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Is Curcumine Effect Comparable to 5-Aminosalicylic Acid or Budesonide on a Rat Model of Ulcerative Colitis Induced by Trinitrobenzene Sulfonic Acid?

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***Abstract*—**Inflammatory bowel disease (IBD) is a chronic relapsing-remitting condition that afflicts millions of people throughout the world and impairs their daily functions and quality of life. Treatment of IBD depends largely on 5-aminosalicylic acid (5-ASA) and corticosteroids. The present study aimed to clarify the effects of 5-aminosalicylic acid, budesonide and currcumin on 90 male albino rats against trinitrobenzene sulfonic acid (TNB) induced colitis. TNB was injected intrarectally to 50 rats. The other 40 rats served as control groups. Both 5-ASA (in a dose of 120 mg/kg) and budesonide (in a dose of 0.1 mg/kg) were administered daily for one week whereas currcumin was injected intraperitonially (in a dose of 30 mg/kg daily) for 14 days after injection of either TNB in the colitis rats (group B) or saline in control groups (group A). The study included estimation of macroscopic score index, histological examination of H&E stained sections of the colonic tissue, biochemical estimation of myeloperoxidase (MPO), nitric oxide (NO), and caspase-3 levels, in addition to studying the effect of tested drugs on colonic motility. It was found that budesonide and curcumin improved mucosal healing, reduced both NO production and caspase-3 level. They had the best impact on the disturbed colonic motility in TNBS-model of colitis.

***Keywords*—**Colitis, curcumin, nitric oxide.

I. Introduction

INFLAMMATORY bowel disease (IBD) is a chronic relapsing-remitting inflammation of the gastrointestinal tract that produces debilitating symptoms of diarrhea, abdominal pain and weight loss. It comprises primarily two disorders – ulcerative colitis (UC) and Crohn's disease (CD) [1]. Aminosalicylates and corticosteroids remain the standard for induction and maintenance therapies of UC. Immunomodulators such as 6-mercaptopurine and azathioprine are effective steroid-sparing and maintenance therapies. Nevertheless, these standard treatments, especially the corticosteroids, have proven to be a double-edged sword in the treatment of IBD where the need for corticosteroids is associated with a worse prognosis; including increased risk of surgery [2],[3].

Although the exact etiology of IBD remains unknown, the pathogenesis is gradually being unraveled, seeming to be the result of a combination of environmental, genetic, and immunological factors in which an uncontrolled immune response within the intestinal lumen leads to inflammation. Alterations in the production of many cytokines, reactive oxygen species (ROS), nitric-oxide (NO) and the process of apoptosis have been also involved in the pathogenesis of IBD [4], [5].

The important role attributed to NO and the process of apoptosis in IBD prompted us to study whether the beneficial effects of tested drugs on TNBS-induced colitis could be attributed to their effects on these factors.

NO plays physiological roles in the control of smooth muscle tone and motility, maintenance of adequate perfusion, and regulation of the immune response. On the other hand, excessive production of NO is associated with the initiation and maintenance of inflammation. Various studies based on animal models as well as in humans, suggested a pathogenic role of NO in active UC. However, the exact role of NO overproduction in intestinal inflammation remains obscure [6].

Apoptosis is important in regulating overall cell number in virtually all tissues, including the epithelium of the gastrointestinal tract, so that epithelial cell numbers remain constant and tissue homeostasis is maintained. Recently, evidence has increased that cell death by apoptosis plays an important role in the pathogenesis of mucosal damage in patients with IBD [7]. Several mediators have been proposed to explain the pathway leading to apoptosis of crypt epithelial cells in inflamed colonic mucosa, such as p53, the perforin-granzyme system, TNF-α, and NO. However, the interactions among the multiple proteins and pathways that regulate apoptosis during colitis still remain unclear [8], [9].

Abnormalities in colonic motor function in patients with IBD were first documented in the 1950s [10]. Both increased and decreased smooth muscle contractility have been observed in such cases. The mechanisms underlying the motility dysfunction are unclear but may involve changes in colonic smooth muscle function, or enteric neurotransmission [11],

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[12]. Changes in quality (atrophy and vacuolization) and quantity of intramuscular and myenteric interstitial cells of Cajal (ICC) are also reported in human and animal models of colitis.

In view of such considerations, the aim of the present work was to study the possible effects of certain relevant drugs on the colonic mucosa of rats with TNBS induced colitis. The study also aimed to assess the effect of these drugs on colonic NO level, MPO activity, and on the colonic apoptotic rate. Additionally, the present study aimed to study the effect of tested drugs on colonic motility.

II. Materials and Methods

A. Drugs and Chemicals

5ASA: (Pentasa 1% - Ferring), Budesonide : (Enemacort 0.02mg/1ml - MUP), Curcumin : (Sigma Chemical Company),TNB: 2,4,6-trinitrobenzenesulfonic acid (TNBS) (Fluka). All other chemicals in vitro studies were of the A.R and purchased from Sigma Aldrich Company.

B. Research Protocol

This followed and was accepted by the Alexandria Faculty of Medicine Ethical Committee. In which 90 male albino rats weighed 150-200gms were acclimatized in standard cages for two weeks before study in Pharmacology Department Animal House, Faculty of Medicine Alexandria University. Housing was and maintained under a 12 h light/ 12 h dark cycle at a constant temperature (22 ± 2ºC) and humidity, water and food were allowed *ad libitum* throughout the study. Animals were randomly divided into the following groups:

Control groups A (40 rats): animals in this group had received 1 ml saline intrarectally, served as a control group, they were subsequently subdivided into the following subgroups (each of 10 rats):

A1: animals in this group received 1 ml saline intrarectally daily for 7 days.

A2: animals in this group received 5-aminosalicylic acid in a dose of 120 mg/kg intrarectally daily for 7days [13].

A3: animals in this group received budesonide in a dose of 0.1 mg/kg intrarectally daily for 7 days [13].

A4: animals in this group received curcumin in a dose of 30 mg/kg by intraperitonial injection (i.p) daily for 14 days [14].

Colitis groups B (50 rats): Colitis was induced in these animals by trinitrobenzene sulfonic acid (TNBS). Animals were anesthetized with xylazine (20 mg/kg) and ketamine (100 mg/kg) intramuscularly, TNBS was administered intrarectally; once, in a dose of 100 mg/kg in 50% ethanol [15]. Then, they were subdivided into the following subgroups (each of 10 rats), both B1 and B1II are injured control groups received saline:

B11: Received 1 ml saline intrarectally daily for 7 days.

B1II: Received 1 ml saline intrarectally daily for 14 days.

B2: Received 5-aminosalicylic acid in a dose of 120 mg/kg intrarectally daily for 7days.

B3: Received budesonide in a dose of 0.1 mg/kg intrarectally daily for 7 days.

B4: Received curcumin in a dose of 30 mg/kg by intraperitonial injection (i.p) daily for 14 days.

At the end of study period; rats were sacrificed under light ether anesthesia, the distal colon was removed and examined macroscopically for signs of inflammation. Then, three representative specimens were obtained from the colon from a region 2 cm proximal to the anus. [13] One segment was taken for motility experiments, another specimen was frozen in 0.05 M phosphate buffer containing 0.5% hexadecyl-trimethylammonium bromide for analysis of myeloperoxidase chemically [13], nitric oxide (nitric oxide assay kit-Biodiagnostic), and caspase-3 (Caspase-3/CPP32 activity Colorimetric Assay Kit) [14]. A third specimen piece from each specimen was fixed in 10% formalin overnight, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H & E) for histological examination [15].

C. In Vitro Contractility

The distal colon was opened along the mesenteric border, muscle strips (1.0 × 0.4 cm) were cut along the circular axis, mounted in individual 8-ml organ baths, and maintained in oxygenated Krebs' solution at 37°C. The smooth muscle contractions were recorded using an isometric tension transducer [16].

Colonic specimens from control and treated groups were examined for the contractile response to Ach (10-8 to 10-4 M), and concentration-dependent response curve to Ach was constructed. To evaluate the role of NO on basal contractile activity of both control and treated groups, spontaneous contractile activity was examined in the presence of the nitric-oxide synthase inhibitor N$^{\omega}$-nitro-L-arginine (L-NNA; 10 μM). The full effect of L-NNA was obtained after 10 min of incubation.

D. Analysis of Results

Results of the present study were tabulated and analyzed using the ANOVA statistical test, expressed as the mean of individual response ± standard Error. Differences among group were compared using one-way ANOVA through the Statistical Package of Social Science (SPSS/version 16.0) soft ware. LSD test was used as a post hoc test for comparison among groups. Statistical significance was set at P values lower than 0.05.Log ED50 were estimated by Graph Pad Prism 5 program.

III. Results

A. Macroscopic Score Index in Normal Control Group

Results of the present study revealed that animals of group A showed no evidence of macroscopic mucosal damage, adhesions, or ulceration of the colon.

Examination of the colon from TNBS-treated rats revealed the presence of multiple mucosal erosions and ulcerations in all experimental animals of both groups (macroscopic damage score were 7.2±1.47for group BI).

Treatment with 5-ASA was associated with a non significant decrease of the mean value of the macroscopic

damage score (with a mean value of 3.80±1.22) in comparison to group B1.

Treatment with budesonide was associated with a highly significant decrease in the mean value of ulcer score index (2.20±1.03) in comparison to group B1.

Treatment with curcumin was associated with a significant decrease in the mean value of ulcer score index (2.30±1.05) in comparison to group B1.

B. Colonic Contractile Activity

Muscle strips from the rat colon spontaneously demonstrated regular rhythmic contractions. The mean force of contraction of the normal untreated group was 1.80±0.37 gm tension.

Our results revealed that muscle strips from the rat colon of group B1 showed a significant reduction of the force of spontaneous contractions compared to the normal control group. The mean values were 0.72±0.28 gm tension for group B1 compared to 1.80±0.37 gm tension group A1.

Treatment with 5-aminosalicylic acid was associated with a non significant increase of the spontaneous contraction in comparison to the TNBS-control group (B1), with a mean value of 1.00±0.26 gm tension. Treatment with budesonide was associated with a significant increase in the spontaneous contraction in comparison to B1, with a mean value of 1.62±0.23 gm tension.

Similarly, treatment with curcumin was associated with a significant increase in the spontaneous contraction in comparison to B1 with a mean value of 1.54±0.36 gm tension.

L-NNA (10 μM) induced an increase in the amplitude of spontaneous contractile activity which peaked after 5-10 min. The mean value after L-NNA application was 2.52±0.44 gm tension, for group A1.

L-NNA did not significantly increase the amplitude of spontaneous contraction in group B1, with a mean value of 0.92±0.28 gm tension.

L-NNA induced a significant increase in the amplitude of spontaneous contractile activity (in groups B2-B4) as compared to the amplitude of spontaneous contractions before L-NNA administration (mean values after L-NNA application were 1.44±0.35 gm tension, 2.18±0.25 gm tension, 1.86±0.32 gm tension) for 5-aminosalicylic acid treated group, budesonide treated group, curcumin treated group, respectively. (Not represented)

Data in table demonstrate that Ach stimulation produced a concentration-dependent increase in the force of contraction in all muscle strips of group A, starting with a low concentration of Ach, increasing gradually with the maximal response developed to 100μm Ach. Log EC50 of Ach response were -6.30, -6.22, -5.62 for 5-aminosalicylic acid treated group, budesonide treated group, and curcumin treated group, respectively, meaning that treatment with these drugs did not significantly alter the response to Ach.

TNBS administration induced a significant reduction in the sensitivity to acetylcholine. A greater concentration of Ach was required to stimulate contractions, and a rightward shift in the dose response curve was observed. Log EC50 = -4.45 for group B1, compared to Log EC50= -6.30 of normal control group (demonstrated in the Fig. 6).

Ach stimulation produced a dose dependent increase in the amplitude of contraction in these groups. Log EC50 of Ach response were -4.58, -6.01, -5.95 for 5-aminosalicylic acid treated group, budesonide treated group, and curcumin treated group, respectively.

C. Myeloperoxidase Level

In this study, the mean value of MPO level in the normal control group was 0.20±0.04 Units/mg tissue..

TNBS administration was associated with a significant increase of the myeloperoxidase level in the colonic tissue when it was measured 1 week following TNBS rectal administration. The mean values for MPO level was 1.63±0.46 Units/mg tissue in groups B1.Treatment with 5-aminosalicylic acid, budesonide, or curcumin were associated with a significant decrease of the mean value of MPO in the colonic tissues in comparison to the TNBS-control group (mean values were 0.45±0.16 Units/mg tissue, 0.41±0.18 Units/mg tissue, 0.32±0.09 Units/mg tissue, respectively).

D. Nitric Oxide Level

The NO level of the normal control group was 33.73 ± 3.42 μmol/gm tissue. There was a significant increase of the NO level in the colonic tissue when it was measured 1 week following TNBS administration. The mean values of NO level were 40.84±3.24 μmol/gm tissue, in groups B1.

Treatment with 5-aminosalicylic acid was associated with a non significant decrease of the mean value of NO level in the colon compared to the TNBS-control group, with a mean value of 38.79±3.47 μmol/gm tissue.

Treatment with budesonide or curcumin were associated with a significant decrease in the mean value of NO level in the colon compared to TNBS-control groups, with a mean value of 23.91±2.89 μmol/gm tissue, 25.48±2.64 μmol/gm tissue respectively.

E. Caspase-3 Level

The level of caspase-3 in the colonic tissue 7.07 in the normal control group was ±1.24 nmol/mg protein/min.

Results of the present study revealed that TNBS administration was associated with a significant increase of the caspase-3 level in the colonic tissue with mean values of 37.76±1.90 nmol/mg protein/min in group B1.

Treatment with 5-aminosalicylic acid was still associated with a non significant decrease of the mean value of caspase-3 in the colon (mean value of 34.76±2.59 nmol/mg protein/min), in comparison to TNBS-control group.

Treatment with budesonide or curcumin was associated with a significant decrease in the mean value of caspase-3 in the colon (mean value of 7.11±1.59 nmol/mg protein/min, 6.54±1.49 nmol/mg protein/min respectively) compared to TNBS-control group.

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TABLE I
EFFECT OF (± SE) 5-AMINOSALICYLIC ACID (120 MG/KG INTRARECTALLY, DAILY FOR 7 DAYS), BUDESONIDE (0.1 MG/KG INTRARECTALLY, DAILY FOR 7 DAYS) AND CURCUMIN (30 MG/KG I.P, DAILY FOR 14 DAYS), ON COLONIC ULCER SCORE INDEX, MPO LEVEL (UNITS/MG TISSUE), NITRIC OXIDE LEVEL (µMOL/GM TISSUE) , CASPASE-3 LEVEL (NMOL/MG PROTEIN/MIN) AND SPONTANEOUS CONTRACTIONS(GM TENSION) IN CONTROL RATS (A GROUPS) AND TNBS-COLITIS RATS (B GROUPS) (100 MG/KG IN 50% ETHANOL INTRARECTALLY)

Groups	Ulcer score index	MPO level Units/mg tissue	NO level µmol/gm tissue	Caspase-3 level	Spontaneous contractions gm tension
A1	0	0.20±0.01	22.65±0.65	7.07±0.39	1.80±0.12
B1I	7.20±0.46	1.63±0.14*	40.84±1.02*	37.76±0.60*	0.72±0.09*
B1II	7.10±0.48	1.52±0.11*	40.77±1.07*	37.47±0.68*	0.78±0.06*
A2	0	0.27±0.01	23.62±0.77	6.37±0.48	1.80±0.09
B2	3.80±0.38**	0.45±0.05Δ**	38.79±1.09Δ	34.76±0.81Δ	1.00±0.08Δ
A3	0	0.21±0.02	33.73±1.08	5.33±0.43	1.20±0.10
B3	2.20±0.32**	0.41±0.05**	23.91±0.91**	7.11±0.50**	1.62±0.07**
A4	0	0.18±0.01	23.86±0.52	6.44±0.51	1.80±0.10
B4	2.30±0.33**	0.32±0.03**ΔΔ	25.48±0.83**	6.54±0.47**	1.54±0.11**
P value	<0.0001	0.01	0.0001	0.0001	>0.05

Group A1: normal untreated control group. Group A2: 5-aminosalicylic acid treated group. Group A3: Budesonide acid treated group. Group A4: Curcumin treated group. Group B1I: TNBS control group after 1 week. Group B1II: TNBS control group after 2 week. Group B2: TNBS+5-aminosalicylic acid. Group B3: TNBS+Budesonide. Group B4: TNBS+Curcumin. S.E.: Standard error.

F. Histological Results

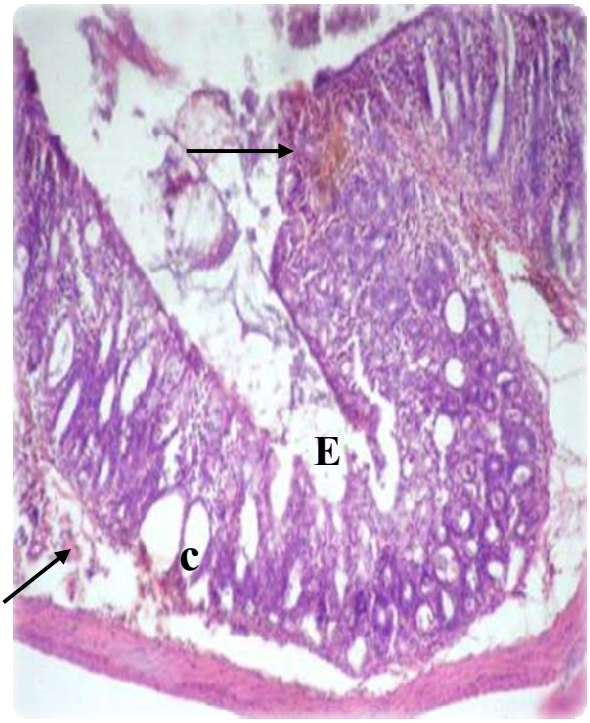


Fig. 1 Light micrograph of the colon of TNBS control colitis group, showing multiple epithelial erosions (E), and necrotic hemorrhagic areas surrounded by inflamed connective tissue lamina propria of both mucosa and submucosa (arrows). Some crypts are cystically dilated with abscess formation (C). (H&E stain, Mic. Mag.X 100)

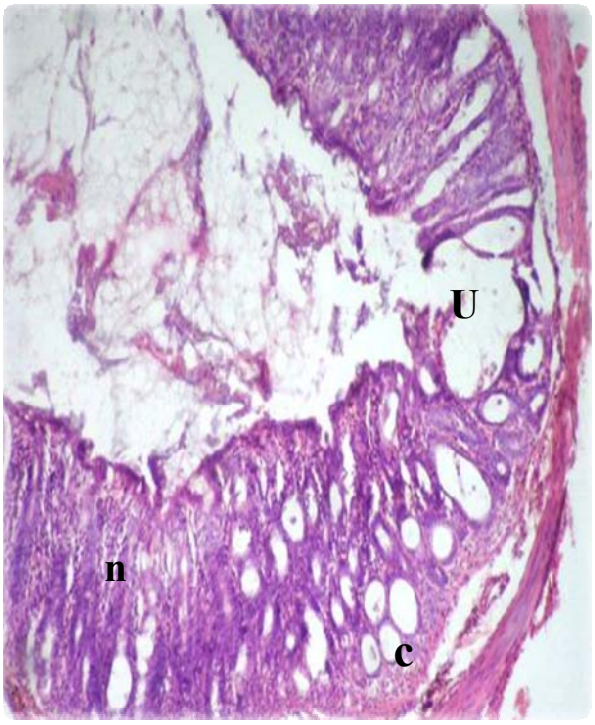


Fig. 2 Light micrograph of the colon of TNBS control colitis group, showing localized colonic ulcer (U) involving almost the whole mucosa disrupting the muscularis mucosa deep to apparently intact musculosa. The lumen contains sloughed necrotic fragments mixed with inflammatory debris. Short narrow crypts with necrotic columnar and goblet cells (n) are seen, other crypts appeared cystically dilated with crypt abscess formation (C). (H&E stain, Mic. Mag.X 100)

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Light microscopic examination of H&E stained sections of TNBS-control non treated groups showed that the lumen was filled with sloughed necrotic tissues mixed with excess mucus. The general histologic architecture of the colonic mucosal glands was disturbed. Multiple mucosal erosions of varying depths were observed ranging from sloughed out surface mucosal cells to wide ulcers involved the whole mucosa and disrupting the muscularis mucosa deep to the musculosa

Other features of colonic damage were observed in the form of evident glandular atrophy with short narrow crypts lined by necrotic columnar and goblet cells, while other areas exhibited swollen crypts forming cystically dilated abscesses surrounded by hemorrhagic inflammatory cellular infiltrate. (Figs. 1 and 2).Light microscopic examination of H&E stained sections of 5-aminosalicylic acid + TNBS treated group revealed incomplete healing of the ulcerated mucosa. The general architecture of the colonic glands was almost resorted; however some crypts appeared shorter with sloughing of their apices into the lumen, others showed narrow lumen. The lining epithelium of the crypts was generally of low columnar cells, while goblet cells were less frequently encountered compared to the negative control sections. Moderate inflammatory infiltrate was observed in some areas between the crypts (Fig. 3).

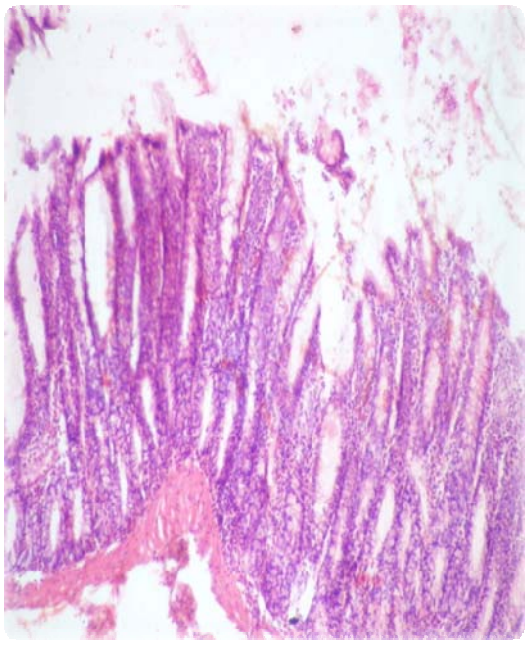


Fig. 3 Light micrograph of **5-aminosalicylic acid** + TNBS treated group (120 mg/kg intrarectally, daily for 7 days) showing nearly normal architecture of the crypts. Some crypts appeared with sloughing of their apices into the lumen (A), others showed narrow lumen (L). moderate inflammatory infiltrate in the lamina propria (H&E stain, Mic. Mag.X 100)

Light microscopic examination of H&E stained sections of **budesonide+TNBS** treated group revealed good healing of the ulcerated mucosa. The general architecture of the colonic glands was resorted; the lumen appeared free from necrotic or inflammatory debris. The crypts almost regained their full length and cellularity with some intervening cellular infiltrate (Fig. 5).

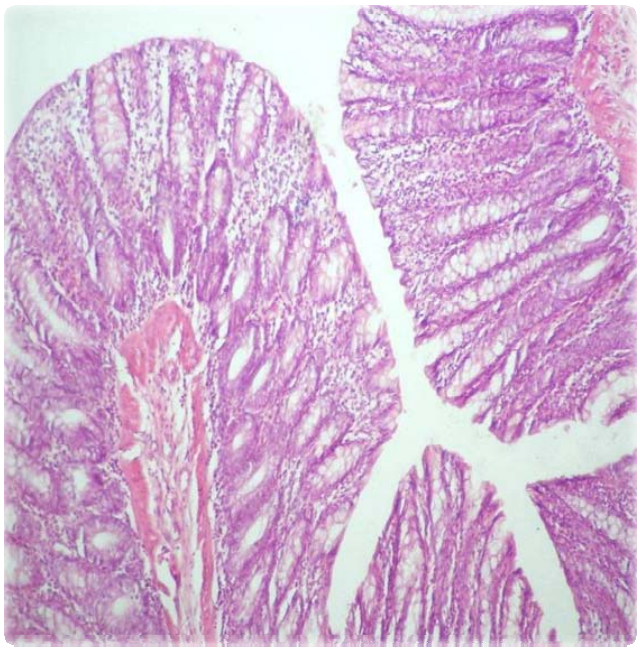


Fig. 4 Light micrograph of **budesonide+TNBS** treated colon showing almost normal architecture of the mucosa. Nearly all crypts regained their full length and cellularity with some intervening cellular infiltrate between them (arrows). Notice, the lumen is free from necrotic or inflammatory debris. (H&E stain, Mic. Mag.X 100)

Light microscopic examination of H&E stained sections of **curcumin** + TNBS treated group revealed good coverage of the ulcerated mucosa. The mucosal crypts showed adequate cellularity and their lumina appeared of moderate length and width of compared to the control sections. The regenerating surface columnar cells were observed covering the luminal surface of the mucosa. Mild cellular infiltration was seen in the connective tissue of the lamina propria and submucosa (Fig. 4).

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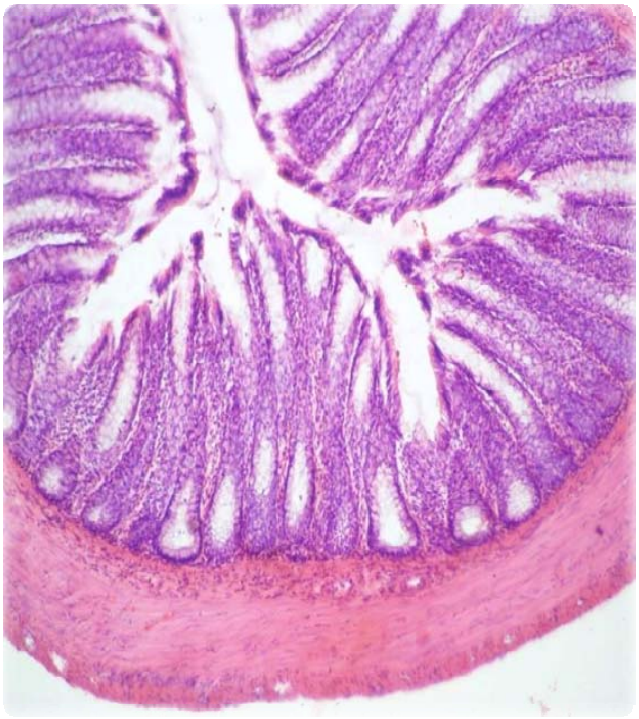


Fig. 5 Light micrograph of **curcumin** + TNBS treated colon. Almost all crypts appear intact (C) with adequate length and cellularity. The surface epithelial cells are well observed covering the mucosa (E). Note, mild cellular infiltration (arrows). (H&E stain, Mic. Mag.X 100)

IV. Discussion

Ulcerative colitis is a chronic relapsing-remitting condition that afflicts millions of people throughout the world and impairs their daily functions and quality of life. While most reports on animal models of colitis focused predominantly on the pro inflammatory mediators that may initiate inflammatory process, only few studies have addressed the link between NO production, apoptosis and the impact of treatment on colonic motility. The present study had the interest to investigate the effect of some drugs on these parameters. TNBS-model was chosen in the present study to characterize the morphological and functional changes associated with colitis [17]-[20].

In the present study, tested drugs (in absence of TNBS-induced colitis) produced no changes on spontaneous contractions as compared to the normal untreated control group. The effect of Ach and L-NNA were also unaltered in these groups. On the other hand, spontaneous contraction of the colon was reduced significantly ($P<0.01$) when it was assessed 1 week after TNBS administration. The maximum response and the sensitivity to Ach were reduced. Furthermore, there was no significant increase in the amplitude of spontaneous contractions after L-NNA application, suggesting loss of nitrergic control after TNBS-induced inflammation.

Our results were in consistent with previous studies where a decrease in the amplitude of contractile activity was observed in patients with UC as well as in animal models of inflammation (Fig. 6). The response to cholinergic stimulation is also decreased in muscle strips taken from UC patients and from animal models of colitis [20]-[22].

The decreased contractility is suggested to be developed independent of the manner in which the colitis is induced. The mechanism(s) of altered motility in IBD is complex [(Konturek PC 2011). It was suggested that the changes in colonic contractions are not due to initial mucosal injury of the inducing agent but due to the subsequent inflammatory response [23]. Of particular importance, the induction of NOS in the mucosal and neuromuscular layers was previously suggested to play an important role in the hypocontractility response in cases of IBD [24].

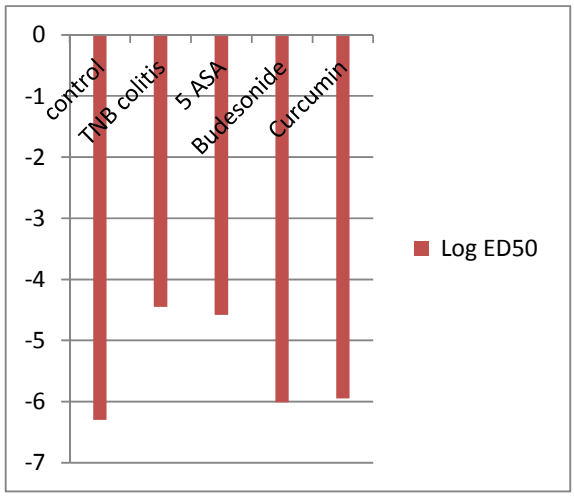


Fig. 6 Effect of 5-aminosalicylic acid (120 mg/kg intrarectally, daily for 7 days), budesonide (0.1 mg/kg intrarectally, daily for 7 days) and curcumin (30 mg/kg i.p, daily for 14 days), on ED50 to Acetyl choline in control rats (A group) and TNBS-colitis rats (B groups) (100 mg/kg in 50% ethanol intrarectally)

Results of the present study revealed that administration of 5-ASA (after TNBS administration) didn't significantly increase the amplitude of spontaneous contraction in this group. Ach produced a dose dependent increase in the amplitude of contraction, however, treatment with this drug failed to reverse the rightward shift in the dose response curve towards normal. On the other hand, there was an increase in the amplitude of spontaneous contractions after L-NNA applications, suggesting that even mild amelioration of tissue inflammation could result in restoration of nitrergic control of the colonic tissue.

The inability of 5-ASA to restore normal motility pattern may be explained by many reasons. First the degree of anti-inflammatory effect of 5-ASA is not sufficient to produce complete healing of the colon as observed in the macroscopic and microscopic examination. Second, the increased level of NO is known to have an inhibitory effect on motility. Lastly, lack of any effect on apoptosis may be another contributing factor to the suppressed motility [12]-[14].

Results of the present study revealed that budesonide and curcumin treatment were associated with a significant

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improvement in spontaneous and Ach-induced contractions, associated with an increase in the spontaneous contractions after L-NNA applications. Similar improvement was previously demonstrated with other drugs that have anti inflammatory effects [14]-[16].

The present study showed that 5-ASA treatment (after TNBS administration) produced incomplete healing of the ulcerated mucosa evident both macroscopically and microscopically. Our results were in consistence with previous studies demonstrating that pre-treating rats with 5-ASA resulted in significantly less inflammation after the induction of colitis, whereas giving this treatment one day after TNBS did not completely ameliorate colitis [7], [25], [26], [27].

Our results also revealed that 5-ASA produced a significant decrease in the level of MPO. This was in consistence with other studies that demonstrate similar effects of 5-ASA on MPO level. [28](Egan LJ 1999). The mechanisms of anti-inflammatory action of 5-ASA are numerous and not entirely understood. It has a potent inhibitory effect on a number of pro-inflammatory mediators released by the mucosa.

Recently, 5-ASA is known to be a ligand for PPAR γ. which plays an important role in the maintenance of mucosal integrity in the intestine. Evidence also indicates that PPAR-γ negatively interferes with the NF-kB and AP-1 signaling pathways, and this mechanism is thought to be its primary mode of anti-inflammatory action [28], [29].

In the present study there was a non significant decrease of the mean value of NO level in the colon in the group that received 5-ASA after TNBS administration compared to the TNBS-control group. Previous studies showed controversial effects of 5-ASA on NO level. Sandoval et al; demonstrated that mesalamine has no effect on NO levels. Alternatively, another study found that 5-ASA can decrease the expression of iNOS. This effect may be attributed to its effect on PPAR γ which can inhibit the production of nitric oxide, by decreasing the expression of iNOS [30]. However this effect appeared only in an in vitro study [31].

The present study also showed that the rate of apoptosis, as assessed by caspase-3 level, was not affected by 5-ASA treatment (with or without TNBS administration) compared to their respective control groups. Rust et al; reported that sulfasalazine, but not its metabolites 5-ASA and sulfapyridine, is a potent inhibitor of bile acid induced hepatocyte apoptosis in vitro and in the intact liver, with significant reduction of caspases 9 and 3 [32].

Effect of budesonide treatment:

Our results revealed that treatment with budesonide (after TNBS administration) led to a significant improvement of the macroscopic and microscopic pictures. Our results also revealed a decrease in MPO level in the same group. These results are in accordance with previous studies that demonstrate the beneficial anti-inflammatory effects of corticosteroids in IBD which was largely attributed to their ability to reduce the expression of proinflammatory genes, non-transcriptional activation of eNOS. In addition to the direct effect of glucocorticoids on iNOS expression, induction of iNOS indirectly through inhibition of NF-κB which is known to inhibit many cytokines known to induce iNOS expression [33]-[36].

In the present study, budesonide treatment led to a significant decrease in caspase-3 level in TNBS treated group. The level of caspase-3 was nearly similar before and after TNBS administration. The effect of corticosteroid on the process of apoptosis in different cell types is different. Corticosteroid were shown to induce apoptosis in lymphocytes, on the other hand, corticosteroid had been reported to suppress apoptosis in other cell types e.g. alveolar epithelial cells, hepatoma cells, mammary gland epithelial cells, and in glioma cells [37]-[38]. However, the main mechanism by which corticosteroids regulate apoptosis is poorly understood. Recently, Wang et al; found that dexamethasone inhibit the expressions of NOS and Caspase-3 mRNA in an animal model with lipopolysaccharide (LPS)-induced brain damage [39] (Wang Z 2006). They suggested that the anti-apoptotic effect and neuroprotective effects of dexamethasone may be partially realized by inhibiting the expression of NOS mRNA. So, the inhibition of caspase-3 observed in the present study may be attributed to inhibition of deleterious effect of excessive NO production.

Effect of curcumin treatment:

Interest in curcumin and its promising medicinal value is growing, especially since it does not appear to be significantly toxic [40]. Curcumin is known to exhibit a variety of pharmacological effects including anti-inflammatory, antitumor, and anti-infectious activities [41].

In the present study curcumin treatment was continued for 2 weeks; as previous studies reported that treatment of experimental colitis in rats with curcumin for 1week could not improve the survival rate. [42] The present study revealed that curcumin treatment led to a significant reduction of the macroscopic score index. Histological results revealed good healing of the ulcerated mucosa, with restoration of the general architecture of the colonic mucosal glands, and mild cellular infiltration. Curcumin treatment also was associated with a significant decrease of both MPO and NO levels. Additionally it was associated with a decrease in caspase-3 level. The positive effects of curcumin could be explained by many mechanisms. Curcumin inhibits COX-2 and iNOS expression, inhibits arachidonic acid metabolism, modulates cellular signal pathways, and inhibits certain hormonal, growth factor, and oncogene activities. It is also a potent inducer of cytoprotective heat shock proteins (HSP). Curcumin is also a potent inhibitor of TGF-β and fibrogenesis. Moreover, curcumin can intercept and neutralize active oxygen species including superoxide, hydroxyl radical, in addition to NO and peroxynitrite [43]-[45].

Compared to budesonide, there was no significant difference between budesonide-treated group and curcumin-treated group in all parameters assessed in this study. As compared to 5-ASA, the present study revealed that curcumin was superior to 5-ASA treatment in reducing tissue inflammation as assessed macroscopically and

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microscopically. Reference [46] (Jian et al 2005) reported that treatment with curcumin was similar to sulfasalazine treatment in prevention and treatment of TNBS-induced intestinal inflammation. This discrepancy could be attributed to the time of administration and the duration of sulfasalazine treatment, where in the study of Jian et al, they gave the drugs for 3 days before, and 2 weeks after TNBS administration. Curcumin is reported to inhibit apoptosis in a variety of cell types. The anti-apoptotic mechanisms of curcumin specifically include: inhibition of AP-1, c-Jun N-terminal kinase, Akt, and NF-κB signaling pathways. Specifically, increased activity of NF-κB has been associated with evasion of apoptosis, malignant transformation, sustained cell proliferation, metastasis, and angiogenesis [47], [48]. The inhibitory effect of curcumin on iNOS gene expression provides another mechanism for its anti-apoptotic effect. As activation of the tumor suppressor gene p53 or the DNA repair enzyme poly (ADP-ribose) polymerase (PARP). It is known that activation of p53 or PARP is often associated with apoptotic cell death. These results provide further support for the link between curcumin and inhibition of apoptosis [49]. Although curcumin has bright prospects in the treatment of IBD, randomized controlled clinical investigations in large cohorts of patients are needed to fully evaluate the clinical potential in IBD patients. Clearly, changes in contractility observed *in vitro* cannot be extrapolated directed into the *in vivo* situation. Consideration must be made of the influences of enteric nerves, the interstitial cells of Cajal, and endocrine factors. In addition, the roles of circular and longitudinal muscle differ in terms of their contribution to peristaltic activity. Nevertheless, assessment of *in vitro* muscle contractility is an acceptable and convenient method of assessing how inflammatory and immune cells alter the intestinal motility [24], [50].

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