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RESEARCH ARTICLE

A Comparative Study on the Efficacy of Natural and Synthetic NF-κB Inhibitors on the Treatment of Experimentally Induced Ulcerative Colitis in Rats

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Abstract

Background and Purpose: Ulcerative Colitis (UC) is a long-term, inflammatory condition characterized by episodes of relapse and remission, affecting millions of people worldwide. The Nuclear Factor kappa B (NF-κB) serves as a key factor and biomarker in the inflammatory context, significantly influencing the course of mucosal inflammation in UC. This work aims to explore the relative potency and compare the efficacy of curcumin and resveratrol (natural NF-κB inhibitors) to mesalazine, a standard treatment for ulcerative colitis (synthetic inhibitor of NF-κB), in a rat model of acetic acid-induced colitis. **Methods:** Forty male Wistar rats were randomly divided into 5 groups, with eight animals in each group. Group I was the standard control group, and the remaining 32 rats received a rectal injection of 2 mL of 4% acetic acid solution to induce UC. They were then randomly subdivided into 4 groups: Group IIA (untreated ulcerative colitis group), Group IIB (mesalazine-treated ulcerative colitis group), Group IIC (resveratrol-treated ulcerative colitis group), and Group IID (curcumin-treated ulcerative colitis group). Oxidative stress and pro-inflammatory markers were assessed one week after UC induction or treatments. **Results:** This study showed that the treatment with natural NF-kB inhibitors, namely curcumin and resveratrol, was superior to the conventional mesalazine therapy in lowering malondialdehyde (MDA) and inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 17 (IL-17), and NF-kB in the colonic tissue. **Conclusion:** Natural NF-kB inhibitors, namely curcumin and resveratrol, are superior to the conventional mesalazine therapy in the treatment of UC.

Major Findings: Curcumin was superior to resveratrol in reducing oxidative stress and inflammatory markers associated with the disease. Histological examination of the colonic tissue demonstrated almost normal mucosa architecture with no inflammation or ulceration in the curcumin-treated group.

Keywords: Curcumin, NF-kB Inhibitors, Resveratrol, Ulcerative Colitis

1. Introduction

Ulcerative Colitis (UC) is one of the Inflammatory Bowel Diseases (IBD) primarily affecting the rectum, ascending colon, or both. Globally, IBD exhibits a widespread presence, with a consistent occurrence in developed regions and a significant rise in incidence and prevalence in newly industrialized regions[1]. Although seldom fatal, severe cases can result in infections, surgeries, and colorectal cancer[2]. This disease significantly affects

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patient's quality of life due to its recurrent, chronic symptoms and relapsing inflammation and ulcers in the mucosa of the colon and rectum. It presents with diarrhea, mucus, pus, bloody stools, and abdominal pain, along with extra-intestinal manifestations involving the skin, mucosa, joints, eyes, lungs, and nervous system[3]. This serious healthcare issue imposes a significant financial burden on the healthcare system, with a rising incidence across all age groups[4].

Multiple factors may contribute to the development of UC, including environmental influences, genetic predispositions, compromised barrier integrity, oxidative stress, defective immune responses, and the induction of pro-inflammatory cytokines, all of which play roles in IBD pathogenesis[5]. An abnormal and ongoing immune reaction to intestinal bacteria plays a crucial role in the development of UC[6]. This immune response involves a modified T-helper-2 (Th2) response, characterized by the activation of specific cytokines including Tumor Necrosis Factor-alpha (TNF-alpha), Interleukin-1 (IL-1), IL-6, and IL-8[7].

Nuclear Factor kappa B (NF-kB) is the key up-regulator of the expression of these cytokines. Under physiological conditions, NF-κB remains inactive in the cytoplasm, bound to inhibitory proteins, IκB. Upon activation by various stimuli, such as microbial products, pro-inflammatory cytokines, and oxidative stress, the degradation of IκB, primarily mediated by IκB kinase, enables NF-κB to translocate to the nucleus and initiate the transcription of target genes involved in inflammation, and immune system activation[8]. Furthermore, TNF-α induced by NF-κB can amplify NF-κB activation, thereby intensifying the ongoing chronic inflammation in the colon[9].

NF-κB is involved in UC pathogenesis, inflammation, and mucosal damage; therefore, many treatment strategies aim to inhibit NF-κB as a key factor in the disease's development[10]. Mesalazine (mesalamine or 5-aminosalicylic acid; 5-ASA) is the first-line treatment for IBD and remains the cornerstone for mild to moderate UC therapy[11]. Mesalazine is effective in controlling active inflammation, maintaining remission, and preventing further progression. It is generally well-tolerated and safe for long-term use with flexible dosing options. The therapeutic effects of mesalazine include the inhibition of NF-κB activation, which leads to the suppression of pro-inflammatory gene expression in the inflamed colonic mucosa. However, the occasional tolerance to mesalazine therapy and the high dose required to control severe UC necessitate exploring other agents with equivalent efficacy[12].

Currently, investigating nontraditional or alternative medicine for treating various chronic inflammatory bowel disorders remains challenging, especially for patients who do not respond to or refuse standard medications. One such approach involves using food derivatives, which are generally considered less toxic. Nonetheless, the limited scientific evidence supporting their effectiveness and the insufficient understanding of their mechanisms has hindered their integration into mainstream therapy.

Resveratrol is a natural polyphenolic compound that present in grapes, berries, peanuts, and other plant sources[13]. It exhibits various pharmacological effects, including free radical scavenging, inhibition of platelet aggregation, anticancer, and anti-inflammatory actions, along with cardioprotective properties. Research shows that resveratrol successfully blocks NF-κB signaling by inhibiting the functions of NF-κB and IκB kinase[14]. Therefore, resveratrol may provide a novel approach to treating inflammation-associated diseases as UC.

The food derivative curcumin, which is the main component of *Curcuma longa*. Curcumin is used as a spice to provide the distinctive flavor and yellow color to curry[15]. In its countries of origin, turmeric has been used for centuries as a traditional remedy to treat inflammatory conditions[16]. Curcumin is widely studied for its anti-inflammatory, anti-cancer, and antioxidant properties[17]. Curcumin's anti-inflammatory effects are achieved by inhibiting NF-κB expression through regulation of the NF-κB/IkB pathway and reducing the expression of pro-inflammatory cytokines like TNF-α, IL-17. Previous research has shown its ability to attenuate inflammation in the colonic tissue of UC patients[18].

The aim of this work is to compare the efficacy of natural NF-κB inhibitor as curcumin and Resveratrol with other synthetic modalities of NF-κB inhibitors as Mesalazine to treat ulcerative colitis in rat model of acetic acid induced colitis.

2. Materials and Methods

2.1 Laboratory Animals

The study involved 40 male Wistar albino rats, 8 weeks old and weighing 200-250 grams, sourced from the

Physiology Department Animal House at Alexandria Faculty of Medicine. One week prior to and throughout the experiments, the rats were housed under standard conditions for temperature, humidity, and feeding. All procedures adhered to the guidelines approved by the Institutional Ethics Committee at the Faculty of Medicine, Alexandria University, for laboratory animal handling.

2.2 The Study Design

A total number of 40 rats were randomly allocated into 5 groups (8 animals per group). Group I: Normal Control group (NC) 8 healthy rats served as untreated controls and received 1 ml of normal saline rectally. The remaining 32 rats were injected rectally with 2 mL of 4 % acetic acid solution[19]. The rats were maintained horizontally for 2 min to avoid leakage of acetic acid. Subsequently, these rats with induced ulcerative colitis were randomly allocated into 4 groups; Group IIA (untreated-UC) rats received 2 ml of saline by oral gavage daily and served as the positive control group. Group IIB (mesalazine-treated ulcerative colitis group; Mes-UC) rats received oral mesalazine (SalofalkR 500 tab, Minapharm Egypt) in a dose of 300 mg/kg/day[20]. Group IIC (Resveratrol-treated UC group; Res-UC) rats received oral resveratrol at 10 mg/kg/day (Resveratrol MetaDrops 200 mg/ml)[21]. Group IID (Curcumin-treated UC group; Cur-UC) rats received oral curcumin (MaryRuth organics turmeric curcumin liquid drops) at 50 mg/kg/day[22]. Normal control and untreated UC groups received 2 ml of saline by oral gavage once daily in the morning. In all groups, treatment lasted for 7 days after the induction of colitis.

2.3 Animal Sacrifice and Sampling

By the 8th day, rats were anesthetized using ketamine-xylazine cocktail, then animals were sacrificed and blood samples were collected from the rats retro-orbital plexus. Blood was collected in plain non-heparinized test tubes for separation of serum by centrifugation at 3000 rpm for 15 minutes. The serum was then stored at -80^{o} C. All animals were euthanized, and a 6 cm section of the distal colon was removed from each. Approximately 1 cm of this tissue was homogenized in phosphate buffer (pH 7.4) for biochemical analysis, while the remaining colon portions were fixed in 10% formalin for histopathological examination.

Frozen parts of the colons were washed with ice-cold TBS containing tris-HCl (10 mM), NaCl (133 mM) at pH 7.4. They were then homogenized in a lysis buffer with NaCl (100 mM), EDTA (100 mM), Nonidet P-40 (0.5%), Na-deoxycholate (0.5%), and Tris (pH 7.5, 10 mM) that included a protease inhibitor cocktail. The homogenate was centrifuged for 10 minutes at 2000 g at 4° C, and the supernatant was used for protein concentration assays[23].

2.4 Macroscopic Assessment of Colonic Damage

Macroscopically visible colonic lesions, including mucosal edema, thickening, bleeding, hyperemia, erosions, shortening, and necrosis, were evaluated. The lesions were categorized as normal, mild, moderate, or severe[24]. The weight-to-length ratio of the colon was assessed.

Specimens were immediately examined under a surgical microscope (DF Vasconcelos, Sao Paulo, Brazil, 10 x magnifications). A pathologist blinded to the groups evaluated the damages using a 0-5 scale. The macroscopic colon lesion scores were defined as follows: 0, indicating no lesion; 1, for mucosal hyperemia without ulcers; 2, for linear ulcers without colonic stenosis; 3, for linear ulcers with isolated colon stenosis; 4, for ulcers in multiple colon areas; and 5, for large ulcers with necrosis and perforation.

2.5 Histopathological Examination

Colon samples from all studied groups were fixed in 10 % formalin, processed and embedded in paraffin, and 5 µm thick sections were retrieved and stained with Hematoxylin and Eosin stain. Changes related to ulcerative colitis histopathology were assessed as regards (mucosal ulceration, cryptitis, crypt abscess, dysplasia, lamina propria, submucosal involvement by inflammatory infiltrate, presence of granuloma, crypt distortion, and atrophy)[25].

2.6 Biochemical Studies

2.6.1 Assessment of Oxidative Stress Markers

Determination of lipid peroxides in the form of Malondialdehyde (MDA) in the colon. MDA was assessed in the colonic tissue homogenates. The colorimetric thiobarbituric acid reaction method was used to measure MDA, and the concentration was represented as (nmol/g protein)[26].

2.6.2 Determination of Antioxidant Markers in Tissue Homogenates

Total Antioxidant Capacity (TAC) was measured calorimetrically by inhibiting the formation of Thiobarbituric Acid Reactive Substances (TBARS) in the presence of antioxidants, with results expressed in mM/mg protein[27]. Meanwhile, GSH (mg/mg tissue), an indicator of antioxidant defense, was quantified using the Ellman method[28] .

2.6.3 Determination of Pro-inflammatory Markers in Tissue Homogenates

2.6.3.1 Quantitative Assessment of TNF-α

TNF-α levels (pg/mg) were measured in tissue homogenates using Enzyme-Linked Immunosorbent Assay (ELISA) kits (Catalog # CS-E11987r). This assay employs a sandwich enzyme immunoassay method, with sample results obtained from the standard curve[29].

2.6.3.2 Quantitative Assessment of IL-17

IL-17 levels (pg/mg) were quantified in colonic tissue homogenate using R and D systems Quantikine rat immunoassay kits (Catalog #: DY8410-05). This sandwich enzyme immunoassay method used the standard curve to read sample values[30].

2.6.3.3 Quantitative Assessment of NFkB

The quantification of NFkB level (ng/mg) was performed in tissue homogenates using ELISA kits (Catalog # MBS453975) according to the manufacturer's protocol[31].

2.7 Statistical Analysis

Data analysis was conducted using IBM SPSS version 24. Results are presented as mean ± SD. Normality was assessed via skewness and kurtosis Z-values. One-way ANOVA was employed for data comparison, followed by Tukey's post hoc tests for pairwise group analysis. A significance level of < 0.05 was used. Pearson correlation analysis was performed to compute the 'r' value for each pair of parameters[32].

3. Results

3.1 Body Weight Assessment

Weight gain was evidenced in the normal control and Cur-UC groups when compared at the start of the study (D0) and at the end of the study before rat scarification (D7). In the other groups, untreated-UC, Mes-UC, and Res-UC, there is significant net weight loss, $P < 0.001$ (Table 1).

3.2 Gross Examination of the Colon

Macroscopic examination of the colonic mucosa shows significant variations between all the studied groups. In the normal group, the mucosa is entirely normal in all colons. The untreated-UC group showed areas of edema and mucosal ulcers of various sizes, along with well-defined dark regions of necrosis, and dilation of the colonic wall was observed. In the Mes-Uc group, the colonic mucosa also exhibited multiple ulcers of different sizes and edema. Only one rat displayed intense inflammation and necrosis of the mucosa. In Res-UC rats, the mucosa was edematous with areas of inflammation, but no ulceration or necrosis was seen. Cur-UC group had colonic mucosa similar to that of the normal control group, but with infrequent, sparse areas of edema and inflammatory response.

3.3 Histopathological Assessment

Histological examination of the colonic tissue from the different groups revealed that the normal control group had unremarkable mucosa with no ulceration. The untreated-UC group showed focal mucosal ulceration, cryptitis, and transmural mixed acute and chronic inflammatory infiltrate (lymphoplasmacytic mixed with neutrophils). In the Mes-UC group, colonic mucosa exhibited focal ulceration; one colonic

Table 1. Results of the body weight of the animals, comparing day zero (D0) and D7 of the experiments.

Time course	I (NC)	IIA (UC)	IIB (Mes-UC)	IIC (Res- UC)	IID (Cur-UC)
D0(gm) Mean ± SD	225.12±3.31	226.0± 4.44	227.12 ± 5.05	227.0± 2.62	224.5 ± 4.27
D7 (gm) Mean ± SD	233.62 ± 3.99	218.0 ± 3.7	221.37 ± 3.85	225.5 ± 2.61	228.87 ± 3.56
Gain/loss %	3.77 ± 1.6 %	-3.54 ± 1.8 %**	-2.53 ± 1.2 %**	-0.66 ± 1.4%**	1.94 ± 1.9 %

NC: normal control; UC: ulcerative colitis; Mes: mesalazine; Res: resveratrol; Cur: curcumin, (** P< 0.001).

fragment displayed mucosal ulceration with transmural inflammation extending to the serosa. In Res-UC rats, the mucosa showed focal minor erosion and ulceration with mixed inflammatory infiltrate in the lamina propria, with no cryptitis. The Cur-UC group rats had colonic mucosa that appeared nearly normal, with no ulceration or inflammation (Figure 1a-j).

3.4 Assessment of the Biochemical Parameters

3.4.1 Determination of Oxidative Stress Parameters (Colonic MDA, TAC, and GSH Levels)

In this study, colonic MDA levels were notably higher in the untreated UC group compared to the normal control. Treatment markedly reduced colonic MDA levels compared to the untreated UC group. Additionally, significant differences were observed among all treated groups, with the lowest values in the Cur-UC group, followed by Res-UC, and then Mes-UC (Table 2, Figure 2a). Conversely, colonic TAC and GSH levels were significantly lower in the untreated UC group relative to the normal control. Treatment with mesalazine, resveratrol, or curcumin significantly increased the levels of both TAC and GSH compared to the untreated UC group. No significant difference was detected in GSH and TAC levels between Res-UC and Cur-UC groups (Table 2, Figure 2 b,c).

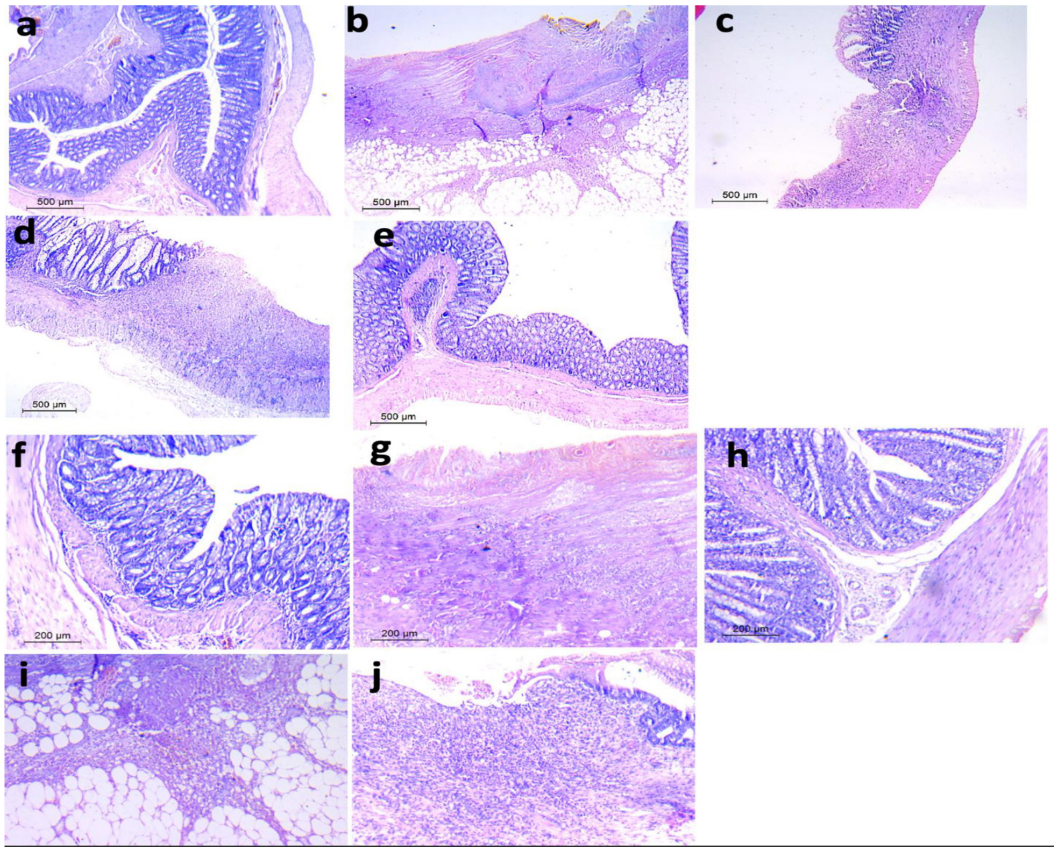


Figure 1. Hematoxylin and eosin stained sections from different studied groups **(a, f)** Colonic section from the normal control group, revealed unremarkable mucosa findings with no ulceration (x400, x 100 respectively) **(b, g)** Colonic section from the UC-untreated group-II: revealed focal mucosal ulceration, cryptitis, and transmural mixed acute and chronic inflammatory infiltrate of lymphoplasmacytic admixed with neutrophils (x400, x100 respectively) **(c, h)** Colonic sections from the mesalazine-treated group reveal focal ulceration, one colonic fragment features mucosal ulceration with transmural inflammation extending to serosa in "J" (x400, x100 respectively) **(d, i)** Colonic sections from the resveratrol-treated group revealed focal small erosion and ulceration with mixed inflammatory infiltrate in the lamina propria with no cryptitis (x400, x100 respectively) **(e, j)** Colonic sections from the treated group by curcumin: near normal mucosa with no ulceration, no inflammation. (x400, x100 respectively).

3.4.2 Assessment of Pro-inflammatory Cytokines (TNF-α, IL-17, and NF-κB)

The acetic acid induction of UC resulted in a significant increase in the levels of TNF-α, IL-17, and NF-κB in the colonic tissue of UC rats. Treatment with various NF-κB inhibitors (mesalazine, resveratrol, or curcumin) significantly reduces all cytokine levels as compared to untreated UC rats. The inhibitory effect on cytokines level was superior in the Cur-UC group, followed by the Res-UC group, and least in the Mes-UC group. The difference between the different treated groups was highly significant (P < 0.001) (Table 2, Figure 3).

3.4.3 Correlation Studies

Correlation studies revealed a Significant negative correlation between TAC and MDA, which indicates the redox balance in the disease state and after treatment (r = -0.923, P<0.001) (Figure 2d). Additionally, correlation studies reveal a positive correlation between NF-κB and TNF-α , which are involved in the pathogenesis of ulcerative colitis (r = 0.863, P < 0.001) (Figure 3d).

Table 2. Results of oxidant, antioxidant, and pro-inflammatory tissue markers.

Mean ± SD	I (NC)	IIA (UC)	IIB (Mes-UC)	IIC (Res- UC)	IID (Cur-UC)
MDA (nmol/gm)	16.77 ± 2.15	51.0 ± 4.49**	35.51 ± 1.62**##	24.58 ± 1.48**##	20.76 ± 1.21*##
TAC (mM/mg)	1.33 ± 0.04	0.56 ± 0.15**	1.01 ± 0.09**##	1.22 ± 0.08##	1.35 ± 0.08##
GSH (mg/mg)	49.48 ± 3.23	30.25 ± 3.82**	41.65 ± 2.34**##	44.97 ± 3.68*##	47.68 ± 2.58##
TNF-α (pg/mg)	43.25 ± 1.49	173.12 ± 3.36**	135.37 ± 3.9**##	121.25 ± 2.3**##	63.0 ± 5.45**##
IL-17 (pg/mg)	480.32 ± 32.85	1367.0 ± 37.2**	894.87 ± 50.3**##	687.37 ±22.5**##	518.62 ±11.9##
NFkβ (ng/mg)	2.51 ± 0.13	8.09 ± 0.33**	4.17 ± 0.14**##	3.61 ± 0.15**##	2.92 ± 0.13**##

NC: normal control; UC colitis; Mes: mesalazine; Res: Resveratrol; Cur: curcumin; MDA: Malondialdehyde; TAC: total antioxidant capacity; GSH: reduced glutathione; TNF-a: tumor necrosis factor-a; NFkB: Nuclear factor kappa B *analysis of variance and Tukey's test; **significant difference from NC group, ## significant difference from UC group (P < 0.001).

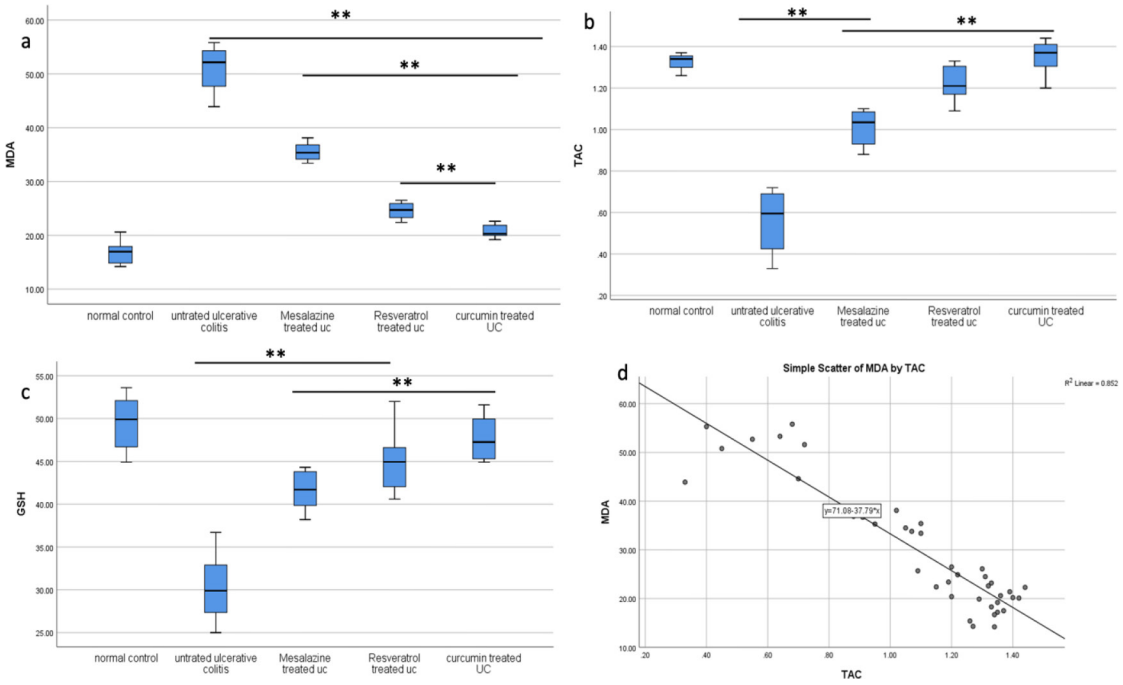


Figure 2. **(a)** The MDA level in the different studied groups (nmol/g) **(b)** The TAC level in the different studied groups (mM/mg) **(c)** The GSH level in the different studied groups (mg/mg). MDA: Malondialdehyde; TAC: total antioxidant capacity; GSH: reduced glutathione; Data are presented as means ± SD using one-way ANOVA test and Tukey's test; ** P < 0.001.

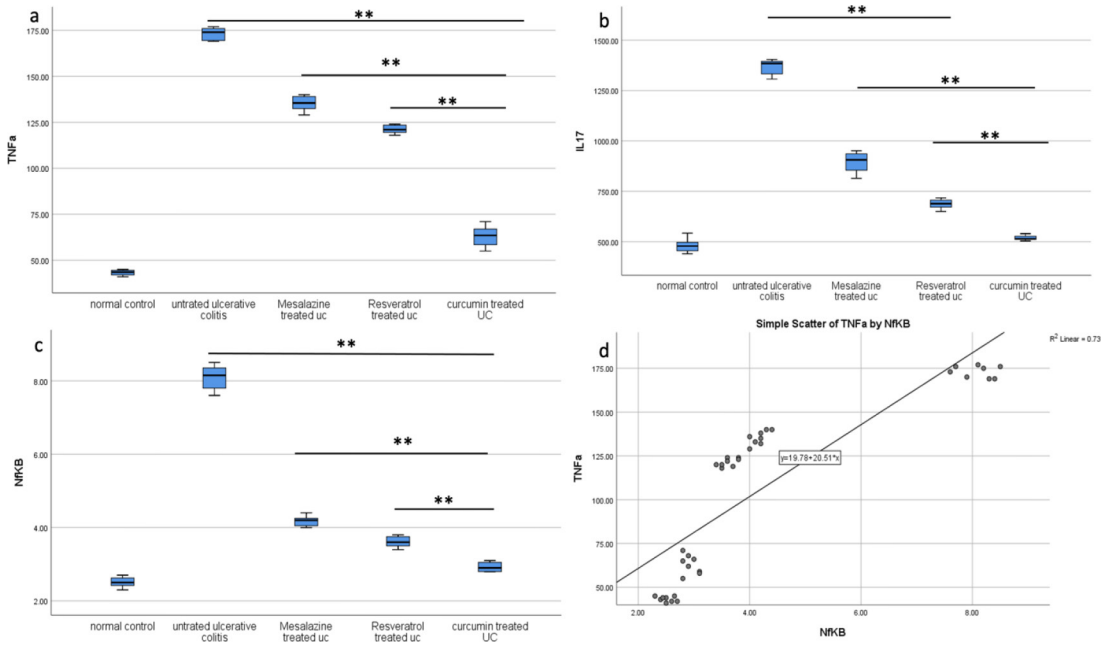


Figure 3. **(a)** The TNF-a level in the different studied group (pg/mg) **(b)** the IL-17 level in the different studied group (pg/mg) **(c)** the NFk-B level in the different studied group (ng/mg). TNF-a: tumor necrosis factor-a; NFkB : Nuclear factor kappa B. Data are presented as means ± SD using one-way ANOVA test and Tukey's test; ** P < 0.001.

4. Discussion

Ulcerative Colitis (UC) is a chronic, inflammatory, relapsing, and remitting disorder that affects millions of people worldwide. It reduces the quality of life and ability to work, while also increasing the economic burden. Conventional medical treatment of UC, including the use of amino-salicylates, corticosteroids, and even immunosuppressive drugs, is not always effective and can cause serious side effects. Clinical challenges in identifying new targets and developing effective intervention strategies are crucial for determining the best course of treatment. NF-kB is a key factor in the inflammatory setting and strongly affects the course of mucosal inflammation in UC[10-12]. The current study aims to evaluate the relative potency and compare the effectiveness of curcumin and resveratrol (the natural NF-κB inhibitors) against mesalazine, a conventional synthetic NF-κB inhibitor used for UC treatment, in a rat model of acetic acid-induced colitis. This study showed that treatment with natural NF-kB inhibitors, namely curcumin and resveratrol, was more effective than conventional mesalazine therapy in lowering MDA and inflammatory cytokines like TNF-α, IL-17, and NF-kB in colonic tissue. Additionally, curcumin was more effective than resveratrol in reducing disease-related oxidant and inflammatory markers. This was also confirmed by histological examination of the colon, which showed near-normal mucosa with no inflammation or ulceration in the Cur-UC group.

Acetic acid-induced colitis is a common method for developing an animal model of UC, as it mimics the pathology observed in humans, characterized by increased inflammatory mediators, localized damage, and epithelial injury[19]. In our study, the untreated colitis group showed severe mucosal ulceration, cryptitis, and transmural infiltration of lymphocytes and neutrophils. Additionally, there was a significant rise in colonic MDA levels, a marker of oxidative stress, indicating neutrophil infiltration and a disrupted inflammatory response. These findings are consistent with observations in both animal models and patients with Inflammatory Bowel Disease (IBD)[33]. Colonic GSH and TAC levels were significantly decreased, indicating increased oxidative stress-mediated injury, which is consistent with previous clinical studies[34]. Pro-inflammatory markers such as TNF-α, IL-17, and NF-kB in the colonic tissue of UC rats were also increased, correlating with disease severity. These markers are known to play vital roles in the development and progression of the disease due to their impact on immune system dysregulation[35].

Mesalazine is the primary treatment for patients with mild to moderate UC and continues to be the standard for

maintenance therapy. However, current use faces several limitations, such as inconvenience, the number of tablets and the dosing frequency (3-4 times daily), limited efficacy across all types of IBD, and low patient adherence[36].

Our study showed that mesalazine treatment in a dose of 300 mg/kg/day significantly reversed the biochemical changes induced by colitis. There was a significant reduction of the pro-inflammatory cytokines (TNF-α, IL-17, and NF-kB) and the oxidative marker (MDA) in the colonic tissue with significant elevations in TAC and GSH levels. However, macroscopic and histopathological findings revealed focal ulceration with one colonic fragment featuring transmural inflammation extending to serosa. Mesalazine (5-aminosalicylic acid, 5-ASA) formulations, as an anti-inflammatory medication, have inhibitory properties similar to other NSAIDs on cyclooxygenase (COX-1 and COX-2), thereby reducing prostaglandin production. Recent experimental and preliminary clinical studies have shown that mesalazine has beneficial effects that are not dependent on COX, such as inhibiting the transcription factor NF-κB[10]. One of the main causes of intestinal mucosal barrier damage is TNF-α, which activates NF-κB, which intensifies barrier impairment by causing the production of several inflammatory cytokines[37]. The inhibitory effect on NF-κB activation occurs through the inhibition of IκB kinase (IKKs-α), which protects IκBα from phosphorylation and degradation. This prevents the activation of NF-κB and contributes to the well-known anti-inflammatory and immunosuppressive effects of sulfasalazine (a 5-ASA used in rheumatoid arthritis)[38]. Furthermore, sulfasalazine's inhibitory effect on TNF-α membrane binding was shown to be dose-dependent[12,37]. Another study showed that sulfasalazine prevents NF-κB/Rel activation triggered by TNF-α[13].

This study also examined the therapeutic effects of resveratrol on acetic acid-induced colitis in rats, as many previous studies have shown it to be particularly effective in treating IBD in experimental models. This study agrees with previous studies that showed that resveratrol is effective in reducing the production of Reactive Oxygen Species (ROS) and increasing the activity of antioxidant enzymes, such as GSH, and increasing the TAC in colonic tissue[39].

The therapeutic effect of resveratrol stems from its ability to lower the production of the pro-inflammatory cytokine IL-17, which was notably reduced in the Res-UC group. Th17 plays a role in the immunopathology and prognosis of IBD by secreting IL-17 and other inflammatory cytokines. Studies have shown that an imbalance between regulatory T cells (Treg) and Th17 cells, as well as levels of IL-10, IL-17, IL-6, and IL-23, plays a key role in the development of UC. The elevated IL-17 in UC is linked to a high Th17/Treg ratio[40,41]. According to Cui *et al*, resveratrol reduces the inflammatory stress state of UC mice by controlling TNF-α-expressing CD3+ T cells and lowering the proportion of neutrophils in the lamina propria and mesenteric lymph nodes. As a result, the incidence of colon cancer associated with colitis is reduced[42]. One of the main causes of intestinal mucosal barrier damage is TNF-α activation of NF-κB, which intensifies barrier impairment by inducing the production of several inflammatory cytokines[36]. Resveratrol was shown to inhibit NF-κB activation and inflammatory factor production in patients with active UC by modulating the inhibitor of NF-κB (IκB) kinase (IKK), resveratrol can stop IκB from being phosphorylated and then degraded[43,44].

Curcumin in our study outperformed Resveratrol and Mesalazine in both biochemical and histopathological assessments for reducing acetic acid-induced colitis. The main mechanism behind curcumin's anti-inflammatory effect is primarily achieved by inhibiting inflammatory pathways and mediators, such as NF-kB and TNF-α[45], both were significantly decreased in the curcumin-treated group in our study. Curcumin inhibits NF-kB expression by affecting the NF-kB/IkB pathway, as it prevents the degradation of IkB, leading to NF-kB inactivation and disrupting the NF-kB-induced kinase signaling pathway. Additionally, Curcumin treatment has been shown to reduce TNF-α levels and decrease oxidative stress driven by TNF-α[46]. TNF-α functions as a positive feedback signal that promotes the production of reactive oxygen and nitrogen species, leading to NF-κB activation. Curcumin has been shown to down-regulate NF-κB expression, which is essential for activating Cyclooxygenase-2 (COX-2); this, in turn, inhibits the expression of COX-2, a key player in the inflammatory process. According to Camasho *et al*., curcumin also suppresses Mitogen-Activated Protein Kinase (MAPK) signaling, resulting in decreased levels of COX-2 and inducible nitric oxide synthase (iNOS), as well as

reduced nitrite production in the colonic mucosa[47]. Additionally, a different study found that giving curcumin in experimental colitis models suppressed Myeloperoxidase (MPO) activity and partially decreased oxidative stress, cytokine cascade, and colitis[15,16,48].

Another mechanism by which curcumin exerts its therapeutic effect on IBD is by restoring the balance of Treg/Th17 cells. The experiments also revealed that curcumin may regulate Treg/Th17 re-balance by inhibiting the IL-23/Th17 pathway, which suppresses inflammatory cytokines such as IL-6, IL-17, and IL-23, while increasing the production of the anti-inflammatory cytokine IL-10[49]. In addition to its anti-inflammatory properties, curcumin also lowers oxidative stress, as evidenced in the current study by decreased MDA levels and increased GSH and TAC in colonic tissue. This effect is due to its enhancement of the Nrf2-Keap1 signaling pathway, which boosts the antioxidant defense system that protects cells from ROS-induced damage. Moreover, curcumin can directly interact with NADPH oxidase and other molecules, leading to increased antioxidant enzymes such as TAC and GSH, and a reduction in MDA levels. These antioxidant and anti-inflammatory pathways of curcumin work together to improve UC[50].

Additionally, individuals with UC often exhibit a skewed CD4/CD8 T cell ratio, characterized by an increase in CD4+ T cells, particularly those from the Th1 and Th17 subsets, which are associated with inflammation. Curcumin and resveratrol, known for their immune-modulating and anti-inflammatory properties, show promise as potential treatments for ulcerative colitis. These compounds can influence T cell function and restore the balance in cytokine production, and immune response dynamics in the gut, potentially leading to reduced inflammation and improved symptoms in UC patients[22,41,51].

5. Conclusion

UC is a debilitating disease that often responds poorly to traditional treatments and newer biologic options. Natural NF-kB inhibitors like curcumin and resveratrol are comparable to conventional mesalazine in treating ulcerative colitis. Curcumin, derived from turmeric, offers several clear advantages, including being effective, affordable, safe, and readily accessible. Consequently, it may serve as an alternative therapy for patients with mild to moderate ulcerative colitis and as an adjunct for treating severe forms.

6. Limitations

A limitation of the study is the small number of outcome variables measured at the end, *e.g.*, inflammatory cytokines such as IL-6 and IL-23, and the anti-inflammatory cytokine IL-10 should have been evaluated at the site of the lesion on gut tissue. Criteria for evaluation of ulcerative colitis, such as 'disease activity index', microscopic scoring, and spleen weight, should also have been included.

7. Ethical Approval

The ethical guidelines of Alexandria University on laboratory animals and the National Research Council's Guide for the Care and Use of Laboratory Animals were adopted. Further, approval was obtained from the Alexandria Faculty of Medicine Ethical Committee (IRB NO:00012098)- FWA: No.00018699. The serial registration number for this study is: 0306810.

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9. Author Contribution

Seham Zakaria Nassar: Conceptualization; methodology; statistical analysis; writing-review and editing. Noha Mohamed Badae: Conceptualization; methodology; visualization; writing-review and editing; project administration. Maram Mohamed Allam: Investigation; resources; data curation; visualization. Eman Ibrahim Anwar: Results interpretation, writing, review, and editing, visual abstract designing, final draft preparation, and manuscript submission. All authors have read and approved the final version of the manuscript.

10. References

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