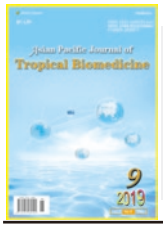

Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org

doi: 10.4103/2221-1691.267638

Impact factor: 1.59

Pyrrolidine dithiocarbamate and saxagliptin ameliorate ulcerative colitis in rats

Berween Mahmoud Elmahmoudy[1]✉, Mai A. Abd El Fattah[1], Mohamed F. Elyamany[1], Laila A. Rashed[2]

[1]*Pharmacology and Toxicology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt*

[2]*Biochemistry and Molecular Biology Department, Faculty of Medicine, Cairo University, Egypt*

ARTICLE INFO

Article history:
Received 18 March 2019
Revision 18 April 2019
Accepted 30 August 2019
Available online 25 September 2019

Keywords:
Ulcerative colitis
Inflammatory mediators
Oxidative stress
Pyrrolidine dithiocarbamate
Saxagliptin
Thioacetamide

ABSTRACT

Objective: To evaluate the antioxidant, immunomodulatory and anti-inflammatory activities of pyrrolidine dithiocarbamate and saxagliptin in rats with thioacetamide-induced ulcerative colitis.

Methods: Animals were orally administered with a vehicle, sulfasalazine (500 mg/kg), pyrrolidine dithiocarbamate (100 mg/kg), and saxagliptin (10 mg/kg) for two weeks. Ulcerative colitis was induced by a single intrarectal instillation of thioacetamide on day 8. Colon samples were collected to assess mitogen-activated protein kinase (MAPK), phosphorylated extracellular signal-regulated kinase (ERK), cAMP response element-binding protein (CREB), interleukin-12 (IL-12), caspase-3, β-defensin, inducible nitric oxide synthase (iNOS) and glucagon like peptide-1 (GLP-1). Moreover, histopathological examination was performed.

Results: Rats treated with thioacetamide caused increases in colonic MAPK, phosphorylated ERK, CREB, caspase-3, IL-12, β-defensin, iNOS, as well as decreases in body weight and GLP-1. In addition, distortion of colonic structure was found by histopathological examination. Pyrrolidine dithiocarbamate and saxagliptin mitigated colitis severity by improving body weight decrease and GLP-1, and reducing colonic MAPK, phosphorylated ERK, CREB, caspase-3, IL-12, β-defensin and iNOS.

Conclusions: Pyrrolidine dithiocarbamate and saxagliptin are efficient against thioacetamide induced colitis through improving inflammatory and oxidative changes.

1. Introduction

The precise etiology of ulcerative colitis (UC) and Crohn's disease which are the two types of inflammatory bowel disease is unclarified, which may be due to a genetic vulnerability, immunologic abnormalities and environmental effects that eventually result in inflammation in the colon, characterized by bleeding, severe diarrhea, abdominal pain, fluid and electrolyte loss[1].

UC is associated with an elevation in specific inflammatory mediators and signs of oxidative stress[2]. The inflammatory response in UC and Crohn's disease is regulated by different cytokines secreted by activated dendritic cells and macrophages, with subsequent activation in the adaptive immune response through triggering and differentiation of many T cells. In UC, impaired T cell regulation is implicated with an imbalance of Treg/Th1, Th2 and Th17 cells in the activated state. The deficiency of proper regulation

✉Corresponding author: Berween Mahmoud Elmahmoudy, Pharmacology and Toxicology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.
Tel: 00201005539039
E-mail: dr_berween@yahoo.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2019 *Asian Pacific Journal of Tropical Biomedicine* Produced by Wolters Kluwer-Medknow. All rights reserved.

How to cite this article: Elmahmoudy BM, Abd El Fattah MA, Elyamany MF, Rashed LA. Pyrrolidine dithiocarbamate and saxagliptin ameliorate ulcerative colitis in rats. Asian Pac J Trop Biomed 2019; 9(9): 373-380.

from T cells, or overproduction of effector T cells, contributes to the development and exacerbation of UC[3].

The definite mechanism of the disease has not been well studied. The mucosa acts as a protective barrier, both microbial pathogens and ingested materials stimulate oxidative stress and gastrointestinal inflammation with the involvement of epithelial immune and inflammatory cells. UC is caused by either ROS overproduction or a decline in antioxidant activity in the inflamed lamina propria[4]. Moreover, inducible nitric oxide synthase (iNOS) causes injury in the colonic tissue, as it stimulates colonic TNF-α generation, which further develops neutrophilic infiltration, promoting tissue proliferation and damage by overproduction of intracellular adhesion molecule and P-selectin[5].

Moreover, the signaling pathways such as mitogen-activated protein kinase (MAPK) are stimulated in oxidative status. MAPKs are protein kinases (extremely conserved both serine/threonine) which play a vital role in many essential cellular processes, such as growth/proliferation, differentiation, motility, and apoptosis/survival, and also stress response[6]. Ordinary MAPKs include several types of extracellular signal-regulated kinases (ERK) such as 1, 2 and 5. Mitogens, stresses, and growth factors stimulate the activation of MAPK kinase (MAPKK) kinases (MAPKKKs) by either receptor-dependent or independent mechanisms, followed by phosphorylation and activation of the produced MAPKK and then MAPKs. The activated MAPKs give rise to phosphorylation and activation of specific target protein kinases[7].

The dithiocarbamates are antioxidants with powerful NF-κB inhibitory influence. Pyrrolidine dithiocarbamate (PDTC) which is the most effective one, attenuates inflammation, as manifested by a marked drop in polymorphonuclear cell infiltration, iNOS activity, with a subsequent decline in NO production, in addition to decrease in lipid peroxidation and interleukin-1β and TNF-α levels[8].

Dipeptidyl peptidase-4 is considered as a key regulator of incretin hormones, which inactivates gut incretin hormone GLP-1 and boosts insulin release *via* enhancing β cell proliferation and diminishing its apoptosis[9]. Dipeptidyl peptidase-4 inhibitors decrease IFN-γ and IL-2 generation so they suppress both mitogen/antigen-stimulated T-cell activation and proliferation[10]. Moreover, they trigger AMP-activated protein kinase signaling[11].

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats (180-200 g) were kept in the standard environmental condition: (23 ± 1) ℃, (55 ± 5)% humidity and 12-h light:12-h dark cycle with free access to water and a standard laboratory diet. Animal care and experimental protocol acceptance was obtained from the Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Cairo. All procedures were approved on December 26, 2016, by the research ethics committee of experimental and clinical studies, Faculty of Pharmacy, University of Cairo, Egypt (PT1886).

2.2. Drugs

Thioacetamide (TAA), saxagliptin, PDTC and sulfasalazine were obtained from Sigma-Aldrich (USA). TAA was freshly prepared by being dissolved in distilled water and was given by rectal instillation (4% solution)[12]. Sulfasalazine, PDTC and saxagliptin were dissolved in 1% methylcellulose, normal saline and ethanol, respectively and given to rats by oral gavage using oral feeding tube in the following doses 500, 100 and 10 mg/kg, respectively[13-15].

2.3. Experimental design

Rats were randomly assigned to five groups, each with eight rats: Group 1 received vehicle; Group 2 received TAA 4% single dose by rectal instillation (TAA group); Group 3 received sulfasalazine (500 mg/kg) orally for 14 d[13]; Group 4 received PDTC (100 mg/kg) orally for 14 d[14], and Group 5 received saxagliptin (10 mg/kg) orally for 14 d[15]. The induction of colitis was performed on day 8 in all groups except for vehicle group by rectal instillation of TAA.

2.4. Induction of colitis

TAA was used to induce experimental colitis, after 36 hours of fasting with free tap water access. Rats were exposed to light anesthesia with ether. About 3 mm diameter polyethylene catheter was fixed in a 1-mL syringe, and inserted into the colon, the catheter's tip was 8 cm proximal to the anus, in a head-down position. About 0.4 mL of TAA, dissolved in distilled water solution (4% solution v/v), was instilled slowly into the lumen of the colon, in a head-down position. The catheter was held in its place for 30 s and then gently removed after delivering the required dose. Each rat was kept in this position for another 30 s in order to prevent the leakage of the solution being instilled. Afterward, treated rats were kept in cages and allowed to have free access to water and food till the day of sacrifice.

2.5. Assessment of body weight change

In order to determine whether the experimental colitis affected body weight or not, rats weights were immediately recorded before colitis induction on day 8 and just before euthanization on the last day of the experiment. The difference between the two weights expresses the change in animal body weight.

2.6. Preparation of tissue samples

The homogenization of the distal colon specimen was performed in ice-cold saline, by glass homogenizer (Heidolph Diax 900, Germany) and 10% (w/v) homogenate was obtained. Cooling centrifuge (Hettich universal 32A, Germany) was used to centrifuge this homogenate at 13 000 rpm for 30 min at 4 ℃ to get rid of cell debris and nuclei. The resultant supernatant was used for the evaluation of biochemical parameters: MAPK, phosphorylated extracellular signal-regulated kinase (PERK), cAMP response element-binding protein (CREB), glucagon-like peptide-1 (GLP-1), and inflammatory markers: interleukin (IL)-12 and β-defensin. in

addition to oxidative stress parameters, iNOS. Moreover, caspase-3, a marker of apoptosis, was also assayed.

2.7. Biochemical assessment

2.7.1. Western blotting analysis for PERK, MAPK and CREB

Protein expressions of MAPK, PERK and CREB were detected using Western blotting technique. Briefly, tissues of colons were homogenized in radioimmunoprecipitation assay buffer (50 mM TrisHCl pH 8, 1% Triton X-100, 150 mM NaCl, 0.5% sodium deoxycholate, and 0.1% SDS) provided with phosphatase inhibitor cocktail. After protein extraction, identical quantities of protein were loaded on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for separation and transported to nitrocellulose membrane and then blocked with 5% skimmed milk. Afterward, each blot was incubated with a primary antibody, including anti-PERK antibody (catalog No. MBS2550028, 1:500), anti-P38 MAPK antibody (catalog No. MBS834557, 1:1 000), anti-CREB antibody (catalog No. MBS475580, 1:2 000) and anti β-actin antibody (catalog No. MBS9400413, 1:3 000) on a roller shaker overnight at 4 ℃. All were supplied by MyBioSource, Inc. USA. Then, they were washed and incubated with HRP-conjugated goat anti-rabbit IgG (Novus Biologicals, USA). Lastly, the blots were constructed using the enhanced chemiluminescence (BIO-RAD, USA catalog No. 170-5060), the band intensity of the target proteins was read by image analysis software after standardization by β actin on the Chemi Doc MP imager (BIO-RAD, USA).

2.7.2. Determination of inflammatory parameters, GLP-1 and caspase-3

The colonic contents of IL-12 and β-defensin, GLP-1 and caspase-3 were quantitatively analyzed by enzyme-linked immunosorbent assay (ELISA) kits which were obtained from MyBioSource, Inc. USA, (catalog No. MBS705214, MBS030876, MBS2501740 and MBS700575, respectively) according to manufacturer's instructions.

2.7.3. Determination of colonic iNOS by quantitative real-time PCR

Qiagen tissue extraction kit (Qiagen, USA, catalog No. PPR75758A) was used to isolate total RNA, and the obtained RNA purity was verified spectrophotometrically at OD 260/280 nm (Beckman, Spectrophotometer, USA). The reverse transcription of an equal amount of extracted RNA into cDNA was performed by using special RT-PCR kit (Fermentas, USA). Quantitative RT-PCR was accomplished by SYBR Green Master Mix (Applied Biosystems, USA) as the manufacturer designed. Briefly, 5 μL of cDNA was mixed with 5.5 μL RNase free water, 12.5 μL SYBR Green mixture and 2 μL of each primer in a 25 μL reaction volume. The used sequences of primers are shown in Table 1.

PCR conditions were adjusted as follows: 95 ℃ (15 s) denaturation, 60 ℃ (60 s) annealing and 72 ℃ (60 s) extension for 40 cycles. Relative expression of the studied mRNA gene was evaluated by comparative Ct method. All values were normalized to *β-actin* (the household gene) and recorded as fold change over background levels.

Table 1. The sequences of primers.

Primer name	Primer sequences
iNOS	
Forward	5′-GACCAGAAACTGTC-′3
Reverse	5′-CGAACATCGAACGTCTCACA-′3
β-actin	
Forward	5′-GGTCGGTGTGAACGGATTTGG-′3
Reverse	5′-ATGTAGGCCATGAGGTCCACC-′3

2.8. Histopathological examination

Randomly selected colons from two to three rats of each group were examined histopathologically. Colons were fixed in 10% formalin overnight and incorporated in paraffin. Serial sections (4 μm thick) were incised, stained with hematoxylin/eosin and subsequently tested under a light microscope. Adobe Photoshop version 8.0 was used for capturing and processing images.

2.9. Statistical analysis

The data were expressed as mean ± SD. In order to compare the means, a one-way variance analysis (ANOVA) was used, followed by Tukey's multiple comparisons test. The significance level was established at $P < 0.05$. Graph Pad Prism software was the chosen program for statistical analysis.

3. Results

3.1. Body weight changes after treatment with PDTC and saxagliptin

Single rectal instillation of TAA (4% solution) caused body weight reduction [(55.00 ± 5.30) g]. Oral treatment with sulfasalazine for two weeks significantly improved TAA-induced decrease in body weight [(38.13 ± 4.50) g]. PDTC and saxagliptin also significantly decreased body weight loss to (33.00 ± 2.07) g and (35.25 ± 1.04) g, respectively ($P < 0.05$).

3.2. PERK, MAPK and CREB levels after treatment with PDTC and saxagliptin

Compared to the normal group, TAA significantly boosted colonic PERK, CREB and MAPK levels, whereas both PDTC and saxagliptin treatments prevented TAA-induced elevation in PERK, MAPK and CREB levels. Moreover, sulfasalazine treatment significantly decreased PERK, MAPK and CREB levels (Figure 1).

3.3. IL-12 and β-defensin levels after treatment with PDTC and saxagliptin

The induction of UC significantly increased colonic IL-12 and β-defensin contents, compared to the normal control group. Treatment with sulfasalazine, PDTC and saxagliptin revealed a marked decline in IL-12 and β-defensin levels compared to TAA group (Figure 2).

3.4. iNOS activity after treatment with PDTC and saxagliptin

TAA-treated group increased iNOS activity significantly compared to the normal control, while treatment with sulfasalazine, PDTC and saxagliptin showed a significant decline in TAA-induced elevation in iNOS activity. Moreover, PDTC and saxagliptin treatments decreased significantly iNOS activity compared to sulfasalazine group (Figure 3).

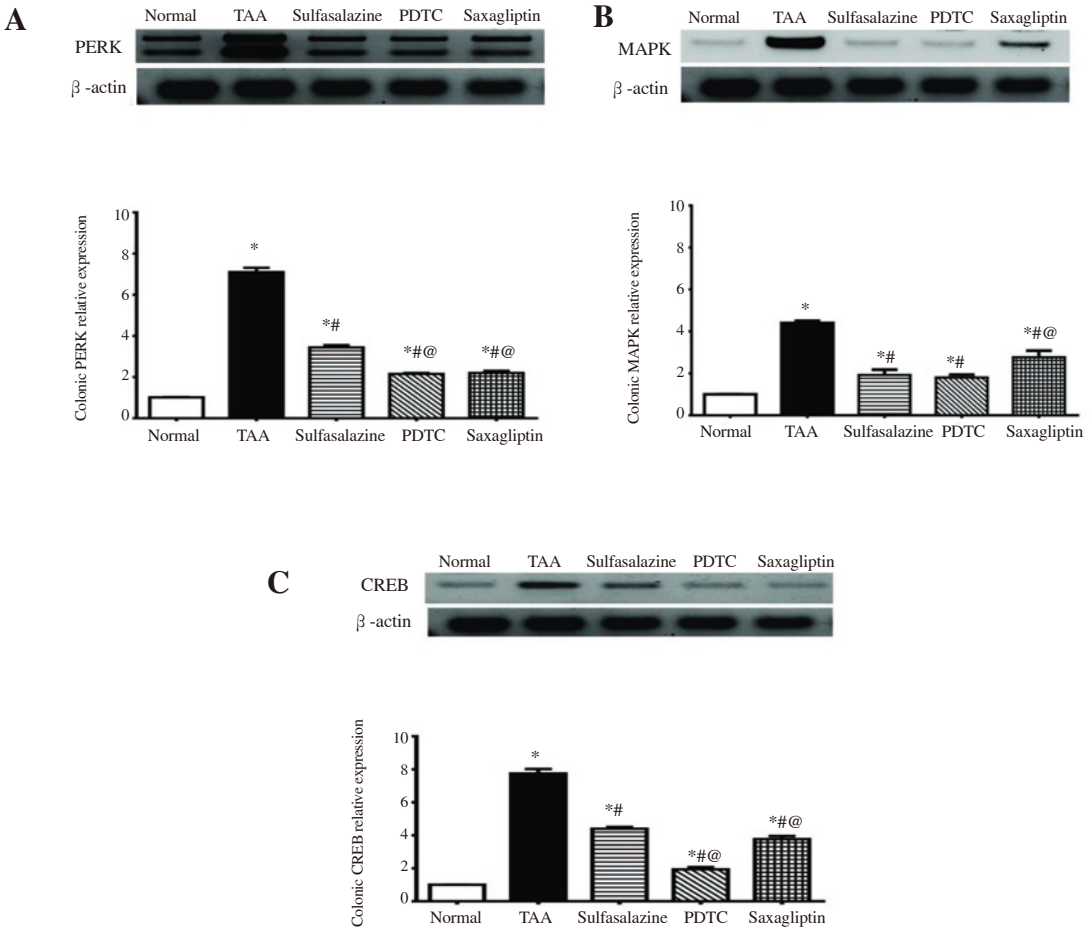

Figure 1. Effect of pyrrolidine dithiocarbamate (PDTC) and saxagliptin treatments on gene expression of phosphorylated extracellular signal-regulated kinase (PERK) (A), mitogen-activated protein kinase (MAPK) (B) and colonic cAMP response element-binding protein (CREB) (C) in thioacetamide (TAA)-induced colitis. β -Actin was used as a loading control. Each value represents mean ± SD (n=8). $^{*}P < 0.05$ *vs* normal control, $^{\#}P < 0.05$ *vs* TAA-treated group and $^{@}P < 0.05$ *vs* sulfasalazine-treated group.

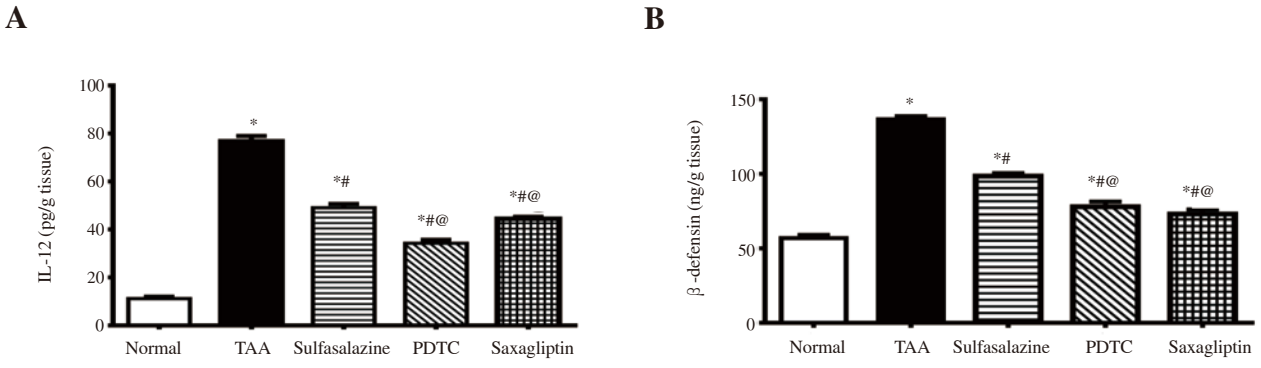

Figure 2. Effect of pyrrolidine dithiocarbamate (PDTC) and saxagliptin treatments on IL-12 (A) and β -defensin (B), in thioacetamide (TAA)-induced colitis. Each value represents mean ± SD (n=8). $^{*}P < 0.05$ *vs* normal control, $^{\#}P < 0.05$ *vs* TAA-treated group and $^{@}P < 0.05$ *vs* sulfasalazine-treated group.

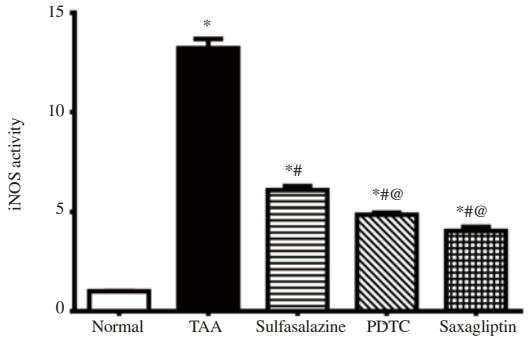

Figure 3. Effect of pyrrolidine dithiocarbamate (PDTC) and saxagliptin treatments on inducible nitric oxide synthase (iNOS) in thioacetamide (TAA)-induced colitis. Each value represents mean ± SD (n=8). $^{*}P < 0.05$ *vs* normal control, $^{\#}P < 0.05$ *vs* TAA-treated group and $^{@}P < 0.05$ *vs* sulfasalazine-treated group.

3.5. GLP-1 content after treatment with PDTC and saxagliptin

TAA decreased GLP-1 content significantly compared to the normal control, whereas all treatment showed an increase in GLP-1 content compared to the TAA-treated group. Moreover, PDTC showed a significant increase in GLP-1 content compared to sulfasalazine group (Figure 4).

3.6. Caspase-3 level after treatment with PDTC and saxagliptin

Caspase-3 level was significantly elevated after instillation of TAA compared to the normal group, while treatments with sulfasalazine, PDTC and saxagliptin resulted in a reduction of caspase-3 content compared to the TAA group. Furthermore, PDTC caused a significant decrement in caspase-3 level compared to sulfasalazine group (Figure 5).

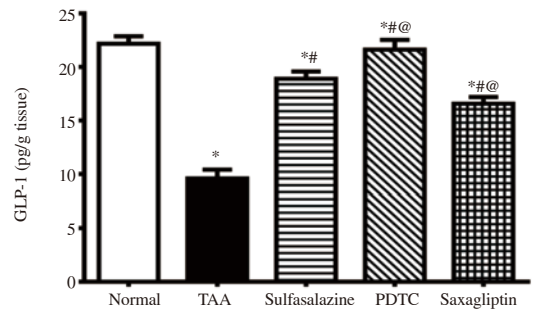

Figure 4. Effect of pyrrolidine dithiocarbamate (PDTC) and saxagliptin treatments on glucagon like peptide (GLP)-1 in thioacetamide (TAA)-induced colitis. Each value represents mean ± SD (n=8). $^{*}P < 0.05$ *vs* normal control, $^{\#}P < 0.05$ *vs* TAA-treated group and $^{@}P < 0.05$ *vs* sulfasalazine-treated group.

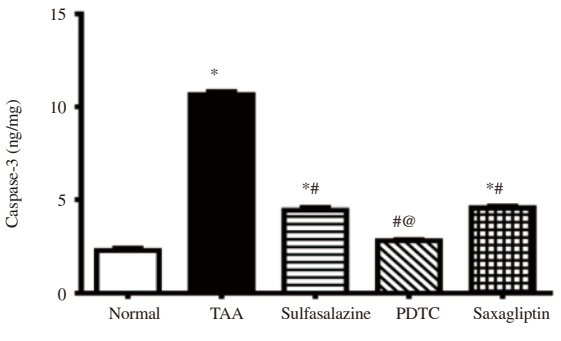

Figure 5. Effect of pyrrolidine dithiocarbamate (PDTC) and saxagliptin treatments on caspase-3 in thioacetamide (TAA)-induced colitis. Each value represents mean ± SD (n=8). $^{*}P < 0.05$ *vs* normal control, $^{\#}P < 0.05$ *vs* TAA-treated group and $^{@}P < 0.05$ *vs* sulfasalazine-treated group.

3.7. Histophathological changes of colon tissue after treatment with sulfasalazine, PDTC and saxagliptin in TAA-induced UC

All treatments ameliorated TAA-induced colonic histopathological changes (necrosis, edema, congestion and ulcer), with the best effects produced by PDTC treatment (Figure 6).

4. Discussion

The first aim of medical remedy for UC is to enhance the quality of life with the least amount of steroids. The effectiveness of PDTC and saxagliptin in UC rat model was studied for 14 d compared to sulfasalazine. One of the symptoms of UC that leads to a decline in body weight is bloody diarrhea. The present study showed a marked drop in body weight following TAA administration, this was in line with a previous study[16].

People with UC suffers from loss of appetite and animal models exhibit body weight loss, loose stool, bloody diarrhea or rectal irritation, and hematochezia[17]. PDTC improved symptoms and significantly increased body weight, as it decreased inflammatory signaling in the muscle. On the other hand, it enhanced protein turnover as it elevated protein synthesis in the muscle that is consistent with previous studies[18,19]. In addition, suppression of NF- κ B activity by PDTC reduces inflammation and enhances cell proliferation and tissue repair[19]. Saxagliptin, which increases the concentration of active GLP-1 and inhibits its degradation, caused a marked increase in body weight. Saxagliptin caused a decline in body weight drop *via* increasing appetite, insulin secretion and decreasing secretion of glucagon[20], as dipeptidyl peptidase-4 inhibitors were found to facilitate the restoration of mucosal damage and the acceleration of healing[21].

Transcription factor NF- κ B has a vital role in inflammation and

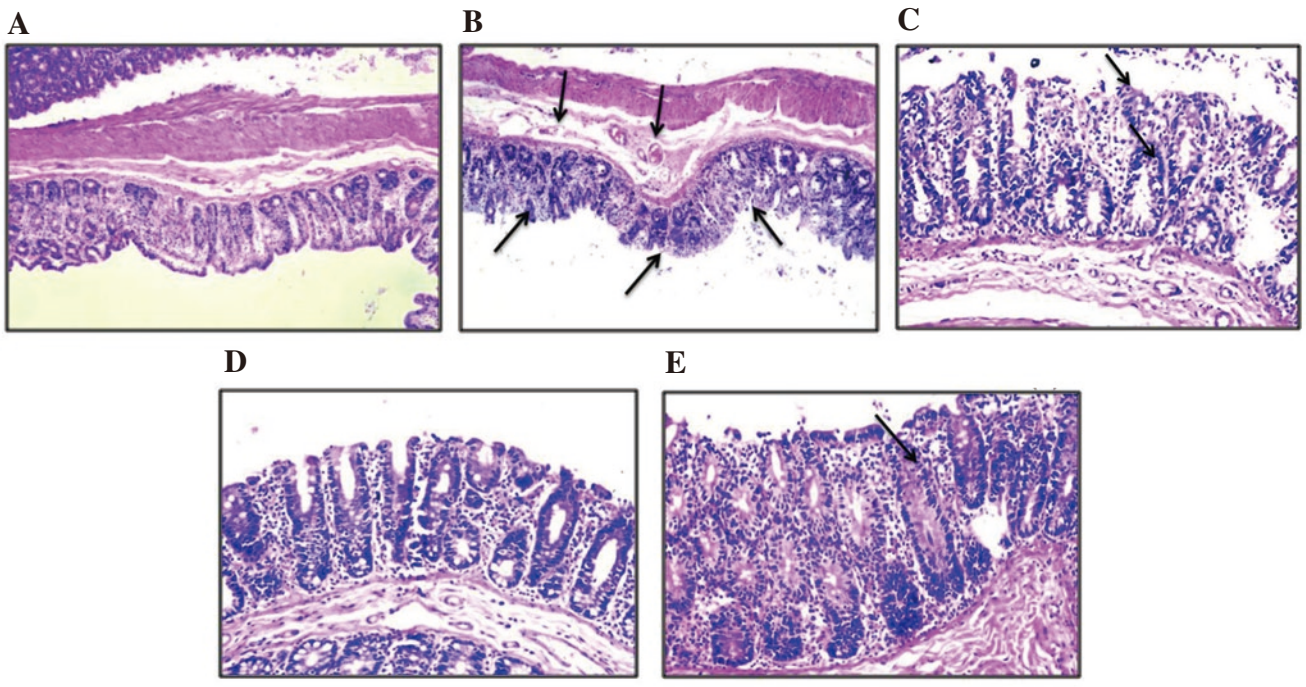

Figure 6. Effect of sulfasalazine, pyrrolidine dithiocarbamate and saxagliptin on histopathological changes in experimental ulcerative colitis in rats' colons. (A) Colon of rats in the normal control group shows no histopathological changes (H & E, × 40). (B) Colon of rats with ulcerative colitis shows ulceration, necrosis, inflammatory cells infiltration and edema with congestion of the blood vessels (H & E, × 16). (C) Colon of rats treated with sulfasalazine shows ulceration in the lining mucosa with inflammatory cells infiltration (H & E, × 40). (D) Colon of rats treated with pyrrolidine dithiocarbamate shows intact mucosal lining epithelium (H & E, × 40). (E) Colon of rats treated with saxagliptin shows inflammatory cells infiltration in the lamina propria (H & E, × 40).

immunity, it is highly activated and expressed in macrophages and epithelial cells of inflamed gut. Besides, it causes transcription of multiple pro-inflammatory genes leading to increased TNF- α , IL-1, IL-6 along with regulation of IL-12 and IL-23 expression[3]. The increased production in pro-inflammatory cytokines and chemokines leads to further exacerbation of inflammation and propagation of oxidative cascade causing DNA and cell damage[22]. NF- κ B induces iNOS mRNA expression, therefore it enhances nitric oxide (NO) production[23]. NF- κ B results in increased ROS production and ROS activates cell-signaling proteins including MAPK and ERK by modifying protein kinases[24]. In cellular processes, ROS oxidizes sulfhydryl (SH) groups of cysteine residues in protein kinases, and then activated protein kinases phosphorylate their target proteins. Additionally, the transcription of various apoptosis-related genes such as caspase-3 mRNAs and caspase-3 protein is mediated by NF- κ B expression[25].

Results of this study revealed a remarkable increase in PERK in UC which is in line with the study of de David *et al*[26], as well as MAPK and CREB contents. Pathogenesis of TAA-induced UC is attributed to oxidative stress (OS), through its metabolism to a reactive metabolite which induces the production of ROS and causes OS[27], this metabolite binds to macromolecules and then increases the production of ROS, which can cause DNA damage and protein dysfunction, gene mutations and finally cell dying. Accumulated ROS behaves as chemical messengers activating different pathways of signaling, like NF- κ B, CREB, p38 MAPK and ERK[7]. The antioxidant properties and consequent inhibition of NF- κ B pathway by PDTC and saxagliptin could be the main mechanism leading to significant declines in MAPK, PERK and CREB levels[18,28,29].

In the current study, TAA instillation increased IL-12 and β -defensin compared to the normal control group. In UC, overproduction of pro-inflammatory cytokines is attributed to NF- κ B pathway activation[1]. However, PDTC and saxagliptin treatment significantly decreased IL-12 and β -defensin levels. A previous study reported that NF- κ B inhibitory effect of saxagliptin resulted in inhibition of both T-cell proliferation and cytokine production[30].

Moreover, the TAA-induced UC showed a remarkable rise in iNOS activity, and this is consistent with that found in the study of Lukivskaya *et al*[31]. iNOS-mediated NO production causes immune response impairment, leading to chronic inflammatory diseases. In epithelial cells, UC is associated with up-regulation of iNOS that causes intestinal inflammation to be initiated and maintained[2]. iNOS activity was decreased by PDTC and saxagliptin, and NO production was found to be decreased by PDTC due to the attenuation of the expression of iNOS[32]. In a recent study, saxagliptin was found to suppress the increased expression of iNOS, NF- κ B pathway activation, along with NO and pro-inflammatory cytokine production[33].

Apoptosis was recognized as a vital key in the UC pathogenesis. Apoptosis starts by activating caspases that cleave specific proteins and trigger programmed cell death[34]. In this study, the group treated with TAA showed an increase in caspase-3 content, which is in line with a previous study[35]. But treatment with PDTC decreased caspase-3 content. Therefore, PDTC decreased apoptotic cell count,

increased the expression of anti-apoptotic factors and the apoptotic protease[25]. In addition, saxagliptin reduced caspase-3 content as previously reported in another study[33]. This effect may be attributed to GLP-1 which inhibited apoptosis in β cells and enhanced its proliferation[36] by increasing anti-apoptotic gene expression, and this might be *via* NF-κB-dependent effect on apoptotic genes transcription[37].

Compared to the normal group, a significant decline in GLP-1 level was found in the current study following TAA administration. Inflammatory bowel disease is linked with elevated interleukins and pro-inflammatory cytokines production[38]. This can be useful for GLP-1 therapy in treating chronic inflammatory disorders since it suppresses inflammatory cytokines, increases anti-inflammatory mediators and suppresses tissues immune cells infiltration[39], including T cell activation gene-3, macrophage colony stimulating factor, TNF-α, IL-1α and stromal cell-derived factor-1[40]. Saxagliptin therapy ameliorates UC by increasing GLP-1 level. These findings are in agreement with recent studies[21,41]. Moreover, PDTC also increased GLP-1 levels. In chronic diseases such as diabetes, NF-κB is activated[42]. An earlier study reported that transfection was increased by incorporating of NF-κB binding sites into GLP-1 plasmid[43], that increasing GLP-1 expression as the binding sites of NF-κB was occupied, so PDTC may increase GLP-1 level by its NF-κB inhibitory effect[44].

In conclusion, the results show that PDTC and saxagliptin can provide anti-inflammatory effects, immunomodulation, and possibly antioxidant activity. Further studies are needed to evaluate and elucidate the effectiveness of PDTC and saxagliptin.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

The authors would like to thank Prof. Dr. Adel Bakeer (Pathology Department, Faculty of Veterinary Medicine, Cairo University, Egypt) for his assistance in the histopathological examinations.

References

[1] Ordas I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet* 2012; **380**(9853): 1606-1619. Doi: 10.1016/S0140-6736(12)60150-0.

[2] Kolios G, Valatas V, Ward SG. Nitric oxide in inflammatory bowel disease: A universal messenger in an unsolved puzzle. *Immunology* 2004; **113**(4): 427-437. Doi: 10.1111/j.1365-2567.2004.01984.x.

[3] Atreya I, Atreya R, Neurath MF. NF-kappaB in inflammatory bowel disease. *J Intern Med* 2008; **263**(6): 591-596. Doi: 10.1111/j.1365-2796.2008.01953.x.

[4] Balmus IM, Ciobica A, Trifan A, Stanciu C. The implications of oxidative stress and antioxidant therapies in Inflammatory Bowel Disease: Clinical aspects and animal models. *Saudi J Gastroenterol* 2016; **22**(1): 3-17. Doi: 10.4103/1319-3767.173753.

[5] Yasukawa K, Tokuda H, Tun X, Utsumi H, Yamada KI. The detrimental effect of nitric oxide on tissue is associated with inflammatory events in the vascular endothelium and neutrophils in mice with dextran sodium sulfate-induced colitis. *Free Radical Res* 2012; **46**(12): 1427-1436. Doi: 10.3109/10715762.2012.732698.

[6] Topcu-Tarladacalisir Y, Akpolat M, Uz YH, Kizilay G, Sapmaz-Metin M, Cerkezkayabekir A, et al. Effects of curcumin on apoptosis and oxidoinflammatory regulation in a rat model of acetic acid-induced colitis: The roles of c-Jun N-terminal kinase and p38 mitogen-activated protein kinase. *J Med Food* 2013; **16**(4): 296-305. Doi: 10.1089/jmf.2012.2550.

[7] Wang Z, Li S, Cao Y, Tian X, Zeng R, Liao DF, et al. Oxidative stress and carbonyl lesions in ulcerative colitis and associated colorectal cancer. *Oxid Med Cell Longev* 2016; **2016**: 15. Doi: 10.1155/2016/9875298.

[8] Cuzzocrea S, Chatterjee PK, Mazzon E, Dugo L, Serraino I, Britti D, et al. Pyrrolidine dithiocarbamate attenuates the development of acute and chronic inflammation. *Br J Pharmacol* 2002; **135**(2): 496-510. Doi: 10.1038/sj.bjp.0704463.

[9] Perry T, Greig NH. The glucagon-like peptides: A new genre in therapeutic targets for intervention in Alzheimer's disease. *J Alzheimers Dis* 2002; **4**(6): 487-496.

[10]Schon E, Demuth HU, Eichmann E, Horst HJ, Korner IJ, Kopp J, et al. Dipeptidyl peptidase IV in human T lymphocytes. Impaired induction of interleukin 2 and gamma interferon due to specific inhibition of dipeptidyl peptidase IV. *Scand J Immunol* 1989; **29**(2): 127-132.

[11]Zeng Y, Li C, Guan M, Zheng Z, Li J, Xu W, et al. The DPP-4 inhibitor sitagliptin attenuates the progress of atherosclerosis in apolipoprotein-E-knockout mice *via* AMPK- and MAPK-dependent mechanisms. *Cardiovasc Diabetol* 2014; **13**: 32. Doi: 10.1186/1475-2840-13-32.

[12]Salem HA, Wadie W. Effect of niacin on inflammation and angiogenesis in a murine model of ulcerative colitis. *Sci Rep* 2017; **7**(1): 7139. Doi: 10.1038/s41598-017-07280-y.

[13]Mustafa A, El-Medany A, Hagar HH, El-Medany G. *Ginkgo biloba* attenuates mucosal damage in a rat model of ulcerative colitis. *Pharmacol Res* 2006; **53**(4): 324-330. Doi: 10.1016/j.phrs.2005.12.010.

[14]Li S, Zhong S, Zeng K, Luo Y, Zhang F, Sun X, et al. Blockade of NF-κB by pyrrolidine dithiocarbamate attenuates myocardial inflammatory response and ventricular dysfunction following coronary microembolization induced by homologous microthrombi in rats. *Basic Res Cardiol* 2009; **105**(1): 139. Doi: 10.1007/s00395-009-0067-6.

[15]Connelly KA, Advani A, Zhang Y, Advani SL, Kabir G, Abadeh A, et al. Dipeptidyl peptidase-4 inhibition improves cardiac function in experimental myocardial infarction: Role of stromal cell-derived factor-1alpha. *J Diabetes* 2016; **8**(1): 63-75. Doi: 10.1111/1753-0407.12258.

[16]Hao H, Zhang L, Jiang S, Sun S, Gong P, Xie Y, et al. Thioacetamide intoxication triggers transcriptional up-regulation but enzyme inactivation of UDP-glucuronosyltransferases. *Drug Metab Dispos* 2011; **39**(10): 1815-1822. Doi: 10.1124/dmd.111.039172.

[17]AlSharari SD, Akbarali HI, Abdullah RA, Shahab O, Auttachoat W,

Ferreira GA, et al. Novel insights on the effect of nicotine in a murine colitis model. *J Pharmacol Exp Ther* 2013; **344**(1): 207-217. Doi: 10.1124/jpet.112.198796.

[18]Narsale AA, Puppa MJ, Hardee JP, VanderVeen BN, Enos RT, Murphy EA, et al. Short-term pyrrolidine dithiocarbamate administration attenuates cachexia-induced alterations to muscle and liver in ApcMin/+ mice. *Oncotarget* 2016; **7**(37): 59482-59502. Doi: 10.18632/oncotarget.10699.

[19]Hirata I, Yasumoto S, Toshina K, Inoue T, Nishikawa T, Murano N, et al. Evaluation of the effect of pyrrolidine dithiocarbamate in suppressing inflammation in mice with dextran sodium sulfate-induced colitis. *World J Gastroenterol* 2007; **13**(11): 1666-1671.

[20]Dave DJ. Saxagliptin: A dipeptidyl peptidase-4 inhibitor in the treatment of type 2 diabetes mellitus. *J Pharmacol Pharmacother* 2011; **2**(4): 230-235. Doi: 10.4103/0976-500X.85934.

[21]Abrahami D, Douros A, Yin H, Yu OHY, Renoux C, Bitton A, et al. Dipeptidyl peptidase-4 inhibitors and incidence of inflammatory bowel disease among patients with type 2 diabetes: Population based cohort study. *BMJ* 2018; **360**: k872. Doi: 10.1136/bmj.k872.

[22]Saxena A, Kaur K, Hegde S, Kalekhan FM, Baliga MS, Fayad R. Dietary agents and phytochemicals in the prevention and treatment of experimental ulcerative colitis. *J Tradit Complement Med* 2014; **4**(4): 203-217. Doi: 10.4103/2225-4110.139111.

[23]Abd Allah ESH, Makboul R, Mohamed AO. Role of serotonin and nuclear factor-kappa B in the ameliorative effect of ginger on acetic acid-induced colitis. *Pathophysiology* 2016; **23**(1): 35-42. Doi: 10.1016/j.pathophys.2015.12.001.

[24]Son Y, Cheong YK, Kim NH, Chung HT, Kang DG, Pae HO. Mitogen-activated protein kinases and reactive oxygen species: How can ROS activate MAPK pathways? *J Signal Transduct* 2011; **2011**: 792639. Doi: 10.1155/2011/792639.

[25]Kan S, Zhou H, Jin C, Yang H. Effects of PDTC on NF-kappaB expression and apoptosis in rats with severe acute pancreatitis-associated lung injury. *Int J Clin Exp Med* 2015; **8**(3): 3258-3270.

[26]de David C, Rodrigues G, Bona S, Meurer L, Gonzalez-Gallego J, Tunon MJ, et al. Role of quercetin in preventing thioacetamide-induced liver injury in rats. *Toxicol Pathol* 2011; **39**(6): 949-957. Doi: 10.1177/0192623311418680.

[27]Koen YM, Sarma D, Hajovsky H, Galeva NA, Williams TD, Staudinger JL, et al. Protein targets of thioacetamide metabolites in rat hepatocytes. *Chem Res Toxicol* 2013; **26**(4): 564-574. Doi: 10.1021/tx400001x.

[28]Cuzzocrea S, Rossi A, Pisano B, Di Paola R, Genovese T, Patel NS, et al. Pyrrolidine dithiocarbamate attenuates the development of organ failure induced by zymosan in mice. *Intens Care Med* 2003; **29**(11): 2016-2025. Doi: 10.1007/s00134-003-1887-8.

[29]Huisamen B, Genade S, Lochner A. Signalling pathways activated by glucagon-like peptide-1 (7-36) amide in the rat heart and their role in protection against ischaemia. *Cardiovasc J Afr* 2008; **19**(2): 77-83.

[30]Yazbeck R, Howarth GS, Abbott CA. Dipeptidyl peptidase inhibitors, an emerging drug class for inflammatory disease? *Trends Pharmacol Sci* 2009; **30**(11): 600-607. Doi: 10.1016/j.tips.2009.08.003.

[31]Lukivskaya O, Patsenker E, Lis R, Buko VU. Inhibition of inducible nitric oxide synthase activity prevents liver recovery in rat thioacetamide-induced fibrosis reversal. *Eur J Clin Invest* 2008; **38**(5): 317-325. Doi: 10.1111/j.1365-2362.2008.01941.x.

[32]Fan YH, Zhao LY, Zheng QS, Dong H, Wang HC, Yang XD. Arginine vasopressin increases iNOS-NO system activity in cardiac fibroblasts through NF-kappaB activation and its relation with myocardial fibrosis. *Life Sci* 2007; **81**(4): 327-335. Doi: 10.1016/j.lfs.2007.05.018.

[33]Nassar NN, Al-Shorbagy MY, Arab HH, Abdallah DM. Saxagliptin: A novel antiparkinsonian approach. *Neuropharmacology* 2015; **89**: 308-317. Doi: 10.1016/j.neuropharm.2014.10.007.

[34]Ola MS, Nawaz M, Ahsan H. Role of Bcl-2 family proteins and caspases in the regulation of apoptosis. *Mol Cell Biochem* 2011; **351**(1-2): 41-58. Doi: 10.1007/s11010-010-0709-x.

[35]Shalkami AS, Hassan M, Bakr AG. Anti-inflammatory, antioxidant and anti-apoptotic activity of diosmin in acetic acid-induced ulcerative colitis. *Hum Exp Toxicol* 2018; **37**(1): 78-86. Doi: 10.1177/0960327117694075.

[36]Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noushmehr H, et al. Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 2003; **144**(12): 5149-5158. Doi: 10.1210/en.2003-0323.

[37]Harkavyi A, Whitton PS. Glucagon-like peptide 1 receptor stimulation as a means of neuroprotection. *Br J Pharmacol* 2010; **159**(3): 495-501. Doi: 10.1111/j.1476-5381.2009.00486.x.

[38]Brestoff JR, Artis D. Immune regulation of metabolic homeostasis in health and disease. *Cell* 2015; **161**(1): 146-160. Doi: 10.1016/j.cell.2015.02.022.

[39]Lee YS, Jun HS. Anti-inflammatory effects of GLP-1-based therapies beyond glucose control. *Mediat Inflamm* 2016; **2016**: 11. Doi: 10.1155/2016/3094642.

[40]Al-Dwairi A, Alqudah TE, Al-Shboul O, Alqudah M, Mustafa AG, Alfaqih MA. Glucagon-like peptide-1 exerts anti-inflammatory effects on mouse colon smooth muscle cells through the cyclic adenosine monophosphate/nuclear factor-kappaB pathway *in vitro*. *J Inflamm Res* 2018; **11**: 95-109. Doi: 10.2147/JIR.S152835.

[41]Zietek T, Rath E. Inflammation meets metabolic disease: Gut feeling mediated by GLP-1. *Front Immunol* 2016; **7**: 154. Doi: 10.3389/fimmu.2016.00154.

[42]Barnes PJ, Karin M. Nuclear factor-kappaB: A pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997; **336**(15): 1066-1071. Doi: 10.1056/NEJM199704103361506.

[43]Mesika A, Grigoreva I, Zohar M, Reich Z. A regulated, NFkappaB-assisted import of plasmid DNA into mammalian cell nuclei. *Mol Ther* 2001; **3**(5): 653-657. Doi: 10.1006/mthe.2001.0312.

[44]Choi S, Oh S, Lee M, Kim SW. Glucagon-like peptide-1 plasmid construction and delivery for the treatment of type 2 diabetes. *Mol Ther* 2005; **12**(5): 885-891. Doi: 10.1016/j.ymthe.2005.03.039.