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Effect of Indole-3-carbinol and/or Metformin on Female Patients with Ulcerative Colitis (Premalignant Condition): Role of Oxidative Stress, Apoptosis and Proinflammatory Cytokines

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Abstract Ulcerative colitis is an inflammatory bowel disease that causes long-lasting inflammation and ulcers in the gastrointestinal tract. It affects the innermost lining of the colon and rectum. The aim of this work was to study the effect of indole-3-carbinol (I3C) or metformin alone and in combination on female patients suffering from ulcerative colitis. Forty female patients were divided as follows: 10 patients represent the normal control group and the other 30 patients with ulcerative colitis were divided into 3 equal groups; metformin treated group, I3C treated group and metformin + I3C treated group. A colonoscopic biopsy was homogenized for determination of tissue tumor necrosis factor alpha (TNF-α), transforming growth factor beta-1 (TGF-β1), malondialdehyde (MDA), catalase (CAT) and myeloperoxidase (MPO). The biopsy was also subjected to histopathological and immunohistochemical examination. Administration of each of metformin or I3C alone and in combination to ulcerative colitis patients induced significant increase in tissue CAT with significant decrease in the colonic endoscopic score, tissue TNF-α, TGF-β1, MDA and MPO and alleviated the histopathological and immunohistochemical changes compared to the same parameters before treatment. Metformin/I3C combination produced significant improvement in the biochemical, histopathological and immunohistochemical parameters compared to the groups that received either metformin or I3C alone. In conclusion, metformin and I3C had protective effects on female patients suffering from ulcerative colitis but their combination had the upper hand.

Keywords: *indole-3-carbinol, metformin, ulcerative colitis, female, patients*

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1. Introduction

Ulcerative colitis (UC) is a premalignant condition of the colon that includes characteristic ulcers [1]. It usually arises due to a combination of environmental and genetic variations together with alteration in bacterial flora which can drive a dysregulated immune response resulting in chronic intestinal inflammation [2]. Several studies reported that the increased production of proinflammatory cytokines together with induction of oxidative stress may play a crucial role in the pathogenesis of UC [3,4]. Treatment of UC usually includes anti-inflammatory drugs, immunosuppressive drugs, biological therapy and even surgical removal of a part of the colon in severe resistant cases [5]. The agents used in treatment of UC may cause serious adverse effects which necessitate the search for alternative drugs with less severe deleterious effects [6].

Metformin is one of the biguanides used widely in treatment of type 2 diabetes mellitus [7]. Recently, Metformin was proven to decrease the harmful effects of the reactive oxygen species in various tissues of the body. Also, metformin has the ability to modulate the expression of the proinflammatory cytokines at both the biochemical and gene expression levels [8]. These properties may create a potential role for metformin in management of UC, possibly through suppression of nuclear factor-κB activation in intestinal epithelial cells and inhibition of the STAT3 signaling pathway [9,10].

Indole-3-carbinol (I3C) is produced by the breakdown of the glucosinolate glucobrassicin, which can be found at relatively high levels in cruciferous vegetables such as broccoli, cabbage, cauliflower and kale. It is also

available in dietary supplements [11]. Recent studies suggested that I3C might have potent protective effects against colitis, possibly due to its antioxidant and anti-inflammatory properties together with its ability to decrease the level of immune cell infiltration found in the large intestine of cases with colitis [12]. Moreover, this effect was suggested to be sex-specific, being more pronounced in females than males [13]. The aim of this work was to study the effect of I3C or metformin alone and in combination on female patients suffering from UC.

2. Patients and Methods

The studied subjects were selected from the inpatient and outpatient clinics of tropical medicine and internal medicine departments, Tanta University Hospital, Tanta, Egypt. This study was conducted in accordance with the World Medical Association Declaration of Helsinki for human subjects and was approved by the local ethics committee. All participants gave us their written informed consent before enrollment in this study.

2.1. Inclusion Criteria

This study was carried out on 30 female patients with ulcerative colitis and 10 individuals whose colonoscopic and histopathologic findings were normal as control. Ulcerative colitis patients were diagnosed on the basis of clinical, endoscopic and histological manifestations according to the criteria of American Gastroenterology Association [14]. The age of the patients ranged from 20 to 46 years with the mean age of 33±4.6 years.

2.2. Exclusion Criteria

The following categories were excluded from the study: Pregnancy, malignancy, heart failure, renal failure, thyroid disorders, acute infection, stroke and patients taking immunosuppressive drugs or traditional drugs used for treatment of UC.

2.3. Drugs Used

Metformin was purchased from Sigma Chemicals (St. Louis MO, USA). I3C was purchased from Sigma-Aldrich (St. Louis, MO). The drugs were then prepared as capsules at Taif University laboratory, Taif, Saudi Arabia. All other chemicals and reagents used were purchased from Sigma Chemical company (St. Louis MO, USA).

2.4. Study Groups

The subjects involved in this study were divided into 4 equal groups as follows:

Group I: Included ten patients with UC treated with metformin in a dose of 850 mg twice daily orally for 3 months [15].

Group II: Included ten patients with UC treated with I3C in a dose of 400 mg twice daily orally for 3 months [16].

Group III: Included ten patients with UC who were treated with metformin in a dose of 850 mg twice daily orally concomitantly with I3C in a dose of 400 mg twice daily orally for 3 months.

Group IV: Included ten individuals whose colonoscopic and histopathologic findings were normal as control.

All the patients and control were subjected to complete history taking and thorough clinical examination. Laboratory investigations including complete blood picture, blood urea and serum creatinine, erythrocyte sedimentation rate (ESR) and stool examination were performed to exclude bacterial causes of colitis.

2.5. Evaluation of Colitis

Colonoscopy was performed in all groups and the severity of the disease was determined. An endoscopic scoring system for UC of Pineton de Chambrun et al. [17] was used. Score O: Normal or inactive disease; Score 1: Mild disease (erythema, decreased vascular pattern and mild friability); Score 2: Moderate disease (marked erythema, increased vascular pattern, friability and erosion); Score 3: Severe disease (spontaneous bleeding and ulceration). Endoscopic findings were recorded before beginning treatment and at the end of the study.

2.6. Histopathological and Immunohistochemical Examination

Multiple biopsies were taken from the colon for histopathological and IL-23 p19 immunohistochemical staining. 4-µm-thick serial sections of formalin fixed, paraffin-embedded tissue were cut and stained by hematoxylin and eosin (H&E) for histopathological evaluation of the groups. The severity of UC was assessed using a histological disease score described by Hirata et al. [18] (Table 1).

Table 1. Histological disease score

Grade 0	Normal colonic mucosa
Grade 1	Loss of one-third of the crypts
Grade 2	Loss of two-thirds of the crypts
Grade 3	The lamina propria is covered with a single layer of epithelium and mild inflammatory cell infiltration is present
Grade 4	Erosions and marked inflammatory cell infiltration are present

Randomly selected 8 fields (magnified 100 times) in each section were inspected and graded as above by a pathologist who was blinded to the treatment protocol. The mean in each section was calculated by scoring the grades in 8 fields.

Apoptotic index, which represents aggregate percentages of apoptotic cells and/or apoptotic bodies per total number of cells (1000 cells counted) in 10 randomly selected high power fields (×400), was counted by one pathologist. In 20% of randomly selected cases, the counting was repeated by another pathologist. In cases with significant disagreement between results, the counting was performed at the multiheaded microscope by both pathologists. The morphological criteria for apoptotic bodies applied in this study were followed according to Staunton and Gaffney [19].

4-µm-thick serial sections of formalin fixed, paraffin-embedded tissue were cut and mounted on positively

charged glass slides. After incubation at 60°C overnight and deparaffinization, sections were placed in 0.01 M sodium citrate buffer (pH 6.0) and heated twice for 5 minutes in a microwave oven. After inactivation of endogenous peroxidase with 0.5% metaperiodic acid in phosphate-buffered saline (PBS) for 10 minutes, sections were incubated with 10% horse serum in PBS for 1 hour. Sections were incubated at 4°C overnight with 100× diluted primary goat anti-IL-23 p19 antibody (R&D Systems, Inc.). The standard avidin-biotin peroxidase complex (ABC) technique was performed using the LabVision Secondary Detection Kit (UltraVision Detection System Anti-polyvalent, HRP). The color was visualized by incubation with chromogen 3, 3' diaminobenzidine for 5 minutes. The slides were then counterstained with Mayer hematoxylin and cover slipped with Permount (StatLab, McKinney, TX). Negative controls were set for each test without the primary antibodies. Results were expressed semi-quantitatively. Positively stained cells were counted by examining at least 10 random fields in each section and expressed as the percentage of positive cells over total cell number [20].

2.7. Assessment of the Biochemical Parameters

Parts of the colonic biopsies were homogenized in 10 volumes of ice-cold 10% trichloroacetic acid and centrifuged at 3000 rpm for 15 minutes at 4°C. The supernatant was removed and recentrifuged at 15000 rpm at 4°C for 8 min. The resulting supernatant was used for determination of tissue catalase (CAT) level according to Higgins et al. [21], tissue malondialdehyde (MDA) level according to the method of Casini et al. [22], tissue myeloperoxidase (MPO) activity according to the method of Bradley et al. [23], tissue TNF-α using ELISA kits according to the instructions of the manufacturer, tissue TGF-β1 using kits supplied by Uscn Life Science Inc. Wuhan, according to the instructions of the manufacturer and tissue total protein content according to the method of Lowry et al. [24].

2.8. Statistical Analysis

Data were presented as mean ± standard error of mean (SEM). Data were statistically analyzed by computer SPSS version 16.0 software. Data were analyzed by one way analysis of normality of variance (ANOVA). Independent sample t-test and Covariate test were used as statistical tests. Comparison of numerical variables between the study groups was performed using Mann–Whitney test to compare independent samples from two groups. Differences between the means of different groups were considered significant at a level of p-value less than 0.05.

3. Results

3.1. Effect of Different Treatments on the Clinical and Laboratory Data of the Studied Group

UC patients showed significant increase in the abdominal pain, diarrhea, blood in the stool and ESR compared to the control group. Administration of metformin and/or I3C to patients with UC resulted in significant decrease in the abdominal pain, diarrhea, blood in the stool and ESR compared to the same data of these groups before treatment. The decrease in the abdominal pain, diarrhea, blood in the stool and ESR was significant in metformin/ I3C combination group compared to the use of either metformin or I3C alone (Table 2).

3.2. Effect of Different Treatments on the Colonic Endoscopic Score

UC patients showed significant increase in the endoscopic score compared to the control group. Administration of metformin and/or I3C to patients with UC resulted in significant decrease in the endoscopic score compared to the endoscopic score of the same groups before treatment. The decrease in the endoscopic score was significant in metformin/ I3C combination group compared to the use of either metformin or I3C alone (Figure 1).

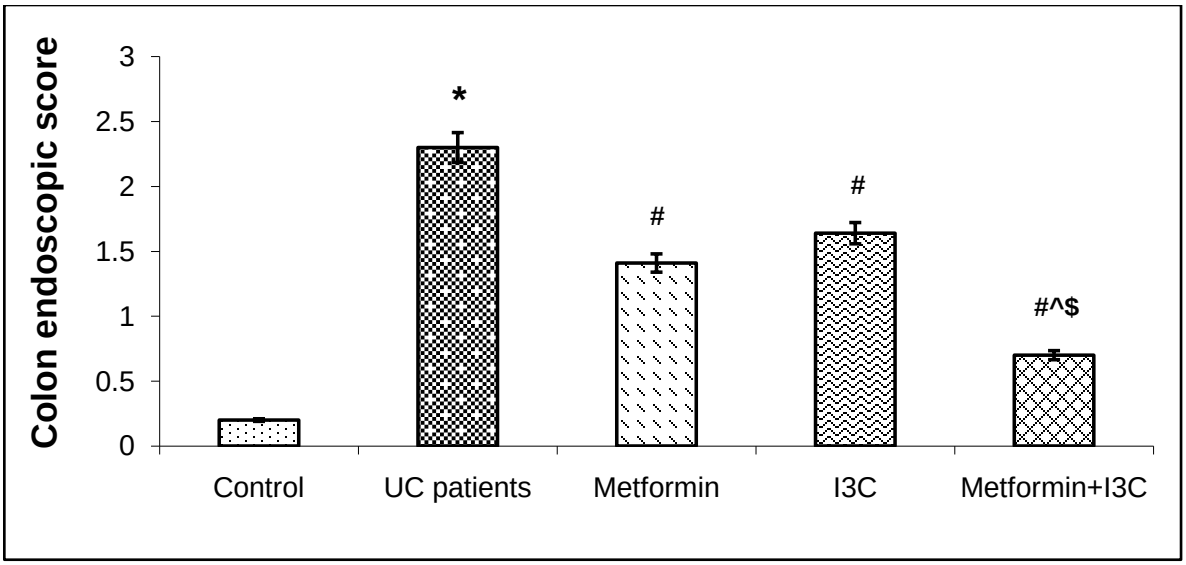
3.3. Effect of Different Treatments on Tissue Antioxidant Status

UC patients showed significant increase in tissue MDA with significant decrease in tissue CAT compared to the control group. Administration of metformin and/or I3C to patients with UC resulted in significant decrease in tissue MDA with significant increase in tissue CAT compared to the same data of these groups before treatment. The decrease in tissue MDA and the increase in tissue CAT were significant in metformin/I3C combination group compared to the use of either metformin or I3C alone (Table 3).

Table 2. Effect of different treatments on the clinical and laboratory data of the studied group

	Control	UC patients before treatment	UC patients treated with metformin	UC patients treated with I3C	UC patients treated with metformin/I3C combination
Abdominal pain	2/10	26/30 [a] (p=0.008)	5/10 [b] (p=0.01)	5/10 [b] (p=0.01)	3/10[bcd] (p_1=0.007; p_2=0.048; p_3=0.048)
Diarrhea	2/10	23/30 [a] (p=0.006)	5/10 [b] (p=0.018)	6/10 [b] (p=0.022)	4/10[bcd] (p_1=0.008; p_2=0.049; p_3=0.045)
Blood in stool	1/10	21/30 [a] (p=0.004)	5/10 [b] (p=0.02)	5/10 [b] (p=0.02)	3/10[bcd] (p_1=0.013; p_2=0.048; p_3=0.048)
ESR (mm/hr)	10.2± 0.5	38.4±0.8[a] (p=0.02)	22.8±0.7[b] (p=0.032)	26.12±0.64[b] (p=0.039)	17.5±0.5[bcd] (p_1=0.024; p_2=0.04; p_3=0.031)

[a] Significant compared to the control group (p<0.05)
[b] Significant compared to UC patients before treatment (p<0.05)
[c] Significant compared to UC patients treated with metformin (p<0.05)
[d] Significant compared to UC patients treated with I3C (p<0.05)
P_1 refers to metformin/I3C combination group compared to UC patients before treatment.
P_2 refers to metformin/I3C combination group compared to UC patients treated with metformin.
P_3 refers to metformin/I3C combination group compared to UC patients treated with I3C.



*Significant compared to the control group (p< 0.05)
Significant compared to UC patients before treatment (p< 0.05)
^ Significant compared to UC patients treated with metformin (p< 0.05)
$ Significant compared to UC patients treated with I3C (p< 0.05)

Figure 1. Effect of different treatments on the colon endoscopic score

Table 3. Effect of different treatments on catalase (CAT), malondialdehyde (MDA), myeloperoxidase (MPO), tumor necrosis factor alpha (TNF-α), transforming growth factor beta 1 (TGF-β1) and histological disease score

	Control	UC patients before treatment	UC patients treated with metformin	UC patients treated with I3C	UC patients treated with metformin/I3C combination
Tissue CAT (μmol/g tissue)	2.23±0.05	0.89±0.04[a] (p=0.03)	1.53±0.05[b] (p=0.029)	1.4±0.06[b] (p=0.033)	1.81±0.05[bcd] (p_1=0.025; p_2=0.043; p_3=0.04)
Tissue MDA (nmol/g tissue)	18.3±0.7	43.1±0.81[a] (p=0.022)	28.51±0.5[b] (p=0.019)	26.4±0.8[b] (p=0.018)	22.2±0.6[bcd] (p_1=0.016; p_2=0.032; p_3=0.038)
Tissue MPO (U/g tissue)	72.2 ±3.6	165.1±5.8[a] (p=0.02)	112.6±4.2[b] (p=0.029)	123.4±4.15[b] (p=0.037)	91.5±5.6[bcd] (p_1=0.021; p_2=0.042; p_3=0.037)
Tissue TNF-α (pg/mg protein)	67.4±4.1	385.6±9.6[a] (p=0.007)	195.3±7.4[b] (p=0.024)	253.2±8.1[b] (p=0.032)	149.8±6.3[bcd] (p_1=0.018; p_2=0.04; p_3=0.032)
Tissue TGF-β1 (pg/μg protein)	7.4±0.4	33.2±0.8[a] (p=0.016)	21.1±0.6[b] (p=0.035)	23.6±0.7[b] (p=0.036)	16.2±0.4[bcd] (p_1=0.016; p_2=0.039; p_3=0.035)
Histological disease score	0.00	2.03±0.06[a] (p<0.001)	0.8±0.03[b] (p=0.023)	1.12±0.04[b] (p=0.03)	0.54±0.03[bcd] (p_1=0.015; p_2=0.03; p_3=0.04)

[a] Significant compared to the control group (p<0.05)
[b] Significant compared to UC patients before treatment (p<0.05)
[c] Significant compared to UC patients treated with metformin (p<0.05)
[d] Significant compared to UC patients treated with I3C (p<0.05)
P_1 refers to metformin/I3C combination group compared to UC patients before treatment; P_2 refers to metformin/I3C combination group compared to UC patients treated with metformin; P_3 refers to metformin/I3C combination group compared to UC patients treated with I3C.

3.4. Effect of Different Treatments on Tissue MPO

UC patients showed significant increase in tissue MPO compared to the control group. Administration of metformin and/or I3C to patients with UC resulted in significant decrease in tissue MPO compared to tissue MPO of the same groups before treatment. The decrease in tissue MPO was significant in metformin/I3C combination group compared to the use of either metformin or I3C alone (Table 3).

3.5. Effect of Different Treatments on Tissue TNF-α and TGF-β1

UC patients showed significant increase in tissue TNF-α and TGF-β1 compared to the control group. Administration of metformin and/or I3C to patients with UC resulted in significant decrease in tissue TNF-α and TGF-β1 compared to tissue TNF-α and TGF-β1 of the same groups before treatment. The decrease in tissue TNF-α and TGF-β1 was significant in metformin/I3C combination group compared to the use of either metformin or I3C alone (Table 3).

3.6. Histopathological and Immunohistochemical Results

UC patients showed significant increase in the histological disease score and IL-23 p19 expression with significant decrease in the apoptotic index compared to the control group. Administration of metformin and/or I3C to patients with UC resulted in significant decrease in the histological disease score and IL-23 p19 expression with significant

increase in the apoptotic index compared to the same data in these groups before treatment. The decrease in the histological disease score and IL-23 p19 expression with the increase in the apoptotic index were significant in metformin/I3C combination group compared to the use of either metformin or I3C alone (Table 3, Table 4; Figure 2, Figure 3).

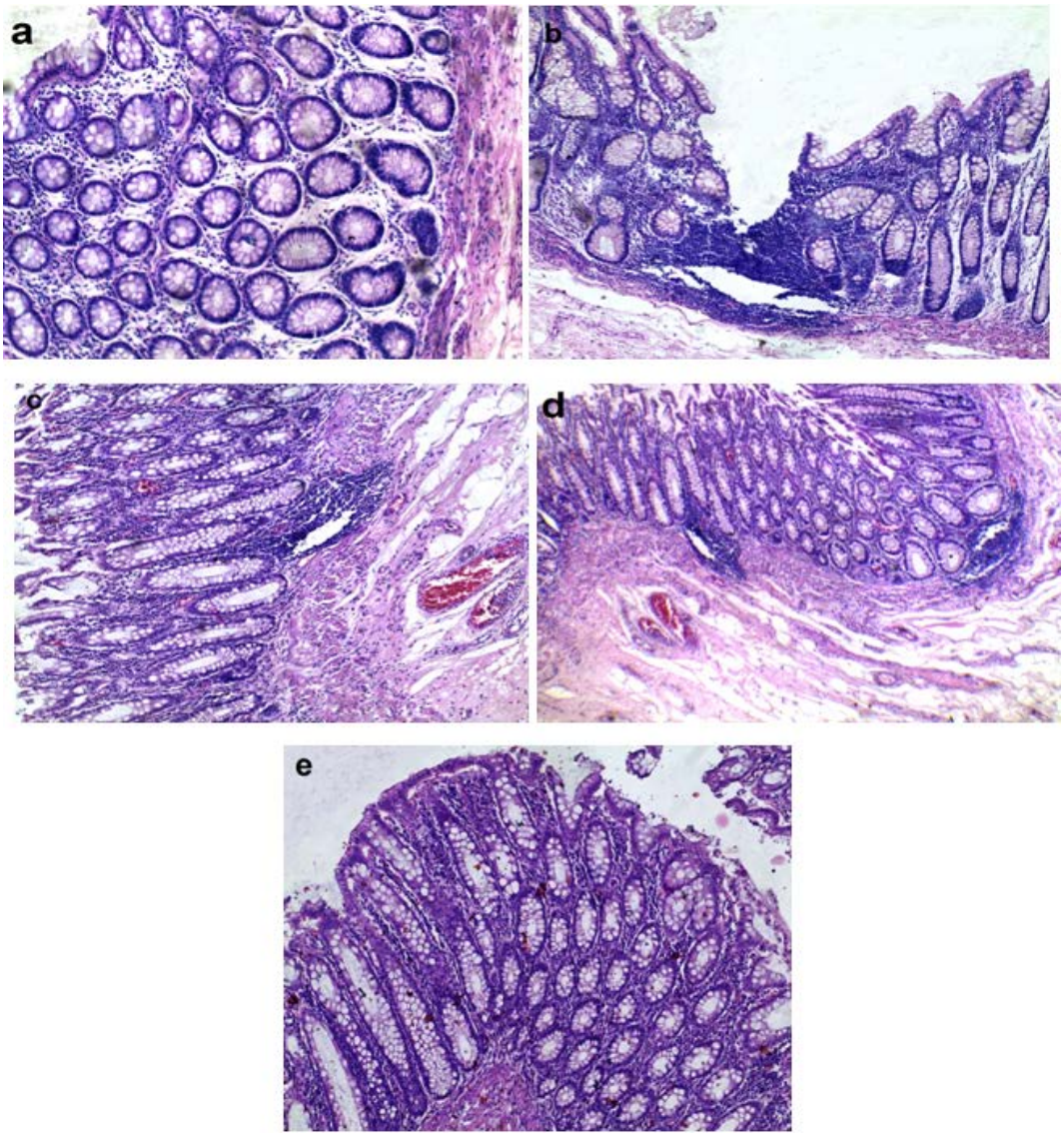


Figure 2. H&E stained sections from the colon of **a)** The control group showing normal appearance of the colonic mucosa, crypts and the mucosal glands; **b)** UC patients before treatment with mucosal ulceration, massive infiltration with neutrophils with severe destruction of the crypts and the mucosal glands; **c)** Metformin treated group with intact mucosal surface, mild to moderate inflammatory cellular infiltration with congested blood vessels in the submucosa; **d)** I3C treated group with minimal mucosal ulceration, increased goblet cell population and moderate inflammatory cellular infiltration; **e)** Metformin/I3C treated group with apparently normal mucosal glands and crypts with marked decrease in the inflammatory cellular infiltration (H&E X200)

Table 4. The effect of different treatments on the apoptotic index in the studied groups.

Group	Apoptotic index (%)
Normal control	7.4±0.2
UC patients before treatment	1.2±0.04[a] (p=0.02)
UC patients treated with metformin	4.7 ± 0.18 [b] (p=0.023)
UC patients treated with I3C	4.2 ± 0.21[b] (p=0.027)
UC patients treated with metformin/I3C combination	5.8 ± 0.25[bcd] (p_1=0.015; p_2=0.04; p_3=0.038)

Apoptotic index (%) was calculated for sections of the colon (values presented as the mean ± SEM of an average of 10 fields).
[a] Significant compared to the control group (p<0.05)
[b] Significant compared to UC patients before treatment (p<0.05)
[c] Significant compared to UC patients treated with metformin (p<0.05)
[d] Significant compared to UC patients treated with I3C (p<0.05)
P_1 refers to metformin/I3C combination group compared to UC patients before treatment.
P_2 refers to metformin/I3C combination group compared to UC patients treated with metformin.
P_3 refers to metformin/I3C combination group compared to UC patients treated with I3C.

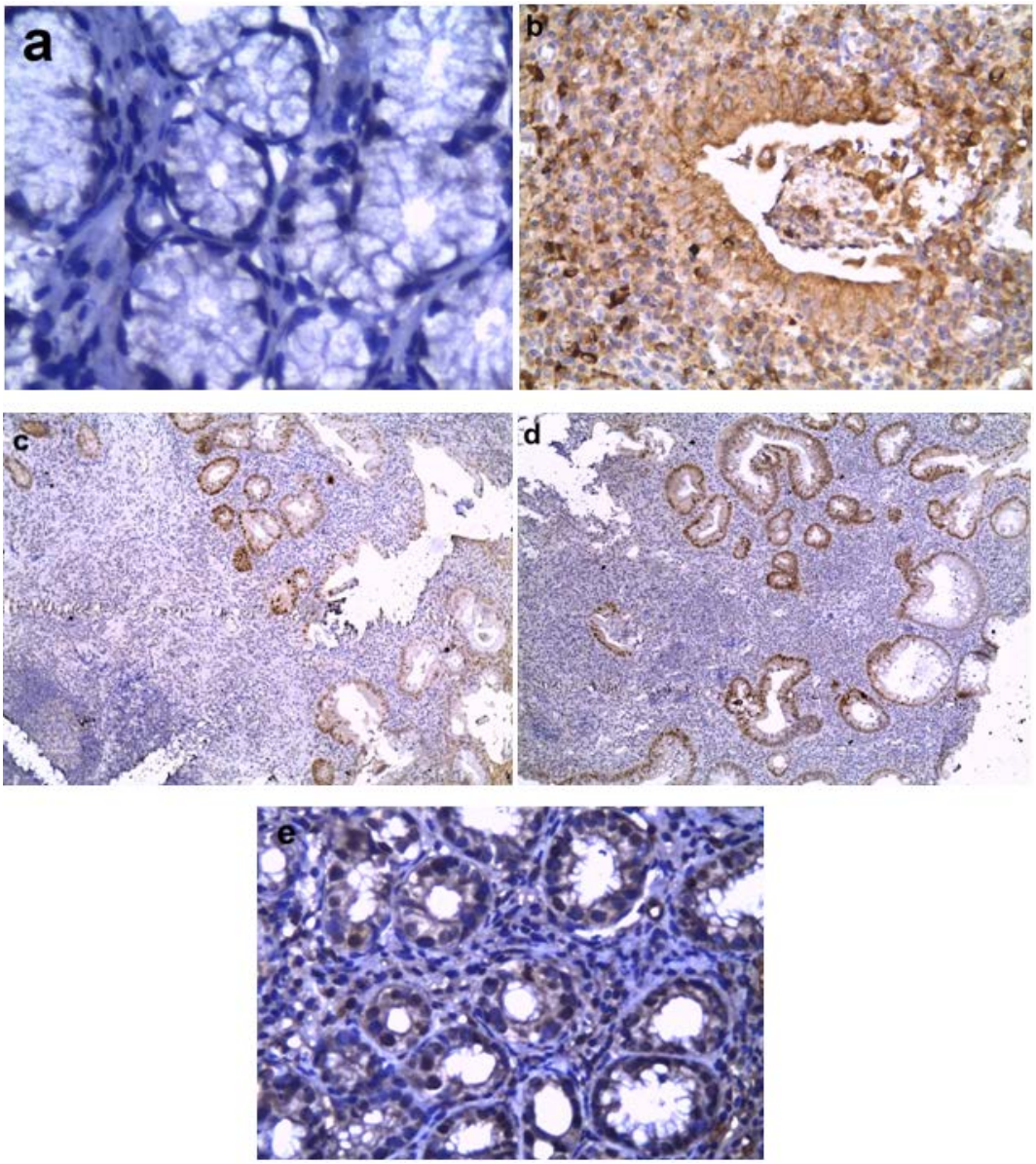


Figure 3. A photomicrograph of the immunohistochemical expression of IL23p19 in **a)** The control group showing very minimal expression; **b)** UC patients before treatment showing extensive expression; **c)** Metformin treated group with moderate expression; **d)** I3C treated group with moderate expression; **e)** Metformin/I3C treated group with mild expression of IL23p19 (Anti-IL-23 p19 X400)

4. Discussion

Ulcerative colitis (UC) is one of the inflammatory bowel diseases that primarily involves the mucosa and submucosa of the colon. The exact etiology of UC is still unknown but overgrowth of the pathogenic microorganisms in the colon, overproduction of the pro-inflammatory cytokines, induction of oxidative stress and affection of the immune system were suggested as predisposing factors [6]. In the present study, patients with UC showed significant decrease in tissue CAT and the apoptotic index with significant increase in the colonic endoscopic score, tissue TNF-α, TGF-β1, MDA, MPO, tissue IL-23 p19 expression and the histological disease score compared to the control group which support the role of oxidative stress and inflammation in the pathophysiology of UC.

Oxidative stress was thought to play a crucial role in the pathogenesis of UC. Chronic intestinal inflammation found in UC is usually associated with overproduction of both reactive oxygen and reactive nitrogen species (ROS and RNS) leading to oxidative and nitrosative stress, respectively [25]. In UC, there is usually an imbalance between ROS and antioxidant activity which creates oxidative stress. Once they are formed, ROS interact with the molecular complexes inducing cellular oxidative damage which can affect lipids, proteins, and nucleic acids leading to formation of lipid peroxides, enzymatic dysfunction and DNA strand breaks [26]. This was in the same line with the results of the present study where UC patients showed significant increase in tissue MDA with significant decrease in tissue CAT compared to the control group.

TGF-β1 is considered as the cornerstone mediator of UC. It was proven to induce ROS production by suppressing antioxidants defenses such as CAT and reduced glutathione and activates oxidases in cell membranes and mitochondria leading to release of H_2O_2 to the extracellular space which contributes to the development of UC [27]. Moreover, TGF-β1 may induce the production of proinflammatory cytokines such as TNF-α, IL-23 and

IL-17 which in turn contributes to the inflammatory process encountered with UC [28]. This was in agreement with the results of the present study where UC patients showed significant increase in tissue TGF-β1 with in turn increases the levels of tissue TNF-α and IL-23 compared to the control group.

Interleukin 23 (IL-23) is a member of the proinflammatory cytokines, consisting of a p19 subunit and a common p40 subunit. IL-23 plays a critical role in the pathogenesis of a number of immune-mediated inflammatory diseases including UC by recruitment of several inflammatory cells [29]. IL-23 is essential for the differentiation of Th17 lymphocytes which are implicated in chronic inflammatory/autoimmune diseases. IL-23 was proven to promote TNF-α, IL-17 and IL-6 production by Th17 cells which are associated with the induction of autoimmune inflammation [30]. Our study showed significant increase in the expression of IL-23p 19 protein associated with significant increase in tissue TNF-α in patients with UC compared with the control group which was similar to the results reported by Liu et al. [20] and El-Bassat et al. [31].

MPO is an enzyme contained within neutrophils, monocytes and macrophages. It is a member of the peroxidases that produces hypochlorous acid from hydrogen peroxide (H_2O_2) and chloride anion during the neutrophil's activity. Hypochlorous acid is cytotoxic and may cause oxidative damage in host tissues [32]. Moreover, the release of MPO in UC was correlated to an enhanced release of the neutrophil activating peptide interleukin-8 (IL8) which plays a vital role in the inflammatory bowel diseases. The levels of MPO in active UC patients were increased significantly and correlated with laboratory parameters and endoscopic grade of inflammation. A paired analysis showed a decrease in MPO levels after the resolution of disease exacerbation [33]. This was in accordance with the results of our study where patients with UC showed significant increase in tissue MPO compared with the control group.

Metformin is one of the biguanides that is considered the most widely used drug for treatment of type 2 diabetes. In the present study, administration of metformin to UC patients resulted in significant increase in tissue CAT with significant decrease in colonic endoscopic score, tissue TNF-α, TGF-β1, MDA and MPO and alleviated the histopathological and immunohistochemical changes compared to the same parameters before treatment. These effects might be attributed to the antioxidant, anti-inflammatory and apoptosis inducing properties of metformin [34]. Lee et al. [10] reported that metformin ameliorates inflammatory bowel disease by suppression of the STAT3 signaling and regulation of the balance between Th17/Treg. Park et al. [35] found that metformin, by activating AMP kinase enzyme, reduces TGF-β1 expression which in turn may affect the expression of TNF-α and IL-23 leading to amelioration of the inflammatory process encountered in UC. Moreover, metformin may inhibit the inflammatory angiogenesis through attenuation of the main components of the fibrovascular tissue and inhibition of the expression of TGF-β1 and MPO [36].

I3C is one of the phytochemicals that is found in large amounts in cruciferous vegetables. In the present study, I3C administration to UC patients resulted in significant increase in tissue CAT with significant decrease in colonic endoscopic score, tissue TNF-α, TGF-β1, MDA and MPO and alleviated the histopathological and immunohistochemical changes compared to the same parameters before treatment. These results were in agreement with Busbee et al. [12] and Benson et al. [13] who reported that I3C has antioxidant, anti-inflammatory, antiproliferative and apoptosis inducing properties.

I3C was reported to have anti-inflammatory effects by inhibiting production of the proinflammatory cytokines, thereby inhibiting the expression of nuclear factor-κB, TNF-α and inducible nitric oxide synthase [37]. Moreover, I3C has the ability to act as a scavenger of free radicals and to induce the activity of various antioxidant enzymes such as CAT, superoxide dismutase and glutathione peroxidase [38]. Also, I3C was reported to inhibit MPO activity and TGF-β1 expression which may contribute to its anti-inflammatory effects in cases of UC [39,40].

In the present study, the improvement in the biochemical, histopathological and immunohistochemical parameters was significant in the group that received metformin/I3C combination compared to the groups that received either metformin or I3C alone. This might be due to the synergistic anti-inflammatory and antioxidant properties of metformin/I3C combination together with their ability to inhibit TGF-β1 and IL-23 p19 expression and to affect apoptosis of colonic mucosal cells.

5. Conclusion

The present study demonstrated that metformin and I3C have protective effect in female patients with UC due to their anti-inflammatory and antioxidant properties together with their inhibitory effects on TGF-β1 and IL-23 p19 expression in the colon with affection of apoptosis but metformin/I3C combination had the upper hand. So, it is recommended to use metformin/I3C combination as an adjuvant agent in treatment of UC to increase the efficacy and decrease the adverse effects of the traditional drugs.

Conflict of Interest

The authors declare that there is no conflict of interest.

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