

Mucuna pruriens extract as Prebiotic and Lactobacillus rhamnosus as Probiotic Intervention mitigated histological changes in DNCB-Induced Colitis via GLP-1/Nrf2/NF- κ B Axis Regulation

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Abstract

Ulcerative colitis (UC) is a debilitating inflammatory bowel disorder characterized by epithelial damage, oxidative stress, and dysregulated immune responses. Current pharmacological treatments often present limitations in efficacy and safety. This study investigates the synergistic therapeutic potential of *Mucuna pruriens* extract (MPE), a polyphenol-rich prebiotic, and *Lactobacillus rhamnosus* (LGG), a probiotic strain, in a DNCB-induced colitis rat model. MPE was profiled using LC-MS/MS to identify bioactive constituents, and its anti-inflammatory efficacy was assessed in vitro using Caco-2 cells. In vivo, rats were administered MPE and LGG, individually and in combination, following DNCB-induced colitis. Biomarkers, including GLP-1, NF- κ B, IL-6, IL-1 β , Nrf2, and SCFAs, were quantified via ELISA, immunoblotting, and HPLC. Histological and immunohistochemical analyses evaluated mucosal integrity and protein expression. Results demonstrated that MPE reduced intracellular ROS and inhibited NF- κ B nuclear translocation. Combined treatment with MPE and LGG significantly restored colon morphology, reduced spleen hypertrophy, and suppressed pro-inflammatory cytokines. Notably, GLP-1 and Nrf2 expression were upregulated, and SCFA levels were elevated, indicating enhanced gut barrier function and microbial homeostasis. These findings suggest that MPE and LGG exert complementary effects through improved intestinal mucosal lining and epithelial damage via modulation of oxidative and inflammatory pathways, offering a promising biotherapeutic approach for UC management and functional food development.

Introduction

Ulcerative colitis (UC) is a gastrointestinal inflammatory illness that is non-specific, recurring, and chronic inflammatory bowel disease that affects the Colon and rectum, including immunological dysregulation, genetic susceptibility, and interactions with the gut microbiota, which affects individuals of all ages. (Fanizzi et al., 2024) Its primary pathogenesis is abnormal Th2-type immune activation, which is represented by inappropriate pro-inflammatory cytokine release and impairs the structural strength of the epithelial barrier. Current treatments, including immunomodulators and biologics, provide remission for some individuals; nevertheless, 30% have secondary therapy failure. Infection and cancer risks are also increased by prolonged usage (Noor et al., 2022). These restrictions emphasize the immediate need for safer treatment approaches that focus on advanced molecular mechanisms. Although the incidence rate is rather high among Asians, recent epidemiological research has indicated that North Americans have the highest prevalence of it (Jing et al., 2019). Because UC burdens society, its growing occurrence worldwide is cause for concern. Consisting of several systemic symptoms such as bloody stools, diarrhoea, and abdominal pains, UC is a complicated illness (Collaborators, 2020). *Mucuna pruriens* (Linn.), popularly referred to as the "velvet bean," is indigenous to tropical Central and South America as well as several Asian nations, including Thailand, India, and China (Lampariello et al., 2012) Its seeds were previously utilized in traditional medicine to treat a range of illnesses, such as Parkinsonism and sexual dysfunction (Pathak-Gandhi and Vaidya, 2017). Still, the anti-inflammatory effects of *Mucuna pruriens* seeds in UC are unclear.

The primary components, short-chain fatty acids (SCFAs), are created when gut microbiota ferment dietary fibre like prebiotics, and produce acetate, butyrate, and propionate. SCFAs bind GPR43, and the interactions between SCFAs and GPR43 significantly impact inflammatory responses. A decrease in SCFAs is linked to UC both clinically and experimentally, treating colitis with more SCFA consumption is helpful. Furthermore, evidence links colonic lumen SCFAs to a lower incidence of diabetes (Tian et al., 2018). The human digestive system has around 5000 different bacterial species and, on average, 10 microorganisms/ml of luminal material. Roughly 90% of all bacterial species are members of the phyla Bacteroidetes, which is mostly made up of Gram-negative bacteria, and Firmicutes, primarily Gram-positive bacteria (Holmes et al., 2011). Moreover, it's been suggested that modifications to the gut microbiome promote intestinal permeability and the mucosal immune response, which in turn aid in the onset of diabetes. Pre and Probiotics have the potential to improve the management of IBD and diabetes by altering the gut microbiota (Floch and Montrose, 2005). Prebiotics and Probiotics have been found to be useful adjuvants in insulin resistance therapy and may contribute in maintaining a healthy gut microbiome. Elevated intestinal permeability might potentially aid in the assimilation of antigens that may cause harm to the epithelial lining (Vehik and Dabelea, 2011). GLP-1RA uses a variety of techniques to provide anti-inflammatory effects. Semaglutide treatment for T2D and cardiovascular disease resulted in a considerable reduction in high-sensitivity C-reactive protein (hs-CRP), which is a systemic inflammatory biomarker. These findings suggest that GLP-1RA may have the ability to control inflammation in a range of medical disorders (Balogh et al., 2023). (TNF- α) and (IL-6) are examples of inflammatory cytokines that are prevented from generating and releasing via immune signalling pathway regulation (Bertocchini and Baroni, 2021). Second, GLP-1RAs support the start of anti-inflammatory processes that help reduce inflammation, such as the AMP-activated protein kinase (AMPK) pathway. Moreover, GLP-1RA has proven to be able to lessen the activation of nuclear factor-kappa B (NF- κ B), an essential regulator of inflammation, which in turn reduces the production of inflammatory chemicals (Wei et al., 2016). In this work, we made a hydroalcoholic extract of *Mucuna pruriens* and used UHPLC-Q-Orbitrap HRMS to thoroughly examine its functional metabolites. Here, in this study we have examined the potential prebiotic function of *Mucuna pruriens* along with *Lactobacillus rhamnosus* strain bacteria (probiotic) and the potential protective impact of GLP-1 in DNCB-induced rats in relation to DNCB-mediated colitis and by analyzing intestinal pathological conditions, pro-inflammatory cytokines levels, and the extent of oxidative stress in Caco2 cells, we assessed the therapeutic effectiveness of MPE in treating ulcerative colitis.

Materials and Methods

2.1. Materials

We acquired sulfasalazine (CAT No. S0580), propionic acid (CAT No. P0500), and standard butyric acid (CAT No. B0754) from TCI Chemicals Ltd., Japan. We procured acetic acid (CAT No. 32532) from SRL Pvt. Ltd. Sigma–Aldrich Pvt Ltd., Munich, Germany, provided the glutathione peroxidase (GPx; CAT No. G6137-100UN), malondialdehyde (MDA; CAT No. MAK085), Griess reagent (CAT No. G4410-10G) for

evaluating antioxidant biomarkers, 2,7-Dichlorodihydrofluorescein diacetate (CAT No. D6883), and dinitrochlorobenzene (CAT No. 237329-50G). Rat TNF-alpha (CAT No. MBS2507393), IL-6 rat ELISA kit (CAT No. MBS355410), and a GLP-1 active ELISA kit (CAT No. EGLP-35 K), superoxide dismutase (CAT No. E-BC-K020-M) were purchased from My Bio Source Inc. and Elabscience, respectively.

2.2. Extraction method

The seeds were procured from reputable local merchants and analysed by Dr. P.S. Nagar, an associate professor in the botany department of the Faculty of Science at the Maharaja Sayajirao University of Baroda, Vadodara, 390 002, Gujarat, India. *Mucuna pruriens* was extracted using a modified version of a process that was previously published in the literature. The seeds were dried at room temperature (26°C) and then mechanically milled into a fine powder. *Mucuna pruriens* hydroalcoholic extract was made by using ethanol: water (1:1v/v) and shaking for 48 hours at room temperature. After filtering out the leftover material, Rota vapour was used to pool and concentrate it. The samples were lyophilised for 24 hours at 80°C and 0.02 bar of pressure to get the powdered MP extract (Tavares et al., 2020).

2.3. Phytochemical Screening

2.3.1. Total phenol estimation:

The Folin-Ciocalteu method, outlined in a previous study, was used to quantify phenolic content in a water-based extract. Gallic acid at 5 mg/mL in methanol served as the reference. Different concentrations of gallic acid were prepared for testing. MPE at 1 mg/mL in methanol was also included. After incubation at 40°C for 30 min, the absorbance was measured at 765 nm. Phenolic content was calculated using the formula $y = 0.0049x$ ($R^2 = 0.997$), where C represents concentration, M represents mass, and V represents volume of the extract. (Jimoh et al., 2020)

2.3.2. Total flavonoids estimation:

To calculate the flavonoid content, we used an adapted aluminium chloride spectrophotometric method involving a colorimetric reaction with quercetin standards (Jimoh et al., 2020). Methanolic extracts and standard samples were prepared in a 1:1 ratio. MPE extract and graded quercetin standards were mixed with NaNO₂ and then with AlCl₃ and NaOH before settling and measuring absorbance at 430 nm after a 20-minute incubation. The amount of flavonoids was measured as milligrams of quercetin equivalent per gram of extract using a standard curve with a good fit.

2.4. Phytoconstituent analysis

Using established techniques, a preliminary investigation was done on the MP seed extracts to determine which phytoconstituents were present (Balamurugan et al., 2019; Kancherla et al., 2019). Upon accomplishing the subsequent chemical evaluations, the findings are summarized in Table 1.

Table 1
Estimated Concentration of Total Phenolic and Flavonoid
Components in respective MPE Extract.

S.No.	Phytochemical test	Quantity
1.	Total phenol	782.31 ± 8.49 (mg GAE/g)
2.	Total flavonoid	479.59 ± 4.08 (mg QE/g)

Alkaloids

The presence of alkaloids was confirmed by Dragendorff's test. An orange-red precipitate was produced when 1 mL of Dragendorff's solution was added to 2 mL of the extract, suggesting the existence of alkaloids.

Flavonoids

Flavonoids were detected in the extract using the Shinoda test. A rich pink tone was produced by adding ten drops of diluted hydrochloric acid and a piece of magnesium to one millilitre of extract, indicating the presence of flavonoids.

Terpenoids

Liebermann-Burchard test confirmed the presence of terpenoids. Briefly, filtering 0.5 g of plant extract diluted in 2 ml of chloroform allowed us to detect the presence of terpenoids. A drop of concentrated H_2SO_4 was put into an equivalent amount of acetic acid to filter. There were terpenoids present when the ring was blue-green.

Tannins

Ferric chloride test determined the presence of tannins. It was done by boiling and filtering 0.25 g of plant extract with 10 ml of distilled water. To the filtrate, 1% $FeCl_3$ was subsequently added. Tannins were detected by a brownish-green or blue-black staining (Shanmugavel and Krishnamoorthy, 2018).

Steroids

The Salkowski test was performed to detect steroids. A crimson color was seen, indicating the existence of steroids, after shaking the plant extract with chloroform and adding conc H_2SO_4 along the perimeter of a test tube.

Saponins

The Frothing test detected the presence of saponins. A solution of 0.5 g of plant extract and 5 ml of distilled water was heated to identify saponins. The mixture was aggressively agitated after cooling in order to create a stable, continuous froth (Maryam et al., 2012).

Carbohydrates

These were detected by Molisch's test. Briefly, to 2 millilitres of extract, just a few drops of an alcoholic α -naphthol solution were poured. Afterwards, a small amount of concentrated H_2SO_4 was applied along the test tube's walls. A violet color ring formed, demonstrating the occurrence of carbohydrates at the interface of two liquids.

2.5. UHPLC-Q-Orbitrap HRMS Analysis for characterization

MP extract qualitative profiles and metabolite separation were carried out using the UHPLC system (Dionex UltiMate 3000, Thermo Fisher Scientific, Waltham, MA, USA) outfitted with an autosampler device, a Quaternary UHPLC pump operating at 1250 pressure, and a degassing system. A 0.2 μm PTFE membrane filter was used to filter 1.0 mg of dried extract after it had been diluted in 1 ml of LC-MS-grade methanol with 0.1% formic acid (v/v). Using a thermostated ($T = 40^\circ\text{C}$) Thermo Hypersil GOLD TM C18 column (100 mm $\times$ 2.1 mm, 1.9 μm) and an injection volume of 10 μL at a constant flow rate of 0.4 mL/min. Separation by chromatography was done. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Elution took place using the gradient elution program. The DAD detector captured the UV signals between 190 and 800 nm. The Orbitrap Exploris 240 mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) was assessed for mass spectrometry. It had ESI ionization probes and could operate in both negative and positive ion modes. The following were the HESI (heated electrospray ionization probe) parameters: The temperature of the vaporizer was set at 320°C , and the sheath gas flow rate, auxiliary gas flow rate, and sweep gas flow rate were set at 40, 15, and 0 arbitrary units, respectively. The spray voltage was set at 3500 V for the positive and 2500 V for the negative. A temperature of 270°C was chosen for the ion transfer tube. The RF lens was set at 70% and the mass range was 100 to 3000 m/z with a resolution of 120000. The rate of data gathering was 2 Hz. All MS data acquisition was done in full-scan mode with Thermo Scientific Xcalibur.

2.6. *In-vitro* cell culture Experimentation

2.6.1. Cytotoxicity assay

The intestinal epithelial cell line of humans (Caco-2 cells) was procured from the NCCS in Pune. They were kept at 37°C in a humidified incubator with 5% CO_2 in Dubelcco's modified Eagle's medium (DMEM) incorporated with 10% FBS and an antibiotic–antimycotic solution. 1X trypsin-EDTA was used to passage the cells at a confluency of 70–80%. MPE was evaluated for cytotoxicity in Caco2 cells using the MTT assay in accordance with standard protocol at five serially diluted concentrations: 1000 $\mu\text{g/mL}$, 800 $\mu\text{g/mL}$, 600 $\mu\text{g/mL}$, 400 $\mu\text{g/mL}$, and 200 $\mu\text{g/mL}$.

2.6.2. Assessment of intracellular ROS level

10,000 Caco-2 cells were seeded per well on a 6-well plate to measure intracellular ROS scavenging activity. The cells were cultured for 24 h after being infected with 0.5% DSS, either with or without MPE treatments (250, 500, and 1000 $\mu\text{g/mL}$). When cells were exposed to H2DCFDA dye, ROS oxidation

caused it to be deacetylated by cellular esterases to a non-fluorescent molecule (5(6)-carboxy-2'-7'-dichlorofluorescein). This was then transformed into the fluorescent molecule 2'-7'-dichlorofluorescein (DCF), and following PBS washing, was used to get rid of any remaining dye. After that, the cells were examined and captured on camera using a fluorescent microscope (Zeiss Axio vert. A1) and quantified using Image J software to measure the fluorescence intensity normalized to the control (Zhou et al., 2024)

2.6.3. Immunofluorescence

Immunofluorescence has detected GLP-1 overexpression and the presence and migration of the molecular markers NF- κ B from the cytoplasm to the nucleus. In short, Caco-2 cells were plated in a DMEM high-glucose medium with a density of 10,000 cells per well. Both cells were incubated for 24 hours before being challenged with DSS 0.5% with or without MPE treatments (250, 500, and 1000 μ g/mL). They were then incubated for another 24 hours. The cells underwent two PBS washes after the treatments were completely removed, and then they were fixed for 15 minutes using 4% paraformaldehyde. 0.1% Triton X-100 was used for 30 minutes of permeabilisation, followed by 4% BSA and 0.1% Triton X-100 for an hour of blocking. After rinsing with PBS and incubating the cells overnight with primary antibodies at 4°C, the cells were probed with the relevant fluorescence-conjugated secondary antibody Alexa flour 488, and kept at room temperature for two hours. After staining the nuclei with DAPI antifade solution, the cells were incubated for 20 minutes, twice washed with 0.1% Triton-X 100 in PBS, and finally mounted on slides. A fluorescent microscope (Zeiss Axiovert A1) was used to view the slides.

2.7. *In-vivo* Experimentation

2.7.1. Animals and Experimental Design

The study design received approval from the Institutional Animal Ethics Committee (IAEC) of the National Institute of Pharmaceutical Education and Research (NIPER) with IAEC certificate No. NIPER-H/IAEC/35/22. Each experiment was conducted in accordance with the Committee for Control and Supervision of Experiments with Animals' (CCSEA) rules. The female SD rats, weighing 150–180 g, were bought from NIN, Hyderabad, Telangana. Every animal was acclimated for a week in the animal housing facility of NIPER-Hajipur, Bihar, using sterile husk bedding with a carrying capacity of six rats per cage. All rats were housed in sterile polyacrylic cages with a 24-hour light and day cycle, regulated humidity ($60 \pm 10\%$) and temperature ($25 \pm 2^\circ\text{C}$). Rats were grouped into five groups at random. ($n = 6$ per group): (i) Control group; (ii) DNCB (disease control) group (20 g/l DNCB 300 μ l on nap daily for 14 days); (iii) DNCB + Standard group (sulfasalazine 100 mg/kg). (iv) DNCB + MPE (100 mg/kg as prebiotic), (v) DNCB + *L. rhamnosus* group (1×10^9 CFU/0.2 mL/rat/day) administered by oral route.

2.7.2. Assessment of Colon length, weight and Spleen Index

Every group of animals was sacrificed, and the spleen and Colon were taken out. To get rid of all the trash, PBS was used for cleaning. We measured and compared the colons' length in centimetres and the spleen's weight in grams for each group. Standard weighing devices were used to determine the Colon's weight in grams. Spleen weight (g) was normalised to the body weight (g) in order to compute the spleen weight index.

2.7.3. Proinflammatory cytokines assessment

An ELISA kit was used to quantify the amounts of TNF- α and IL-6 in the colon homogenate sample from My BioSource, Inc. This ELISA was created utilizing the biotin double antibody sandwich method to measure TNF- α and IL-6. IL-6 and TNF- α were measured by adding and treating biotin-labelled anti-IL-6 & anti-TNF- α antibodies. Together with streptavidin-HRP, this combination created an immune complex. After incubation, the unattached enzyme was washed. When substrates A and B were combined, the blue solution became yellow as a result of the acid's reaction. Significant associations were found between the color intensity of the solution and the rat IL-6 and TNF- α levels. (Yuan et al., 2023).

2.7.4. Estimation of SOD

SOD activity in homogenates of colon tissue was evaluated using an ELISA kit from Elabscience. The capacity of SOD to stop phenazine methosulphate from reducing the nitroblue tetrazolium dye was examined. A 1:10:1 ml ratio was used to mix the sample (0.02 ml) with the working solution (50 mM/l phosphate buffer pH 8.5, 1 mM/l nitroblue tetrazolium, and 1 mM/l NADH). The mixture was mixed with 0.02 millilitres of the enzyme working solution to initiate the reaction. Following a 20-minute incubation period at 37°C, the absorbance at 450 nm was determined.

2.7.5. Estimation of Glutathione Peroxidase Activity

Utilizing a glutathione peroxidase test kit from Sigma-Aldrich Pvt Ltd, USA, assessed the glutathione peroxidase activity in homogenates of colon tissue. By converting an organic peroxide to oxidised glutathione (GSSG), glutathione peroxidase activity was indirectly assessed in this procedure. This product was returned to its reduced state by glutathione reductase using NADPH as a cofactor. In terms of protocol, the manufacturer's recommendations were followed. The following formula was utilised to calculate the enzyme activity: $\mu\text{mole/mg}$ of protein.

$$\text{GSSG} = \text{Total GSH} - \text{Free GSH}/2$$

2.7.6. Assessment of oxidative stress and antioxidant parameters

2.7.6.1. Assessment of ROS

The 2,7-dichlorodihydrofluorescein diacetate (H2DCFDA) dye technique was utilised to measure ROS production. Using this technique, colon homogenate samples were treated with H2DCFDA, a fluorescent chemical that, when exposed to ROS, transforms into DCF. In order to perform the test, 10 μl of 10 mM

H2DCFDA was added to 100 µl of colon homogenate. The sample was incubated for 15 minutes in the dark in a 96-well plate. ROS like H₂O₂, OH⁻, and ONOO⁻ oxidised DCFH to form fluorescent DCF. DCF fluorescence was quantified using a Spectramax iD5 multimode ELISA reader and Softmaxpro 7 software at 488 nm excitation and 530 nm emission. (Chattopadhyay et al., 2015)

2.7.6.2. Assessment of MDA

Lipid peroxidation is an accurate indicator of oxidative stress, which is the breakdown of lipids caused by oxidative damage. The oxidative attack on polyunsaturated lipids, which is primarily caused by reactive oxygen species, results in a well-defined chain reaction with end products such as malondialdehyde (MDA). Through the MDA Colorimetric Assay Kit (TBA Method), Thiobarbituric acid (TBA) and the catabolite of lipid peroxide, can react to form a red molecule (Malondialdehyde, MDA) having a maximum absorption peak at 532 nm and read by using a multimode ELISA reader. (Kakkar et al., 1995)

2.7.6.3. Assessment of Nitric oxide (NO)

Griess Reagent was applied to colon homogenate samples in order to convert nitrite to nitrogen oxide. After that, the Griess Reagent and Nitrogen Oxide mix to create a stable product, which was detectable at 540 nm.

2.7.7. Western Blot Analysis

Cell lysate was separated using RIPA lysis buffer and protease inhibitors, and then centrifuged for the *in-vitro* test in Caco-2 cells. The protocol followed for Colon tissues for the *in-vivo* test included sacrificing the rats and separating the colons of each group's animals, processing separately with RIPA buffer and a protease inhibitor cocktail, and then homogenising. After keeping it for 20 minutes at 4°C, the homogenised mixture was centrifuged for 15 minutes at 10,000 g. Various tubes collected the supernatant, and its protein content was quantified using the BCA kit. The tissue and cell lysate were divided into 50 µg portions, received SDS loading dye and underwent centrifugation after boiling. The supernatant was placed onto a 10% SDS page gel. Then, the protein in the gel was moved to a PVDF membrane (0.45 mm) for 1.5 h (Yoo et al., 2008). The PVDF membrane was blocked for an hour with 3% BSA in 1% TBST. It was then incubated overnight at 4°C with primary antibodies (Nrf-2, 1:2000, Thermo Fisher), followed by washing with TBST the next day, and it was then incubated with goat anti-rabbit IgG (1:10,000, Thermo Fisher) for an hour, conjugated with horse redox peroxidase (HRP). Using a Bio-Rad ChemiDoc system, the blots were imaged after being treated with an ECL chemiluminescent substance to visualise the protein bands. Image J analysis Software was employed to measure the blots, and the results were standardised to the housekeeping protein β-actin expression levels.

2.7.8. Measurement level of Insulin

The rat insulin test kit (Elabscience) was used to determine the amount of insulin. After homogenizing 100 mg of tissue with 500 µL of 1x cell extraction buffer, the sample was allowed to cool for 20 minutes. After that, it was centrifuged for 20 minutes at 4°C at 18,000 g. After being collected, the supernatant was used to further estimate the absorbance at 450 nm through a multimode reader.

2.7.9. Measurement Serum GLP-1 level

This assay involves capturing active GLP-1 from the serum using a GLP-1 active ELISA kit that contains a monoclonal antibody. When reference standards with known active GLP-1 concentrations are used with an excitation/emission wavelength of 355 nm/460 nm, were used in conjunction with reference curves made in the same assay, the quantity of active GLP-1 in the unknown sample directly correlated with the amount of fluorescence produced.

2.7.10. Short-chain fatty acid determination from faeces using HPLC

The SCFA standards, acetic acid, propionic acid, and butyric acid, were obtained from TCI chemicals to make a standard plot. All of the groups' mice's faecal pellets were gathered, and faecal SCFA level samples were measured using HPLC. In order to extract faecal SCFAs from every mouse, 100 mg of faecal sample was centrifuged with 1 ml of methanol, followed by 3000× g centrifugation at 20°C for a duration of min (Bihan et al., 2022). A C-18 column and an Agilent 1260 Infinity II were used for HPLC. Acetonitrile: 0.1% orthophosphoric acid (20:80) was the mobile phase ratio, and orthophosphoric acid was used to correct the pH. The measurements were made at 215 nm wavelength, 1 mL/min flow rate, and 20°C column temperature.

2.7.11. Histopathological Examination

Hematoxylin and eosin staining (H&E) was performed using the techniques outlined in previous studies (Nama et al., 2025). Each rat colon was dissected, preserved in 10% formalin for an entire day, and then dehydrated with alcohol. Sections of the colonic tissues, each 5 µm thick, were cut and preserved in paraffin, inspected and photographed specific colonic areas using a fluorescence light microscope set to 10X resolution. The colon histology was inspected in three independent samples per group. Each specimen was given a number that represented the degree of tissue damage and narcotic epithelium destruction.

2.7.12. Immunohistochemical staining

In order to prepare the colon tissues for immunohistochemical examination, animals were anesthetised. Then, 4% paraformaldehyde (pH 7.4) was infused throughout the body of each rat via the left ventricle. After removing the Colon from each rat, they were stored for a full night at 4°C in 4% paraformaldehyde. Then, for three days, the Colon was serially maintained in 10%, 20%, and 30% sucrose solution. Colon sectioning was stored in a 24-well plate and was completed using a Leica cryostat at 30 microns. After being deparaffinized, bovine serum albumin was used to block the samples. The primary antibodies GLP-1 and NF-κβ (1:500, Thermo Fisher) were used in this study. Incubation was done overnight at 4°C. The next day, phosphate-buffered saline (PBS) was used to wash the wells that contained colon portions. The slices were then treated for 30 minutes at 37°C with secondary antibodies (Alexa fluor 488). The nuclei were then specifically labelled using DAPI staining. The parts were then installed after being carefully cleaned. Pictures were taken using a fluorescent microscope (Zeiss Axiovert A1, Germany).

After selecting ten random shots from each rat, at least thirty images from each group of rats were examined.

3. Statistical analysis

To analyse the data, GraphPad Prism version 8 evaluation was used. Following a one-way analysis of variance (ANOVA) to determine the group differences, the Tukey multiple comparisons test was applied. Statistical significance was defined as a p-value of less than 0.05, and the results were presented as mean $\pm$ SD.

Result

4.1. Determination of Total Phenolic and Flavonoid content in hydroalcoholic extract of MPE

For estimation of total phenolic content, the standard curve equation ($y = 203.95x + 1.2669$) was used to describe the phenolic content in gallic acid equivalents (GAE), and the correlation coefficient (R^2) was 0.9987 [Fig. 1A]. The hydroalcoholic extract had the most substantial amount of total phenolic components, measuring 782.31 ± 8.49 mg GAE/g. Following the standard curve equation in ($y = 0.0014x - 0.0351$), the extract's total flavonoid concentration was represented in terms of quercetin equivalents (QE), with a correlation coefficient (R^2) of 0.9956 [Figure 1B]. In this case, the MPE extract had the greatest flavonoid concentration, measuring 479.59 ± 4.08 mg QE/g [Table 1].

4.2. Preliminary phytoconstituent analysis

Preliminary phytochemical screening was performed on freshly prepared extracts to determine whether the MPE extract contained alkaloids, tannins, phenolics, flavonoids, steroids, saponins and carbohydrates as depicted in Table 2. Similar phytoconstituent profiles were presented by hydroalcoholic extracts of the MPE, which also showed positive results for alkaloids, flavonoids, terpenoids, tannins, steroids, saponins, and carbohydrates in the Dragendroff reagent test, Shinoda test, Liebermann Burchard test, Ferric chloride test, Salkowski test, Frothing test and Molisch's test, respectively.

Table 2

Positive results were obtained for all qualitative criteria in the qualitative study of the prebiotic *Mucuna pruriens* extract. + indicates the presence of the respective phytoconstituent in MPE.

Phytochemical constituents	Test	Result
Alkaloid	Dragendroff reagent test	++
Flavonoid	Shinoda test	+
Terpenoids	Liebermann Burchard test	++
Tannins	Ferric chloride test	++
Steroids	Salkowski test	+
Saponins	Frothing test	+
Carbohydrates	General Molisch test	++

Table 2

Positive results were obtained for all qualitative criteria in the qualitative study of the prebiotic *Mucuna pruriens* extract.+ indicates the presence of respective phytoconstituent in MPE.

S.No.	Phytochemical test	Quantity
1.	Total phenol	782.31 ± 8.49 (mg GAE/g)
2.	Total flavonoid	479.59 ± 4.08 (mg QE/g)

4.3. FT-IR Analysis of MPE Extracts

The functional group of active components in the extract of *Mucuna pruriens* was further demonstrated using the band values found in the FT-IR spectrum. Figure 2 shows the numerical values associated with each potential functional group band. Five prominent bands were visible in the flavonoid-enriched MPE extract (between 400 and 4000 cm^{-1}). Peak 1 with 83.95% transmittance shows an O-H stretch in alcohols or phenols, which was usually indicated by a prominent, wide IR absorption band at 3252 cm^{-1} . Peak 2 shows stretching vibrations in the 1600–1400 cm^{-1} region for the carbon-carbon double bonds (C = C), with a band frequency of 1578.6 cm^{-1} . Peak 3 at 1388.16 cm^{-1} was linked to symmetric C-H bending or scissoring vibrations in alkanes, which were a common and strong band in alkane spectra that extended from 1470 to 1350 cm^{-1} . Peak 4 at 1041.66 cm^{-1} was linked to alkyl aryl ether stretching and also represented C–O stretching of the tertiary alcohol. The most likely reason for peak 5 at 514.03 cm^{-1} was a C-X stretch, more precisely a C-Br stretch in alkyl halides, commonly observed C-Br stretch ranges from 690 to 515 cm^{-1} . (Fig. 2)

4.4. Characterization by LC-HRMS

LC-HRMS was used to identify phytoconstituents in the hydroalcoholic extract of *Mucuna pruriens* based on metabolite class, retention time, experimental m/z, MS/MS fragments, and database differences (library). In both the positive and negative ionization modes, MS data were acquired. The majority of the m/z readings in the MPE extract occurred between 170 and 593. It was observed that the prevalent active ingredients in the LC-FTMS-ESI-MS positive and negative modes were N-Acetylneuraminic acid, Bis(4-octylphenyl) amine, 12-Oxo-20-carboxy-leukotriene B4 (LTB4), 5 α -androst-16-en-3-one (androst-16-en-3-one) and Methyl 6-O-[1-methylpropyl]-beta-d-galactopyranoside (Fig. 3). Additionally, both the positive and negative chromatograms were shown (Fig. 3) (Supplementary Table 1). Alkaloids, tannins, phenolics, flavonoids, steroids, saponins, and carbohydrates were found to be abundant in the hydroalcoholic extract of *Mucuna pruriens*, which might account for its biological activity.

4.5. Cytotoxicity evaluation of MPE in Caco-2 cells

The MTT assay was used to evaluate the potential cytotoxic effects of MPE in Caco-2 cells. It was evident from Fig. 4A that MPE by itself did not cause any cytotoxicity in the Caco-2 cell lines up to 1000 μ g as compared to the control group (0 μ g), with an IC₅₀ of 1178 μ g. For the following studies, non-toxic dosages of MPE 250 μ g, 500 μ g, and 1000 μ g were selected based on the previously reported results.

4.6. MPE attenuated intracellular ROS levels in DSS-induced Caco-2 cells

As inflammation occurs, activated macrophages substantially accelerate oxygen absorption, which causes a significant ($p < 0.05$) release of ROS and oxidative damage in DSS 0.5% treated cells compared to the control group. ROS activity inside cells increased as a result of DSS exposure. MPE's antioxidant activity was examined in vitro utilizing a fluorescence microscope and the H2DCFDA staining assessment. With DSS-induced cells, the mean fluorescence intensity of MPE 250 μ g, 500 μ g, and 1000 μ g pre-treated groups can be observed in Fig. 4C. At 250, 500, and 1000 μ g doses, the results showed that MPE significantly ($p < 0.05$) reduced intracellular production of ROS in a dose-dependent manner.

4.7. MPE stimulated GLP-1 activation and diminished NF- κ B nuclear translocation in in vitro

An immunofluorescence experiment was performed in grown human epithelial Caco-2 cells to determine whether MPE's activity affects NF- κ B and GLP-1. It was evident from the findings that MPE treatment significantly ($p < 0.05$) reduced the NF- κ B nuclear translocation in Caco-2 cells at 250, 500, and 1000 μ g doses compared to DSS alone-treated cells. Using the fluorescence-labelled antibody, the translocation status of NF- κ B subunits in Caco-2 cells was observed in Fig. 5C and Fig. 5D. With the findings that MPE decreased NF- κ B activation, this study also tried to determine if MPE can alter GLP-1 signaling in Caco-2 cells. Treatment with MPE at 250, 500, and 1000 μ g doses significantly ($p < 0.05$) elevated the level of GLP-1 in a dose-dependent manner compared to DSS-treated cells and demonstrated its antioxidant effect, as seen in Figs. 5A and 5B.

4.8. MPE suppressed inflammation and elevated GLP-1 through immunoblotting in Caco-2 cells

In order to verify the possible impacts of MPE on the GLP-1/ NF- κ B pathway in Caco-2 cells, the immunoblotting technique was used to assess the protein levels of NF- κ B, GLP-1, IL-1 β , and TNF- α . The levels of these inflammatory proteins, NF- κ B, IL-1 β , and TNF- α , were significantly ($p < 0.05$) increased by DSS 0.5% treated cells. Following MPE therapy, TNF- α , IL-1 β , and NF- κ B protein expression levels were significantly ($p < 0.05$) reduced in a dose-dependent manner when compared to DSS alone-treated cells, as seen in Fig. 6A and 6C&D. From the findings, the level of GLP-1 protein was significantly ($p < 0.05$) reduced in DSS 0.5% treated cells and upregulated the level of GLP-1 in MPE treatment of 250, 500 and 1000 μ g in a dose-dependent manner as compared to DSS alone-treated cells. (Fig. 6B)

4.9. DNCB-induced colitis model

4.9.1. Impact of Prebiotics and Probiotics on Spleen Index, Colon Length and Weight Parameters

A DNCB-induced inflammatory model was established in order to investigate the positive effects of probiotics (LGG) and prebiotics (MPE) on colitis. It demonstrated that topical DNCB application causes the usual symptoms of IBD colitis, including colon weight, length, reduced food intake, diarrhoea, and even hematochezia. Additionally, over-activated immune activity and the severity of chronic colitis could show up as splenomegaly and shortened colon length. According to our study's findings, the rats in the DNCB-treated group had enlarged spleens and shorter colons (Fig. 7). Nonetheless, MPE and LGG therapy effectively ($p < 0.05$) reduced the Colon's length and the spleens' expansion, which were comparable to those of the DNCB group. When compared to the normal control group, the 20g/l of 300 μ l DNCB therapy significantly ($p < 0.05$) increased colon weight and decreased colon length. Whereas oral co-treatments of MPE (100 mg/kg) and LGG (1×10^9 CFU/0.2 mL/rat/day) increased the colon length and lowered the colon weight significantly ($p < 0.05$) in rats as compared to the DNCB group, demonstrating the protective effect of both MPE and LGG in the DNCB model.

4.9.2. Prebiotic and probiotic decreased the level of proinflammatory cytokines and upregulated antioxidant enzyme activity

Pro-inflammatory cytokine levels, such as TNF- α and IL-6, significantly affect how severe inflammation is in IBD. The disease control group's colonic homogenate concentration levels of pro-inflammatory cytokines, IL-6, and TNF- α were significantly ($p < 0.05$) increased as compared to the normal control group, as seen in (Fig. 8. A&B). In contrast, as compared to the DNCB group, the levels of these pro-inflammatory cytokines were significantly ($p < 0.05$) decreased in the prebiotic (MPE) and probiotic (LGG) treated groups. Similarly, the group that received 100 mg/kg of sulfasalazine (standard) likewise showed an attractive impact (Fig. 8). Low levels of glutathione and SOD, as well as a change in the activity of

antioxidant enzymes, are characteristics of DNCB-induced UC, which harms the intestinal mucosa. GSH and SOD levels were significantly ($p < 0.05$) reduced in DNCB-treated groups than in the normal control group, as seen in (Fig. 8.C&D). However, compared to the DNCB group, prebiotic (MPE) and probiotic (LGG) therapy significantly ($p < 0.05$) raised GSH and SOD levels.

4.9.3. Effect of Prebiotic and Probiotic on Oxidative stress and lipid peroxidation

Oxidative stress is linked to the pathophysiology of chronic UC-related colorectal cancer and plays a role in the development and maintenance of intestinal inflammation in UC. MDA levels had significantly ($p < 0.05$) increased in rats administered DNCB in comparison to the control group. whereas MDA levels had been successfully lowered by probiotic (LGG) and prebiotic (MPE) treatment when compared to the DNCB group of rats (Fig. 9A). The administration of DNCB increased NO levels and exacerbated nitrative stress in comparison to the control group ($p < 0.05$). However, compared to the disease control group, the prebiotic (MPE) and probiotic (LGG) treatments significantly ($p < 0.05$) decreased NO activity (Fig. 9B). The ROS levels in the colon regions of DNCB-induced rats were significantly ($p < 0.05$) increased as compared to the normal control group. In contrast to the disease control group, ROS levels were significantly ($p < 0.05$) lower in the prebiotic (MPE) and probiotic (LGG) treatment groups (Fig. 9C).

4.9.4. Effect of Pre and Probiotic on the expression of NF- κ B, Nrf2, IL-1 β , IL-6, GLP-1R, GPR-43/41 through Western Blotting

The production of inflammatory cytokines is triggered by extracellular stimuli, including DNCB, which also causes oxidative stress and further activates the NF- κ B pathway. Consequently, immunoblotting analysis was carried out to determine if MPE and LGG suppress the NF- κ B signalling pathway. Rats with colitis treated by DNCB showed a substantial ($p < 0.001$) increase in NF- κ B, IL-1 β , and IL-6 ($p < 0.001$) (Fig. 10). whereas probiotic and MPE therapy significantly ($p < 0.001$) reduced NF- κ B phosphorylation and decreased IL-1 β and IL-6 levels. Inflammatory cell survival in UC may be considerably increased by Nrf2. Furthermore, an inverse correlation between Nrf2 and NF- κ B activation has been identified. Therefore, we next looked at Nrf2's potential function in this colitis model. Nrf2 was expressed at far lower levels in DNCB control rats, whereas MPE and probiotic therapy considerably ($p < 0.001$) increased that expression. In colitis rats, MPE and probiotics markedly ($p < 0.001$) increased the expression of the GLP-1R protein, GPR 41 and GPR43 protein expression as compared to the disease control group. (Fig. 10)

4.9.5. Insulin assessment and GLP-1 level of rat serum

Rat serum insulin levels in the DNCB group were substantially ($p < 0.05$) reduced compared to those in the normal control group. Conversely, compared to the DNCB group, the level of insulin was significantly ($p < 0.05$) elevated in the prebiotic (MPE) and probiotic treatment groups. GLP1 levels in the DNCB groups were found to be significantly ($p < 0.05$) lower compared to the normal control group of rats. GLP-1 was quantified using an ELISA kit, and it was discovered that the treatment group, which included

Mucuna pruriens (100 mg/kg) and the standard group, had far higher levels of GLP-1 than the DNCB group. Probiotics by themselves raise GLP1 in comparison to the DNCB group. (Fig. 11)

4.9.6. Assessment of SCFA in faeces

The results of the analysis of faecal samples revealed a substantial ($P < 0.05$) increment in the concentrations of SCFA in treatment with both prebiotic and probiotic as compared to the disease control group (Fig. 12). The levels of SCFA in the disease control group were considerably ($P < 0.05$) reduced as compared to the normal control group. The calibration curves for butyric acid, propionic acid, and acetic acid ($n = 3$) that were created using the standard solutions showed acceptable linearity for all standards using HPLC. The calibration curves for the typical short-chain fatty acids and regression analysis showed high correlations with the regression line for all criteria, with a correlation value of 0.995.

4.9.7. Histopathological analysis

From the findings with histopathology of the Colon, normal control rats' colons showed intact epithelial surfaces and normal mucosa. Following DNCB treatment, there was significant ($P < 0.05$) necrotic epithelium damage, which was indicative of colitis. Probiotic (1×10^9 CFU/0.2 mL/day/rat) and MPE (100 mg/kg) treatment reduced the degree of damage and demonstrated a protective effect by strengthening the epithelial layer. Standard sulfasalazine (100 mg/kg) had a comparable protective impact on disease control. A common semi-quantitative grading approach for colonic inflammation score (often a 0–4 scale) based on epithelium damage, infiltration, and architectural distortion [Fig. 13F]. They found that the colonic mucosa in healthy control animals had normal architecture, with an intact epithelial lining and a score of 0 for inflammation [Fig. 13A]. Significant ($P < 0.05$) inflammatory infiltration and epithelial necrosis were seen in animals given DNCB, which is suggestive of a severe inflammatory response with an inflammation score of 3–4 [Fig. 13B]. With an inflammation score of 1, the MPE group's treatment demonstrated improved mucosal protection, low inflammatory infiltration reflecting mild inflammation, and notable preservation of mucosal structure [Fig. 13D]. The probiotics group significantly ($P < 0.05$) reduced mucosal damage and partially restored epithelial integrity, suggesting a moderate anti-inflammatory impact compared to the DNCB group, which had an inflammation score of 1–2 [Fig. 13. E].

4.9.8. Immunohistochemical analysis

An immunofluorescence assay was completed for GLP-1 and NF- κ B expression levels. Present results showed that the GLP-1 expression levels of rats given DNCB were significantly ($P < 0.05$) lower than those of the control group (Fig. 14A). These results implied a role for the GLP-1 pathway in the colonic inflammation brought on by exposure to DNCB. Crucially, GLP-1 expression was significantly raised by prebiotic and probiotic treatment ($P < 0.05$). Contradictory DNCB-treated rats showed a substantial ($p < 0.05$) increase in NF- κ B expression (Fig. 14C). whereas probiotic and MPE therapy significantly ($p < 0.05$) reduced NF- κ B phosphorylation, suggesting that treatment might alter this particular pathway to provide its colonoprotective benefits.

Discussion

Globally, there have been 6.8 million recorded cases of UC, with a frequency that ranges from 79.5 to 84.3 instances per 1,00,000 people. 5-Amino salicylic acid, glucocorticoids, immunomodulators, and antibiotics are being used to treat UC. However, many medications have serious adverse effects like gastrointestinal problems, glaucoma, high blood sugar, nausea, vomiting, and even the emergence of treatment resistance over time, so finding a workable treatment is still a key concern (Feuerstein et al., 2019). Consequently, there is a natural medical necessity for ulcerative colitis treatment that is suitable for prolonged use. A significant medicinal plant, *Mucuna pruriens*, belongs to the Fabaceae family. It is indigenous to regions of tropical Central and South America as well as several Asian nations, such as Thailand, India, and China (Sathiyarayanan and Arulmozhi, 2007). According to several studies, *Mucuna pruriens* extracts have demonstrated potent antioxidant and anti-inflammatory properties in several types of in vivo models of Parkinsonism and neuroinflammation. Plant components include naturally occurring substances called flavonoids, phenolic compounds, and other metabolites, which have a significant antioxidant activity. Reactive oxygen species can donate an electron to phenolic and flavonoid molecules, creating far more stable phenoxyl radicals. Therefore, it is important to identify those substances in plants. (Pandey and Rizvi, 2009). We found the greatest flavonoid concentration in the MPE extract, measuring 479.59 ± 4.08 mg QE/g and phenolic content 782.31 ± 8.49 mg GAE/g. Primary phytoconstituent analysis of MPE also found positive results for the occurrence of alkaloids, flavonoids, terpenoids, tannins, steroids, saponins, and carbohydrates. A range of primary and secondary plant metabolites known as essential phytochemicals, which have anti-inflammatory, anti-diabetic, anti-microbial, and other recognized biological properties, are abundant in medicinal plants and herbs (Saiful Yazan and Armania, 2014; Seth and Sharma, 2004). LC-HRMS analysis of MPE, we estimate the common metabolites N-Acetylneuraminic acid, Bis(4-octylphenyl) amine, 12-Oxo-20-carboxy-leukotriene B4 (LTB4), 5 α -androst-16-en-3-one (androstenone) and Methyl 6-O-[1-methylpropyl]-beta-d-galactopyranoside in both positive and negative ionization mode, which have potential biological activity. While the UC molecular mechanism involves the production of free radicals and the elevation of inflammatory cytokines, substances with anti-inflammatory and antioxidant properties may be useful in treating DNCB-induced ulcerative colitis. (Han et al., 2022) In addition to regulating blood sugar, the gut hormone GLP-1 also possesses anti-inflammatory, anti-apoptotic, and antioxidative qualities. While some studies have shown that GLP-1 alleviates UC symptoms and decreases inflammation brought on by macrophages, the underlying mechanism is still unknown (Mahdy et al., 2025).

In this work, we evaluated the anti-inflammatory properties of *Mucuna pruriens* seeds and *Lactobacillus rhamnosus* in a rat model of ulcerative colitis produced by DNCB and the anti-inflammatory activity of MPE in Caco-2 cells *in-vitro*. The pathophysiology of UC is now well understood because of research on oxidative stress and inflammatory pathways. The Caco-2 cell line is another widely used model for inflammatory research that mimics the structure and functionality of intestinal epithelial cells in humans. (Singh et al., 2018) Comparing the Caco-2 cells to the control (0 μ g), the administered molecule MPE shows no cytotoxicity up to 1000 μ g. The pathophysiology of ulcerative colitis is significantly influenced by the increased production of ROS, which damages cellular DNA, proteins, lipids, and organelles while

also impairing the integrity of the cell membrane. In order to assess ROS levels and ascertain MPE's antioxidant properties, we have used the H2DCFDA test in DSS-stimulated Caco-2 cells. MPE scavenged intracellular ROS levels dose-dependently, demonstrating strong antioxidant qualities. (Wang et al., 2023) We discovered that the only fraction of MPE that significantly inhibits NF- κ B translocation is the hydroalcoholic fraction. The NF- κ B and GLP-1 immunofluorescence test in DSS-stimulated Caco-2 cells further confirmed these results by demonstrating that MP extract administration resulted in downregulated NF- κ B expression and nuclear translocation and activated the GLP-1 pathway (Joshi et al., 2020). As a major mediator of inflammation, NF- κ B interacts with a variety of immunological receptors. The release of inflammatory cytokines is triggered by extracellular stimuli like DSS, which causes oxidative stress and further activates the NF- κ B pathway. To verify the strong validation of MPE on the GLP-1/ NF- κ B pathway in Caco-2 cells, immunoblotting was used to assess the protein levels of NF- κ B, GLP-1, IL-1 β , and TNF- α . When compared to the DSS group, MPE treatment significantly decreased the levels of TNF- α , IL-1 β , and NF- κ B protein expression and upregulated GLP-1 in a dose-dependent manner. These *in-vitro* anti-inflammatory effects inspired the selection of MPE for additional investigation of its anti-inflammatory, antioxidant, and anti-colitis characteristics in an *in-vivo* model of ulcerative colitis induced by DNCB in SD rats.

The DNCB, by working as a hapten, triggers a secondary immune reaction when applied topically. Constant use speeds up the immune response by changing colonic antigens, which ultimately results in tissue necrosis and edema. (Rabin and Rogers, 1978) Treatment with DNCB causes the immune system to become hyperactive and generates a lot of inflammatory mediators, which negatively affect the colonic mucosal epithelial lining and cause ulcerative colitis to develop on its own. Rats given DNCB showed an enormous rise in colon weight, a noticeable decrease in colon length, a loss of stool consistency, damage that was obvious at the macroscopical level, and a rise in the spleen weight index. (Habbas et al., 2025) These findings were further validated by histopathologically examining colon tissue with elevated epithelial and mucosal destruction as compared to the normal control group. A 14-day treatment with prebiotic and probiotic therapy at doses of 100 mg/kg and (1×10^9 CFU/0.2 mL/rat/day), respectively, restored colon weight, reduced colon length loss, and increased spleen weight index. In addition to preventing the colonic epithelium damage caused by inflammatory cell infiltration in colon tissue, further prebiotic and probiotic therapy restored the histological changes. Gastrointestinal inflammation positively correlates with oxidative stress, which alters the intestinal tract's redox equilibrium. However, persistent and long-term production of ROS leads to an imbalance between antioxidant defence systems and free radicals, which results in oxidative damage. (Mundhe et al., 2019) According to our findings by oxidative stress measures, the MPE and probiotic significantly reduced the ROS, MDA, and nitrite levels in UC caused by DNCB. In patients with ulcerative colitis, inflammation usually begins in the rectum, where it is most active, and progresses down the Colon's varying length. Increased inflammatory mediator levels have been reported in the literature, suggesting that pro-inflammatory cytokines such as IL-6, TNF- α , IL-1 β , and associated signaling pathways are essential for the development of ulcerative colitis. (Fan et al., 2009) From our study, further MPE and probiotic

administration considerably reduced the generation of cytokines that promote inflammation, such as IL-6 and TNF- α .

The present work demonstrated that by preventing colonic inflammation, preserving the intestinal barrier, and controlling the gut microbiota, oral infusion of prebiotics and probiotics as GLP-1 agonists lessens the impact of tissue damage in DNCB-induced colitic rats *in vivo* and the anti-inflammation activity of MPE in Caco-2 cells *in vitro*. GLP-1 appears to provide protection against DNCB in the Colon of rats. Interestingly, GLP-1 reduces inflammatory reactions *in vivo* by preventing AKT/NF- κ B and MAPK signalling molecules from becoming phosphorylated. According to the present research, GLP-1 may be a viable treatment option for UC prevention (Anbazhagan et al., 2017). Inflammatory cytokines, including TNF- α , IL-1 β , and IL-6, can initiate the pathophysiology and accelerate the advancement of UC. Clinical research suggested that monoclonal antibodies against TNF- α and anti-IL-6 may have some ameliorative impact on colitis patients. It was suggested that UC is relieved by GLP-1's function in suppressing inflammation. In this work, GLP-1 impacted the protein expressions of TNF- α , IL-6, and IL-1 β in UC rats and suppressed the activity of MDA (Krasner et al., 2014). Furthermore, from western blotting findings, probiotic and MPE therapy significantly reduced NF- κ B phosphorylation and decreased IL-1 β and IL-6 levels with increased GLP-1, GPR-41, and GPR-43, which showed GLP-1 can inhibit the translocation of NF- κ B from the cytosol to the nucleus. GLP-1 is the key marker that suppresses oxidative stress via inhibition of the NF- κ B pathway. Macrophages participate in the innate immune response, cause tissue damage, generate proinflammatory cytokines, and aid in the onset of UC (Yan et al., 2018). From the immunofluorescence study of GLP-1 and NF- κ B, we found that MPE and Probiotic treatment upregulate the GLP-1 expression and suppress the phosphorylation of NF- κ B in colon tissue as compared to the DNCB group. These results imply that the protective function of GLP-1 is associated with its capacity to control cytokines that promote inflammation. By balancing cellular homeostasis and triggering a number of signalling pathways that target inflammation, including the NF- κ B pathway, Nrf2 activation can reduce inflammation (Wardyn et al., 2015). Several investigations have demonstrated a connection between the NF- κ B and Keap1/Nrf2 pathways. The first finding suggests that Nrf2 may indirectly regulate NF- κ B activity via suppressing MafK protein (Ahmed et al., 2017). Secondly, Keap1 can prevent IKK β from being ubiquitinated and degraded, which in turn prevents NF- κ B from being activated. Third, by producing inflammatory mediators and then interacting with Keap1 to stimulate the production of Nrf2, the inflammatory response can suppress NF- κ B activity (Liu et al., 2008). Thus, we next examined the possible role of Nrf2 in this model of colitis. To achieve this, we examined Nrf2 expression by immunoblotting in colon tissue from rats. MPE and probiotic therapy significantly increased Nrf2 expression, while DNCB control rats showed much lower levels of this protein. In summary, Nrf2 activation in the gut can minimise intestinal damage and inflammatory action by inhibiting inflammatory pathways or lowering the overreaction of oxidative stress.

In UC, destruction of the intestinal barrier and inflammation of the intestinal mucosa frequently coexist with a decline in the no. of bacterial flora in the gut that have historically been linked to beneficial effects on the host (Dou et al., 2020). GLP-1 improved the variety and richness of the gut microbiota in colitic animals caused by DNCB. DNCB stimulation had the greatest effect on the relative abundance of

Lactobacillaceae; however, GLP-1 administration allowed Lactobacillaceae to endure and thrive. By changing the makeup of the intestinal microbiota and regulating immune responses, *Lactobacillus rhamnosus* can help relieve the symptoms of inflammatory bowel disease. Lactobacillus was crucial in controlling the diversity of the gut flora. Because anaerobes like Lactobacillaceae may create short-chain fatty acids, which are isolated by faeces analysis by HPLC, from the HPLC analysis, we found significantly increased levels of SCFA (acetic acid, propionic acid and butyric acid) in the MPE and probiotic treatment group as compared to the DNCB group. They are linked to creating good bacteria by raising the gut's concentration of SCFA (Zhai et al., 2018). According to a recent study, there is a connection between the risk factors for the development of UC and the makeup of the intestinal microbiota. GLP-1 therapies were shown to enhance Lactobacillaceae and Bifidobacteriaceae while decreasing Ruminococcaceae and Bacteroides. Ruminococcaceae was shown to be abundant in UC patients' faeces in clinical investigations (Jiang et al., 2020).

According to analyses conducted using Western blotting, GLP-1 and GPR-41, GPR-43 are implicated in pathways linked to translation of NF- κ B/Nrf2, immune system abnormalities, environmental adaptability, and cell motility. (Shen et al., 2018). According to our research, taking GLP-1 to maintain the equilibrium of energy and protein to reduce intestinal inflammation with the epithelial membrane, these metabolic pathways may also have some anti-inflammatory properties. (Chen et al., 2021). This study concentrated on the protective as well as beneficial effects of GLP-1 in rats with colonic inflammation produced by DNCB. Intestinal microbiota regulation and membrane damage reduction are two benefits of GLP-1. GLP-1 is therefore a potentially effective marker for intestinal inflammation.

Conclusion

According to the data obtained, *Mucuna pruriens* can be employed as a prebiotic supplement for *Lactobacillus* strains. When *Mucuna pruriens* hydroalcoholic extract was tested for its anti-inflammatory properties, it was found to lessen DSS-induced inflammation in Caco-2 cells and DNCB-induced colitis in rats. The findings suggest that *Mucuna pruriens* hydroalcoholic extract and *Lactobacillus rhamnosus* can restore gut microbiota by lowering inflammation and SCFA synthesis. GLP-1 markers GLP-1R, GPR43, and GPR41 analysis demonstrated that *M. pruriens* modulates Colon GLP-1 levels. All of the data together showed that *Mucuna pruriens* and *Lactobacillus rhamnosus* administration significantly prevented colitis in rats with DNCB-induced colitis model by regulating aberrant microbiota-gut-brain axis, with a GLP-1/Nrf2/NF- κ B axis modulation-based anti-colitis mechanism. *Mucuna pruriens* might be a cutting-edge prebiotic ingredient used in food to treat colitis.

Declarations

Conflict of interest

The authors state that they have no financial or commercial relationships that may be interpreted as a conflict of interest during the study.

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Nil

Author Contribution

L.N. wrote the original draft, collected the data, and performed all experimentation. R.D. and A.B. reviewed the manuscript. R.G., A.R., P.K. and K.M. validated the method and reviewed the manuscript. G.G., V.R. and N.K. designed the study, performed the Data analysis, supervised the study, and reviewed the final draft. All authors reviewed the final draft.

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Tables

Table 1. Estimated Concentration of Total Phenolic and Flavonoid Components in respective MPE Extract.

S.No.	Phytochemical test	Quantity
1.	Total phenol	782.31± 8.49 (mg GAE/g)
2.	Total flavonoid	479.59 ± 4.08 (mg QE/g)

Table 2. Positive results were obtained for all qualitative criteria in the qualitative study of the prebiotic *Mucuna pruriens* extract. + indicates the presence of the respective phytoconstituent in MPE.

Phytochemical constituents	Test	Result
Alkaloid	Dragendroff reagent test	++
Flavonoid	Shinoda test	+
Terpenoids	Liebermann Burchard test	++
Tannins	Ferric chloride test	++
Steroids	Salkowski test	+
Saponins	Frothing test	+
Carbohydrates	General Molisch test	++

Figures

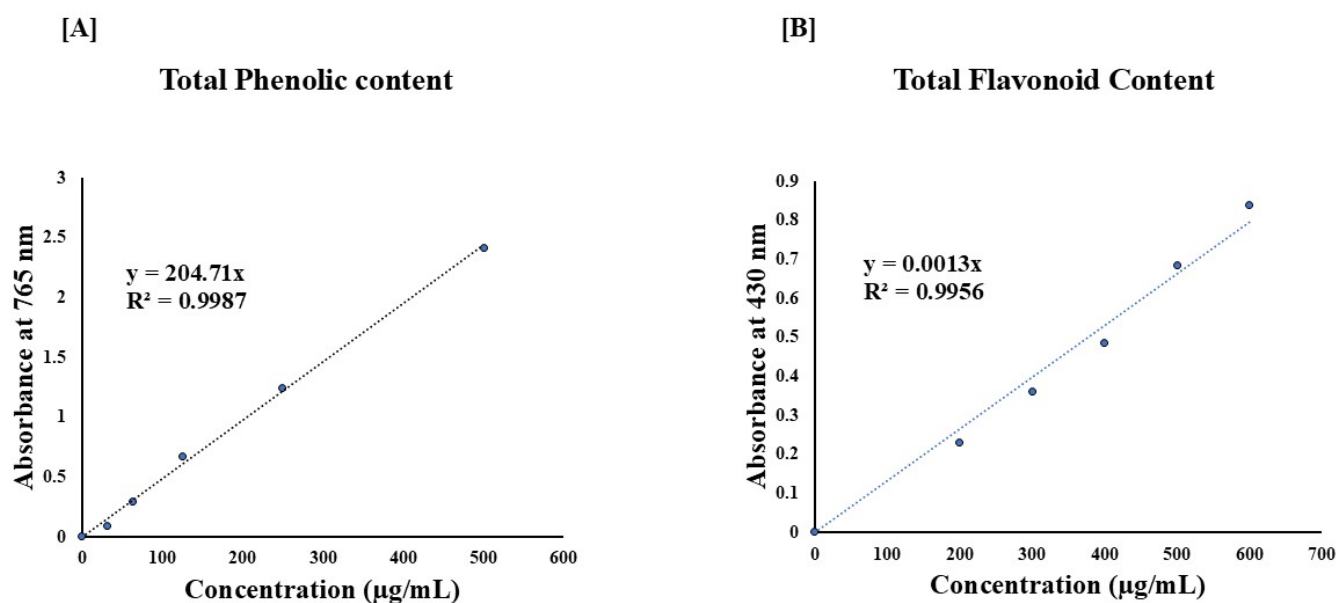


Figure 1

(A) Calibration curve for the estimation of the total phenolic content in the MPE extracts using gallic acid as a reference component. (B) calibration curve for estimating the total flavonoid content of MPE extracts using quercetin as a reference component. All results are presented as means $\pm$ SD for the two tests in the table mentioned (Table 1).

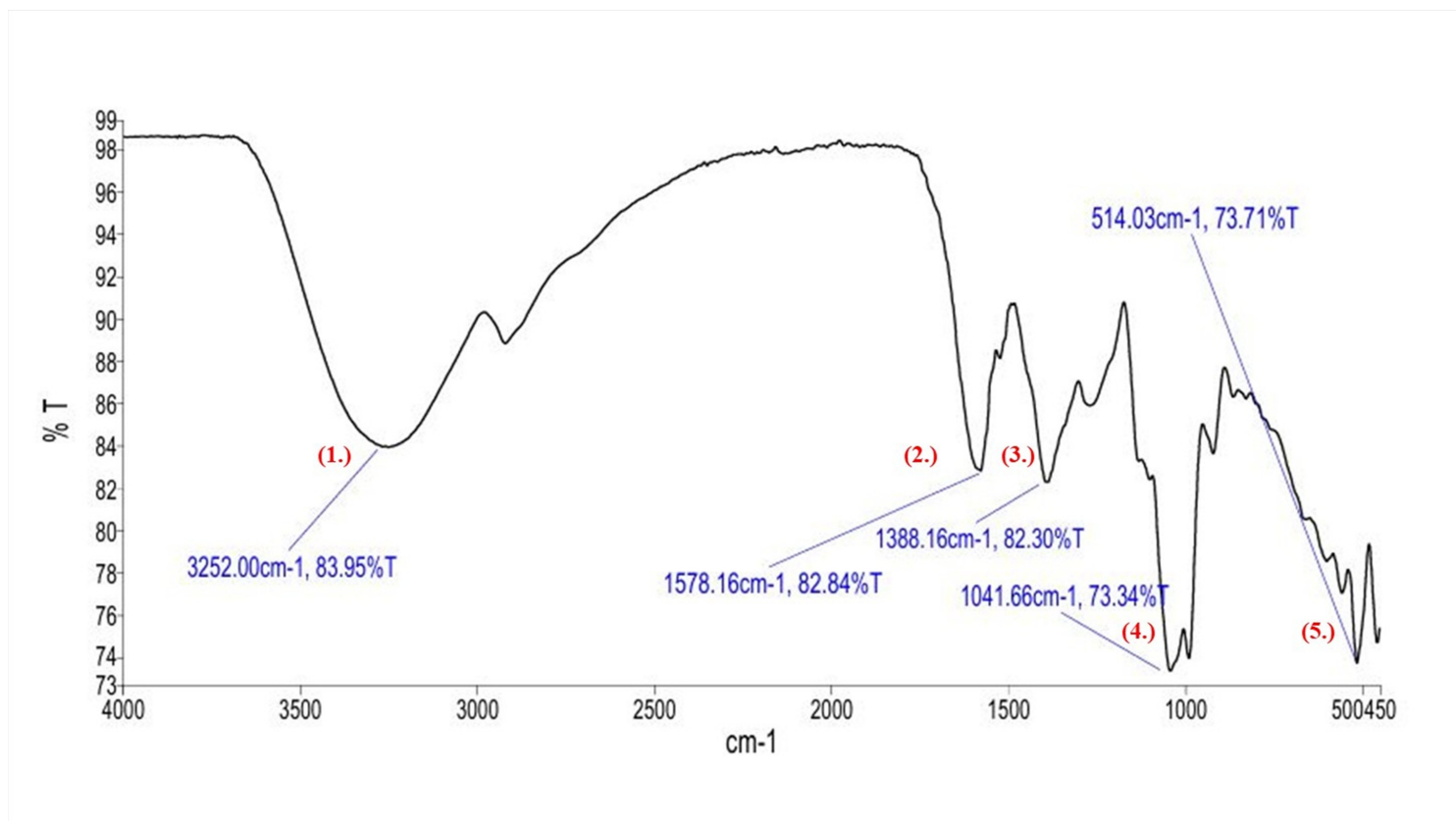


Figure 2

FT-IR spectrum representing potential bands in the *Mucuna pruriens* extract.

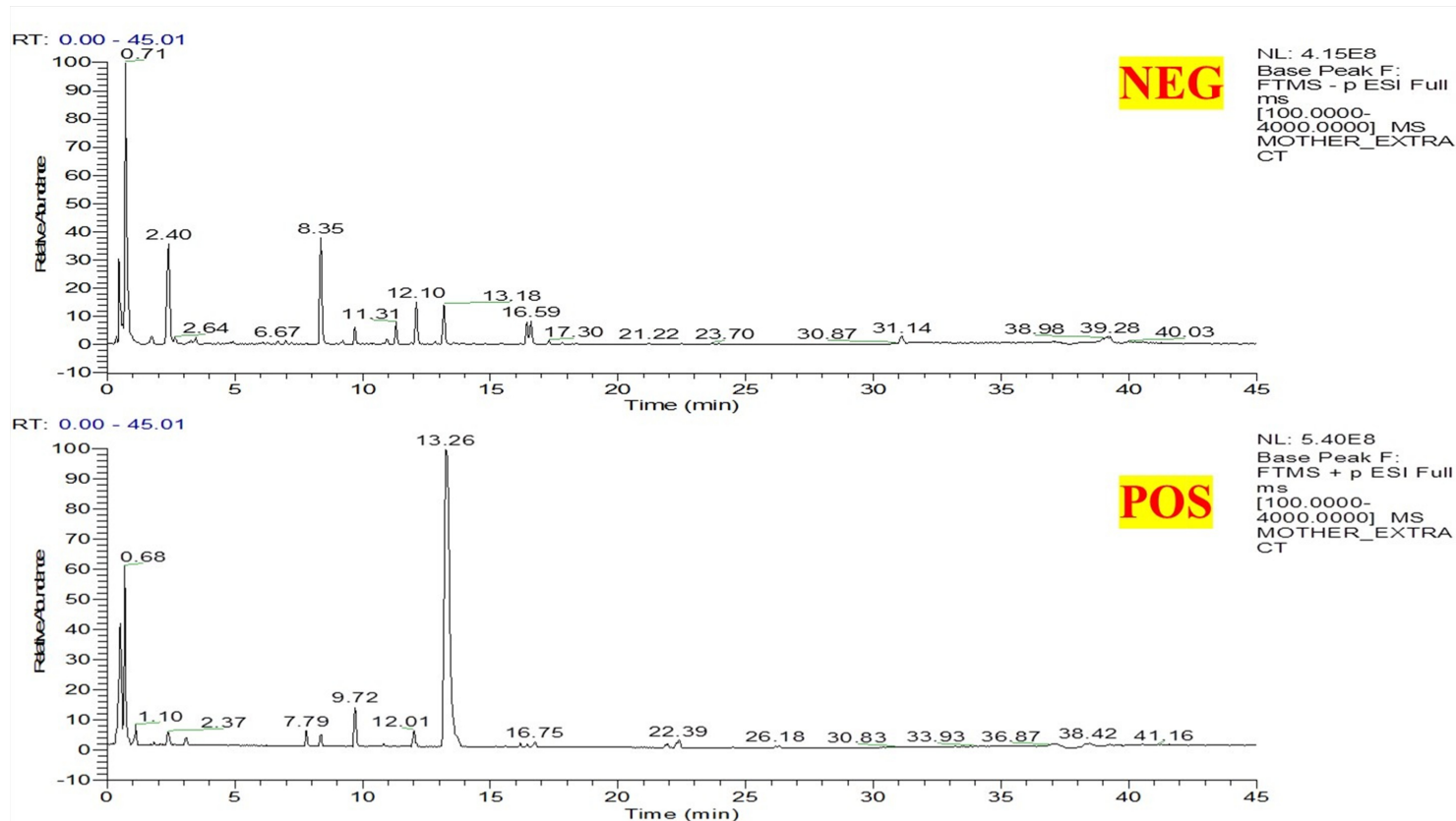


Figure 3

Mucuna pruriens FTMS-LC/MS/MS investigation. *Mucuna pruriens* chromatograms from FTMS-LC/MS/MS operate in both positive (POS) and negative (NEG) ionization modes.

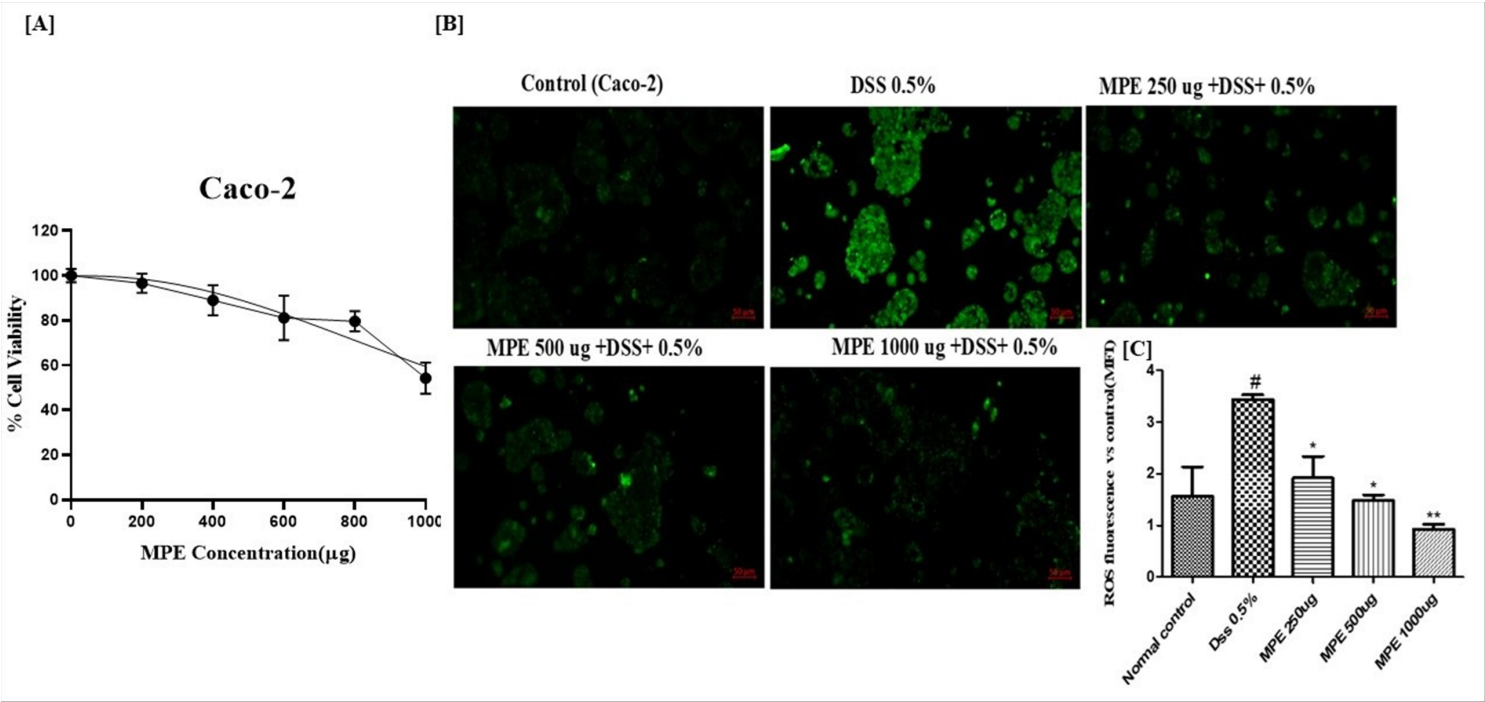


Figure 4

In vitro, MPE shows strong anti-inflammatory properties. [A] Potential cytotoxicity of MPE in Caco-2 cells at concentrations ranging from 0 µg to 1000 µg.[B] H2DCFDA staining indicates the oxidative stress caused by ROS production in DSS-driven Caco-2 cells treated with 250 µg, 500 µg, and 1000 µg of MPE. [C] Graphical representation of ROS fluorescence quantification. Values are expressed as mean ± SD. #p < 0.05 vs control *p < 0.05 vs DSS 0.5% control, **p < 0.01 vs DSS 0.5%.

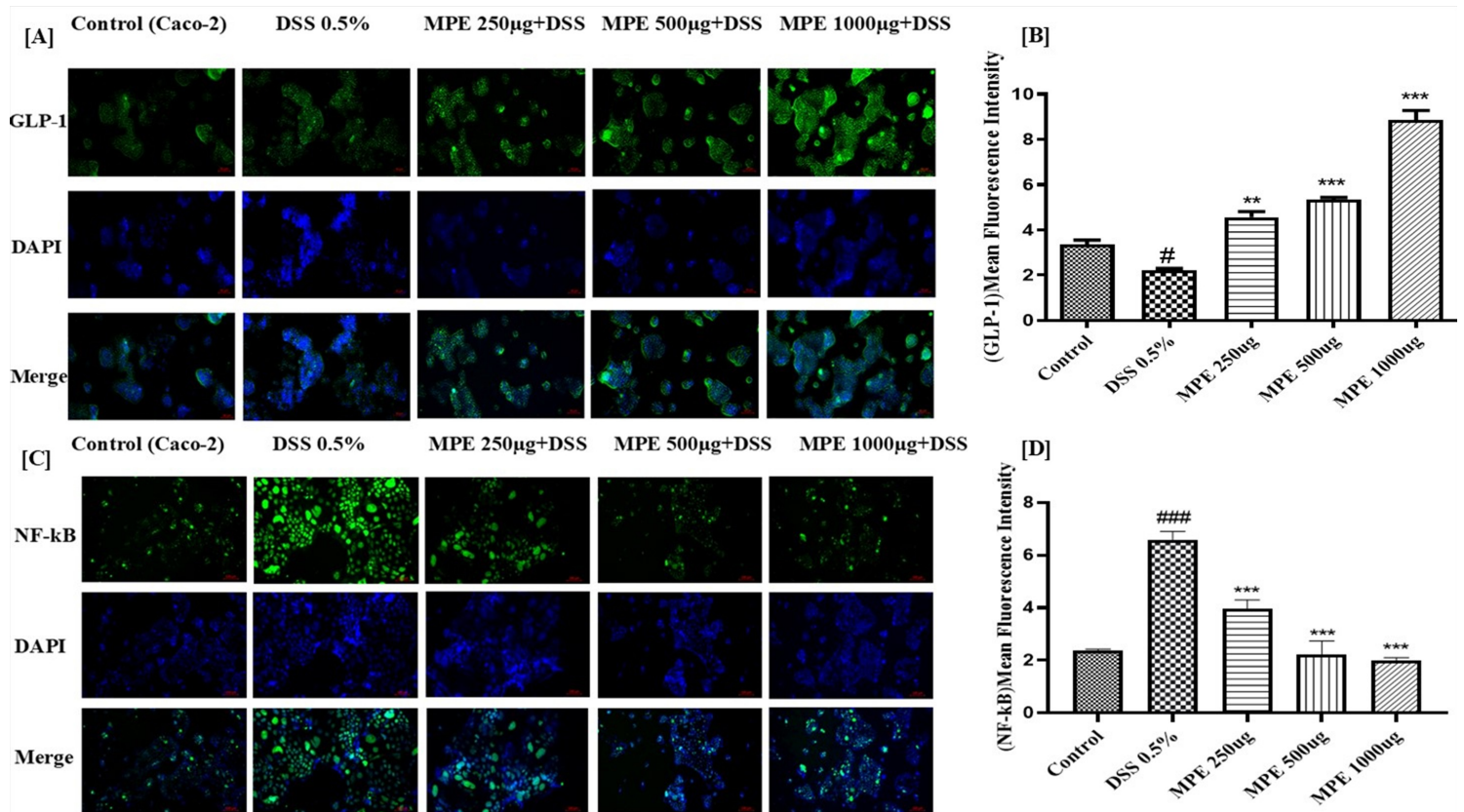


Figure 5

[A&C] Immunofluorescence of the GLP-1 and NF- κ B proteins revealed representative images of oxidative stress and inflammation in DSS-induced Caco-2 cells. MPE at 250 μ g, 500 μ g, and 1000 μ g triggered the GLP-1 pathway and prevented NF- κ B nuclear translocation. [B&D] Activation of GLP-1 pathway and Co-localization of NF- κ B in DSS-driven Caco-2 cells treated with MPE and probiotic is shown graphically. Magnification at 10X and Values are expressed as mean $\pm$ SD. ###p < 0.001 vs control, #p < 0.05 vs control ***p < 0.001 vs DSS 0.5%, **p < 0.01 vs DSS 0.5%.

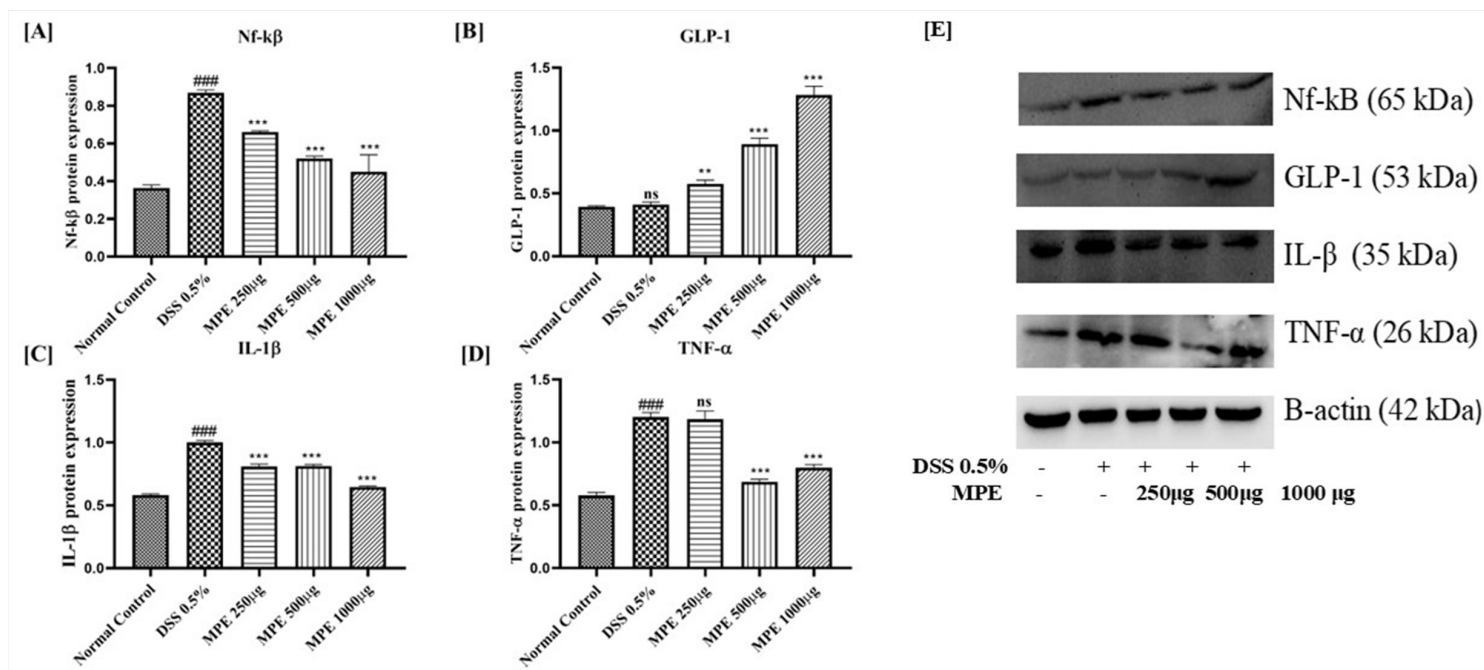


Figure 6

MPE and Probiotic therapy triggered GLP-1 anti-inflammatory pathways and suppressed NF-κβ-mediated inflammation in Caco-2 cells. [A]. Quantification of NF-κβ levels using western blots, shown graphically [B]. The GLP-1 levels Using Image J software, band intensities were analyzed and normalized using β-Actin. Probiotics and MPE affect the levels of pro-inflammatory cytokines in Caco-2 cells, including [C]. IL-β levels [D]. TNF-α levels [E] representative blot of NF-κβ, GLP-1, IL-β, TNF-alpha, and β-Actin. Values are expressed as mean ± SD and analysis was done by one-way ANOVA followed by Tukey's multicomparison test. ###p < 0.001 vs control, ***p < 0.001 vs DSS 0.5%, **p < 0.01 vs DSS 0.5%.

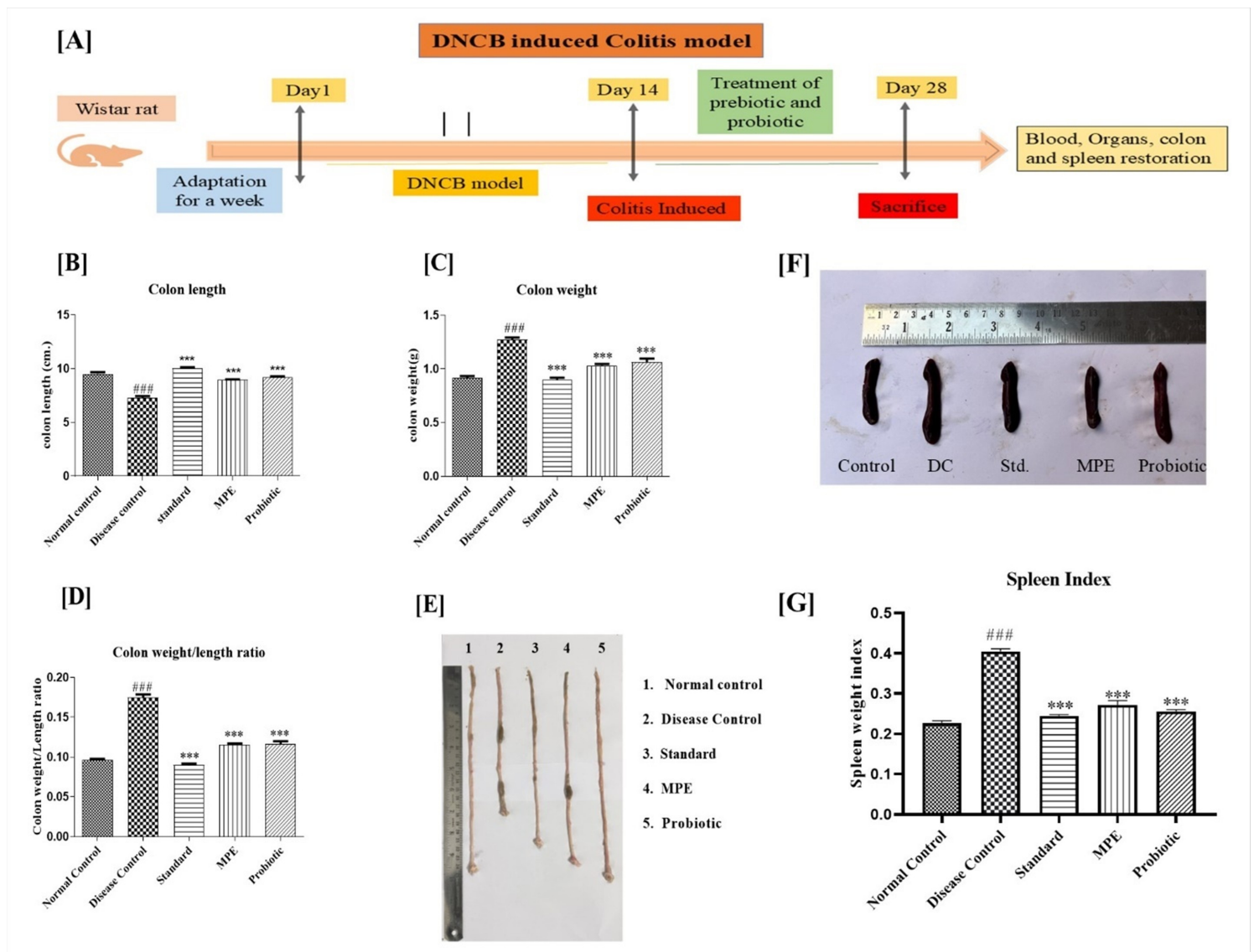


Figure 7

[A] Diagrammatic representation of the animal model induction and treatment program experimental design. The centimetre scale was used to measure the colon's length. [B] demonstrates a substantial reduction in colon length in the DNCB group compared to the control group at ($p < 0.05$). In [C], standard weighing devices were used to determine the colon's weight in grams. The disease control group had a significantly higher colon weight ($p < 0.05$) than the standard group. A significant increase ($p < 0.05$) in Colon weight/ length ratio [D] was found in the disease control group as compared to the treatment group. [E] Changes in the colon of each group by image. [F&G] Representative pictures of spleen weights and quantification plot. Values are expressed as mean $\pm$ SD. ### $p < 0.001$ vs Normal control, *** $p < 0.001$ vs Disease control, ** $p < 0.01$ vs Disease control.

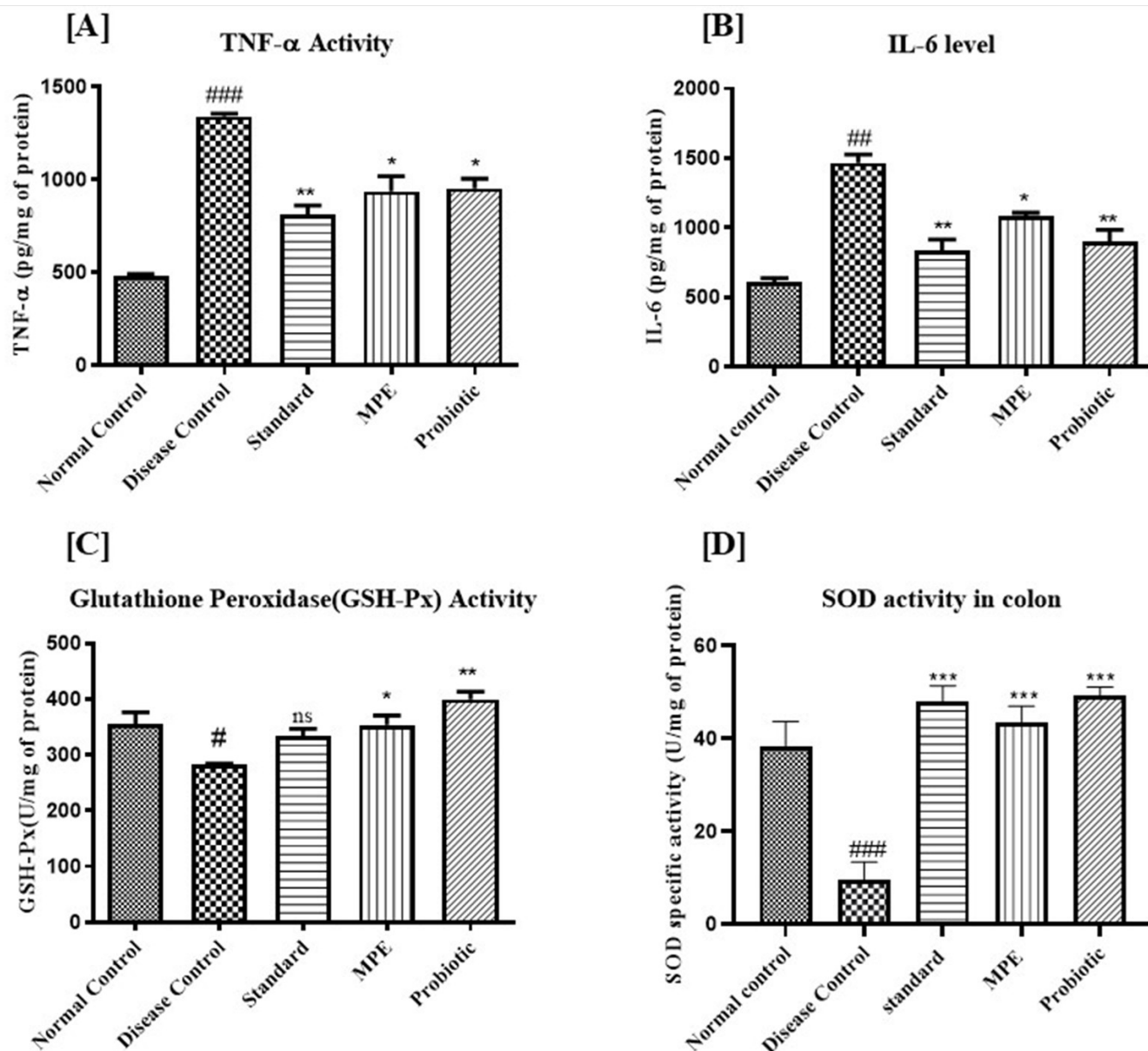


Figure 8

Impact of probiotics (LGG) and prebiotics (MPE) on pro-inflammatory cytokine levels in colonic homogenate samples. [A] TNF- α levels, [B] IL-6 levels. [C] Estimating GSH-Px activity using assay kit ELISA. [D] Superoxide dismutase level in colonic homogenate through ELISA. Data are represented as mean $\pm$ SD, where ###p < 0.001 vs Normal control, ***p < 0.001 vs Disease control.

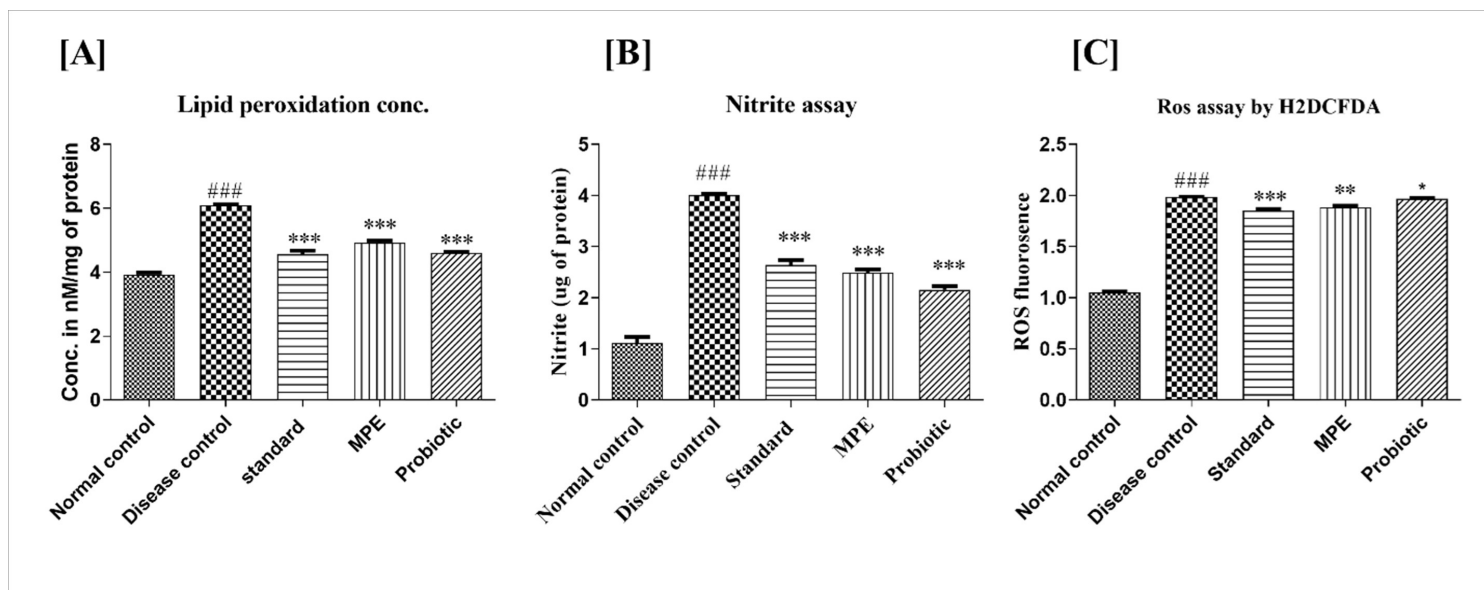


Figure 9

Prebiotic and probiotic effects on oxidative stress and lipid peroxidation that promote inflammation. (A) Lipid peroxidation level estimation. (B) Measuring the nitrite content in colon homogenate. (C) Estimating ROS using H2DCFDA assay kit to assess oxidative stress. The data were reported as mean $\pm$ SD, with significance levels of # $p < 0.05$ vs Normal control, * $p < 0.05$ vs Disease control, ** $p < 0.01$, ### $p < 0.01$, and *** $p < 0.001$.

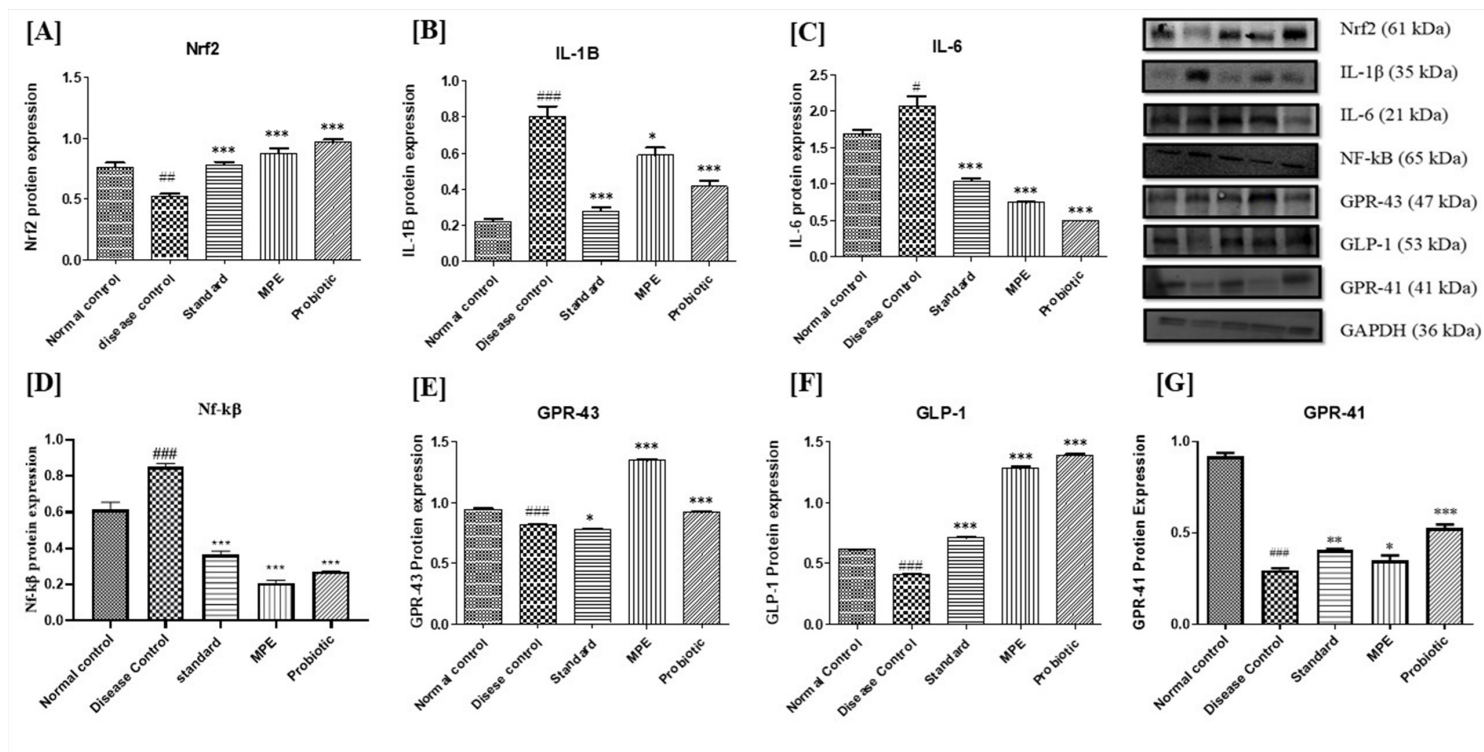


Figure 10

GLP-1 inhibits the translocation of NF- κ B and activates phosphorylation of KEAP-1/Nrf2 pathway in DNCB rat colitis model. The protein expression of Nrf2(A), IL-6(B), IL-1 β (C), NF- κ B (D), GPR-43 (E), GLP-1 (F), and GPR-41(G) was estimated by western blotting. The values shown here are the mean value $\pm$ SD of three independent experiments. For Prebiotic *mucuna pruriens* and probiotic increased the expression levels of Nrf2 ($p < 0.001$), GLP-1 ($p < 0.001$), GPR-43 ($p < 0.001$), and GPR-41 ($p < 0.001$) as compared to the Disease control (DNCB) group. As a loading control, GAPDH was used.

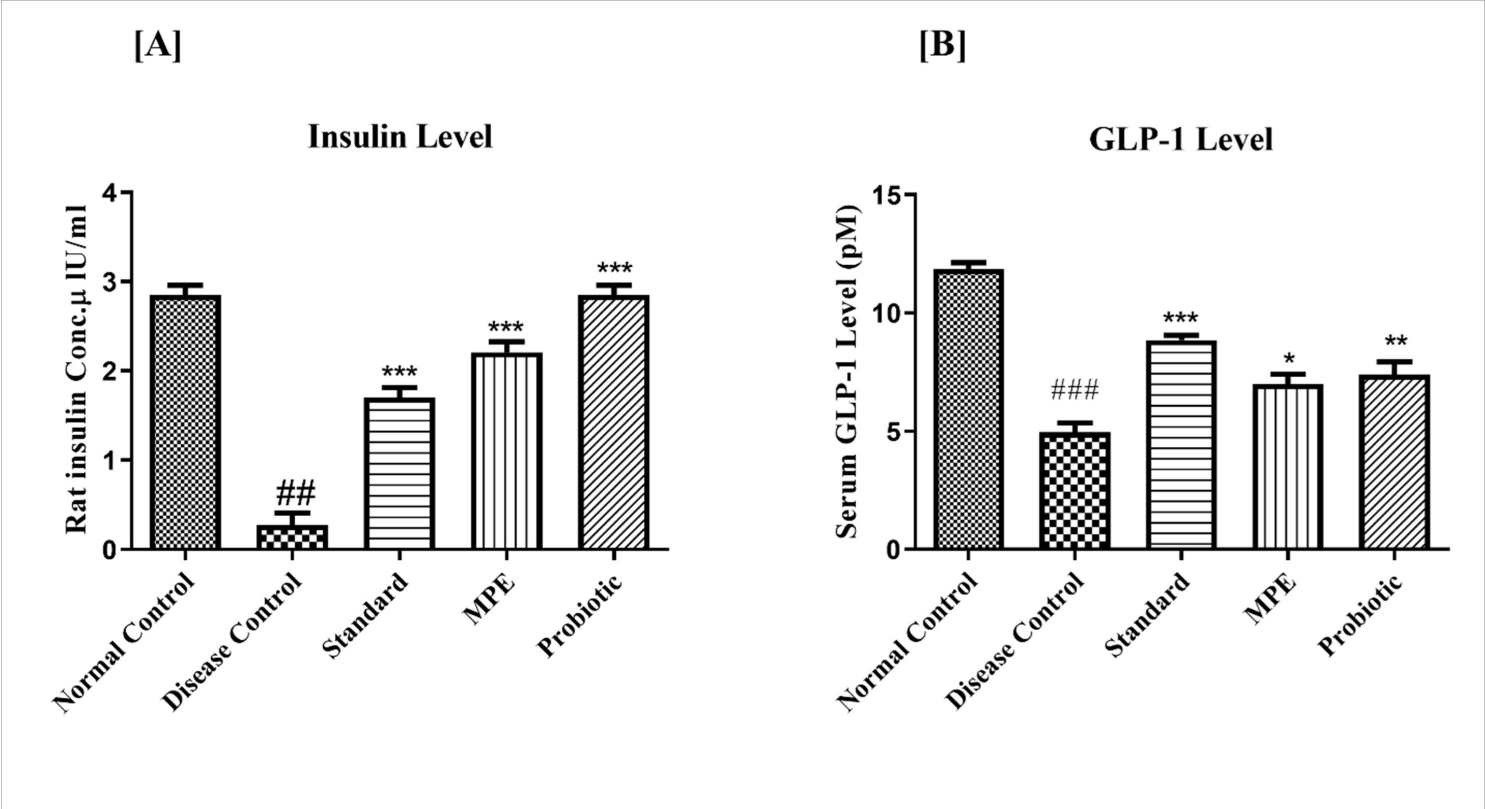


Figure 11

[A] insulin level of Prebiotic *Mucuna pruriens* and probiotic on DNCB-treated rats (in serum). Rat insulin level (μ l. U/ml). [B] Estimation of GLP-1 level in serum sample. Values are expressed as mean $\pm$ SD. ## $p < 0.01$ vs Normal control, *** $p < 0.001$ vs Disease control.

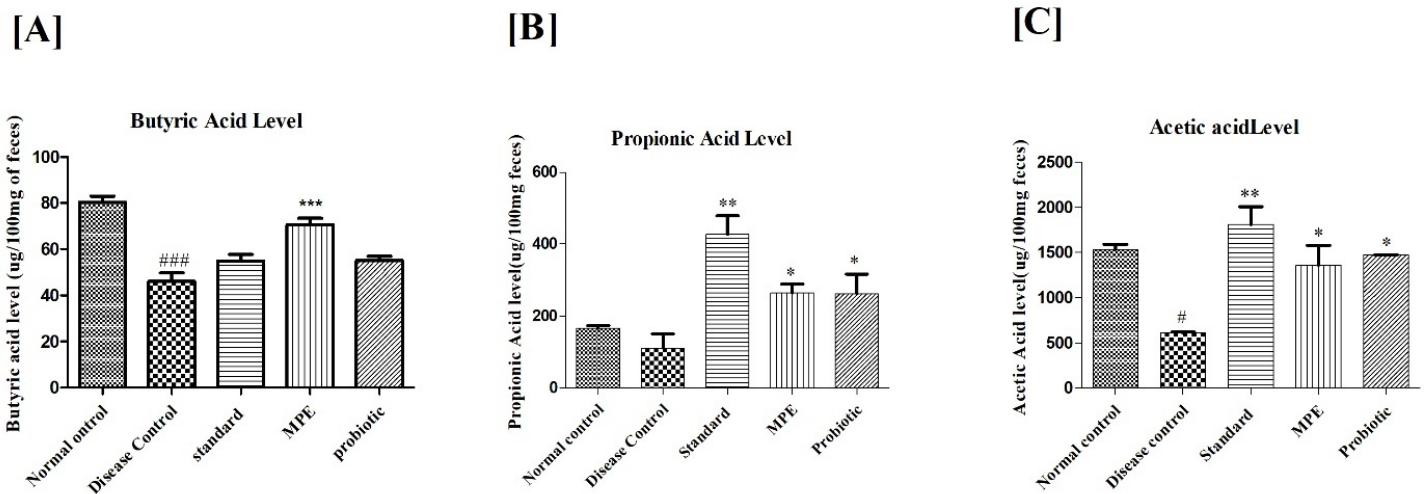


Figure 12

[A] Butyric acid level in faeces sample, [B] Propionic acid level in faeces, [C] Acetic acid level in 100 mg of faeces through HPLC analysis. The data is represented as Mean $\pm$ SD. Bars which are significantly different are represented as ($p < 0.05$).

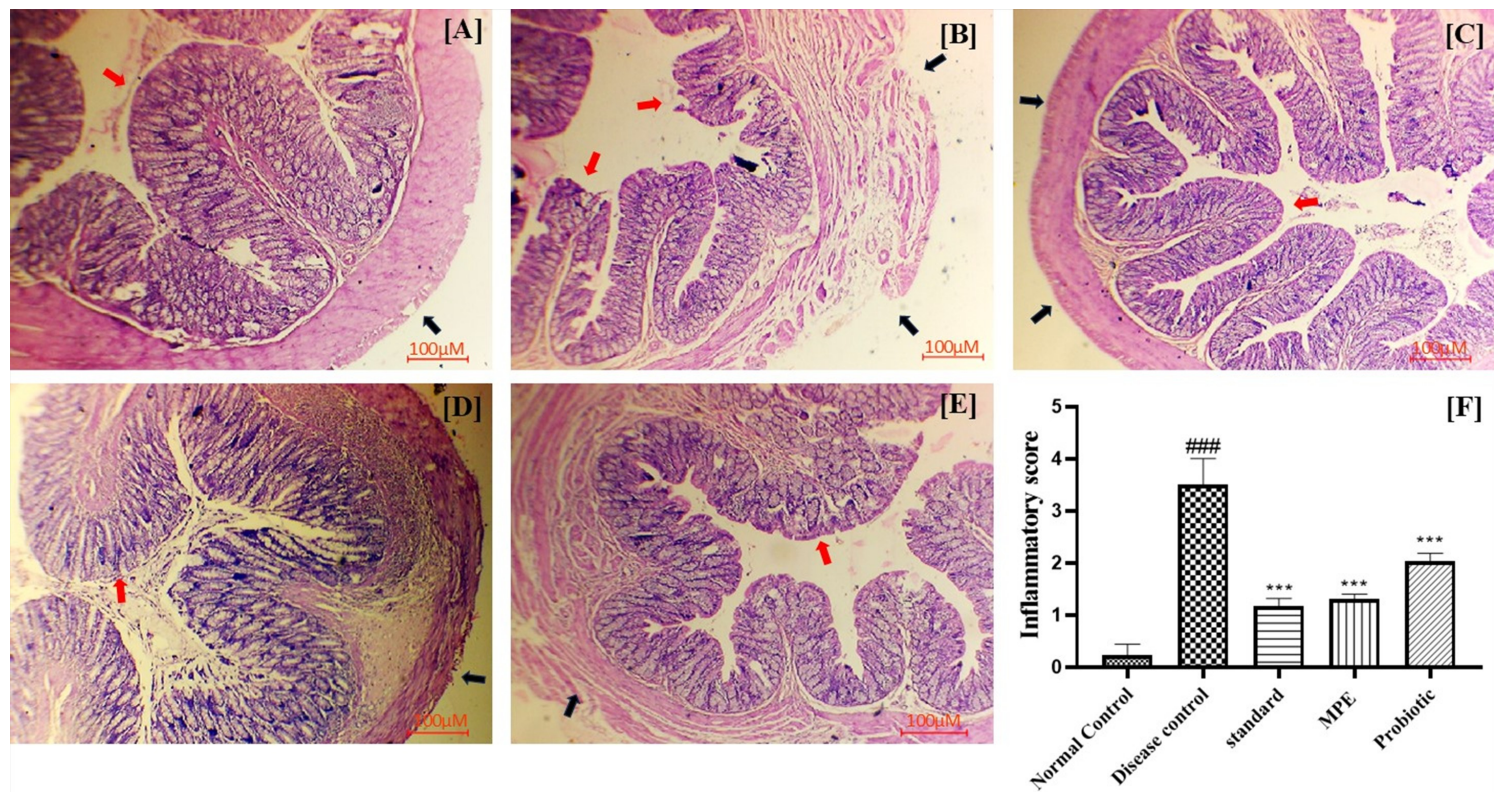


Figure 13

Histopathology study of colon tissues stained with hematoxylin and eosin. [A] shows a healthy mucosa and epithelial layer with a surface that is intact. [B] Rats given DNCB alone had increased disruption of

the intestinal mucosal lining (black arrow) and severe necrotic epithelial damage (red arrow). [D&E] Treatment with prebiotics (MPE) and probiotic improved the intestinal mucosal lining, epithelial damage and decreased ulcers and inflammation. It has a 100X magnification. The data (N = 3) is displayed as Mean $\pm$ SD. Standard sulfasalazine (100 mg/kg) [C] demonstrates an improved epithelial layer and mucosa lining than disease control. [F] Inflammation scoring (original magnification 100X, H and E staining).

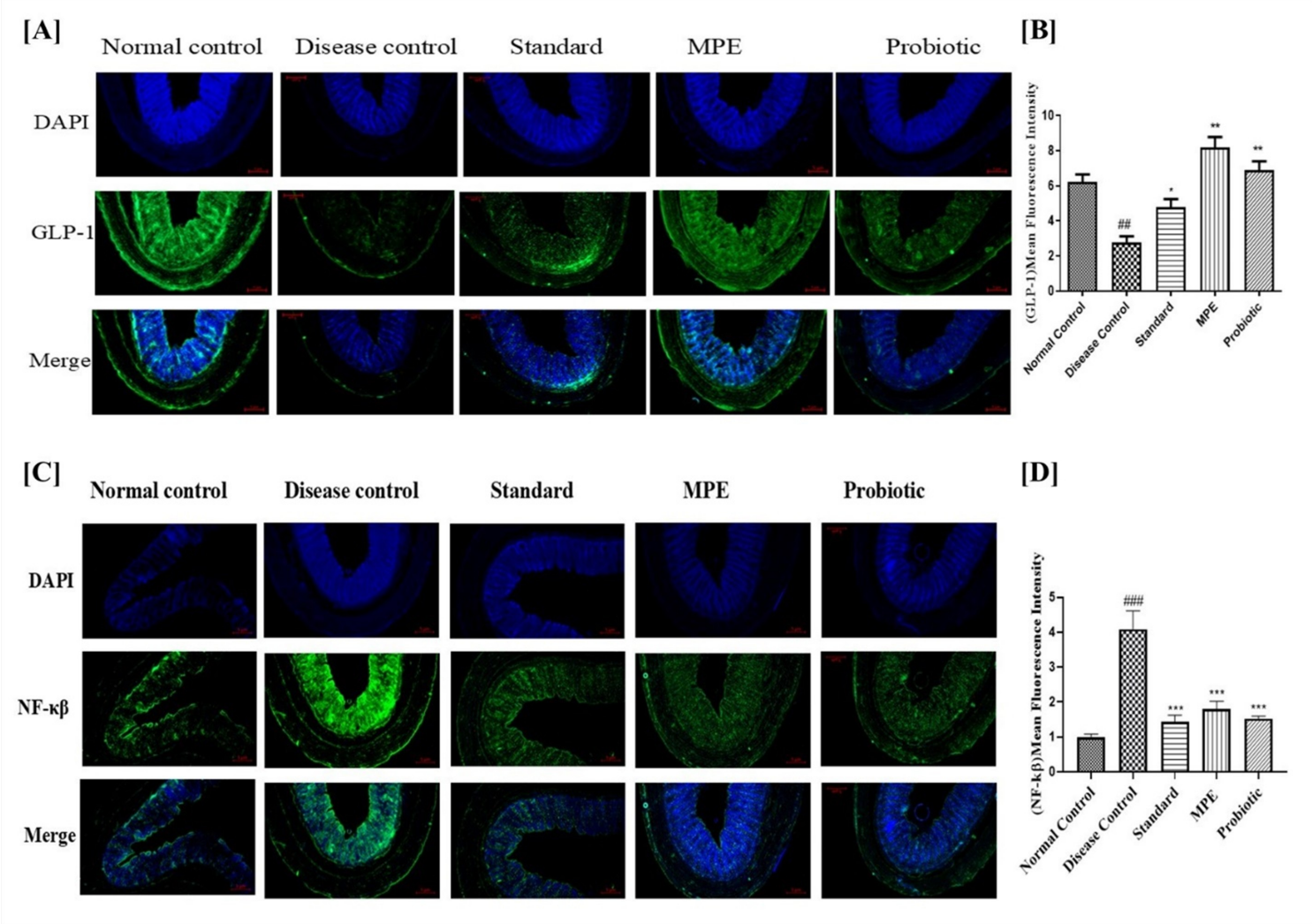


Figure 14

Effect of MPE and Probiotic on expression of GLP-1 (in colon of rat). (A) Immunofluorescent staining of GLP-1 (green) and DAPI (blue) in the colon (scale bar = 50μm). (B) represents the Mean fluorescent intensity by Image J software. Data are the mean $\pm$ SD, and analysis was done by one-way ANOVA followed by Tukey's multicomparison test. ##p-value < 0.01 compared to the control group, **P-value < 0.01 compared to the disease control group.

Supplementary Files

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