cognitive performance, and brain pathology—including glial activation, neuronal integrity, oxidative stress, and neuroinflammation—alongside intestinal barrier markers and gut microbiota composition. Our findings suggest that (-)-epicatechin alleviates colitis-driven brain functional impairment by reducing systemic inflammation, restoring gut microbial balance, and enhancing intestinal barrier integrity. This protective cascade subsequently attenuates neuroinflammation, oxidative stress, and neuronal damage, ultimately

preserving cognitive function. Through integrated analysis of gut microbiota and barrier integrity, this study provides novel mechanistic insights into the microbiota-mediated neuroprotective effects of (–)-epicatechin and supports its therapeutic potential for comorbid gut and brain disorders.

2. Material and methods

2.1 Animals and experimental design

Male C57BL/6J mice (6–8 weeks) were purchased from Charles River Laboratories (Beijing, China). All mice were housed under specific pathogen-free conditions with a 12-h light-dark cycle and provided with standard chow and water ad libitum, with temperature kept at 21–24°C, and humidity at 40–70%. After one week of adaptation, mice were randomized to three groups, including the normal group (Ctrl), the model group (DSS), and the (–)-epicatechin-treated group (Epi). The model group was given 0.2 mL of PBS, and the Epi group was given (–)-epicatechin (>98%, Aladdin, USA) 100 mg kg^{–1} per day for 1 week. The intervention dose of (–)-epicatechin chosen for this study is based on previous reports [11, 23]. Then 2.8% (w/v) DSS (molecular weight: 36,000–50,000, MP Biochemical) was added to the drinking water from day 7 to 14 for the DSS and Epi groups. Weight change refers to the percentage obtained by dividing the weight on the corresponding number of days by the weight on day 7. Mice body weight and feces were recorded and scored daily during DSS treatment. The disease activity index (DAI) was scored daily for each mouse according to criteria indicated previously [24]. Briefly, the DAI was the average of the scores of body weight loss (0, <1%; 1, 1–5%; 2, 5–10%; 3, 10–20%; 4, >20%), feces occult blood (0–4, normal to gross bleeding) and diarrhea severity (0–4, normal to obvious watery feces). On day 14, the mice were sacrificed, and samples were collected for further analyses. All animal experiments were performed under the Guidelines for Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Experimental Animal Care and Use Committee of Nanchang University, number IACUC-20250529001.

2.2 Biochemical analysis

The TNF- α , IL-6, IL-10 and IL-1 β levels in the brain tissue were determined by ELISA kits (Elabscience Biotechnology Co., Ltd., Wuhan, China) according to the manufacturer's instructions. The total antioxidant capacity (T-AOC) and total superoxide dismutase (SOD) in the brain tissue were measured by a commercially available kit (Beyotime, Jiangsu, China) according to the manufacturer's manual.

2.3 Intestinal permeability

Intestinal permeability was determined as previously described with small modifications [25]. Briefly, all mice were fasted for 6 h and then orally given oral with 4kDa FITC-dextran (200 mg kg^{–1} body weight). After 90 min, 100 μ L of blood was collected from the tail vein. The blood sample was kept in the dark and centrifuged at 3000 g for 10 min to isolate serum. The fluorescence in the serum samples was measured using the Varioskan Flash reader ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 485/520$ nm).

2.4 Histopathology analysis

Colons fixed in 4% paraformaldehyde were paraffin-embedded and sectioned at a thickness of 5 μm and stained with HE. The paraffin sections were scored according to the criteria indicated previously [26]. The total histologic score was calculated as the sum of all index scores. Tissue antigens were repaired using the microwave, left to cool naturally, and washed by shaking on a decolorizing shaker. Immunostaining was performed with anti-ZO-1, and anti-Occludin primary antibodies (1:500; Servicebio, China), followed by incubation with secondary antibodies (HRP, Goat Anti-mouse IgG, 1:200; Servicebio, China) and then DAB color rendering. Next, cell nuclei were re-stained using hematoxylin and then dehydrated and sealed. The sections were imaged by Aperio LV1 (Leica, Germany) and the images were processed with ImageJ.

2.5 Immunofluorescence

After euthanasia, the brain tissues of mice were fixed overnight with 4% PFA in PBS at 4°C. Subsequently, samples were embedded in paraffin in cryomolds and stored at room temperature until further use. The brains were cut into 5 μm slices of paraffin sections. For immunofluorescence staining, sections were blocked with 5% normal goat serum or 3% BSA in PBS at room temperature for 1 h and then incubated with primary Abs in blocking buffer at 4°C overnight. After washing with PBS, sections were stained with fluorophore-conjugated secondary Abs in PBS at room temperature for 1.5 h. Counterstaining was done with DAPI reagent in PBS for 10 min. Primary antibody IBA1 (cat. no. GB12015); GFAP (cat. no. GB11096); Synaptophysin (cat. no. GB11553); PSD-95 (cat. no. GB11277) and NeuN (cat. no. GB11138) from Wuhan Servicebio Technology Co.,Ltd. were used for immunostaining. A Panoramic MIDI II scanner (3DHitech) was used for imaging and the images were pre-prepared for further evaluation with CaseViewer (3DHitech).

2.6 Imaging and quantification

For glial cell proliferation and interaction analysis, brain sections were immunostained with the anti-GFAP and anti-IBA1 antibodies. Images were processed using ImageJ, and the number of GFAP⁺ astrocytes and IBA1⁺ microglia was quantified. In addition, the percentage of IBA1⁺ microglia overlapping with GFAP⁺ astrocytes and GFAP⁺ astrocytes overlapping with IBA1⁺ microglial cells was quantified.

For glial cell activation analysis, Sholl analysis was performed in ImageJ on the IBA1⁺ microglia and GFAP⁺ astrocytes. A radius was drawn from the center of the cell body to the end of the longest branch to set the upper and lower limits for concentric circle placement. The first circle was set as close to the edge of the cell body as possible to ensure the cell body was not counted as an intercept on the circle. The distance between each circle was set at 2 μm for all cells. The summarized intersects, ramification index, and ending radius were calculated.

For neuronal function analysis, brain sections were co-immunostained with the anti-SYP and anti-PSD-95 antibodies for synaptic puncta colocalization analysis or immunostained with the anti-NeuN antibody for neurodegeneration analysis. Images were processed using ImageJ, and the number of colocalized puncta per section was quantified with the Synapse Counter plugin for ImageJ.

2.7 Genomic DNA extraction and 16S-rRNA sequencing of colon content

Bacterial DNA was extracted from mouse colon content using an OMEGA Soil DNA Kit extraction kit (Omega Bio-Tek, Norcross, USA). The quantity and quality of extracted DNAs were measured using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA) and agarose gel electrophoresis, respectively. The V4 region of the 16S rRNA gene was amplified using (338 F 5'-barcode-ACTCCTACGGGAGGCAGCA-3' and 806 R 5'-GGACTACHVGGGTWTCTAAT-barcode-3') PCR. After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2×250 bp sequencing was performed using the Illumina NovaSeq platform with NovaSeq 6000 SP Reagent Kit (500 cycles). Microbiome bioinformatics were performed with R 4.4.2. The DADA2 was used for quality filtering, de-noising, splicing, and chimera removal. The sequences obtained above were merged according to 100% sequence similarity, and the characteristic sequence ASVs and abundance data tables were generated. The Greengenes reference (v13.8) database was used for assigning taxonomy. The files of Illumina sequencing data were uploaded to the China National Center for Bioinformation under the accession number CRA027259.

2.8 Extraction and real-time quantitative PCR (RT-qPCR) analysis of colon tissue

After the RNA extraction from the mice colon tissue using the TRIzol method, the RNA was reversed to cDNA according to the instructions of PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa, China). The cDNAs were amplified as described in the manual of TB Green® Premix Ex Taq™ II FAST qPCR kit (TaKaRa, China). The sequences of primers are shown as follows: *Zo1* (F AGGACACCAAAGCATGTGAG; R GGCATTCTGCTGGTTACA), *Occludin* (F TTGAAAGTCCACCTCCTTACAGA; CCGGATAAAAAGAGTACGCTGG), *Claudin5* (F GCAAGGTGTATGAATCTGTGCT; R GTCAAGGTAACAAAGAGTGCCA), *Muc2* (F CTCCAACCTCCAGATCCGAG; R TCCACAGTCTTTACCAGATGT). The expression levels of targeted genes were calculated using the $2^{-\Delta\Delta C_t}$ method.

2.9 16S rRNA-sequencing data analysis

The 16S rRNA data analysis was mainly executed in R (4.4.2, R Core Team 2020) and RStudio (2024.4.1.748). The ASV and taxonomy tables generated on the DADA2 workflow were further processed to calculate the compositional abundance at different taxonomic levels using microeco package (V 1.11.0) [27]. Subsequently, the microeco package was also employed to visualize the ternary plot, Upset plot, compositional bar plot, donut plot, alluvial plot and Venn plot. At the ASV level, the distribution of the top 10 ASVs among three groups was compared using circlize (V 0.4.16) and microeco packages [28]. The function `estimate_richness` in phyloseq package (V 1.50.0) was used to calculate the α -diversity of microbial communities and the following statistics were conducted by Kruskal-Wallis's test with multiple comparisons corrected by Dunn's test. The package of psych (V 2.4.12), pheatmap (V 1.0.12) and reshape2 (V 1.4.4) packages were introduced to calculate Spearman's correlation coefficient, visualize the plot and integrate the output data, respectively. Redundancy Analysis (RDA) was performed on microeco object and visualized by ggplot2 (V 3.5.1). In addition, the microeco object was also used to further calculate the Mantel's p and r value

using the linkET (V 0.0.7.4). As with α -diversity, the statistics of relative abundance for *Escherichia* *Shigella* and *Mucispirillum* were conducted using Kruskal-Wallis's test, with multiple comparisons corrected by Dunn's test. We implemented a random forest classifier using the microeco package with Mean Decrease Accuracy metric for feature importance. Feature selection was optimized through Boruta algorithm (maxRuns=300, pValue=0.01) followed by permutation testing (1,000 repetitions, α =0.05).

2.10 Y-maze spontaneous alternation test

The Y-maze apparatus was made of gray plastic, consisted of three compartments (3 cm (W) bottom and 10 cm (W) top, 40 cm (L) and 12 cm (H)) radiating out from the center platform ($3 \times 3 \times 3$ cm triangle), and positioned 60 cm above the floor. In this test, each mouse was placed in the center of the maze facing toward one of the arms and then allowed to explore freely for 5 min. We recorded and analyzed the spontaneous behavioral alternations of the mice using Visutrack (Shanghai, China). A reduction in behavioral alternations corresponds to memory impairment. To exclude the existence of olfactory cues, the apparatus was thoroughly cleaned with 20% ethanol after each trial. The successive entries into arms were recorded and the spontaneous behavioral alternations were calculated as follows: percentage of time spent in the novel arm (%) = ((number of alternations)/(total arm entries-2)) $\times$ 100%.

2.11 Novel object recognition test

The mice were placed in a white, acrylic box ($40 \times 40 \times 40$ cm). During the training trial, mice were allowed to explore two objects placed at two opposite positions at the same distance from the two nearest box corners. Then, the mice were returned to their home cage for 15 min (short-term memory). Subsequently, mice were placed back in the acrylic box for 5 min after replacing one of the previous objects with a new one. Exploration of the objects by the mice was defined as touching the object with their nose or mouth. All trials were recorded and scored manually using Visutrack (Shanghai, China). The percentage of time was calculated using the following formula: percentage of novel object preference = (novel object exploration time/(novel object exploration time + familiar object exploration time)) $\times$ 100%; A value $> 50\%$ indicates preference for the novel object. The percentage of time for the familiar object was calculated in the same way. To exclude the existence of olfactory cues, the box and the objects were thoroughly cleaned with 20% ethanol after each trial.

2.12 Statistical analysis

Graphical data were analyzed with GraphPad Prism 9.0 and presented as mean $\pm$ SEM. Typically, each set of data contains six replicates, while several measurements and tests did not include mice with insufficient tissue samples or mice with overly severe symptoms, as indicated by the figure caption. All data were assumed to be normally distributed for statistical analysis. Statistical significance was determined using two-tailed Student's t-tests to compare two groups and one-way ANOVA followed by Dunn's test for the multiple comparisons of three groups. $p < 0.05$ was considered statistically significant.

3. Results

3.1 (-)-Epicatechin ameliorates DSS-induced colitis

In this study, mice received a two-week oral administration of (–)-epicatechin, with colitis induced during the second week via the provision of 2.5% (w/v) dextran sulfate sodium (DSS) in drinking water (**Fig. 1A**). Compared to the normal control (Ctrl) group, mice in the DSS model group exhibited a pronounced loss of body weight, an elevated disease activity index (DAI), shortened colon length, and severe histopathological damage, confirming the successful establishment of colitis model (**Fig. 1B–H**). Notably, (–)-epicatechin (Epi) supplementation significantly reduced DAI and inhibited body weight loss of colitis mice (**Fig. 1B–D**). Furthermore, the shortening of colon length observed in DSS-treated mice was markedly attenuated by Epi treatment (**Fig. 1E, F**).

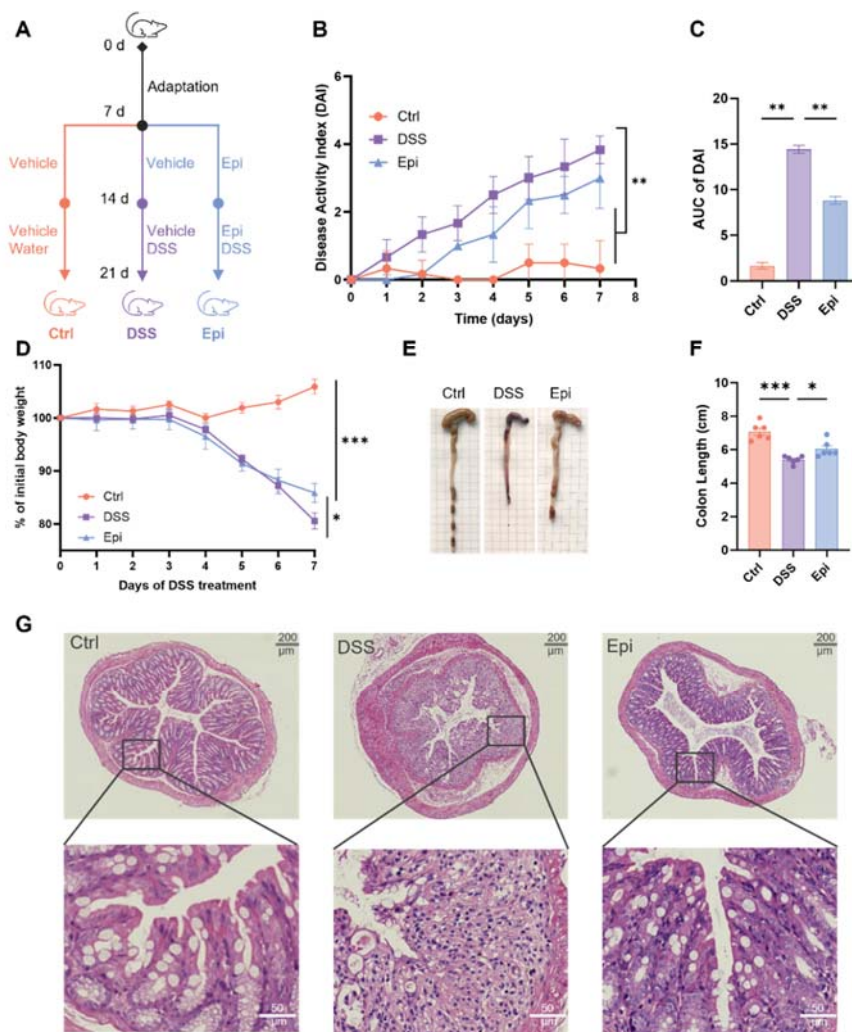


Figure 1. Effects of (–)-epicatechin treatment on colitis severity. (A) Schematic representation of the experimental groups. (B) Disease activity index (DAI) scores were the mean of scores of body weight loss, feces occult blood and diarrhea severity. (C) Area Under the Curve (AUC) of DAI from day 0 to day 7 upon DSS treatment. (D) Body weight change (%) refers to the percentage obtained by dividing the weight of the corresponding number of days by the weight on day 0 upon DSS treatment. (E) Representative images of colon length. (F) Quantification of colon length. (G) Representative images of HE staining of the colon tissue. Data are displayed as the mean $\pm$ SEM ($n=6$). Statistics were performed with one-way ANOVA with Tukey's post hoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Assessment of intestinal inflammation via HE staining revealed that the control group exhibited intact colonic architecture, characterized by well-organized epithelial cells and preserved crypt structures (**Fig. 1G**). In contrast, the DSS-treated group displayed severe colonic damage, including disorganized or sloughed

epithelial cells, marked inflammatory cell infiltration in the lamina propria and submucosa, and disrupted crypt architecture. These pathological alterations were significantly alleviated by (–)-epicatechin intervention (**Fig. 1G**). These findings demonstrate that (–)-epicatechin effectively attenuates colitis-associated phenotypes, histopathological damage and inflammatory levels in the colon.

3.2 (–)-Epicatechin improves disruption of the intestinal barrier in colitis mice

Given that impairment of the mucosal and epithelial barrier is a key element in the etiology of ulcerative colitis [29], we further assessed the expression of mucin and tight junctions in intestinal epithelial cells, as well as functional intestinal barrier leakage. Consistent with previous studies [26, 30], immunohistochemistry analysis confirmed lower expression of *ZO-1* and *occludin* in the epithelial barrier of colitis mice compared with normal controls. Retrograded levels of ZO-1 and occluding in colitis mice were largely restored by (–)-epicatechin intervention (**Fig. 2A–C**). RT-qPCR results further showed that (–)-epicatechin treatment reversed the downregulation of gene expression of *Zo1*, *Occludin* and *Claudin5* in colitis mice, bringing them close to levels observed in normal mice (**Fig. 2D**). Since *Muc2* is the dominant secretory mucin maintaining the protective mucus bilayer, we also quantified its expression level in the colon tissue [31]. Similarly, the (–)-epicatechin treatment evaluated the *Muc2* expression level in DSS-treated mice (**Fig. 2E**). The elevated serum FITC-dextran concentration in DSS mice was markedly reduced by (–)-epicatechin intervention (**Fig. 2F**). Together, these results indicate that (–)-epicatechin effectively restores intestinal barrier function in colitis mice.

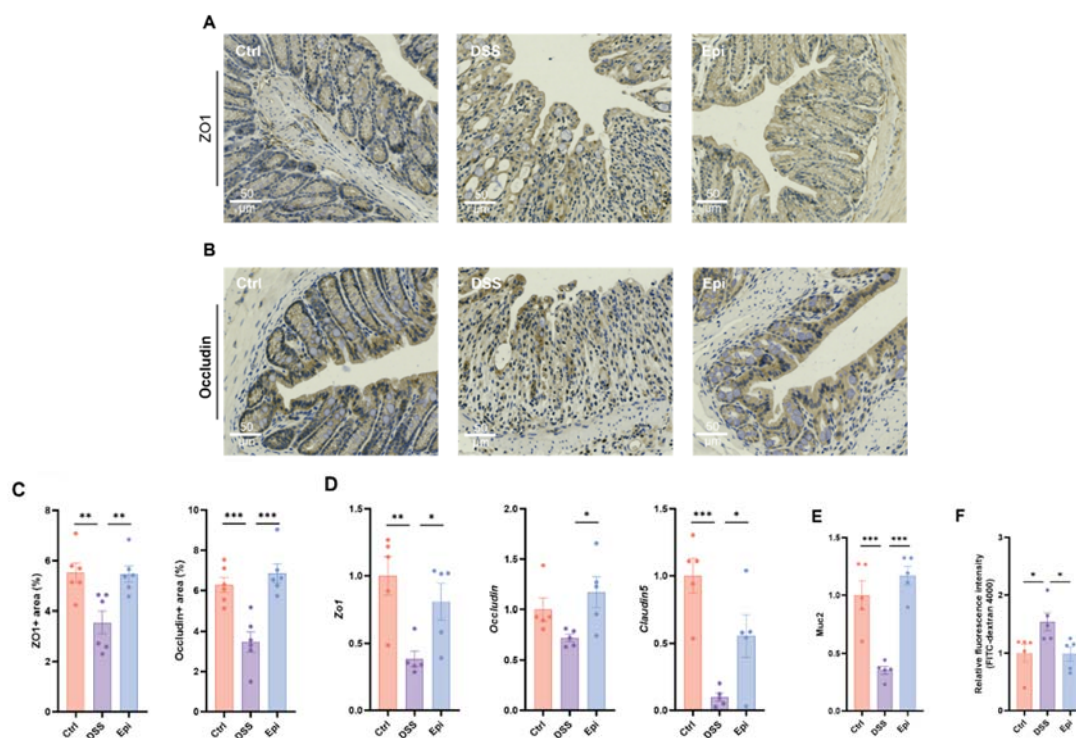


Figure 2. Effects of (–)-epicatechin treatment on intestinal barrier integrity. (A–B) Representative images of tight junction protein ZO-1 (A) and Occludin (B) by immunohistochemical staining in colon tissue. Scale bar: 50 μm. (C) Quantification of ZO-1 (left) and Occludin (right) positive area. (D) The mRNA expression levels of *Zo1*, *Occludin* and *Claudin5* in the colon. (E) The mRNA expression levels of *Muc2* in the colon. (F) The FITC-dextran leakage of the intestinal barrier. Data are presented as the mean ± SEM (n=6, n=5 when the sample tissues of some mice were insufficient). Statistics were performed with one-way ANOVA with Tukey's post hoc test. *P < 0.05, **P < 0.01, ***P < 0.001.

3.3 Effect of (-)-epicatechin on gut microbiota

As the gut microbiota play an important role in the gut-brain axis and provide essential signals to microglia, astrocytes, and oligodendrocytes [32], we first evaluated the differences in microbial community structure among groups. As illustrated in **Fig. 3**, microbial composition was analyzed across phylum, genus and ASV levels. At the phylum level, the gut microbiota composition of normal controls was dominated by Bacillota (formerly known as Firmicutes) and Bacteroidota. In contrast, DSS-induced colitis mice exhibited a marked reduction in Bacillota abundance concomitant with a marked expansion of Proteobacteria, ultimately establishing the latter as a dominant phylum. Notably, (-)-epicatechin administration effectively counteracted these microbial community shifts (**Fig. 3A, S1A**). Upset and Venn analyses revealed 472 core ASVs shared among all three groups, with the DSS group exhibiting reduced ASV diversity compared to controls. Following (-)-epicatechin intervention, both unique and shared ASVs with the control group increased in colitis mice (**Fig. 3B, S1B**).

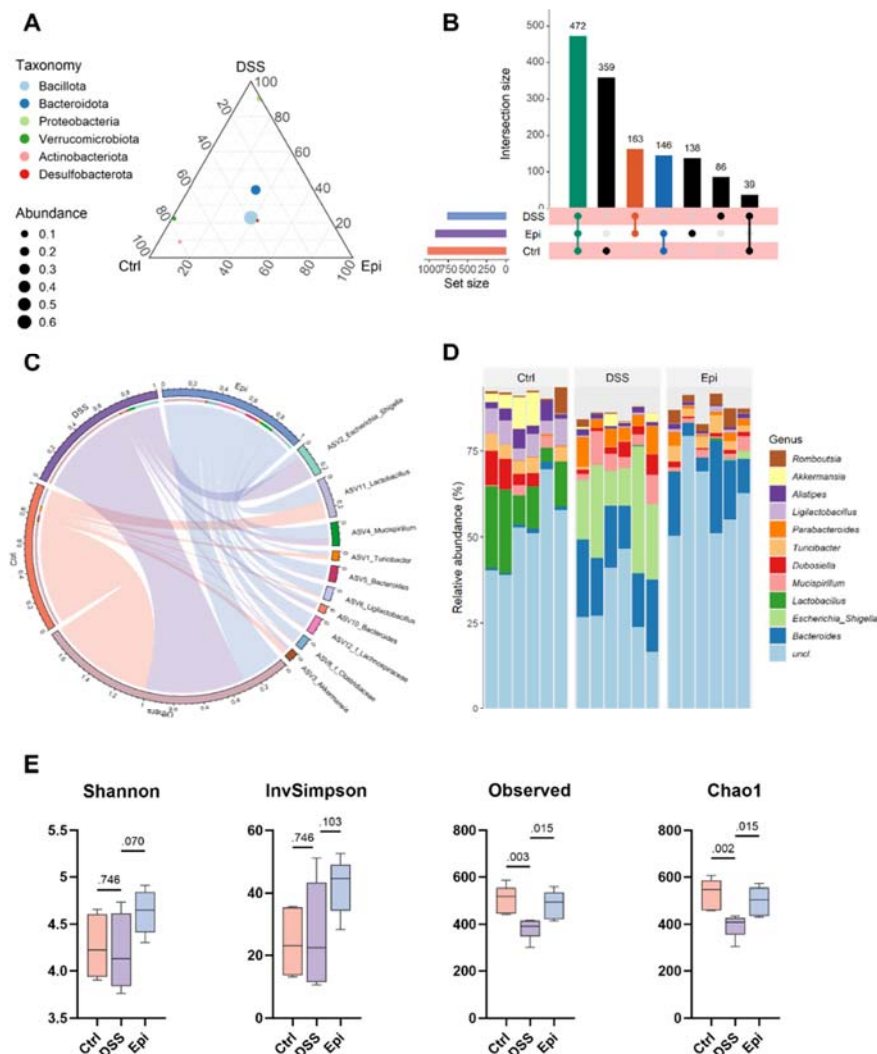


Figure 3. Effects of (-)-epicatechin on colonic microbiota. (A) Ternary displaying the relative abundance of bacterial phyla in each group. (B) UpSet diagram of gut microbiota at ASV level in all three groups. (C) Circos plot displaying the abundance of the top 10 ASVs. (D) Community bar-plot of the relative abundance of gut microbiota on genus level. (E) The alpha diversity indices of gut microbiota in different groups. Data are presented as the mean $\pm$ SEM (n=6). Statistics were performed with Kruskal-Wallis test and multiple comparisons were corrected with Dunn's test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

At the genus level, the relative abundances of beneficial bacteria *Akkermensia* and *Lactobacillus*—known as (next-generation) probiotics—were significantly higher in normal mice compared to colitis mice (**Fig. 3C, S1C, S2C**). Conversely, *Escherichia-Shigella*, the highly destructive bacterium that can accelerate the development of IBD [33], became the dominant genus in DSS-induced colitis mice at both genus and ASV levels (**Fig. 3C-D, S2A, S2C**). The gut dysbiosis in colitis mice could be partly restored by (–)-epicatechin, as evidenced by decreased *Escherichia-Shigella* colonization and increased abundance of ASV11_ *Lactobacillus* and *Tomboutsia*, a genus associated with probiotics-mediated changes in gut microbiota [34] (**Fig. 3C-D, S2A, S2C**). Furthermore, although the Shannon and InvSimpson indices of the microbial communities showed no significant differences, both the Observed and Chao1 indices were significantly reduced in DSS-treated mice. (–)-Epicatechin treatment specifically reversed this reduction in species richness (**Fig. 3E**), suggesting the prevention of species extinction or promotion of recolonization without the full restoration of community evenness. Subsequently, the NMDS analysis was employed to assess the overall similarity of gut microbiota composition between groups. As shown in **Fig. S2B**, the (–)-epicatechin could narrow the distribution distance between the separate clusters of normal mice and colitis mice. Taken together, the above findings indicate that the (–)-epicatechin exhibits therapeutic potential by mitigating colitis-associated gut dysbiosis.

3.4 (–)-Epicatechin alleviates colitis-associated cognitive deficits

Clinical studies have shown that IBD patients often exhibit deficits in attention and executive function, particularly in working memory, suggesting that cognitive impairment is a potential extraintestinal manifestation of IBD [35]. To evaluate cognitive function in mice, we performed a novel object recognition test (NOR) and Y-maze for assessment of spatial working and reference memory in mice (**Fig. 4A, D**). The DSS-induced colitis mice showed cognitive impairment, as indicated by a reduced percentage of spontaneous alternation in the Y-maze and decreased exploration of the novel object in the NOR test (**Fig. 4B-F**). Notably, (–)-epicatechin treatment effectively prevented the occurrence of colitis-associated cognitive impairments as shown in the NOR and Y-maze tests (**Fig. 4B-F**). These observations demonstrate that (–)-epicatechin intervention can significantly mitigate cognitive impairment in DSS-induced colitis mice.

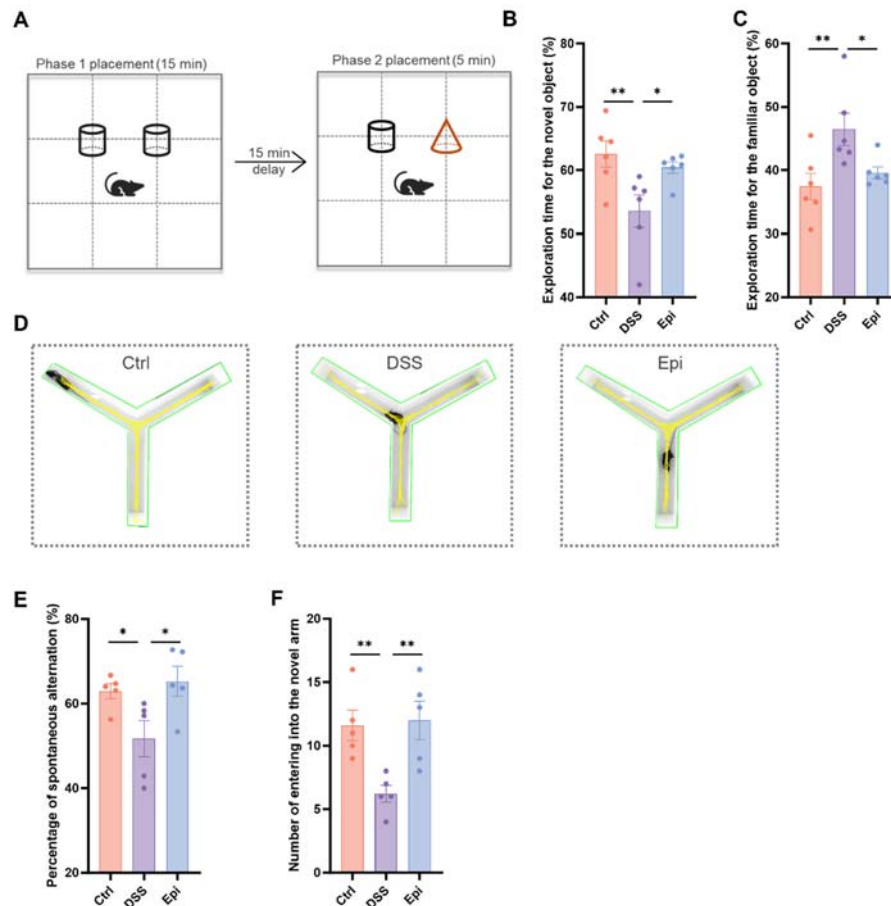


Figure 4. Effects of (–)-epicatechin treatment on cognitive functions. (A) Schematic representation of the experimental design of NOR test. (B) The percentage of the time for the novel object of NOR test was calculated as follows: (novel object exploration time/total exploration time) × 100%. (C) The percentage of the time for the familiar object of NOR test was calculated as follows: (familiar object exploration time/total exploration time) × 100%. (D) Representative maps of tracks in Y-maze test. (E) Quantification of Y-maze alternation percentage calculated as follows: (number of alternations/total arm entries–2) × 100%. (F) Number of mice entering into the novel arm in Y-maze test. Data are presented as the mean ± SEM (n=6, n=5 in Y-maze test since mice with overly symptoms were excluded). Statistics were performed with one-way ANOVA with Tukey's post hoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.5 Effect of (–)-epicatechin on oxidative stress and neuroinflammation

It is well established that key aspects of the pathogenesis of colitis involve oxidative stress and inflammation alterations [36], both of which are also implicated in the pathogenesis of neurological disorders [37]. Thus, we evaluated the total antioxidant capacity (T-AOC) and total superoxide dismutase (SOD) in the brains of mice. As shown in **Fig. 5A-B**, both T-AOC and SOD activity were significantly reduced in DSS-induced colitis mice compared to normal controls. These decreases were effectively reversed by (–)-epicatechin treatment.

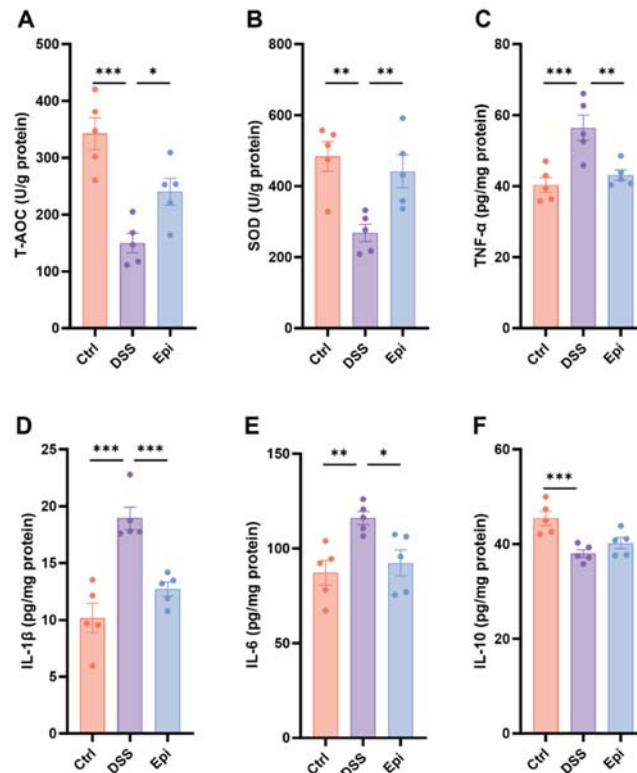


Figure 5. Effects of (–)-epicatechin treatment on oxidative stress and inflammation in brain tissue. (A) T-AOC activity in brain tissue. (B) SOD activity in brain tissue. (C–E) The levels of pro-inflammatory cytokines TNF- α (C), IL-1 β (D) and IL-6 (E) in the brain. (F) The level of anti-inflammatory cytokine IL-10 in the brain. Data are presented as the mean $\pm$ SEM (n=5 due to insufficient tissue of some mice). Statistics were performed with one-way ANOVA with Tukey's post hoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

With respect to neuroinflammation, elevated levels of the pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6 were observed in DSS-induced colitis mice (**Fig. 5C–E**). In contrast, (–)-epicatechin treatment markedly attenuated these pro-inflammatory responses, approaching levels detected in controls (**Fig. 5C–E**). Meanwhile, the anti-inflammatory IL-10 was significantly suppressed in colitis mice and this downregulated trend was inhibited by (–)-epicatechin intervention (**Fig. 5F**). Together, these findings suggest that (–)-epicatechin alleviates colitis-associated brain pathology by reducing oxidative stress and attenuating neuroinflammation.

3.6 Effect of (–)-epicatechin on glial cell functions

Neuroinflammation is an orchestrated process involving multiple types of glial cells in CNS and peripheral immune cells [38]. First, we investigated the effects of DSS-induced colitis and (–)-epicatechin on microglial functions using immunostaining for the microglial cell marker IBA1. Hippocampal microgliosis was evident in DSS mice, as evidenced by the elevated IBA1⁺ cell numbers compared to normal controls (**Fig. 6A, B**). Strikingly, the (–)-epicatechin treatment effectively alleviated this microglial proliferation. Detailed microglia analysis revealed that microglia from DSS mice exhibited reduced complexity, including decreased sum of intersections, ramification index, and ending radius, consistent with an activated microglial phenotype (**Fig. 6C–F**).

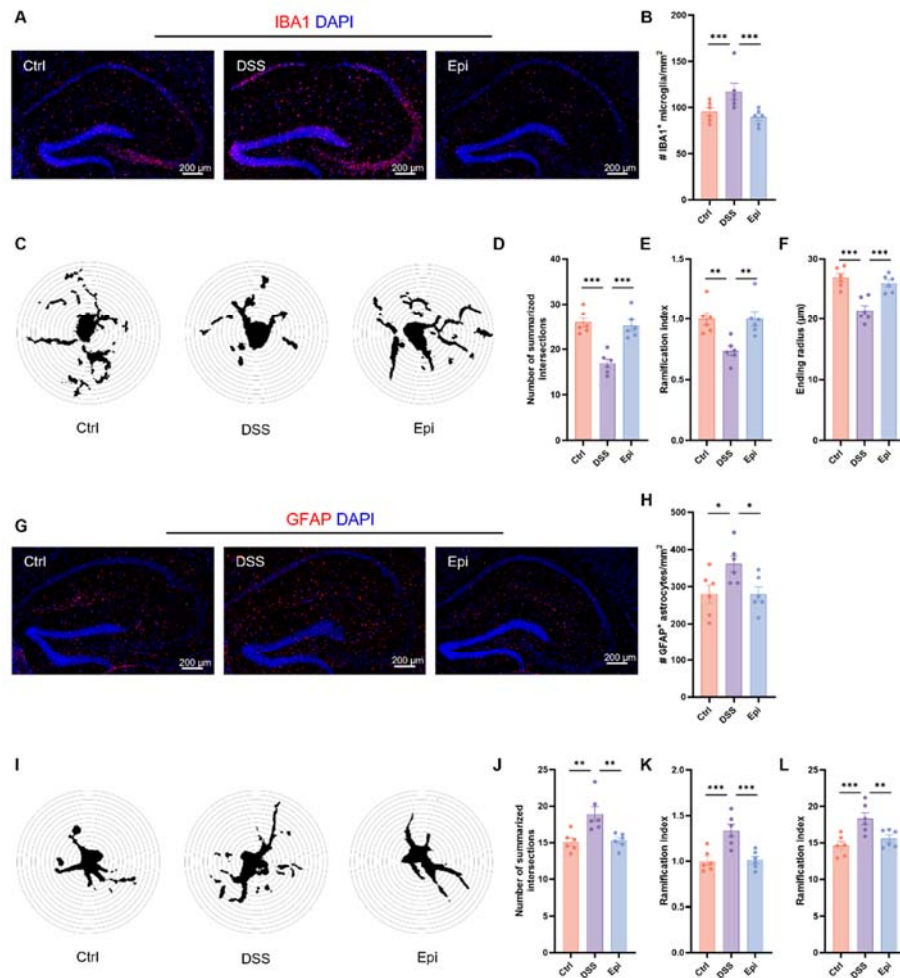


Figure 6. Effects of (–)-epicatechin supplementation on cognitive functions in DSS-induced colitis mice. (A) Representative images of immunostainings for IBA1 in hippocampus. Scale bar: 200 μm . (B) Quantification of the number of IBA1⁺ microglia in hippocampus. (C) Representative image for Sholl analysis of microglia in the hippocampus. The interval of the concentric circles is 2 μm . Quantification of summarized intersects (D), ramification index (E), and ending radius (F) by Sholl analysis from immunostained IBA1 signals. (G) Representative images of immunostainings for GFAP in hippocampus. Scale bar: 200 μm . (H) Quantification of the number of GFAP⁺ astrocytes in hippocampus. (I) Representative image for Sholl analysis of astrocytes in the hippocampus. The interval of the concentric circles is 2 μm . Quantification of summarized intersects (J), ramification index (K), and ending radius (L) by Sholl analysis from immunostained GFAP signals. Data are presented as the mean $\pm$ SEM (n=6). Statistics were performed with one-way ANOVA with Tukey's post hoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

In parallel, the effects of colitis and (–)-epicatechin on astrocytic functions were also evaluated by immunostaining with the astrocyte marker GFAP. Quantitative evaluation revealed a pronounced elevation in hippocampal GFAP⁺ astrocyte density in DSS-induced colitis mice compared with normal mice. Notably, this colitis-associated astrogliosis was significantly attenuated by (–)-epicatechin administration (**Fig. 6G, H**). Sholl analysis further showed that astrocytes in colitis mice displayed heightened complexity, reflected by increased sum of intersections, ramification index, and ending radius, indicative of astrocyte activation. These morphological changes were notably suppressed in (–)-epicatechin-treated colitis mice (**Fig. 6I-L**). These findings collectively demonstrate that (–)-epicatechin effectively counteracts neuroglial activation in the hippocampus induced by intestinal inflammation, suppressing both microglial hyperactivation and astrocytic reactivity.

3.7 Effect of (–)-epicatechin on neuronal functions

As key regulators of CNS homeostasis, astrocytes and microglia play essential roles in maintaining neuronal function [39]. To investigate neuropathological manifestations, we systematically assessed the neuronal integrity and synaptic architecture among groups. Immunofluorescence quantification demonstrated marked reductions in the intensity of presynaptic proteins synaptophysin (SYP) and postsynaptic density protein 95 (PSD-95) in the hippocampus of colitis mice compared to normal controls. This loss of synaptic proteins was substantially inhibited by (–)-epicatechin treatment (**Fig. 7A, B**). Consistent with these findings, high-resolution co-immunostaining images of SYP and PSD-95 revealed significant depletion in synaptic puncta density in colitis mice compared with normal controls, which was also effectively prevented by (–)-epicatechin administration (**Fig. 7A, C**). Moreover, the abnormal activation of microglia actively participates in the phagocytosis of live neurons [40]. Corroborating this mechanism, quantitative analysis indicated substantial neuronal loss in the CA1 and CA3 regions of the hippocampus in colitis mice. Notably, (–)-epicatechin treatment markedly preserved neuronal numbers in these regions (**Fig. 7D, E**). Altogether, these analyses establish (–)-epicatechin as a potent inhibitor against gut inflammation-driven synaptic and neuronal deficits.

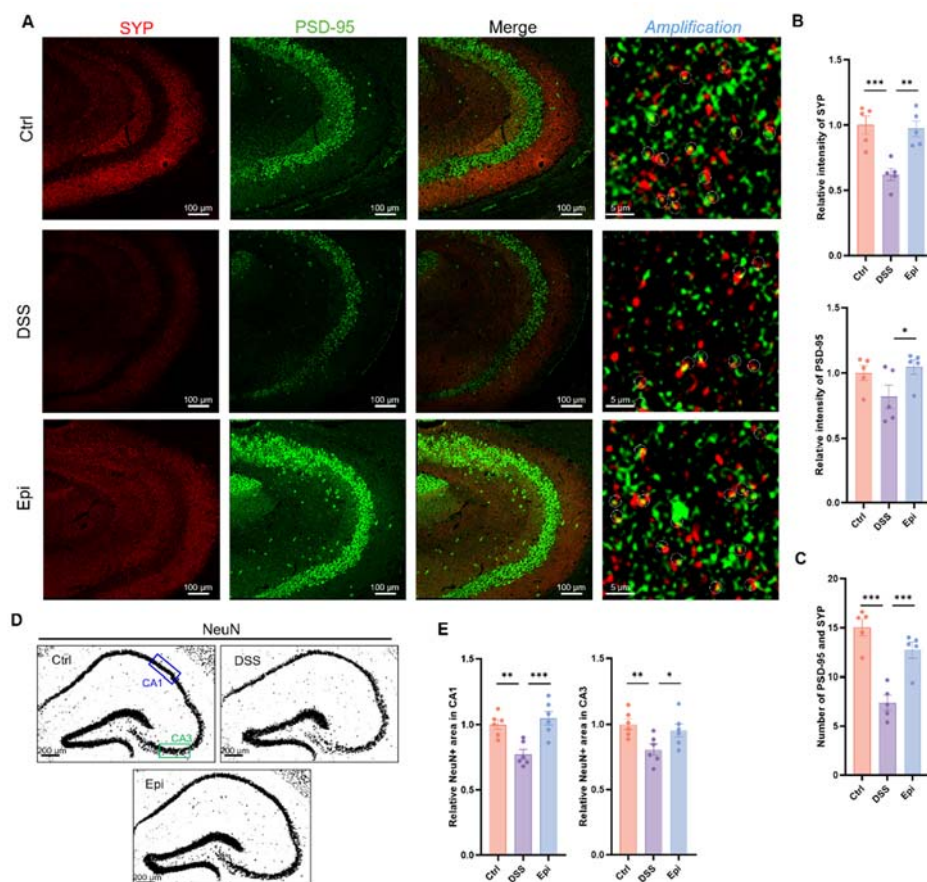


Figure 7. Effects of (–)-epicatechin on neuronal functions. (A) Representative SYP and PSD-95 coimmunostaining and high magnification images in hippocampus. Scale bars: 100 μ m (left) and 5 μ m (right). (B) Quantification of the relative intensity of SYP (upper) and PSD-95 (lower). (C) Quantification of the number of colocalized puncta of SYP and PSD-95. (D) Representative NeuN immunostaining images in hippocampus. Scale bar: 200 μ m. (E) Quantification of the area of NeuN⁺ neurons in hippocampus in CA1 (left) and CA3 (right) regions. Data are presented as the mean $\pm$ SEM. Data are presented as the mean $\pm$ SEM (n=6, n=5 when the sample tissues of some mice were insufficient). Statistics were performed with one-way ANOVA with Tukey's post hoc test. * P < 0.05, ** P < 0.01, *** P < 0.001.

3.8 Gut microbiota correlation with intestinal and brain function upon (–)-epicatechin treatment

Given that gut dysbiosis and compromised intestinal barrier integrity are recognized as primary contributors to cognitive impairment in IBD patients, this study further investigated the interrelationships among gut microbiota composition, intestinal barrier function, and brain behavioral outcomes (**Fig. 8**). Consistent with previous findings, exploration time of the familiar object in the NOR test and GFAP⁺ astrocytes were positively correlated with FITC-dextran leakage and negatively correlated with Muc2 (a key mucus barrier component) (**Fig. 8C**). These results strongly support that intestine barrier dysfunction drives weakened cognition performance in colitis model as previous reports [41].

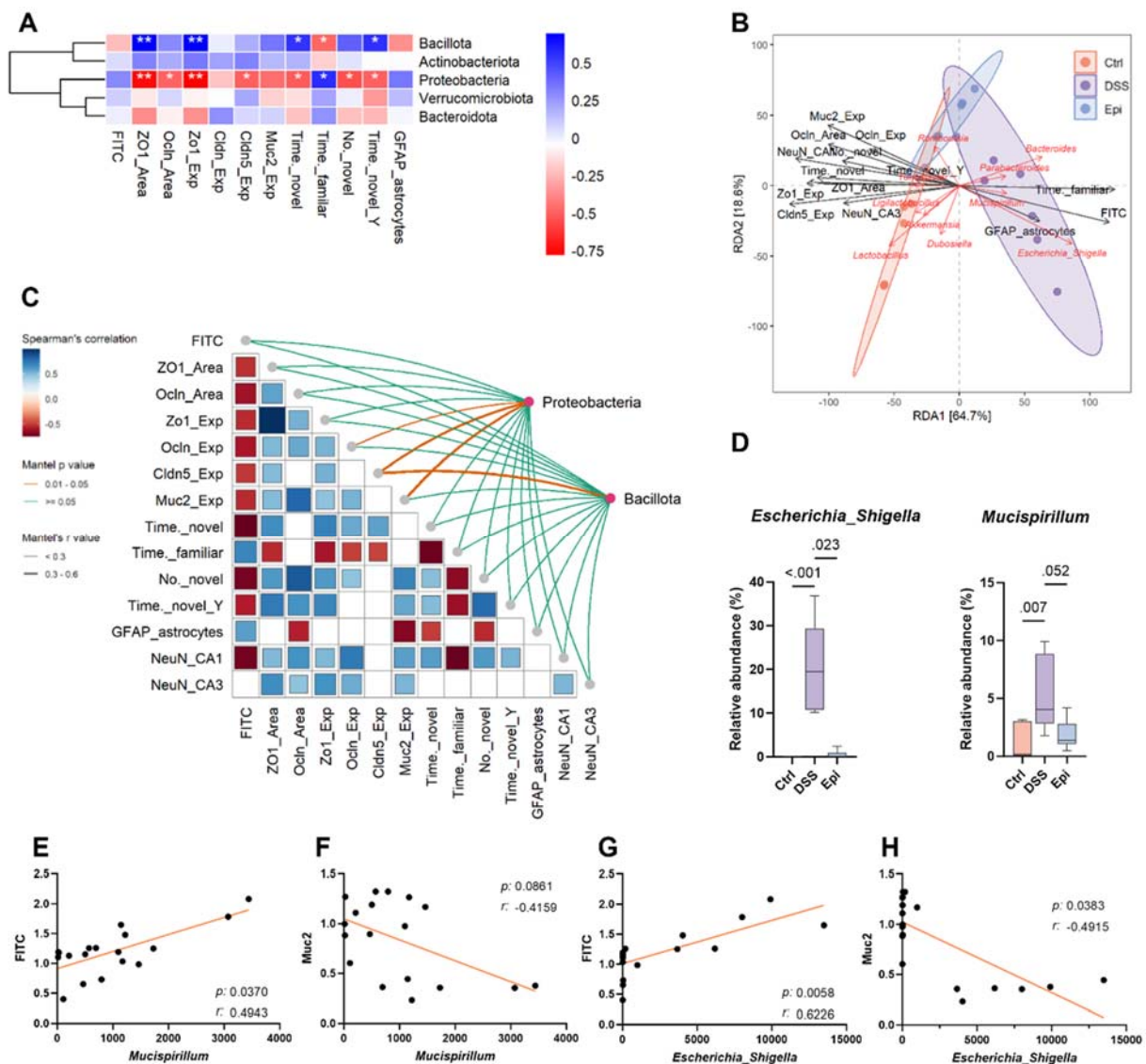


Figure 8. The relationship between gut microbiota, intestinal barrier and brain function. (A) Correlation heatmap of 5 dominant bacterial phyla with indicators of intestine barrier and brain function. (B) RDA analysis diagram of gut microbiota and indicators of intestine barrier and brain function. (C) Spearman correlation and Mantel's analysis among phyla Proteobacteria and Bacillota and indicators of intestine barrier and brain function. The lower triangle matrix displays the Spearman correlation coefficients between indicators (blue for positive, red for negative). Lines represent the significant relationships identified by Mantel's test, where line color indicates the p value type (≥ 0.05 or $0.01-0.05$) and line thickness represents the Mantel's r value (< 0.3 or $0.3-0.5$). (D) Relative abundance of *Escherichia_Shigella* and *Mucispirillum* in the mice of three groups. (E) Correlation between *Escherichia_Shigella* and *Mucispirillum* with indicators of intestinal barrier function. Data are presented as the mean $\pm$ SEM (n=6). Statistics were performed with Kruskal-Wallis test and multiple comparisons were corrected with Dunn's test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

As for gut microbiota, phyla Bacillota and Proteobacteria emerged as dominant taxa linked to both intestine barrier disruption and cognition deficits (**Fig. 8A**). Specifically, Spearman correlation and Mantel test analyses further identified Proteobacteria as strongly correlated with Muc-2 and tight junction proteins (**Fig. 8C**). Redundancy analysis (RDA) identified FITC-dextran levels and tight junction protein expression as explanatory variables of the increased abundance of *Escherichia_Shigella* (a Proteobacteria member) in colitis mice, with opposing directional effects (positive and negative correlations, respectively). Likewise, *Mucispirillum*, the indicator bacterium for DSS-induced colitis, showed a similar pattern in the RDA model (**Fig. 8B**). Relative abundance analysis confirmed that both *Escherichia_Shigella* and *Mucispirillum* were enriched in DSS intervention but restored to near-normal levels following (–)-epicatechin intervention (**Fig. 8D**). Subsequent Spearman correlation analysis revealed that FITC-dextran (positively) and Muc2 (negatively) were significantly correlated with the abundance of these bacterial taxa (**Fig. 8E**). Altogether, all these findings highlight the cognitive benefits of (–)-epicatechin in colitis models are highly likely mediated through the modulation of gut microbiota, particularly via suppression of Proteobacteria (e.g., *Escherichia_Shigella*) and *Mucispirillum*.

4. Discussion

Increasing evidence indicates that green tea and cocoa, along with their derived products, exert beneficial effects on brain function, including anxiety reduction, attention enhancement, and cognitive improvement [42, 43]. Both are recognized as rich sources of flavonoids, particularly flavanols, which exhibit potent antioxidant and anti-inflammatory properties with demonstrated health benefits [44, 45]. Among these flavonoids, the (–)-epicatechin, a major constituent in green tea and cocoa, has been shown to alleviate colitis severity [23, 46]. The gut serves as a gateway for external factors that trigger systemic inflammation and tissue damage. Increased intestinal barrier permeability and dysregulation of the gut microbial ecosystem are pivotal in the pathogenesis of multiple systemic diseases, making the gut a potential therapeutic target [47]. As documented previously, (–)-epicatechin can ameliorate the damage of the intestinal barrier and modulate the gut dysbiosis [10, 11]. In addition, we also observed that (–)-epicatechin can improve colitis-related brain dysfunctions, which was correlated with the changes of residents in the gut microbiota, especially the intestinal barrier-associated bacteria. Our results verified the health-promoting effects of (–)-epicatechin in the gut-brain axis and provided clues regarding relationships among the dietary nutrition, gut microbiota and brain health.

Disruption of the intestinal epithelial barrier is one of the key factors in the etiology of IBD [29, 48]. A compromised intestinal barrier facilitates the translocation of gut-derived substances into systemic circulation, triggering chronic inflammation that contributes to brain pathology [4, 5]. Additionally, oxidative stress plays a significant role in the pathogenesis of IBD [49]. Indeed, we found that DSS-induced colitis impaired tight junction expression and compromised intestinal barrier integrity. Also, we observed elevated neuroinflammation in colitis mice, likely linked to intestinal damage and microbial dysbiosis. Under physiological conditions, cellular antioxidant defenses, including enzymatic antioxidants such as T-AOC and

SOD, protect against oxidative damage to proteins, lipids, and nucleic acids [50]. In this regard, the brain is particularly vulnerable to oxidative stress due to its relatively lower antioxidant capacity [51]. Our results showed markedly elevated oxidative stress levels in colitis mice, which strongly correlated with inflammation levels. (–)-Epicatechin treatment not only attenuated intestinal inflammation relieved, but also significantly reduced neuroinflammation and oxidative stress in the brain.

Astrocytes and microglia, as resident immune cells of the central nervous system (CNS), play crucial roles in both CNS immunity and the maintenance of neuronal homeostasis [39]. Neuroinflammation, one of the earliest manifestations, is the overactivation of astrocytes and microglia [52]. Accordingly, the effects of (–)-epicatechin on glial cells and neuronal cells in DSS-induced colitis mice were explored based on the immunostaining-based morphology assays. We observed that colitis led to hyperactivation of astrocytes and microglia, which was attenuated by (–)-epicatechin treatment. This inhibition aligns with studies demonstrating (–)-epicatechin's ability to dampen Nrf2, AP-1 and NF-κB signaling pathways [53, 54]. Chronic microglial activation may induce excessive synaptic pruning and eventually lead to synaptic loss [55]. Consistent with this, synaptic density was reduced in colitis mice, which may further contribute to the cognitive decline, as we have previously reported [5, 56]. Interestingly, (–)-epicatechin treatment ameliorated these neuronal and cognitive deficits, likely through modulation of glial immune responses and attenuation of oxidative stress. While the specific mechanism of (–)-epicatechin entry into the central nervous system has been rarely investigated, existing literature suggests it may cross the blood-brain barrier directly or via specific transporters, possibly in the form of bioactive metabolites [57, 58]. However, the neuroprotective and anti-neuroinflammatory effects observed in this study are most consistent with an indirect mechanism mediated as reflected by altered microbiota composition, enhanced intestine barrier and modulated oxidative stress and inflammation [59]. Further research is warranted to definitively establish the exact pathway and extent of its penetration into the brain.

Colitis development involves immune dysregulation, genetic susceptibility and environmental factors, but gut microbiota dysbiosis is also one of the key triggers [60]. The gut microbiota is an important participant in gut-brain axis communication and brain health [61, 62]. Previous studies indicate that (–)-epicatechin can ameliorate metabolic diseases such as type 2 diabetes mellitus and hyperlipidemia via reshaping gut microbiota [8, 63]. Therefore, we investigated whether gut microbiota modulation contributes to the alleviation of brain dysfunction by (–)-epicatechin in colitis mice.

DSS-induced colitis led to significant gut microbiota dysbiosis, marked by the expansion of pathogenic taxa such as *Escherichia_Shigella*, depletion of beneficial genera (e.g., *Lactobacillus*) and altered alpha diversity as reflected by decreased Observed and Chao1 indices. These unfavorable changes were partially reversed after (–)-epicatechin treatment in colitis mice. It is noteworthy that the enrichment of key pathobionts *Escherichia_Shigella* and *Mucispirillum* in DSS mice were inhibited by (–)-epicatechin treatment. Both bacteria are known to thrive in inflamed environments and exploit a compromised barrier [64, 65]. This study revealed that the abundances of pathogenic genera *Escherichia_Shigella* and *Mucispirillum* showed

significant positive correlations with gut barrier impairment markers (e.g., elevated FITC values, downregulated tight junction proteins). Therefore, we hypothesize that (–)-epicatechin's primary action is to create an unfavorable environment for these pathogens. On the other hand, current evidence supports that *Escherichia/Shigella* disrupts the barrier via epithelial invasion and pro-inflammatory responses [66], while *Mucispirillum schaedleri* compromises mucus layer integrity [67]. Here, we found that ameliorated colitis-associated cognitive dysfunction in (–)-epicatechin-treated mice was accompanied by restored gut barrier integrity and modulated microbiota composition. Thus, (–)-epicatechin likely attenuates cognitive deficits by suppressing these pathobionts, thereby interrupting their synergistic barrier disruption. This mechanism may underlie the observed reductions in neuroinflammation, hippocampal damage, and oxidative stress, ultimately improving cognitive function. Our findings underscore the "microbiota-barrier-brain axis" as a therapeutic target for gut-brain communication disorders [68], though causality requires validation via fecal microbiota transplantation studies.

5. Conclusion

In conclusion, our study demonstrates that (–)-epicatechin acts as a multifaceted therapeutic agent that alleviates colitis-induced cognitive impairment by targeting the gut microbiota–intestinal barrier interface. Specifically, (–)-epicatechin counteracts gut-brain axis dysfunction in IBD by reversing microbial dysbiosis—notably suppressing pathobionts such as *Escherichia/Shigella* and *Mucispirillum*—restoring intestinal barrier integrity, and attenuating neuroinflammatory responses. These actions collectively facilitate preserved neuronal viability and synaptic function, leading to improved cognitive behaviors. Our findings deepen the mechanistic understanding of catechin-mediated neuroprotection and underscore the potential of dietary catechins as prebiotic adjuvants in managing systemic inflammatory conditions involving gut-brain communication.

Competing interests

The authors declare no competing interests.

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Data availability

Data will be made available on request.

Reference

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