

# Effects of Adding Dexpanthenol to Prednisolone in an Experimental Model of Inflammatory Bowel Disease

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## Article Info

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## ABSTRACT

**Background & Objective:** Inflammatory bowel diseases (IBDs) represent serious chronic auto-inflammatory conditions, affecting the alimentary tract. The beneficial effect of dexpanthenol has been observed on some inflammatory conditions. Here, the therapeutic potential of combined dexpanthenol and prednisolone in alleviating the symptoms of the animal model of IBD was investigated.

**Materials & Methods:** Luminal instillation of 4% acetic acid (2 ml for each rat) was used to induce IBD. Rats in the treatment groups received dexpanthenol (500 mg/kg), prednisolone (2 mg/kg), or a combination of both (half doses of each drug) by oral gavage for 11 consecutive days.

**Results:** Dexpanthenol could regress the clinical scores of the IBD model more than prednisolone. More importantly, combination therapy with half doses of dexpanthenol and prednisolone caused more considerable improvement in the disease activity index (DAI) compared to IBD rats received monotherapy. Both monotherapies promoted a remarkable decrease in the messenger RNA (mRNA) expression of NF- $\kappa$ Bp65, as well as the levels of interleukin 6 (IL-6) and TNF- $\alpha$  activity in the inflamed colon. Dexpanthenol could regress the intensity of malondialdehyde (MDA), myeloperoxidase (MPO), and nitric oxide (NO) in the inflamed colon more than prednisolone. More importantly, our results demonstrated that combination therapy resulted in a much more prominent decrease in the level of TNF- $\alpha$  and NF- $\kappa$ Bp65, as well as the level of superoxide dismutase (SOD) and total gut protein content, than monotherapy. Conversely, combination therapy resulted in a much more pronounced decrease in NO and MDA levels than those recorded in IBD rats received individual treatment. Finally, the mRNA level of I $\kappa$ B $\alpha$  did not show any remarkable discrepancy between the experimental groups.

**Conclusion:** The combination of dexpanthenol and prednisolone could be used as a promising strategy to alleviate the signs of IBD.

**Keywords:** Dexpanthenol, Prednisolone, Inflammatory bowel diseases, Combination therapy



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## Introduction

Inflammatory bowel diseases (IBDs) represent serious chronic auto-inflammatory conditions, affecting the alimentary tract in dogs and humans (1). However, the principal etiology of IBD is uncertain, and the progression of the disease is thought to originate as a consequence of aberrant immune responses and continuous inflammation against the microbiome in the gut (2).

Diverse classes of immunomodulatory or anti-inflammatory drugs, e.g., steroids, 5-aminosalicylates, cyclosporine, azathioprine, cyclophosphamide, and chlorambucil, can be administered to control extensive inflammatory reactions in IBD (1–3). Despite the relative effectiveness, these medications have significant side effects, such as infections, indigestion,

other alimentary disorders, skin thinning, and visual disorders (2, 3). Due to the complexity of auto-inflammatory diseases, it does not appear that a monotherapy could be useful in all patients during all periods of IBD. Therefore, it is quite logical to use the combination of new medications or the existing drugs for better IBD control or reduction of the side effects (4, 5). Fortunately, the animal model of IBD, e.g., acetic acid-induced colitis in rats, has provided an excellent opportunity to test new drug compounds (3).

Pantothenol (also called panthenol) is biologically active amino alcohol and a monocarboxylic acid amide. Dexpanthenol (D-panthenol, provitamin B5) is the only active form of pantothenol, which is quickly oxidized to pantothenic acid (PA; vitamin B5) in an

organism (6, 7). PA is a fundamental component of coenzyme A, which acts as an essential cofactor in metabolic reactions (8). Interestingly, some studies have documented the antioxidant and anti-inflammatory benefits of dexpanthenol (6, 8, 9). For example, the beneficial effect of dexpanthenol has been observed on testicular and renal ischemic damages, diabetic nephropathy, necrotizing enterocolitis, ulcerative colitis, acute lung injury, and experimentally-induced endometriosis (8–10). Also, dexpanthenol can potentiate wounds healing via moisturizing and skin barrier enhancing. In skin irritations and wounds, Dexpanthenol stimulates not only metabolic activity in skin cells, but also gene expression for optimum wound healing (11).

Nonetheless, there is no or limited information on the potential benefits of combined dexpanthenol and prednisolone on IBD. Accordingly, the present investigation was done to assess the potential benefits of co-administering dexpanthenol and prednisolone.

## Materials and Methods

### Reagents

The enzyme-linked immunosorbent assay (ELISA) kits were prepared from PeptoTech (UK). Other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### Animals

In total, 50 male Wistar albino rats, aged 6–7 weeks, weighing 200–250 g were prepared from Empirical Animal Care Center, Urmia University, Urmia, Iran. The rats were maintained under stable situations (i.e., 25°C and a 12-hour dark/light cycle) and rendered water and food *ad libitum*. Ethical considerations concerning research with the laboratory animals were observed, as stated in the regulations of the Ethics Committee of Faculty of Veterinary Medicine, Urmia University, Urmia, Iran (Ref.IR-UU-AEC-142/DA3).

### In-vivo Experimental Design

IBD was induced by intra-rectal instillation of acetic acid, as described previously (12). Briefly, all the Wistar rats maintained under the fast condition for 36 hr. Next, a pediatric catheter was inserted into the anus of the rats flowing light ether anesthesia in a way that the tip of the catheter was inserted about 8 cm into the end part of the gut. Afterward, a solution of acetic acid diluted to 4% (4 mL) was administrated into the lumen of the colon by enema. The healthy group was also instilled with normal saline 0.9% with a volume of 4 ml. Rats were put in the head-down vertical status for 1 min to prevent acetic acid leakage.

Rats were randomly appointed within four groups of 10 rats as follows: the healthy group, control colitis rats, colitis rats receiving dexpanthenol (500 mg/kg every day, oral gavage), colitis rats receiving prednisolone (2 mg/kg every day, oral gavage), and colitis rats receiving a combination of both dexpanthenol and prednisolone (half doses of each drug, oral gavage). The control colitis rats were treated with an equal volume of PBS. Healthy rats were intrarectally received PBS and treated with an equal volume of PBS with the same schedule of IBD rats. The pharmacotherapy was initiated after the induction of colitis and followed all over the investigation until the 11<sup>th</sup> day when the animals were sacrificed. Afterward, the gut tissues were harvested for further examination.

### Evaluation of Disease Severity

Clinical signs (rectal bleeding, stool blood, and stool consistency) were monitored every day. The disease severity score was monitored; the sum of the scores of the parameters is shown in Table 1 (13). The sum of the scores of all the criteria was used to calculate the disease activity index (DAI). To monitor the length/weight ratio, the length and weight of the colon between the ileocecal junction and rectum were measured. One of the common features of colonic instillation of acetic acid is the enlargement of the spleen. Therefore, the ratio of the spleen weight/body weight of each rat was calculated as the spleen weight index

**Table 1. Scoring system for the evaluation of colitis**

Score	Weight loss	Stool consistency	Blood feces
0	Negative	Normal	Negative
1	1-9%	Soft	Red
2	10-19%	Very Soft	Dark Red
3	< 20%	Diarrhea	Black

Sum of scores of all criteria was used to calculate DAI

Note. DAI: Disease activity index.

### Preparation of Colonic Homogenate

First, 10 cm of distal colon's tissue was dissected and washed with normal saline. The same amount of gut was homogenized at 10 volumes of ice-cold normal saline. Then, homogenates were centrifuged at 1200 g for 10 min at 4°C (14).

### Biochemical Analysis

The activity of (Cu–Zn and Mn) superoxide dismutase (SOD; EC 1.15.1.1) was monitored, similar to the procedure qualified by Sun *et al.* (15). The reality of this assay was the blockage of the reduction of nitro-blue tetrazolium by the xanthine–xanthine oxidase system as a superoxide anion ( $O_2^{\bullet-}$ ) generator. In this system, one unit of SOD was calculated as the enzyme content promoting 50 % occlusion in the rate of nitro-blue tetrazolium reduction. The SOD activity was reported as units per mg of protein.

The activity of glutathione peroxidase (GPx; EC 1.6.4.2) was calculated by the procedure described by Paglia and Valentine (16). In brief, reduced glutathione (GSH), NADPH, and sodium azide were added to a tube. Then, hydrogen peroxide ( $H_2O_2$ ) was added to initiate the enzymatic reaction. The shift in absorbance at 340 nm was spectrophotometrically counted. The activity of GPx was presented as units per g of protein.

Malondialdehyde (MDA) evaluation was followed, similar to the scheme explained earlier (17). In a short, 2.5 mL of reaction buffer (0.25 M HCl, 0.37% thiobarbituric acid, and 15% trichloroacetic acid, 1:1:1 ratio) was mixed with 100  $\mu$ L of colon homogenate and heated at 95°C for about 1 hr. Afterward, the mixture was centrifuged at 4000 g for 10 min. Eventually, the absorbance of the supernatant was spectrophotometrically monitored at 540 nm. Findings were reported as nM of MDA/mg protein.

To evaluate myeloperoxidase (MPO) activity, 10  $\mu$ L of homogenized gut sample was coupled with 80  $\mu$ L of 0.75 mM  $H_2O_2$  and 110  $\mu$ L of reaction dilution (2.9 mM 3,3',5,5'-tetramethylbenzidine [TMB] in 14.5% dimethyl sulfoxide plus 150 mM of sodium phosphate buffer at pH 5.4). The absorbance was monitored at 450 nm (reference: 620 nm), and samples were maintained for 15 min at 37°C. Thereupon, sulfuric acid (50  $\mu$ L of 2 M) was mixed to stop the reaction, and the optical-absorbance was recorded at 450 nm. As the standard, 10  $\mu$ L of horseradish peroxidase (2.5 and 25 milliunit/mL) was applied. Finally, MPO activity was reported as the discrepancy of the absorbance in accordance with the standard curve of horseradish peroxidase. Data were presented in milliunits per milliliter (mU/mL) (3).

The Griess method was used to monitor nitric oxide (NO) levels in colonic tissues. Shortly, 100  $\mu$ L of Griess reagent (0.1% naphthyl ethylenediamine, 3% phosphoric acid, and 0.1% sulphanilamide) was admixed with 100  $\mu$ L of the homogenized colonic specimen. The mixture was kept in the dark at 25°C for

10 min. Finally, the absorbance was spectrophotometrically recorded at 540 nm. The nitrite level was reported in accordance with the standard curve (18).

The Lowry protein assay was used to assess the total protein concentration in the gut homogenate.

### Evaluation of Inflammatory Cytokines in Colonic Homogenate

The production intensity of TNF- $\alpha$  and interleukin 6 (IL-6) in the gut samples was calculated by using a commercially available enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's guidelines.

### Expression of mRNA of I $\kappa$ B $\alpha$ and NF- $\kappa$ B p65 in the Gut Samples

The content of messenger RNA (mRNA) in the hind part of colonic tissue was drawn out by a standard kit according to the producer's guidelines. In short, a real-time reverse transcription-polymerase chain reaction (RT-PCR) procedure was applied after synthesizing complementary DNA (cDNA). The  $\beta$ -actin gene was employed for normalizing mRNA expression. The sense and anti-sense primers are as follows: PCR forward set for NF- $\kappa$ B p65: 5'-TGCAGGCTCCTGTGCGAGTG-3'; PCR reverse set for NF- $\kappa$ B p65: 5'-TCCGGTGGCGATCGTCTGTGT-3'; PCR forward set for I $\kappa$ B $\alpha$ : 5'-CGTGTCTGCACCTAGCCTCTATC-3'; PCR reverse set for I $\kappa$ B $\alpha$ : 5'-GCGAAACCAGGTCAGGATTC-3'; PCR forward set for  $\beta$ -actin: 5'-GCAGGAGTACGATGAGTCCG-3'; and PCR reverse set for  $\beta$ -actin: 5'-ACGCAGCTCAGTAACAGT CC-3' (13).

### Statistical Surveys

DAI was evaluated by using the non-parametric Kruskal–Wallis exam confirmed by the Mann–Whitney U evaluation with the Bonferroni adjustment. The rest of the data were examined by one-way analysis of variance (ANOVA) plus Dunnett's *post-hoc* test. Data were represented as means  $\pm$  SD. The P-values of under 0.05 were assumed statistically significant.

## Results

### Monitoring the Disease Activity

The well-being and weight change of the animals were recorded daily after the instillation of acetic acid into the gut lumen. As shown in Figure 1, there was no significant difference between the outcomes of IBD rats received dexpanthenol or prednisolone at the end of the study. However, regarding the average mean cumulative disease score, the reduction in IBD animals received dexpanthenol was significantly more prominent than in IBD rats treated with prednisolone (Figure 2A). More importantly, combined dexpanthenol and prednisolone in half doses caused

more considerable improvement in the cumulative disease score compared to IBD rats received both monotherapies (Figure 1).

The statistical analysis of clinical scores indicated that, compared to the IBD group, combination therapy and treatment with dexpanthenol led to a significant reduction in the cumulative disease score from the fourth day after the induction of the disease (Figure 1). However, at the end of the study, the average mean cumulative disease score was considerably lessened in IBD animals treated with combination therapy compared with the dexpanthenol-treated IBD group (Figure 2A). For IBD rats treated with prednisolone, this period was repeated from day 8 after the disease induction (Figure 1).

The ratio of colon weight/length was monitored to evaluate the condition of colonic mucosal damage. The increased weight/length ratio was observed with a significant difference in colitis rats compared to healthy rats (Figure 2B). Treatment with dexpanthenol showed a non-significant decrease in the colon weight/length ratio compared to the treatment with prednisolone (Figure 2B). However, combination therapy showed a significantly lower weight/length ratio than both monotherapies (Figure 2B).

The spleen weight index was increased in the rats with colitis compared to the healthy ones, while all the therapies significantly reduced it (Figure 2C). Treatment with prednisolone promoted a notable decrease in the spleen weight index compared to the treatment with dexpanthenol (Figure 2C). Combination therapy with dexpanthenol and prednisolone led to a remarkable decrease in the spleen weight index compared to the IBD rats received both monotherapies, so that there was no difference in this index between combined treated colitis rats and healthy ones (Figure 2C).

#### Evaluation of Biochemical Parameters

The attained data showed marked promotion in MDA content and reduction in GSH content, and SOD activity was noticed in the gut tissues of the colitis rats, when compared with healthy rats (Table 2). Our results

indicated that the MDA content in dexpanthenol or combined IBD treated rats was more pronounced than in IBD rats treated with prednisolone. Conversely, dexpanthenol or combination therapy could mount the levels of SOD and GSH more profoundly than prednisolone in IBD rat (Table 2). Also, the obtained data demonstrated that combination therapy resulted in a much more pronounced decrease in MDA levels and a more profound increase in SOD levels than the levels recorded in IBD rats received individual treatment (Table 2).

NO activity, as well as MPO in the gut homogenate of animals with colitis, was markedly mounted compared to healthy rats (Figures 3A and 3B). Treatment with dexpanthenol promoted marked regression in MPO activity and the level of NO output in the colonic homogenate of rats with colitis compared with prednisolone-treated IBD rats (Figures 3A and 3B). More importantly, combination therapy resulted in a much more profound decrease in NO than NO levels recorded in IBD rats treated with each medication alone (Figures 3A and 3B).

As shown in Figure 3C, a significant decrease in protein concentration in gut homogenates was observed in colitis rats compared to healthy ones. Both monotherapies could reverse the decreasing pattern of protein content in gut tissue without any significant difference (Figure 3C). Combination therapy caused more considerable improvement in the protein content of colonic tissues compared to both monotherapies (Figure 3C).

#### Monitoring the Level of Pro-inflammatory Cytokines

As shown in Figure 3, the levels of IL-6 and TNF- $\alpha$  in the gut specimens were significantly increased in vehicle-treated colitis rats compared to healthy animals. All the therapies caused a significant and marked reduction in the content of these cytokines in the gut homogenate of the animals with colitis (Figure 4). Furthermore, our data demonstrated that combination therapy resulted in a much more noticeable decrease in TNF- $\alpha$  level than monotherapy (Figure 4).

**Table 1. Scoring system for the evaluation of colitis**

	MDA (mM/mg)	SOD (U/mg protein)	GSH ( $\mu$ mol/g)
Healthy	16.07 $\pm$ 3.01	0.9 $\pm$ 0.07	2.89 $\pm$ 0.22
Colitis	66.54 $\pm$ 6.38 <sup>\$</sup>	0.41 $\pm$ 0.03 <sup>\$</sup>	1.76 $\pm$ 0.13 <sup>\$</sup>
Colitis+Dexp.	28.48 $\pm$ 4.27 <sup>\$*</sup>	0.76 $\pm$ 0.05 <sup>\$*</sup>	2.49 $\pm$ 0.19 <sup>\$*</sup>
Colitis+Pred.	44.86 $\pm$ 3.97 <sup>\$*&amp;</sup>	0.61 $\pm$ 0.05 <sup>\$*&amp;</sup>	2.1 $\pm$ 0.15 <sup>\$*&amp;</sup>
Colitis+Com.	22.78 $\pm$ 3.81 <sup>*&amp;#</sup>	0.83 $\pm$ 0.06 <sup>*&amp;@</sup>	2.53 $\pm$ 0.19 <sup>\$*@</sup>

Data were reported as mean  $\pm$  SD (<sup>\$</sup> $P$ <0.05 versus healthy rats; <sup>\*</sup> $P$ <0.05 versus PBS-treated IBD rats; <sup>&</sup> $P$ <0.05 versus dexpanthenol-treated RA rats; <sup>@</sup> $P$ <0.05 versus prednisolone-treated rats).

Note. PBS: Phosphate-buffered saline  
Dexp: Dexpanthenol-treated IBD group.

IBD: Inflammatory bowel disease.  
Pred: Prednisolone-treated IBD group.

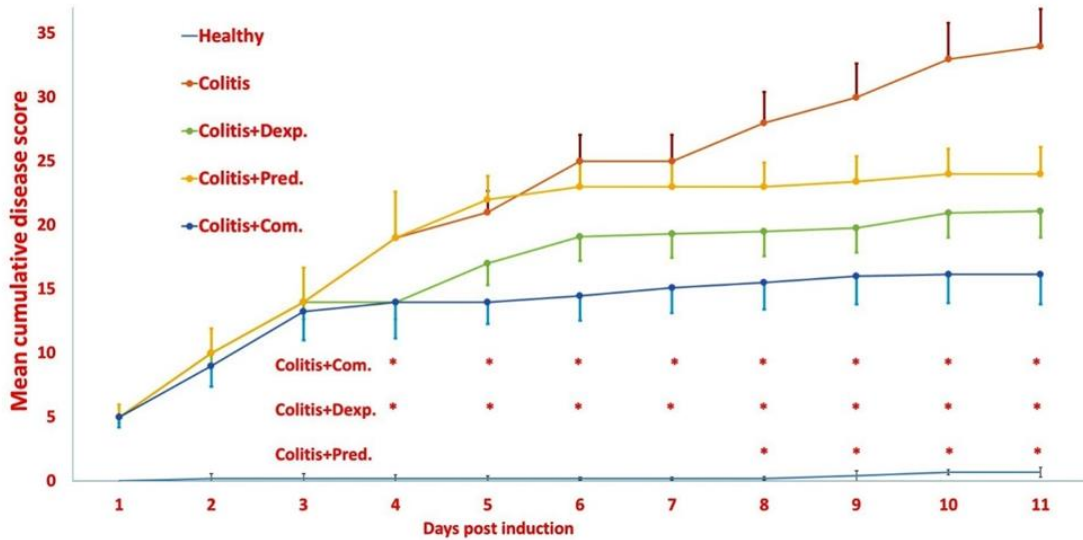
RA: Rheumatoid arthritis  
Com: Combination-treated IBD group.



**Results of RT-PCR Analysis**

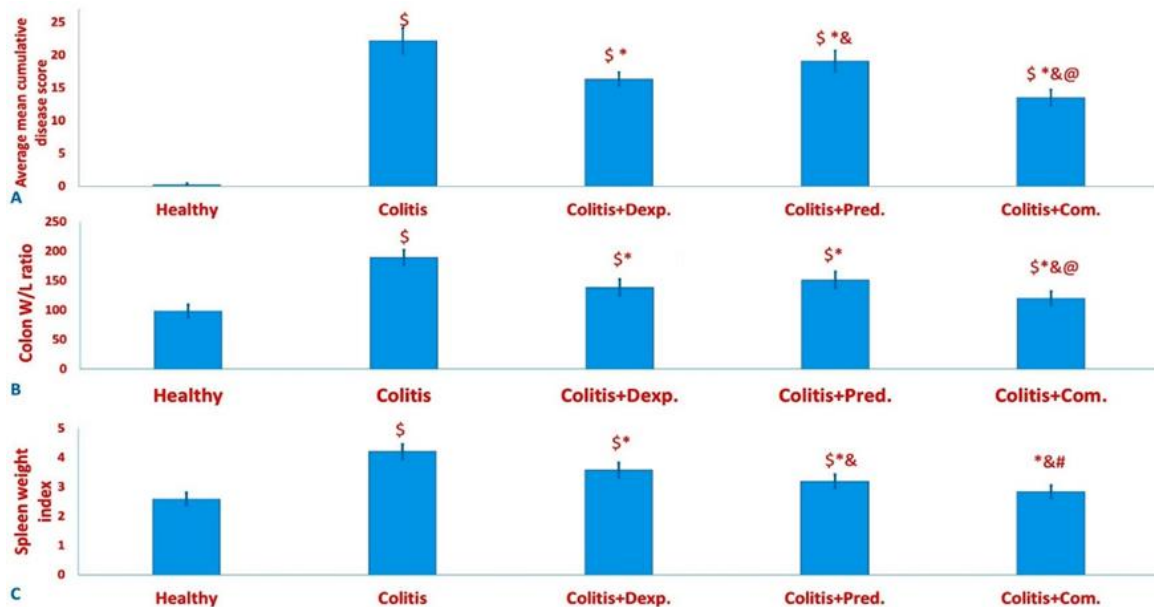
As expected, the expression of NF- $\kappa$ Bp65 (nuclear factor kappa-light-chain-enhancer of activated B cells) mRNA in the hindgut samples was mounted in the animals intrarectally received acetic acid compared to healthy animals (Figure 5A). Both treatments led to a

significant reduction in the mRNA level of NF- $\kappa$ Bp65. However, combination therapy caused a more considerable reduction (Figure 5A). Besides, the mRNA level of I $\kappa$ B $\alpha$  (inhibitor of NF- $\kappa$ B) showed no statistically significant discrepancy between the experimental groups (Figure 5B).



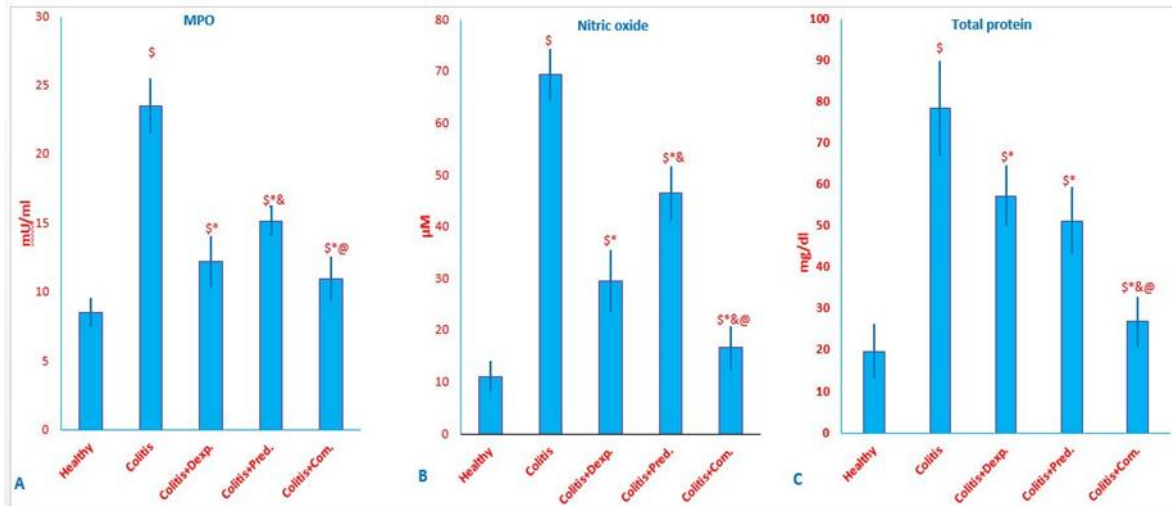
**Figure 1.** The assessment of the mean cumulative disease score in IBD rats. IBD rats were treated with dexpanthenol and prednisolone alone and in combination. The combination of half doses of dexpanthenol and prednisolone improved clinical outcomes of colitis better than the treatment with either drug alone in full doses. Results are presented as mean  $\pm$  SD (\$ $P$ <0.05 versus healthy rats; \* $P$ <0.05 versus PBS-treated IBD rats; & $P$ <0.05 versus dexpanthenol-treated RA rats; @ $P$ <0.05 versus prednisolone-treated rats).

Note. PBS: Phosphate-buffered saline      IBD: Inflammatory bowel disease.      RA: Rheumatoid arthritis  
 Dexp: Dexpanthenol-treated IBD group.      Pred: Prednisolone-treated IBD group.      Com: Combination-treated IBD group.



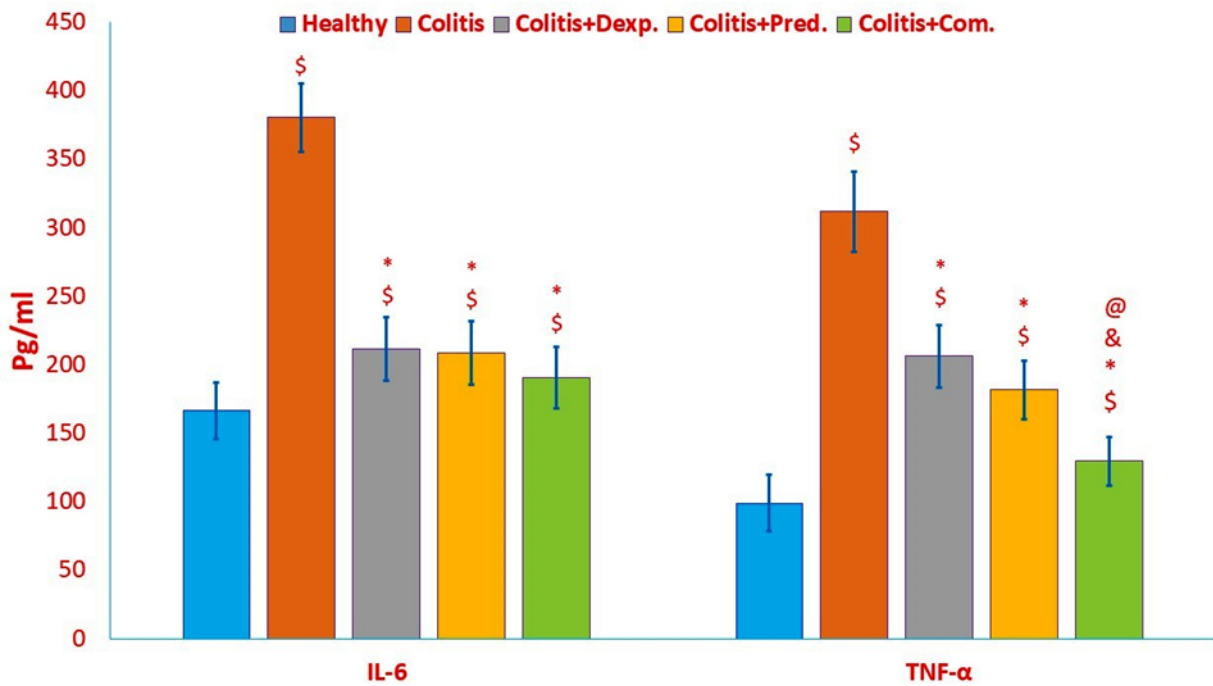
**Figure 2.** The assessment of clinical features in IBD rats. Results are presented as mean  $\pm$  SD (\$ $P$ <0.05 versus healthy rats; \* $P$ <0.05 versus PBS-treated IBD rats; & $P$ <0.05 versus dexpanthenol-treated RA rats; @ $P$ <0.05 versus prednisolone-treated rats).

Note. PBS: Phosphate-buffered saline      IBD: Inflammatory bowel disease.      RA: Rheumatoid arthritis  
 Dexp: Dexpanthenol-treated IBD group.      Pred: Prednisolone-treated IBD group.      Com: Combination-treated IBD group.



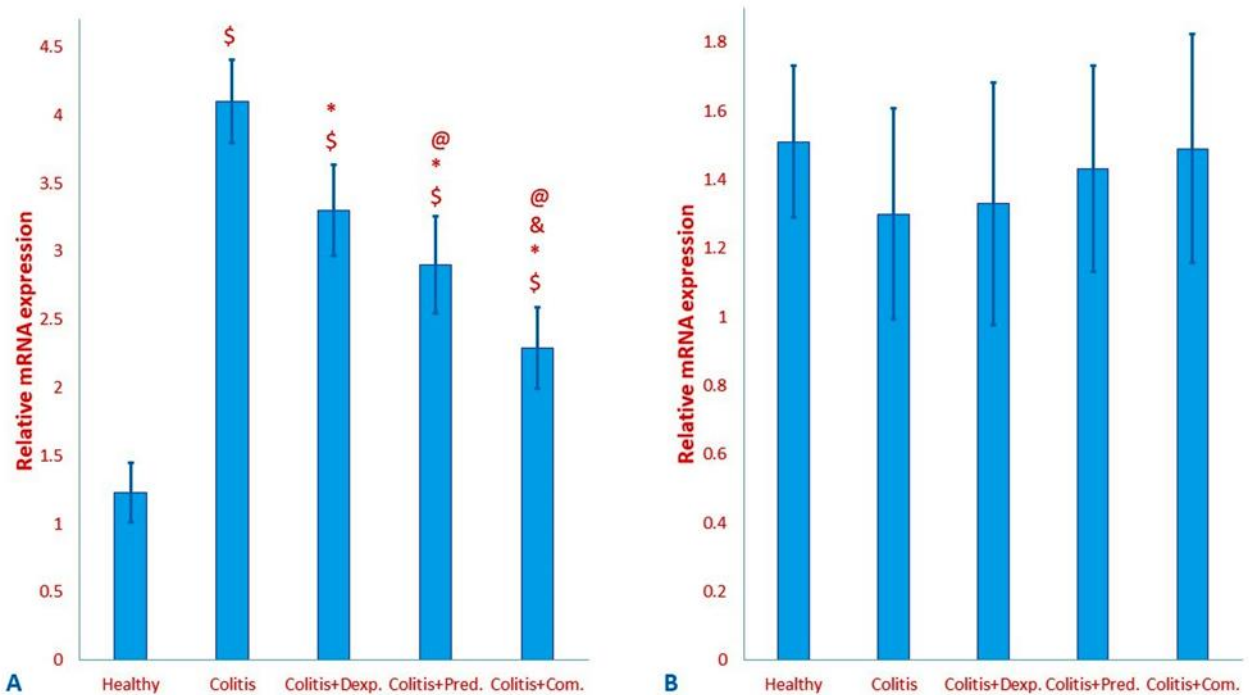
**Figure 3.** Alteration in some biochemical parameters in the colonic homogenate of colitis rats. Data are reported as mean ± SD (\$*P*<0.05 versus healthy rats; \**P*<0.05 versus PBS-treated IBD rats; &*P*<0.05 versus dexpanthenol-treated RA rats; @*P*<0.05 versus prednisolone-treated rats).

*Note.* PBS: Phosphate-buffered saline IBD: Inflammatory bowel disease. RA: Rheumatoid arthritis  
 Dexp: Dexpanthenol-treated IBD group. Pred: Prednisolone-treated IBD group. Com: Combination-treated IBD group.



**Figure 4.** The effect of therapeutic approaches on the levels of pro-inflammatory cytokines in the colonic homogenate of experimental rats. The findings are shown as mean ± SD (\$*P*<0.05 versus healthy rats; \**P*<0.05 versus PBS-treated IBD rats; &*P*<0.05 versus dexpanthenol-treated RA rats; @*P*<0.05 versus prednisolone-treated rats).

*Note.* IL-6: Interleukin 6. TNF-α: Tumor Necrosis Factor Alpha PBS: Phosphate-buffered saline IBD: Inflammatory bowel disease. RA: Rheumatoid arthritis Dexp: Dexpanthenol-treated IBD group. Pred: Prednisolone-treated IBD group. Com: Combination-treated IBD group.



**Figure 5.** The relative expression of the mRNA of NF-κBp65 (A) and IκB (B) in the colonic specimen. Results are presented as mean ± SD (\$ $P < 0.05$  versus healthy rats; \* $P < 0.05$  versus PBS-treated IBD rats; &  $P < 0.05$  versus dexamethasone-treated RA rats; @ $P < 0.05$  versus prednisolone-treated rats).

*Note.* mRNA: Messenger RNA. NF-κBp65: p65 subunit of nuclear factor kappa-light-chain-enhancer of activated B cells IκB: inhibitor of NF-κB PBS: Phosphate-buffered saline IBD: Inflammatory bowel disease. RA: Rheumatoid arthritis Dexp: Dexamethasone-treated IBD group. Pred: Prednisolone-treated IBD group. Com: Combination-treated IBD group.

## Discussion

Combination therapy, along with a different anti-inflammatory mechanism for controlling the complicated situation induced by immunological disorder, is a logical decision (19, 20). Dexamethasone is an alcohol with biological activity analog of PA, which transforms into PA within cells (6). PA performs its anti-oxidative benefits by augmenting the synthesis of reduced GSH and related peroxidase enzymes, which render as the utmost protective machinery versus oxidative stress and lipid peroxidation in the inflammation PA performs its anti-oxidative benefits by augmenting the synthesis of reduced GSH and related peroxidase enzymes (8). Moreover, PA merges into the infrastructure of coenzyme A, promotes anti-inflammatory benefits, and exerts epithelization (7, 8). On the other hand, prednisolone is a famous glucocorticoid, which up-regulates the expression of anti-inflammatory proteins and, simultaneously, down-regulates the expression of pro-inflammatory proteins (21).

The main propose of combination therapy is to promote significant regression in the clinical outcomes, which is superior to pharmacotherapy with each drug alone (20). The findings of the current investigation suggested that the combined dexamethasone and

prednisolone in half doses led to a remarkable decrease in the adversity of IBD, which was more pronounced than each medication alone. The current study also indicated that combination therapy could enforce gut-healing because combined medications can turn out the severity of diminution in total protein levels.

Moreover, it is necessary that each medication prescribed for combination therapy possesses a good safety margin and does not induce further toxicities when used simultaneously (19, 20). In this investigation, no bright adverse incident was reported in experimental groups. Also, combined therapy increased weight-gaining in rats.

According to IBD pathogenesis, just after the initial mucosal damage in the gut, inflammatory cells produced a large number of reactive oxygen and nitrate substances, such as H<sub>2</sub>O<sub>2</sub>, hydroxyl radical, superoxide, NO, and peroxynitrite (22). MDA is a good predictor of lipid peroxidation (13). MPO enzyme is a peroxidase that catalyzes the production of hypochlorous (or hypobromous) acid and oxidizes tyrosine to the tyrosyl radical by H<sub>2</sub>O<sub>2</sub>.

Both tyrosyl radical and hypochlorous acid are potentially cytotoxic, so that they may induce oxidative

damage in the host tissue (13, 23). Tissues exposed to inflammation defend against reactive nitrogen species and reactive oxygen species via endogenous enzymatic antioxidants (such as SOD) and endogenous non-enzymatic antioxidants (such as GSH) (24). Previous documents have also indicated that dexpanthenol markedly attenuates MDA contents and MPO activity and also mounts GSH and SOD activity in the LPS-challenged lung tissue and cisplatin-induced hepatotoxicity model (6, 9).

Although the infiltration of inflammatory cells could be controlled relatively by corticosteroids, tissue oxidative and nitrative damages are not directly influenced by these agents (13, 23). It has been documented that dexpanthenol possesses antioxidative benefits via enhancing the expression of reduced GSH and its related peroxidase enzymes (25). Our results indicated that the intensity of MDA, MPO, and NO was suppressed in dexpanthenol or combined IBD treated rats more profoundly than in IBD rats received prednisolone. Conversely, dexpanthenol or combination therapy could increase the levels of SOD and GSH more profoundly than prednisolone in IBD rats.

It shall be noted that MPO can be applied as an indirect indicator to assess the severity of neutrophil infiltration into inflamed tissue, such as a colon in IBD condition (3). As described previously, dexpanthenol can accelerate re-epithelization and promote wound healing (8). Also, damage to the mucosal cell and loss of mucosal cell integrity is the first stage in the development of IBD, such as acetic acid-induced colitis (3, 13, 23). Therefore, a part of the benefit observed in combination therapy may be due to the potent anti-inflammatory effect of prednisolone together with the antioxidant properties and epithelial repair features of dexpanthenol. In this regard, the results indicated that combination therapy led to a much more pronounced decrease in NO and MDA levels and also more profoundly increased the level of SOD than the levels recorded in IBD rats received individual treatment.

IL-6 and TNF- $\alpha$  have vital roles in the pathology of IBD (22, 26). Medication with the biological inhibitors of TNF- $\alpha$  substantially regresses the signs of IBD (27). TNF- $\alpha$  also participates in pyrexia, algasia, and cachexia (26). IL-6 promotes the production of acute-phase proteins, such as C-reactive protein (CRP) (28). NF- $\kappa$ B, as a master vigorous inflammatory transcription factor, can promote the secretion of pro-inflammatory mediators, such as IL-6 and TNF- $\alpha$  (13). Recently, the NF- $\kappa$ B pathway and their downstream signal regulators, including IL-6 and TNF- $\alpha$ , have been considered as new promising anti-inflammatory strategies for controlling IBD (29).

The findings of this survey established that both monotherapies led to a remarkable reduction in the mRNA expression of NF- $\kappa$ Bp65, production intensity of IL-6, and TNF- $\alpha$  in the inflamed colon. Previous

data have also shown that TNF- $\alpha$  and IL-6 in the fluid of the bronchoalveolar lavage of mice with acute lung injury were decreased by dexpanthenol administration (6). TNF- $\alpha$  levels were also significantly decreased in a rat endometriosis model treated with dexpanthenol compared to the healthy group (7). More importantly, our results demonstrated that combination therapy resulted in a much more prominent reduction in the levels of TNF- $\alpha$  and NF- $\kappa$ Bp65 than both monotherapies. A further decrease in the level of TNF- $\alpha$  may be the reason for the better weight gain in the combination therapy group. I $\kappa$ B $\alpha$  can suppress NF- $\kappa$ B signaling by blocking nuclear localization signals (30). The obtained data of this study indicated that the mRNA level of I $\kappa$ B $\alpha$  did not show any significant diversity between the experimental groups.

Collectively, this research represented for the first time that treatment with dexpanthenol could subtract the clinical schema of the animal model of IBD more than prednisolone. Our data also indicated for the first time that combined therapy with a half dose of dexpanthenol and prednisolone could alleviate the clinical and laboratory indices of the animal model of IBD more significant than the treatment with each drug alone. Combination therapy promoted a reduction in the levels of oxidative and nitrative stress marker concurrent with the levels of TNF- $\alpha$  and NF- $\kappa$ Bp65 more impressively than each therapy alone.

Accordingly, a combination of these medications can be suggested as a potential strategy for better IBD management. Nevertheless, the present study was only a preliminary investigation conducted in only one animal model. Gut inflammation induced by Acetic acid were most similar to the ulcerative colitis form of human IBD. Hence, further research is required to recognize the accurate mechanism of the reported synergistic benefit between dexpanthenol and prednisolone. Also, if the results of the research on other animal models are appropriate, it is necessary to begin the initial phase of clinical studies in humans.

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## Conflict of Interest

Authors declared no conflict of interest.

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