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# Research Article
# Ameliorative Potential of Kaempferol in TNBS-Induced Colitis in Experimental Rats: A Role of Activation of PPARγ Pathway

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## Abstract

**Background and Objective:** Ulcerative colitis (UC) is a chronic condition mainly characterized by inflammatory and ulcerative interstitial mucosa of the colon. Kaempferol has been reported for its antioxidant, antiinflammation, antiulcer and wound healing effects. The objective of the study was to evaluate the potential of kaempferol against UC induced by Trinitrobenzene Sulfonic Acid (TNBS) in experimental rats. **Materials and Methods:** The TNBS (100 mg/kg, in 50% ethanol) was used to induce colitis in overnight fasted Sprague-Dawley rats (180-220 g) on day 0 and they received (n = 18, in each group) either vehicle (DMSO) or 5-aminosalicylic acid (500 mg/kg), or kaempferol (50 or 100 or 200 mg/kg), orally for 14 days. Various macroscopic, biochemical, molecular and histopathological analysis was assessed to evaluate the efficacy of kaempferol against UC. **Results:** The TNBS-induced spleen enlargement, colonic damage and ulceration were effectively and dose-dependently ($p<0.01$ and $p<0.001$) ameliorated by kaempferol (100 and 200 mg/kg). Elevated colonic oxido-nitrosative (SOD, GSH, MDA and nitric oxide) stress and MPO levels were also markedly ($p<0.01$ and $p<0.001$) reduced by kaempferol treatment. The TNBS-induced elevated cytokine levels (TNF-α and IL-1β) were significantly ($p<0.01$ and $p<0.001$) decreased after kaempferol administration. Western blot analysis also suggested that altered colonic PPARγ and collagen-1 protein expressions were effectively ($p<0.01$ and $p<0.001$) down-regulated by kaempferol administration. Furthermore, kaempferol treatment markedly reduced histopathological alteration induced by TNBS in the colon ($p<0.01$ and $p<0.001$). **Conclusion:** The results of the present study suggested that kaempferol exerts its colono-protective effect via activation of the PPARγ pathway to inhibit the release of inflammatory cytokines, oxidative stress, neutrophil infiltration and collagen-1 formation during TNBS-induced colitis.

Key words: Collagen-1, IL-1β, kaempferol, peroxisome proliferator-activated receptor gamma, trinitrobenzene sulfonic acid, tumour necrosis factor-α, ulcerative colitis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Inflammatory Bowel Disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract with two main types: Crohn's disease and ulcerative colitis[1,2]. Ulcerative colitis (UC) primarily affects the colon and the rectum and is mainly characterized by inflammation and ulceration of the interstitial mucosa of the colon[3]. Symptoms of UC include abdominal pain, diarrhea, rectal bleeding, weight loss and fatigue. Due to its chronic nature and symptoms, UC significantly impacts the quality of life[4]. The incidence of UC was 9-20 cases per 100,000 persons per year, whereas the estimated prevalence of UC was 5 million cases worldwide in 2023, which is expected to increase by Berre *et al.*[1]. In 2021, mean direct and indirect costs for IBD management were $11,668.68 and $74.90 in China, suggesting a significant economic burden of UC in these patients[5].

Although, the exact cause of UC is not fully understood, it is believed to involve a combination of genetic, environmental and immune system factors[6,7]. The pathophysiology of UC involves an abnormal immune response, intestinal barrier disruption and gut microbiome changes[8]. The immune system mistakenly attacks the lining of the colon, leading to inflammation and the formation of ulcers[1]. Additionally, research has indicated that the pathophysiology of the disease is connected to intestinal inflammation, which encourages bacterial invasion, thereby resulting in cellular destruction and necrosis in the colon and intestine[9]. Various immune cells, including T cells, B cells and macrophages, release inflammatory mediators such as cytokines (Tumor Necrosis Factor-alpha (TNF-α) and Interleukins (ILs)) contribute to tissue damage[10-12]. Furthermore, environmental triggers, including infections, dietary factors and exposure to pollutants, may contribute to the onset or exacerbation of colitis in genetically predisposed individuals. Chronic and extensive colitis may lead to complications such as perforation of the colon, toxic megacolon and an increased risk of colorectal cancer[13].

Current targeted therapies aim to modulate the immune response, restore the intestinal barrier and address microbial dysbiosis[14]. Treatment strategies often involve a combination of medications, including anti-inflammatory drugs (aminosalicylates such as mesalamine and sulfasalazine, corticosteroids such as prednisone), immunosuppressants (azathioprine, 6-mercaptopurine and methotrexate) and biologics (infliximab, adalimumab, vedolizumab and ustekinumab), along with lifestyle modifications and in some cases, surgical intervention is needed. However, it has been noted that the extensive use of these medications is linked to decreasing microbial diversity and may aggravate colitis because of diminished microbial variety in the gut, which may amplify the effects of one dominating species of microorganisms[15]. Thus, the present management and treatment of UC have remained controversial and continue to be difficult due to the limited usage of antibiotics. Researchers have thus made deliberate efforts to find innovative, affordable and safer drugs while considering evidence-based complementary and alternative therapies.

Experimental animal models are important for evaluating mechanisms of various new therapeutic moieties in colitis management[16]. Numerous researchers have documented UC by intracolonic instillation of 2,4,6-Trinitrobenzene Sulfonic Acid (TNBS) is a well-established, widely accepted and commonly used animal model of IBD[7,17-20]. The TNBS serves as a hapten and when transmitted into the colon wall, it interacts with high molecular weight tissue proteins that induce immunogenic Th1 inflammation response[17]. This immune-inflammatory reaction causes the activation of inflammatory mediators, which results in colonic damage, including necrosis and ulcerations that resemble the characteristics of human UC[21].

The use of active phytochemicals to prevent or treat illness has gained popularity in recent years. These phytochemicals are regarded as effective medicine since they may have minimal or no toxicity on healthy tissues[22]. Based on their historical worth, many contemporary medications are derived from natural sources. Kaempferol ((3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one)) widely found in various fruits including broccoli, beans, tea, leeks and onions, well documented for its potential against oxidative stress, inflammation, microbial infection, cancer, cardiotoxicity, neurotoxicity, diabetes, convulsion, arthritis and allergy[2,23]. Researchers recently reported kaempferol's wound-healing potential in diabetic and nondiabetic rats[24]. Kaempferol exerts its antiulcer effect via a reduction in TNF-α, IL-1β, IL-6, Myeloperoxidase (MPO) and nitric oxide (NO) levels in ethanol-induced gastric ulcers[25]. Additionally, Yeon *et al.*[26] revealed that kaempferol inhibited the elevated expression of pro-inflammatory cytokines (TNF-α, IL-1β and IL-8) in *Helicobacter pylori*-induced gastric ulcers. However, more thorough research is needed to identify its molecular mechanisms of action in ameliorating the damage induced by TNBS on the colon. Thus, the present study aimed to evaluate the potential of kaempferol against ulcerative colitis induced by TNBS in experimental rats.

## MATERIALS AND METHODS

**Study area:** The study was conducted in Department of Pharmacology of People's Hospital of Dongxihu District from August to December, 2023.

**Animals:** Sprague-Dawley rats (adult male, 180-220 g, n = 108) were procured from the Laboratory Animal Center of the People's Hospital of Dongxihu District. The housing conditions for rats throughout the experimental protocol were: Temperature: 24±1°C, relative humidity: 45-55%, dark/light cycle: 12:12 hrs, food: Standard pellet chow, water: Filtered (*ad libitum*). While 09:00 to 17:00 hrs were considered to carry out all the experiment protocols.

**Ethical consideration:** This experiment was approved by the Institutional Animal Ethics Committee (IAEC, People's Hospital of Dongxihu District, China). A guideline outlined in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the ARRIVE (Animal Research: Reporting of *in vivo* Experiments) guidelines were followed to perform all the experiments[27].

**Chemical and kits:** Kaempferol and TNBS (Sigma Chemical Co., St. Louis, Missouri, USA), TNF-α and IL-1β rats ELISA kits (Bethyl Laboratories Inc., Montgomery, Texas, USA), primary antibodies of PPARγ and collagen-1 (Abcam, Cambridge, Massachusetts, USA) were purchased from respective manufacturers.

**Induction of colitis and drug treatment schedule:** Colitis was induced by using TNBS (100 mg/kg, in 50% ethanol) in overnight fasted Sprague-Dawley rats[28]. The TNBS administered rats were randomly divided (n = 18, each) and received following treatments viz., 1% DMSO (Dimethyl Sulfoxide; TNBS control group), kaempferol (50 or 100 or 200 mg/kg) and 5-ASA (5-aminosalicylic acid, 500 mg/kg) orally for 14 days. Another group of rats i.e., normal group who received 1% DMSO also maintained separately. Body weight, spleen weight, hematological evaluation (complete blood cell count), colonic damage and ulceration was determined as per previously reported methods by Kandhare *et al.*[29] and Kandhare *et al.*[30].

**Biochemical assays:** At the end of the study (on 15th day) rats were sacrificed by cervical dislocation and the levels of colonic oxido-nitrosative stress (MPO, nitric oxide, SOD (Superoxide Dismutase), GSH (Glutathione)) and MDA (lipid peroxidation) content were estimated according to previously described methods by Kandhare *et al.*[29] and Kandhare *et al.*[30]. The levels of TNF-α and IL-1β in colon tissue were evaluated using respective rats ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, Texas, USA) as per the manufacturer's instructions[31].

**Western blot assay:** Whereas protein expressions of PPARγ and collagen-1 were estimated in lung tissue according to the method described elsewhere[29,30].

**Histological analysis:** Histopathological analysis of colon tissue was carried out using Hematoxylin and Eosin (H&E) stain and photographs were captured by a light compound microscope with a Zeiss intravital microscopy setup (Zeiss Axioscope A1, Carl Zeiss MicroImaging, Jena, Germany) as described previously by Kumar *et al.*[32].

**Statistical analysis:** GraphPad Prism 5.0 software (GraphPad, San Diego, California) was used for data analysis. Data are expressed as Mean±Standard error mean (SEM) and analyzed by using one-way ANOVA followed by Tukey's multiple range *post hoc* analysis (for parametric tests) as well as the Kruskal-Wallis test for *post hoc* analysis (non-parametric tests). A value of $p<0.05$ was considered to be statistically significant.

## RESULTS

**Body weight and spleen weight:** There was a significant ($p<0.001$) decrease in the body weight, whereas there was an increase in the spleen weight of TNBS-induced control rats compared to the normal rats. Body weight of kaempferol (100 and 200 mg/kg) treated rats significantly ($p<0.01$ and $p<0.001$) increased, whereas spleen weight significantly ($p<0.01$ and $p<0.001$) decreased in a dose-dependent manner compared to TNBS-induced control rats. Similarly, 5-ASA (500 mg/kg) treated rats also showed a significant increase ($p<0.001$) in body weight and a decrease in spleen weight compared to TNBS-induced control rats (Table 1).

**Colonic damage and ulceration:** There was a significant ($p<0.001$) increase in colon weight and colon weight-to-length ratio in TNBS-induced control rats compared with the normal group. Colon weight and colon weight to length ratio of kaempferol (100 and 200 mg/kg) treated rats significantly ($p<0.01$) and dose-dependently decreased compared to TNBS-induced control rats. Similarly, 5-ASA (500 mg/kg) treated rats also showed a significant decrease ($p<0.001$) in colon weight and colon weight-to-length ratio compared to TNBS-induced control rats (Table 1 and Fig. 1a-f).

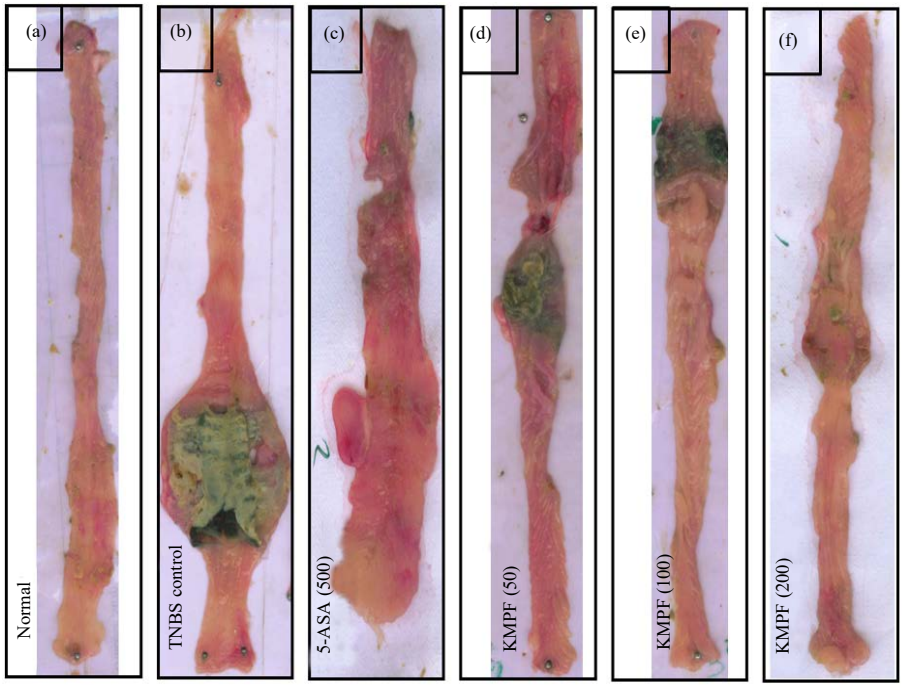


Fig. 1(a-f): Morphological representation of colons from (a) Normal, (b) TNBS control, (c) 5-ASA (500 mg/kg), (d) KMPF (50 mg/kg), (e) KMPF (100 mg/kg) and (f) KMPF (200 mg/kg) treated rats

Table 1: Effect of kaempferol on TNBS-induced alterations in body weight, spleen weight, colon weight, colon weight to length ratio, ulcer area and ulcer index in rats

| Treatment | Body weight (gm) | Spleen weight (gm) | Colon weight (gm) | Colon weight to length ratio (gm/cm) | Ulcer area ($mm^2$) | Ulcer index |
|---|---|---|---|---|---|---|
| Normal | 214.40±9.75 | 1.51±0.12 | 1.14±0.13 | 0.17±0.02 | - | - |
| TNBS control | 157.90±6.46$^{###}$ | 2.33±0.11$^{###}$ | 2.69±0.13$^{###}$ | 0.30±0.04$^{###}$ | 42.23±3.58 | 49.52±7.84 |
| 5-ASA (500) | 206.20±8.54*** | 1.53±0.08*** | 1.28±0.08*** | 0.18±0.02*** | 15.92±2.94*** | 23.07±3.85*** |
| KMPF (50) | 156.80±5.82 | 2.30±0.09 | 2.51±0.14 | 0.30±0.01 | 38.81±1.77 | 41.78±1.39 |
| KMPF (100) | 190.70±6.66** | 1.95±0.11** | 1.99±0.14** | 0.26±0.02** | 30.26±2.79* | 34.31±1.26*** |
| KMPF (200) | 200.50±8.24*** | 1.65±0.17*** | 1.46±0.12*** | 0.20±0.03*** | 16.60±2.27*** | 32.87±1.95*** |

Data are expressed as Mean±SEM (n = 6) and analyzed by one-way ANOVA followed by Tukey's multiple range test, data of macroscopical score and stool consistency was analyzed using non-parametric Kruskal-Wallis ANOVA followed by Tukey's multiple range test, *p<0.05, **p<0.01 and ***p<0.001 as compared to TNBS control group and $^{###}$p<0.05 as compared to normal group, TNBS: 2,4,6-Trinitrobenzene Sulfonic Acid, 5-ASA: 5-Aminosalicylic Acid and KMPF: Kaempferol

Ulcer area and ulcer index were increased significantly (p<0.001) in TNBS-induced control rats compared to normal rats. Treatment with kaempferol (100 and 200 mg/kg) significantly and dose-dependently decreased ulcerated area (p<0.05 and p<0.001) and ulcer index (p<0.01 and p<0.001) when compared with the TNBS-induced control rats. The 5-ASA (500 mg/kg) also produced a significant (p<0.001) reduction in ulcerated areas and index when compared with the TNBS-induced control rats (Table 1).

**Hematological alterations:** The TNBS-induced control rats showed significantly decreased (p<0.001) WBC, RBC, Hb, HCT and PLT levels in blood compared to normal rats. Kaempferol (100 and 200 mg/kg) showed significant and dose-dependent increases (p<0.01 and p<0.001) in WBC, RBC, Hb, HCT and PLT levels in blood compared to vehicle control rats. Additionally, treatment with 5-ASA (500 mg/kg) showed a significant increase (p<0.001) in WBC, RBC, Hb, HCT and PLT levels in blood compared to the TNBS-induced control rats (Table 2).

**Colonic oxide-nitrosative damage:** There was a significant decrease (p<0.001) in SOD and GSH, whereas there was an increase (p<0.001) in MDA and nitric oxide of TNBS-induced control rats compared to normal rats. Kaempferol (100 and 200 mg/kg) significantly increased (p<0.001) SOD, significantly and dose-dependently increased (p<0.01 and p<0.001) GSH levels compared to TNBS-induced control rats. When

Table 2: Effect of kaempferol on TNBS-induced alterations in WBC, RBC, Hb, HCT and PLT in rats

| Treatment | WBC ($\times 10^3$/μL) | RBC ($\times 10^6$/μL) | HGB (g/dL) | HCT (%) | PLT ($\times 10^5$/μL) |
|---|---|---|---|---|---|
| Normal | 18.83±2.18 | 14.33±1.71 | 19.00±2.54 | 53.67±3.51 | 10.67±1.65 |
| TNBS control | 5.33±1.80### | 4.33±1.20### | 4.50±1.31### | 33.00±2.32### | 2.83±0.54### |
| 5-ASA (500) | 15.50±1.12*** | 14.00±1.63*** | 18.33±2.57*** | 52.67±2.46*** | 9.67±0.84*** |
| KMPF (50) | 7.83±1.58 | 3.50±1.03 | 4.17±1.80 | 32.17±1.83 | 3.00±0.93 |
| KMPF (100) | 17.17±3.36** | 13.00±2.65*** | 15.17±2.73** | 44.17±2.44** | 8.33±1.69** |
| KMPF (200) | 19.17±2.79*** | 13.33±2.29*** | 18.00±1.57*** | 49.17±1.85*** | 10.00±1.44*** |

Data are expressed as Mean±SEM (n = 6) and analyzed by one-way ANOVA followed by Tukey's multiple range test, **p<0.01 and ***p<0.001 as compared to TNBS control group and ###p<0.05 as compared to normal group, TNBS: 2,4,6-Trinitrobenzenesulfonic Acid, 5-ASA: 5-Aminosalicylic Acid, KMPF: Kaempferol, WBC: White Blood Cell, RBC: Red blood cell, Hb: Hemoglobin, HCT: Hematocrit and PLT: Platelet

Table 3: Effect of kaempferol on TNBS-induced alterations in colonic SOD, GSH, MDA, NO, MPO, TNF-α and IL-1β in rats

| Treatment | Colonic SOD (U/mg of protein) | Colonic GSH (μg/mg protein) | Colonic MDA (nM/mg of protein) | Colonic NO (μg/ml) | Colonic MPO (U/g of tissue) | Colonic TNF-α (pg/mg wet tissue) | Colonic IL-1β (pg/mg wet tissue) |
|---|---|---|---|---|---|---|---|
| Normal | 15.81±1.32 | 26.32±1.85 | 24.65±2.57 | 27.38±3.48 | 5.35±1.50 | 108.40±7.05 | 24.71±1.51 |
| TNBS control | 3.00±0.70### | 11.64±1.88### | 70.80±3.32### | 75.44±3.48### | 21.64±2.32### | 203.30±13.46### | 146.30±2.23### |
| 5-ASA (500) | 14.40±2.27*** | 25.98±1.95*** | 24.71±2.15 | 35.27±4.31*** | 6.65±1.15*** | 129.80±8.03*** | 60.16±1.65*** |
| KMPF (50) | 3.79±1.42 | 17.21±2.52 | 69.64±2.11 | 76.23±4.33 | 18.75±2.28 | 198.30±8.92 | 142.00±2.20 |
| KMPF (100) | 13.55±2.39*** | 20.18±1.26*** | 58.92±3.71* | 54.38±7.48** | 12.33±1.79** | 157.20±9.42** | 111.40±3.21** |
| KMPF (200) | 14.06±2.06*** | 25.41±1.26*** | 34.90±2.99*** | 39.05±4.52*** | 9.01±2.12*** | 138.40±9.48*** | 76.76±2.41*** |

Data are expressed as Mean±SEM (n = 6) and analyzed by one-way ANOVA followed by Tukey's multiple range test, *p<0.05, **p<0.01 and ***p<0.001 as compared to TNBS control group and ###p<0.05 as compared to normal group, TNBS: 2, 4, 6-Trinitrobenzene Sulfonic Acid, 5-ASA: 5-Aminosalicylic Acid, KMPF: Kaempferol, SOD: Superoxide dismutase, GSH: Glutathione, MDA: Malondialdehyde, NO: Nitric oxide, MPO: Myeloperoxidase, TNF-α: Tumor Necrosis Factor-alpha and ILs: Interleukins

compared to TNBS-induced control rats, MDA and nitric oxide levels were decreased significantly and dose-dependently (p<0.05 and p<0.001, p<0.01 and p<0.001) in kaempferol (100 and 200 mg/kg) treated rats. Treatment with 5-ASA (500 mg/kg) also significantly increased (p<0.001) the SOD and GSH levels, whereas decrease (p<0.001) in MDA and nitric oxide levels compared to TNBS-induced control rats (Table 3).

**Colonic MPO levels:** There was a significant increase (p<0.001) in MPO levels of TNBS-induced control rats compared to normal rats. Kaempferol (100 and 200 mg/kg) significantly and dose-dependently decreased (p<0.01 and p<0.001) MPO levels compared to TNBS-induced control rats. Treatment with 5-ASA (500 mg/kg) also significantly reduced (p<0.001) the elevated MPO levels compared to TNBS-induced control rats (Table 3).

**Cytokine levels:** There was a significant increase (p<0.001) in colonic TNF-α and IL-1β levels of TNBS-induced control rats compared to normal rats. Kaempferol (100 and 200 mg/kg) significantly and dose-dependently decreased (p<0.01 and p<0.001) colonic TNF-α and IL-1β levels compared to TNBS-induced control rats. Treatment with 5-ASA (500 mg/kg) also significantly decreased (p<0.001) the elevated colonic TNF-α and IL-1β levels compared to TNBS-induced control rats (Table 3).

**Colonic PPARγ and collagen-1 protein expressions:** There was a significant down-regulation (p<0.001) in colonic PPARγ protein expression, whereas there was a considerable up-regulation (p<0.001) in colonic collagen-1 protein expression of TNBS-induced control rats compared to normal rats. Kaempferol (100 and 200 mg/kg) significantly and dose-dependently (p<0.01 and p<0.001) up-regulated colonic PPARγ protein expression, whereas down-regulated colonic collagen-1 protein expression compared to TNBS-induced control rats. Treatment with 5-ASA (500 mg/kg) also significantly (p<0.001) up-regulated colonic PPARγ protein expression and down-regulated colonic collagen-1 protein expression compared to TNBS-induced control rats (Fig. 2a and b).

**Histopathology of colon:** Colon tissue from normal rats showed intact epithelial crypts of the mucosal layer without any evidence of necrosis; however, it showed the presence of mild infiltration of inflammatory cells (Fig. 3a). The histopathological features of TNBS control rat showed significant (p<0.001) transmural necrosis, edema, diffused inflammatory cell infiltration in the mucosa, desquamated areas and loss of the epithelium (Fig. 3b). Kaempferol (100 and 200 mg/kg) significantly (p<0.001) attenuated the extent and severity of the histological signs of cell damage that were associated with intrarectal instillation of TNBS (Fig. 3c-e).

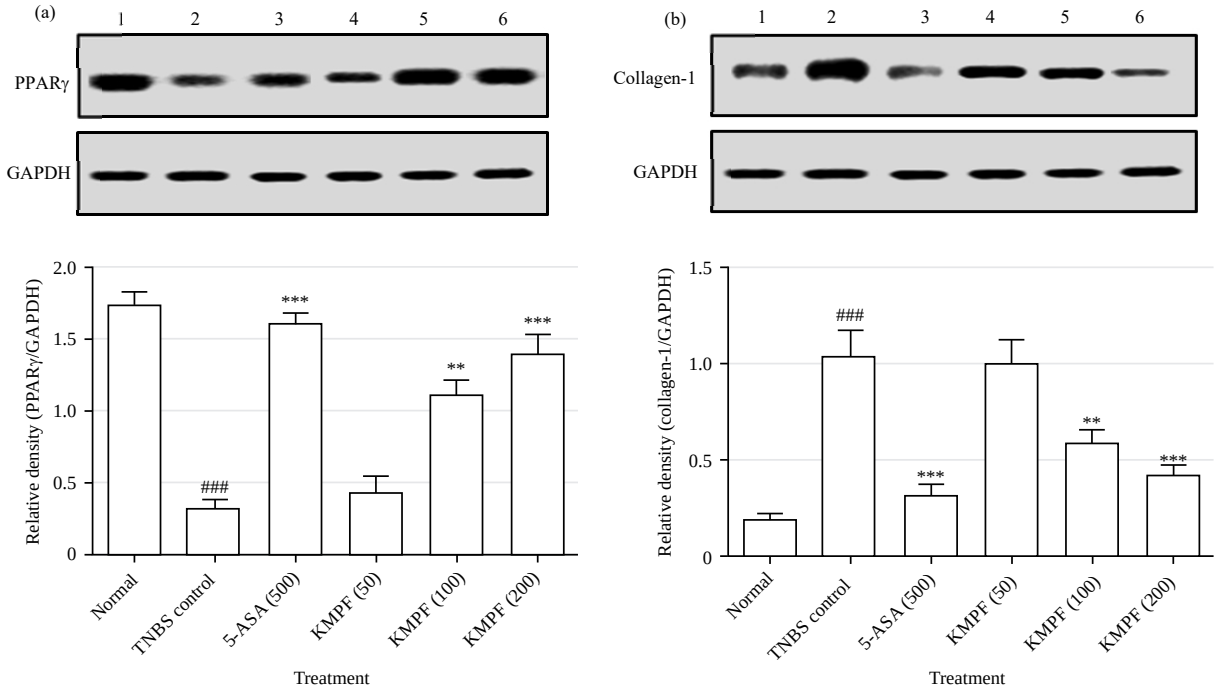


Fig. 2(a-b): Effect of kaempferol on TNBS-induced alterations in colonic (a) PPARγ and (b) Collagen-1 protein expression in rats

Data are expressed as mean±SEM (n = 6) and analyzed by one-way ANOVA followed by Tukey's multiple range test, *p<0.05, **p<0.01 and ***p<0.001 as compared to TNBS control group and ###p<0.05 as compared to normal group. Representative protein expression of Normal (Lane 1), TNBS control (Lane 2), 5-ASA (500 mg/kg) (Lane 3), kaempferol (50 mg/kg) (Lane 4), kaempferol (100 mg/kg) (Lane 5) and kaempferol (200 mg/kg) (Lane 6) treated rats, TNBS: 2,4,6-Trinitrobenzenesulfonic Acid, 5-ASA: 5-Aminosalicylic Acid, KMPF: Kaempferol, PPARγ: Peroxisome Proliferator-Activated Receptor Gamma and GAPDH: Glyceraldehyde 3-Phosphate Dehydrogenase

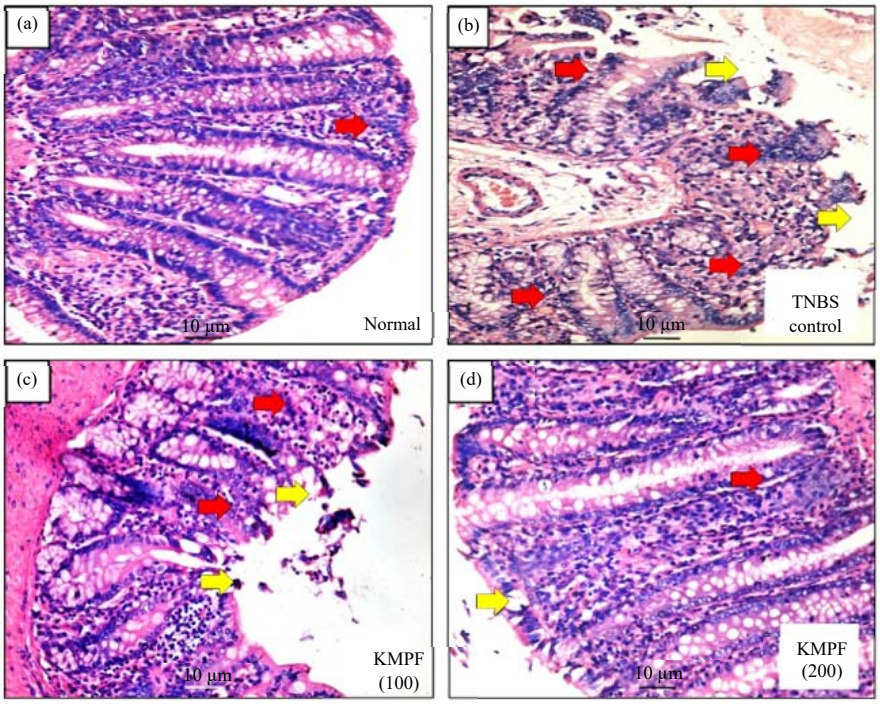


Fig. 3(a-e): Continue

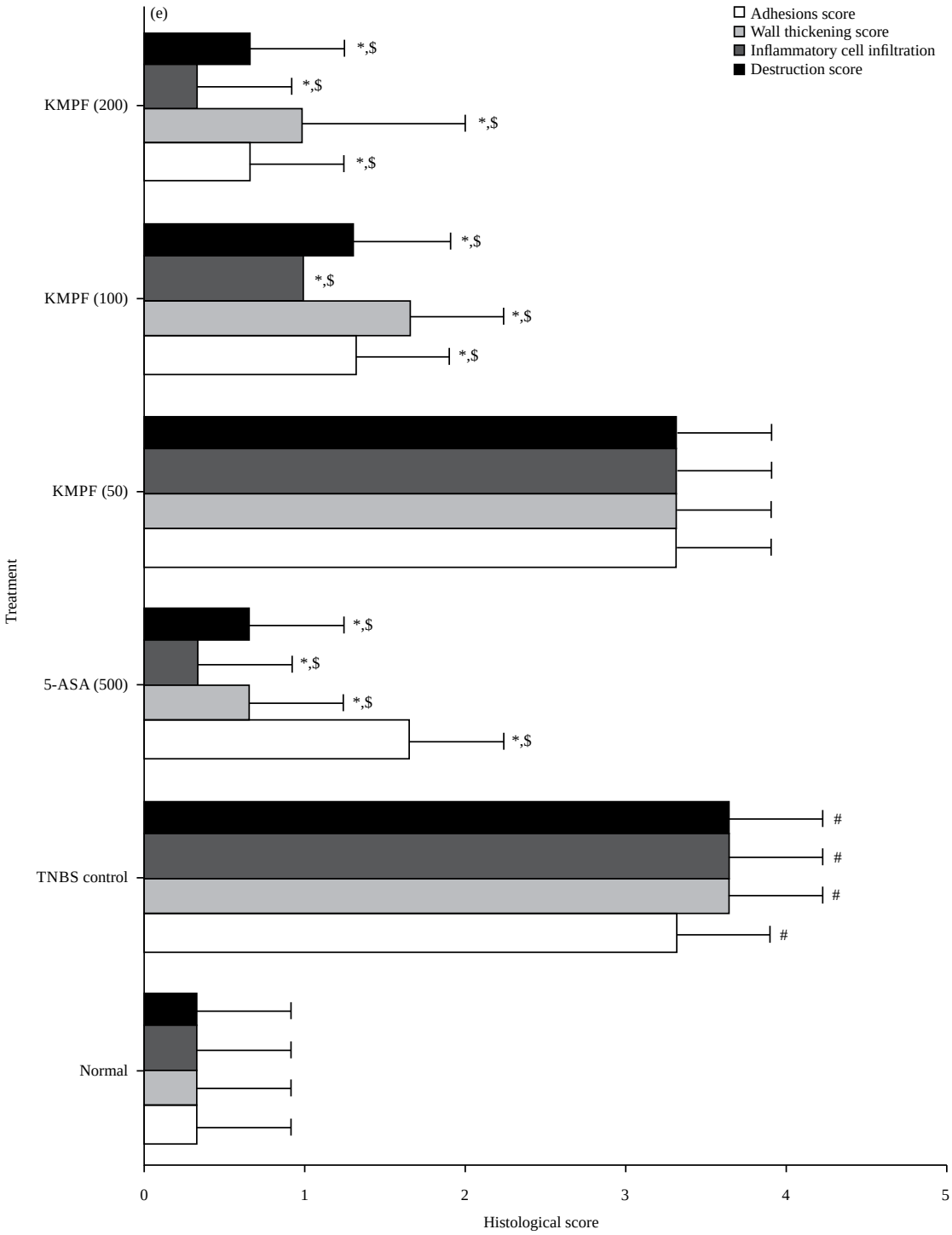


Fig. 3(a-e): Effect of kaempferol on TNBS-induced alterations in colon histopathology, photomicrograph of sections of colon tissue from (a) Normal, (b) TNBS control, (c) KMPF (100 mg/kg), (d) KMPF (200 mg/kg) treated rats stained with H&E stain and (e) The quantitative representation of histological score

Data are expressed as Mean±SEM (n = 3) and analyzed by one-way ANOVA followed by the Kruskal-Wallis test applied for *post hoc* analysis, *p<0.05, **p<0.01 and ***p<0.001 as compared to TNBS control group and ###p<0.05 as compared to normal group, TNBS: 2,4,6-Trinitrobenzenesulfonic Acid, 5-ASA: 5-Aminosalicylic Acid, KMPF: Kaempferol, The red arrow indicated inflammatory infiltration and the yellow arrow indicated necrosis and images (×40 magnification) are typical and represent each study group

## DISCUSSION

Ulcerative colitis is a chronic complication of gastrointestinal disease that significantly affects an individual's quality of life[1]. Inflammation damages the colon's epithelial cells, resulting in the loss of the protective barrier function. This allows bacteria and other luminal contents to encounter the underlying tissue[33,34]. To the best of our knowledge, this work was the first to extensively investigate the pharmacological action of kaempferol in an experimental model of TNBS-induced colitis with a diverse mechanism of action. The finding suggested that kaempferol exerts its protective efficacy via activation of the PPARγ pathway to inhibit the release of inflammatory cytokines, oxidative stress, neutrophil infiltration and collagen-1 formation during TNBS-induced colitis.

In the present investigation administration of TNBS is associated with weight loss, ulcer formation and rectal bleeding in TNBS control rats. However, administration of kaempferol showed amelioration in TNBS-induced decreased body weight and ulceration. It has been reported that inflammation can lead to crypt abscesses, where inflammatory cells accumulate within the crypts of the colonic mucosa[19,35]. Continuous inflammation results in ulcers forming in the colon's lining, a hallmark of UC[21,35]. The inflammatory changes in the colon and rectum contribute to the clinical manifestations of UC, including diarrhea, rectal bleeding, abdominal pain, urgency and weight loss[1,29]. Moreover, studies have shown a connection between UC and symptoms such as fatigue, compromised growth due to decreased appetite and insufficient weight gain[2,20].

Administration of rats with TNBS resulted in altered splenic immune responses, as evidenced by a substantial increase in the spleen weight in the TNBS control rats, as observed in current study findings. Kaempferol intervention mitigated this increase, underscoring its role in modulating immune responses. According to the Principles of Chinese Traditional Medicine, the spleen is the main organ affected by UC as it is crucial in acquiring immunity[20,28]. Impairment of the spleen's capacity to transport and metabolize nutrients results in pathological substances, including water dampness and accumulated phlegm. The spleen, which is a component of the mononuclear phagocyte system, often enlarges in response to infection or immunological responses[36]. Numerous research emphasize the importance of splenic immune responses during the development of UC, as the spleen is the primary organ for the destruction of RBCs[37,38]. Increased spleen weight indicates systemic immune function and spleen enlargement sometimes implies global immune suppression[1,21]. The results of the current study were in accordance with findings from a previous study, which suggested that kaempferol exerted its immunoinflammatory potential via modulating immune response through the spleen[39].

In the current study, the observed elevation in SOD and GSH concentrations in the TNBS control group suggested that the TNBS induction may augment the progression of oxidative stress, which abruptly destroys the antioxidant potency. However, kaempferol administration significantly enhanced and restored the concentration of GSH and SOD in the intestinal tissue, thereby counteracting the action of oxidative stress in TNBS-treated rats. It has been well documented that high quantities of nitric oxide generated by inducible NO synthase during intestinal inflammation enhance the permeability of endothelium and gut barriers through S-nitrosylation and protein nitration[33,40]. Furthermore, both the enzymatic (SOD, CAT and GPx) and non-enzymatic (GSH) antioxidants effectively regulate the production of free radicals in a healthy colon[41]. However, the intestinal membrane integrity is compromised during oxidative stress induced by TNBS, resulting in reduced antioxidant defense[7,28]. Both SOD and GSH have distinctive mechanisms of action in the colon. A high level of SOD has been shown to protect against colonic damage, whereas GSH can fend off harmful oxidative stress metabolites in the colon[18,20]. According to the literature, major plant metabolites such as flavonoids either directly scavenge hydroxyl radicals or indirectly enhance antioxidant enzymes[42-45]. Even though the therapeutic actions of many flavonoids in disease prevention/treatment have been postulated, little is known about their antioxidant activities[46-48]. Kaempferol, on the other hand, is a natural flavonoid with a well-defined antioxidant activity, as evident from scientific data available in the literature[24,25,49]. Therefore, current findings were consistent with the previous report, which revealed that kaempferol possessed potential antioxidant effects by enhancing the SOD and GSH concentration during colonic damage[24,25,49]. Additionally, a recent study in experimental models discovered that kaempferol protects the intestine from oxidative stress damage brought on by dextran sulfate sodium-induced UC[34,50].

To determine the protective effects of kaempferol against UC, pro-inflammatory cytokines levels were examined in the rat colon and findings suggested that kaempferol intervention groups demonstrated protective effects against the inflammatory cascade induced by TNBS. This implies that kaempferol may play a crucial role in modulating the levels of molecular pro-inflammatory biomarkers throughout the

TNBS-induced inflammatory process. Furthermore, H&E staining was used to detect the intestinal damage and modification induced by inflammatory infiltration in the colon histology. Examined colon from TNBS control rats depicted the incidence and severity of the rat colon after induction with TNBS reflected by a high degree of intestinal edema, inflammation, necrosis and swelling of the epithelial cells. However, the administration of kaempferol ameliorated this TNBS-induced colonic damage via its anti-inflammatory potential. Pro-inflammatory cytokines are potential regulators and key players associated with various stages of inflammation[51,52]. In chronic inflammation conditions such as ulcerative colitis, pro-inflammatory cytokines play a significant role in the induction and maintenance of this disease[53,54]. The TNF-α is a major pro-inflammatory cytokine involved in the immune response and its elevated levels have been reported clinically in the inflamed mucosa of the colon in UC patients[55]. The TNF-α promotes inflammation, induces the production of other inflammatory mediators and contributes to the recruitment of immune cells to the site of inflammation[56,57]. The IL-1β and IL-6 are pro-inflammatory cytokines contributing to the inflammatory cascade[58,59]. These cytokines activate and recruit immune cells, further amplifying the inflammatory response[8,60,61].

In the present investigation, TNBS-induced colon showed diminished PPARγ and elevated collagen-1 expressions, however, kaempferol treatment ameliorated TNBS-induced alterations in PPARγ and collagen-1 expressions. Researcher documented that Peroxisome Proliferator-Activated Receptor gamma (PPARγ) is a nuclear receptor that plays a crucial role in regulating various physiological processes, including inflammation and immune responses during UC[6,35]. Activation of PPARγ has been shown to inhibit the production of inflammatory mediators, such as TNF-α, IL-1β and IL-6, by downregulating the NF-κB (Nuclear Factor-kappa B) pathway and, thus, suppress the immune response[12,62]. The PPARγ activation has also been implicated in promoting the integrity of the intestinal barrier by influencing the expression of tight junction proteins[1,12]. Because of its anti-inflammatory and immunomodulatory effects, PPARγ has been considered a potential target for treating UC[12,35]. Treatment with PPARγ agonist in *ex-vivo* colonic biopsies from UC patients showed activation of anti-inflammatory PPARγ activity[35]. Furthermore, transcriptional activation of Transforming Growth Factor-β (TGF-β) inhibits PPARγ transcription, which further promotes collagen-1 production[6,63-65]. These processes lead to a rapid increase in fibroblasts, epithelial-mesenchymal transition and fibrocyte accumulation, which ultimately results in dysregulated wound repair and extracellular matrix accumulation in the inflamed colon[66,67]. Elevated collagen-1 expression is accountable for excess collagen deposition in the intestinal wall, which may contribute to fibrosis, thus impacting the function and integrity of the mucosal barrier[49,68]. Studies reported that kaempferol exerts its potential via suppression of collagen synthesis[49,68]. The results of the present study were in accordance with the findings of previous investigators suggesting the potential of kaempferol during the wound healing process[49,68].

Current study has several limitations. Firstly, due to acute nature of inflammation induced by TNBS in colon, current study unable to evaluate the effect of kaempferol against chronic inflammation. Secondly, IBD is well known to affect the various segment of colon however, in the present study we could able to evaluate the effect of kaempferol in distal colon only resulting into ameliorative effect in localized Inflammation than systemic. Thus, further investigation is needed to evaluate the potential of kaempferol against chronic and systemic inflammation.

## CONCLUSION

In present investigation, results demonstrated that kaempferol exerts its anti-inflammatory and colonoprotective effect in experimental model of ulcerative colitis. This protective efficacy of kaempferol against TNBS-induced UC was likely due to activation of the PPARγ pathway to inhibit the release of inflammatory cytokines, oxidative stress, neutrophil infiltration and collagen-1 formation. To the best of our knowledge, this is the first evidence reporting a direct link between kaempferol and PPARγ activation in UC. It is important to note that findings of current study may contribute to the effective utilization of kaempferol in managing clinical IBD. However, further studies are warranted to confirm the effectiveness of kaempferol during UC in clinical settings.

## SIGNIFICANCE STATEMENT

To the best of our knowledge, the findings of the present study first time reported potential of kaempferol against ulcerative colitis induced by Trinitrobenzene Sulfonic Acid (TNBS) in experimental rats. The present findings will deliver valuable information to researchers and physicians to find the alternative healthcare product to manage ulcerative colitis. Thus, these new findings on the possible role of kaempferol may offer promise to better healing outcomes during colitis.

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