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Exploiting the role of various types of ion-channels against chemically induced inflammatory bowel disease in male Wistar rats

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ABSTRACT

Objective: To evaluate the role of nifedipine (L-type of calcium channel blocker), glibenclamide [cystic fibrosis transmembrane conductance regulator (CFTR) channel blocker], niflumic acid (chloride channels blocker) and 2-aminoethyldiphenyl borate (calcium release-activated channels channel blocker) against chemically induced inflammatory bowel disease in male Wistar rats.

Methods: Inflammatory bowel disease was induced by the administration of 2,4-dinitrobenzene sulfonic acid intracolonically. Animals were divided into 7 groups by keeping 6 animals in each group. Group one and two were treated as normal control and disease control, respectively and seven pretreated with sulfasalazine and remaining groups were treated with test drugs. Animals were pretreated for 5 days before administration of 2,4-dinitrobenzene sulfonic acid. Various parameters were recorded during and after treatment period like disease activity index (body weight, food intake, diarrhea score, occult blood score), biomarkers study in peritoneal macrophages and histopathology of colon.

Results: Glibenclamide had offered significant protective effect on disease activity index, biochemical parameters confirmed by histopathological examination. 2-Aminoethyldiphenyl borate and niflumic acid had also shown insignificant protection in few parameters.

Conclusions: Protection observed by pretreatment of glibenclamide is may be of its blocking effect of CFTR channels of the mast cells, which regulate chloride influx and efflux phenomenon.

1. Introduction

Inflammatory bowel disease (IBD) is an idiopathic disease characterized by chronic recurrent ulceration caused mostly because of dysregulated immune response to the host intestinal microflora. Two major types of IBD are ulcerative colitis and Crohn's disease. Ulcerative colitis involves the colon alone, but Crohn's disease is transmural involving any segment of the gastrointestinal tract from the mouth to the anus with "skip lesions"[1].

Inflammatory mediators are known to play crucial role in the pathologic and clinical features of IBD. In response to various antigenic stimuli, cytokines are released by macrophages and bind to respective receptors and produce autocrine, paracrine, and endocrine effects[2].

Mast cells and their mediators are known to involve in the pathogenesis of IBD[3]. The amount of mast cell tryptase, the number of mast cells in the lamina propria and submucosa are significantly increased in the colorectal mucosa of IBD patients[4]. Increased numbers of mast cells in the colonic mucosa of IBD patients are accompanied by increased expression of tumor necrosis factor (TNF)-α, interleukin (IL)-16 and substance P. In mucosa of IBD patients, highly elevated histamine and tryptase levels were detected, which is strongly suggesting that mast cell degranulation is involved in the pathogenesis of IBD. Mast cell mediators like tryptase, histamine, platelet activating factor, prostaglandins and leukotrienes have the capacity to produce a variety of cytokines which are responsible for various acute and chronic inflammatory responses and thus IBD[5].

Immunoglobulin E (IgE)-dependent activation of both human and rodent mast cells is characterized by an influx of extracellular

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All experimental procedures involving animals were conducted in accordance to Nations Animal Ethical Guidelines and approved by Institutional Animal Ethical Committee and Committee for the Purpose of Control and Supervision on Experiments on Animals.

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Ca^{2+} which is essential for subsequent release of both preformed (granule-derived) mediators and newly generated autacoids and cytokines. Recently, various types of ion-channels are identified on the surface of the mast cells but exact role of these channels in the degranulation process is yet not been fully established. Flow of ions such as K^+ and Cl^- regulate cell membrane potential and thus influence Ca^{2+} influx which is important in activation responses[6].

Ca^{2+} selective calcium release activated calcium current is the well characterized mechanism for Ca^{2+} influx via store operated calcium entry (SOCE) which is first described in rat mucosal-type (line RBL-2H3) mast cells[7,8]. L-type Ca^{2+} channels were thought to be a characteristic feature of excitable cells and also exist in mast cells to mediate non-SOCE, which is crucial for protecting mast cells against activation-induced mitochondrial cell death[9]. Though the evidence is indirect and not definitive, chloride channels (CICs) have also been found to be involved in regulating membrane polarity and enhancing Ca^{2+} influx and degranulation of mast cells. A number of CICs, including CIC-2, -3, -4, -5, and -7 and cystic fibrosis transmembrane conductance regulator (CFTR), have been characterized electro-physiologically or by RT-PCR in rodent and human mast cell lines[10]. Calcium channel blockers such as nifedipine, amlodipine, verapamil and diltiazem suppress the activation of various immune reactions, including T cells, mast cells and macrophages indicating their immunosuppressant effect[11]. This suppression may be via decreasing intracellular Ca^{2+} availability which is crucial for the mast cell degranulation process and hence mediator release. Mast cell activation requires Cl^- flux, which maintains the driving force for entry of extracellular calcium and initiates release of mediators such as histamine and cytokines. It is well known that rat mast cells express a functional CFTR, which is crucial in mediator release. Mast cells express several members of the CIC family and these CIC channels are important in mast cell functions[12]. Glibenclamide, tolbutamide reversibly affects exclusively open state of the CFTR channels to interrupt Cl^- conduction[13].

By considering all above facts, the present study was undertaken to find out the role of these ion-channels against chemically induced IBD. In the present study, four test drugs *i.e.*, nifedipine (L-type of calcium channel blocker), glibenclamide (CFTR channel blocker), niflumic acid (CIC channel blocker) and 2-aminoethyldiphenyl borate (2-APB) [calcium release-activated channels (CRAC) channel blocker] were tested and data was compared with standard salfasalazine treated group by considering various parameters like disease activity index (DAI), biomarkers and histopathology examination of colon *etc*.

2. Materials and methods

2.1. Animals

Forty-two male Wistar albino rats weighing in between 180–200 g were used in this study. All the animals were bred at central animal house at Northern Border University, Rafha, Saudi Arabia and used in this experiment. National Animal Ethical Guidelines were strictly followed for animal handling, maintenance and care. This project was approved by Institutional Animal Ethical Committee (IAEC) and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) bearing project license number (CPCSEA/1657/ IAEC/CMRCP/Col-11-13/14). By observing health status of the animals for 10 days, healthy animals were selected for this study.

2.2. Chemicals

Nifedipine and glibenclamide were purchased from Azine Healthcare, Ahmedabad, India, 2-aminoethyldiphenyl borate, niflumic acid and 2,4-dinitrobenzene sulfonic acid were procured from Sigma Aldrich, USA, sulfasalazine was purchased from IPCA Lab, Mumbai, India, σ-dianisidine obtained from Loba Chemie, Mumbai, India, thiobarbituric acid and 5,5'-dithiobis-(2-nitrobenzoic acid) were purchased from Sisco Research Lab, Mumbai, India.

2.3. Experimental design

Animal grouping, treatment schedule and doses were mentioned in Table 1. All the experiments were carried out between 9:00–16:00 h. In the present study, seven groups of male Wistar rats containing 6 rats in each group, were used. Colitis was induced in all groups except first group which was kept as sham control group that received distilled water instead of 2,4-dinitrobenzene sulfonic acid (DNBS). Colitis was induced by administration of DNBS by intra-colon route.

All the treatment groups including standard were pretreated for 5 days before DNBS administration. The doses of test drugs were selected based on the "highest safe dose" previously used in preclinical experiments[14-17].

2.3.1. Induction of IBD

IBD was induced by the administration of DNBS intracolonically using the method described previously by Morris (1989). Briefly, fasted rats were lightly anesthetized with the cocktail of ketamine, 80 mg/kg and xylazine, 50 mg/kg *i.p.* After inducing anesthesia, a rubber catheter was inserted rectally and advanced such that the tip of the catheter was approximately 8 cm proximal to the anus. Then 0.5 mL of 50% (v/v) ethanol containing DNBS (100 mg/kg) was injected. Thereafter, the animals were retained for 15 min in a Trendelenburg position to avoid any kind of lockage and oozing of DNBS[18].

Animals were observed for three days after induction of IBD. During observation days, no test/standard drug was administered to any of the groups. Body weight, food intake, diarrhea and occult blood in feces of the rats were observed daily as part of DAI. On Day 4, animals were sacrificed and abdomen was opened by a midline incision. The colon was removed, freed from surrounding tissues, opened along the anti-mesenteric border, rinsed, weighed and processed for biochemical analysis and histopathology study.

2.3.2. Measurement of DAI

Depending on the severity of condition, DAI was measured as scores from 0-4. Each animal was scored daily from 0–4 for the changes in growth rate, stool consistency and presence of gross bleeding or occult blood in feces. By using benzidine test, the presence of occult blood in feces was determined. The total

Table 1
Drug doses of different groups.

Group	Treatment	Category	Dose	ROA
Group I	Distilled water	-	0.5 mL	Intracolonically
Group II	DNBS	Hapten	100 mg/kg	Intracolonically
	Distilled water	-	1% of body weight	*p.o.*
Group III	DNBS	Hapten	100 mg/kg	Intracolonically
	Nifedipine	Calcium channel blocker	10 mg/kg	*p.o.*
Group IV	DNBS	Hapten	100 mg/kg	Intracolonically
	Glibenclamide	CFTR channel blocker	0.5 mg/kg	*p.o.*
Group V	DNBS	Hapten	100 mg/kg	Intracolonically
	2-APB	CRAC channel blocker	20 mg/kg	*i.p.*
Group VI	DNBS	Hapten	100 mg/kg	Intracolonically
	Niflumic acid	CIC channel blocker	10 mg/kg	*p.o.*
Group VII	DNBS	Hapten	100 mg/kg	Intracolonically
	Sulfasalazine	-	100 mg/kg	*p.o.*

ROA: Route of administration.

score was the sum of the three subscores and the score of food intake[19].

2.3.3. Gross morphological changes and wet weight on colon

The distal colon was isolated, opened by a longitudinal incision and was immediately examined under a stereomicroscope and visible damage was scored on a 0–5 scale by the criteria previously described by Morris with slight modification[18].

2.3.4. Assay of myeloperoxidase (MPO) activity

The mucosal tissue was homogenized in 3 mL of 0.05 mol/L phosphate buffer (pH 6.0) containing 0.5% w/v cetyl trimethyl ammonium bromide. At 1 700 r/min, the homogenate was centrifuged for 20 min at 4 ℃ (REDMI CM-12). The supernatant was diluted five-fold with potassium phosphate buffer. A volume of 1.4 mL of 0.00107% H_2O_2 diluted with potassium phosphate buffer was added to 0.05 mL of the diluted sample. Later, 0.05 mL of 0.03 mol/L O-dianisidine solution was added to the above mixture and the tissue MPO activity was determined from the increase of absorbance at 450 nm for 60 s[20].

2.3.5. Estimation of malonaldehyde (MDA) level from phenazine methosulphate (PMS)

To 0.5 mL of PMS, 0.5 mL of Tris hydrogen chloride buffer was added and incubated at 37 ℃ for 2 h and then 1 mL of ice cold trichloroacetic acid was added, centrifuged at 1 000 r/min for 10 min. To 1 mL of supernatant taken from above, 1 mL of thiobarbituric acid was added and the tubes were kept in boiling water for 10 min. The tubes were removed and brought up to room temperature and 1 mL of distilled water was added and the absorbance was measured at 532 nm by using UV spectrophotometer [21].

2.3.6. Estimation of reduced glutathione (GSH) from PMS

A total of 0.75 mL of PMS was mixed with 0.75 mL of 4% sulfosalicylic acid and then centrifuged at 1 200 r/min for 5 min at 4 ℃ (REDMI CM-12). From the above, 0.5 mL of supernatant was taken and added with 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[22].

2.3.7. Estimation of histopathological score

In a blinded manner, the tissue damage and mucosal integrity were assessed and quantified on 0–3 by two independent pathologists with respect to loss of epithelium, damage of crypts, depletion of globlet cell, infiltration of inflammatory cells using the method by Iba *et al.* with slight modification[23].

2.4. Statistical analysis

All the results were expressed as mean ± SEM. Data analysis was performed using GraphPad Prism 5.0 software (GraphPad, San Diego, CA). Statistical comparisons were made between drug-treated groups and disease control animals. Data of biochemical parameters were analyzed using One-way ANOVA; Dunnett's multiple range test was applied for *post hoc* analysis. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effect of ion-channel blockers on DAI

Body weight, food intake, diarrhea and occult blood in feces of the rats were observed daily as part of DAI.

3.2. Body weight

Animal weight was recorded daily for 5 days after 2 h of treatment period and on Day 3 and 4 of the study, there was significant ($P < 0.05$) decline in body weight of the disease control animals compared to normal control. This decrease in the body weight was protected by the pretreatment with glibenclamide (0.5 mg/kg) and sulfasalazine (100 mg/kg) but non-significant compared to disease control group (Figures 1 and 2).

3.3. Food intake

After DNBS administration, food intake was significantly ($P < 0.001$) reduced in the disease control group compared to normal control group. By the pretreatment with glibenclamide, food intake was significantly ($P < 0.001$) increased compared to disease control for all 4 days of the study. But at the same

time, other test groups like nifedipine and niflumic acid have not shown any effect on food intake but pretreatment with 2-APB has shown minor increase in food intake compared to disease control group. Standard sulfasalazine has caused significant ($P < 0.01$) increase in food intake compared to disease control group but less than normal control group (Figure 3).

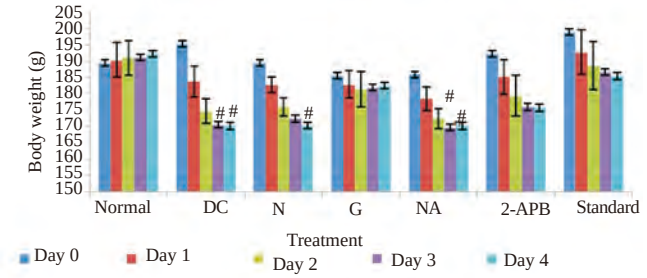

Figure 1. Effect of various types of ion-channel blockers on body weight against DNBS induced IBD in male Wistar rats.
DC: Disease control; N: Nifedipine; G: Glibenclamide; NA: Niflumic acid; #: Comparison of disease control and all test groups with normal control group.

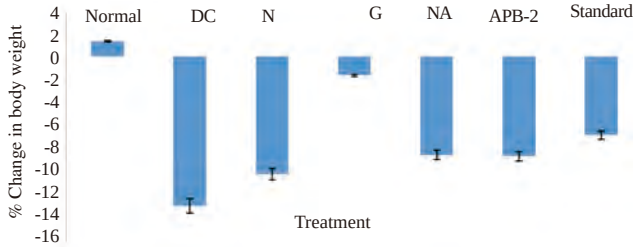

Figure 2. Effect of various types of ion-channel blockers on change in body weight against DNBS induced IBD in male Wistar rats.
DC: Disease control; N: Nifedipine; G: Glibenclamide; NA: Niflumic acid.

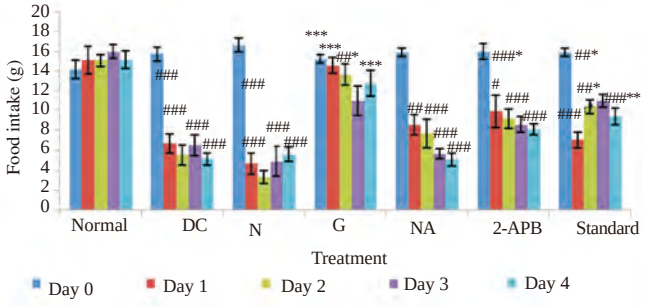

Figure 3. Effect of various types of ion-channel blockers on food intake against DNBS induced IBD in male Wistar rats.
DC: Disease control; N: Nifedipine; G: Glibenclamide; NA: Niflumic acid. #: Comparison of disease control and all test groups with normal control group; *: Comparison of test groups with disease control group. Values are significant at *#: $P < 0.05$, **##: $P < 0.01$ and ***###: $P < 0.001$.

3.4. Diarrhea and occult blood in feces

The treatment with DNBS caused significant severe diarrhea ($P < 0.001$) and occult blood in feces ($P < 0.001$) in the disease control group compared to normal control group where there was no diarrhea. Diarrhea and occult blood in feces significantly ($P < 0.001$) controlled by the pretreatment with glibenclamide compared to diseased control group for all 4 days (Figures 4 and 5).

3.5. Effect of ion-channel blockers on gross morphology and wet weight of colon

There was significant rise in gross morphology score ($P < 0.001$) in the disease control group compared to normal, but in the pretreatment with glibenclamide, there was significant ($P < 0.001$) high protective effect. 2-APB and niflumic acid also had little but significant protective effect ($P < 0.001$) (Figure 6).

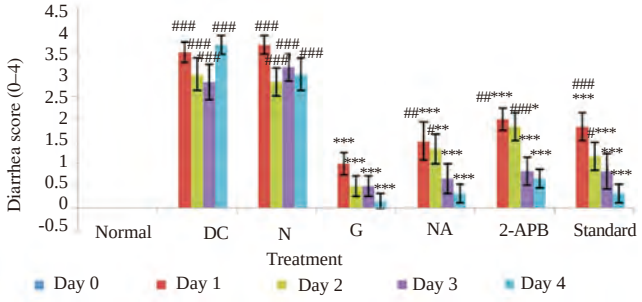

Figure 4. Effect of various types of ion-channel blockers on diarrhea score Against DNBS induced IBD in male Wistar rats.
DC: Disease control; N: Nifedipine; G: Glibenclamide; NA: Niflumic acid. #: Comparison of disease control and all test groups with normal control group; *: Comparison of test groups with disease control group. Values are significant at *#: $P < 0.05$, **##: $P < 0.01$ and ***###: $P < 0.001$.

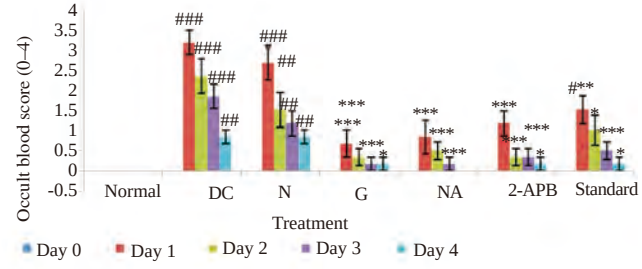

Figure 5. Effect of various types of ion-channel blockers on occult blood score against DNBS induced IBD in male Wistar rats.
DC: Disease control; N: Nifedipine; G: Glibenclamide; NA: Niflumic acid. #: Comparison of disease control and all test groups with normal control group; *: Comparison of test groups with disease control group. Values are significant at *#: $P < 0.05$, **##: $P < 0.01$ and ***###: $P < 0.001$.

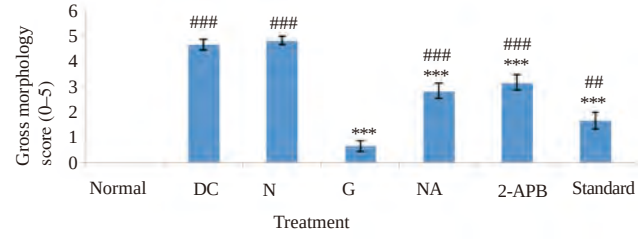

Figure 6. Effect of various types of ion-channel blockers on morphological score against DNBS induced IBD in male Wistar rats.
DC: Disease control; N: Nifedipine; G: Glibenclamide; NA: Niflumic acid. Values are significant at ***###: $P < 0.001$. #: Comparison of disease control and all test groups with normal control group; *: Comparison of test groups with disease control group.

There was significant increase in the wet weight of colon ($P < 0.001$) and in the disease control group compared to the normal control. In the pretreatment with the glibenclamide, there was significant reduction in the wet weight of colon ($P < 0.001$) (Figure 7).

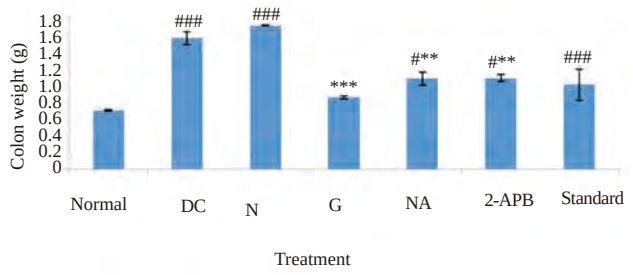

Figure 7. Effect of various types of ion-channel blockers on colon weight against DNBS induced IBD in male Wistar rats.
DC: Disease control; N: Nifedipine; G: Glibenclamide; NA: Niflumic acid. Values are significant at $^{\#}$: $P < 0.05$, **: $P < 0.01$ and $^{***\#\#\#}$: $P < 0.001$. $^{\#}$: Comparison of disease control and all test groups with normal control group; *: Comparison of test groups with disease control group.

3.6. Effect of ion-channel blockers on biochemical parameters

In the present study, we have found that there was significant increase in MPO ($P < 0.001$) and MDA level ($P < 0.001$) and fall in GSH level in disease control group compared to sham control. By the pretreatment with glibenclamide, these MPO ($P < 0.001$), MDA ($P < 0.001$) and GSH levels were reversed significantly compared to disease control group (Figure 8).

3.7. Effect of ion-channel blockers on histopathology features

There was significant increase in the scores of colon histopathology features *i.e.*, loss of epithelium ($P < 0.001$), damage of crypts ($P < 0.001$), depletion of goblet cells ($P < 0.001$) and infiltration of inflammatory cells ($P < 0.001$) in the disease control group compared to the normal control. By the pretreatment with the glibenclamide, there was significant reduction in the scores of colon histopathology features *i.e.*, loss of epithelium ($P < 0.01$), damage of crypts ($P < 0.001$), depletion of goblet cells ($P < 0.001$) and insignificant reduction in infiltration of inflammatory cells (Figures 9-12).

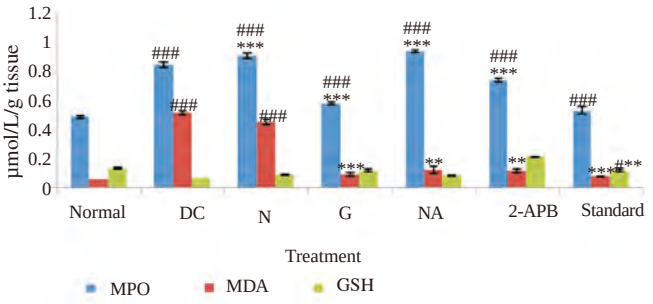

Figure 8. Effect of various types of ion-channel blockers on MPO activity, MDA level and reduced GSH level against DNBS induced IBD in male Wistar rats.
DC: Disease control; N: Nifedipine; G: Glibenclamide; NA: Niflumic acid. Values are significant at $^{\#}$: $P < 0.05$, **: $P < 0.01$ and $^{***\#\#\#}$: $P < 0.001$. $^{\#}$: Comparison of disease control and all test groups with normal control group; *: Comparison of test groups with disease control group.

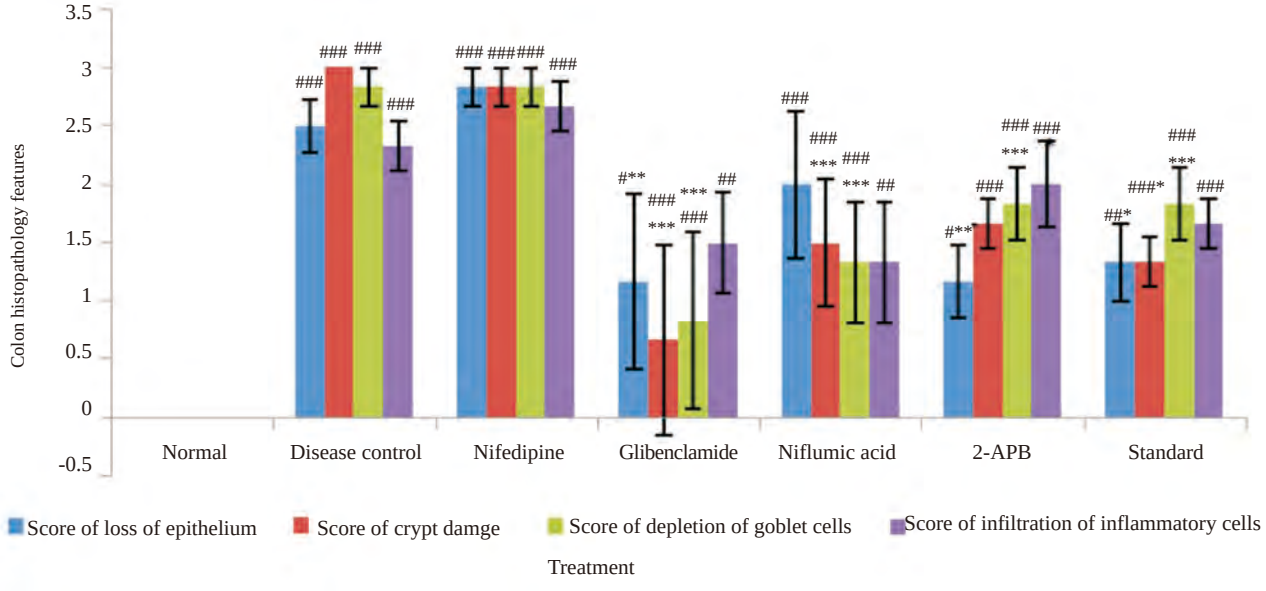

Figure 9. Effect of various types of ion-channel blockers on the colon histopathology features against DNBS induced IBD in male Wistar rats.
Values are significant at $^{*\#}$: $P < 0.05$, $^{**\#\#}$: $P < 0.01$ and $^{***\#\#\#}$: $P < 0.001$. $^{\#}$: Comparison of disease control and all test groups with normal control group; *: Comparison of test groups with disease control group.

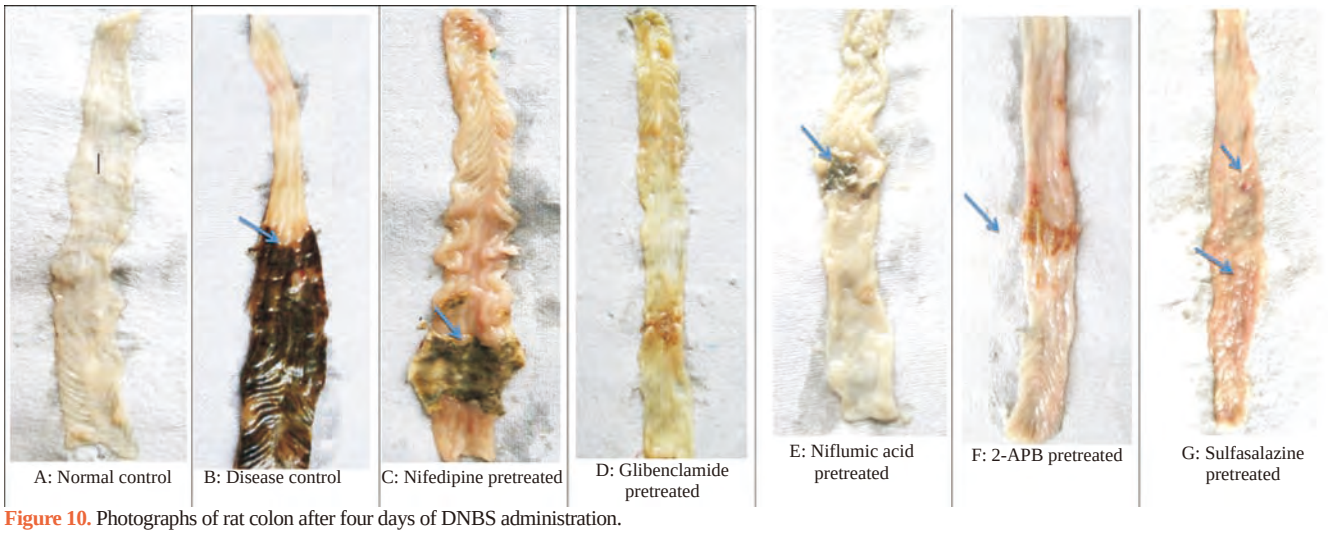

Figure 10. Photographs of rat colon after four days of DNBS administration.

A: Normal control group pretreated with distilled water (10 mL/kg) not showing any hyperemia, ulcers or inflammation; B. Disease control group pretreated with distilled water (10 mL/kg) showing lesion area with severe inflammation > 2 cm length; C: Nifedipine (10 mg/kg) pretreated group showing lesion area with severe inflammation > 1 cm length; D: Glibenclamide (0.5 mg/kg) pretreated group not showing any lesion area or inflammation but showing small ulcer spot; E: Niflumic acid (10 mg/kg) pretreated group showing lesion area with severe inflammation; F: 2-APB (20 mg/kg) pretreated group showing hyperemia, ulcers, and inflammation; G: Sulfasalazine (100 mg/kg) pretreated groups not showing any lesion area or inflammation but showing hyperemia and small ulcer spot.

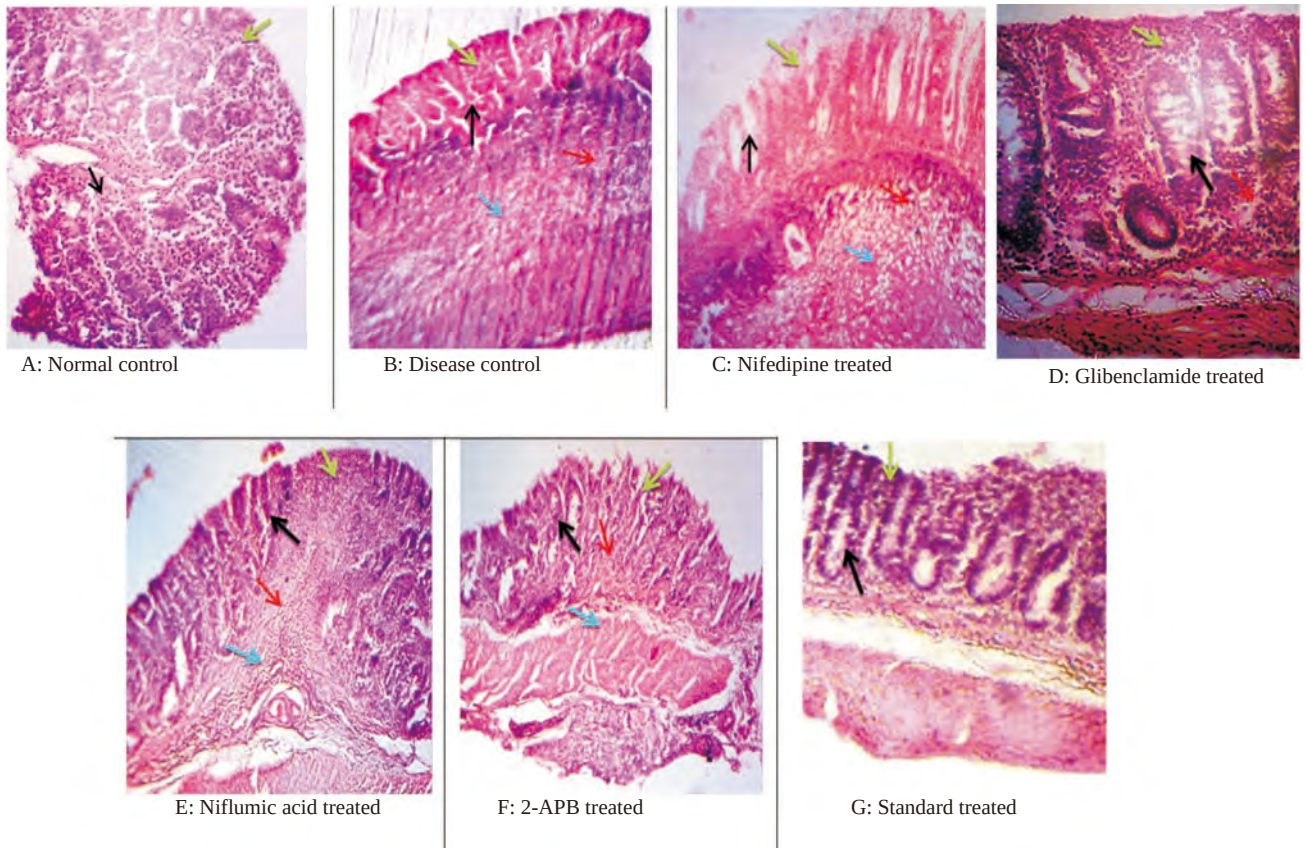

Figure 11. Histopathology sections of rat colon stained with haematoxylin and eosin stain.

Images (× 50 magnification) are typical and representative of each study group. A: Normal control group pretreated with distilled water (10 mg/kg) showing intact epithelial, normal mucosal architecture (green arrow) and normal crypts (black arrow); B: Disease control group pretreated with distilled water (10 mg/kg) showing severe loss of epithelium and ulceration (green arrow), disappearance of crypts (black arrow), severe edema in submucosa (blue arrow) and infiltration of inflammatory cells (red arrow); C: Nifedipine (10 mg/kg) pretreated group showing severe loss of epithelium and ulceration (green arrow), disappearance of crypts (black arrow), severe edema in submucosa (blue arrow) and infiltration of inflammatory cells (red arrow); D: Glibenclamide (0.5 mg/kg) pretreated group showing results similar to normal control group *i.e.*, intact epithelial, normal mucosal architecture (green arrow) and normal crypts (black arrow) but with very minor infiltration of inflammatory cells (red arrow); E: Niflumic acid (10 mg/kg) pretreated group showing loss of epithelium (green arrow), architectural disarray of crypts and disappearance of crypts (black arrow), severe edema in submucosa (blue arrow) and infiltration of inflammatory cells (red arrow); F: 2-APB (20 mg/kg) pretreated group showing severe loss of epithelium and ulceration (green arrow), architectural disarray of crypts (black arrow), severe edema in submucosa (blue arrow) and infiltration of inflammatory cells (red arrow); G: Sulfasalazine (100 mg/kg) pretreated group showing intact epithelial, normal mucosal architecture (green arrow) and normal crypts (black arrow).

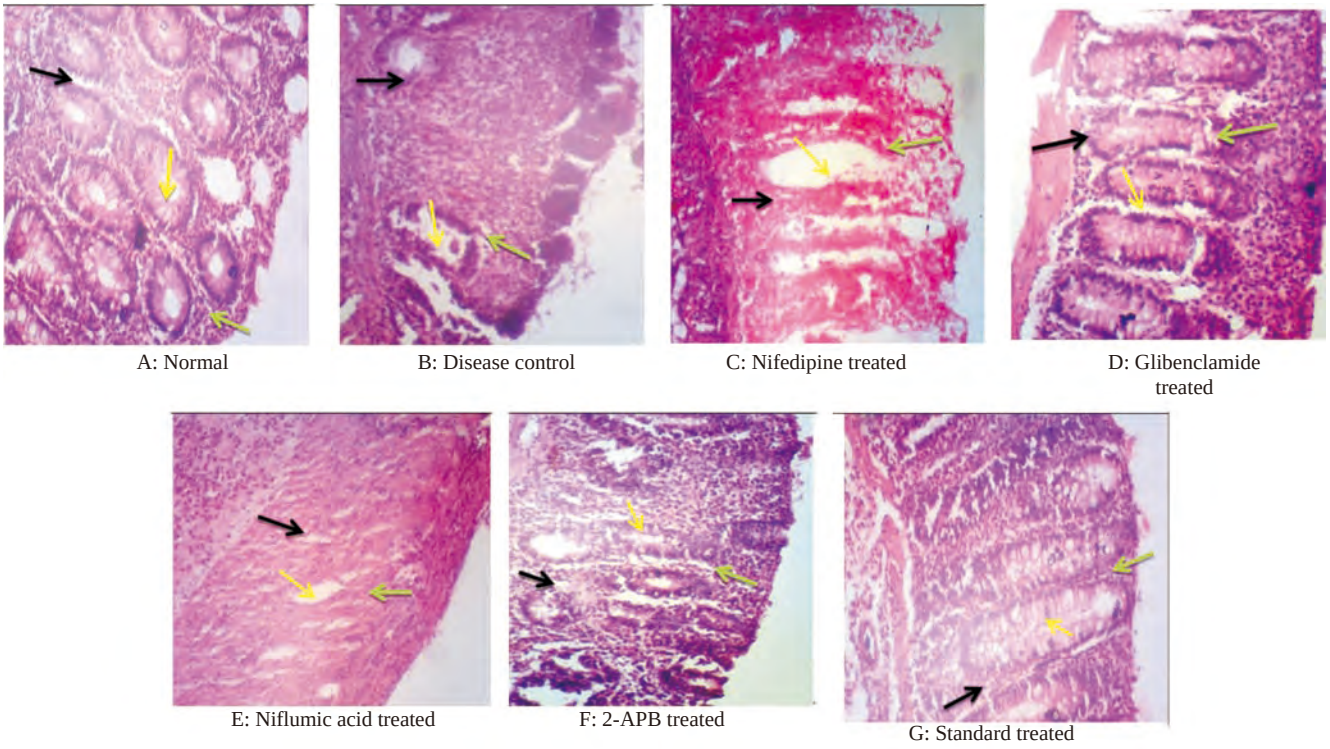

Figure 12. Histopathology sections of rat colon stained with haematoxylin and eosin.
Images (100× magnification) are typical and representative of each study group. A: Normal control group pretreated with distilled water (10 mg/kg) showing intact epithelial, normal mucosal architecture (green arrow), normal crypts (black arrow) and normal goblet cells (yellow arrow); B: Disease control group pretreated with distilled water (10 mg/kg) showing severe loss of epithelium and ulceration (green arrow), disappearance of crypts (black arrow), and severe depletion of goblet cells (yellow arrow); C: Nifedipine (10 mg/kg) pretreated group showing severe loss of epithelium and ulceration (green arrow), disappearance of crypts (black arrow), and severe depletion of goblet cells (yellow arrow); D: Glibenclamide (0.5 mg/kg) pretreated group showing results similar to normal control group *i.e.*, intact epithelial, normal mucosal architecture (green arrow), normal crypts (black arrow) and normal goblet cells (yellow arrow); E: Disease control group pretreated with distilled water (10 mg/kg) showing severe loss of epithelium and ulceration (green arrow), disappearance of crypts (black arrow), and severe depletion of goblet cells (yellow arrow); F: 2-APB (20 mg/kg) pretreated group showing severe loss of epithelium and ulceration (green arrow), architectural disarray of crypts (black arrow) but goblet cells were not much affected (yellow arrow); G: Sulfasalazine (100 mg/kg) pretreated group showing intact epithelial, normal mucosal architecture (green arrow) and normal crypts (black arrow), normal goblet cells (yellow arrow).

4. Discussion

According to the currently accepted hypothesis, IBD is resulted from a dysregulated response of the mucosal immune system toward intraluminal antigens of bacterial origin in genetically predisposed persons[24-27].

In chronic IBD, not only increased numbers of mast cells can be found, but also the cellular content of these cells is greatly changed. For example, granules contained a higher expression of IL-16, TNFα, and substance P[28-31]. Moreover, there is abundant evidence for mast cell degranulation in the intestinal mucosa of IBD patients. Increased concentrations of mast cell mediators in the gastrointestinal tract have been detected in these patients[32,33].

As a proinflammatory mediator, histamine can increase ion transport across the epithelial barrier and therefore contribute to diarrhea found in IBD[34]. Mast cells are recognized as an important cellular source for TNFα in human IBD[35]. Also TNFα was demonstrated to be an important mediator for diarrhea[36,37]. Furthermore, after mast cell degranulation, several mediators, like proteases, cytokines, prostaglandins and chemokines have shown to induce micro-vascular leakage and recruit inflammatory cells to the site of inflammation and therefore enhance the inflammatory response[38,39].

Ion-channels are transmembrane pore-forming proteins involved in many physiological processes like of cell volume regulation, action potential, heart rate, muscle contraction and fluid volume regulation in urine secretion *etc*. Ion-channels were said to play an important role in mast cell activation and subsequent degranulation and release of mast cell mediators. IgE-dependent activation of human and rodent mast cells is characterized by an influx of extracellular Ca^{2+}, which is essential for subsequent release of both preformed (granule-derived) mediators and newly generated autacoids and cytokines.

However, flow of ions such as K^+ and Cl^- are likely to play an important role in activation responses because they regulate cell membrane potential and thus influence Ca^{2+} influx.

IgE-dependent stimulation of the high affinity IgE receptor results in rapid secretion of various proinflammatory chemical mediators and cytokines. All of the outputs depend to certain degrees on an increase in the intracellular Ca^{2+} concentration and influx of Ca^{2+} from the extracellular space is often required for their full activation. There is strong evidence that high affinity IgE receptor stimulation induces two different modes of Ca^{2+} influx, SOCE and non-SOCE, which are activated in response to endoplasmic reticulum Ca2+ store depletion and independently of Ca^{2+} store depletion, respectively, in mast cells. The best characterized SOC channels are known as "CRAC" channels.

The recent data suggest that L-type Ca^{2+} channels, which were thought to be a characteristic feature of excitable cells, exist in mast cells to mediate non-SOCE, which is critical for protecting mast cells against degranulation and subsequent activation induces granulation and mitochondrial cell death. So blocking of Ca^{2+} channels may found to be beneficial in IBD treatment[40].

From the literature survey, it was found that Cl^- channel have a functional role in mediator secretion from the mast cells and Cl^- channel blockers were proved to inhibit mediator secretion[41]. Mast cell CFTR is a functional Cl^- channel which might be important in mediator release. An inhibitor of CFTR-dependent Cl^- flux, diphenylamine-2-carboxylate found to downregulates mast cell mediator release[42].

Based upon this, it is hypothesized that the blocking of the Ca^{2+} and Cl^- channels may protect the mast cells against activation and subsequent degranulation, release of mast cell mediators.

In present study, four drugs *i.e.*, nifedipine (L-type of calcium channel blocker), 2-APB (CRAC channel blocker), glibenclamide (CFTR channel blocker, at low doses) and niflumic acid (CIC channel blocker) were tested to explore their role against chemically induced IBD where sulfasalazine was used as a standard drug[43].

DNBS induce IBD in male Wistar rats as it is one of the most accepted models resembling to human pathology.

IBD was induced by the administration of DNBS intracolonically using the method described previously by Morris. Induction of colitis was confirmed by observing body weight, food intake, diarrhea and occult blood in feces of the rats daily as part of DAI which are important symptoms of IBD.

DAI features including weight loss, fall in food intake, diarrhea and occult blood in feces are important symptoms of IBD. The protection of glibenclamide against weight loss may be of increased insulin secretion from pancreatic β-cells. Glibenclamide also offered protection against fall in food intake due to prevention of degranulation of the mast cells and thereby subsequent release of IL-1 and 5-hydroxytryptamine from mast cell store sites regulates hypothalamic paraventricular nucleus (the food control center in hypothalamus). The protection of glibenclamide against diarrhea may be of its CFTR channel blocking activity which has crucial role in diarrhea of inflammatory origin because of its importance in ionic secretion and additional regulatory actions on NaCl absorption[44,45]. Other test drugs like niflumic acid and 2-APB show minor protection against these parameters, but nifedipine has not shown any protection but has enhanced severity of these symptoms.

Biochemical parameters like MPO, MDA and GSH levels are important biomarkers in evaluating IBD. MPO is an enzyme that functions in the oxygen dependent killing of microorganisms which is released from the primary granules of neutrophils during acute inflammation[46,47]. Pretreatment with glibenclamide has shown protection against rise in MPO level. MDA is the major reactive aldehyde resulting from the peroxidation of biological membranes. Pretreatment with glibenclamide has shown protection against rise in MDA level. The tripeptide GSH (L-glutamyl-L-cysteinyl glycine, is the most important intracellular antioxidant which is very much dependent on GSH synthesis. Fallen GSH levels eventually causes mucosal damage[48]. Glibenclamide has also shown good protection against fall in GSH level. This protective effect of glibenclamide against rise in MPO, MDA and fall in GSH is might be because of its mast cell stabilizing as well as free radical scavenging activity.

Glibenclamide is a sulphonylurea drug that is widely used to treat non-insulin-dependent diabetes mellitus[49]. At nanomolar concentrations, it binds to the sulphonyl urea receptor of pancreatic β-cells to cause the inhibition of ATP-sensitive K^+ channels (K_{ATP} channels) and promote insulin secretion[50]. A molecular explanation for the functional similarities between CFTR and K_{ATP} channels has emerged following the identification of the genes that encode K_{ATP} channels in pancreatic β-cells, and cardiac and skeletal muscle[51,52].

Rat mast cells express functional CFTR Cl^- channels, which might be important in mediator release. Mast cell activation requires Cl^- efflux, which maintains the driving force for entry of extracellular Ca^{2+} and initiates release of cytokines, mediators such as histamine. The sulfonylurea drug glibenclamide is widely used as inhibitor of the CFTR. Glibenclamide inhibition of CFTR Cl^- channel was voltage dependent and enhanced when the external Cl^- concentration was decreased. By the blocking of Cl^- efflux from CFTR channels, glibenclamide can block the mast cell degranulation and may protect the mast cells against DNBS induced colitis.

All the above finding is also supported by the gross morphology and histopathological section of colon. Treatment with glibenclamide has not shown any lesion area or inflammation but showing small ulcer spot compared to disease control group which showed lesion area with severe inflammation > 2 cm

length. Moreover, pre-treatment with glibenclamide has shown to maintain intact epithelial, normal mucosal architecture, normal crypts and normal goblet cells compared to disease control group with severe loss of epithelium and ulceration, disappearance of crypts and severe depletion of goblet cells.

Other test drugs like nifedipine, niflumic acid and 2-APB have not shown significant protections in most of the parameters. But 2-APB and niflumic acid have shown some degree of protection in DAI, colon weight, spleen weight and biochemical parameters like MPO, MDA and histopathology features. At our laboratory conditions, nifedipine has not shown any protection against DNBS induced IBD.

Among all the test drugs, only glibenclamide has offered protection against DNBS induced IBD; other test drugs like niflumic acid and 2-APB have also offered protection in DAI but not as good as glibenclamide. Surprisingly, nifedipine has accentuated IBD symptoms. On the basis of this study, we concluded that glibenclamide has potent anti-IBD effects by its various mechanisms.

The advantage of these finding is that in many individuals, IBD and diabetes co-exist together and tackling those patients may consume many drugs together, because of multiple drugs combinations possibility of drug interaction increases several times and may show horrifying effects. And some time, it is fatal too, so mono therapy with glibenclamide might be enough to control both the conditions simultaneously.

Conflict of interest statement

I declare that I have no conflict of interest.

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References

[1] Corridoni D, Arseneau KO, Cominelli F. Inflammatory bowel disease. *Immunol Lett* 2014; **161**(2): 231-5.

[2] Cohen LB, Nanau RM, Delzor F, Neuman MG. Biologic therapies in inflammatory bowel disease. *Transl Res* 2014; **163**(6): 533-56.

[3] De Winter BY, van den Wijngaard RM, de Jonge WJ. Intestinal mast cells in gut inflammation and motility disturbances. *Biochim Biophys Acta* 2012; **1822**(1): 66-73.

[4] Zare-Mirzaie A, Lotfi M, Sadeghipour A, Haghi-Ashtiani MT. Analysis of colonic mucosa mast cell count in patients with chronic diarrhea. *Saudi J Gastroenterol* 2012; **18**(5): 322-6.

[5] Lakhan ES, Kirchgessner A. Neuro inflammation in inflammatory bowel disease. *J Neuroinflammation* 2010; **7**: 37.

[6] Rice LV, Bax HJ, Russell LJ, Barrett VJ, Walton SE, Deakin AM, et al. Characterization of selective calcium-release activated blockers in mast cells and T-cells from human, rat, mouse and guinea-pig preparations. *Eur J Pharmacol* 2013; **704**(1-3): 49-57.

[7] Freichel M, Almering J, Tsvilovskyy V. The role of TRP proteins in mast cells. *Front Immunol* 2012; **3**: 150.

[8] Hoth M, Penner R. Calcium release-activated calcium current in rat mast cells. *J Physiol* 1993; **465**: 359-86.

[9] Suzuki Y, Inoue T, Ra C. Calcium signaling in mast cells: focusing on L-type calcium channels. *Adv Exp Med Biol* 2012; **740**: 955-77.

[10] Bradding P. Mast cell ion channels. *Chem Immunol Allergy* 2005; **87**: 163-78.

[11] Liu W, Matsumori A. Calcium channel blockers and modulation of innate immunity. *Curr Opin Infect Dis* 2011; **24**(3): 254-8.

[12] Qu C, Liang F, Smythe NM, Schulte BA. Identification of ClC-2 and CIC-K2 chloride channels in cultured rat type IV spiral ligamentfibrocytes. *J Assoc Res Otolaryngol* 2007; **8**(2): 205-19.

[13] Cui G, Song B, Turki HW, McCarty NA. Differential contribution of TM6 and TM12 to the pore of CFTR identified by three sulfonylurea-based blockers. *Pflugers Arch* 2012; **463**(3): 405-18.

[14] Cao QR, Cui JH, Park JB, Han HK, Lee J, Oh KT, et al. Effect of food components and dosing times on the oral pharmacokinetics of nifedipine in rats. *Int J Pharm* 2010; **396**(1-2): 39-44.

[15] Ahangarpour A, Zamaneh HT, Jabari A, Nia HM, Heidari H. Antidiabetic and hypolipidemic effects of *Dorema aucheri* hydroalcoholic leave extract in streptozotocin-nicotinamide induced type 2 diabetes in male rats. *Iran J Basic Med Sci* 2014; **17**(10): 808-14.

[16] Ballough GPH, Kan RK, Nicholson JD, Fath DM, Tompkins CP, Moffa GM, et al. Brain damage from soman-induced seizures is greatly exacerbated by dimethyl sulfoxide (DMSO): modest neuroprotection by 2-aminoethyl diphenylborinate (2-APB), a transient receptor potential channel inhibitor and inositol 1,4,5-triphosphate receptor antagonist. *J Med CBR Def* 2008; **6**: 1-20.

[17 Gupta R, Mehra NK, Jain NK. Development and characterization of sulfasalazine loaded fucosylated PPI dendrimer for the treatment of cytokine-induced liver damage. *Eur J Pharm Biopharm* 2014; **86**(3): 449-58.

[18] Morampudi V, Bhinder G, Wu X, Dai C, Sham HP, Vallance BA, et al. DNBS/TNBS colitis models: providing insights into inflammatory bowel disease and effects of dietary fat. *J Vis Exp* 2014; (84): e51297.

[19] Cho EJ, Shin JS, Noh YS, Cho YW, Hong SJ, Park JH, et al. Anti-inflammatory effects of methanol extract of *Patrinia scabiosaefolia* in mice with ulcerative colitis. *J Ethnopharmacol* 2011; **136**(3): 428-35.

[20] Haddadi R, Mohajjel Nayebi A, Brooshghalan SE. Pre-treatment with silymarin reduces brain myeloperoxidase activity and inflammatory cytokines 6-OHDA hemi-parkinsonian rats. *Neurosci Lett* 2013; **555**: 106-11.

[21] Patel MA, Patel PK, Patel MB. Effect of ethanol extract of *Ficus bengalensis* (bark) on inflammatory bowel disease. *Indian J Pharmacol* 2010; **42**(4): 214-8.

[22] Tipple TE, Rogers LK. Methods for the determination of plasma or tissue glutathione levels. *Methods Mol Biol* 2012; **889**: 315-24.

[23] Iba Y, Sugimoto Y, Kamei C, Masukawa T. Possible role of mucosal mast cells in the recovery process of colitis induced by dextran sulfate sodium in rats. *Int Immunopharmacol* 2003; **3**(4): 485-91.

[24] Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol* 2003; **3**(7): 521-33.

[25] Ko JK, Auyeung KK. Inflammatory bowel disease: etiology, pathogenesis and current therapy. *Curr Pharm Des* 2014; **20**(7): 1082-96.

[26] Denmark VK, Mayer L. Current status of monoclonal antibody therapy for the treatment of inflammatory bowel disease: an update. *Expert Rev Clin Immunol* 2013; **9**(1): 77-92.

[27] Palmer KR, Penman ID, Paterson-Brown S. Alimentary tract and pancreatic disease. In: Haslett C, Chilvers ER, Boon NA, Colledge NR, Hunter JAA, editors. In: *Davidson's principles and practice of medicine*. Edinburgh: Churchill-Livingstone; 2002, p. 747-831.

[28] Kurosawa M, Nagai H. Accumulation of mast cells in the lesions and effects of antiallergic drugs on the patients with inflammatory bowel disease. *Ulcers* 2013; **2013**: 714807.

[29] He SH. Key role of mast cells and their major secretory products in inflammatory bowel disease. *World J Gastroenterol* 2004; **10**(3): 309-18.

[30] Lilja I, Gustafson-Svärd C, Franzen L, Sjodahl R. Tumornecrosis factor-alpha in ileal mast cells in patients with Crohn's disease. *Digestion* 2000; **61**(1): 68-76.

[31] Stoyanova II, Gulubova MV. Mast cells and inflammatory mediators in chronic ulcerative colitis. *Acta Histochem* 2002; **104**(2): 185-92.

[32] Ramsay DB, Stephen S, Borum M, Voltaggio L, Doman DB. Mast cells in gastrointestinal disease. *Gastroenterol Hepatol (N Y)* 2010; **6**(12): 772-7.

[33] Baratelli F, Le M, Gershman GB, French SW. Do mast cells play a pathological role in neurofibromatosis type 2 and ulcerative colitis? *Exp Mol Pathol* 2014; **96**(2): 230-4.

[34] Rijnierse A, Nijkamp FP, Kraneveld AD. Mast cells and nerves tickle in the tummy implication for inflammatory bowel disease andirritable bowel syndrome. *Pharmacol Ther* 2007; **116**(2): 207-35.

[35] Oprins JC, van der Burg C, Meijer HP, Munnik T, Groot JA. Tumour necrosis factor alpha potentiates ion secretion induced by histamine in a humanintestinal epithelial cell line and in mouse colon: involvement of the phospholipase D pathway. *Gut* 2002; **50**(3): 314-21.

[36] Patil HP, Patil L, Kadam VJ. TNF-α: a potential therapeutic target for inflammatory bowel disease. *Asian J Pharm Clin Res* 2011; **4**: 158-66.

[37] Mendoza JL, Abreu MT. Biological Markers in inflammatory bowel disease: practical consideration for clinicians. *Gastroenterol Clin Biol* 2009; **33**: S158-73.

[38] Rijnierse A, Redegeld FA, Blokhuis BR, Van der Heijden MW, Te Velde AA, Pronk I, et al. Ig-free light chains play a crucial role in murine mast cell-dependent colitis and are associated with human inflammatory bowel diseases. *J Immunol* 2010; **185**(1): 653-9.

[39] Kurashima Y, Kiyono H. New era for mucosal mast cells: their roles in inflammation allergic immune responses and adjuvant development. *Exp Mol Med* 2014; **46**: e83.

[40] Murat Z, Korkut M, Akgün E, Firat O, Aynaci M, Içöz G, et al. The comparative effects of calcium channel blockers in an experimental colitis model in rats. *Turk J Gastroenterol* 2004; **15**(4): 243-9.

[41] Ashmole I, Bradding P. Ion channels regulating mast cell biology. *Clin Exp Allergy* 2013; **43**(5): 491-502.

[42] Kulka M, Gilchrist M, Duszyk M, Befus AD. Expression and functional characterization of CFTR in mast cells. *J Leukoc Biol* 2002; **71**(1): 54-64.

[43] Szalai Z, Kupai K, Veszelka M, Pósa A, Török S, Berkó AM, et al. Novel features of the rat model of inflammatory bowel disease based on 2,4,6-trinitrobenzenesulfonic acidinduced acute colitis. *Acta Biol Szeged* 2014; **58**(2): 127-32.

[44] Sheppard DN, Robinson KA. Mechanism of glibenclamide inhibition of cystic fibrosis transmembrane conductance regulator Cl^- channels expressed in a murine cell line. *J Physiol* 1997; **503**(Pt 2): 333-46.

[45] Martinez-Augustin O, Romero-Calvo I, Suarez MD, Zarzuelo A, de Medina FS. Molecular bases of impaired water and ion movements in inflammatory bowel diseases. *Inflamm Bowel Dis* 2009; **15**(1): 114-27.

[46] Turkay C, Kasapoglu B. Noninvasive methods in evaluation of inflammatory bowel disease: where do we stand now? An update. *Clinics (Sao Paulo)* 2010; **65**(2): 221-31.

[47] Vaca CE, Wilhelm J, Harms-Ringdahl M. Interaction of lipid peroxidation products with DNA. A review. *Mutat Res* 1988; **195**(2): 137-49.

[48] Martensson J, Jain A, Meister A. Glutathione is required for intestinal function. *Proc Natl Acad Sci U S A* 1990; **87**(5): 1715-9.

[49] Loubatières A. Effects of sulfonylureas on the pancreas. In: Volk BW, Wellman KE, editors. *The diabetic pancreas*. London: Bailliere Tindall; 1977, p. 489-515.

[50] Zhou Q, Garin I, Castaño L, Argente J, Muñoz-Calvo MT, Perez de Nanclares G, et al. Neonatal diabetes caused by mutations in sulfonylurea receptor 1: interplay betweenexpression aand Mg-nucleotide gating defects of ATP-sensitive potassium channels. *J Clin Endocrinol Metab* 2010; **95**(12): E473-8.

[51] Sturgess NC, Kozlowski RZ, Carrington CA, Hales CN, Ashford ML. Effects of sulphonylureas and diazoxide on insulin secretion and nucleotide-sensitive channels in an insulin secreting cell line. *Br J Pharmacol* 1988; **95**(1): 83-94.

[52] Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP 4th, Boyd AE 3rd, Gonzalez G, et al. Cloning of the β-cell high-affinity sulphonylurea receptor: a regulator of insulin secretion. *Science* 1995; **268**: 423-6.