



Hesperidine promotes recovery of ulcerative colitis and inhibit inflammatory responses in acetic acid-treated rat

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ABSTRACT

The present study was designed to investigate the protective effect of hesperidin extract on some blood and biochemical profile in addition interleukin-1 (IL-1) and zonula occluding -1(ZO-1) 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) was received no drugs. Group II (AA) group (Acetic acid induced colitis group) rats were administrated with 2 ml of acetic acid (AA) 4% intracolonic as a single dose. Group III (HMC) group (Hesperidin methyl chalone group) rats were administrated hesperidin orally (200 mg/kg, b.wt/day) for 14 days. Group IV (HMC+AA) group (Hesperidin protected + acetic acid 4% induce colitis group) rats were administrated orally (200 mg/kg, b.wt/day) for 14 days and at the 11th day were administrated intracolonic with 2 ml AA 4% as a single dose. The obtained results showed that significant decrease in neutrophils to lymphocytes ratio (N/LR) and increase in lymphocytes to monocytes ratio (L/MR) in (HMC+AA) group when compared with (AA) group. In addition, ALP in serum was significantly decrease with significant down-regulation in IL-1 β , inducible nitric oxide (iNOS), cyclooxygenase-2 (COX2) and ZO-1 gene expression level in colon tissue of (HMC+AA) group when compared with (AA) group. The results revealed that hesperidin has protective, anti-inflammatory and antioxidant effects in experimental model of ulcerative colitis induced in rats.

Keywords: IL-1 β , ZO-1, hesperidin, acetic acid, rats.

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1. INTRODUCTION

Inflammatory bowel disease (IBD) comprises a group of chronic relapsing disorder characterized by inflammation of the gastrointestinal tract, which includes Crohn's disease (CD) and ulcerative colitis (Uc) (Khor *et al.*, 2011). Although the exact cause of IBD is still not clear, but two broad hypotheses have arisen regarding the fundamental nature of the pathogenesis of IBDs. The first contends that primary deregulation of the mucosal immune system leads to excessive immunologic responses to normal microbiota (Stecher and Hardt,

2008). The second one is abnormal gastrointestinal (GI) tract luminal factors such as microorganisms of GI tracts, oxidative stress, and defects in the GI mucosal barrier which allow the penetration of luminal factors into mucosa (Rahimi *et al.*, 2010).

UC occurs in the inner lining of the colon (large intestine) or rectum and the common symptoms are diarrhea, abdominal cramps and rectal bleeding (Debnath *et al.*, 2012). Furthermore, ulcerative colitis is characterized by significant thickening and dense infiltration of neutrophils,

monocytes, macrophages and T cells typically on the mucosal layer of the bowel wall (*Ellrichmann et al., 2014*).

The intestinal epithelium is a first line of defense against luminal bacteria, which consists of absorptive and secretory cells such as goblet cells and small intestinal Paneth cells (*Beisner et al., 2010*). The integrity of layer is maintained by several proteins which include tight junctions (TJs), adherens junctions and desmosomes. It does not only provide mechanical barrier but also release number of microbe-killing molecules (*Beisner et al., 2010*). Acetic acid-induced colitis is a model used to screen various drugs for colitis (*Mohsen et al., 2011*).

Hesperidin is a flavanone-type flavonoid that can be found in abundance in citrus fruits (oranges and lemons). It has natural anti-oxidative and anti-inflammatory effects (*Parhiz et al., 2015*). Consequently, the aim of the present study was to investigate the protective effects, an anti-inflammatory, and antioxidant properties of hesperidin extracts against acetic acid induce UC in rats *via* determinations of the levels of IL-1 β , IL-10, iNOS, COX-2, and ZO-1.

2. MATERIALS AND METHODS

2.1. Experimental animal:

The present study was carried out on forty (40) Wistar rats of 6 weeks age, weighing

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. Hesperidin Methyl Chalcone:

Hesperidin was purchased from I Herb Company, USA, Hesperidin was dissolved in distilled water and administrated orally once daily at a dose of 200 mg/kg body weight (*Acipayam et al., 2013*) by gastric tube for 10 days before intracolonic injection with acetic acid and for 4 days after intracolonic injection with acetic acid.

100-120 g obtained from the Animal House, Faculty of Veterinary Medicine, Benha University, Egypt.

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.	
AA	Acetic acid 4% given <i>via</i> intracolonic route at a dose of 2 /ml as a single dose.	Whole blood, Serum and tissues samples of colon at 2, 4 day of intracolonic injection with diluted acetic acid4% for:
HMC	Hesperidin Methyl Chalcone given <i>via</i> intragastric route at a dose of 200mg/kg B.W once daily by gastric tube for 14 days.	- Total leukocytic count and differential leukocytic count. - Biochemical studies: Measurement of total protein, albumin, ALP in serum. - Determination of mRNA expression of inflammatory cytokines: IL-1 β , IL-10, ZO-1 COX-2 and iNOs in tissue. - Histopathological examination: Colonic segments were stained by H&E.
HMC+AA	Hesperidin Methyl Chalcone given <i>via</i> intragastric route at a dose of 200mg/kg B.W once 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. Determination of Total proteins:

Principle:

Serum total proteins were determined according to Biuret method of *Vassault et al.*, (1986). In this method, copper ions in alkaline media react with peptide bonds of proteins producing violet color, which is proportional to the amount of protein present in the serum sample when measured colorimetrically at 550 nm.

2.6. Determination of Albumin:

Principle:

Colorimetric determination of albumin was performed according to the method of *Doumas*, (1971). At a pH (4.1), albumin binds with bromocresol green (BCG) to form blue -green complex. The color intensity of the blue - green color is directly proportional to the albumin concentration in the sample. It is determined by monitoring the increase in absorbance at 583 nm.

2.7. Determination of serum alkaline phosphatase activity (ALP):

Principle:

Kinetic determination of ALP activity was performed according to the method of *Tietz*, (1983). Alkaline phosphate in the sample catalyzes the hydrolysis of colorless *p*-nitrophenyl phosphate (*p*-NPP) to give *p*-nitrophenol and inorganic phosphate. Optimized concentrations of zinc and magnesium ions are present to activate the

alkaline phosphatase in the sample. The *p*-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance at 409 nm.

2.8. 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 26SYBR green Master mix and 200 ng of each primer. PCR reactions included 10 min at 95uC for activation of AmpliTaq Gold DNA polymerase, followed by 40 cycles at 95uC for 15 sec (denaturing) and 60uC 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*).

2.9. Histopathological examination:

Specimens were taken from the colon of rats and fixed in neutral buffered formalin saline (10%) 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*).

2.10. 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.

On the other hand, there was a significant increase in TLC of hesperidin protected plus acetic acid-treated group after 2 days when compared with acetic acid-treated group. Meanwhile, no significant change in TLC of hesperidin protected plus acetic acid-treated was observed after 4 days when compared with acetic acid-treated group. Also, no significant in eosinophil and basophil in hesperidin protected plus acetic acid-treated group was observed after 2 and 4 days when compared with acetic acid-treated group. Significant increases were obtained in lymphocytes and L/MR in hesperidin protected plus acetic acid-treated after 2 and 4 days, and there were significant decreases in neutrophils, monocytes, and N/LR after 2 and 4 days when compared with acetic acid-treated group.

Our data in (table 3,4), concerning to biochemical parameters, alkaline phosphatase (ALP) results revealed significant elevations in acetic acid-treated group after 2 and 4 days when compared with control group. Meanwhile, total protein (TP) showed no significant alteration after 2 days, and TP showed significant decrease in acetic acid-treated group after 4 days when compared with control group. Additionally, significant decreases in albumin and albumin/globulin ratio (A/GR) was observed in acetic acid-treated group after 2 and 4 days, but a

significant increase in globulin in acetic acid-treated group after 2 and 4 days when compared with control group.

Regarding to, Hesperidin protected plus acetic acid treated rats showed significant reduction in ALP after 2 and 4 days when compared with acetic acid-treated group. Significant elevations were observed in TP, albumin, and A/GR in hesperidin protected plus acetic acid treated group after 2 and 4 days when compared with acetic acid-treated group. Meanwhile, there was a significant decrease in globulin of hesperidin protected plus acetic acid treated group after 2 days when compared with acetic acid-treated group and no significant change in globulin after 4 days when compared with acetic acid treated group.

The results present in fig. (1) concerning to cytokines changes, in acetic acid treated group showed significant increase in IL-1, INOs, COX-2, and ZO-1 after 4 days when compared with control group, while no significant change was observed in IL-10 in acetic acid-treated group after 4 days when compared with control group.

Conversely, hesperidin protected plus acetic acid-treated group showed significant decrease in IL-1, INOs, COX-2, and ZO-1 after 4 days when compared with acetic

acid-treated group, but IL-10 showed a non-significant change in hesperidin protected plus acetic acid-treated group after 4 days when compared with acetic acid-treated group.

Histopathological examination:

The middle part of colon of normal animal showed normal intestinal crypts with normal lining epithelium fig. (2). The middle part of the colon of rat that received acetic acid after two days from administration showing degenerative changes in the epithelial cell lining of the villi fig. (3). Meanwhile after 4 days sever degenerative changes and desquamation of the epithelial cell that lining the intestinal villi were seen. Moreover heavy leukocytic infiltrations in the degenerated villi were also detected fig. (4). Middle part of the colon of the rat treated with hesperidin and acetic acid after 2 days from administration showing mild degenerative changes in the intestinal villi which manifested by mild desquamation of epithelial cell lining of this villi with mild intervillus leukocytic infiltration fig. (5). Meanwhile after 4 days the affected colon nearly showing normal histological structure except mild leukocytic infiltration around some of villi was detected fig. (6).

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

Animals 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 \pm 0.32 ^c	2.08 \pm 0.05 ^c	6.92 \pm 0.64 ^{cd}	0.21 \pm 0.01 ^c	0.19 \pm 0.02 ^b	20.70 \pm 1.52 ^{bc}	0.29 \pm 0.01 ^a	0.01 \pm 0.01 ^a
AA _(D2)	9.57 \pm 0.10 ^c	3.78 \pm 0.17 ^a	5.59 \pm 0.05 ^d	0.34 \pm 0.02 ^a	0.62 \pm 0.03 ^a	12.64 \pm 0.58 ^d	0.32 \pm 0.03 ^a	0.01 \pm 0.01 ^a
HMC _(D2)	11.17 \pm 0.05 ^b	2.28 \pm 0.01 ^c	7.35 \pm 0.03 ^b	0.21 \pm 0.01 ^c	0.21 \pm 0.02 ^b	19.55 \pm 1.31 ^{bc}	0.28 \pm 0.08 ^a	0.02 \pm 0.00 ^a
HMC+AA _(D2)	12.85 \pm 0.44 ^a	2.85 \pm 0.04 ^b	8.82 \pm 0.37 ^a	0.24 \pm 0.01 ^{bc}	0.22 \pm 0.01 ^b	17.87 \pm 0.18 ^c	0.30 \pm 0.05 ^a	0.02 \pm 0.00 ^a

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

Animals groups	WBCs ($\times 10^3/\mu\text{l}$)	Neutrophil s ($\times 10^3/\mu\text{l}$)	Lymphocyte s ($\times 10^3/\mu\text{l}$)	Monocytes ($\times 10^3/\mu\text{l}$)	N/L Ratio	L/M Ratio	Eosinophi l ($\times 10^3/\mu\text{l}$)	Basophil ($\times 10^3/\mu\text{l}$)
C _(D4)	12.44 \pm 0.40 ^b	1.60 \pm 0.15 ^c	7.21 \pm 0.10 ^b	0.49 \pm 0.03 ^a _b	0.17 \pm 0.01 ^d	23.90 \pm 0.66 ^b	0.29 \pm 0.01 _a	0.02 \pm 0.01 _a
AA _(D4)	12.65 \pm 0.46 ^a _b	3.95 \pm 0.34 ^a	5.60 \pm 0.17 ^c	0.52 \pm 0.01 ^a	0.50 \pm 0.03 ^a	18.71 \pm 0.01 ^c	0.32 \pm 0.01 _a	0.02 \pm 0.01 _a
HMC _(D4)	13.00 \pm 0.08 ^a _b	2.07 \pm 0.06 ^c	8.27 \pm 0.27 ^a	0.42 \pm 0.01 ^b _c	0.22 \pm 0.01 ^c _d	21.97 \pm 0.14 ^b _c	0.27 \pm 0.01 _a	0.02 \pm 0.01 _a
HMC+AA _(D4)	13.54 \pm 0.11 ^a	2.85 \pm 0.01 ^b	8.81 \pm 0.06 ^a	0.42 \pm 0.02 ^b _c	0.29 \pm 0.02 ^c	33.50 \pm 2.50 ^a	0.34 \pm 0.01 _a	0.02 \pm 0.00 _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, HMC: hesperidin group, HMC+AA: plus acetic acid group

Table (3): Biochemical parameters after 2 days in acetic acid treated (AA) group, hesperidin group (HMC), and hesperidin protected plus acetic acid group (HMC+AA).

Animals groups	ALP (U/L)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio
C _(D2)	482±12 ^b	6.01±0.32 ^{ab}	4.35±0.01 ^{bc}	1.55±0.05 ^d	2.25±0.04 ^b
AA _(D2)	514±0.10 ^a	5.36±0.01 ^b	3.27±0.13 ^d	2.26±0.07 ^a	1.71±0.26 ^c
HMC _(D2)	382±5.10 ^c	6.67±0.03 ^a	5.00±0.03 ^a	1.70±0.03 ^c	2.98±0.01 ^a
HMC+AA _(D2)	469±0.15 ^b	6.61±0.35 ^a	4.40±0.04 ^{bc}	1.76±0.01 ^c	2.90±0.06 ^a

Table (4): Biochemical parameters after 2 days in acetic acid treated (AA) group, hesperidin group (HMC), and hesperidin protected plus acetic acid group (HMC+AA).

Animals groups	ALP (U/L)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio
C _(D4)	410±23.50 ^b	5.57±0.16 ^{bc}	4.14±0.21 ^a	1.17±0.11 ^c	2.84±0.06 ^{bc}
AA _(D4)	460±8.05 ^a	4.28±0.53 ^d	3.50±0.09 ^c	1.90±0.09 ^a	2.22±0.09 ^d
HMC _(D4)	372±11.35 ^{bc}	6.80±0.01 ^a	4.60±0.12 ^a	1.64±0.05 ^{bc}	3.36±0.12 ^a
HMC+AA _(D4)	400±7.20 ^{bc}	5.73±0.30 ^{bc}	4.25±0.10 ^{ab}	1.68±0.02 ^{ab}	3.21±0.02 ^{ab}

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 , HMC: hesperidin group, HMC+AA: plus acetic acid group

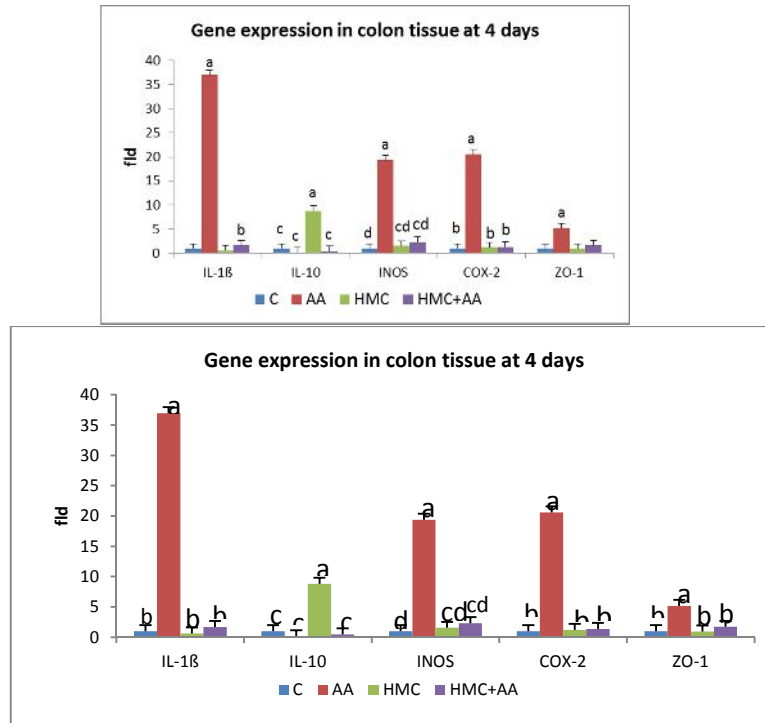
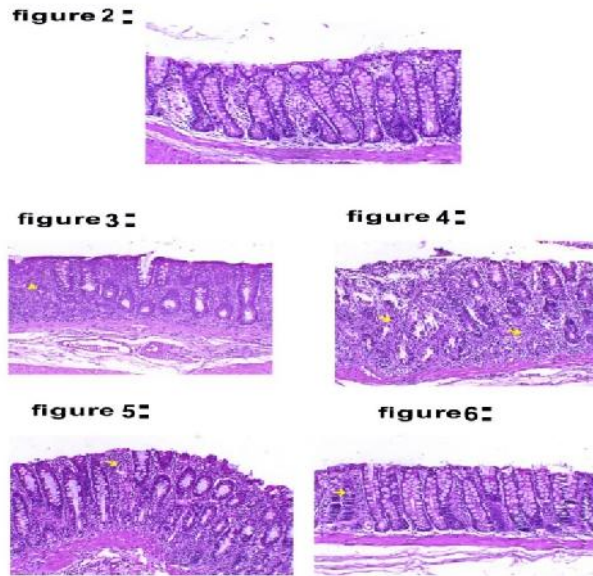


Figure (1): Changes in cytokines after 4 days of intracolonic injection with acetic acid in acetic acid (AA) group, Hesperidin (HMC) group, and hesperidin protected plus acetic acid (HMC+AA) group.



Figures 2-6: Figure 2. Middle colon of normal animal showing normal intestinal crypts with normal lining epithelium. Figure 3. Colon of animal traeted with acetic acid sacrificed at 2 days. Middle part of colon is showing colitis associated with crypt degeneration and necrosis of the most upper and heavy leukocytic infiltration (arrowhead).Figure 4. Middle colon of acetic acid-treated animal (sacrificed at 4 days) showing diffuse colitis. Figure 5. Animal treated with HMC +AA (sacrificed at 2 days) Middle colon is showing moderate degree of colitis (arrowhead indicates marked leukocytic infiltration between crypts). Figure 6. Animal treated with AA+HMC (sacrificed at 4 days) showing mild degree of colitis (arrow indicates area focal degenerated crypts), H&E, X200.

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 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. Regarding to TLC showed significant increase in hesperidin protected plus acetic acid after 2 days when compared with acetic acid-treated group. Additionally, significant increases in lymphocytes and L/MR was observed in hesperidin protected plus acetic acid-treated group after 2 and 4 days when compared with acetic acid-treated group. Our results agree with Camps-Bossacoma et al., (2017) who reported that hesperidin does possess immunoregulatory properties in the intestinal immune response. Hesperidin administration changed mesenteric lymph node lymphocyte (MLNL) composition in the intestinal epithelium (TCR + cells). Lymphocytes represented primarily by the TCR $\gamma\delta$ + T cells, provide signals that enhance barrier function and intercalate between intestinal epithelial cells on the basolateral side of epithelial tight junctions (Dalton et al., 2006). Meanwhile, there were significant decreases in neutrophils, monocytes, and N/LR in hesperidin

protected plus acetic acid-treated group after 2 and 4 days when compared with acetic acid-treated group. Our results agreed with Ohtsuki et al., (2002) who explained hesperidin have anti-inflammatory properties. Our result is confirmed by histopathological examination of colon tissue which revealed marked decrease inflammatory cells infiltration on the 4th days of colitis. Concerning to biochemical parameters in acetic acid-treated group, alkaline phosphatase (ALP) results revealed significant elevations after 2 and 4 days when compared with control group. These results agree with that observed by Kumar et al., (2014) who reported that Colonic inflammation was additionally portrayed by an increase in ALP level, which has been attributed principally to leucocyte. Meanwhile, a significant decrease in TP was observed in acetic acid treated group after 4 days when compared with control group. Significant decreases in albumin and A/GR was observed in acetic acid-treated group after 2 and 4 days when compared with control group with significant increase in globulin of acetic acid treated group was observed after 2 and 4 days when compared with control group. Our results agree with Ungaro et al., (2012). Abnormal integrity of intestinal wall in colitis consequently defected in the adsorptive function of the intestine leading to malabsorbance of protein and fats with anorexia (malnutrition of protein and fat) which caused decrease of protein and lipid synthesis in liver and weight loss during the first periods of experiment (Minaiyan et al., 2015). Decrease of total proteins and albumin in colitis groups could be caused by the direct effects of acetic acid on intestinal wall integrity and oxidative stress induction (Wang et al., 2016). A/G ratios decrease due to globulin levels rising during inflammation (Suh et al., 2014). Globulin family has several members including alpha, beta, and gamma globulins. Among them, gamma globulin accounts for the largest proportion. It is known as immunoglobulin or antibody and secreted

by B cells of the adaptive immune system. Therefore, it plays an important role in immunity. An increased level of globulin has been proved to be related to inflammation (Gabay and Kushner, 1999; Wang et al., 2016).

Regarding to Hesperidin protected plus acetic acid treated rats; significant reduction in ALP was observed after 2 and 4 days when compared with acetic acid-treated group. Meanwhile, significant elevations were observed in TP, albumin, and A/GR after 2 and 4 days in hesperidin protected plus acetic acid-treated with significant decrease in globulin in hesperidin protected plus acetic acid treated group after 2 days when compared with acetic acid-treated group. These results agree with Li and Schluesener, (2017) who explained that the therapeutic activity of hesperidin in several pathological conditions that might be attributed to the anti-inflammatory and antioxidant effects of these constituents. Additionally, Hesperidin improves the colonic absorptive function greatly compromised in experimental colitis, leading to fewer diarrhea symptoms, which are frequent in intestinal inflammation (Crespo, et al., 1999).

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 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. Meanwhil, 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- 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- and INF- 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).

Concerning to IL-1 β , INOs, COX-2, and ZO-1, they showed significant decrease in hesperidin protected plus acetic acid-treated group when compared with acetic acid group. These results agree with Shin et al., (2009); Bruckner et al., (2012); Camuesco et al., (2004); Ren et al., (2015); Kwon et al., (2005); Dou et al., (2013). One preliminary study reported that the hesperidin presents antioxidant and anti-inflammatory properties (Sezer et al., 2011). Hesperetin, derived from the hydrolysis of the aglycone, and hesperidin have previously been reported to be capable of scavenging peroxynitrite (ONOO-) (Kim et al., 2004), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and reactive oxygen species (ROS) (Jung et al., 2003), so it has been suggested that hesperetin promotes cellular defence activation in the protection against ONOO--involved diseases, such as inflammatory conditions (Kim et al., 2004).

The inhibition of COX-II gene by hesperidin may have blocked the production of prostaglandins leading to the suppression of inflammatory response. The NF- κ B arouses proinflammatory pathway by stimulating the expression of various inflammatory cytokines, chemokines and adhesion molecules (Lawrence, 2009). Therefore, inhibition of NF- κ B by hesperidin may have blocked the NF- κ B-induced inflammatory pathway and subsequently invoked anti-inflammatory action (Ghorbani et al., 2012). In addition,

The Northern blot assay showed that the LPS-induced gene expression of COX-2 was clearly inhibited by hesperetin and hesperidin at a concentration of 250 μ M, suggesting that these compounds possess an anti-inflammatory effect (Murakami et al., 2005).

5. CONCLUSION

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

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