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ORIGINAL ARTICLE

D-Pinitol-Attenuated Trinitrobenzene Sulfonic Acid-Induced Ulcerative Colitis in Experimental Rats: Possible through Inhibition of Nuclear Factor-κB/Nuclear Factor of Kappa Light Polypeptide Gene Enhancer In B-Cell Inhibitor-Alpha Pathway and Activation of Colonic Tight Junction Proteins

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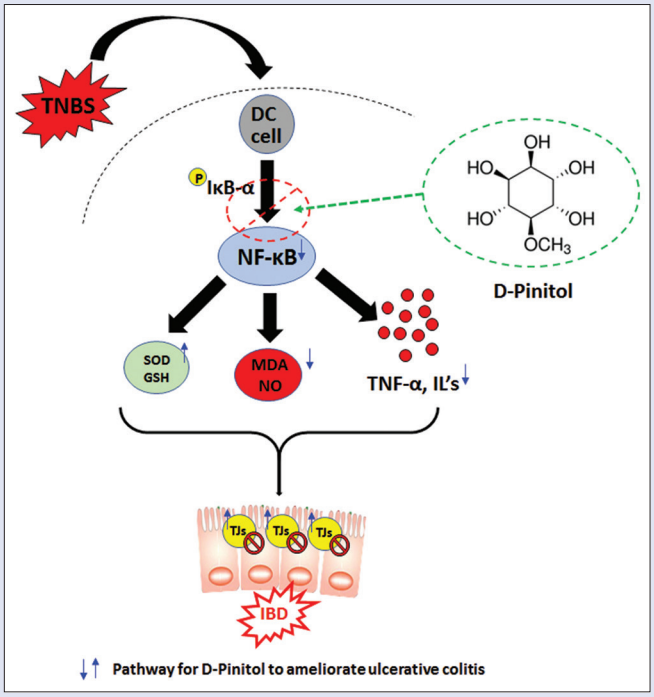
ABSTRACT

Background: Ulcerative colitis (UC) is a complex, chronic, and relapsing inflammatory disorder categorized by chronic inflammation followed by colonic damage. D-Pinitol has been recognized for its numerous pharmacological properties, counting antioxidant, antiulcer, and anti-inflammatory potential. **Aim:** The aim of the study was to measure the plausible mechanisms of action of pinitol on an experimental model of (2, 4, 6-trinitrobenzene sulfonic acid [TNBS])-induced UC in rats. **Materials and Methods:** TNBS (100 mg/kg, in 50% ethanol) was employed to induce UC in overnight fasted Sprague–Dawley rats. The rats have received either vehicle or (5-aminosalicylic acid [5-ASA]) or pinitol (5 or 10 or 20 mg/kg), p.o. for 14 days. Innumerable biochemical and molecular analysis were achieved in colon tissue. **Results:** Rectal instillation of TNBS resulted in the induction of colonic damage reproduced by marked ($P < 0.05$) reduced in colonic total antioxidant capacity (TOC) and significant ($P < 0.05$) surge in colonic oxido-nitrosative, myeloperoxidase, and hydroxyproline levels. However, administration of pinitol (10 and 20 mg/kg) effectively inhibited these TNBS-induced colonic damages. Real-time polymerase chain reaction (PCR) analysis recommended that TNBS-induced upregulated cytokine (Tumour necrosis factor-α [TNF-α], (interleukins)-1 [ILs] β, and IL-6), and nuclear factor-κB (NF-κB) messenger ribonucleic acid expressions were effectively ($P < 0.05$) condensed by pinitol. Western blot analysis recommended that pinitol conspicuously ($P < 0.05$) augmented tight junction proteins (claudin-1, occludin, and Zonula occludens-1 (ZO-1)) expression to improve colon functions. TNBS-induced histopathology modification in the colon was significantly ($P < 0.05$) diminished by pinitol. **Conclusion:** D-Pinitol ameliorated TNBS-induced UC through inhibition of raised oxidative stress (TOC, superoxide dismutase, glutathione, and Malondialdehyde) and inflammatory release (TNF-α, ILs, and NF-κB), which further progresses the intestinal barrier through activation of colonic tight junction proteins (ZO-1, claudin-1, and occludin).

Key words: Claudin-1, D-pinitol, trinitrobenzene sulfonic acid, ulcerative colitis, zonu

SUMMARY

- The current work has attentive for the first time on appraising the effect of d-Pinitol against trinitrobenzene sulfonic acid (TNBS)-induced ulcerative colitis (UC) in rats. The present study studied certain important outcomes of UC, counting biochemical, behavioral, molecular, and histological analysis. The results of the present search recommended that pinitol ameliorated TNBS-induced UC through inhibition of elevated oxidative stress (TOC, superoxide dismutase, glutathione, and Malondialdehyde) and inflammatory release (tumor necrosis factor-α, interleukins, and NF-κB), which further advances the intestinal barrier through activation of colonic tight junction proteins (Zonula occludens-1, claudin-1, and occludin). Ia occludens-1



d-Pinitol inhibited Nuclear Factor-κB/nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-alpha pathway to ameliorate trinitrobenzene sulfonic acid-induced ulcerative colitis.

Abbreviations used: ANOVA: Analysis of variance; 5-ASA: 5-aminosalicylic acid; COX-2: Cyclooxygenase-2; DAI: Disease Activity Index; DMSO: Dimethyl sulfoxide; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; GSH: Glutathione; HP: Hydroxyproline; IAEC: International Animal Ethics Committee; IBD: Inflammatory Bowel Disease; ILs: Interleukin's; IκBα: Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-alpha; iNOs: Inducible nitric oxide synthase; MDA:

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Malondialdehyde; MPO: Myeloperoxidase; NO: Nitric Oxide; NF-κB: Nuclear Factor-κB; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase; TNBS: Trinitrobenzene Sulfonic Acid; TNF-α: Tumour necrosis factor-α; T-AOC: Total Antioxidant Capacity; UC: Ulcerative Colitis, ZO-1: Zonula occludens-1.

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INTRODUCTION

Inflammatory Bowel Disease (IBD) is a complex, chronic, and relapsing inflammatory disorder of the Gastrointestinal Tract (GIT) encompass of Ulcerative Colitis (UC) and Crohn's disease.[1,2] UC is a mutual inflammatory and ulcerative condition that largely affects the colon and rectum, which suggestively adjusts the quality of life and causes the induction of colon cancer.[3-5] It has been well recognized that mucosal ulceration, low-grade fever, commotion of the epithelial barrier, fatigue, mucous stool, bloody diarrhea, abdominal pain, reduced appetite, and rectal bleeding are the clinical characteristics of UC.[1,6] Epidemiological studies recommended that, with augmented modernization, UC incidence also amplified significantly, with 6.8 million IBD cases worldwide, among which UC is 0.5–24.5/100,000 individual/years.[7] Thus, UC is the utmost frequently encountered clinical issue necessitating urgent courtesy for a safe and effective therapeutic regimen.

Numerous studies recognized that variation in the microbial flora of the intestine, external environment factors, genetic influences, and changed immune response are the putative mediators in the induction and upkeep of pathogenesis of UC.[1,8] The malfunction of mucosal barriers results in augmented intestinal permeability through destruction of tight junction (TJ), leading to mucosal injury. Researchers have stated the important role of claudin-1, ZO-1, and occludin, which are vital intestinal TJ barrier proteins during the pathogenesis of UC.[9] Furthermore, macrophages that are major innate immune cells have been recognized as important protagonists during the UC. Activation of macrophages induces the release of various inflammatory mediators such as tumour necrosis factor-α (TNF-α), interleukins (ILs), inducible nitric oxide (NO) synthase (iNOS), and Cyclooxygenase-2 (COX-2), which further subsidized to colonic damage.[10] The researcher distinguished the importance of inflammatory mediators in the pathogenesis of IBD; thus, a large number of investigators employed the various therapeutic agents bearing anti-inflammatory property, counting corticosteroids, and 5-aminosalicylates acid (5-ASA) for the management of UC.[11,12] However, the high treatment cost and long-term use-related side effects of these pharmaceutical agents cannot be overlooked, which limits their clinical application. Therefore, there is an urgent need to classify a newer therapeutic moiety preferably from the herbal origin with momentous safety and efficacy for the management of UC.

An array of researchers has explored the potential of polyphenolic moieties from the herbal origin using 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced UC.[10,13] D-Pinitol (3-O-methyl-chiro-inositol) is a naturally occurring plant polyphenol that offerings a number of functional foods such as pinewood, legumes, soy, and alfalfa.[14,15] The pharmacological potential of pinitol contains antihyperlipidemic, immunomodulatory, cardioprotective hepatoprotective, antidiabetic, antioxidant, anti-inflammatory, anticancer, anti-aging, neuroprotective, and wound healing.[16-18] Lee *et al.* (2007) described the inhibitory potential of pinitol against Th2 immune response through inflammatory influx.[17] D-Pinitol exerts its antiarthritic property through downregulation in the expression of inflammatory mediators (TNF-α, ILs, COX-II, and vascular endothelial growth factor).[19] Furthermore, an *in vitro* study recommended that the inhibition of release of TNF-α, IL-1 β, IL-6, and iNOS by pinitol contributes to its anti-inflammatory potential.[20] Notably, the anti-inflammatory property of pinitol attributes to the inhibitory potential of pinitol against phosphorylation of NF-κB (nuclear factor kappa B), which further weakened the release of inflammatory mediators.[16] A recent double-blind, randomized, placebo-controlled clinical study testified the hepatoprotective efficacy of pinitol through attenuation of elevated oxidative stress in non-alcoholic fatty liver disease patients.[21] Treatment of pinitol in Type 2 diabetes mellitus patients also established its efficacy in reducing blood glucose levels.[15] Recently, pinitol-rich fraction of *Bruguiera gymnorrhiza* (L.) Lam. Fruit extract conveyed its antiulcer potential through activation of the Keap1/Nrf2 signaling pathway and inhibition of inflammatory mediators (TNF-α and ILs).[22] Despite extensive studies on pinitol, no studies have entirely discovered its potential against TNBS-induced UC. Thus, considering this background, the current search was designed to measure the plausible mechanisms of action of pinitol on an experimental model of TNBS-induced colitis in rats.

MATERIALS AND METHODS

Animals

Sprague–Dawley rats (adult male, 180–220 g, 6–7 weeks) were acquired from the Second Affiliated Hospital of Xi'an Jiaotong University. Throughout the experimental protocol, the housing conditions for the rats were temperature: 24 ± 1°C, relative humidity: 45%–55%, normal dark/light cycle, food: standard pellet chow, and water: filtered (*ad libitum*). A time of 09:00–17:00 h were measured to carry out all the experiments protocol approved by the Institutional Animal Ethics Committee of Xi'an Honghui Hospital (approved No. XJDHH-202007). A guidelines delineated in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the (Animal Research: Reporting of *In-vivo* Experiments [ARRIVE]) guidelines (http://www.nc3rs.org/ARRIVE) were followed to accomplish all the experiments.

Chemical and kits

D-Pinitol (purity: 97%) and TNBS (Sigma Chemical Co., St Louis, MO, USA), Total ribonucleic acid (RNA) Extraction kit and One-step Real-time–PCR (RT-PCR) kit (MP Biomedicals India Private Limited, India), primary antibodies of Claudin-1, occludin, Caspase-1, and ZO-1 (Abcam, Cambridge, MA, USA) were procured from respective manufacturers.

Induction of colitis and drug treatment schedule

TNBS (100 mg/kg, in 50% ethanol) was employed to induce colitis in overnight fasted rats according to a method report elsewhere.[11] Further, they were alienated randomly into the various groups (n = 24), namely, normal group (treated with 1% aqueous Dimethyl sulfoxide (DMSO), p.o.),

TNBS control group (received DMSO, p.o.), 5-ASA treated group (received 5-aminosalicylic acid (500 mg/kg, p.o.), and d-Pinitol-treated group (received d-Pinitol [5 or 10 or 20 mg/kg, p.o.]). The rats were treated with either vehicle or d-Pinitol or 5-ASA for 14 days. D-Pinitol dosage, i.e. 5 or 10 or 20 mg/kg, was based on earlier stated methods.[17-19]

Macroscopic assessment

On day 15, the animals were forewent through cervical dislocation, and the colon was isolated to determine colonic damage, ulcer area, and ulcer index according to formerly reported methods.[23,24] Previously stated methods were used to assess the stool consistency, macroscopic score, and disease activity index.[23,24]

Biochemical assays

The levels of myeloperoxidase (MPO), glutathione (GSH), superoxide dismutase (SOD), NO (NO content), lipid peroxidation (i.e. Malondialdehyde [MDA]), hydroxyproline (HP), 5-Hydroxytryptamine (5-HT), and T-AOC (total antioxidant capacity) were assessed in scratched colon mucosa of the colon tissue according to earlier described methods.[23-25] Colon mucosa was mixed with 0.1M phosphate buffer and homogenized on ice for 60 s at 10,000 rpm in a homogenizer (Remi Equipment Pvt. Ltd. and Remi Motors Ltd., Mumbai, India) and then further applied for these biochemical assays.

The T-AOC was measured using cyclic voltammetry (Autolab, PGSTA 101, Metrohm, Switzerland). Briefly, a three-electrode system was used for the study. The working electrode: glassy carbon (Autolab) 8 mm in diameter was polished before each measurement; the platinum wire aided as an auxiliary electrode and saturated calomel electrode as the reference electrode. The cell confined 2 mL of phosphate-buffered liver homogenate. All cyclic voltammogram measurements were achieved in the range (−0.2) – (1.3) V at a scan rate of 400 mV/s. Each sample was examined thrice.

Real-time-polymerase chain reaction and western blot assay

The messenger RNA (mRNA) expressions of TNF-α, IL-1 β, IL-6, NF-kB, and β-actin were investigated in colon tissue using RT-PCR according to manufacturer's instructions (MP Biomedicals India Private Limited, India).[26,27] Whereas protein expressions of Claudin-1, occludin, ZO-1 (Zonula occludens-1 or TJ protein-1), and (Glyceraldehyde 3-phosphate dehydrogenase) were assessed in colon tissue according to the method labeled elsewhere.[23,24]

Histological analysis

Histopathological analysis of colon tissue was carried out using hematoxylin and eosin (H and E) stain, and photographs were apprehended by a light compound microscope with a Zeiss intravital microscopy setup (Zeiss Axioscope A1, Carl Zeiss MicroImaging, Jena, Germany) as designated previously.[28] The colon histology was scored as none (0), mild (1), moderate (2), and severe (3).

Statistical analysis

GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA) was used to achieve data analysis. Data are articulated as mean ± standard error mean (SEM) and analyzed using One-Way analysis of variance (ANOVA) followed by Tukey's multiple range *post hoc* analysis (for parametric tests) as well as Kruskal–Wallis test for *post hoc* analysis (nonparametric tests). A value of $P < 0.05$ was measured to be statistically significant.

RESULTS

Body weight, colon index, disease activity, colonic damage, ulceration, and stool consistency

There was a momentous diminution ($P < 0.05$) in body weight and colon index, whereas disease activity, colonic damage, ulceration, and stool consistency were decidedly augmented ($P < 0.05$) in the TNBS control group as compared to the normal group. However, the administration of 5-ASA effectively ($P < 0.05$) lessened TNBS-induced alterations in body weight, colon index, disease activity, colonic damage, ulceration, and stool consistency compared to the TNBS control group. Treatment with pinitol (10 and 20 mg/kg) markedly amplified ($P < 0.05$) body weight and colon index, whereas disease activity, colonic damage, and ulceration were suggestively lessened ($P < 0.05$) when compared to the TNBS control group [Table 1 and Figure 1].

Colonic total antioxidant capacity

Total Antioxidant Capacity (T-AOC) in the colon was evidently declined ($P < 0.05$) in the TNBS control group when compared with the normal group. 5-ASA effectively ($P < 0.05$) augmented colonic T-AOC as compared to the TNBS control group. Pinitol (10 and 20 mg/kg) treatment also conspicuously ($P < 0.05$) attenuated TNBS-induced variations in colonic T-AOC as compared to the TNBS control group [Figure 2].

Colonic oxido-nitrosative damage

When compared with the normal group, colonic SOD and GSH levels were distinctly lessened ($P < 0.05$), whereas MDA and NO levels were markedly increased ($P < 0.05$) in the TNBS control group. 5-ASA treatment blatantly ($P < 0.05$) inhibited TNBS-induced elevated oxido-nitrosative stress compared to the TNBS control group. Administration of pinitol (10 and 20 mg/kg) also noticeably enlarged ($P < 0.05$) colonic SOD and GSH levels, whereas MDA and NO levels were significantly reduced ($P < 0.05$) when compared with the TNBS control group [Table 2].

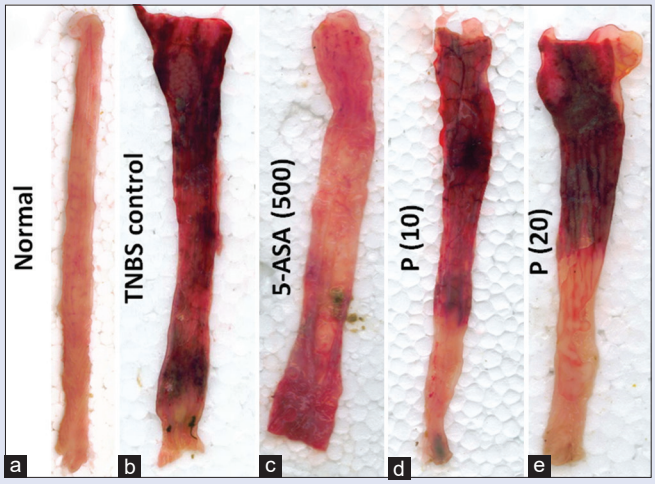

Figure 1: Morphological representation of colons from Normal (a), trinitrobenzene sulfonic acid control (b), 5-aminosalicylic acid (500 mg/kg) (c), Pinitol (10 mg/kg) (d), and Pinitol (20 mg/kg) (e) treated rats

Table 1: Effect of pinitol on 2, 4, 6-trinitrobenzenesulfonic acid induced alterations in body weight, colon weight, colon weight to length ratio, ulcer area, ulcer index, disease activity index, macroscopic score, and stool consistency score in rats

Treatment	Body weight (g)	Colon weight (g)	Colon weight to length ratio (g/cm)	Ulcer area (mm²)	Ulcer index	Percentage inhibition	DAI scores	Macroscopic score	Stool consistency score
Normal	252.60±2.84	1.98±0.18	0.19±0.02	1.60±0.81	3.40±1.77	-	0.20±0.20	0.00±0.00	0.00±0.00
TNBS control	233.20±5.31$^{\#}$	4.39±0.17$^{\#}$	0.43±0.02$^{\#}$	30.17±3.81$^{\#}$	65.15±7.45$^{\#}$	-	3.80±0.20$^{\#}$	6.60±0.24$^{\#}$	2.60±0.24$^{\#}$
5-ASA (500)	244.80±3.77*,$	2.89±0.17*,$	0.28±0.02*,$	6.00±2.04*,$	12.86±4.36*,$	80.94	1.00±0.32*,$	1.80±0.37*,$	0.40±0.24*,$
P (5)	236.20±3.34	4.19±0.17	0.43±0.02	29.25±2.17	60.22±5.67	2.05	3.40±0.24	6.20±0.37	2.40±0.24
P (10)	246.40±2.79*,$	3.49±0.17*,$	0.34±0.02*,$	21.07±3.05*,$	42.28±5.92*,$	28.34	2.60±0.24*,$	5.00±0.45*,$	1.80±0.20*,$
P (20)	251.20±3.15*,$	2.69±0.17*,$	0.27±0.02*,$	14.01±1.19*,$	29.33±2.51*,$	52.77	1.80±0.20*,$	2.20±0.20*,$	1.20±0.37*,$

*P<0.05 as compared to TNBS control group, $^{\#}P$<0.05 as compared to normal group and, $^{\$}P$<0.05 as compared to one another (pinitol and 5-ASA). Data are expressed as mean±SEM (n=6) and analyzed by one-way ANOVA followed by Tukey's multiple range test. The macroscopic score and stool consistency data were analyzed using nonparametric Kruskal–Wallis ANOVA followed by Tukey's multiple range test. TNBS: 2, 4, 6-Trinitrobenzenesulfonic acid, 5-ASA: 5-aminosalicylic acid, P: Pinitol, SEM: Standard error of mean, ANOVA: Analysis of variance, DAI: Disease activity index

Table 2: Effect of pinitol on 2, 4, 6-trinitrobenzenesulfonic acid induced alterations in colonic superoxide dismutase, glutathione, malondialdehyde, nitric oxide, hydroxyproline, 5-hydroxytryptamine, and myeloperoxidase in rats

Treatment	Colonic total protein (mg/gm)	Colonic SOD (U/mg of protein)	Colonic GSH (μg/mg protein)	Colonic MDA (nM/mg of protein)	Colonic NO (μg/ml)	Colonic HP (μg/mg tissue)	Colonic 5-HT (ng/g of tissue)	Colonic MPO (U/g of tissue)
Normal	8.97±0.90	8.15±0.47	2.71±0.40	9.56±0.53	369.40±49.80	11.27±1.20	26.06±7.977	3.13±0.24
TNBS control	21.07±0.84$^{\#}$	1.85±0.19$^{\#}$	1.19±0.11$^{\#}$	23.18±2.31$^{\#}$	752.80±63.03$^{\#}$	19.85±1.61$^{\#}$	49.52±11.88$^{\#}$	12.72±1.03$^{\#}$
5-ASA (500)	15.57±1.30*,$	4.40±0.76*,$	2.19±0.11*,$	18.86±2.70	538.80±97.47*,$	17.33±1.79	59.24±7.22	9.39±0.38*,$
P (5)	20.46±1.24	2.60±0.51	1.16±0.08	20.02±1.61	679.90±49.70	17.36±1.40	49.84±10.65	12.01±0.84
P (10)	15.90±1.68*,$	4.14±0.53*,$	1.59±0.24*,$	16.69±2.03*,$	477.20±87.49*,$	13.27±2.28*,$	50.14±12.46	9.84±0.53*,$
P (20)	15.20±1.01*,$	4.95±0.43*,$	2.10±0.27*,$	13.85±1.42*,$	532.40±61.81*,$	13.42±2.28*,$	48.54±13.84	10.15±0.46*,$

*P<0.05 as compared to TNBS control group, $^{\#}P$<0.05 as compared to normal group and, $^{\$}P$<0.05 as compared to one another (pinitol and 5-ASA). Data are expressed as mean±SEM (n=6) and analyzed by one-way ANOVA followed by Tukey's multiple range test. TNBS: 2,4,6-trinitrobenzenesulfonic acid, 5-ASA: 5-aminosalicylic acid, P: Pinitol, SOD: Superoxide dismutase, GSH: Glutathione, MDA: Malondialdehyde, NO: Nitric oxide, HP: Hydroxyproline, 5-HT: 5-hydroxytryptamine, MPO: Myeloperoxidase, SEM: Standard error of mean, ANOVA: Analysis of variance

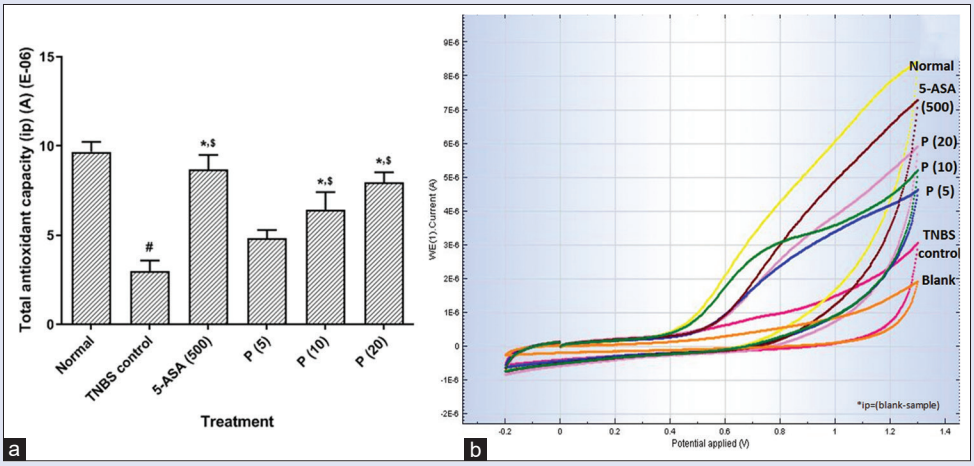

Figure 2: Effect of pinitol on trinitrobenzene sulfonic acid induced alterations in colonic total antioxidant capacity in rats (a) and representative total antioxidant capacity curve from each group (b). Data are expressed as mean ± standard error mean (n = 6) and analyzed by one-way analysis of variance followed by Tukey's multiple range test. *P < 0.05 as compared to trinitrobenzene sulfonic acid control group, $^{\#}P$ < 0.05 as compared to normal group and $^{\$}p$ < 0.05 as compared to one another (pinitol and 5-aminosalicylic acid). TNBS: 2, 4, 6-Trinitrobenzenesulfonic acid; 5-ASA: 5-Aminosalicylic acid; P: Pinitol

Colonic Myeloperoxidase, 5-Hydroxytryptamine, and hydroxyproline levels

Colonic MPO, 5-HT, and HP were amplified effectively (P < 0.05). TNBS control group after intrarectal instillation of TNBS compared to the normal group. Administration of 5-ASA effectively (P < 0.05) condensed colonic MPO but failed to meaningfully cut colonic 5-HT and HP compared to the TNBS control group. However, pinitol (10 and 20 mg/kg) treatment markedly reduced (P < 0.05)

colonic MPO and HP compared with the TNBS control group. The elevated level of colonic 5-HT did not condense after treatment with pinitol [Table 2].

Cytokine and nuclear factor-κb levels

The mRNA expressions of colonic TNF-α, IL-1 β, IL-6, and NF-kB were strikingly upregulated ($P < 0.05$) in the TNBS control group compared with the normal group. Treatment with 5-ASA significantly ($P < 0.05$) downregulated TNBS-induced raised pro-inflammatory cytokine (TNF-α, IL-1 β, and IL-6) and NF-κB mRNA expressions as compared to TNBS control group. Pinitol (10 and 20 mg/kg) administration flagrantly attenuated ($P < 0.05$) pro-inflammatory cytokine and NF-κB mRNA expressions as compared to the TNBS control group [Table 3].

Tight junctions proteins expression

Intrareactal instillation of TNBS resulted in noteworthy downregulation ($P < 0.05$) of expressions of colonic TJs proteins (claudin-1, occludin, and ZO-1) expressions in the TNBS control group as compared to the normal group. 5-ASA efficiently ($P < 0.05$) upgraded TNBS-induced downregulation in claudin-1, occludin, and ZO-1 protein expressions compared to the TNBS control group. Administration of pinitol (10 and 20 mg/kg) also obviously upregulated ($P < 0.05$) claudin-1, occludin, and ZO-1 protein expressions when compared with the TNBS control group [Figure 3].

Histopathology of colon

TNBS induces clear obliteration in the colon tissue reflected by elevated inflammatory infiltration, disruption of colon and goblet cell architecture, and thickening of the colon wall, which were apparent in the colon tissue from the TNBS control group [Figure 4b]. Histological analysis of colon tissue from normal group portrayed normal colonic architecture with evidence of mild inflammatory infiltration [Figure 4a]. Conversely, 5-ASA treatment effectively ($P < 0.05$) inhibited TNBS-induced destruction of colon tissue imitated by reduced inflammatory infiltration and thickening of the colon as compared to the TNBS control group [Figure 4c]. Administration of pinitol (10 and 20 mg/kg) also noticeably lessened ($P < 0.05$) TNBS-induced histological modifications in colon tissue when compared with the TNBS control group [Figure 4d-f].

DISCUSSION

UC is a complex relapsing illness of the GIT considered by chronic inflammation followed by colonic damage.[1,6] Increasing evidence

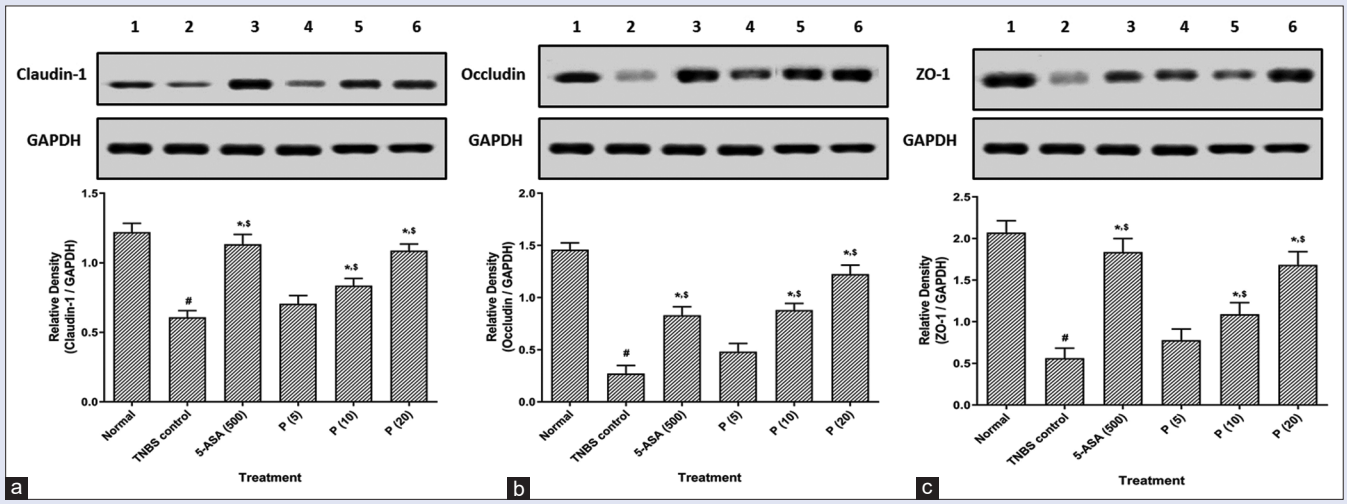

Figure 3: Effect of pinitol on trinitrobenzene sulfonic acid induced alterations in colonic claudin-1 (a), occludin (b), and Zonula occludens-1 (c) protein expression in rats. Data are expressed as mean ± standard error mean ($n = 6$) and analyzed by one-way analysis of variance followed by Tukey's multiple range test. *$P < 0.05$ as compared to trinitrobenzene sulfonic acid control group, #$P < 0.05$ as compared to normal group and $p < 0.05$ as compared to one another (pinitol and 5-aminosalicylic acid). Representative protein expression of Normal (Lane 1), trinitrobenzene sulfonic acid control (Lane 2), 5-aminosalicylic acid (500 mg/kg) (Lane 3), Pinitol (5 mg/kg) (Lane 4), Pinitol (10 mg/kg) (Lane 5), and Pinitol (20 mg/kg) (Lane 6) treated rats. TNBS: 2, 4, 6-Trinitrobenzenesulfonic acid; 5-ASA: 5-Aminosalicylic acid; P: Pinitol; ZO-1: Zonula Occludens-1

Table 3: Effect of pinitol on 2, 4, 6-trinitrobenzenesulfonic acid induced alterations in colonic tumor necrosis factor-alpha, interleukins - 1β, interleukins - 6 and nuclear factor kappa B messenger ribonucleic acid expressions in rats

Treatment	TNF-α/β-actin ratio	IL-1β/β-actin ratio	IL-6/β-actin ratio	NF-kB/β-actin ratio
Normal	0.51±0.05	0.048±0.007	0.61±0.05	0.48±0.04
TNBS control	1.96±0.08#	0.192±0.005#	1.08±0.06#	0.90±0.05#
5-ASA (500)	0.79±0.06*,$	0.090±0.003*,$	0.64±0.08*,$	0.55±0.03*,$
P (5)	1.80±0.12	0.174±0.01	0.98±0.07	0.92±0.07
P (10)	1.32±0.07*,$	0.116±0.006*,$	0.88±0.04*,$	0.65±0.06*,$
P (20)	0.93±0.08*,$	0.104±0.009*,$	0.74±0.06*,$	0.67±0.05*,$

*$P<0.05$ as compared to TNBS control group, #$P<0.05$ as compared to normal group and, $$P<0.05$ as compared to one another (pinitol and 5-ASA). Data are expressed as mean±SEM ($n=6$) and analyzed by one-way ANOVA followed by Tukey's multiple range test. TNBS: 2, 4, 6-trinitrobenzenesulfonic acid, 5-ASA: 5-aminosalicylic acid, P: Pinitol, TNF-α: Tumor necrosis factor-alpha, ILs: Interleukins, p-NF-kB: Phospho nuclear factor kappa B, mRNA: Messenger ribonucleic acid

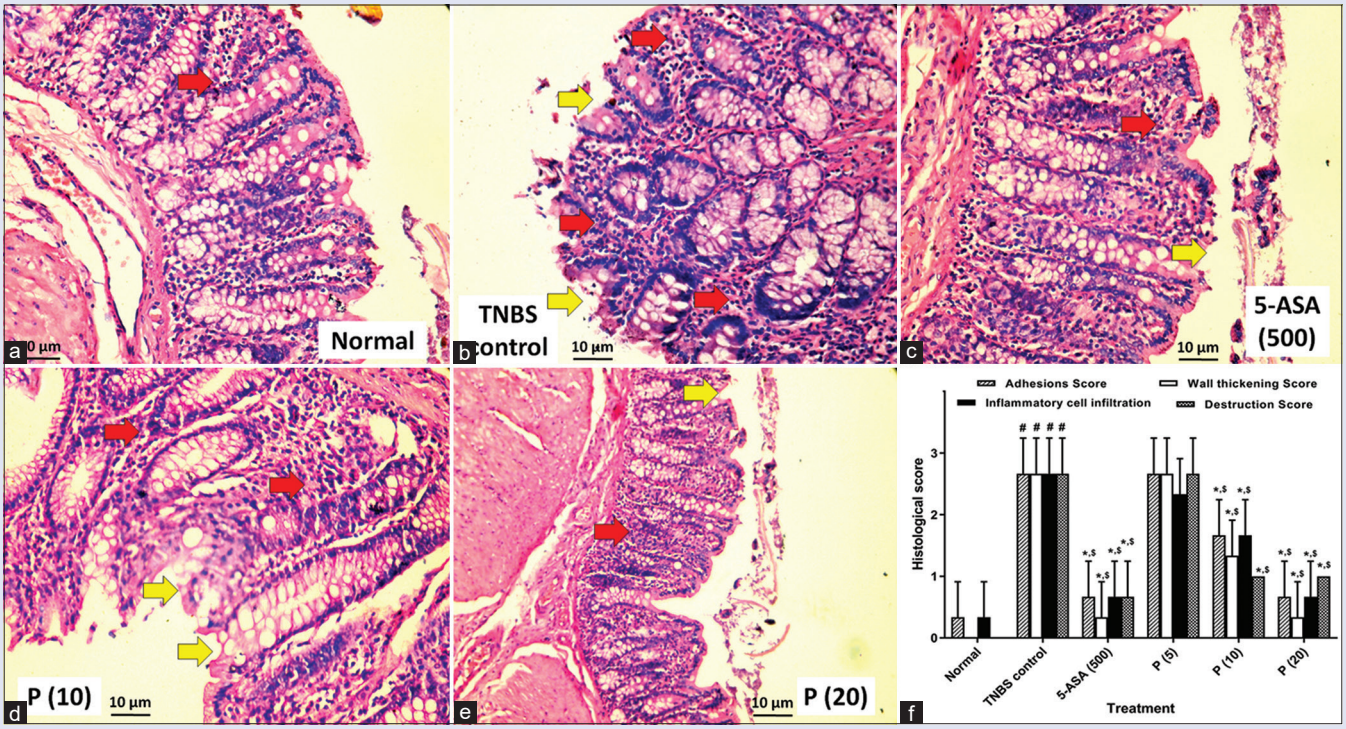

Figure 4: Effect of pinitol on trinitrobenzene sulfonic acid induced alterations in colon histopathology. Photomicrograph of sections of colon tissue from Normal (a), trinitrobenzene sulfonic acid control (b), 5-aminosalicylic acid (500 mg/kg) (c), Pinitol (10 mg/kg) (d), and Pinitol (20 mg/kg) (e) treated rats stained with H and E stain. The quantitative representation of histological score (f) Data are expressed as mean ± standard error mean (n = 3) and analyzed by one-way Analysis of variance followed by the Kruskal–Wallis test was applied for *post hoc* analysis. $^{*}P < 0.05$ as compared to trinitrobenzene sulfonic acid control group, $^{\#}P < 0.05$ as compared to normal group and $^{\$}p < 0.05$ as compared to one another (pinitol and 5-aminosalicylic acid). TNBS: 2, 4, 6-Trinitrobenzenesulfonic acid; 5-ASA: 5-Aminosalicylic acid; P: Pinitol. The red arrow indicated inflammatory infiltration, and the yellow arrow indicated necrosis. Images (×40 magnification) are typical and are representative of each study group

confirmed elevated production of reactive oxygen and nitrogen species during UC, which further originated the vicious cycle of inflammatory release, including NF-κB, TNF-α, and ILs.[1,6] Thus, inflammation plays a prodigious role in the pathogenesis of UC. Notably, earlier research indicated that pinitol inhibited the release of inflammatory mediators to exert its anti-inflammatory potential.[17,19,20] Thus, in the present study, we have measured the potential of pinitol against UC induced by TNBS. Rectal instillation of TNBS along with ethanol makes ease of interaction of TNBS with colonic protein through snowballing mucosal barrier, which further induces injury to the colonic that resemble the human colitis. Interestingly, treatment with pinitol ameliorated TNBS-induced UC through inhibition of elevated oxidative stress (TOC, SOD, GSH, and MDA) and inflammatory release (TNF-α, ILs, and NF-κB), which further recovers the intestinal barrier through activation of colonic TJ proteins (ZO-1, claudin-1, and occludin) (Pictorial abstract).

Suggestion proposed that unexplained loss of body weight, amplified clinical score, and stool consistency are the primary features of UC, which provide visions into a disease state.[6,23] The existing study also rectal instillation of TNBS caused diminished body weight, increased ulcer area, clinical score, and stool consistency, which are reliable with findings of previous researchers.[13] However, treatment with pinitol improved body weight and reduces ulcer area and clinical score detected after TNBS administration. Similarly, histopathological analysis of colon tissue from pinitol-treated rats displayed amelioration of TNBS-induced microscopic alteration, counting inflammatory infiltration, and disruption of architecture of colonic and goblet cells. Increasing indication has stated the strong anti-inflammatory potential of pinitol,[17,19,20] which might subsidize to its colono-protective potential during TNBS-induced colitis.

An array of studies recommended that raised generation of reactive oxygen species induces oxidative stress and inflammatory release, contributory to the development, and maintenance of UC.[2,23,24,29] Furthermore, oxidative stress activates activation of NF-κB, which results in the release of pro-inflammatory mediators.[30-34] abnormal production of pro-inflammatory cytokines (such as TNF-α and ILs) and activation of inflammatory signaling pathways induce a systematic and low-grade inflammation in colonic tissue closely linked with colonic damage.[1,35] TNF-α activates T-cell proliferation and differentiation, thus surge the permeability of the intestinal epithelial layer through disturbing the TJ and modulate the colonic functions.[1] Whereas IL-1 β induces sustain inflammatory responses in the colon and IL-6 has been closely concerned in the pathogenesis of colitis.[1] Moreover, activation of various hypertrophic signaling pathways such as NF-kB and mitogen-activated protein kinase upsurge the accumulation of extracellular matrix protein such as collagen Type I, which further contributes to UC.[6,36] Thus, the NF-κB signaling pathway is energetic for regulating inflammation in the colon. In resting state, IκB-α (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-alpha), an NF-κB inhibitor, keep it in the inactive form in the cytoplasm through formation of NF-κB/IκB-α complex with of p50 and p65 heterodimers of NF-κB.[37,38] However, degradation of IκB-α through oxidative phosphorylation fallouts in activation and translocation of NF-κB to the nucleus. This translocation consequences in the transcription of an array of genes responsible for cell proliferation, inflammation, cell necrosis, and cell death.[37,38] In the present search, instillation of TNBS associated with elevated inflammatory

response (NF-κB, TNF-α, and ILs) and collagen (HP) synthesis, which was bettered by pinitol treatment. Sethi *et al.*(2008) also recommended that pinitol suppresses the activation of NF-κB to inhibit the cellular inflammatory responses;[16] thus, the results of the contemporary study corroborate the outcomes of the earlier researcher.[16]

During the last decade, the role of TJ proteins (such as ZO-1, Occludin, and Claudin-1) and the intestinal barrier has been well recognized to preserve healthy colonic architecture and function, including a cellular update of nutrients, water, and electrolytes.[39] However, upregulated inflammatory markers pledge the inflammatory response that causes disruption of TJ and intestinal barrier, leading to colonic dysfunction.[9] Studies have shown that the number and composition of claudins decide the tightness of TJ, whereas assembling and maintenance of these TJ are occludin dependent.[40,41] ZO-1 has also been recommended as an important TJ protein for the intestinal barrier's normal function such as epithelial proliferation and repair.[9] Moreover, recent results exposed that intestinal epithelial ZO-1 protein expressions were abridged in patients with IBD than healthy volunteers.[9] Thus, existing research emphases on improving TJ proteins to manage UC.[9] In line with the findings of the earlier investigator, the results of the current study displayed that TNBS-induced colitis was related with reduced expressions of ZO-1, occludin, and claudin-1.[9] Interestingly, the administration of pinitol significantly enhanced the diminished expressions of TJ proteins, signifying its role in improving colon functions.

5-ASA, inhibitions of NF-κB, which is extensively employed pharmacotherapy for the management of UC; however, the studies have exposed that, when it is taken orally, a major part of it undergoes absorption in the stomach and upper small intestine before reaching to colon; thus, it has a very fewer local effect at colon site. In addition, it covers several adverse events, counting fever, loss of appetite, headache, vomiting, nausea, abdominal pain, and rash. Thus, most of the colitis patients favored therapeutic moieties of herbal origin for the treatment of UC. Treatment regimens from herbs such as *Boswellia serrata* and *Aloe vera* have shown some useful effects in ameliorating symptoms of UC with negligible side effects in patients with chronic colitis.[40,42] Pinitol has also been well established clinically to accomplish various disorders, counting hepatotoxicity, diabetes, obesity, and allied metabolic disorders.[15,21,41] Thus, considering this obtainable scientific information, pinitol can be measured a potential therapeutic moiety of herbal origin for further clinical development against UC management.

CONCLUSION

The result of the contemporary inquiry specified that D-Pinitol perfected TNBS-induced colitis in experimental rats. D-Pinitol applied its colonoprotective effect probably through the inhibition of activation of NF-κB/Iκ B-α pathway, thus inhibited the raised production of oxidative stress (TOC, SOD, GSH, and MDA) and inflammatory release (TNF-α and ILs) consequently progresses the intestinal barrier through improving activity of colonic TJ proteins (ZO-1, claudin-1, and occludin).

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Conflicts of interest

There are no conflicts of interest.

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