



Anti-inflammatory and antioxidant effects of pomegranate on acetic acid-induced ulcerative colitis in rats

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ABSTRACT

The present study was designed to investigate the protective effect of pomegranate extract on some blood and antioxidant parameters in addition to interleukin-1 (IL-1) and inducible nitric oxide (iNOS) gene expression of colon tissue in acetic acid-induced ulcerative colitis in rats. Forty rats were divided into four equal groups. Group I (c) group (control normal group) received no drugs. Group II (AA) group (Acetic acid induced ulcerative colitis group) rats were administrated with 2 ml of acetic acid (AA) 4% intracolonic as a single dose. Group III (PGFE) group (Pomegranate group) rats were administrated with pomegranate orally (500 mg/kg, b.wt/day for 14 days. Group VI (PGFE+AA) group (PGFE protected plus AA 4% induced ulcerative colitis group) rats were administrated with PGFE orally (500 mg/kg, b.wt/day for 14 days and at the 11th day was administrated intracolonic as a single dose with 2 ml of AA 4%. The obtained results showed that a significant decrease in neutrophils to lymphocytes ratio (N/LR) and increase in lymphocytes to monocytes ratio (L/MR) in (PGFE+AA) group when compared with (AA) group. Meanwhile, malondialdehyde (MDA) and nitric oxide (NO) were significantly decreased with significant down-regulation in IL-1 β and iNOS gene expression levels were obtained in colon tissue of (PGFE+AA) group when compared with (AA) group. The results revealed that pomegranate has protective, anti-inflammatory and antioxidant effects in experimental model of ulcerative colitis in induced rats.

Keywords: *IL-1 β , iNOS, pomegranate, acetic acid, rats.*

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1. INTRODUCTION

Inflammatory bowel disease (IBD) results from dysregulation of intestinal mucosal immune responses to microflora in genetically susceptible hosts (*Yan et al., 2012*), which includes Crohn's disease (CD) and ulcerative colitis (UC) (*Khor et al., 2011*).

Pathogenesis of IBD wasn't fully demystified today, however, risk factors may include a variety of environmental, immunologic, genetic, microbial, and psychological factors combined with

unnatural autoimmune response to some stimuli, involvement of cells and proteins, with immune dysfunction, oxidative stress, and the inflammatory mediators playing a major role in IBD development (*Szandruk et al., 2017*).

Ulcerative colitis is characterized by significant thickening and dense infiltration of neutrophils, monocytes, macrophages, T cells typically on the mucosal layer of the bowel wall (*Ellrichmann et al., 2014*). Infiltration of white globules and macrophages into intestinal mucosa is a

salient sign of IBD. Activated neutrophils on mucosal surface produce free radicals, such as peroxide ion, radical hydroxyl, and hydrogen peroxide. These elements cause lipid peroxidation, increased vascular permeability, higher levels of neutrophils on intestinal mucosa, and chronic inflammation (*Tertychnyi et al., 2017*).

During the progression of IBD, disruption of intestinal epithelial barrier is regarded as the central event in IBD pathogenesis which is followed by robust immune responses towards intestinal flora in a context of genetic predisposition (*Sanchez-Munoz et al., 2008*). These barrier defects are attributed to enhanced activity of pro-inflammatory cytokines like tumor necrosis factor alpha (TNF α), interferone gamma (INF γ), IL-1 β and IL-13, which are highly expressed in the chronically inflamed intestine (*Hering et al., 2012*).

Acetic acid-induced colitis is a model used to screen various drugs for colitis (*Mohsen et al., 2011*) which is characterized by severe inflammation, oxidative stress, neutrophil infiltration and release of different inflammatory mediators that are the hallmarks of this model (*Carty et al., 2000*).

Punicalagin and ellagic acid, the main bioactive constituents in the pomegranate husk, have been reported to exert diverse actions such as antioxidant, (*Madriral-Carballo et al., 2009*), immunoprotection (*Reddy et al., 2007*), and produce a beneficial effect in ulcerative colitis (*Ogawa et al., 2002*). Consequently, the aim of the present study was to investigate the protective effects, an anti-inflammatory, and antioxidant properties of Pomegranate in acetic acid induce UC in rats *via* determination of MDA, NO, IL-10, iNOS,

Cyclooxygenase (COX-2) , and Zonula Occluding-1(ZO-1).

2. MATERIALS AND METHODS

2.1. Experimental animal:

The present study was carried out on forty (40) Wistar rats on 6 weeks age at weighing 100-120 g obtained from the Animal House, Faculty of Veterinary Medicine, Benha University, Egypt. All animals were caged and maintained on a standard diet, with free access to tap water and were acclimatized for 1 week before the starting of the experiment.

2.2. Acetic acid solution (4% v/v):

The acetic acid 4% was prepared by diluting glacial acetic acid 99.9% with 0.9 physiological saline.

Induction of colitis:

Experimental ulceration in colon tissue was done, according to the method described by *Mousavizadeh et al., (2009)* with slight modification. In brief, under light ether anesthesia rats were administered 2 mL of 4% acetic acid solution (v/v) intracolonic using polyurethane tube (external diameter 2mm). After AA administration, rats were hold horizontally for 2 min to prevent AA leakage from anal opening. Control animals underwent the same procedure using equal volume of normal saline instead of AA solution.

2.3. Pomegranate fruit extract (PGFE):

Pomegranate fruit extract was purchased from I Herb Company, USA, and PGFE was dissolved in distilled water and administrated orally once daily at a dose of 500 mg/kg body weight with according to *Rosillo et al., (2012)* by gastric tube for 10 days before intracolonic injection with acetic acid and for 4 days after acetic acid.

2.4. Experimental design:

Groups	Chemical /Route/ Dose/Duration	Type and Time of sampling / Parameters measured
C	Saline given intracolonic at a dose of 2ml as single dose.	<ul style="list-style-type: none"> ● Whole blood, Serum and tissues samples of colon at 2, 4 day of intracolonic injection with diluted acetic acid 4% for: Total leukocytic count and differential leukocytic count. Biochemical studies: Measurement of GPX, MDA and NO in tissue. Determination of mRNA expression of inflammatory cytokines: IL-1β, IL-10, ZO-1 COX-2 and iNOs in tissue. Histopathological examination: Colonic segments were stained by H&E.
AA	Acetic acid 4% given <i>via</i> intracolonic route at a dose of 2 /ml as a single dose.	
PGFE	Pomegranate fruit extract given <i>via</i> oral route at a dose of 500mg/kg B.W once daily by gastric tube for 14 days.	
PGFE +A.A	Pomegranate fruit extract given <i>via</i> oral route at a dose of 500mg/kg B.W daily by gastric tube for 14 days. On 11th day of experimental period administrated with 2 ml of acetic acid 4% intracolonic as a single dose.	

2.5. Colon lipid peroxidation and enzymatic antioxidant:

Briefly, Colon tissues were divided into suitable portions, tissue was rinsed with 1×PBS to remove excess blood, homogenized in 20 mL of 1×PBS and stored overnight at ≤ -20 °C. After two freezes –thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. Remove the supernate and assay immediately. The resultant supernatant will be used for determination of the following parameters according to the kit manufacture's instructions. For MDA the Rat Malondialdehyde ELISA Kit (Mybiosource, USA), NO the Rat Total Nitric Oxide (NO) ELISA Kit (Mybiosource, USA), and GPX the Rat Glutathione Peroxidase 1, GPX1 ELISA Kit (Mybiosource, USA) were used.

The ELISA is based on the competitive binding enzyme immunoassay technique. The microtiter plate provided in this kit has

been pre-coated with an antibody specific to MDA or NO or GPX, during the reaction, MDA or NO or GPX in the sample or standard competes with a fixed amount of biotin-labeled for sites on a pre-coated onto a microplate. Excess conjugate and unbound sample or standard are washed from the plate. Next, Avidin conjugate to horseradish peroxidase (HRP) is added to each microplate well and incubated. Then a tetramethylbenzidine (TMB) substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm \pm 2nm. The concentration of MDA or NO or GPX in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2.6. Real-time polymerase Chain Reaction (PCR) protocol:

Total RNA was extracted from colon tissues using RNeasy Mini kit (Qiagen,CA, USA)

and the purity of obtained RNA was verified spectrophotometrically at 260/280 nm. Equal amounts of RNA (2 mg) were reverse transcribed into cDNA using Superscript Choice systems (Life Technologies, USA) according to the manufacturer's instructions. To assess the expression of inflammation and apoptosis-associated target genes, quantitative realtime PCR was performed using SYBR green PCR Master mix (Applied Biosystems, CA, USA) as described by the manufacturer. Briefly, in a 25 ml reaction volume, 5 ml of cDNA was added to 12.5 ml of SYBR green Master mix and 200 ng of each primer. PCR reactions included 10 min at 95°C for activation of AmpliTaq Gold DNA polymerase, followed by 40 cycles at 95°C for 15 sec (denaturing) and 60°C for 1 min (annealing/extension). The expression level was calculated from the PCR cycle number (CT) where the increased fluorescence curve passes across a threshold value. The relative expression of target genes was obtained using comparative CT (DDCT) method. The DCT was calculated by subtracting GAPDH CT from that of target gene whereas DDCT was obtained by subtracting the DCT of calibrator sample (control gp) from that of test sample. The relative expression was calculated from the 2-DDCT formula (Livak and Schmittgen, 2001).

2.7. Histopathological examination:

Autopsy samples were taken from the colon of rats and fixed in neutral buffered formaline solution for twenty four hour. Washing was done in tap water then ascending grades of ethyl alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven. Paraffin bees wax tissue blocks were prepared for sectioning at microns by slide microtome. The

obtained tissue sections were collected on glass slides, deparaffinized and stained by hemotoxylin and eosin stains for histopathological examination (Banchroft et al., 1996).

2.8. Statistical analysis:

Statistical analysis was performed using the statistical software package for social science (SPSS) for Windows (Version 16.0; SPSS Inc., Chicago, IL). The significance of differences between the experimental groups was evaluated by one-way analysis of variance (ANOVA). If one-way ANOVA indicated a significant difference, then differences between individual groups were estimated using Duncan as a post hoc. Results are expressed as the mean \pm standard error of mean. A *P*-value of less than 0.05 was considered significant (Kinnear and Gray, 2006).

3. RESULTS

The results presented in (Table 1,2) showed that there were no significant changes in TLC, lymphocytes, eosinophil and basophil in acetic acid-treated group after 2 days when compared with control group. Meanwhile, significant increases in neutrophil and neutrophil/lymphocytes ratio (N/LR) were obtained in acetic acid-treated group after 2 and 4 days when compared with control group. Also, a significant increase in monocytes was observed in acetic acid-treated group after 2 days, but a significant decrease in lymphocytes/monocytes ratio (L/MR) was observed in acetic acid-treated group after 2 and 4 days when compared with control group. Rats treated with acetic acid showed no significant changes in TLC, monocytes, eosinophil, and basophil after 4 days when compared with control group. Meanwhile, a significant decrease in lymphocytes was observed in acetic acid-treated group after 4 days when compared with control group.

Concerning to the results of TLC in pomegranate protected plus acetic acid, a significant increase was observed after 2 days with significant decrease after 4 days when compared with acetic acid-treated group. Additionally, there were significant reductions in neutrophils, monocytes and N/LR in pomegranate protected plus acetic acid-treated group after 2 and 4 days when compared with acetic acid-treated group. Meanwhile, lymphocytes, and L/MR showed significant increase in pomegranate protected plus acetic acid-treated group after 2 and 4 days when compared with acetic acid-treated group. No significant changes were revealed in eosinophil and basophils in pomegranate protected plus acetic acid-treated group after 2 and 4 days when compared with acetic acid group. Concerning to antioxidant changes and oxidative stress biomarkers in colon tissue (Table 2,3), acetic acid-treated group revealed a significant decrease in glutathione peroxidase (GPX) activity and significant increase in malondialdehyde (MDA) and nitric oxide (NO) concentrations after 2 and 4 days when compared with control group. On the other hand, there was a significant increase in GPX activity in pomegranate protected plus acetic acid-treated group after 2 days when compared with acetic acid-treated group. Meanwhile significant decrease in MDA and NO concentration were observed in pomegranate protected plus acetic acid-treated group after 2 and 4 days when compared with acetic acid-treated group. Concerning to cytokines changes (fig.1), there was no significant change in IL-10 gene expression level in colon tissue and significant up-regulation in IL-1 β , iNOS, COX-2, and ZO-1 gene expression level in colon tissue of acetic acid treated group were observed after 4 days when compared

with control group. On contrast, there was significant down-regulation in IL-1 β , INOs, COX-2, and ZO-1 gene expression level in colon tissue of pomegranate protected plus acetic acid-treated group after 4 days when compared with acetic acid-treated group.

Histopathological examination:

The distal part of colon of normal animal showed normal intestinal crypts with normal lining epithelium (fig.2). The distal part of the colon of rat received acetic acid after 2 days from administration showing heavy leukocytic infiltrations in between intestinal villi especially intestinal mucosa fig. (3). Meanwhile after 4 days from administration the distal part showing heavy replacement of the intestinal villi by leukocyte fig. (4). The distal part of the colon of rat treated with PGFE and administrated with acetic acid revealed marked decrease the acetic acid-associated colitis which mostly seen within the mild to moderate degree of colitis either on the 2nd or 4th days of colitis fig. (5, 6).

4. DISCUSSION

Concerning to acetic acid-treated group, there were significant increases in neutrophil and neutrophil/ lymphocytes ratio (N/LR) after 2 and 4 days when compared with control group. These results agree with *Cherfane et al., (2015)* who reported that Neutrophil/Lymphocyte ratio (NLR) has been generally observed in inflammatory disease such as ulcerative colitis. An increase in N/LR means systemic inflammation responses (*Xue et al., 2014*). Meanwhile, lymphocytes showed significant decrease in acetic acid-treated group after 4 days when compare with control. These results agree with *Meek and Morton, (2016)* who demonstrated that the ulcerative colitis is an inflammatory reaction disease that associated with mobilization and recruitment some types of

Table (1): Leukogram after 2 days in acetic acid treated (AA) group, pomegranate group (PGFE), and pomegranate protected plus acetic acid group (PGFE+AA).

Animal groups	WBCs ($\times 10^3/\mu\text{l}$)	Neutrophils ($\times 10^3/\mu\text{l}$)	Lymphocytes ($\times 10^3/\mu\text{l}$)	Monocytes ($\times 10^3/\mu\text{l}$)	N/L Ratio	L/M Ratio	Eosinophil ($\times 10^3/\mu\text{l}$)	Basophil ($\times 10^3/\mu\text{l}$)
C _(D2)	9.33±0.32 ^c	2.08±0.05 ^c	6.92±0.64 ^{cd}	0.21±0.01 ^c	0.19±0.02 ^b	20.70±1.52 ^{bc}	0.29±0.01 ^a	0.01±0.01 ^a
AA _(D2)	9.57±0.10 ^c	3.78±0.17 ^a	5.59±0.05 ^d	0.34±0.02 ^a	0.62±0.03 ^a	12.64±0.58 ^d	0.32±0.03 ^a	0.01±0.01 ^a
PGFE _(D2)	10.25±0.10 ^{bc}	1.28±0.08 ^d	7.28±0.09 ^b	0.23±0.01 ^{bc}	0.19±0.01 ^b	30.36±1.60 ^a	0.30±0.01 ^a	0.02±0.00 ^a
PGFE+AA _(D2)	11.04±0.47 ^b	1.53±0.11 ^d	7.43±0.02 ^b	0.26±0.01 ^{bc}	0.22±0.03 ^b	30.29±0.45 ^a	0.28±0.04 ^a	0.02±0.00 ^a

Table (2): Leukogram after 4 days in acetic acid treated (AA) group, pomegranate group (PGFE), and pomegranate protected plus acetic acid group (PGFE+AA).

Animal groups	WBCs ($\times 10^3/\mu\text{l}$)	Neutrophils ($\times 10^3/\mu\text{l}$)	Lymphocytes ($\times 10^3/\mu\text{l}$)	Monocytes ($\times 10^3/\mu\text{l}$)	N/L Ratio	L/M Ratio	Eosinophil ($\times 10^3/\mu\text{l}$)	Basophil ($\times 10^3/\mu\text{l}$)
C _(D4)	12.44±0.40 ^b	1.60±0.15 ^c	7.21±0.10 ^b	0.49±0.03 ^{ab}	0.17±0.01 ^d	23.90±0.66 ^b	0.29±0.01 ^a	0.02±0.01 ^a
AA _(D4)	12.65±0.46 ^{ab}	3.95±0.34 ^a	5.60±0.17 ^c	0.52±0.01 ^a	0.50±0.03 ^a	18.71±0.01 ^c	0.32±0.01 ^a	0.02±0.01 ^a
PGFE _(D4)	9.48±0.18 ^d	1.51±0.11 ^c	7.08±0.04 ^b	0.36±0.02 ^c	0.20±0.02 ^d	20.36±0.60 ^{bc}	0.27±0.02 ^a	0.02±0.01 ^a
PGFE+AA _(D4)	10.55±0.15 ^c	2.05±0.43 ^c	7.43±0.20 ^b	0.34±0.03 ^c	0.28±0.02 ^c	22.55±0.15 ^b	0.31±0.01 ^a	0.02±0.01 ^a

Mean values with different superscripts letters (a, b, c and d) within the same column are significantly different at ($p < 0.05$). D₂ mean 2 days, D₄ mean 4 days. AA :acetic acid treated group, PGFE: pomegranate group, PGFE+AA: pomegranate protected plus acetic acid group.

Tables (3): Changes in GPX, MDA , and NO in colon tissues after 2 days in acetic acid treated (AA) group, pomegranate group (PGFE), and pomegranate protected plus acetic acid group (PGFE+AA).

Animal groups	GPX (U/g. tissue)	MDA (mmol/g. tissue)	NO (MmoI/g. tissue)
C _(D2)	12.99±0.61 ^a	41.17±3.99 ^d	36.89±1.72 ^d
AA _(D2)	7.85±0.79 ^c	104.09±7.96 ^a	75.59±2.65 ^a
PGFE _(D2)	10.68±0.48 ^{ab}	61.44±2.31 ^c	40.94±4.76 ^{cd}
PGFE+AA _(D2)	10.72±0.63 ^{ab}	81.74±4.56 ^b	64.20±2.55 ^b

Tables (4): Changes in GPX, MDA , and NO in colon tissues after 4 days in acetic acid treated (AA) group, pomegranate group (PGFE), and pomegranate protected plus acetic acid group (PGFE+AA).

Animal groups	GPX (U/g. tissue)	MDA (mmol/g. tissue)	NO (MmoI/g. tissue)
C _(D4)	12.41±0.77 ^a	58.84±2.39 ^d	35.94±2.48 ^c
AA _(D4)	7.66±0.60 ^b	108±7.81 ^a	77.61±4.03 ^a
PGFE _(D4)	9.95±0.69 ^{ab}	68.27±3.25 ^{cd}	54.56±3.54 ^b
PGFE+AA _(D4)	9.94±0.91 ^{ab}	82.52±3.11 ^{bc}	56.26±3.52 ^b

Mean values with different superscripts letters (a, b, c and d) within the same column are significantly different at ($p < 0.05$). D₂ mean 2 days, D₄ mean 4 day. AA :acetic acid treated group, PGFE: pomegranate group, PGFE+AA: pomegranate protected plus acetic acid group.

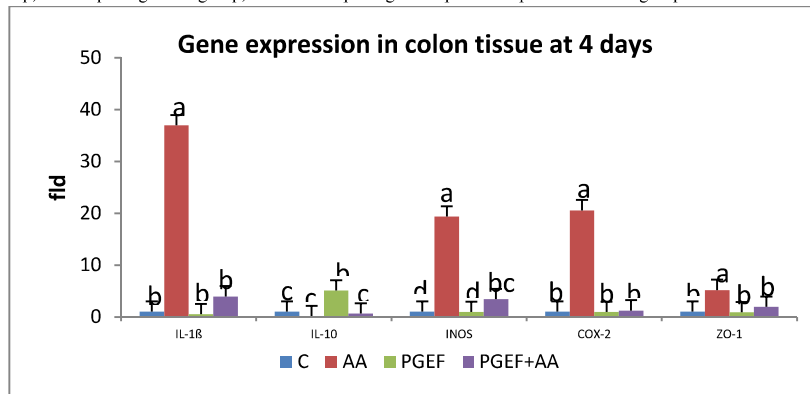
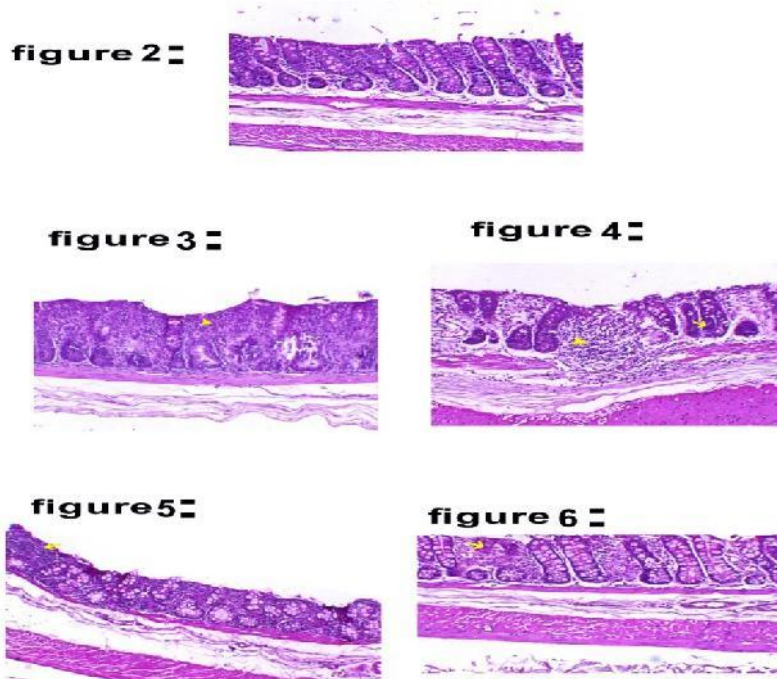


Figure (1): Changes in cytokines after 4 days of intracolonic injection with acetic acid in acetic acid (AA) group, Pomegranate (PGFE) group, and Pomegranate protected plus acetic acid (PGFE+AA) group.



Figures 2-6: Figure 2, proximal colon of normal animal showing normal intestinal crypts with normal lining epithelium. Figure 3. Colon of animal treated with acetic acid sacrificed at 2 days. Distal part of colon is showing colitis associated with crypt degeneration and necrosis of the most upper and heavy leukocytic infiltration (arrowhead). Figure 4. Colon of animal treated with acetic acid sacrificed at 4 days. Distal part is showing lymphocytic cell infiltrations (arrow). Figure 5. Colon of animals treated with PGFE+AA sacrificed at 2 days. Distal part of colon is showing mild to moderate degree of colitis (arrowhead indicates interstitial inflammatory cell infiltration). Figure 6. Colon of animals treated with PGFE+AA sacrificed at 4 days. Distal part of colon is showing mild degree of colitis (arrowhead) H&E, X200.

white blood cells (WBCs) such as lymphocytes, neutrophils, and macrophages from the circulation to the colon mucosa. Meanwhile, monocytes showed significant increase after 2 days, and significant decrease in lymphocytes/monocytes ratio (L/MR) in acetic acid-treated group after 2 and 4 days when compared with control. The obtained results are in agreement with *Chen et al., (2017)* who found that elevated monocyte counts and decreased lymphocytes to monocytes ratio (L/MR) values were significantly differed between active UC and UC in remission and performed better than other leukocyte profiles. On contrary, there were significant reductions in neutrophils, monocytes and N/LR in pomegranate protected plus acetic acid-treated group after 2 and 4 days when compared with acetic acid-treated group. Meanwhile, there were significant increases

in lymphocytes and L/MR in pomegranate protected plus acetic acid-treated group after 2 and 4 days when compared with acetic acid-treated group. The current results agreed with *Aruna et al., (2016)*. Pomegranate seed oil (PSO) was shown to exhibit *in vivo* antioxidant and anti-inflammatory activities by limiting neutrophil-activation and lipid peroxidation consequences, which may be useful in the prevention and treatment of several inflammatory diseases, such as inflammatory bowel disease (*Boussetta et al., 2009*). Regarding to antioxidant parameters, acetic acid-treated group revealed a significant decrease in glutathione peroxidase (GPX) activity with marked increase in malondialdehyde (MDA) and nitric oxide (NO) concentration in acetic acid-treated group after 2 and 4 days when compared with control group. These

results go in harmony with that of *Balmus et al., (2016)*. Oxidative stress is considered to be a causative factor of AA-associated alterations in the colon tissue. Experimental studies have indicated that oxidative stress results from the shift of equilibrium between the pro-oxidant and anti-oxidant systems in favor of the pro-oxidant system as a result of excessive production of free oxygen radicals (*Halliwell and Chirico, 1993*). On the other hand, there was a significant increase in GPX in pomegranate protected plus acetic acid-treated group after 2 days, and significant decrease in MDA and NO concentration in pomegranate protected plus acetic acid-treated group after 2 and 4 days when compared with acetic acid-treated group. These results agree with they observed by *Larrosa et al., (2010)*. Anti-inflammatory effect of pomegranate fruit extract is achieved by the inhibition of the inflammatory cytokine-induced production of PGE₂ and +NO (*Shukla et al., 2008*). Punic acid (Pomegranate seed oil) is effective against ROS/MPO-mediated tissue damage and reduces the neutrophil-activation (*Yuan et al., 2009*).

Concerning to cytokines, IL-1 β in acetic acid-treated group observed significant up-regulation gene expression level in colon tissue after 4 days when compared with control group. Our results agree with *Al-Rejaie et al., (2013)*. An excess of free radicals leads to the impairment of the local intestinal mucosal barrier, and immune activation causes a transient production of inflammatory cytokines IL-1 β contributing to the inflammatory process of ulcerative colitis (*Pravda, 2005*). Meanwhile, no significant change in IL-10 gene expression level in colon tissue of acetic acid was observed after 4 days when compared with control group. These results agree with

Togawa, (2002) who showed no significant change in IL-10 in colitis and disagree with *Tomoyose et al., (1998)*; *Barada, (2007)* who reported that a rise of IL-10 was observed in colitis. Meanwhile, *Ishizuka et al., (2001)* reported that decreased production of IL-10 was associated with severe cases of IBD. IL-10 deficiency leads to spontaneous colitis (*Huber, 2011*; *Rubtsov, 2008*). Polymorphisms in the genes that encode IL-10 or IL-10 receptor are linked to increased incidence of IBD (*Rubtsov, 2008*; *Franke, 2008*). A significant up-regulation gene expression level in colon tissue in iNOs of acetic acid-treated group was observed after 4 days when compared with control group. Our results agree with *Piechota-Polanczyk and Fichna, (2014)*. INOS-derived NO stimulates TNF- α production in the middle and distal colon, which promotes the infiltration of neutrophils for example through stimulation of synthesis of intracellular adhesion molecule (ICAM) and P-selectin, therefore leading to colonic tissue damage (*Yasukawa et al., 2012*).

Regarding to COX-2, significant up-regulation gene expression level in colon tissue was observed in acetic acid-treated group after 4 days when compared with control group. These results agree with *Tsubouchi et al., (2006)*. There is two isoforms of COX: constitutive COX-1 and inducible COX-2. COX-1 has been considered crucial for mucosal integrity since it produces cytoprotective and anti-inflammatory prostaglandins such as PGE₂ (*Dubois et al., 1998*). On the contrary, the expression of COX-2 can be induced by a variety of stimuli related to the inflammatory response. This isoform is responsible for an increased production of prostaglandins involved in IBD (*Morita, 2002*; *Willoughby and Moore, 2000*).

Concerning to ZO-1 in acetic acid-treated group a significant up-regulation gene expression level in colon tissue was observed after 4 days when compared with control group. These results agree with *Xavier and podolsky, (2007)* who exhibit a dysfunctional intestinal epithelium barrier with increased tight junction permeability was occurred in genetically predisposed IBD. The release of TNF- α and INF- γ induces intestinal damage in IBD, such as changes in tight junction (TJ) structures, apoptosis and enhanced bacterial translocation (*Hering et al., 2012*), that can be reproduced in cell and animal experimental models.

In addition, several studies suggest that the impairment of the epithelial barrier function can be considered as one of the early events that occur in intestinal inflammation, since it facilitates the entry of antigens from the intestinal lumen to the mucosa that may prompt the uncontrolled and exacerbated immune response (*Vivinus-Nebot et al., 2014*).

Conversely, IL-1 β , INOs, COX-2, and ZO-1 was observed significant down-regulation gene expression level in colon tissue of pomegranate protected plus acetic acid-treated group after 4 days when compared with acetic acid-treated group. Our results agreed with *Lian et al., (2009)*; *Singh et al., (2009)*; *Larrosa et al., (2010)*; *Umesalma and Sudhandiran, (2010)*; *Rosillo et al., (2011)*; *Rosillo et al., (2012)*. *Punica granatum L.* (Lithraceae) extract and its ellagic acid-rich fraction have suggested that it reduced the damage in colitis, alleviated the oxidative events and reduced pro-inflammatory protein to basal levels probably through preventing MAPKS and NF-kB signaling pathways (*Rosillo et al., 2011*). The individual effect of ellagic acid and their microbiota are derived metabolites

uroolithins (Uro) on colon fibroblasts upon IL-1 β treatment as an *in vitro* inflammation model. Uro-A down regulated COX-2 and PGE synthase -1 (Mpges-1) mRNA expression and protein levels (*González-Sarrías et al., 2010*). The results suggest that Uro-A is the main compound responsible for the pomegranate anti-inflammatory properties (*Manach et al., 2005*).

A mixture of urolithins A, B and ellagic acid completely abolished IL-1 β induction by PGE, and this activity was mainly due to urolithin A (*Gimenez-Bastida, et al., 2012*). Colonic mucus plays an important protective role against chemically induced ulceration which may also facilitate the repair of the damaged epithelium (*Al-Rejaie et al., 2012*). The peel extracts of *Punica granatum* have substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid (*Barzegarl et al., 2007*). Ellagic acid also increased the amount of mucus in colon mucosa. The protective effect of mucus as an active barrier may be largely attributed to its viscous and gel forming properties which are derived from mucin glycoprotein constituents. This viscous mucus seems to be associated with regenerative processes of the mucosa (*Alarcon et al., 1994*).

5. CONCLUSION

In conclusion, the results of present study revealed that ulcerative colitis induced by acetic acid as well as protection and treatment with pomegranate in ulcerative colitis could be reflected by alterations in antioxidant enzyme and pro-inflammatory cytokines, indicating the anti-inflammatory and antioxidant properties of pomegranate extract as powerful agents in ulcerative colitis.

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