



Protective effects of *Pluchea indica* aqueous leaves tea extract against oxidative stress and inflammation in a dextran sulfate sodium-induced colitis rat model

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ABSTRACT

Ulcerative colitis (UC) is a chronic inflammatory gastrointestinal disease associated with excessive reactive oxygen species, colonic injury, and increased cancer risk. *Pluchea indica* leaves exert antioxidant and anti-inflammatory properties; however, their effects on UC remain unknown. This study investigated the effects of *P. indica* leaves tea extract (PIE) in dextran sulfate sodium (DSS)-induced UC in rats. Rats were divided into five groups: control, PIE only (150 mg/kg), DSS, and DSS with PIE (50 or 150 mg/kg). Distilled water or PIE was administered orally for 14 days, with 3% DSS given from days 7 to 14. DSS rats showed elevated disease activity index, shortened colon length, increased spleen weight, and colonic injury. It also increased malondialdehyde (MDA) levels, decreased superoxide dismutase and catalase activities, and upregulated interleukin (IL) -1 β and IL-6 mRNA expression. Pretreatment with PIE (50 or 150 mg/kg) alleviated colitis severity, decreased MDA levels, restored antioxidant enzyme activities, and downregulated pro-inflammatory cytokines, although its effects on spleen weight reversal were not significant. PIE alone showed no significant differences from the control. These findings suggest that PIE exhibits protective effects against DSS-induced colitis and may serve as a potential alternative for UC prevention, although further studies are needed to support these findings.

1. INTRODUCTION

Ulcerative colitis (UC) represents a chronic inflammatory bowel disease (IBD) marked by continuous inflammation of the colonic mucosa, typically beginning in the rectum and extending proximally to segments of the colon [1]. Over recent decades, UC has evolved into a major global health burden, with a significant incidence in developing countries and an alarming increase in cases across developed regions [2,3]. It is considered one of the most prevalent gastrointestinal (GI) disorders and is strongly correlated with an elevated risk

of colorectal cancer approximately 8–10 years after diagnosis [4]. The exact etiology and pathogenesis of UC remain unclear, but the disease is believed to result from multiple factors that contribute to its development, including genetic variations, altered gut microbiota composition, and environmental factors that disrupt colonic mucosal homeostasis and activate immune responses, leading to severe intestinal inflammation [5,6]. In addition, evidence from experimental models and clinical studies suggests that oxidative stress, elevated reactive oxygen species (ROS), and an imbalance in the activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), play essential roles in the initiation of UC pathogenesis [7]. This oxidative imbalance can increase membrane permeability by damaging the mucosal layer in the GI tract, allowing bacterial invasion, activating immune responses, and sustaining chronic inflammation [8].

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Furthermore, oxidative stress can induce the activation of various inflammatory cells that are recruited to the injured colonic mucosa and secrete pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , IL-6, and IL-8. The upregulation of these cytokines promotes tissue infiltration, thereby increasing the production and release of inflammatory mediators during chronic mucosal inflammation, which further contributes to the progression and development of UC [9–11]. Therefore, reducing the increase in ROS by enhancing cellular antioxidants such as SOD and CAT, as well as modulating inflammatory cytokines, could be useful in alleviating UC. Although currently available drugs, such as 5-aminosalicylates, sulfasalazine, corticosteroids, and immunomodulatory drugs that can reduce intestinal inflammation and help maintain remission, their long-term use is often associated with various adverse effects [12]. Interestingly, compounds derived from natural products are being explored as potential therapeutic strategies for preventing and treating UC. Various active compounds, such as polyphenols, flavonoids, and alkaloids, possess multiple biological effects, including antioxidant, anti-inflammatory, anticancer, and antidyslipidemic activities, as demonstrated in both *in vivo* and *in vitro* studies [13]. *Pluchea indica* (L.) Less (“Khlu” in Thai) is a shrub belonging to the family Asteraceae and is one of the most well-known indigenous medicinal plants of Southeast Asia. In the traditional folk medicine of Thailand, Khlu has been used for the treatment of fever, kidney stones, ulcers, and hemorrhoids [14]. *P. indica* is abundant in bioactive substances with potential pharmacological effects, with its leaf extract exhibiting higher phenolic content than the stems and flowers [15]. The leaves of Khlu have been shown to contain significant health-promoting compounds, including dietary fiber, calcium, β -carotene, chlorogenic acid, caffeic acid, and quercetin. In Thailand, it has been developed into a commercially available as health-promoting tea. To date, many pharmacological evidences have demonstrated that both aqueous and alcoholic extracts of Khlu leaves exhibit a wide range of pharmacological properties, which include antioxidant, anti-inflammatory, anti-diabetic, and anticancer activities [16–18]. *P. indica* leaves tea demonstrate antioxidant activity by scavenging free radicals involved in lipid peroxidation, reducing oxidative damage, and decreasing malondialdehyde (MDA) levels, while promoting oxidative balance by enhancing SOD and CAT activities in the streptozotocin (STZ)-induced diabetic rat model [18]. The aqueous extract of *P. indica* herbal tea leaves also reported to its antioxidant and anti-inflammatory activities by inhibiting the production of inflammatory mediators, including nitric oxide (NO) and prostaglandin E2 (PGE2), possibly through the downregulation of NF- κ B activation in LPS-stimulated inflammation in RAW 264.7 macrophages. Furthermore, the aqueous extract of *P. indica* tea demonstrated anti-hemorrhoidal effects by attenuating inflammation and tissue damage in hemorrhoidal conditions induced by croton oil in a rat model [19]. In addition, ethanolic extracts of *P. indica* leaves have been shown to inhibit ethyl phenylpropionate-induced ear edema and carrageenan-induced paw edema in acute inflammation animal models [17,20,21]. However, limited data are available regarding the inhibitory effects of *P. indica* leaf tea extract

on colonic inflammation and oxidative stress, both of which contribute to the severity of UC. Therefore, this study was to investigate the antioxidant and anti-inflammatory effects of the aqueous extract of *P. indica* leaves tea on colonic inflammation in DSS-induced UC model through animal experimentation. Our findings demonstrated the efficacy of *P. indica* leaves and suggested that their potential may serve as promising nutraceuticals benefits, particularly as therapeutic agents for GI disorders, specifically on UC.

2. MATERIALS AND METHODS

2.1. *P. indica* aqueous leaves tea extract (PIE) preparation

P. indica leaves tea was obtained from Bann Khounthain, Thailand. About 10 g of dried leaves tea was soaked in 100 ml of boiled water (70°C) for 5 minutes, and the aqueous part was filtered through multiple layers of cotton gauze. The extraction procedure was repeated five times. The aqueous extract was then concentrated using a rotary evaporator and freeze-dried by lyophilization to obtain a dry powder. The percentage yield (3.59% (w/w)) of PIE was calculated using the following equation: percentage yield (%) = (final weight of the extract (g)/initial weight of dried leaves (g) \times 100. The dry powdered extract was freshly dissolved in distilled water before being used.

2.2. Determination of antioxidant activity of *P. indica* aqueous leaves tea extract

2.2.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The scavenger activity of PIE was evaluated using the DPPH assay as previously described [22], with slight modifications. The reaction mixtures (200 μ l) consisted of 100 μ l of 0.1 mM DPPH solution in ethanol and 100 μ l of various concentrations of PIE (2, 4, 8, 16, 31, and 62.5 μ g/ml) or L-ascorbic acid (1.5, 3, 6, 12.5, 25, and 50 μ g/ml) as a reference. The absorbance of the mixture (indicated by a color change from deep violet to light yellow) was detected at 517 nm using a microplate reader (Synergy H, BioTek, VT). The radical scavenging activity was expressed as the percentage inhibition of DPPH activity using the following formula: $(A_0 - A_1)/A_0 \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2.2.2. Ferric reducing antioxidant power (FRAP) assay

The ability to reduce ferric ions (Fe³⁺) to the ferrous ions (Fe²⁺) was measured as previously described with some modifications [23]. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-Tris (2-pyridyl)-1,3,5-triazine) dissolved in 40 mM HCl, and 20 mM FeCl₃·6H₂O in a ratio of 10:1:1 (v/v). The reaction mixture (200 μ l), consisting of 100 μ l of FRAP reagent and 100 μ l of various concentrations of PIE (2, 4, 8, 16, 31, and 62.5 μ g/ml), was incubated at room temperature for 30 minutes. The absorbance was measured at 593 nm using a microplate reader (Synergy H, BioTek, VT). The reducing power of PIE or the FRAP value was expressed as μ mol of Trolox equivalents per gram of extract.

2.3. Animals and ethical consideration

Male Wistar rats (5 weeks old, 150–200 g body weight (BW)) were obtained from Nomura Siam International Co., Ltd, Pathumwan, Bangkok, Thailand. Rats were housed in stainless steel cages under controlled conditions ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$) with a 12 hours light: 12 hours dark cycle at the Laboratory Animal Service Center, Prince of Songkla University, with free access to a standard chow diet (Chareon Pokapan Co. Ltd., Thailand) and drinking water ad libitum. All rats were acclimatized to the laboratory room for 1 week prior to the experiments. All the animal procedures complied with the standards for the care and use of experimental animals, and the study was approved by the Institutional Animal Care and Use Committee of Prince of Songkla University (MHESI 68014/894, Ref. AR032/2022).

2.4. DSS-induced UC in the rats model and experimental design

After 1 week of acclimatization, the rats were randomly allocated into five groups, each consisting of six animals. All groups were administered orally for 14 consecutive days, as illustrated in Fig. 1A. The groups were as follows: (1) control group received distilled water, (2) PIE group received PIE at 150 mg/kg BW/day, (3) DSS group received distilled water, (4) DSS + 50 mg/kg PIE group received PIE at 50 mg/kg BW/day, and (5) DSS + 150 mg/kg PIE group received PIE at 150 mg/kg BW/day. The selected doses of 50 and 150 mg/kg BW of PIE were based on previous studies reporting significant antioxidant and anti-inflammatory effects at these levels in rodent models, without observable toxicity [19]. From days 7 to 14 of treatment, 2 hours after being force-fed, rats were induced with colitis through free access administration of 3% DSS solution freshly prepared daily in drinking water (DSS molecular weight: 40 kDa, Sigma-Aldrich, USA), as previously described [24], except for the control and PIE groups.

Throughout the experiment, indicators of colitis severity, including BW loss, stool consistency, and the presence of blood in the stool, were monitored and recorded daily and expressed as the disease activity index (DAI). All rats were euthanized with an intraperitoneal injection of thiopental sodium (150 mg/kg BW). The colon and spleen were immediately removed, with colon length (cm) and spleen weight (mg) measured. Colon tissues were then fixed in 10% neutral-buffered formalin for histopathological analysis and washed with ice-cold 0.9% normal saline solution (NSS) and stored at -80°C for further analysis.

2.5. Determination of DAI

The symptoms indicating severity of the disease were assessed using the DAI as previously described [25], which was determined based on daily scoring of (1) bodyweight loss, (2) stool consistency, and (3) the presence of blood in the stool (Helena Laboratories, Beaumont, Texas). BW changes were classified into five distinct levels: 0 = no weight loss or weight gain; 1 = 1%–5% loss; 2 = 5%–10% loss; 3 = 10%–20% loss; and 4 = >20% loss. Stool consistency was graded as 0 = normal, 2 = loose, and 4 = diarrhea. The presence of blood in stool was scored as 0 = negative, 2 = positive (moderate), and 4 = positive (severe). The total scores from three parameters were combined and represented as the DAI.

2.6. Histopathological examination

After sacrifice, colonic tissue samples from the distal portion were directly fixed in 10% neutral buffered formalin. Colonic tissues were embedded in a paraffin block, transversely sectioned into a thickness of 5 μm using a rotary microtome (Leica Microsystems, Wetzlar, Germany), and stained with hematoxylin and eosin (H&E) using standard procedures. The H&E-stained sections were examined under a light microscope (Olympus, BX53; Melville, NY). All histological changes were

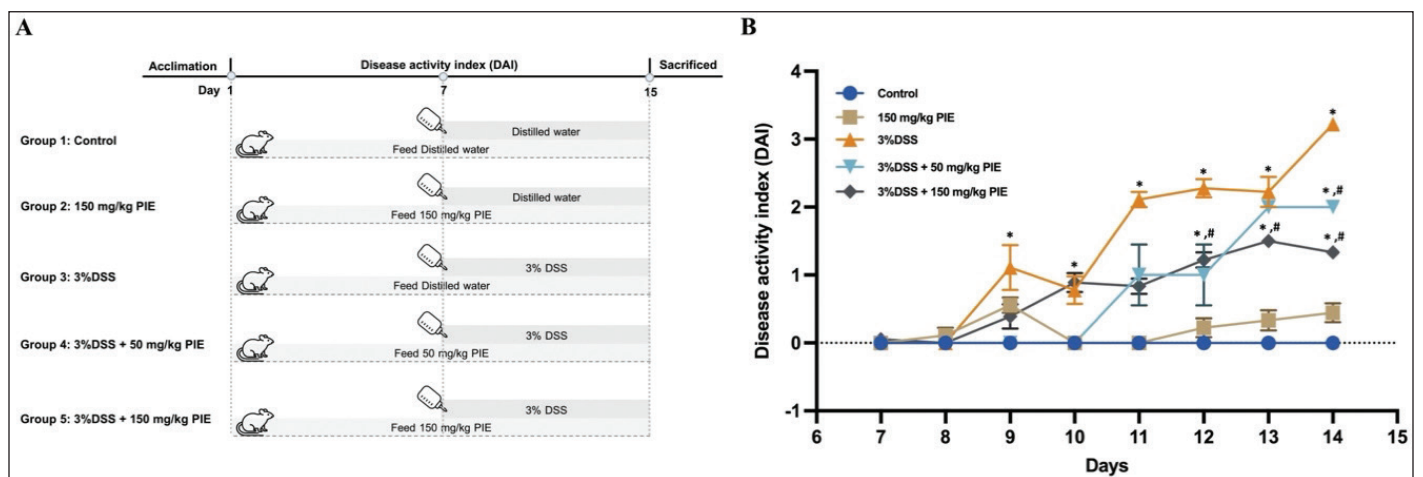


Figure 1. Effects of PIE on DSS-induced colitis. (A) Schematic diagram of the animal experimental design. (B) Disease activity index (DAI), which include a BW loss, stool consistency and the presence of blood in the stool. All data presented indicate the mean \pm SEM ($n = 6$). Statistical significance was assessed using one-way ANOVA with Tukey's test, with significance considered at $p < 0.05$. * $p < 0.05$ when compared with the control group and # $p < 0.05$ when compared with the DSS group; DSS, dextran sulfate sodium; PIE, *P. indica* aqueous leaves tea extract; 150 mg/kg PIE, rats treated with 150 mg/kg BW PIE; 3%DSS, DSS-induced UC rats without any treatment; 3%DSS + 50 mg/kg PIE, DSS-induced UC rats with 50 mg/kg BW PIE; 3%DSS + 150 mg/kg PIE, DSS-induced UC rats with 150 mg/kg BW PIE.

evaluated based on four parameters: epithelial cell loss, crypt damage, goblet cell depletion, and inflammatory cell infiltration. Epithelial cell loss was graded as 0 (none), 1 (0%–5% loss), 2 (5%–10% loss), and 3 (>10% loss of epithelium). Crypt damage was scored as 0 (none), 1 (0%–10% loss), 2 (10%–20% loss), and 3 (>20% loss of crypt). Goblet cell depletion was graded as 0 (none), 1 (0%–5% loss), 2 (5%–10% loss), and 3 (>10% loss of goblet). Inflammatory cell infiltration was assessed as 0 (none), 1 (0%–5%), 2 (5%–10%), and 3 (>10%) as previously described [26]. Histological scoring of colonic tissues was performed using Aperio ImageScope 12.3.3 software by two blinded pathologists, and the results were expressed as total histological scores.

2.7. Measurement of oxidative stress markers and activity of antioxidant enzymes

2.7.1. Preparation of colonic tissue homogenate

Colonic tissue from each group was collected and rinsed in ice-cold 0.9% NSS. Colonic tissues were homogenized in 0.1M phosphate-buffered saline (PBS, pH 7.4) and centrifuged at 12,000 rpm at 4°C for 20 minutes. The supernatant was collected and stored at –80°C for subsequent determination of oxidative stress biomarkers.

2.7.2. Determination of SOD activity in the rat's colon

SOD activity in colonic tissue was measured using the SOD Assay Kit (Code No. 19160, Sigma-Aldrich, Darmstadt, Germany) following the manufacturer's instructions. Briefly, xanthine and xanthine oxidase were used to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The absorbance of the reaction mixture was measured at 450 nm using a spectrophotometer (BMG Labtech, Offenburg, Germany). Enzymatic activity was presented in units of SOD activity/mg protein, where one unit was defined as the amount of enzyme required to cause 50% inhibition of INT reduction.

2.7.3. Determination of CAT activity in the rat's colon

CAT activity was determined based on the formation of a stable complex between hydrogen peroxide and ammonium molybdate, as previously described [27]. The reaction mixture, consisting of the sample, 0.01M phosphate buffer (pH 7.4), and

hydrogen peroxide (H₂O₂), was loaded into a 96-well plate. The plate was then incubated at 37°C for 1 minute, and the reaction was stopped by adding 32.4 mM ammonium molybdate. The absorbance was subsequently measured at 405 nm using a spectrophotometer (BMG Labtech, Offenburg, Germany). CAT activity was expressed as CAT activity/mg protein by measuring the decrease in color intensity of the complex formed between H₂O₂ and molybdenum salts.

2.7.4. Evaluation of MDA content in the rat's colon

The concentration of MDA, a marker of lipid peroxidation, was determined as previously described [28]. Briefly, 150 µl of colonic tissue homogenate was interacted with 10% TCA, 125 µl of 5 mM EDTA, 125 µl of 8% SDS, and 10 µl of 0.5 µl/ml of BHT. After incubating for 10 minutes, an equal volume of 0.6% TBA was added, and the mixture was heated at 95°C for 30 minutes, cooled in an ice bath, and centrifuged at 10,000 rpm at 25°C for 5 minutes. The absorbance of the MDA-TBA complex, forming a pink reaction product, was measured at 532 nm using spectrophotometry (BMG Labtech, Offenburg, Germany). MDA levels were quantified using a 1,1,3,3-tetramethoxypropane standard curve and expressed as nmol/mg protein.

2.8. Real-time PCR analysis

The total mRNA extraction from the homogenized colonic tissue sample was performed using TRIzol[®] reagent (Invitrogen, Carlsbad, CA) and quantified using a nanodrop spectrophotometer (Eppendorf, Hamburg, Germany). Then, complementary DNA (cDNA) was synthesized from 1.5 µg of total RNA using the RScript cDNA synthesis kit (Bio-Helix, Taiwan) at 65°C for 5 minutes, 55°C for 50 minutes, and 70°C for 15 minutes as a final step by a PTC-200 Thermal Cycler (Marshall Scientific, Hampton, USA). The mRNA expression levels of IL-1β and IL-6 were analyzed using an iTaq Universal SYBR Green Supermix qPCR kit (Bio-RAD, Hercules, CA) and utilized in a real-time detection system (BIOER, Hangzhou Bioer Technology Co. Ltd., Hangzhou, China). The relative mRNA expression of genes was calculated based on the 2^{-ΔΔCt} method and expressed as the x-fold change in gene expression change compared with the control group. GAPDH served as an internal control for normalization. The primer sequences used for the RT-PCR are shown in Table 1.

Table 1. Primer sequences used in this study.

Gene	Sequence	Tm (°C)	Product size (bp)
IL-1β			
Forward	5'CCTATGTCTTGCCCGTGGAG3'	63.7	118
Reverse	3'CACACACTAGCAGGTCGTCA5'	61.3	
IL-6			
Forward	5'CACTTCACAAGTCGGAGGCT3'	65	138
Reverse	3'TCTGACAGTGCATCATCGCT5'	60.9	
GAPDH			
Forward	5'GACTCTACCCACGGCAAGTT3'	62	139
Reverse	3'CGACATACTCAGCACCAGCA5'	61.2	

2.9. Statistical analysis

Data are presented as mean \pm SEM. Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test using GraphPad Prism (GraphPad Software, San Diego, CA). A p -value < 0.05 was considered statistically significant.

3. RESULTS

3.1. Antioxidant activity of *P. indica* aqueous leaves tea extract

The DPPH assay demonstrated that the aqueous leaf tea extract of *P. indica* exhibited concentration-dependent radical scavenging activity, with inhibition values of 10.98%, 20.01%,

33.00%, 41.03%, 63.32%, and 74.36% at concentrations of 2, 4, 8, 16, 31, and 62.5 $\mu\text{g/ml}$, respectively. Specifically, ascorbic acid exhibited an IC₅₀ value of 13.90 $\mu\text{g/ml}$, whereas PIE had an IC₅₀ value of 30.23 $\mu\text{g/ml}$. In addition, the FRAP assay showed an antioxidant capacity of 268 μmol Trolox equivalents per gram of extract, supporting that PIE possesses antioxidant activity.

3.2. PIE attenuated DSS-induced UC symptoms in rats

UC symptoms were measured by evaluating the DAI scores. From the 3%DSS treatment period, all rats survived after receiving DSS. No significant reduction of BW was

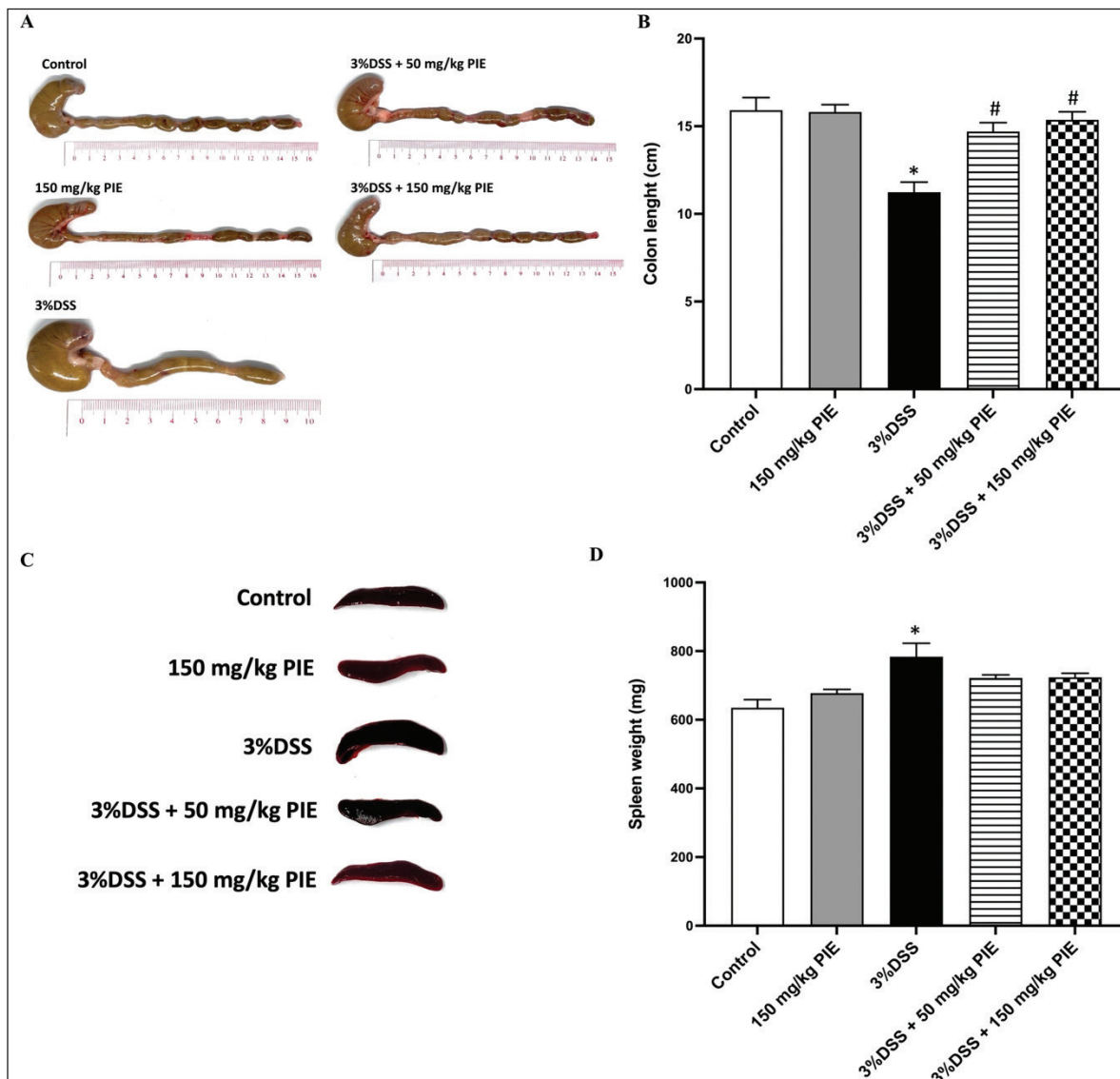


Figure 2. PIE antagonized the colon length and spleen weight in DSS-induced UC rats. (A,B) Representative image of colon tissue from each group and measured length of the colon. (C,D) Representative image of spleen tissue from each group and measured weight of the spleen. All data presented indicate the mean \pm SEM ($n = 6$). Statistical significance was assessed using one-way ANOVA with Tukey's test, with significance considered at $p < 0.05$. * $p < 0.05$ when compared with the control group and # $p < 0.05$ when compared with the DSS group. DSS, dextran sulfate sodium; PIE, *P. indica* aqueous leaves tea extract; DSS, dextran sulfate sodium; PIE, *P. indica* aqueous leaves tea extract; 150 mg/kg PIE, rats treated with 150 mg/kg BW PIE; 3%DSS, DSS-induced UC rats without any treatment; 3%DSS + 50 mg/kg PIE, DSS-induced UC rats with 50 mg/kg BW PIE; 3%DSS + 150 mg/kg PIE, DSS-induced UC rats with 150 mg/kg BW PIE.

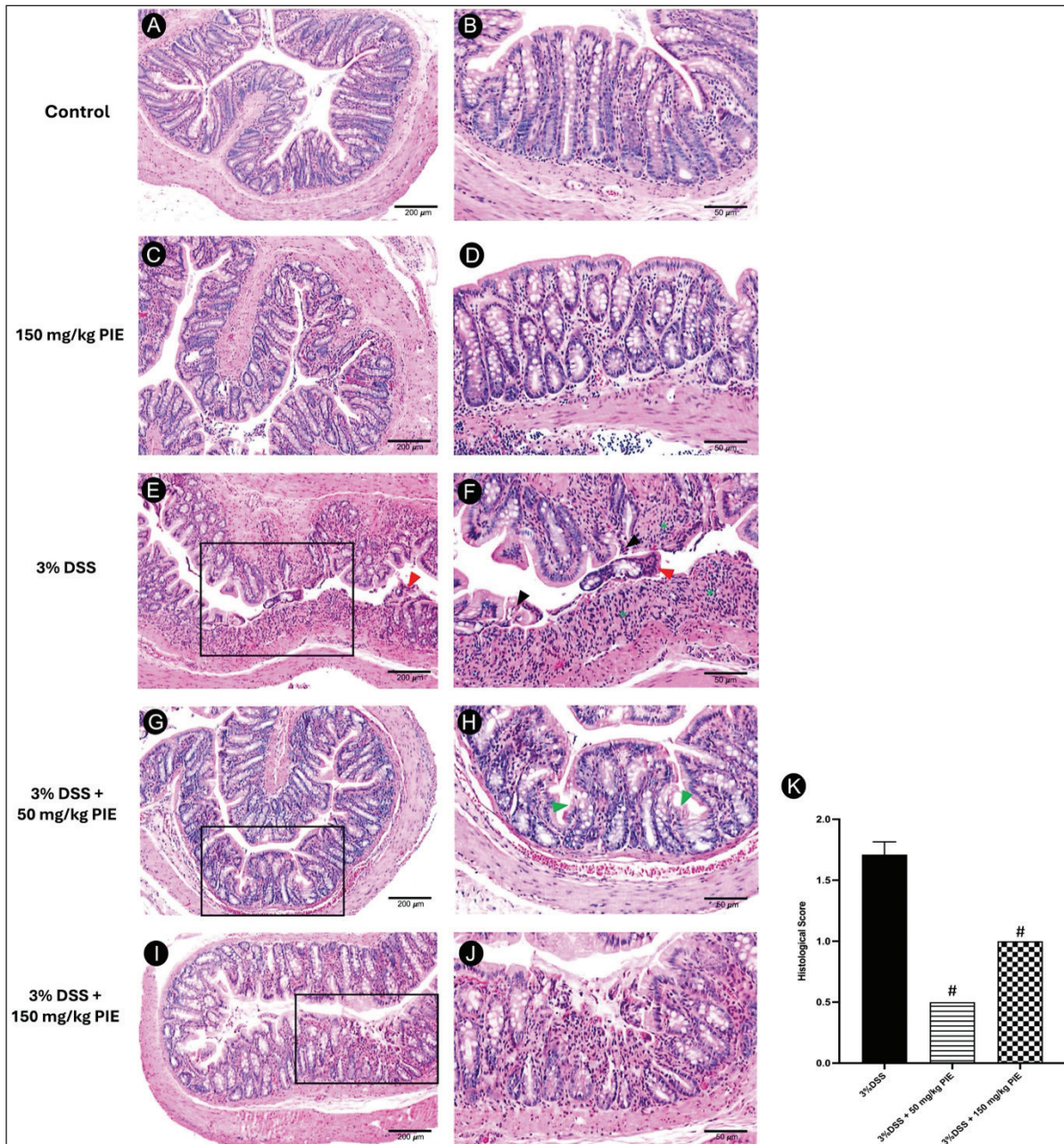


Figure 3. Effects of PIE on histological changes and scores in the colonic tissues of rats with DSS-induced UC. Colon sections were stained with H&E and shown at 10x and 40x magnification (scale bars = 200 and 50 μ m). (A–J) Representative images of H&E staining of colonic tissue from each group and (K) histological scores in the colonic tissues. All data presented indicate the mean \pm SEM ($n = 6$). Statistical significance was assessed using one-way ANOVA with Tukey's test, with significance considered at $p < 0.05$. # $p < 0.05$ when compared with the DSS group. DSS, dextran sulfate sodium; PIE, *P. indica* aqueous leaves tea extract. Black arrows indicate loss of the epithelium and intact basement membrane; green asterisks mark vessels penetrating the muscularis propria; green arrowheads indicate epithelial injury; red arrows indicate epithelial detachment from the basement membrane.

observed in the control and 150 mg/kg PIE alone groups. Rats receiving 3%DSS showed signs and symptoms of UC as seen by a significant increase in DAI, which included % BW loss, stool consistency, and the presence of blood in the stool when compared to the control group ($p < 0.05$). Interestingly, pretreatment with PIE (50 and 150 mg/kg) could significantly reduce, although not completely reverse, the severity of DSS-induced UC compared to the DSS-treated group ($p < 0.05$)

(Fig. 1B), suggesting that PIE could ameliorate inflammation severity in DSS-induced UC in rats.

3.3. PIE antagonized the effects on colon length and spleen weight in DSS-induced UC rats

Compared to the control group, DSS-induced UC was clearly associated with a significantly marked decrease in colon length ($p < 0.05$), suggesting that a rat model of UC

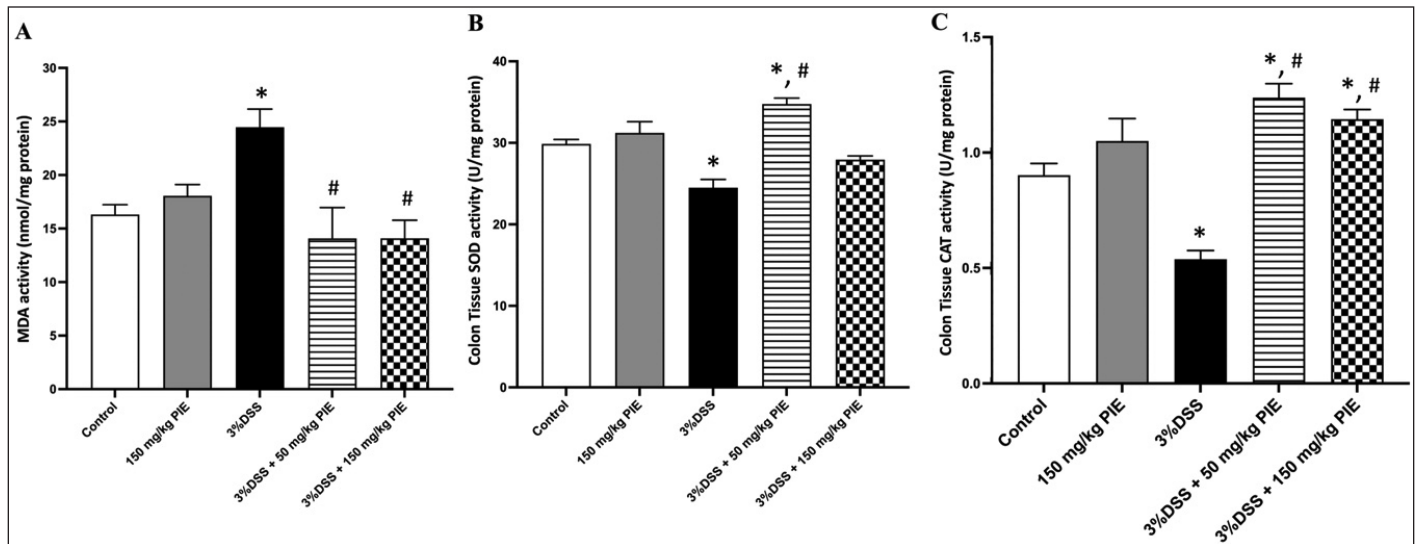


Figure 4. Effects of PIE on oxidative stress parameters in rats with DSS-induced UC. (A) MDA levels, (B) SOD activity, and (C) CAT activity in colonic tissue. All data presented indicate the mean \pm SEM ($n = 6$). Statistical significance was assessed using one-way ANOVA with Tukey's test, with significance considered at $p < 0.05$. * $p < 0.05$ when compared with the control group and # $p < 0.05$ when compared with the DSS group. DSS, dextran sulfate sodium; PIE, *P. indica* aqueous leaves tea extract; DSS, dextran sulfate sodium; PIE, *P. indica* aqueous leaves tea extract; 150 mg/kg PIE, rats treated with 150 mg/kg BW PIE; 3%DSS, DSS-induced UC rats without any treatment; 3%DSS + 50 mg/kg PIE, DSS-induced UC rats with 50 mg/kg BW PIE; 3%DSS + 150 mg/kg PIE, DSS-induced UC rats with 150 mg/kg BW PIE.

was successfully constructed. Interestingly, pretreatment with PIE (50 and 150 mg/kg) was significantly able to antagonize the effect of DSS on colon length when compared to the DSS-treated group ($p < 0.05$) (Fig. 2A,B). In addition, rats in the DSS-treated group showed a significant increase in spleen weights when compared to the control group, which implied the presence of an inflammation state. Co-treatment of DSS with PIE (50 and 150 mg/kg) were increased in spleen weight, but did not significantly differ among the groups (Fig. 2C,D). However, rats given 150 mg/kg of PIE alone showed no significant differences in colon length or spleen weight compared to the control group. Taken together, these findings indicate that PIE extract attenuates DSS-induced colonic inflammation.

3.4. PIE ameliorated histological changes in the colonic tissues of DSS-induced UC rats

The histological change of colonic injury was examined in sections stained with H&E. Colonic tissue sections from both the control and PIE-treated group exhibited a normal appearance (Fig. 3A–D). In contrast, the DSS-treated group showed marked histopathological lesions (Fig. 3E,F), including significant epithelial cell damage (Fig. 3F; black arrow), detachment of the epithelium from the basement membrane (Fig. 3E,F; red arrow), crypt cell destruction, depletion of goblet cells, and increased inflammatory cell infiltration into the lamina propria of the colonic mucosa (Fig. 3F; green asterisks). These changes were associated with significantly elevated histological scores (Fig. 3K). However, rats administered with PIE 50 mg/kg (Fig. 3G,H) and 150 mg/kg (Fig. 3I,J) showed mitigation of DSS-induced damage to epithelial cells, crypts, and goblet cells, along with reduced immune cell infiltration, related with the histological scores were significantly decreased

($p < 0.05$), when compared to the DSS-treated group. These findings indicate that PIE, particularly at 50 mg/kg, effectively attenuates mucosal epithelial injury and reduces inflammation severity in the colonic tissue of a colitis rat model.

3.5. PIE affects oxidative stress parameters in the colonic tissues of DSS-induced UC rats

Excessive oxidative responses, which lead to colonic tissue damage, are one of the contributing factors in UC. The effects of PIE on colonic oxidative stress were assessed by measuring MDA levels, as well as SOD and CAT activities. The results showed a significant ($p < 0.05$) increase in MDA levels in the DSS-treated group, while SOD and CAT levels in colonic tissue were notably decreased compared to the control group. However, no significant differences were observed in any of these parameters in the PIE-treated group alone. Interestingly, PIE treatment significantly enhanced the antioxidant activities of both SOD and CAT and significantly ($p < 0.05$) decreased MDA levels in colonic tissue when compared to the DSS-treated group (Fig. 4A–C). These findings suggest that PIE may alleviate oxidative stress and protect against oxidative damage in colonic tissue in the DSS-induced UC rat model.

3.6. PIE downregulated the mRNA expression levels of pro-inflammatory cytokines in the colonic tissues of DSS-induced UC rats

IL-1 β and IL-6 are key inflammatory mediators that play significant roles in leukocyte infiltration and the progression of UC. The mRNA expression levels of IL-1 β and IL-6 were significantly ($p < 0.05$) increased in the DSS group when compared with the control group (Fig. 5A,B). In contrast, DSS-induced UC rats treated with 50 or 150 mg/kg

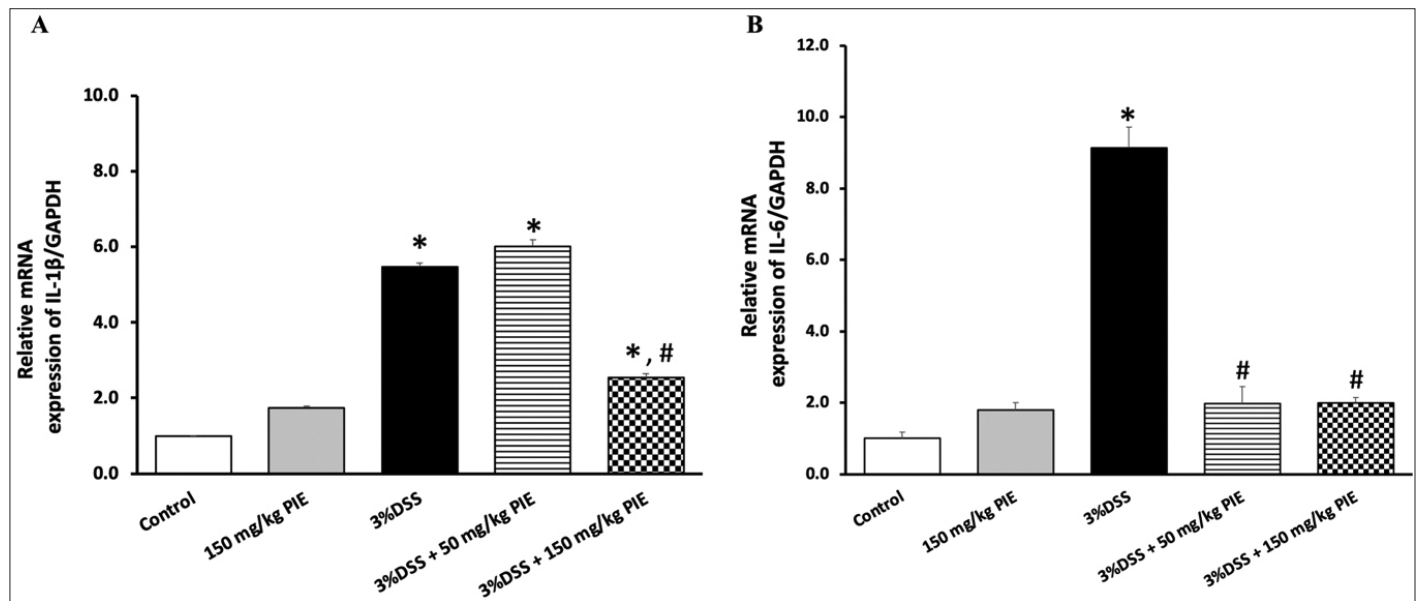


Figure 5. Effects of PIE on the expression of (A) IL-1 β and (B) IL-6 mRNA in colonic tissues in rats with DSS-induced UC. Relative gene expression values were normalized to GAPDH levels and expressed as fold change compared with control group. All values are presented as mean \pm SEM ($n = 6$). * $p < 0.05$ when compared with the control group and # $p < 0.05$ when compared with the DSS group, using one-way ANOVA with Tukey's test. DSS, dextran sulfate sodium; PIE, *P. indica* aqueous leaves tea extract; DSS, dextran sulfate sodium; PIE, *P. indica* aqueous leaves tea extract; 150 mg/kg PIE, rats treated with 150 mg/kg BW PIE; 3%DSS, DSS-induced UC rats without any treatment; 3%DSS + 50 mg/kg PIE, DSS-induced UC rats with 50 mg/kg BW PIE; 3%DSS + 150 mg/kg PIE, DSS-induced UC rats with 150 mg/kg BW PIE.

PIE exhibited a significant ($p < 0.05$) suppression of IL-1 β and IL-6 expression levels (Fig. 5A,B). Although the PIE low-dose group significantly increased IL-1 β expression compared to the control group, its levels were comparable to the DSS group, with no significant rise observed. This suggests that at the low dose, PIE may not substantially modulate IL-1 β in this acute colitis model, possibly due to the severity of DSS-induced colitis and the short duration of the acute model. Interestingly, no significant changes in the expression of these inflammatory mediators were observed in the 150 mg/kg PIE-treated group alone compared to the control group. These findings suggest that PIE treatment can alleviate colonic tissue damage and suppress the inflammatory process in the colonic tissues during an inflammatory condition.

4. DISCUSSION

The leaves of *P. indica* have been reported to possess various therapeutic properties. In this study, we demonstrated that the aqueous leaf tea extract of *P. indica* (PIE) exhibited antioxidant activity, as confirmed by the DPPH and FRAP assays, which may contribute to its pharmacological effects, including antioxidant and anti-inflammatory activities in DSS-induced UC rats, and further highlighted its protective effect against colonic inflammation. Our results indicated that pretreatment with PIE significantly ameliorated the severity and progression of UC in DSS-induced rats. In addition, PIE decreased MDA levels and increased SOD, CAT activities. Moreover, PIE alleviated colonic inflammation, possibly by downregulating the expression of inflammatory cytokines, including IL-1 β and IL-6.

Among chemically induced IBD models, DSS is widely used as a standard chemical agent for establishing the UC animal model [29,30]. DSS is a toxic substance to colonic epithelial cells, causing erosions that compromise the integrity of the mucosal barrier. This damage increases the permeability of the colonic epithelium, allowing pathogenic bacteria to penetrate the tissues, triggering the release of proinflammatory cytokines and then mucosal injury. Notably, DSS-induced colitis is a condition that closely mimics UC, primarily affecting the colon rather than the small intestine, and shares similar morphological, histopathological, and symptomatic features with human colitis [31–33]. Here, the DAI was monitored as the first step to assess the severity of the inflammatory process in UC models. In this study, DSS-induced UC rats showed a significant increase in DAI, including BW loss, altered stool consistency, and the presence of blood in the stool, thereby confirming successful induction of colitis [34]. Furthermore, a significant reduction in colon length and an increase in spleen weight were observed, in agreement with previous studies [33,35–37] and reflecting mucosal damage and systemic inflammation. Interestingly, PIE significantly alleviated the symptoms of UC, as evidenced by a marked decrease in DAI, preservation of colon length, and reduction in spleen weight, although it did not significantly reverse DSS-induced UC. Together, these findings demonstrate that PIE alleviates clinical symptoms, protects colonic tissue, and modulates systemic inflammatory responses in UC. According to the histological changes in colonic tissues, the DSS group revealed severe lesions characterized by extensive damage to epithelial and crypt cells and a high level of inflammatory infiltration in the mucosal and submucosal areas. Interestingly, pretreatment with PIE, particularly at 50 mg/kg

BW, ameliorated both the histological structure and histological scores of colonic tissues in the DSS-induced UC rat model. In our preliminary experiments, which were not part of the present study, PIE was directly compared with sulfasalazine (30 mg/kg BW). No statistically significant differences were observed in UC severity, which was assessed daily based on BW loss, fecal consistency, bleeding, and histopathological alterations. These findings suggest that PIE may confer protective effects comparable to sulfasalazine, at least in this acute experimental model. In accordance with prior studies, it is noteworthy that a dose of 50 mg/kg BW of PIE also significantly reduced disease severity in both croton oil-induced hemorrhoid and indomethacin-induced colitis models [19]. It was previously reported that *P. indica* leaves comprised of many biologically active compounds, such as phenolic acids, flavonoids, and glycosides with antioxidant activity [15,18,20]. In this study, the aqueous leaf tea extract of *P. indica*, although prepared by using hot water, still showed quite high antioxidant. The IC50 of the PIE in DPPH assay was 30.23 µg/ml (ascorbic acid was 13.90 µg/ml), and the reducing power or FRAP value of the PIE was calculated to be 268 µmol Trolox equivalents per gram of extract, which might be responsible for the pharmacological activity. Furthermore, previous studies have revealed that *P. indica* leaves contain active phytoconstituents, particularly phenolic acids and flavonoids, which are well known for their potent antioxidant and anti-inflammatory properties in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-, DSS-, and acetic acid-induced UC models in animal experiments [38–42]. These results also suggest that PIE exerts a preventive effect, as PIE could reduce the severity and progression of UC, as evidenced by all the parameters observed in the DSS-induced UC rat model.

Evidence is increasingly showing that mucosal tissue injury and inflammation are associated with excessive ROS production and decreased activity of antioxidant enzymes, including SOD, CAT, and GPx, which in turn leads to oxidative stress. The overproduction of ROS plays a crucial role in the progression and severity of colitis, contributing to cellular damage in proteins, DNA, and lipids, impairing the intestinal epithelial barrier, and activating proinflammatory signaling pathways, which may represent an early critical event in models of experimental colitis [43–46]. Previous studies have demonstrated that exposure to DSS leads to elevated levels of MDA, an indicator of lipid peroxidation in the colonic tissue, which is considered a product of free radicals generated during oxidative stress. In addition, the activities of antioxidant enzymes such as SOD, CAT, and GPx are significantly decreased in the colonic mucosa of colitis experimental models [47–50]. The results from this study agreed with previous findings, showing a significant increase in MDA levels and a marked decrease in the activity of antioxidant enzymes, including SOD and CAT, in rats with DSS-induced UC. Treatment with PIE resulted in a marked decrease in MDA levels and enhanced the activity of SOD and CAT in the colonic tissues of rats with DSS-induced UC. Previous reports have shown that *P. indica* herbal tea leaves exhibited antioxidant activity in *in vitro* studies by exerting free-radical scavenging properties [21,51]. Similarly, an *in vivo* study demonstrated that *P. indica* leaves

extract possesses potent antioxidant activity and can alleviate liver injury in STZ-induced diabetic animals by significantly increasing SOD and CAT levels, while decreasing MDA levels [18]. In addition, it has been shown to increase CAT enzyme activity in rats induced with carbon tetrachloride [52]. Although the active compounds were not investigated in the present study, the antioxidant assays conducted demonstrated that PIE possesses antioxidant activity, implying that these substances may reduce colonic mucosal injury through their antioxidant and free radical scavenging properties, particularly ameliorating the pathological consequences associated with UC.

The activation of the immune system, along with both acute and chronic inflammation in the mucosal tissue, is mediated by the pro-inflammatory cytokines. Among these cytokines, IL-6 and IL-1β seem to be the principal cytokines involved in colitis development [53,54]. IL-6 plays a crucial role in promoting inflammation by stimulating the production of acute-phase proteins and mediating intestinal inflammation, which can be used as markers for inflammation processes. IL-6 is primarily secreted by intestinal epithelial cells, smooth muscle cells, CD4+ T cells, and macrophages. It further enhances the recruitment and activation of neutrophils and other immune cells to the site of inflammation, contributing to the chronic inflammation within the gut. IL-1β is involved in several aspects during the progression of IBD by inducing pro-inflammatory responses and promoting the recruitment and activation of immune cells within the gut mucosa. In addition, during acute UC, IL-1β is implicated in the disruption of the intestinal barrier, leading to increased permeability of the intestinal epithelial tight junctions. Regarding patients with IBD as well as in experimental animal models, IL-6 and IL-1β production and signaling are upregulated in both plasma and inflamed colonic mucosal tissue [54–58]. Consistent with previous studies on the role of IL-6 and IL-1β, we found increased expression of both IL-6 and IL-1β genes in rats treated with DSS. Compared to the model group, pretreatment with PIE was able to suppress the mRNA expression of IL-6 and IL-1β in colonic tissue, which were reversed by PIE to near normal levels. Moreover, the previous study reported that the hot water extract from *P. indica* herbal tea leaves exhibits potent inhibitory effects on LPS-induced NO and PGE2 production in RAW 264.7 macrophages, as well as in a rat model of acute inflammation in carrageenan-induced paw edema [17,20]. These findings suggest that PIE has potential and can be a one-therapeutic agent for UC, as its anti-inflammatory properties and antioxidant activities may also play some part in suppressing pro-inflammatory cytokine expression.

5. CONCLUSION

In summary, this study demonstrated the protective effects of PIE against DSS-induced UC in a rat model, due to its activities in reducing DAI, alleviating colonic inflammation, reducing oxidative stress through the inhibition of lipid peroxidation and the enhancement of antioxidant enzyme activities, and suppressing the mRNA expressions of inflammatory cytokines. Thus, the results of this study provide preliminary evidence that PIE may exert antioxidative and

anti-inflammatory effects against colonic mucosal injury, with potential protective effects for the prevention and management of UC. Therefore, PIE may serve as a potential candidate and alternative for supplementation in the management of colonic inflammation in the near future. Further investigations are needed to identify the active compounds of PIE and to elucidate the underlying mechanisms of action in the context of colonic inflammation. Studies using long-term and chronic models are also required to substantiate its efficacy and safety.

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

The study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Science, Prince of Songkla University, Songkhla, Thailand, and was conducted in accordance with the Ethics of Animal Experimentation guidelines of the National Research Council of Thailand (Ethics Registry: MHESI 68014/894; Ref. AR032/2022).

10. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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