The Impact of Quercetin on Sirtuin1, High Mobility Group Box 1 and Selected Oxidative Stress Indices in Ulcerative Colitis Induced by Oxazolone in Rats

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Abstract: Background: Ulcerative colitis (UC) is a chronic relapsing inflammatory bowel disease (IBD) characterized by superficial mucosal ulceration, rectal bleeding, diarrhea, and abdominal pain. UC is restricted to the colon. The cause of UC is still unknown, but several factors have been documented. These include environmental factors, genetic factors, microbial pathogens, altered levels of inflammatory mediators, oxidative stress. Recently, antioxidant supplementation has been the major focus of attention across the world among the health professionals to explore it as a strategy to protect against the injurious effects of oxidative stress. **The aim of this study is to** investigate the beneficial effects of quercetin in a rat model of oxazolone induced ulcerative colitis. **Material and method:** The study was conducted on 60 male albino rats divided into four groups; group I(control group), group II (oxazolone induced colitis group) and group III (co-treated group) which was subjected to intra-rectal injection of a single dose of 1.1ml / rat of oxazolone solution which was dissolved in 40% (v/v) aqueous ethanol to a final concentration of 7.5 mg/mL at the day of induction were given Quercetin orally in a dose of 5mg/kg / day for 14 days. Group IV (prophylaxis & treated group): which was given quercetin orally in a dose of 5 mg /kg / day for 7 days before induction of ulcerative colitis then quercetin was given orally in a dose of 5 mg /kg / day for 14 days after induction of ulcerative colitis. All groups were subjected to estimation of Peroxinitrite level, Sirtuin 1 (SIRT1) Level, High mobility group box 1 (HMGB1) level and FRAP (ferric reducing antioxidant power) assay respectively. **Results:** This study showed that quercetin significantly decreased HMGB1 and peroxinitrite level. Also it increase SIRT1 and FRAP level. **Conclusion:** On basis of these results it could be concluded that quercetin exhibits anti-inflammatory, antioxidant effects in experimentally induced UC in rats.

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1. Introduction

Inflammatory bowel disease (IBD) is a group of inflammatory relapsing autoimmune conditions of the gastrointestinal system. The two major types of IBD are Ulcerative colitis (UC), which is limited to the colon, and Crohn's disease(CD), which can involve different parts of the gastrointestinal tract [(1)].

Ulcerative colitis (UC) is an idiopathic chronic inflammatory disease of the rectum and colon that follows a course of relapse and remission. Ulcerative colitis mainly affects the population in the Western countries. Now it becomes common in rest of the world due to the adoption of Western lifestyle [(2)]. The symptoms of UC include frequent bowel movements, diarrhea with bloody stool, rectal bleeding and weight loss [(3)]. The long-term repeated attacks of the disease not only makes it refractory, but also result in a higher risk of developing colitis-associated colorectal cancer [(4)].

High mobility group box-1 (HMGB1) is a highly conserved, non-histone nuclear protein that functions to stabilize nucleic acid structure and modulate gene transcription [(5)]. HMGB1 can be released into the cytoplasm and even the extracellular milieu from activated inflammatory cells or necrotic cells, which then triggers inflammation [(6)] . HMGB1 is a cytokine-like mediator associated with many severe inflammatory and autoimmune diseases, such as sepsis , acute pancreatitis, rheumatoid arthritis , and systemic lupus erythematosus [(7)]. Its cytoplasmic translocation can be regulated by post-translational modifications such as acetylation and methylation. Hyperacetylation of HMGB1 affects its DNA binding and redirects it toward the cytoplasm. Also HMGB1 expression increased significantly in the serum and colon of inflammatory bowel disease mice. These findings suggest that HMGB1 might be an important mediator of IBD as well as a new target for therapy [(8)].

Sirtuins are a family of histone deacetylases (HDACs) that catalyse deacetylation of both histone and none-histone lysine residues [(9)]. Sirtuins regulate important metabolic pathways in prokaryotes and eukaryotes and are involved in many biological process such as cell survival, senescence, proliferation, apoptosis and DNA repair [(10)].

Sirtuin1 was the first SIRT family member to be discovered. It needs cellular nicotinamide adenine dinucleotide (NAD) as a cofactor for deacetylation reactivity. SIRT1 deacetylates the lysine residues of various proteins, which have a role in the progression of immune-inflammatory processes. SIRT1 can act as an inhibitor of inflammatory signals, at least in specific cell types [9,10].

Oxidative stress represents an imbalance between net level of reactive oxygen species (ROS) and the antioxidant capacity of the body in favor of ROS potentially leading to cellular damage [11]. ***Peroxynitrite*** which is the product from the diffusion-controlled reaction of nitric oxide (NO) with the superoxide radical, is capable of oxidising and nitrating a wide range of biomolecules, such as proteins, lipids and DNA, leading to cellular damage. For instance, peroxynitrite-dependent modification of proteins affect enzymatic function in several cell types [12].

Ferric reducing/antioxidant power (FRAP) assay is a recently developed, direct test of "total antioxidant power."FRAP assay is sensitive, simple, and speedy and facilitates experimental and clinical studies investigating the relationship among antioxidant status, dietary habits, and risk of disease [13].

Flavonoids play an important role for adjunct nutritional therapy of chronic intestinal inflammation. Quercetin is an important dietary flavonoid present in different vegetables, fruits, nuts, and beverages such as coffee, tea, and red wine [14]. Quercetin is reported to have antioxidant properties associated with antithrombic, antihypertensive, anticarcinogenic and anti inflammatory effects [15].

2. Materials and Methods

Chemicals:

Quercetin (>95 purity), oxazolone and other chemicals and solvents used unless otherwise described were purchased from Sigma (Sigma, St Louis, USA). All chemicals and solvents were of high analytic grade.

Induction of experimental colitis:

Induction of ulcerative colitis was performed as follows:

Oxazolone solution was prepared by dissolving in 40% (v/v) aqueous ethanol to a final concentration of 7.5 mg/mL and administered once in a dose of 1.1 mL /rat into the colon through a rubber catheter inserted 4 cm inside anal verge under light ether anesthesia. Then, the catheter was removed, and the rat was held vertically for 30 seconds to ensure distribution of the oxazolone within the entire colon and cecum [16,17].

Study design and animal grouping:

The current study was carried out in Medical Biochemistry Department, Faculty of Medicine, Tanta University, Egypt in accordance to the guidance of ethical committee of Medical Research, Faculty of Medicine, Tanta University, Egypt (Approval code code31801\ 10\17). This study comprised 60 male albino rats of approximately 120 - 150 g body weight obtained from experimental animal colony of Tanta University. During the study, animals housed in wire mesh cages, were fed standard rat chew and allowed free access to water. They were kept under constant environmental conditions (25 ºC and lighting regimen of 12-h dark/12-h light cycle). They were fasted for 24 hours before the experimental procedure but allowed free access to water.

The studied animals were divided randomly into four equal groups as follows:

(A)Group I (control group): This group included 15 rats which were subjected to intra-rectal injection of40% aqueous ethanol once in a dose of 1.1 ml/rat into the colon through a rubber catheter under light ether anesthesia.

(B) Group II (oxazolone induced colitis group): This group included 15 rats which were subjected to intra-rectal injection of a single dose of 1.1ml / rat of oxazolone solution which was prepared by dissolving in 40% (v/v) aqueous ethanol to a final concentration of 7.5 mg/mLvia rubber catheter under light ether anesthesia [16,17].

(C) Group III (concomitant or co- treatment group): This group included 15 rats which were subjected to intra-rectal injection of a single dose of 1.1ml / rat of oxazolone solution which was dissolved in 40% (v/v) aqueous ethanol to a final concentration of 7.5 mg/mLvia rubber catheter under light ether anesthesia then at the day of induction were given Quercetin orally, Firstly a stock solution of quercetin was prepared (10 mg mL−1) in DMSO; and then the stock solution of quercetin in DMSO (1 mL) was diluted with 10 mM phosphate buffer saline (pH 7.4) to produce a20 mL suspension. The dose of quercetin was 5mg/kg / day for 14 daysby oral administration [18].

(D)Group IV (prophylaxis & treated group): quercetin was given orallyin a dose of 5 mg /kg / day for 7 days before induction of ulcerative colitis then quercetin was given orally in a dose of 5 mg /kg / day for 14 days after induction of ulcerative colitis [19].

Blood and tissue sampling

At the end of the experiment, All rats were anaesthetized by ether, then rats were sacrificed. Abdomen and thorax were opened and the distal 8 cm of the colon were dissected, opened longitudinally along its mesenteric border and rinsed with ice cold saline to remove extraneous materials, examined for mucosal injury and assessed macroscopically. Then

tissue samples were divided into small pieces. Some pieces were fixed in 10% formosaline solution for histopathological examination. The remaining pieces were weighted and homogenized with a Potter-Elvenhjem tissue homogenizer in phosphate buffer saline (PBS) 10 mM (pH 7.4) then were centrifuged at 12,000 rpm for 20 minutes at 4◦C and the resultant supernatant was separated and stored in aliquots at -80ºC till used for different estimations.

Disease activity index

DAS was represented by the sum of highest score of the followed each criterion **Weight loss**: 1; 1–5%, 2; 5–10%, 3; 10–20%, 4; more than 20%. **Fecal consistency**: 2; loose feces, 3; mild diarrhea, 4; severe diarrhea/ **Rectal bleeding**: 3; mild bleeding, 4; severe bleeding) for 3 days (day 8–10) [20].

Biochemical assessment

1. Colonic tissue High mobility group box 1 (HMGB1) level was measured using commercial ELISA kit supplied by sunred Company, Cat. number (201-11-0258) (USA), according to the manufacturer's instructions. The corresponding levels were expressed as ng/ mg tissue protein [21]

2. Colonic tissue sirtuin1 (SIRT1) level was measured using commercial ELISA kit supplied by sunred Company, Cat. number (201-11-1498) (USA), according to the manufacturer's instructions. The corresponding levels were expressed as ng / mg tissue protein.

3. Colonic tissue peroxynitrite level was determined according to the method described by **(Beckman et al., 1992)** [22], cited by **(Van Uffelen et al.,1998)** [23]. Peroxynitrite mediated nitration of phenol, resulting in nitrophenol, which can be measured spectrophotometrically at 412 nm. results were presented as µmol /g. tissue.

4. Colonic tissue ferric reducing antioxidant power (FRAP): [24] The procedure described by Benzie and Strain was followed.

It is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored form in the presence of antioxidants, an intense blue colour with an absorption maximum at 593 nm develops, and the rate limiting factor of (Fe^{II} –TPTZ), and hence colour formation is the reducing ability of the sample**.** Results were expressed as Fe^{II} µmol/gram wet tissue.

5. Protein content was measured according to the method of Bradford. This method of protein estimation involves the binding dye Coomassie Brilliant Blue G-250 to proteins. A standard curve was plotted, and the protein content of the unknown samples was read from the constructed curve at wavelength of 595 nm [25].

Histopathological examination:

Macroscopic scoring: For each animal, the distal 8 cm portion of the colon was removed, cut longitudinally down its mesenteric border, cleaned in physiological saline to remove faecal residues. Mucosal injury was assessed macroscopically using the grading scale by Morris and colleagues ranging from 0-5.

The grading scale comprises: 0 = No macroscopic changes.1 = Mucosal erythema only or focal hyperaemia with no ulcers.2 = Single site of ulceration with no significant inflammation.3 = Linear ulcer with inflammation at one site. 4 =Two or more sites of ulceration and inflammation.5 =Major site of injury or inflammation extending 1-2cm along length of colon [26].

Microscopic scoring: From each group, samples were taken from 5 randomly selected rats, preserved in 10 % formalin for 24 hours. Trimming was done on formalin fixed samples and washed in tape water for 12 hours. Serial alcohols (methyl, ethyl and absolute) were used for dehydration of tissue samples. Then tissue samples were cleared in xylene and embedded in paraffin. Paraffin blocks were sectioned at 3 µ thickness by slide microtome. The obtained tissue sections were collected on glass slides and stained by haematoxylin and eosin (H & E) for histopathological examination by light microscope.

The degree of severity of colonic inflammation was graded semi quantitatively from 0-11: (Loss of mucosal architecture (score 0-3), Cellular infiltration (score 0-3), Muscle thickening (score 0-3), Crypt abscess formation (score 0-1), Goblet cell depletion (score 0-1)) [27].

Statistical Analysis

Statistical analysis was conducted as mean and standard deviation using Statistical Package for Social Sciences (SPSS), version 16.0 for Windows (SPSS, Chicago, IL). One-way analysis of variance (ANOVA) was used for multiple comparisons to evaluate the statistical significance between experimental groups followed by post hoc test. The correlation study was calculated using Pearson's correlation. P value < 0.05 was considered significant.

3. Results

Table 1 summarized the comparative statistics of studied biomarkers between all groups. There was statistically significant increase of colonic tissue HMGB1 and tissue Peroxinitrite levels in group II when compared to control and treated groups. Also There was statistically significant decrease of colonic tissue SIRT1 and FRAP levels in group II when compared to control and treated groups.

Table (1): Comparative statistics of studied biomarkers between all groups

Parameters/Groups	Group I[a] (n=15)	Group II[b] (n=15)	group III [c] (n=15)	group IV [d] (n=15)	ANOVA	
					F	P-value
Disease activity index	–	5.95 ± 1.05[cd]	4.69 ± 0.99 [b]	3.83 ± 1.2[b]	14.38	<0.001*
Colonic tissue HMGB 1 ng/mg tissue protein	80.77 ± 8.72[bc]	144.16 ± 10.24[acd]	116.96 ± 14.46[abd]	100.39 ± 16.17[bc]	29.21	<0.001*
Colonic tissue SIRT 1 ng/mg tissue protein	187.12 ± 13.16[bc]	133.53 ± 18.23[acd]	154.31 ± 16.17[ab]	166.77 ± 15.3[b]	18.59	<0.001*
Colonic tissue peroxinitrite µmol/g tissue	15.15 ± 4.71[b]	27.37 ± 9.2[ac]	20.67± 6.89[b]	18.10± 5.76	8.66	<0.001*
Colonic tissue (FRAP) (µmol of Fe^{II}equivelant/g tissue)	19.79 ±4.08[bc]	8.73 ± 1.38[acd]	13.07 ± 3.03[abd]	16.97 ± 4.45[bc]	29.21	<0.001*

Data are mean± standard deviation of a group of 15 rats. Statistical analysis is carried out using one-way ANOVA with Tukey's post hoc test, SPSS computer program. HMGB 1: High mobility group box1, SIRT1: Sirtuin1, FRAP: ferric reducing antioxidant power. [a.b.c.d] represent Significant difference between groups: [a]: significance from group I, [b] significance from group II, [c] significance from group III; [d]: significance from group IV. *P<0.05 is significant.

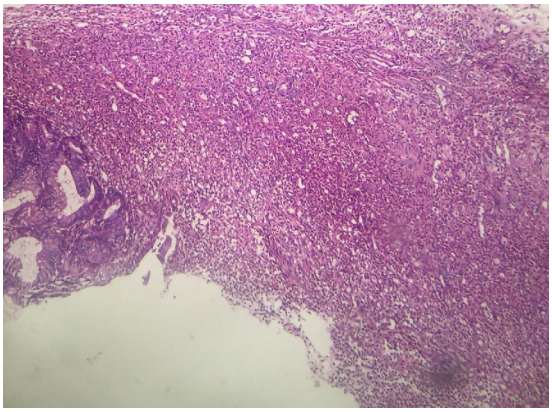

Fig. (1): Colonic tissue section of oxazolone induced colitis group (group II) with heavy mucosal infiltration and areas of mucosal ulceration and distortion of crypt architecture (H. & E. 100 x).

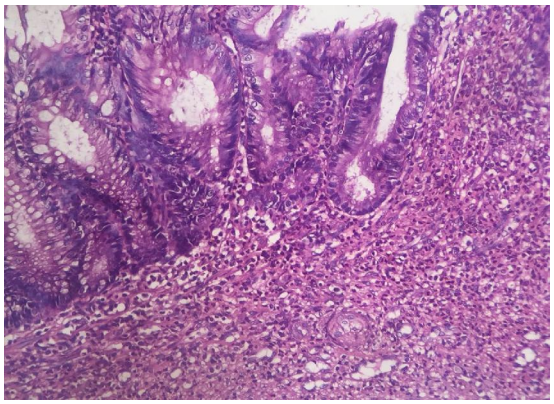

Fig. (2): Colonic tissue section of oxazolone induced colitis group (group II). (H. & E. 100 x)

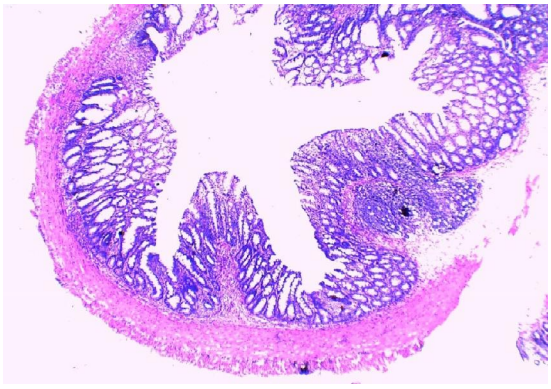

Fig. (3): Colonic tissue section of control group with normal colonic mucosal lining (H. & E. 400x).

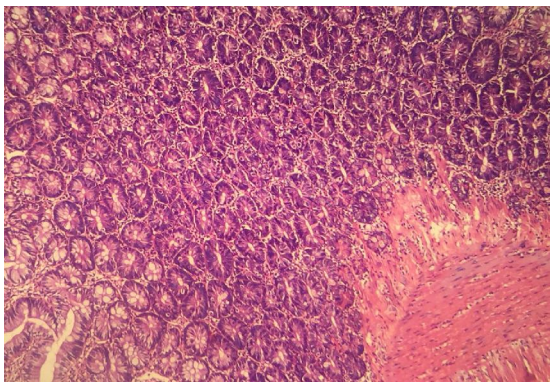

Fig. (4): Colonic tissue section of co- treated group III with lesser inflammatory cellular infiltration, no mucosal ulceration or glandular dysplasia (H. & E. 100 x).

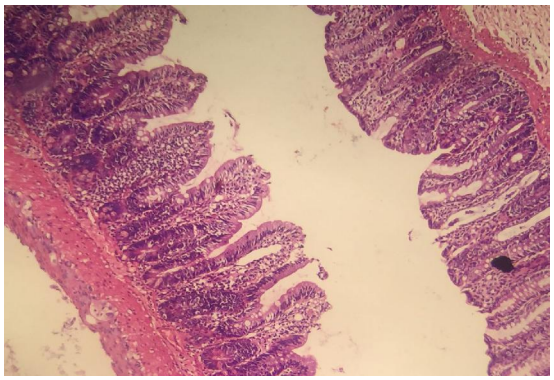

Fig. (5): Colonic tissue section of prophylactic and treated group (group IV) with lesser mucosal inflammatory infiltration, no mucosal ulceration or glandular dysplasia (H. & E. 400 x).

Histopathological examination results

Histopathological examination of colonic tissue of rats of oxazolone induced colitis group (group II) showed heavy mucosal inflammatory infiltration, mild glandular dysplasia, ulceration and distortion of crypt architecture as shown in (**Fig.1**) and (**Fig.** 2)**.** While control groups showed normal mucosal glands, no inflammation, ulceration and dysplasia as shown in (**Fig.3).** Also, co-treated group in this study (group III) and prophylactic and treated group (group IV) showed lesser inflammatory cell infiltration compared to oxazolone induced colitis group, no mucosal ulceration or glandular dysplasia as shown in (**Fig.4,5).**

Table 4 showed comparative statistics of both macroscopic and microscopic grading of all studied groups. There was statistically significant increase of both grading in group II when compared to treated groups which showed improvement under the effect of quercetin treatment.

Table (4): Comparative statistics of both macroscopic and micriscopic grading of all studied groups

Grading/Groups	Group I[a]	Group II[b]	group III [c]	Group IV[d]	ANOVA	
					F	P-value
Macroscopic grading	0 ± 0^{b}	3.0 ± 1.58^{acd}	0.40 ± 0.55^{b}	0.20 ± 0.45^{b}	13.244	<0.001*
Microscopic grading	0 ± 0^{b}	4.0 ± 2.35^{acd}	1.20 ± 0.45^{b}	0.20 ± 0.45^{b}	11.514	<0.001*

Data are mean± standard deviation of a group of 15 rats. Statistical analysis is carried out using one-way ANOVA with Tukey's post hoc test, SPSS computer program. [a.b.c.d.] Significant difference between groups: [a]: significance from group I, [b] significance from group II, [c] significance from group III; [d]: significance from group IV. *P<0.05 is significant.

Table (5): Correlation studies between macroscopic grading and studied parameters in oxazolone induced colitis group.

Matrix correlation	Macroscopic score	
	r	**P value**
Colonic Tissue Peroxynitrite Level	0.914	0.017*
Colonic Tissue HMGB 1 Level	0.935	0.020*
Colonic Tissue Homogenate FRAP Level	- 0.986	0.002*
Colonic Tissue Homogenate SIRT 1 Level	- 0.987	0.002*
Disease Activity Index (DAI)	0.981	0.003*

Correlation study was carried out using Pearson correlation. *Significant at p value < 0.05.**;** HMGB 1: High mobility group box1, SIRT1: Sirtuin1, FRAP: ferric reducing antioxidant power. disease Activity Index (DAI)

Table (6): Correlation studies between macroscopic grading and studied parameters in treated colitis group.

Matrix Correlation	Macroscopic score	
	r	**P value**
Colonic TissuePeroxynitriteLevel	0.725	0.048*
Colonic TissueHMGB 1 Level	0.729	0.048*
Colonic TissueHomogenate FRAP Level	-0.791	0.045*
Colonic TissueHomogenate SIRT 1 Level	-0.778	0.046*
disease Activity Index (DAI)	0.776	0.046*

Correlation study was carried out using Pearson correlation. *Significant at p value < 0.05.**;** HMGB 1: High mobility group box1, SIRT1: Sirtuin1, FRAP: ferric reducing antioxidant power. disease Activity Index (DAI)

Tables 5 and 6 showed correlation studies between macroscopic grading and studied parameters in oxazolone induced colitis group and treated colitis group respectively. There were *significant positive correlations* between macroscopic grading and each of Colonic Tissue Peroxynitrite Level, HMGB 1 Level and Disease Activity Index in both oxazolone induced colitis group and treated group. Also there were *significant negative correlations* between macroscopic grading and each of Colonic Tissue FRAP Level and Colonic Tissue SIRT 1 Level in oxazolone induced colitis group and treated group.

Tables 7 and 8 showed correlation studies between microscopic grading and studied parameters in oxazolone induced colitis group and treated colitis group respectively. There were significant positive correlations between microscopic grading and each of Colonic Tissue Peroxynitrite Level, Colonic Tissue HMGB 1 Level and Disease Activity Index (DAI) in both oxazolone induced colitis group and treated group. Also there were significant negative correlations between microscopic grading and each of Colonic Tissue FRAP Level and Colonic Tissue SIRT 1 Level in both oxazolone induced colitis group and treated group.

Table (7): Correlation studies between microscopic grading and studied parameters in oxazolone induced colitis group.

Matrix correlation	Microscopic score	
	r	**P value**
Colonic Tissue Peroxynitrite Level	0.958	0.001*
Colonic Tissue HMGB 1 Level	0.803	0.033*
Colonic Tissue Homogenate FRAP Level	-0.978	0.004*
Colonic Tissue Homogenate SIRT 1 Level	-0.900	0.037*
Disease Activity Index (DAI)	0.934	0.020*

*Correlation study was carried out using Pearson correlation. *Significant at p value < 0.05.;* HMGB 1: High mobility group box1, SIRT1: Sirtuin1, FRAP: ferric reducing antioxidant power

Table (8): Correlation studies between microscopic grading and studied parameters in treated colitis group

Matrix correlation	Microscopic score	
	r	**P value**
Colonic Tissue Peroxynitrite Level	0.742	0.047*
Colonic Tissue HMGB 1 Level	0.780	0.046*
Colonic Tissue Homogenate FRAP Level	-.957	0.006*
Colonic Tissue Homogenate SIRT 1 Level	-0.766	0.046*
Disease Activity Index (DAI)	0.778	0.046*

*Correlation study was carried out using Pearson correlation. *Significant at p value < 0.05.;* HMGB 1: High mobility group box1, SIRT1: Sirtuin1, FRAP: ferric reducing antioxidant power

4. Discussion

UC is a chronic inflammatory bowel disease of unclear etiology, with mucosal inflammation and ulceration of the colon. There is a rising incidence and prevalence worldwide [(28)]. Rectal administration of the hapten reagent oxazolone dissolved in ethanol induces a severe colitis in rats or mice [(29)]. Oxazolone initiates a typical picture of ulcerative colitis mimics that occurs in human. Moreover, histological examination showed morphological similarities between this model and human UC [(30)]. Quercetin is a polyphenolic bioflavonoid possesses various biological, pharmacological, and medicinal activities including antioxidant, antiviral, and anti-inflammatory properties. It is present in different vegetables, fruits, nuts, and beverages such as citrus fruit, buckwheat, and onions [(31)]. Using Disease activity index, inflammation was assessed based on the changes in the following parameters over time: weight of the animal, stool consistency and presence of rectal bleeding. Final score is sum of the assessed parameters [(32)]. In this study, oxazolone induced colitis group (group II) showed a significant increase in disease activity index compared with the control group (group I). These results are supported by **Lee et al., 2012[(33)] and Abdin et al., 2008[(34)].** Also this study demonstrated that rats treated with quercetin (group III and group IV) showed significantly lower disease

activity index compared with oxazolone induced colitis group (group II). Similar results obtained by **Castangia et al., 2015**[35] revealed that after quercetin-loaded coated vesicle administration, rats started to gain weight and have normal stools without bleeding. **HMGB1** is an important non-histone nuclear protein, the biological activity of HMGB1 is closely associated with its location. Inside cells, HMGB1 binds to DNA and modulates chromosomal architecture, stabilize nucleic acid structure and modulate gene transcription and expression. Once HMGB1 translocates outside of the cell, it can serves as a mediator of inflammation to drive various autoimmune and inflammatory diseases such as inflammatory bowel disease [36]. The results of the present study showed significant increases of colonic tissue **HMGB1** levels in oxazolone induced colitis group (group II) when compared to the other studied groups. These results are supported by **(Zhongliang et al 2014)** [36] and **(Xianghua Guo et al., 2015)** [37]**.**

Significant decrease of HMGB1 levels was detected in quercetin treated groups either co-treated or prophylactic and treated (group III and IV respectively). Similar results obtained by **(Xi Li et al., 2016)** [38]**.** They explained this effect of quercetin on the basis of that quercetin treatment decreased HMGB1 cytoplasmic translocation. Also The up-regulated HMGB1 levels were significantly inhibited by quercetin administration through a molecular mechanism. Moreover, extracellular HMGB1 exerts its effects by binding to cell surface receptors, including receptors for RAGE, TLR2, TLR4, and TLR9. It was found that co-treatment with quercetin decreased the expression levels of TLR2 and TLR4 mRNA and protein. **(Xi Li et al., 2016)** [38]**. Sirtuin 1 (SIRT1)** is aNAD-dependent class III histone deacetylase (HDAC), plays important roles in many physiological processes, including oxidative stress, inflammation, gene transcription and energy metabolism [39]. SIRT1regulates negatively the expression of various proteins involved in the control of immune-inflammatory pathways through deacetylation of transcription factors, such as NF-κB and activator protein-1[40]. The present study revealed that the oxazolone induced colitis group (***group II)*** showed significant decrease in the level of colonic tissue **Sirtuin 1 (SIRT 1)** when compared to the other studied groups. Significant increase of SIRT1 levels was detected in quercetin treated groups either co-treated or prophylactic and treated (group IIIand IV respectively) with no significant difference between them. In accordance with our results **Xionga et al., 2017** [41] revealed that, significant decrease of colonic tissue SIRT1 level in rat colitis model. Defective expression of SIRT1 mediated endoplasmic reticulum stress, leading to dysfunction of epithelial immune responses. Also **Xionga et al., 2017** [41] showed that a significant increase in SIRT1 level in treated group via down-regulation of miR-132.3.

Reactive oxygen and nitrogen species (RONS) have been implicated in the pathogenesis of UC. There has been extensive focus on nitric oxide (NO) and reactive oxygen species (ROS) as possible etiological factors in the initiation and/or propagation of the inflammatory process [42]**.** Interactions between excessive NO and oxygen radicals are detrimental for vital tissues. NO interfaces with oxygen radicals such as superoxide to produce peroxynitrite. Several studies assessed nitrosative stress in UC in terms of finding significant high serum levels of NO, nitrate and nitrite **(Sgambato,2017)** [43]. The present study revealed that the diseased group (group II) had significant increase in colonic tissue levels of peroxynitrite when compared to control group. This increase can be due to stimulation of iNOS during inflammation by cytokines such as TNF-α. Peroxynitrite, a redox derivative of NO, enhances the inflammatory response by sustaining the nuclear localization of NFκB. Moreover, the oxidation of amino acids by peroxynitrite increases the antigenicity of immunoglobuin-G, generating ligands for which autoantibodies show higher activity. A number of studies suggest high levels of serum peroxynitrite in chronic inflammatory conditions. **Amna and Sanan, 2014**[44] reported that there is a link between inflammation and nitrative free radicals. Inflammation induces an increase in free radicals and subsequently promotes oxidative stress. The treated groups in the current study (group IIIand Group IV) showed decrease in colonic tissue homogenate levels of peroxynitrites compared to diseased group (group II). In agreement with this study, **Sotnikova et al., (2013)** [45] stated that Quercetin belongs to the most potent scavengers of ROS, including superoxide, peroxyl, alkoxyl and hydroxylradicals, and reactive nitrogen species like NO•. and ONOO–**.** Also Quercetin and other flavonoids dramatically reduced oxidative stress in lymphocytes from IBD patients and healthy individuals. The present study showed that the diseased group (group II) showed significant decrease in thevalues of colonic tissue Ferric Reducing Antioxidant Power when compared to control group. These results are in agreement with **Esmaily et al., 2009**[46] they reported a significant decrease in FRAP value of diseased group when compared to control group due to colonic damage and inflammation. These results could be explained by infiltration of immune cells to the site of inflammation, leading to the production of a large amount of harmful components including ROS. ROS contribute to oxidative damages to various cellular components resulting in cell death or apoptosis, as a consequence of imbalance between

production of oxidative components and anti-oxidant defence system. This confirms the role of toxic stress as a contributing factor in developing IBD. The treated groups in the current study (group III and Group IV) showed increase in colonic tissue levels of FRAP as compared to diseased group (group II). In agreement with this study, **Lotito et al., (2006)** [47]stated that a significant increase in the total antioxidant capacity of plasma has often been observed after the consumption of flavonoid-rich foods by humans. These observations lead to the hypothesis that dietary flavonoids play a significant role as antioxidants in vivo, thereby reducing chronic disease risk. Histopathological study supported the biochemical findings in the present study as there were significant positive correlation between histopathological scoring and the biochemical markers in both oxazolone induced colitis and quercetin treated groups.

Colonic tissue of oxazolone induced colitis rats showed heavy mucosal inflammatory infiltration, mild glandular dysplasia, ulceration and distortion of crypt architecture. while control groups showed normal mucosal glands, no inflammation, ulceration and dysplasia. Also, co-treated group in this study (group III) and prophylactic and treated group (group IV) showed lesser inflammatory cell infiltration compared to oxazolone induced colitis group, no mucosal ulceration or glandular dysplasia. Similar results obtained by **Abdin et al 2008**[16].

5. Conclusion

On the basis of these results, the current study highlights evidences for the promising protective effects of quercetin in oxazolone induced UC model which resembles the histopathological appearance of the disease in human. These favorable actions were linked with its modulatory effect in maintaining adequate intracellular redox environment as evidenced by reducing some oxido-inflammatory makers and also reducing HMGB1 level and with subsequent improvement of histopathological abnormalities of colonic tissues. These findings presented here also imply that quercetin has therapeutic potential in the prevention of UC and represent a promising therapeutic strategy.

Recommendations

In view of the assessed data, *quercetin* may be a promising drug in prevention and treatment for UC. The safety and efficacy of *quercetin* for UC and its underlying mechanisms deserve further studies.

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