Original article

Defining the role of CFTR channel blocker and ClC-2 activator in DNBS induced gastrointestinal inflammation

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A R T I C L E   I N F O

A B S T R A C T

In the present study, we have investigated and/or compared the role of glibenclamide, G as cystic fibrosis transmembrane conductance regulator (CFTR) inhibitor, and lubiprostone, L as chloride channel-2 (ClC-2) activator in the 2,4-dinitrobenzene sulfonic acid (DNBS)-induced gastrointestinal inflammation. GI inflammation was induced by intrarectal administration of DNBS. Rats were randomly allocated in 5 groups as sham control, distilled water + DNBS, sulfasalazine (S) + DNBS, G + DNBS, and L + DNBS. All the groups were pre-treated successively for five days before the induction of colitis. One day before and the first four days after DNBS administration various parameters were studied. Later, blood chemistry, colon's gross structure, histology, and the antioxidant load was examined. Pre-treatment with G significantly protected the change induced by DNBS concerning the change in body weight, food intake, diarrhea, occult blood in the feces, wet weight of the colon, and spleen. G because of its anti-inflammatory property down-regulated the neutrophil and WBC count and up-regulated the lymphocyte number. Moreover, G efficiently ameliorates the oxidative stress in the colon and declines the level of myeloperoxidase and malondialdehyde and up-regulated the level of superoxide dismutase and glutathione. Lubiprostone has not shown any promising effects, in fact, it causes an increase in diarrheal frequency. Our findings from this study represent that G has good potential to ameliorate GI inflammation induced by DNBS by its multiple actions including CFTR blockage and reducing the release of inflammatory markers from the MCs, anti-inflammatory and free radical scavenging property.

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1. Introduction

Inflammation is a well-organized process that has a few common properties but different tissue-specific responses. Gastrointestinal (GI) tract inflammation is often related to the damage of the intestinal barrier as it is the first contact place for the attack of pathogens and food antigens (Cucu and Dima, 2011). GI inflammation is associated with ulcerative colitis (UC) and Crohn's disease (CD) collectively called the inflammatory bowel disease (IBD) (Anbazhagan et al., 2018). To tackle the inflammatory process GI tract is armed with lymphoid organs and other complex protective mechanisms. One such mechanism is balancing essential ions and water content across the intestinal epithelium. Thus, the transport function and barriers of GI tract work in harmony to maintain the normal health of the GI tract. The ion-channels are transmembrane pore-forming proteins that regulate the flow of the ions across the cell membrane and are considered as the main modulator of GI barrier function. Ion-channels are activated by various stimuli like voltage across the membrane, stretch, ligand, etc. The ultimate goal of ion-channels and barrier proteins is to enable

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the digestive system to maintain its physical and chemical environment to overcome the effects of the inflammatory processes (Cucu and Dima, 2011).

It's a well-known fact that inflammatory mediators play a crucial role in the pathologic and clinical features of IBD and involvement of mast cells (MCs) is central. In humans and rodents, MCs antigenic response is IgE mediated and flow of the ions (cation and anions) across the MCs membrane plays a key role in the degranulation process. Release of preformed mediators (histamine, heparin, tryptase, chymase) and newly generated autacoids which can produce a variety of cytokines and initiate the acute and chronic inflammatory conditions by cascades of interdependent reactions (Hamilton et al., 2014; Boeckxstaens, 2015). Earlier clinical study shows that colorectal mucosa of IBD patients represents an elevated level of MCs, mast cell tryptase and histamine specifically in the region of lamina propria and submucosa, suggesting MC degranulation is one of the essential processes in the pathogenesis of IBD (Zare-Mirzaie et al., 2012). A vast variety of ion-channels exists on the GI tract and plays many critical functions to maintain homeostasis but the exact role of a few ion-channels is not clearly defined; one such is the chloride channel. Chloride channels represent a relatively under-explored target class for drug discovery and its role in the GI inflammation is not yet fully defined (Verkman and Galietta, 2009). Recent research has shown that ClC-2 is involved in Cl⁻ secretion and also associated with re-structuring of tight junctions within the injured epithelium and has a critical role in the regulation of tight junctions against murine models of intestinal dysfunction but the exact role of this channel is not been studied (Jin and Blikslager, 2015). Lubiprostone is a ClC-2 channel activator that has been approved for the treatment of chronic idiopathic constipation (Black and Ford, 2018).

CFTR (Cystic fibrosis transmembrane conductance regulator) is the product of cystic fibrosis gene which regulate Cl⁻ channel in the apical layer of epithelial secretory cells (Csanády et al., 2019). Although Cl⁻ transport has been generally implicated in the modulation of membrane potential in several cell types and recent studies have shown that Cl⁻ conductance is an important component of MC activation, secretion and degranulation process (Kulka et al., 2002). Now it is well understood that MCs express functional CFTR channels, play a crucial role in the degranulation process and release of mediators. Glibenclamide is one such drug which reversibly interrupts Cl⁻ conduction in the open state of the CFTR channels (Cui et al., 2012).

Based upon above facts we have selected glibenclamide as CFTR blockers and lubiprostone as ClC-2 activator and studied/compared their roles against DNBS induced IBD in Wistar rats by considering various parameters like disease activity index (DAI- food intake, change in the body weight, diarrhea, and occult blood score), gross morphology and histopathology of the colon and biochemical examination.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and lab wares

Sulfasalazine, glibenclamide and lubiprostone were procured from the local market. Biochemical kits were purchased from the Span diagnostics. 2,4-dinitrobenzene sulfonic acid (DNBS) was procured from Santa Cruz, USA supplied by Bio Medical Sciences Est. Saudi Arabia. All the chemicals used in this study are of AR grades. Tomos scientific cooling centrifuge (5427 R), Labomed Inc LB271 trinocular microscope, Shimadzu UV visible Spectrophotometer (UV1800), rotary microtome- Slee Germany, and, Stat Fax autoanalyzer (2000), digital balance (ER 180A) were used from our lab facilities.

2.1.2. Experimental animals

Thirty male Wistar rats with a mean age of 4 months and the bodyweight in-between 200 and 220 g were used in this study. Before starting the experiments, formal permission was obtained from the Institutional Animal Ethical Committee (IAEC) and National Committee of Bio Ethics (NCEB) guidelines were strictly followed in the study.

Animals were obtained from the central animal house facility at Northern Border University, under biosafety level 2. After procuring, the animals were acclimatized for the next 10 days in our laboratory conditions of constant temperature (21 ± 1 °C) and relative humidity of 50–55%, 12:12 Hr light/dark cycle, *ad libitum* access to chow diet. Animals showing any signs of illness and distress were replaced by healthy animals. All the experiments were carried out in between 9:00–16:00 hrs.

2.2. Methods

2.2.1. Animals grouping and dose

Animals were divided into 5 groups (n = 6). Group 1 was labelled as sham control (DW); group 2 as disease control (DC) (DW + DNBS); group 3 served as standard (S) (sulfasalazine + DNBS); group 4 and 5 were labelled as test groups, treated with glibenclamide (G) + DNBS; lubiprostone (L) + DNBS respectively.

All the tests and standard drugs were administered per orally (*p.o.*) whereas DNBS was administered intra-colon route.

Dose of the glibenclamide, sulfasalazine and DNBS were referred from the earlier literatures whereas dose of lubiprostone was calculated by the formula derived by Nair and Jacob, and expressed in mg/kg/bw (Nair and Jacob, 2016).

$$Dose of the Animal = \frac{Surface Area of the Animal}{Surface Area of the Human} x Human Dose$$

Rat Surface Area = 0.025 m^2
Human Surface Area = 1.6 m^2

The doses were taken as glibenclamide (0.5 mg/kg, *p.o.*), lubiprostone (0.75mcg, *p.o.*), sulfasalazine (100 mg/kg, *p.o.*) and DNBS (100 mg/kg, intrarectally).

2.2.2. Experimental protocol

Colitis was induced by intrarectal administration of DNBS (100 mg/kg) suspended in 0.5 mL of 50% (v/v) of ethanol (EtOH) in PBS to the entire test and standard groups while the normal control group was injected with equivalent amounts of the 50% (v/v) EtOH solution intrarectally considered as a sham control group, handled in a similar way as like other groups. All the animal groups were pretreated consecutively for 5 days with the respective compounds before DNBS administration.

Animals were fasted (drinking water was supplied) for 48 hrs before the induction of colitis. Rats were anesthetized using a combination of 0.1% ketamine and 0.06% of xylazine by intraperitoneal (*i.p.*) injection. After conforming anesthesia, fecal material was removed by applying a gentle pressure on the lower abdomen. A silicon rubber catheter of 3 mm internal diameter was introduced into the colon via the rectum and pushed 8 cm proximal to the anus, and DNBS (prepared as described above) was injected (only once) by using a tuberculin syringe. Later, animals were injected with normal saline (5% body weight, *s.c.*) for the next 5 days to avoid dehydration. Subsequently, animals were kept in trendelenburg position until the recovery from anesthesia (Morampudi et al., 2014) (Figs. 1a and 1b).
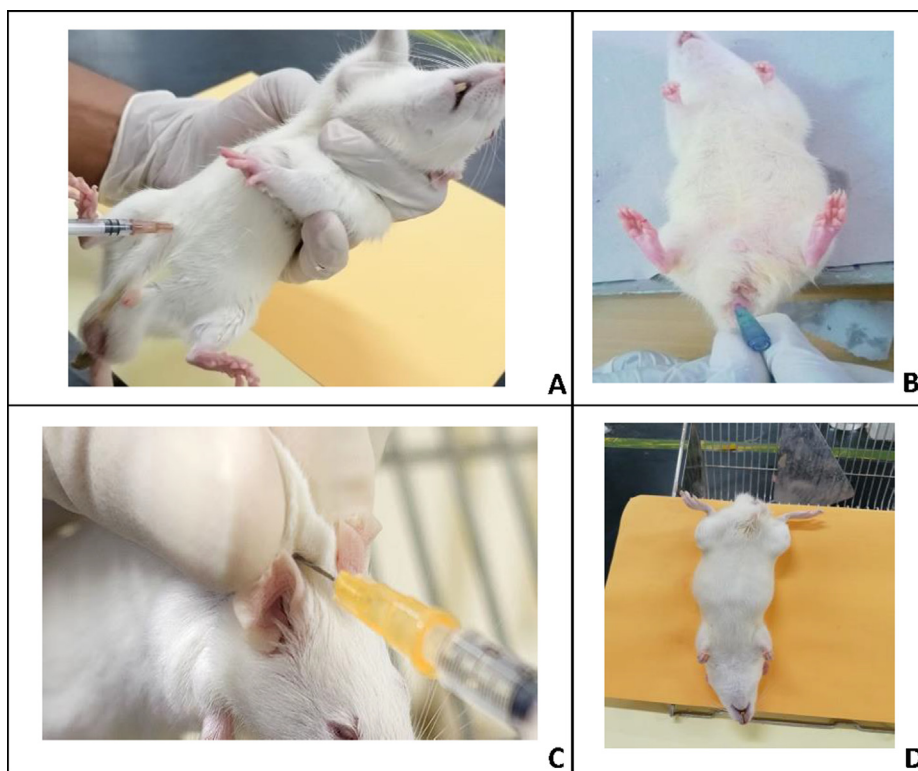
Fig. 1a. Induction of DNBS induced gastrointestinal inflammation. A. Rats were anesthetized by the cocktail ketamine + xylazine, *i.p.* injection. B. The DNBS solution/control solution administration by using a tuberculin syringe and a flexible plastic tube and inserted 8 cm into the colon. C. After DNBS administration, all rats received injected with normal saline (5% body weight, *s.c.*) to prevent dehydration. D. Trendelenburg position of rats till the recovery from anaesthesia.

Animals were kept under observation period for the next four days and no drugs were administered during this period. During this period, as a part of disease activity index (DAI) a few parameters were recorded like food consumption, recording of body weight, frequency of stool passing, consistency of fecal material, and blood in the feces. On day 5, all the animals were sacrificed by cervical dislocation, a mid-line incision was made and colon was taken out, cleaned, and washed with the PBS and wet weight of the colon and spleen was recorded. Later, the colon was opened longitudinally by cutting through the anti-mesenteric border, pictures were captured (gross morphology study), and stored in the 10% formalin solution for biochemical and histopathological studies.

2.2.3. Recording of Disease activity index (DAI)

All the parameters of DAI were caried out for 5 times i.e., before DNBS administration (day 0) and for the next 4 days.

2.2.3.1. Animal weight. Animal weight was recorded in all the groups before DNBS administration (considered as day 0) and for the next 4 days at 9.00 hrs. To get the exact weight food was deprived in all animals 2 hrs before weighing.

2.2.3.2. Food intake. Food consumption study was undertaken as per the method prescribed by Chidrawar, 2012 (Chidrawar et al., 2012). Food spillage was considered as 0.1 g with correction and quantity of food consumed per 20 ± 3 gm of the body weight.

2.2.3.3. Study on stool. Diarrhea, stool consistency and presence of gross bleeding or occult blood in feces were also recorded and severity was scored from 0 to 4. Occulted blood in feces was determined as per the method prescribed by Borges et al. 2018 (Borges et al., 2018).

2.2.4. Hematological study

Briefly, 48 hrs after DNBS administration (i.e., 8th day of the study) blood was withdrawn (1% of bodyweight only) through the orbital venous sinus puncture under the cocktail of ketamine (75 mg/kg) + xylazine 10 mg/kg), *i.p.* anesthesia and collected in pre-labeled heparinized tubes. The RBC, WBC, neutrophil, and lymphocyte count was carried by using Hematology Analyzer, Diacell, (Diacell AD 690).

2.2.5. Biochemical analysis

Biochemical analysis was carried out on the 5th day after the DNBS administration in all groups. Colon was homogenized in chilled phosphate buffer (pH 7.4) and centrifuged at 800 rpm for 5 min at 4 °C (REMI C-24) to separate the molecular debris. The supernatant was separated and centrifuged at 10000 rpm for 20 min at 4 °C (REMI CM-12) to get the post-mitochondrial supernatant (PMS). PMS was used to assay myeloperoxidase (MPO), malonaldehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) levels.

2.2.5.1. MPO activity. MPO, a marker of neutrophil migration was estimated by measuring H_2O_2 dependent oxidation of O-dianisidine. The mucosal tissue (n = 6) from the entire experimental group was isolated, homogenized and PMS was prepared as described above. The supernatant was collected and assayed for the MPO activity by the colorimetric method as described by Souza et al. 2003 (Souza et al., 2003).

2.2.5.2. MDA level. MDA levels were measured as a marker of lipid peroxidation status in all animals. In a test tube, 1 mL supernatant of the tissue homogenate sample was mixed with 0.2 mL of 4% w/v of sodium dodecyl sulfate, 1.5 mL 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 1.5 mL of 0.8% thiobarbituric acid
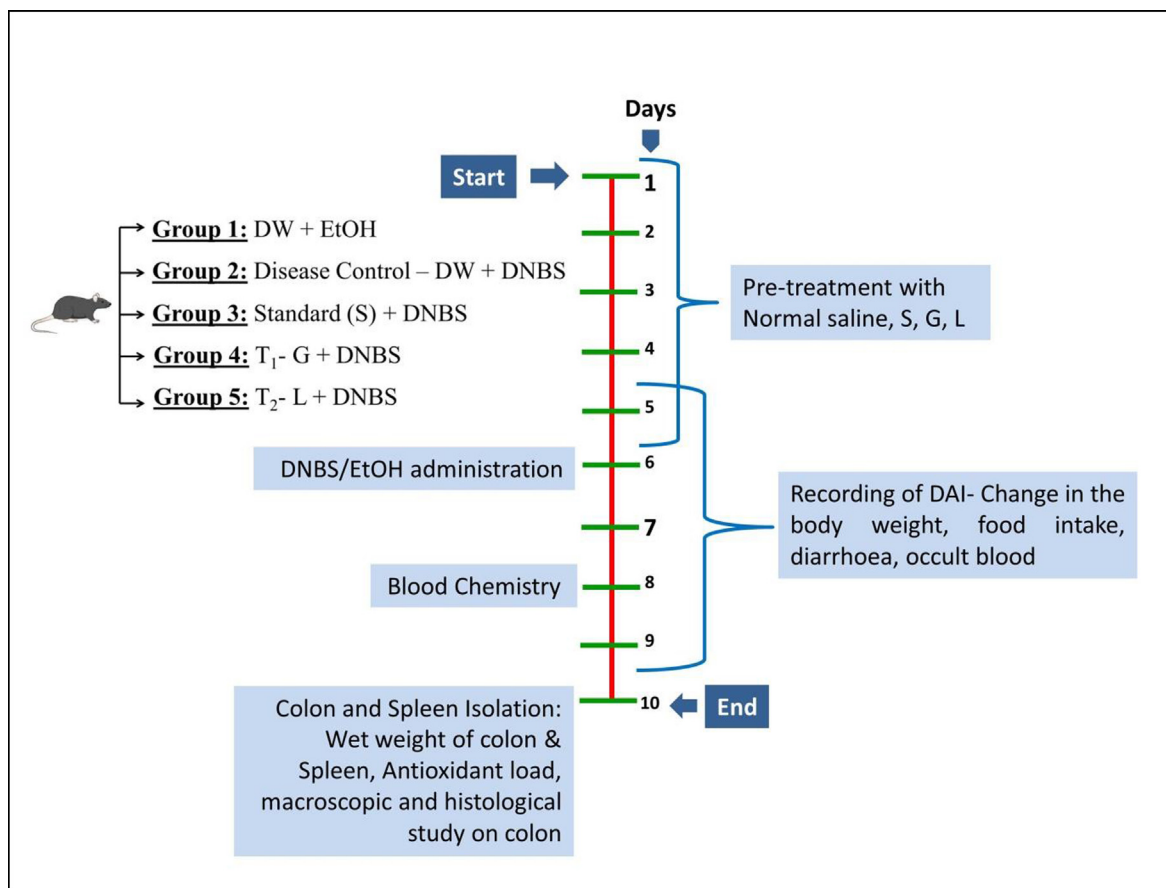
Fig. 1b. Study flow chart- animal grouping and time-line.

(TBA) was added in the same tube. The tube was heated in a water bath at 85 °C for 60 min. A pick color was developed which was read against a blank at 532 nm. Malondialdehyde was calculated using a standard curve and reported as a microgram of MDA/mL (Ohkawa et al., 1979).

2.2.5.3. GSH level. 0.75 mL of 4% sulfosalicylic acid was added to the equal amount of PMS and centrifuged at 1200 xg for 5 min at 4 °C (REDMI CM-12). From this mixture, 0.5 mL of supernatant was added to the 4.5 mL of 0.01 mol/L 5,5′-dithiobis-(2- nitrobenzoic acid). Absorbance was measured at 412 nm by using a UV–vis spectrophotometer (Tipple and Rogers, 2012).

2.2.5.4. SOD level. The assay was carried out as per the method prescribed by Misra and Fridovich. 200 µL of the lysate, 2.5 mL of buffer, 30 mM EDTA and 300 µL of 2 mM of pyrogallol was added in the PMS. An increase in the absorbance was recorded at 420 nm for 3 min by spectrophotometer. One unit of enzyme activity is equivalent to 50% inhibition of the rate of auto-oxidation pyrogallol as determined by the change in the absorbance/min, expresses in U/mg protein (Misra and Fridovich, 1972).

2.2.6. Macroscopic changes, wet weight of colon and spleen
On the 5th day after the DNBS administration spleen and colon was isolated and wet weight was recorded by using 0.01 mg analytical balance. Macroscopic changes on the luminal portion of the colon were recorded by opening the colon longitudinally and mildly cleaned with PBS to removes waste debris. Arbitrary scale ranging from 0 to 5 was assigned based upon the severity of the damage (Table 1) (Olamilosoye et al., 2018).

Table 1
Severity of damage (Morphological study- scaled from 0 to 5).

Score	Macroscopic changes
0	No observable changes
1	Presence of mucosal erythema only
2	Minor bleeding, mucosal edema but no erosions
3	Modest (active) bleeding, ulcers &/or erosions and edema
4	Reasonable tissue necrosis, edema and erosions
5	Severe erosions &/or perforation, necrosis and edema

2.2.7. Histopathological score of colons
Briefly, a 10 cm portion of the distal colon which was earlier stored in the formaldehyde was embedded in the paraffin and 5 µm sections were prepared by using a microtome. Later, tissues were stained with hematoxylin and eosin and were checked under Labomed Inc LB271 trinocular microscope at 100X magnification. The severity of damage and anatomical integrity of the colon was scaled from 0 to 3 by two independent observers to avoid any errors (Table 2). Various microscopic parameters like loss of epithelium, damage of crypts, and depletion of goblet cell and neu-

Table 2
Severity of damage (histopathological study- scaled from 0 to 3).

Score	Microscopic changes
0	Normal appearance and no observable changes
1	Inflammation on mucosa and submucosa
2	One side inflammation on the entire wall of the bowel
3	Linear ulceration and necrosis on both side of the wall

trophil infiltration of inflammatory cells were recorded from the histopathological slides (El-Akabawy and El-Sherif, 2019).

2.3. Statistical analysis

All the data was tabulated and expressed as mean ± SEM. Data was compared for statistical differences in between the groups by one-way analysis of variance and Dunnett's multiple range tests was implemented for post hoc analysis. Data was considered statistically significant if the p value is < to 0.05 and highly significant at $p < 0.001$. Data was analyzed by using SPSS version 24.

* Sham control vs Disease control (DC), Standard (S), Test 1 (G) and Test 2 (L)

^ Disease control (DC) vs Standard (S) and Test groups Test 1 (G) and Test 2 (L)

EndNote[x9] version was used for inserting references.

3. Results

3.1. Effects of drug/s treatments on changes in body weight

A decrease in the body weight was observed in all the treatment groups including the standard and test groups. DC group showed significant ($p < 0.01$) weight loss as revealed by a higher reduction in body weight (-12.92%) compared to normal control (1.31%). Pretreatment with glibenclamide (-1.65%) and sulfasalazine (-6.78%) has shown a significant ($p < 0.01$; $p < 0.05$) attenuation declining in the body weight compared to the DC (Fig. 2).

3.2. Food consumption

Food consumption was reduced in all 4 days (day 1 to 4) after DNBS administration in DC group compared to the sham control group, significantly on day 1 ($p < 0.00$), day 2, ($p < 0.01$) and day 4 ($p < 0.001$) of the study. Glibenclamide treated group has shown a modest increase in the food intake on day 2 ($p < 0.01$), day 3, and day 4 ($p < 0.01$) compared to the DC group. Treatment with sulfasalazine also protected the decrease in the food consumption on day 2 ($p < 0.01$) 3 and 4 compared to the DC group but food intake was not more or equal to the sham control group (Fig. 3).

3.3. Effect on diarrhea

The frequency of stool passing and its consistency was recorded on all 4 days after DNBS administration. After DNBS treatment there was significant (day 1 $p < 0.001$; day 2 $p < 0.01$; day 3 $p < 0.01$ and day 4 $p < 0.001$) increase in diarrhea in DC group compared to sham control on all 4 days. Diarrhea was more significant in the lubiprostone treated group and scores were very similar to the plain DNBS treated group. By the treatment with glibenclamide, there was a significant ($p < 0.001$) decrease in the passing of the stools on days 2, 3, and 4 compared to DC group (Fig. 4).

3.4. Mean occluded blood score

Occluded blood was scored from arbitrary scale 0–4 based upon the severity. The score was significantly ($p < 0.001$) high in DC group on day 1 compared to group 1. Later, the score was reduced gradually on days 2, 3, and 4 compared to day 1. Glibenclamide treatment has significantly protected diarrhea induced DNBS on days 2 ($p < 0.05$) and day 4 ($p < 0.01$ respectively (Fig. 5).

3.5. Hematological study

Plain DNBS treated group has represented a significant increase in WBC ($p < 0.001$) and neutrophil ($p < 0.01$) count whereas, there was significant ($p < 0.001$) decline in lymphocyte count compared to the sham control group. These changes were protected by the pre-treatment with glibenclamide compared to group 2. There was no noticeable effect were observed concerning RBC count by any of the treatment groups (Fig. 6).

3.6. Biomarkers study

Remarkable changes in the biochemical parameters were induced by the DNBS. Group 2 has shown significant incline in MPO ($p < 0.001$) and MDA ($p < 0.01$) levels while decline in SOD ($p < 0.01$) and GSH ($p < 0.01$) levels compared to the group 1.

Sulfasalazine has significantly ameliorated the changes induced by DNBS in all four biomarkers (MPO, $p < 0.001$; MDA, $p < 0.01$; GSH, $p < 0.001$; SOD, $p < 0.01$). Pretreatment with glibenclamide
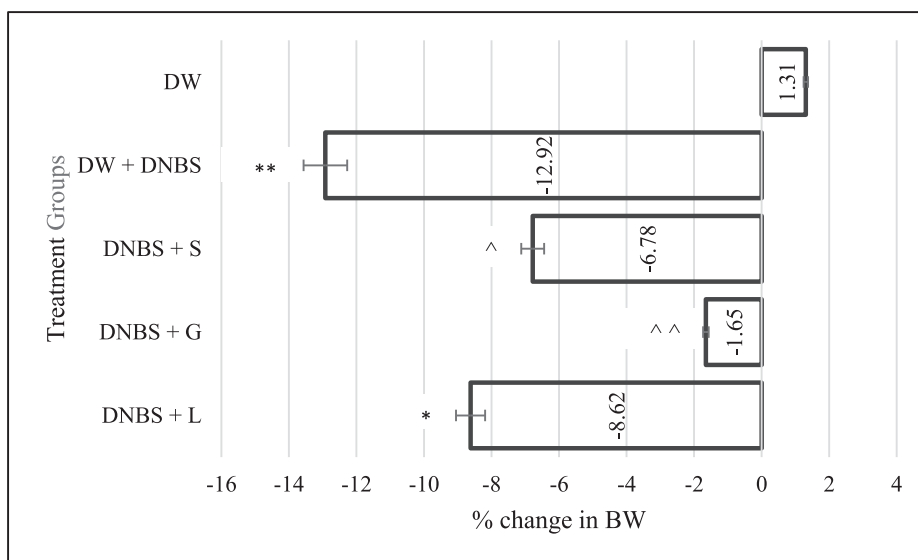
Fig. 2. Effect of CFTR blocker and ClC-2 activator on % change in body weight against DNBS induced GI inflammation in male Wistar rats. Data is presented as mean ± SEM; n = 6; *p < 0.05, **p < 0.01, ***P < 0.001 Sham control vs Disease control (DC), Standard (S), Test 1 (G) and Test 2 (L). ^p < 0.05, ^^p < 0.01, ^^^P < 0.001 Disease control (DC) vs Standard (S) and Test groups Test 1 (G) and Test 2 (L).
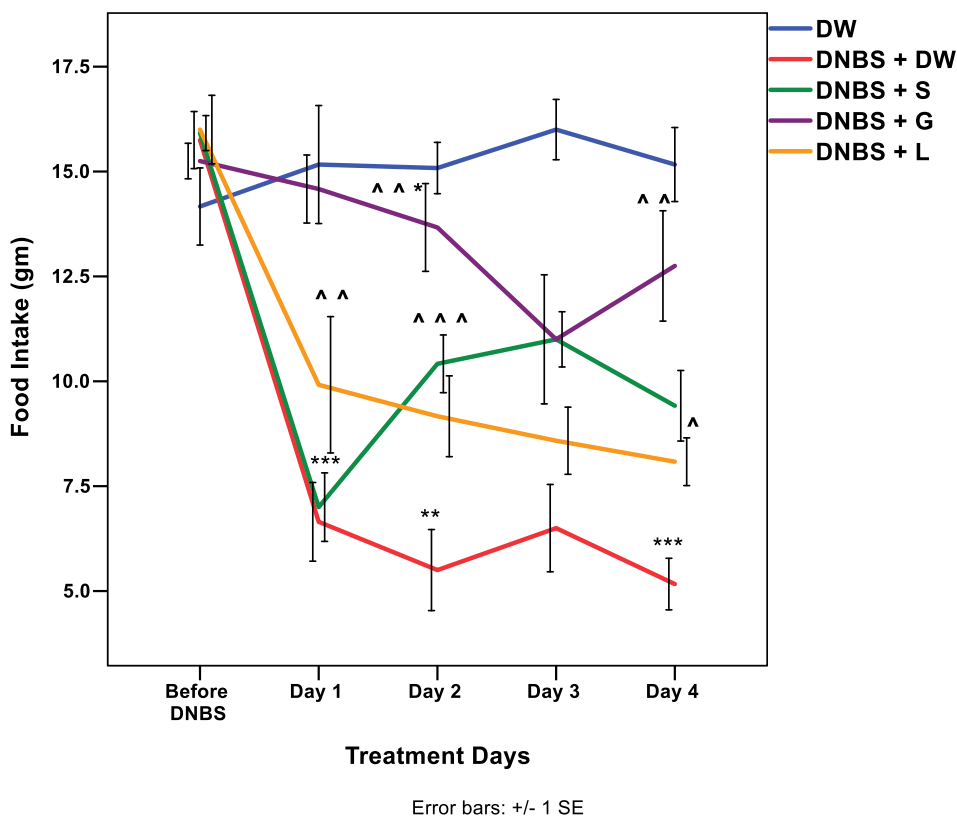
Fig. 3. Effect of CFTR blocker and ClC-2 activator on food intake against DNBS induced GI inflammation in male Wistar rats. Data is presented as mean ± SEM; n = 6; *p < 0.05, **p < 0.01, ***P < 0.001 Sham control vs Disease control (DC), Standard (S), Test 1 (G) and Test 2 (L) ^p < 0.05, ^^p < 0.01, ^^^P < 0.001 Disease control (DC) vs Standard (S) and Test groups Test 1 (G) and Test 2 (L).
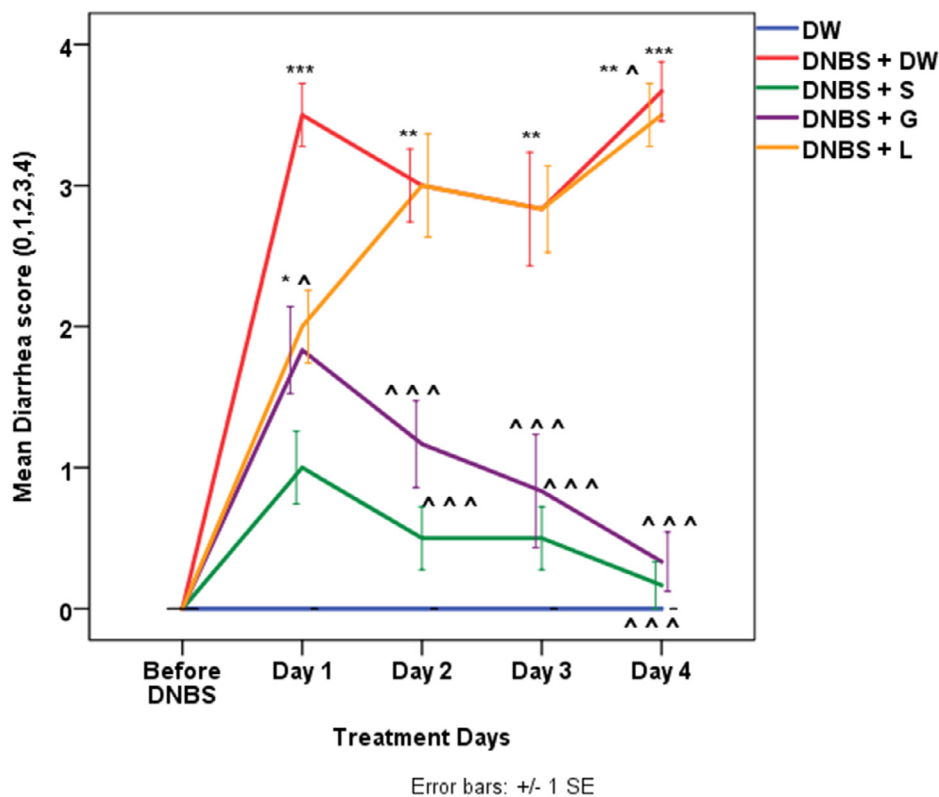

Fig. 4. Effect of CFTR blocker and ClC-2 activator on mean diarrhea score against DNBS induced GI inflammation in male Wistar rats. Data is presented as mean ± SEM; n = 6; *p < 0.05, **p < 0.01, ***P < 0.001 Sham control vs Disease control (DC), Standard (S), Test 1 (G) and Test 2 (L). ^p < 0.05, ^^p < 0.01, ^^^P < 0.001 Disease control (DC) vs Standard (S) and Test groups Test 1 (G) and Test 2 (L).
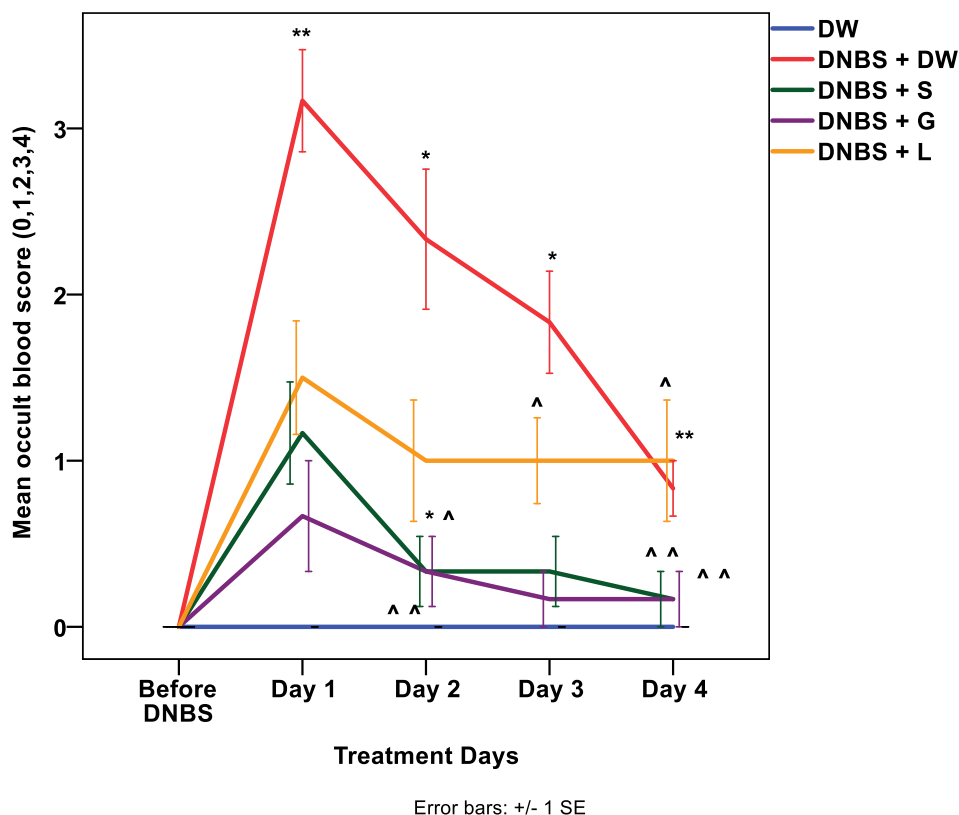
Fig. 5. Effect of CFTR blocker and ClC-2 activator on mean occult blood score against DNBS induced GI inflammation in male Wistar rats. Data is presented as mean ± SEM; n = 6; *p < 0.05, **p < 0.01, ***P < 0.001 Sham control vs Disease control (DC), Standard (S), Test 1 (G) and Test 2 (L). ^p < 0.05, ^^p < 0.01, ^^^P < 0.001 Disease control (DC) vs Standard (S) and Test groups Test 1 (G) and Test 2 (L).

has also shown promising results by revering the changes induced by the DNBS in all 4 biochemical parameters. (MPO, p < 0.01; MDA, p < 0.05; GSH, p < 0.01; SOD, p < 0.001). Lubiprostone also offered mild protection but the data was not significant compared to the glibenclamide and sulfasalazine treated groups (Fig. 7).

3.7. Gross morphology of colon

The gross macroscopic score was significantly (p < 0.001) high in plain DNBS treated group. Damage produced by the DNBS was significantly protected by glibenclamide compared to the DC group (p < 0.01) but the score was higher than the sham control group (p < 0.01). There was partial but significant protection offered by the lubiprostone (p < 0.05) compared to the DC group (Figs. 8A and 8B).

3.8. Mean wet weight of colon and spleen

Wet weigh of colon and spleen was significantly (p < 0.01) high in the DC group compared to sham control. Pre-treatment with glibenclamide (p < 0.01) and lubiprostone (p < 0.05) the colon and spleen weight was significantly reduced compared to the DC group. Sulfasalazine has shown most promising results; significantly (p < 0.001) reduced the wet weight of colon and spleen (Fig. 9).

3.9. Histopathological features of colon

Patchy loss of epithelium (p < 0.01), crypt damage (p < 0.001), depletion of goblet (p < 0.01) cells and neutrophil infiltration

(p < 0.01) was observed in DC compared to group 1. Treatment with glibenclamide and sulfasalazine significantly attenuated the extent and severity of the histological signs at various extend. In fact, glibenclamide has offered better protection compared to standard sulfasalazine treated group, while the effect of lubiprostone was modest (Figs. 10A and 10B).

4. Discussion

Treatment of GI inflammation including CD and UC remains an unsolved question because of its overlapping clinical symptoms and unknown etiology (Bray et al., 2016). Many treatment strategies were implemented but there is no satisfactory outcome. The drugs like 5-aminosalicylates includes mesalamine, sulfasalazine, and balsalazide are in practice to keep the patients in the remission phase but unfortunately, these drugs are partially effective only in mild to moderate cases (Bray et al., 2016).

It has been known from decades that ion-channels plays a vital role to maintain the homeostasis by controlling many physiological functions including nerve depolarization, muscle contraction regulation of heart rhythm, etc (Strickland et al., 2019). A handful of drugs are in clinical use which acts by modulating the ion-channel function. Compared to cationic channels anionic channels are relatively under-explored target class for drug discovery and chloride channels are one of them (Verkman and Galietta, 2009). ClC are involved in numerous physiological functions including regulation of cell volume, neuronal excitation, fluid secretion, contraction of smooth muscles, etc. CFTR channels were identified on the MCs and ClC-2 (a type of voltage-gated chloride channel) studied to be involved in Cl⁻ secretion and also associated with re-
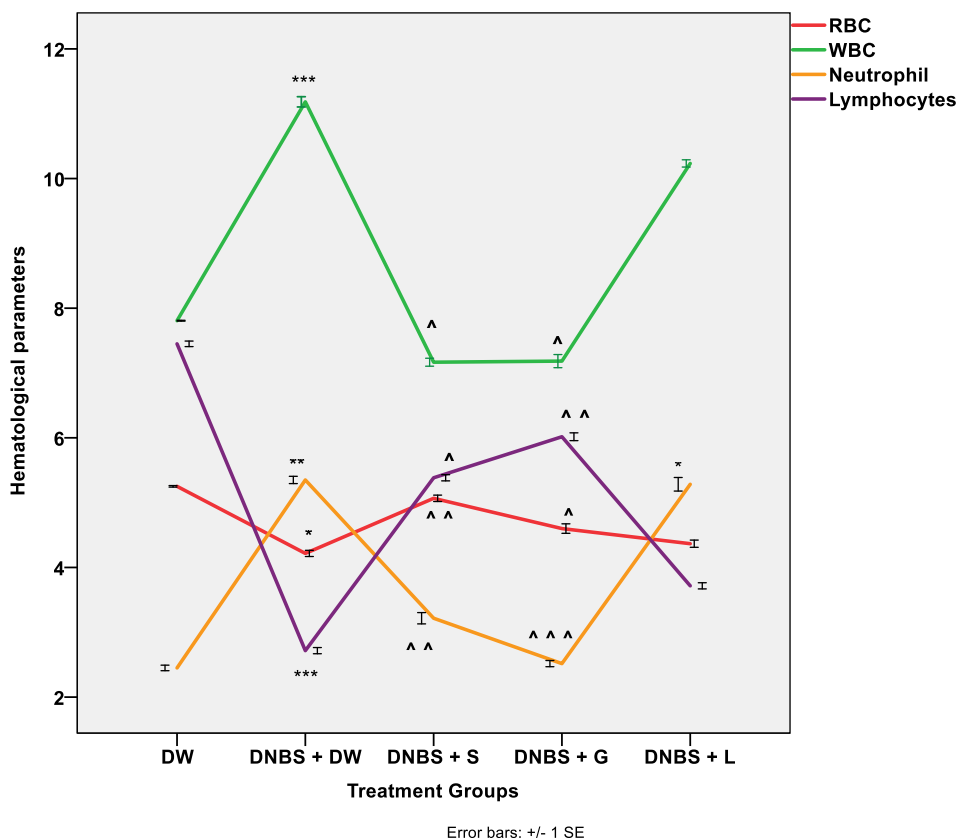
Fig. 6. Effect of CFTR blocker and ClC-2 activator on hematological parameters against DNBS induced GI inflammation in male Wistar rats. Data is presented as mean ± SEM; n = 6; *p < 0.05, **p < 0.01, ***P < 0.001 Sham control vs Disease control (DC), Standard (S), Test 1 (G) and Test 2 (L). ^p < 0.05, ^^p < 0.01, ^^^P < 0.001 Disease control (DC) vs Standard (S) and Test groups Test 1 (G) and Test 2 (L).

structuring of tight junctions within injured epithelium but the exact role of these channels in IBD is not been established (Kulka et al, 2002; Jin & Blikslager, 2015).

DNBS induced UC is chemically used *in vivo* model, most commonly used in rodents which recreate the morphological, histopathological, and clinical features of human IBD (Barone et al., 2018).

In the present study, DNBS induced colitis caused up-regulation of DAI (decline in food intake, loss of body weight, an increase in occult blood in the feces, diarrhea) and an increase in the ulcerative areas, wet weight of colon and spleen compared to the sham group. Glibenclamide has ameliorated the DNBS induced DAI changes and many of the parameters considered in the present experiment (Chidrawar, 2016). Lubiprostone also offered considerable protection concerning the DAI but the effects were not as good as glibenclamide. In fact, by the treatment with lubiprostone causes excessive diarrhea comparable to the DC group. Lubiprostone causes activation of ClC-2 channels in the GI epithelium causing an efflux of Cl⁻ ions into the lumen of the intestine causes fluid secretion into the GI lumen which produces softening of stool and increases intestinal transit (Webster et al., 2016).

Its well-known fact that MCs play a key role in systemic anaphylaxis, asthma, IBD, and immunoglobulin E (IgE)-dependent allergic disorders. Activation of MCs leads to the degranulation and release of preformed chemical mediators (Boeckxstaens, 2015; Wouters et al., 2016). MCs express the functional CFTR channels which conduct Cl⁻ ions (Kulka et al., 2002). DNBS acts like a hapten, the immunoglobulin IgE bind to FcεRI on the MCs which triggers the change in the transient receptor potential for which entry of Ca^{2+} ions is essential. An influx of Ca^{2+} is critical for the

fusion of the vesicles with the internal membrane wall of MCs which is partially Cl⁻ion-dependent, requires Cl⁻ efflux, which maintains the driving force for entry of extracellular Ca^{2+} and initiates the release of cytokines, mediators such as histamine (Ashmole and Bradding, 2013). The anti-diabetic drug glibenclamide is known to block CFTR channels, aids blocking of Cl⁻efflux from CFTR channels, and preventing the entry of Ca^{2+} ions and thus MCs degranulation process (Verkman et al., 2013).

DNBS induced changes in the architecture of colon structure were significantly attenuated by the treatment with sulfasalazine and glibenclamide. Glibenclamide significantly down regulated the neutrophil infiltration, affectedly ameliorated histopathological changes towards the healthy side, and represented an anti-inflammatory and anticolitic effect. The above action was supported by the hematological study; glibenclamide has shown a significant decline in WBC and neutrophil count while shot-up the lymphocyte count indicating its anti-inflammatory action (Chukwunonso et al., 2016; Kewcharoenwong et al., 2013). Due to these protective effects, glibenclamide might have prevented excess nutritional and blood loss through diarrhea. Moreover, in the glibenclamide treated group the structure of villi and large goblet cells were intact due to which there might be increased surface area and more rapid absorption of nutrients compared to the disease control group. Because of these possible reasons, there was less weight loss compared to the DC group.

Furthermore, reports about biomarker studies support our findings. MPO is a glycosylated enzyme present in the granules of monocytes and neutrophils. MPO is considered as an index of severity of digestive inflammation (Klebanoff, 2005). In our study MPO level was up-regulated in DC group indicates colonic inflam-
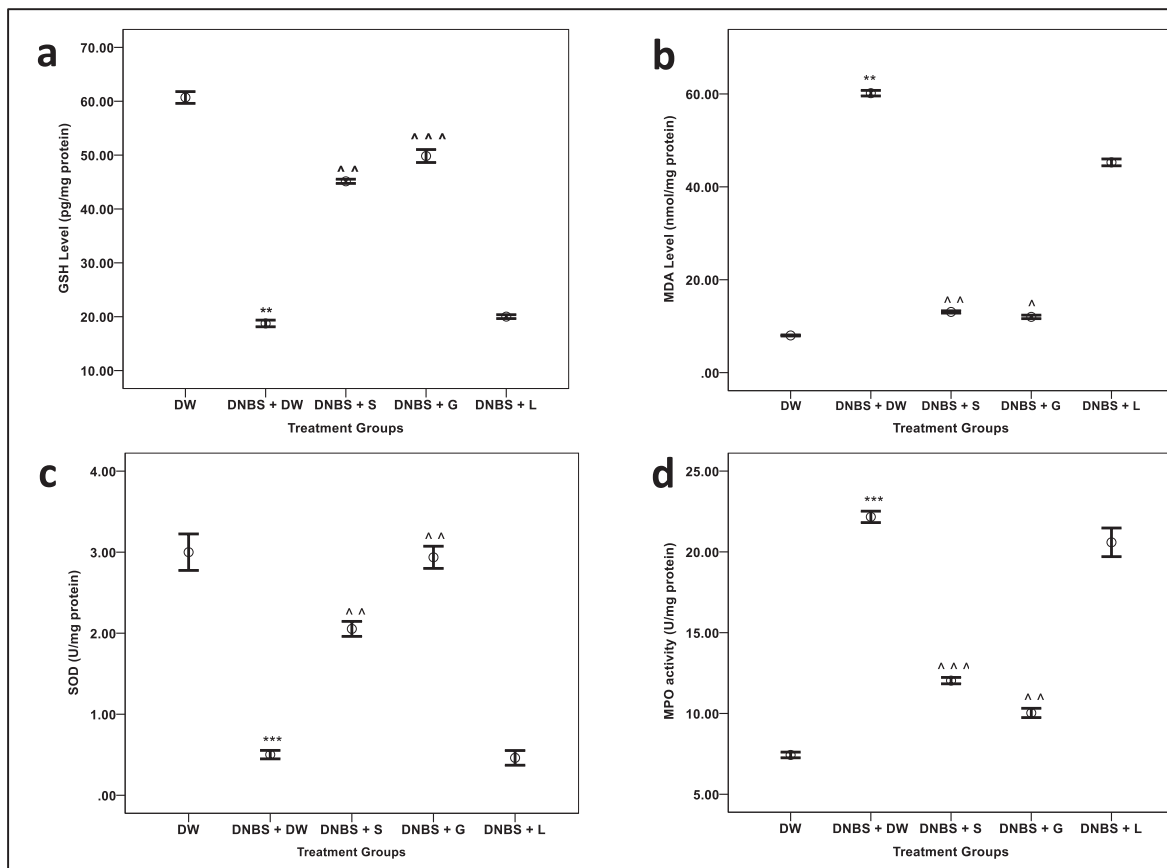
Fig. 7. Effect of CFTR blocker and ClC-2 activator on GSH (Fig. 7a), MDA (Fig. 7b), SOD (Fig. 7c), MPO (Fig. 7d) against DNBS induced GI inflammation in male Wistar rats. Data is presented as mean ± SEM; n = 6; *p < 0.05, **p < 0.01, ***P < 0.001 Sham control vs Disease control (DC), Standard (S), Test 1 (G) and Test 2 (L) ^p < 0.05, ^^p < 0.01, ^^^P < 0.001 Disease control (DC) vs Standard (S) and Test groups Test 1 (G) and Test 2 (L).
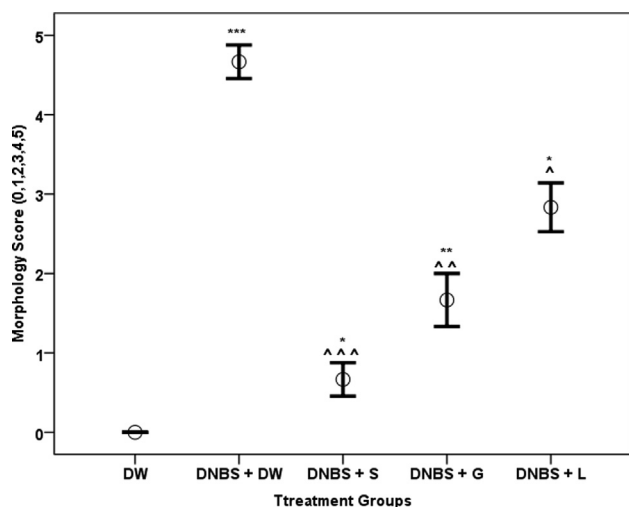

Fig. 8A. Effect of CFTR blocker and ClC-2 activator on mean macroscopic score against DNBS induced GI inflammation in male Wistar rats. Data is presented as mean ± SEM; n = 6; *p < 0.05, **p < 0.01, ***P < 0.001 Sham control vs Disease control (DC), Standard (S), Test 1 (G) and Test 2 (L), ^p < 0.05, ^^p < 0.01, ^^^P < 0.001 Disease control (DC) vs Standard (S) and Test groups Test 1 (G) and Test 2 (L).

mation and damage because of neutrophil infiltration into the colon. The high level of the granulocytes in the colon causes granulocyte induced mucosal damage, disrupts the mucosal barriers,

and propagates an inflammatory response (Mantovani et al., 2001). These effects are significantly ameliorated by the glibenclamide and sulfasalazine treated groups, even the glibenclamide has offered better protection than sulfasalazine but less significantly.

The redox balance is maintained by the SOD, which is abundant in the mitochondria and cytosol (Abreu, 2002; Kandhare et al., 2012). SOD plays an important role by neutralizing highly reactive oxygen free radicals to water and thus preventing damage to the tissue (Erejuwa et al., 2010). In our study, SOD levels were significantly decline in DC group indicating excessive utilization in attenuating the free radicals generated due to DNBS damage. Glibenclamide brought back the SOD to the normal level and restored the oxidative balance in the colonic mucosa.

Mammalians are armed with the free radical scavengers like GSH, involved in the repair of mucosal damage in the gut flora and tackle the inflammation (Chavan et al., 2005). During severe inflammation, the GSH level falls and compromises the defense mechanism, as observed in group 2. However, treatment with glibenclamide up-regulated the colonic tissue GSH level. Treatment with glibenclamide might have neutralized the reactive oxygen species (ROS) level, protected the effect on the antioxidant enzyme, and activated the synthesis of GSH which aided ameliorating the effect of DNBS. Such effects were not been observed in the lubiprostone treated group.

Our findings are in the same line like in the previous study conducted in 2015 by Chukwunonso Obi B and coworkers have revealed the antioxidant potential of glibenclamide in alloxaninduced diabetic rats, in the experiment, they found that gliben-
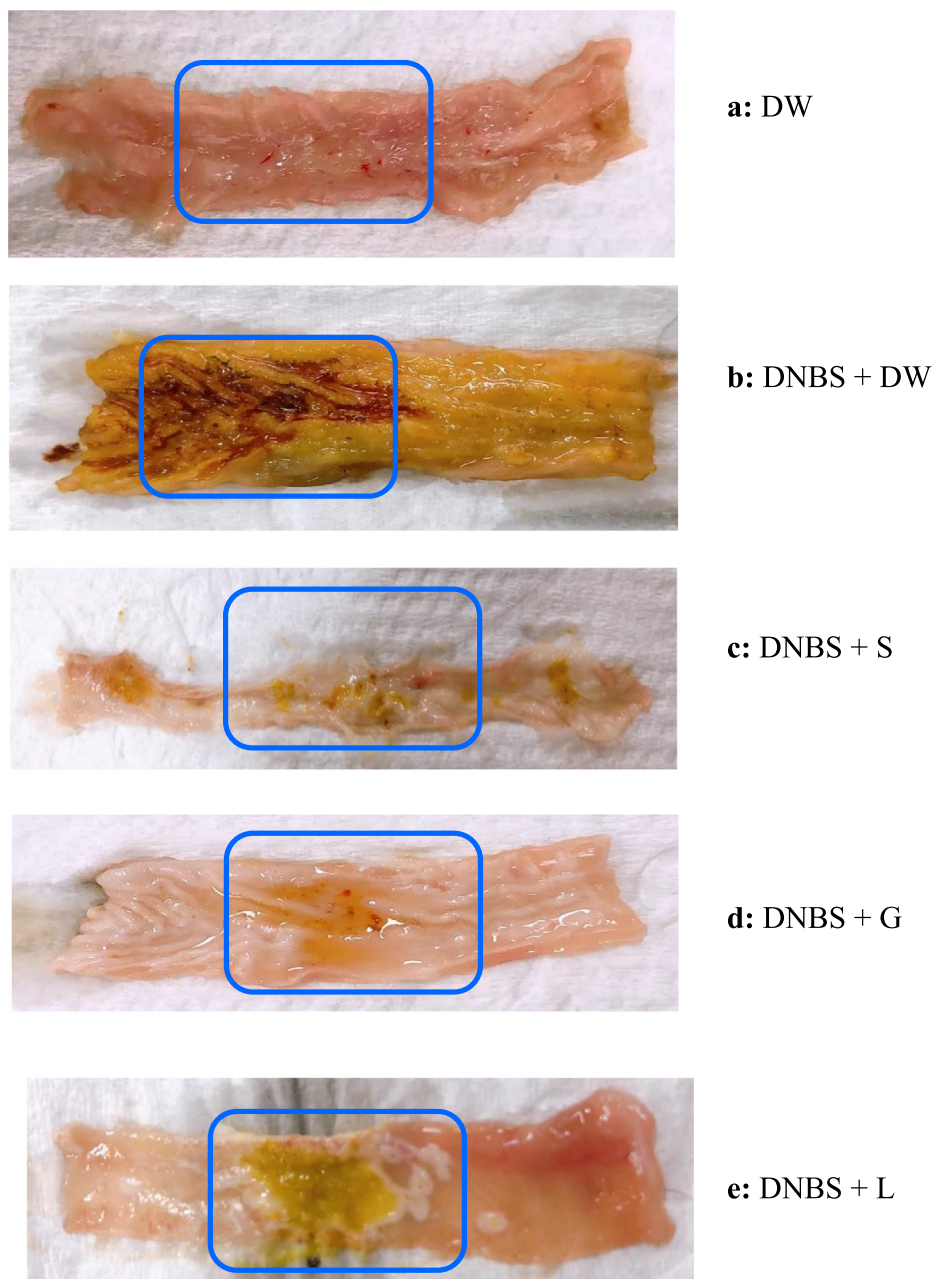
a: DW

b: DNBS + DW

c: DNBS + S

d: DNBS + G

e: DNBS + L

Fig. 8B. Effect of CFTR blocker and ClC-2 activator on macroscopic changes against DNBS induced GI inflammation in male Wistar rats.

clamide declines the MPO and MDA levels whereas elevates the SOD and GSH level (Chukwunonso et al., 2016).

In the present study, glibenclamide has shown promising results against DNBS induced colitis by its multiple actions like blocking the CFTR channels on the MCs, free radical scavenging property, and anti-inflammatory action (Fig. 11). At our laboratory conditions, lubiprostone has not shown any promising effects against DNBS induced colitis compared to the glibenclamide.

There are few shortfalls in the study first, lubiprostone dose reduction might have needed but we have converted the same human dose (recommended for chronic idiopathic constipation) to the animal dose. Second, the estimation of inflammatory mediators study could have been considered which might have supported our findings. Further studies are needed to establish

the dose of glibenclamide for its chronic use to avoid hypoglycemia and *in vitro*, patch-clamp techniques may be implemented to understand the MCs-CFTR channel and glibenclamide interactions.

5. Conclusion

Glibenclamide (as CFTR blocker) treatment effectively ameliorated the oxidative stress, neutrophil infiltration, mast cell degranulation, and histological damage in the DNBS induced colitis while, lubiprostone (CLC-2 activator) has only a modest protective effect. Overall, the results of this study show the usefulness of glibenclamide to prevent DNBS-induced colonic GI inflammation. Glibenclamide will be a good choice for comorbid patients with GI inflammation and DM. Further, in such patients glibenclamide
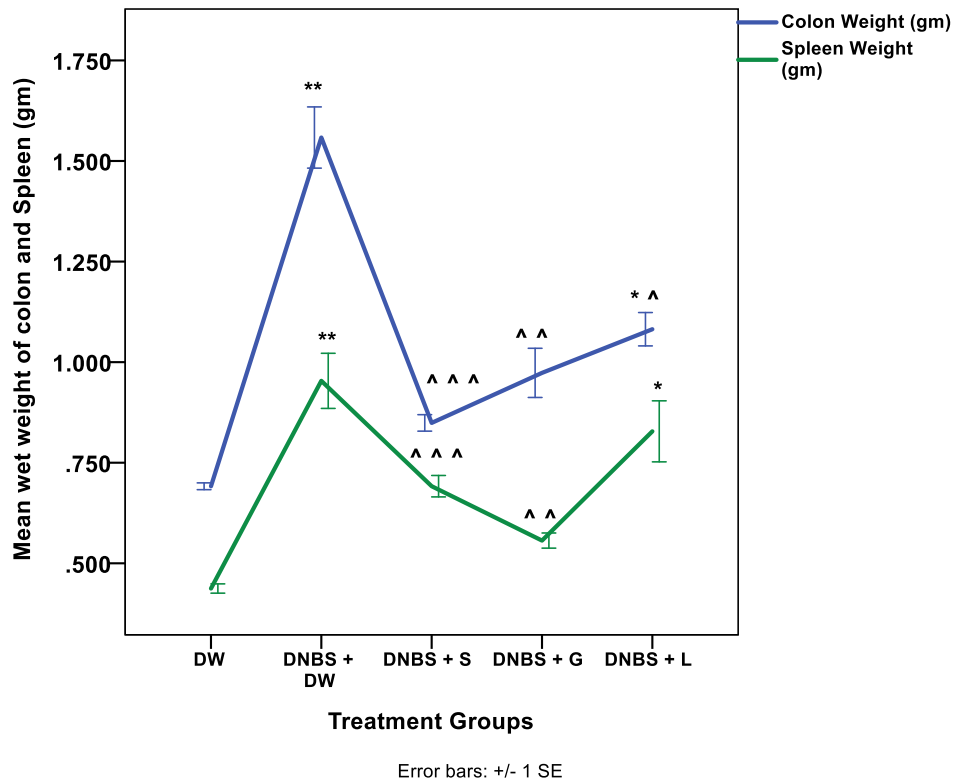
Fig. 9. Effect of CFTR blocker and ClC-2 activator on wet weight (gm) of colon and spleen against DNBS induced GI inflammation in male Wistar rats. Data is presented as mean ± SEM; n = 6; *p < 0.05, **p < 0.01, ***P < 0.001 Sham control vs Disease control (DC), Standard (S), Test 1 (G) and Test 2 (L) ^p < 0.05, ^^p < 0.01, ^^^P < 0.001 Disease control (DC) vs Standard (S) and Test groups Test 1 (G) and Test 2 (L).
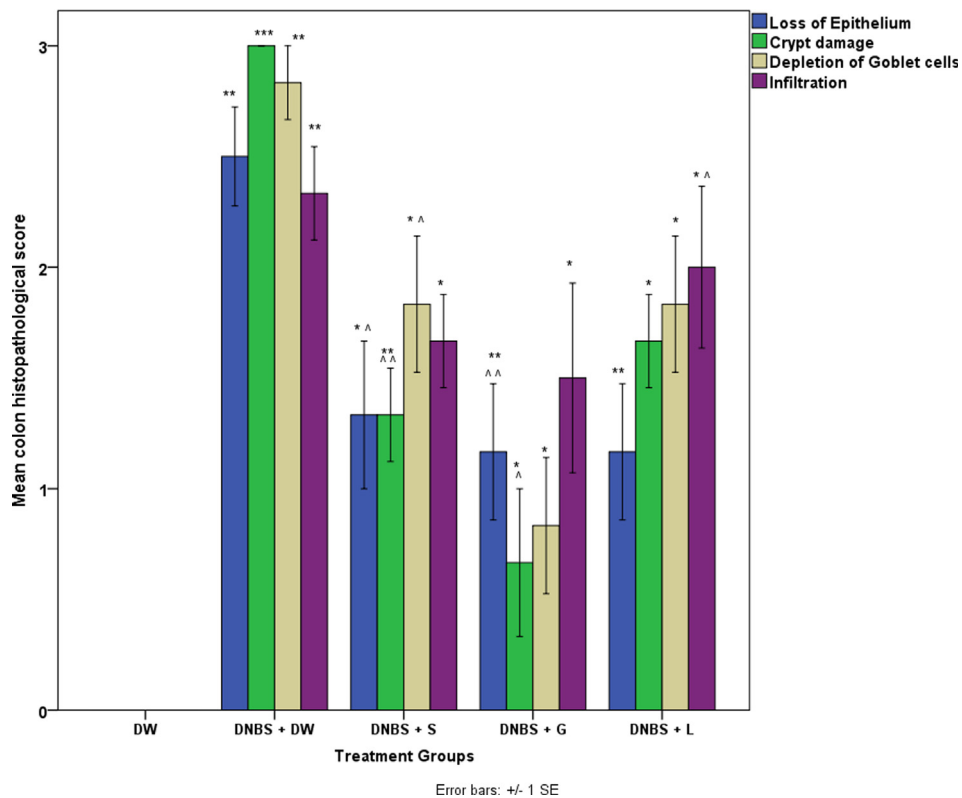

Fig. 10A. Effect of CFTR blocker and ClC-2 activator on mean colon histopathological score against DNBS induced GI inflammation in male Wistar rats. Data is presented as mean ± SEM; n = 6; *p < 0.05, **p < 0.01, ***P < 0.001 Sham control vs Disease control (DC), Standard (S), Test 1 (G) and Test 2 (L) ^p < 0.05, ^^p < 0.01, ^^^P < 0.001 Disease control (DC) vs Standard (S) and Test groups Test 1 (G) and Test 2 (L).
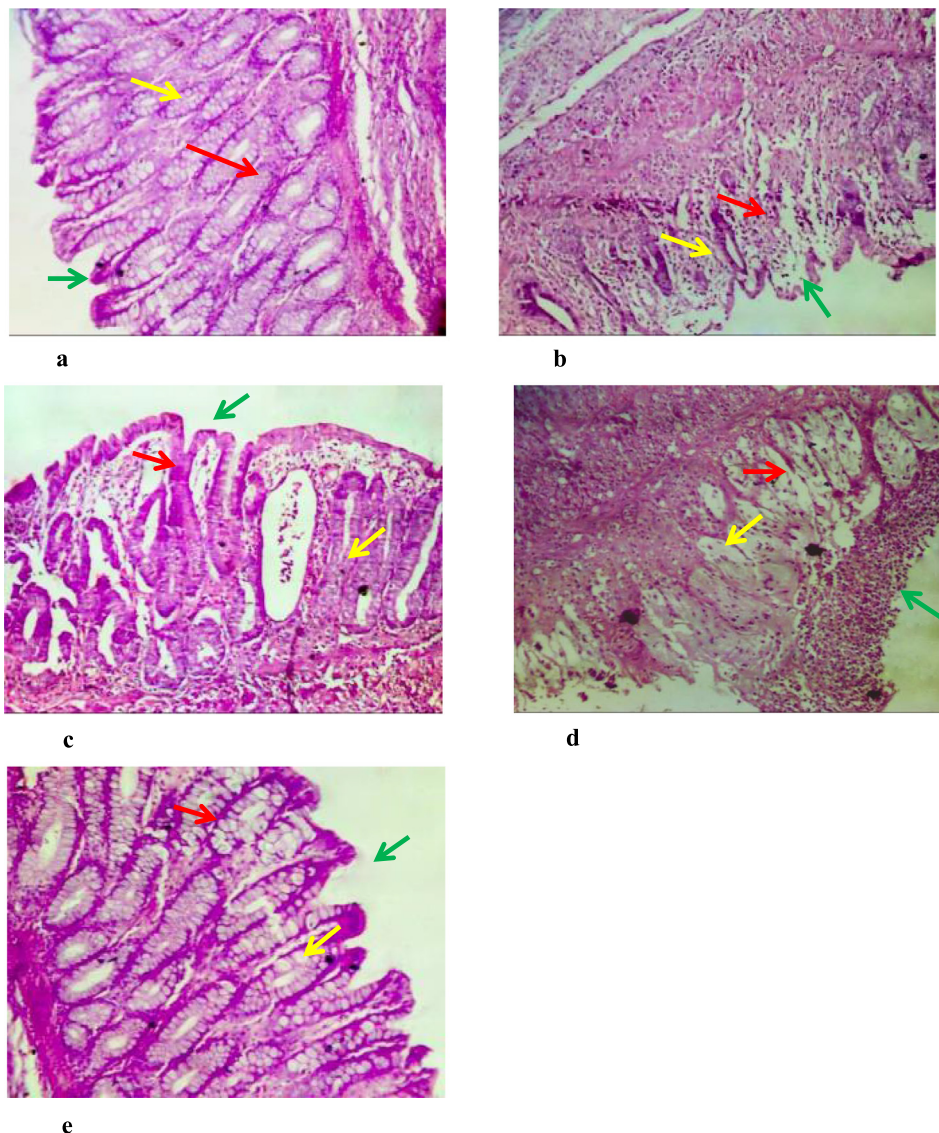
Fig. 10B. Histopathological studies of rat Colon. Stained (H&E) and photographed were captured at 100x magnification. **10B-a** (DW) Undisturbed epithelial cells and mucosal architecture (green arrow), normal crypts (red arrow) and goblet cells (yellow arrow); **10B-b** (DW + DNBS) intracolonic administration of DNBS induced damage to the crypt, disturbed architecture (red arrow) and loss of epithelium in the mucosal layer (green arrow) and goblet cells (yellow arrow); **10B-c** (DW + S) Almost intact epithelium (yellow arrow), crypt structure (red arrow) and goblet cells (yellow arrow) **10B-d** (DW + G) Normal architecture of mucosal and epithelial cells (green arrow), uninterrupted goblet cells (yellow arrow) and crypts (red arrow); **10B-e** (DW + L) Ulceration, severe injury to the epithelium (green arrow), loss of goblet cells (yellow arrow) and crypts (red arrow).

mono-therapy will reduce the cost of the therapy and avoid potential drug interactions due to poly-pharmacy.

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Ethical approval

The present project is approved by IAEC: Institutional Animal Ethical Committee, Faculty of Pharmacy- Northern Border University, Saudi Arabia.

Author(S) contribution

Both the authors have contributed equally for this research. VC: conceptualization, methodology, data acquisition and writing the original draft preparation. BA: investigation, supervision, participated in the experimental procedures, writing and editing the final draft of the manuscript. Both the authors have critically revived and agree the final draft and are accountable for the content and plagiarism of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
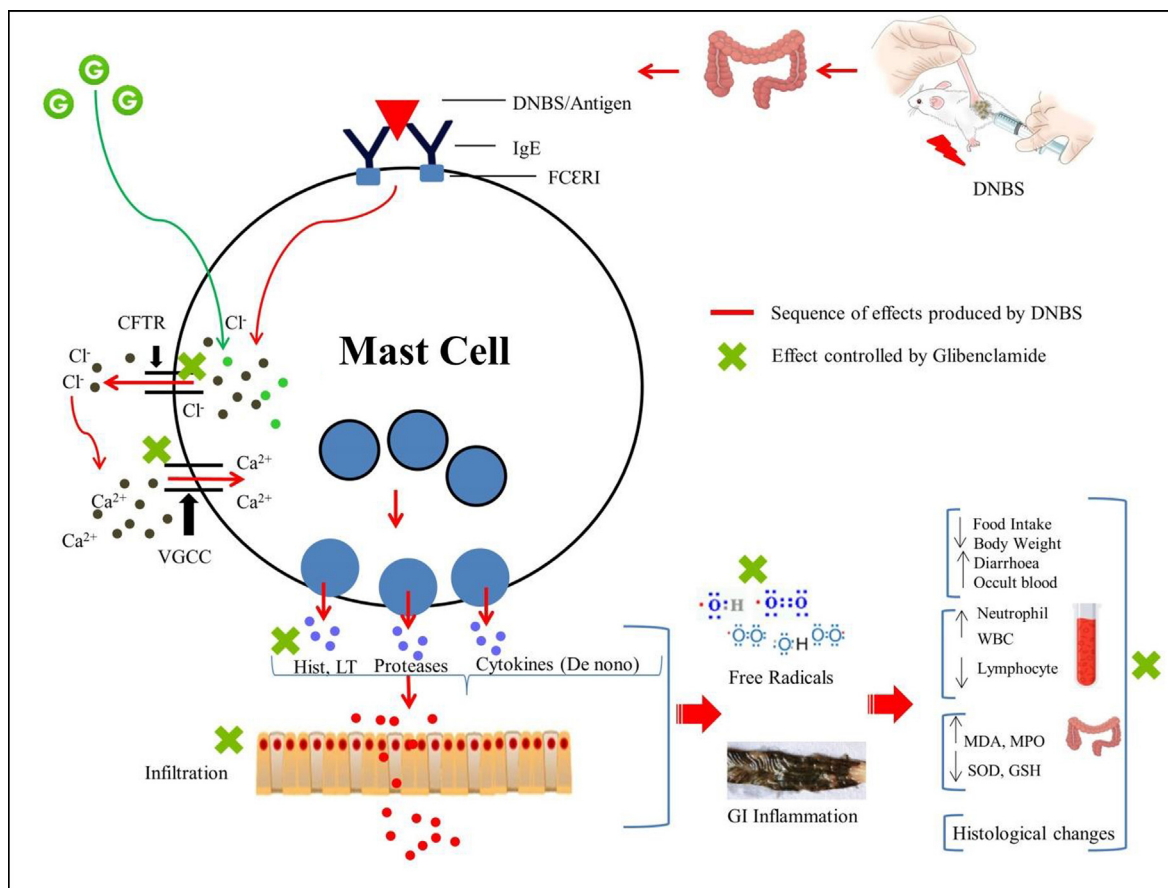
Fig. 11. Proposed Mechanism- Protective effect of Glibenclamide against DNBS induced GI Inflammation. CFTR: Cystic fibrosis transmembrane conductance regulator VGCC: Voltage gated calcium channel.

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