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The potential Anti-Inflammatory and antioxidant activities of Proanthocyanidins in acetic acid-induced ulcerative colitis in rats

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ABSTRACT

The present study was designed to investigate the anti-colitic effect of Proanthocyanidins (Pcs) in acetic acid-induced ulcerative colitis in rats. Forty male albino rats (220-250 g) were divided into five equal groups of 8 rats each. Group I: (Control normal group) rats received no drugs. Group II: (Early ulcerative colitis-induced group): rats received 2 ml (3% v/v) glacial acetic acid intracolically at 21th day from experiment and sacrificed 3 days later of acetic acid administration. Group III: (Late ulcerative colitis-induced group) rats received glacial acetic acid similar to group II for 3 successive days and sacrificed after 21 days. Group IV: (Early ulcerative colitis + Proanthocyanidins protected group) rats received Proanthocyanidins (200 mg/kg body weight/day) orally for 21 days prior to glacial acetic acid administration for 3 days, then the animals were sacrificed. Group V: (Late ulcerative colitis + Proanthocyanidins treated group) rats first administered with glacial acetic acid then after 3 days Proanthocyanidins was administered for 21 days. A significant increase in L-Malondialdehyde (L-MDA) and Myeloperoxidase (MOP) activity with marked decrease in reduced glutathione (GSH) and Catalase (CAT) activity in colon tissue of UC-induced rats as compared with control normal group. However, a significant depletion of colon tissue L-MDA, MOP activity and marked increase in GSH concentrations and CAT activity were observed after Proanthocyanidins treatment compared to ulcerated untreated rats. The quantification real time PCR (qPCR) results revealed a significant up-regulation of mRNA gene expression levels of Tumor necrosis factor alpha (TNF- α), Cyclooxygenase-2 (COX-2) and a significant down-regulation in Transforming growth factor - β 1 (TGF- β 1) in colon of acetic acid-induced colon ulcer in rats. The expression levels of TNF- α , COX-2 were significantly down-regulated and a significant up-regulated in TGF- β 1 in colon tissues after administration of Pcs. Proanthocyanidins (Pcs) protect rats colon mucosa damage against acetic acid-induced ulcerative colitis via anti-inflammatory and anti-oxidative mechanisms.

1. INTRODUCTION

Inflammatory bowel disease (IBD) is an immune mediated disease characterized by inflammation of the gastrointestinal (GI) tract. IBD encompasses both Crohn's disease (CD) and ulcerative colitis (UC), conditions that are chronic and often progressive, but are most commonly associated with intermittent disease flares. The precise cause of IBD remains elusive. However, it is well accepted that IBD occurs at the interactions between luminal immune reactions and environmental factors in genetically susceptible individuals (Celiberto et al., 2018). Urbanization and industrialization are associated with IBD (Kaplan, 2015). Some environmental factors have a different effect on the subtypes of IBD. Smoking and appendectomy is negatively associated with UC, but they are aggravating factors for CD (Legaki and Gazouli, 2016). A westernized high fat diet, full of refined carbohydrates is strongly associated with the development of IBD, contrary to a high in fruit, vegetables and polyunsaturated fatty acid-3 diet that is protective against these diseases (Cope, 2015). High intake of non-

steroidal anti-inflammatory drug and oral contraceptive pills as well as the inadequacy of vitamin D leads to an increased risk for IBD and a more malignant course of disease. Moreover, other factors such as air pollution, psychological factors, sleep disturbances and exercise influence the development and the course of IBD. Epigenetic mechanism like DNA methylation, histone modification and altered expression of micro ribonucleic acid (miRNAs) could explain the connection between genes, dysbiosis of the gut microbiota and environmental factors in triggering the development of IBD (Legaki and Gazouli, 2016). In UC, the inflammatory process solely involves the mucosa and extends by continuity, starting from the rectum. A characteristic symptom of UC is bloody diarrhea, abdominal pain and weight loss, UC is also associated with an increased risk of colorectal cancer (Poggioli and Renzi, 2019). The intra-rectal instillation of diluted Acetic acid (AA) causes 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), which leads to

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production of superoxide anion and initiation of a cascade for the production of various reactive species. This may lead to generation of hydroxyl radicals and peroxides that significantly contribute to the progression of tissue necrosis and mucosal dysfunction (Balmus et al., 2016 and Colombo et al., 2018).

L-Malondialdehyde (L-MDA), the end product of lipid peroxidation, is a good marker of free radical-mediated damage and oxidative stress (Ozturk et al., 2015), the concentration of serum L-MDA was elevated in colitis induced in rats by acetic acid. The lower concentration of anti-oxidants in the colitis group with additional increase in MDA and hydroperoxide levels which are indicator for the tissue injury (Ashry et al., 2016). Also, Myeloperoxidase (MPO) is an enzyme found in primary granules of polymorphonuclear neutrophils and used as an index for the severity of digestive inflammation (Lee et al., 2019). Catalase (CAT) is a key enzyme in the metabolism of hydrogen peroxide (H₂O₂) and reactive nitrogen species, and its expression and localization is significantly change in this diseases, Because of the importance of excess and uncontrollable Reactive Oxygen Species (ROS) in the pathogenesis of ulcerative colitis (Glorieux and Calderon, 2017). The value to determine free radical scavenger such as reduced Glutathione, the endogenous antioxidant GSH was reduced significantly in the colonic tissues of the acetic acid-induced ulcerative colitis in rats (Karakoyun et al., 2018).

As well as some other autoimmune diseases, the over production of tumor necrosis factor alpha (TNF- α) is prevalent in the inflamed tissues and can trigger apoptosis, which plays a significant role in animal colitis models (Nunes et al., 2014). One major consequence of this signaling in the gut is the increased production of prostaglandin E₂ (PGE₂) via cyclooxygenase-2 (COX-2), the inducible isoform of the enzyme, which has been recognized to be a key factor in diverse inflammatory conditions such as IBD (Le Loupp et al., 2015). Transforming growth factor - β 1 (TGF- β 1) plays a critical role in the differentiation and maintenance of T regulatory cells (T-reg cells), especially Tregs generated in the gut (Bauché and Marie, 2017). Dysregulated TGF- β 1 signaling is observed in the intestines of IBD patients (Ihara et al., 2017).

Proanthocyanidins are the most common group of flavonoids in the Western diet, and they are considered the second most abundant group of natural phenolics after lignins. PCs are naturally occurring compounds widely available in fruits, vegetables, nuts and seeds and it is predominantly present in tea, honey, wines, olive oil, cocoa and cereals (Nunes et al., 2016). PCs have potent antioxidants, anti-inflammatory, anti-tumour, cardio-protective, neuroprotective and antimicrobial activities (Ma and Zhang, 2017).

Accordingly, the purpose of the present study was to investigate the effect of Proanthocyanidins against acetic acid induced colitis in rats. Also, to determined whether PCs when administered to ulcerative colitis induced-rats would attenuate the oxidative stress in colon tissue, beneficial for prevention and treatment of colitis complications.

2. MATERIAL AND METHODS

2.1. Experimental animals:

Forty white male albino rats of 12-16 weeks old and weighting 220-250 gm were used in the experimental investigation of this study (Yunusi et al., 2015). The rats were obtained from the Laboratory Animals Research Center, Faculty of Veterinary Medicine, Benha University. Rats were housed in separated wire mesh cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals 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. Proanthocyanidins:

Proanthocyanidin (purity~95%) was obtained from (El-Debeiky pharma company of pharmaceutical industries, Al obour, Cairo, Egypt). Present in red brown powder form. PCs was freshly prepared by dissolved in 7% Dimethylsulfoxide (DMSO) solution then complete to 100 ml isotonic saline solution (0.9% NaCl), and was administered every day orally at a dose of (200 mg/kg b.wt.) for 21 days (Ragab et al., 2013).

2.3. Induction of colitis:

To induce colitis, rats were fasted for 18 hours, and then anaesthetized with an i.p. injection of sodium thiopental (500 mg dissolved in 12.5 ml of normal saline) at the dose level of 0.2 ml/200gm body weight (40 mg/kg b.wt.) (Motavallian-Naeini et al., 2012). Acetic acid (3% v/v) was then administered intra-colonially of rats in a volume of 2 ml, via a polyethylene catheter inserted 8 cm proximal to the anus (Tanideh et al., 2014). 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): received no drugs, served as control non-treated for all experimental groups.

Group II: (Early ulcerative colitis non-treated group): Rats received glacial acetic acid (2ml/rat) at 21 th day from experiment for 3 successive days and on day 24 th the animals were sacrificed and the samples were taken.

Group III: (Late ulcerative colitis non-treated group): Rats received glacial acetic acid (2ml/rat) at first day of experiment for 3 successive days, and the rats were sacrificed after 21 days from induction.

Group IV: (Early ulcerative colitis +PCs protected group): Rats received PCs (200 mg/kg body weight/day) orally for 21 successive days prior to acetic acid administration 3 days after the administration of Acetic acid the animals were sacrificed.

Group V: (Late ulcerative colitis +PCs treated group): Rats received acetic acid (2ml/rat) at the first day of experiment and for 3 successive days, after that PCs treatment (200 mg/kg body weight/day) orally will be started for 21 days, and then the animals were sacrificed on 24 th day of the experiment.

2.5. Sampling:

Colonic tissue specimens were collected at the end of experiment on day 24th for all animal groups (control and experimental groups).

2.5.1. Colonic tissue for biochemical analysis:

After 21 days of treatment with PCs the rats were sacrificed by cervical decapitation. The Colon was quickly removed, and opened along the greater curvature using a scrapper, cleaned by rinsing with cold saline and stored at -20°C for subsequent biochemical analysis.

Briefly, colon tissues were cut, weighed 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 were used for the determination of L-Malondialdehyde (L-MDA), catalase (CAT) and Myeloperoxidase (MPO) 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) (ref. No.: 253-433-4, Sigma-Aldrich, Germany), 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 then the clean supernatant was removed and used for determination of GSH concentration.

2.5.2. Colonic tissue for molecular analysis:

At the end of experiment, and following sacrificing by decapitation, rats colon tissue were immediately excised and frozen in liquid nitrogen and then in -80°C until used for Tumor necrosis factor alpha (TNF- α), Cyclooxygenase-2 (COX-2) and Transforming growth factor - β 1 (TGF- β 1) gene expression analysis by 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 were rinsed in tap water, dehydrated through graded series of alcohols, cleared in xylene and embedded in paraffin wax. 5 μ m 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), Catalase (CAT), reduced glutathione (GSH) and Myeloperoxidase (MPO) were determined according to the methods described by (Mesbah et al., 2004), (Xu et al., 1997), (Patterson and Lazarow, 1955) and (Shi et al., 2005 and Qiu et al., 2015) 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 Tumor necrosis factor alpha (TNF- α), Cyclooxygenase-2 (COX-2) and Transforming growth factor- β 1 (TGF- β 1) 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- $\Delta\Delta$ Ct determined the fold change in gene expression relative to the control.

Forward and reverse primers sequence for real time PCR.

Gene	Forward primer (5'-----/3')	Reverse primer (5'-----/3')
TNF- α	GCATGATCCGCGACGTGGAA	AGATCCATGCCGTTGGCCAG
Cox-2	GATTGACAGCCCACTT	CGGGATGAACCTCTCTCTCA
TGF- β 1	AAGAAGTCACCCGCGTCTA	TGTGTGATGCTTTGGTTTGTCA
β -actin	ACCACACTGTGCCATCTA	CGTCACACTTCATGATG

For Biochemical analysis the obtained data were analyzed and graphically represented using the statistical package for social science (SPSS, 13.0 software, 2009), for obtaining mean and standard deviation and error. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity. However, for molecular analysis the difference between the groups was determined by one way ANOVA using GraphPad Prism 5 (GraphPad 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.

3. RESULTS

Effect of Proanthocyanidin administration on colon tissue L-MDA, CAT, GSH and MPO of acetic acid-induced ulcerative colitis in male rats is presented in table (1). The obtained results showed significant increase L-MDA and MPO with marked decrease 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, MPO and marked increase in CAT and GSH concentrations were observed after Pcs treatment compared to ulcerated untreated rats.

Effect of Pcs administration on the relative mRNA expression of inflammation-related genes, TNF- α , COX-2 and TGF- β 1 in colon of acetic acid -induced ulcerative colitis in rats is shown in table (2). The obtained qPCR results revealed a significant up-regulation of TNF- α , COX-2 and a significant down-regulation of TGF- β 1 gene expression levels in colon of acetic acid-induced ulcerative colitis in rats (G2 and G3) as compared to control normal

group (G1). This expression was significantly down-regulated following administration of Pcs either before (G4) or after (G5) induction of ulcer, with lower expression in

preventive group (G4), and a significant up-regulated in TGF- β 1 in colon tissues.

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

Animal groups	L-MDA (mmol/ g. tissue)	CAT (mmol/min/g. tissue)	GSH (ng / g. tissue)	MPO (U/mg. tissue)
Group I: Normal control	23.86 \pm 0.89 ^d	111.5 \pm 5.11 ^a	12.58 \pm 0.64 ^a	0.07 \pm 0.001 ^e
Group II: Early ulcerative colitis	83.25 \pm 2.430 ^b	19.28 \pm 1.010 ^d	1.0 \pm 0.040 ^d	0.50 \pm 0.030 ^b
Group III: Late ulcerative colitis	95.36 \pm 2.60 ^a	12.61 \pm 0.94 ^e	0.45 \pm 0.02 ^e	0.67 \pm 0.03 ^a
Group IV: Early ulcerative colitis + Proanthocyanidins protected	37.30 \pm 1.02 ^d	79.61 \pm 4.50 ^b	5.2 \pm 0.33 ^b	0.22 \pm 0.02 ^d
Group V: Late ulcerative colitis + Proanthocyanidins treated	50.7 \pm 1.54 ^e	35.8 \pm 2.07 ^c	2.72 \pm 0.17 ^c	0.34 \pm 0.02 ^c

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

Table 2 Effect of Proanthocyanidins administration on the relative expression of TNF- α , COX-2 and TGF- β 1 gene in colon tissue of Acetic acid -induced ulcerative colitis in rats.

Animal groups	Tumor necrosis factor alpha (TNF- α)		Cyclooxygenase-2 (COX-2)		Transforming growth factor - β 1 (TGF- β 1)	
	Fold change mean	SEM	Fold change mean	SEM	Fold change mean	SEM
Group I: Normal control	1.00 ^d	0.08	1.00 ^e	0.08	1.00 ^a	0.06
Group II: Early ulcerative colitis	5.10 ^b	0.31	5.31 ^a	0.34	0.17 ^d	0.02
Group III: Late ulcerative colitis	8.94 ^a	0.44	6.87 ^a	0.4	0.10 ^d	0.01
Group IV: Early ulcerative colitis + Proanthocyanidins protected	2.25 ^c	0.14	2.83 ^b	0.16	0.66 ^b	0.05
Group V: Late ulcerative colitis + Proanthocyanidins treated	4.72 ^b	0.25	3.20 ^b	0.2	0.37 ^c	0.03

Means within the same column carrying different superscript letters are significantly different (P \leq 0.05).

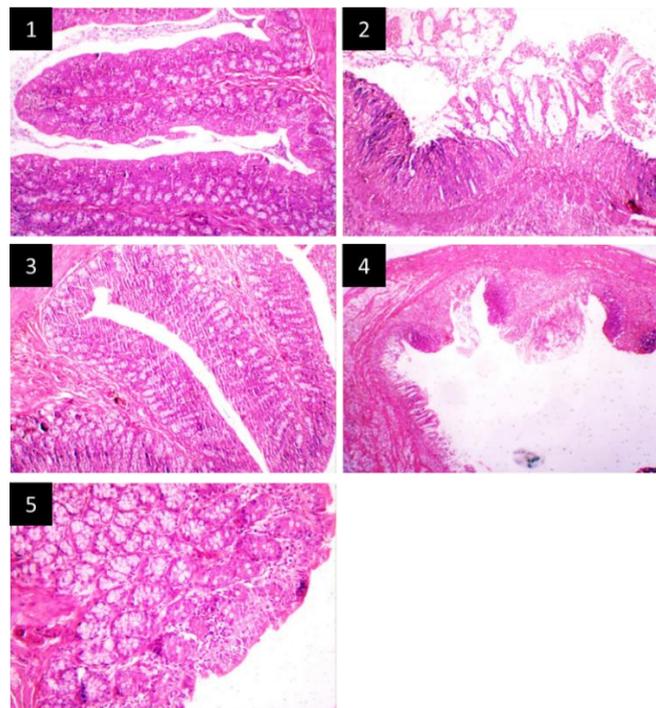


Fig 1 Represent (Group I) Normal control Colon, showing normal histological appearance of mucosal epithelium, lamina propria and muscularis mucosa. H&E stain x 200.

Fig 2 Represent (Group II) Early UC-induced, showing mucosal ulcer characterized by loss of the mucosal crypts of Lieberkühn with replacement by abundant necrotic debris, inflammatory cells and fibrin. H&E stain x 200.

Fig 3 Represent (Group III) Late UC-induced, showing multifocal thickening of the mucosa with elevated hyperplastic crypts and increase in numbers of goblet cells. H&E stain x 100.

Fig 4 Represent (Group IV) Early UC + Proanthocyanidine protected group, showing multifocal ulcers affecting the colonic mucosa. H&E stain x100.

Fig 5 Represent (Group V) Late UC + Proanthocyanidine treated group, showing marked epithelial hyperplasia of the crypts and increase in goblet cells. H&E stain x 200.

4. DISCUSSION

The use of acetic acid for the production of ulcerative colitis in rats is a standardized model (Sadraei *et al.*, 2017), and it has been reported to have similar histological features to that of human. It is usually associated with superficial ulceration and inflammation, enhanced vasopermeability as well as

severe influx of neutrophils and macrophages to the site of injury (Kandhare *et al.*, 2016).

The obtained results showed significant increase L-MDA and MPO with marked decrease in CAT activity and GSH concentrations in colon tissue of acetic acid -induced colon ulcer in rats. Acetic acid produced colon ulcer through different mechanisms including generation of oxidative stress, initiation of lipid peroxidation and inflammation,

infiltration of neutrophils, induction of apoptosis. Moreover, oxidative stress and inflammation are the key mediators in acetic acid colon ulcer pathways (Balmus *et al.*, 2016 and Colombo *et al.*, 2018). Acetic acid provoked free radicals and depletion in antioxidant activities (Tekeli *et al.*, 2018). Malondialdehyde (MDA), the end product of lipid peroxidation, is a good marker of free radical-mediated damage and oxidative stress (Busch and Binder, 2017), CAT is a common antioxidant enzyme present almost in all living tissues that utilize oxygen (Ighodaro and Akinloye, 2018), and protects cells from hydrogen peroxide generated with in them (Kodydková *et al.*, 2014). GSH, the most abundant antioxidant in cells, plays a major role in the defense against oxidative stress-induced cellular injury and is essential for the maintenance of the intracellular redox balance (Zhang and Forman, 2012). Nearly similar results were reported by El-Akabawy and El-Sherif, (2019) who shown a significant increase in L-MDA level and a significant decreased in colon tissue CAT and GSH concentration were observed in colon tissue after acetic acid administration in rats when compared with normal rats. Acetic acid causes severe oxidative stress in colonic tissue, which is manifested as an enhancement in lipid peroxidation that occurs *via* an increase in the MDA content and a decrease in the colonic GSH content (Ashry *et al.*, 2016).

Also, Myeloperoxidase is an important enzyme that catalyses production of reactive oxygen species (Chami *et al.*, 2018). This proinflammatory enzyme is highly expressed by neutrophils and as such is widely used as a neutrophil marker. These oxidants may contribute to host tissue damage at sites of inflammation through reactions with a wide range of biological substrates, including DNA, lipids, and protein amino groups (Russell *et al.*, 2017). In fact, it is well-known that this enzyme is increased in acetic acid induced colitis (Dembiński *et al.*, 2016). Similarly, Rashidian *et al.*, (2019) suggested that, Myeloperoxidase activity was significantly higher in the colonic tissue of the colitis induced rats caused by acetic acid than of the control group. Also, Lee *et al.*, (2019) suggested that, the colitis caused by TNBS was characterized by marked increase in MPO activity, an indicator of polymorphonuclear leukocyte infiltration into the colon.

In the current study a significant depletion of colon tissue L-MDA, and marked increase in CAT activity and GSH concentrations were observed after administration of Proanthocyanidin in colon ulcer- induced rats. Similarly, Bhardwaj *et al.*, (2018) recorded that, rats treated with GSPE produced a significant reduction of MDA levels and increased the anti-oxidant levels like CAT, SOD and GSH. Also, Pcs pretreatment significantly reduced MPO level in ulcerated rats, suggesting the ability of Pcs to prevent neutrophil infiltration in ulcerated colon tissue. These results are nearly similar to those reported by Gil-Cardoso *et al.*, (2019) treatment with Proanthocyanidins (75 mg/kg) lowered the value of MPO activity in a rat model of mild intestinal inflammation and impaired intestinal permeability. Pcs inhibits effectively the lipid peroxidation of cellular membranes, the protein oxidation as well as the DNA damage due its ability to directly scavenge various free radicals, including superoxide radicals and peroxy and hydroxyl radicals (Abhijit *et al.*, 2018).

The obtained qPCR results revealed a significant upregulation of TNF- α , COX-2 and a significant downregulation of TGF- β 1 gene expression levels in colon

of acetic acid-induced colon ulcerative rats as compared to control normal group. Tumor necrosis factor (TNF- α) is a pro-inflammatory cytokine secreted by colonic macrophages increasingly during ulcerative stress (Huang *et al.*, 2015). TNF- α plays a prominent role and the neutralization of this cytokine is accompanied by a remarkable clinical response in patients with IBD (Levin *et al.*, 2016). Similarly, Hosseini *et al.*, (2017) reported that, the levels of TNF- α and IL-1 β in colon tissue were significantly increased in colitis induced by acetic acid in rats in comparison with the control normal group. Also, Matuszyk *et al.*, (2016) suggested that, rectal administration of 3% acetic acid solution led to induction of colitis in all animals, damage of the colonic wall was accompanied by an increase in mucosal concentration of pro-inflammatory IL-1 β and TNF- α . The levels of the proinflammatory cytokines such as TNF- α , IL-1 β and IL-6 are increased significantly in UC (Zhang *et al.*, 2016). Therefore, it has been emphasized that removing these inflammatory triggers and mediators is important in determining the treatment strategies for IBD (Tahan *et al.*, 2011).

Cox-2, which is an inducible enzyme, it is well known that this enzyme is one of the inflammatory proteins expressed excessively in the inflamed regions (Park *et al.*, 2017). Activation of these molecules causes an exaggerated inflammatory response, promoting the progression of intestinal damage, which may affect the intestinal integrity unfavorably (Sanchez-Fidalgo *et al.*, 2015). Eventually, the expression level is increased significantly in experimental colitis models (Yan *et al.*, 2015) and in patients with IBD. This result agrees with earlier studies of Tekeli *et al.*, (2018) reported that, the value of COX-2 protein level in colon tissue was significantly increased in 4% acetic acid was administered intrarectally to induce colitis than normal group.

TGF- β 1 is well known to be a multifunctional cytokine that regulates many biological processes such as cell proliferation, cell differentiation, adhesion, inter cell signaling, and the also production and the degradation of extracellular matrix proteins, thus playing an essential role during wound healing and tissue repair. This indicates that TGF- β 1 expression is part of the normal healing response of colon tissues (Ihara *et al.*, 2017). These findings are in agreement with those reported by Song *et al.*, (2015) recorded that, the colitis induced by acetic acid caused significant decrease of colonic mucosal TGF- β 1 expression when compared to normal rats.

In the current study the obtained qPCR results revealed a significant downregulation of TNF- α , COX-2 and a significant upregulation of TGF- β 1 gene expression levels in colon of acetic acid-induced colon ulcerative rats following administration of Pcs either before or after induction of ulcer.

The interest in dietary polyphenols (PPs) has increased considerably recently due to their antioxidant and anti-inflammatory activities, and their probable role in preventing pathologies associated with oxidative stress, such as cardio-vascular and neurodegenerative diseases (Sakaki *et al.*, 2019).

Pallarès *et al.*, (2013) reported that, endotoxic effects induced by lipopolysaccharide in rats was characterized by an increase of the pro-inflammatory cytokine TNF- α , IL-10, IL-6, IL-1 β In contrast, the levels of this cytokine were significantly lowered in rats treated with Pcs. Moreover,

Chen *et al.*, (2019) recorded that, the anti-inflammatory actions of GSP on pulmonary arterioles initiates and maintains pathological processes in pulmonary arterial hypertension may involve IL-1 β , IL-6 and TNF- α down-regulated in lung tissue. Pcs pretreatment significantly reduced COX-2 level in ulcerated rats, suggesting the ability of Grape seed polyphenols were found to be protective against colitis by downregulation of pro-inflammatory cytokines, COX-2 and reducing translocation of NF- κ B in the colon mucosa (Baliga *et al.*, 2014).

5. CONCLUSION

These findings suggest that oral treatment with Proanthocyanidins shows an effective protection against colitis and oxidative damage in colon mucosal tissue induced by acetic acid in rats. Also, the protective effect of Pcs might be mediated by adjustment of inflammatory mediators and increasing antioxidants as well as attenuating oxidant/antioxidant imbalance. Moreover, Pcs administration may have the potential as an alternative treatment for ulcerative colitis because of its cytoprotective and anti-inflammatory effects.

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