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Lycopene mitigates experimental colitis in rats via inhibiting oxidative stress-mediated inflammation and apoptosis

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ABSTRACT

The potential protective and beneficial effects of Lycopene (LYC) in a rat model of acetic acid-induced ulcerative colitis (UC) were evaluated. Forty male albino rats were divided into five equal groups. Group I: (Control normal) rats received no drugs. Group II: (Early ulcerative colitis): rats received 2 ml (3% v/v) glacial acetic acid intra-colonially at 21thday of experiment and sacrificed 3 days later. Group III:(Early ulcerative colitis + Lycopene protected) rats received Lycopene (10 mg/kg body weight/day, orally) for 21 successive days prior to acetic acid administration. Group IV: (Late ulcerative colitis) rats received acetic acid similar to group II for 3 successive days and sacrificed after 21 days. Group V: (Late ulcerative colitis+ Lycopene treated) rats first administered with acetic acid then after 3 days Lycopene was administered for 21successive days. A significant increase in L-Malondialdehyde (L-MDA) with marked decreases in reduced glutathione (GSH) level and Catalase (CAT) activity were observed in colon tissue of UC-induced rats. Additionally, a significant up-regulation of nuclear factor kappa B (NF-κB), caspase-3 and transforming growth factor-β1 (TGF-β1) and down-regulation of Interleukin -10 (IL-10) and Beta cell lymphoma-2 (Bcl-2) gene expression levels were observed in colon of UC induced rats. However, a significant depletion of colon tissue L-MDA and down-regulation of NF-κB, caspase-3 and TGF-β1 in addition to marked increases in GSH concentration and CAT activity and up-regulation of IL-10 and Bcl-2 were observed after LYC treatment. Lycopene has powerful antioxidants, anti-inflammatory and anti-apoptotic effects against ulcerative colitis.

1. INTRODUCTION

Ulcerative colitis is an intense, local immune reaction characterized by clinical signs such as weight loss, diarrhea, and rectal bleeding; and macroscopic colonic alterations such as ulceration and edema (Matsuoka *et al*., 2018). The intra-rectal instillation of diluted acetic acid (AA) induced acute inflammation restricted to the colon and mimics characteristic features of UC through increase in generation of ulcer, inflammatory mediators, free radicals and neutrophils infiltration (Goyal *et al*., 2014). These lead to production of superoxide anion and initiation of a cascade for the production of various reactive species. Malondialdehyde (MDA) is one of the end products of the lipid peroxidation procedure and increasing in the free radical activity causes overproduction of MDA (Amirshahrokhi and Khalili, 2017). Endogenous antioxidant defenses against reactive oxygen species (ROS) production even in low concentrations influence on two main types: (1) enzymatic (CAT) and (2) non-enzymatic (GSH) (Halliwell and Gutteridge, 2007).

The hallmark of IBD was a perturbed immune system that lead to continuous inflammation in the gut and challenges optimal treatment. Nuclear factor kappa B (NF-κB) is considered to be one of the most important regulators of inflammatory process, which is a widely expressed nuclear transcription factor. El-Shaimaa *et al*. (2020) established that increased expressions of NF-κB was observed in the colonic tissues of the acetic acid-induced ulcerative colitis. One of the main mechanisms involved in colitis function was apoptosis process. Apoptosis and increased expression of caspase-3 were reported in the gut mucosa of ulcerative colitis in animals (Becker *et al*., 2013). Caspase 3 can also be activated by another pathway regulated by the B-cell lymphoma 2 (Bcl-2) family (Sturm *et al*., 2004).

The available treatments for UC mainly include amino salicylates, corticosteroids, and immunosuppressive (Chen *et al*., 2019). However, such therapies were associated with serious side effects (osteoporosis, acute pancreatitis) due to high doses and long-term administration (Jayawardena et al., 2017). Therefore, there is an urgent need to find effective drugs with high therapeutic efficiency and low side effects for UC therapy.

Lycopene (LYC) commonly known natural antioxidants, tend to have protective and antioxidant roles against several oxidative stress-mediated tissue injuries (Hu et al., 2017). Its chemical structure contains many double bonds which have an important role in modulating its ROS scavenging activity. Whereas, these double bonds reduce the high energy required for electron delocalization during reaction with free radicals (Gulcin, 2012). Therefore, the present

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study was designed to investigate the potential antioxidants, anti-inflammatory and anti-apoptotic effects of lycopene on acetic acid-induced colitis in rats.

2. MATERIAL AND METHODS

2.1. Experimental animals:
Forty white male albino rats of 12-16 weeks old and weighting 220-250 g. were used in this study. The rats were fed on constant ration and fresh, clean drinking water was supplied ad-libitum. The animals were left 14 days for acclimatization before the beginning of the experiment.

2. 2. Lycopene:
Lycopene was purchased from Aktin Chemicals, Inc. Company (Nature connecting health), Chengdu, China. Lycopene is present in red powder form. Lycopene (100 mg) was mixed in 2 ml Tween-80 at 25ºC until a homogeneous paste was obtained. Physiological saline at room temperature was added, drop wise and with vigorous stirring, to a final concentration of 10 mg lycopene/ml of suspension (Matos *et al.*, 2000), and was administered orally at a dose of (10 mg/kg b.wt/day) for 21succesive days.

2. 3. Induction of colitis:
To induce colitis, rats were fasted for 18 hours, and then anaesthetized with an intraperitoneal (i.p) injection of sodium thiopental (500 mg dissolved in 12.5 ml of normal saline) at a dose of 0.2 ml/200 g body weight (40 mg/kg b. wt. /i.p) (Motavallian-Naeini et al., 2012). Acetic acid (3% v/v) was administered intra-colonially to rats in a volume of 2 ml, via a polyethylene catheter. Rats were positioned head-down for 30 sec to preclude immediate anal leakage of the instillate and thereafter returned to their cages with access to food and water ad-libitum.

2. 4. Experimental design:
Rats were randomly divided into five main equal groups,8 rats each, placed in individual cages and classified as follow:-
Group I: (Control Normal group): served as control non-treated for all experimental groups.
Group Π: (Early ulcerative colitis-induced group): Rats received AA 3% (2ml/rat) at 21th day of experiment once daily for 3 successive days and on day 24th the animals were sacrificed according to Goyal *et al*. (2014).
Group III: (Early ulcerative colitis +LYC protected group): Rats received LYC (10 mg/kg body weight/day) orally for 21 successive days prior to acetic acid administration for 3 days, at 24th day the animals were sacrificed
Group IV: (Late ulcerative colitis-induced group): Rats received AA 3% (2ml/rat) at the first day of experiment once daily for 3 successive days, and the rats were left and sacrificed after 21 days from induction according to Goyal *et al*. (2014).
Group V:(Late ulcerative colitis +Lycopene treated):Rats first administered with acetic acid 3% (2ml/rat) at the first day of experiment for 3 successive days, then Lycopene was administered orally (10 mg/kg body weight/day) for 21 days, then the rats were sacrificed at 24thday (end of experiment).

2. 5. Sampling:
Colonic tissue specimens were collected once from all animal groups at end of experiment.

2.5.1. Colonic tissue for biochemical and molecular analysis:
Rats were sacrificed by cervical decapitation. The colon was quickly removed and opened using a scrapper, cleaned by rinsing with cold saline and stored at -20°C for subsequent biochemical analysis.
Briefly, colon tissues were cut, weighted and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant was used for the determination of L-Malondialdehyde (L-MDA) concentration and catalase (CAT) activity. Also, 0.2 gm of colon tissues were minced into small pieces homogenized with a glass homogenizer in 0.4 ml of 25% metaphosphoric acid (MPA). Then 1.4 mL of distilled water was added, mixed and incubated for 1 hour and centrifuged for 10 min at 3,000 r.p.m., Finally, the clean supernatant was removed and used for determination of GSH concentration.

2.5.2. Colonic tissue for molecular analysis:
About 0.5 g of rats colon tissue were immediately excised, put in Eppendorf tubes , kept in liquid nitrogen, and then stored at -80°C until used for determination of caspase-3, B-cell lymphoma (bcl-2), nuclear factor kappa B (NF-kB), interleukin-10 (IL-10) and transforming growth factor -β1 (TGF-β1) gene expression analysis using real-time quantitative polymerase chain reaction (RT-qPCR).

2.5.3. Colonic tissue for histopathological examination:
Colon tissue specimens were taken from different parts of the colon for histopathological examination. The specimens were preserved in 10% buffered neutral formalin. The fixed tissue was rinsed in tap water, dehydrated through graded series of alcohols, cleared in xylene and embedded in paraffin wax. 5 µm thick sections were cut and stained with hematoxylin and eosin (H&E) (Bancroft and Stevens, 1996) and then the tissues were examined and evaluated by light microscopy.

2.6. Analysis:
2.6.1. Biochemical analysis:
Colonic tissue L-Malondialdehyde (L-MDA), reduced glutathione (GSH) and Catalase (CAT) were determined according to the methods described by Lahouel et al., 2004; Moron et al., 1979; Weydert and Cullen, 2010, respectively).

2.6.2. Molecular analysis:
Total RNA was isolated from colon tissue of rats using RNeasy Mini Kit (Thermo Qiagen, #74104) according to the manufacturer's protocol. Following determination of RNA concentration and purity by Quawell nanodrop Q5000 (USA),5 mg of total RNA from each sample was reverse transcribed using Quantiscript reverse transcriptase. The produced cDNA was used as a template to determine the relative expression of caspase-3, B-cell lymphoma (bcl-2), nuclear factor kappa B (NF-kB), interleukin-10 (IL-10) and transforming growth factor -β1 (TGF-β1) genes using StepOnePlus real time PCR system (Applied Biosystem, USA) and gene specific primers. The reference gene, β actin, was used to calculate fold change in target genes expression. The thermal cycling conditions, melting curves temperatures, and calculation of relative expression was done. For the treated groups, assessment of 2-ΔΔCt

determined the fold change in gene expression relative to the control (Livak and Schmittgen, 2001).

Forward and reverse primers sequence for real time PCR

Gene	Forward primer (5 ------ 3)	Reverse primer (5 ------ 3)
TGF-B1	AAGAAGTCACCCGCGTGCTA	TGTGTGATGTCTTTGGTTTTGTCA
IL-10	GTTGCCAAGCCTTGTCAGAAA	TTTCTGGGCCATGGTTCTCT
NF-κB	CCTAGCTTTCTCTGAACTGCAAA	GGGTCAGAGGCCAATAGAGA
Caspase3	GGTATTGAGACAGACAGTGG	CATGGGATCTGTTTCTTTGC
Bcl-2	ATCGCTCTGTGGATGACTGAGTAC	AGAGACAGCCAGGAGAAATCAAAC
β-actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC

2.6.3. Statistical analysis

For biochemical analysis the statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS, 18.0 software, 2011 and the individual comparisons were obtained by Duncan's multiple range test (DMRT). All the data were expressed as means ± S.E. Values were considered statistically significant when $p<0.05$. For molecular analysis the difference between the groups was determined by one-way ANOVA using Graph Pad Prism 5 (Graph Pad Software, Inc., LaJolla, CA, USA). Comparison of means was carried out with Tukey's Honestly Significant Difference (Tukey's HSD) test. Data were presented as mean SEM (standard error of mean) and significance was declared at $P < 0.05$ (Steel et al., 1997).

3. RESULTS

Effect of Lycopene administration on colon tissue L-MDA, CAT and GSH of acetic acid-induced ulcerative colitis in male rats was presented in table (1).The obtained results showed significant increase L-MDA concentration with marked decreases in CAT activity and GSH concentrations in colon of acetic acid -induced ulcerative colitis in rats as compared with control normal group. However, a significant depletion of colon tissue L-MDA concentration and marked increase in CAT activity and GSH concentrations were observed after LYC treatment compared to ulcerated untreated rats.

Effect of lycopene administration on the relative expression of NF-κB,IL-10 and TGF-β1 gene in colon tissues of acetic acid -induced ulcerative colitis in rats is presented in table (2).The obtained qPCR results revealed significant up-regulation of NF-κB and TGF-β1 with significant down-regulation of IL-10 gene expression levels in colon of acetic acid-induced ulcerative colitis in rats (G2 and G4) as compared to control normal group (G1). However, NF-κB and TGF-β1 gene expression was significantly down-regulated and IL-10 gene expression level of colon tissues was up-regulated following administration of LYC either before(G3) or after (G5) induction of colitis when compared with early(G2) and late (G4)UC-induced rats, respectively.

Effect of lycopene administration on the relative expression of Caspase-3 and Bcl-2 gene in colon tissue of acetic acid -induced ulcerative colitis in rats was existing in Table (3).The obtained qPCR results showed significant up-regulation of caspase 3 with significant down-regulation of Bcl-2 gene expression levels in colon of acetic acid-induced ulcerative colitis in rats (G2 and G4) as compared to control normal group (G1). Meanwhile, caspase 3 gene in colon tissue was significantly down-regulated and Bcl-2 gene expression level was up-regulated following administration of LYC either before (G3) or after(G5) induction of colitis as compared with early (G2) and late (G4) UC-induced rats, respectively.

Histopathological examinations were performed to determine the effect of lycopene on colons experiencing colitis. Fig. (1) demonstrated the histological results obtained for each group. In the control group, colon tissue showing normal histological appearance of mucosal epithelium and muscularis mucosa.

Table 1 Effect of lycopene administration on colon tissue L-MDA, CAT and GSH of acetic acid -induced ulcerative colitis in rats.

Animal groups	L-MDA (nmol/g. tissue)	CAT (U/g. tissue)	GSH (ng/g. tissue)
Group I: Normal control	33.18^{d}±1.51	6.55^{a}±0.32	32.38^{a}±1.23
Group ΠI: Early ulcerative colitis	96.80^{a} ±3.07	1.22^{e} ±0.11	14.11^{d} ±0.82
Group III: Early ulcerative colitis + Lycopene protected	63.34^{c} ±2.32	4.96^{b}±0.27	24.09^{b}±1.08
Group IV: Late ulcerative colitis	99.75^{a} ±3.46	1.84^{d} ±0.16	12.17^{d} ±0.95
Group V: Late ulcerative colitis + Lycopene treated	75.48^{b} ±2.54	3.22^{c} ±0.21	21.02^{c} ±1.02

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 2 Effect of lycopene administration on the relative expression of NF-κB, IL-10 and TGF-β1 gene in colon tissue of acetic acid -induced ulcerative colitis in rats.

Animal groups	Nuclear factor kappa B (NF-κB)		Interleukin -10 (IL-10)		Transforming growth factor -β1 (TGF-β1)	
	Fold change mean	SEM	Fold change mean	SEM	Fold change mean	SEM
Group I: Normal control	1.00^{d}	0.00	1.00^{a}	0.00	1.00^{d}	0.00
Group ΠI: Early ulcerative colitis	2.28^{a}	0.11	0.22^{d}	0.02	5.24^{a}	0.02
Group III: Early ulcerative colitis + Lycopene protected	1.47^{c}	0.08	0.71^{b}	0.06	3.01^{c}	0.14
Group IV: Late ulcerative colitis	2.27^{a}	0.10	0.37^{c}	0.02	4.08^{b}	0.16
Group V: Late ulcerative colitis + Lycopene treated	1.82^{b}	0.09	0.57^{b}	0.05	3.12^{c}	0.13

Data are presented as (fold change mean ± SEM). SEM = Standard error mean. Means within the same column carrying different superscript letters are significantly different (P≤ 0.05).

Table 3 Effect of lycopene administration on the relative expression of Caspase-3 and Bcl-2 gene in colon tissue of acetic acid -induced ulcerative colitis in rats.

Animal groups	Caspase -3		Beta cell lymphoma-2 (Bcl-2)	
	Fold change mean	SEM	Fold change mean	SEM
Group I: Normal control	1.00^{d}	0.00	1.00^{a}	0.00
Group ΠI: Early ulcerative colitis	4.53^{b}	0.19	0.06^{d}	0.003
Group III: Early ulcerative colitis + Lycopene protected	1.88^{c}	0.09	0.65^{b}	0.04
Group IV: Late ulcerative colitis	5.82^{a}	0.26	0.05^{d}	0.005
Group V: Late ulcerative colitis + Lycopene treated	2.30^{c}	0.12	0.20^{c}	0.02

Data are presented as (fold change mean ± SEM). SEM = Standard error mean. Means within the same column carrying different superscript letters are significantly different (P≤ 0.05).

Histo-pathologically, the colitis group (Figs. 2, 4) showing ulcer replaced by abundant necrotic debris, fibrin, degenerate neutrophils, necrosis and erosion. Cellular infiltration, and crypt abscess formation were decreased in the experimental colitis plus lycopene group in comparison with the colitis group (Figs 3 & 5).

4. DISCUSSION

Because pathogenesis and pathological changes associated with acetic acid-induced acute mucosal inflammatory injury in the distal colon are similar to that are seen in human ulcerative colitis (UC), acetic acid-induced model was commonly used to investigate the pathogenesis of UC and to screen for and evaluate new drugs for treating this condition (Kaulman et al., 2016). Ulcerative colitis is usually associated with superficial ulceration and inflammation, enhanced vascular permeability as well as severe influx of neutrophils and macrophages to the site of injury (Kandhare et al., 2016).

The obtained results showed significant increase in L-MDA concentration with marked decreases in CAT activity and GSH concentration in colon tissue of acetic acid-induced UC in rats. Oxidative injure has been established to have significant serious effects on IBD pathogenesis (Sepehrimanesha et al., 2018).

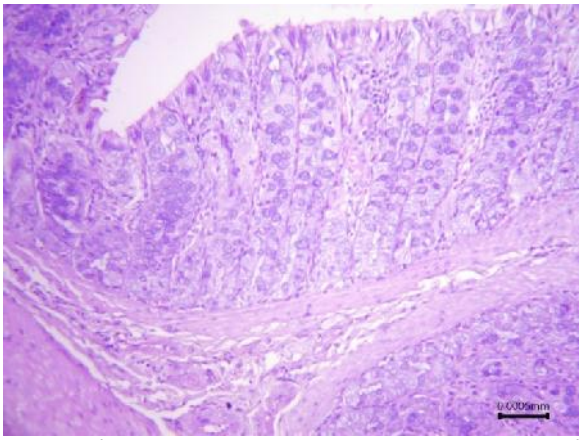

Fig. 1 Colon of normal control rats (Group I) showing normal histological appearance of mucosal epithelium, lamina propria and muscularis. H&E stain x 200.

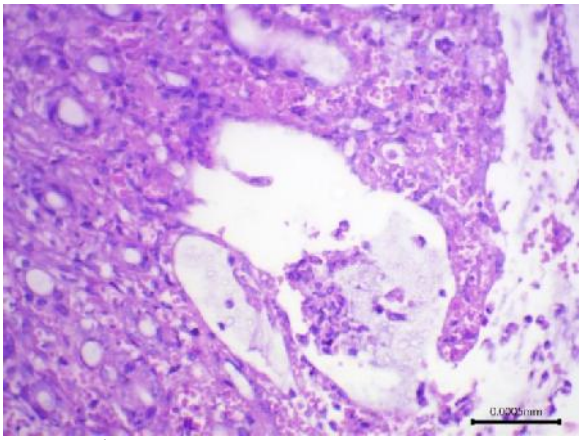

Fig. 2 Colon of early UC-induced rats (Group II) showing ulcer replaced by abundant necrotic debris, fibrin, numerous viable and degenerate neutrophils, macrophages and hemorrhage. H&E stain x 200.

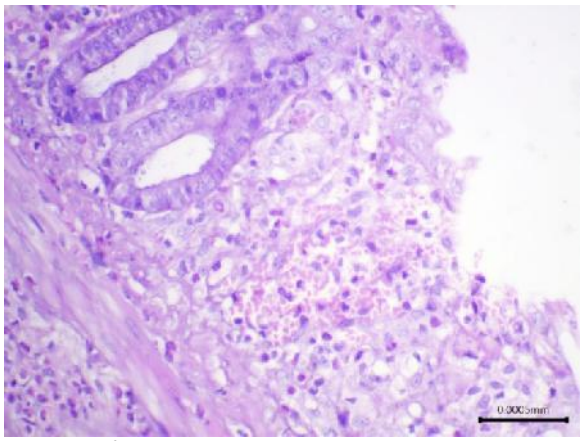

Fig. 3 Colon of early UC plus lycopene protected rats (Group III) showing ulcer characterized by loss of the mucosal epithelium and lamina propria, with replacement by debris, fibrin, inflammatory cells and hemorrhages. H&E stain x 200.

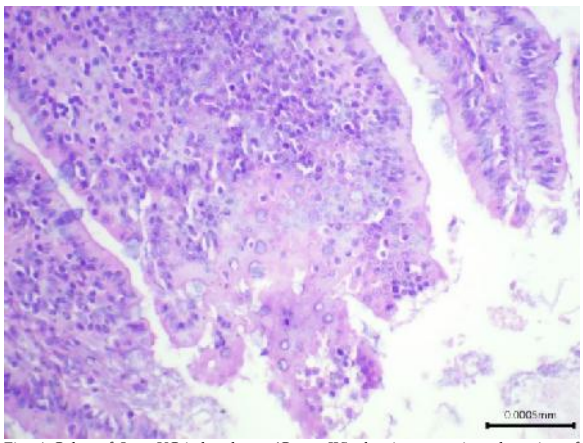

Fig. 4 Colon of Late UC-induced rats (Group IV) showing necrosis and erosion of superficial cells of hyperplastic glands with mononuclear inflammatory cellular infiltration. H&E stain x 200.

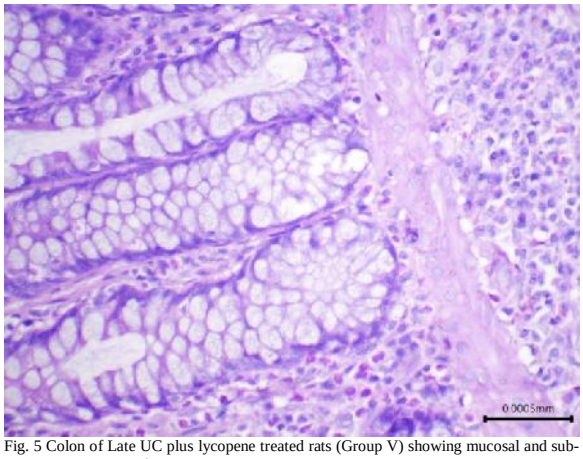

Fig. 5 Colon of Late UC plus lycopene treated rats (Group V) showing mucosal and sub-mucosal aggregates of lymphocytes, viable and degenerate neutrophils and macrophages. H&E stain x 200.

Acetic acid produced colon ulcer through different mechanisms including generation of oxidative stress, initiation of lipid peroxidation, inflammation, infiltration of neutrophils and induction of apoptosis (Tekeli et al., 2018). MDA is a product of the lipid peroxidation that takes place as a consequence of colonic insult (Ozsoy et al., 2017). On the other hand, depletion of tissue GSH has been implicated in the development of UC (Shalkami et al., 2018). GSH is the principal non-peptide sulfhydryl

component (NP-SH) present in mammal cells (Al Asmari et al., 2014) and confers an antioxidant protection against free radicals. CAT is a common antioxidant enzyme present almost in all living tissues that utilize oxygen (Ighodaro and Akinloye, 2018).

In the current study a significant depletion of colon tissue L-MDA and marked increases in CAT activity and GSH concentration were observed after administration of lycopene in colitis- induced rats. Nearly similar results were reported by İbrahim et al. (2019). Lycopene is the most effective antioxidant among the carotenoids and is known to be a highly efficient scavenger of singlet oxygen and other excited species (Atessahin et al., 2010). During quencher of singlet molecular oxygen (1O2), energy is transferred from 1O2 to lycopene, converting it to the energy-rich triplet state. Hence, lycopene may protect in vivo against lipid, protein and DNA oxidation (Drai et al., 2009). These improvements in colon tissue and oxidant/antioxidant balance after lycopene administration may be explained by its free radical scavenging and antioxidant capacities.

The obtained qPCR results revealed a significant up regulation of NF-κB, caspase-3 and TGF-β1 with a significant down regulation of IL-10 and Bcl-2 gene expression levels in colon of acetic acid-induced ulcerative colitis in rats .Increased expressions of NF-κB and its downstream apoptosis hallmark, caspase-3 in the colonic tissues of the acetic acid-induced ulcerative colitis was reported by El-Shaimaa et al. (2020). IL-10 is an important anti-inflammatory cytokine and plays essential role in controlling immune responses in the intestinal mucosa .It inhibits the synthesis of pro-inflammatory cytokines such as TNF-α, IL-1β and IFN-γ and blocks NF-κB activation. These results were nearly similar to those reported by El-Shaimaa et al. (2020), who revealed a significant rise in the inflammatory status of the injured colon with decreased levels of IL-10.Apoptosis is a very important form of programmed cell death that has been conserved throughout evolution .This cellular process is executed by a number of proteases, called caspases, which can be activated in two ways: the extrinsic and the intrinsic pathways (Brahmbhatt et al., 2015). An important increase was observed in the colonic caspase-3 activity animal models of induced colitis (Esra et al., 2020).The extrinsic pathway is triggered by cell-surface death receptors (such as Fas) coupled to extracellular signals, and the intrinsic or mitochondrial pathway is regulated by the Bcl-2 family of proteins. Enhanced expression profile of Bcl-2 was observed in the colonic epithelial cells of IBD patients (Xu et al., 2015).The level of the anti-apoptotic Bcl-2 protein was significantly decreased in AA-treated rats compared with the normal control group (Saber et al., 2019).

In the current study the obtained qPCR results revealed a significant down regulation of NF-κB andcaspase-3 with a significant up regulation of TGF-β1, Bcl-2 and IL-10 gene expression levels in colon of ulcerative rats following administration of LYC either before or after induction of UC. Lycopene has been found to stimulate the production of anti-inflammatory cytokines such as IL-10, which controls the inflammation (Feng et al., 2010). Similarly, Baykalir et al. (2017) reported that, lycopene probably exhibited this protective effect based on its potent antioxidant properties during colitis.

The histological damage in colon disrupts the mucosal barrier function and exacerbates inflammation further by increasing intestinal permeability, which is one of the etio-pathological factors in the development of IBD (Lopetuso et al., 2015). In this current study, it was defined that administration of AA significantly caused histopathological and morphological changes in the colon tissue. Treatment of lycopene caused amelioration in the colon histological view (Figure3,5). Cellular infiltration and crypt abscess formation were decreased in the treatment with LYC group in comparison with the colitis group.

5. CONCULOSIONS

These findings suggest that oral treatment with lycopene showed an effective protection against colitis and oxidative damage in colon mucosal tissue induced by acetic acid in rat. This effect of lycopene may be attributed to interference with three different processes including oxidative stress, inflammation and apoptosis.

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