THE ROLE OF BLACK POMEGRANATE (PUNICA GRANATUM L.) PEEL EXTRACT IN MODULATION OF INFLAMMATORY BOWEL DISEASE IN RATS

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Abstract

The chronicity of inflammatory bowel disease (IBD), the progression of resistant and unpredictable outcomes, and the disruptive nature of bowel tissue with a disability have all made it a noteworthy challenge for gastrointestinal (GI) experts around the world. In addition, Ulcerative colitis (UC) is an incurable, inflammatory disease that affects the colon and rectum. It is one of the two primary kinds of IBD. The goal of the study is to evaluate the efficiency of black pomegranate peel ethanolic extract (Punica granatum L.) (BPPE) in curing colitis induced in rats model by 2, 4 dinitrobenzene sulfonic acid (DNBS). A total 75 female Wistar rats divided into five equal groups. UC was induced by administration of 15mg/kg B.W of DNBS in 0.25 ml of 50% ethanol intrarectally to four groups (control positive and 3 treated groups). The fifth group (control negative) administered 0.25ml of 50% ethanol intrarectally. Six days after DNBS-induced colitis first group (G1) treated with sulfasalazine 25 mg/kg B.W. TID, PO for 30 days, and another treated groups (G2) and (G3) administrated BPPE 100 and 200 mg/kg B.W sid PO for 30 days. Control negative administrated distilled water orally for 30 days. Five rats after 2 ,4 weeks of treatment, and the remaining five rats after one week of treatment withdrawal have been sacrificed from each group. The clinical signs, body weight, colonic tissue macroscopic score, Gross lesion, histopathological changes, the microscopic score, Malondialdehyde (MDA), Myeloperoxidase (MPO) were measured. After rectal administration of DNBS, diarrhea, and bloody stools were observed directly during the experimental periods in control positive group and gradually decreased in the treated groups with BPEE. The result of macroscopic observation of the colon showed there was a significant decrease in treated groups (sulfasalazine, BPPE 100mg/kg B.W, BPPE 200 mg/kg B.W) in comparison with control positive. The gross appearance of colorectal tissue of the experimental groups showed various gross pathological changes in animals that received a single dose of DNBS demonstrated by extensive mucosal damage. Four weeks post-treatment the gross changes of sulfasalazine & BPPE-treated rats (200mg/kg) were less severe compared with lesions of 2 weeks of treatment and characterized by mild thickness with separated small foci of ulcerative and hyperemic mucosa. Histopathological Finding was represented by various histopathological lesions in the colon tissue of experimental groups such as severe loss of entire mucosa and crypts (ulcer). The animals of both groups treated by PBEE 100 and 200 mg/Kg.BW exhibited the colonic columnar re-epithelization was marked with mild to moderate necrotic and cellular debris covering the mucosal surface after four weeks of treatment,, However, one week after stopping treatment, re-epithelization was observed also. It could be concluded that both 100 mg/Kg.BW and 200 mg/Kg.BW of BPPE taken orally for 30 days have the ability to treat ulcerative colitis, however 100 mg/Kg.BW is the superior dosage rate.

Keywords: Black pomegranate, inflammatory bowel disease, Punica granatum L.

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1. Introduction

Inflammatory bowel disease (IBD) is still one of the most challenging and difficult disorders to understand in the twenty-first century (Hadji & Bouchemal, 2022). Ulcerative colitis (UC) is an inflammatory disease that affects the colon and rectum. It is one of the two primary kinds of IBD, the other being Crohn's disease (CD) (Roda et al., 2020; Taku et al., 2020). Ulcerative colitis is associated with a high patient burden; health-related quality of life is negatively impacted at some point for all patients, and persistently for many patients, by ulcerative colitis due to the symptoms, psychological suffering, and interference with social competence that patients experience (Alatab et al., 2020; Kaplan et al., 2019). Despite the fact that over 6.8 million people throughout the world deal with IBD-related issues every year, there are currently no viable medicines available (Jairath & Feagan, 2020). The best treatment approach will maintain remission, prevent disease complications, improve patients' quality of life, and encourage mucosal repair (Rubin et al., 2019). Currently available therapies for inflammatory bowel disease include amino salicylic acid preparations, glucocorticoids, immunosuppressive medicines, biological preparations, surgery(Binienda et al., 2020; Fang et al., 2018; Kohgo, 2000; Lu et al., 2020; Nakase et al., 2021; Nishida et al., 2021; Park et al., 2020). These treatments, however, come with a wide range of adverse reactions, some of which may be very serious (Larussa et al., 2021; Triantafyllidi et al., 2015). Many people all over the world depend solely on alternative medicine. Most of it is derived from plants or uses plant-based active substances. There has been a lot of recent research and financial investment into the field, and it seems likely that medicinal plants will continue to play an important role in the healthcare system continuing forward (Alkhatib et al., 2022). The high cost and side effects of allopathic medications, as well as the rise of resistant microbial strains, have led to an increased interest in medicinal plants among researchers (Konaté et al., 2012).

Pomegranate (**Punica granatum L**.) is a Middle Eastern plant that belongs to the Puricaceae family (Rana et al., 2010), It is one of the top seven fruits with the highest beneficial properties for people (Pereira et al., 2016). Pomegranates have an array of colors, including white, red, and black (Setiawati, 2014). Pomegranates, both red and black, were said to have significant cultural value in Iraq (Morton, 1987). The significant antioxidant activity in black pomegranate peel (Chasanah, 2020) have contributed to their popularity as cure since many decades for various diseases (Ghazaleh et al., 2013). Pomegranates are becoming increasingly prevalent since their fruits are high in nutritional value and offer several health advantages (Melgarejo et al., 2020). Pomegranate has a powerful anti-inflammatory effects, particularly on digestive tract inflammation such as UC (Vučić et al., 2019). It is thought that pomegranate may minimize IBD-related epithelial damage and inflammation (Kusmardi et al., 2022). Moreover, pomegranate peel has historically been used to treat a wide variety of medical conditions including ulcers, inflammation, microbial infection, brain ischemia, Alzheimer's disease, erectile dysfunction, obesity, and cancer (Hou et al., 2019). Also pomegranate have anti-lipid peroxidation effects because of its capacity to reduce platelet aggregation, LDL oxidation, decrease serum cholesterol and macrophage oxidative state (Hussen, 2014; Pérez-Vicente et al., 2002; Rozenberg et al., 2006). Pomegranate peel has been intensively explored in the recent decade due to its significant phytochemical

richness and variety (Balli, Cecchi, et al., 2020; Balli, Tozzi, et al., 2020; Singh et al., 2018). Notably, the most often cited groups are represented by phenolic acids and hydrolysable tannins such as punicalagin and ellagic acid, because they are in responsible of mediating the antioxidant capacity and radical scavenging activity response to a variety of diseases and inflammations (Akhtar et al., 2015; Benchagra et al., 2021; Sorrenti et al., 2019). Black pomegranate has greater therapeutic and nutritional potential. There are just a few reliable references regarding this instance in the literature (Khorrami et al., 2019; Mousavinejad et al., 2009).

Hypothesizing a possible effect of black pomegranate products against IBD, the purpose of this study is to evaluate the efficiency of dry whole pomegranate extract obtained from peel (mesocarp and epicarp) in curing colitis induced in rats model.

2. Methods

Experimental protocols were carried out at the University of Baghdad, Department of Physiology, Biochemistry, and Pharmacology, according to the College of Veterinary Medicine's Scientific Committee on Animal Welfare on the date: 28/3/2023 and in the numbered 710.

Experimental design

Adult female Wistar rats were 75 in total, and they were purchased from an Iraqi cancer research center. The rats ranged in weight from 150 to 225 grams and were 6-8 months old. Animals kept in the animal house of the Veterinary Medicine College University of Baghdad in 15 cages (five rats each cage). Exposed to light for 12 hours per day, with unrestricted access to food and water, and monitored for 2 weeks prior to the experiment at a temperature of 20–23 degrees Celsius in an air-conditioned room to reduce the effects of stress.

Black pomegranate peel extract (BPPE) dosage rates:

BPPE was prepared in our previous experiment (in press) in which all details about the characteristics yield extract and Phytoconstituent have been mentioned. In this experiment, the dosage rates of BPPE used were 100mg/kg. B.W and 200mg/kg. B.W orally (Vanani et al., 2020).

Sulfasalazine dosage rate

Sulfasalazine (Salazopyrin) ® (Pfizer, USA) used at dose 25 mg/kg B.W. orally (Malewska et al., 2011)**.**

The rats were randomly divided into five equal groups of 15 rats each: Control positive (C +ve) induced colitis by 15mg/kg B.W DNBS (BioDuly, China) /0.25 ml 0f 50% ethanol administrated intrarectally. Control negative (C -ve) administered 0.25ml of 50% ethanol intrarectally and given only a vehicle (distilled water) orally by stainless gastric gavage needle for 30 days. Group 1: After DNBS-induced colitis given sulfasalazine 25 mg/kg TID, PO for 30 days. Group 2: After DNBS-induced colitis given 100mg/kg B.W of BPPE sid PO for 30 days. Group 3: After DNBS-induced colitis, given 200mg/kg B.W of BPPE sid PO for 30 days. Five rats after 2 weeks of treatment, after 4 weeks of treatment, and the remaining five rats after one week of treatment withdrawal have been sacrificed from each group.

Colitis induction by 2, 4-Dinitrobenzene sulfonic acid (DNBS)

Colitis was induced by DNBS (BioDuly, China) according to the method described by (Barbara et al., 2000) with negligible changes. DNBS 15mg/kg B.W /0.25ml of 50% ethanol was freshly prepared and administrated intrarectally by using polyethylene catheter (PE-90) (Figure 1).

Figure 1: DNBS administrated intrarectally.

The catheter inserted gently intrarectally, approximately 8 cm proximal to the anus. Inject a small amount of the solution into the catheter to lubricate it and make insertion easier. Slowly inject 0.25 ml of DNBS or an equal volume of 50% ethanol for controls. After injecting the DNBS, the animals were put in Trendelenburg position, which referred to in (figure 2) for 2 minutes to avoid reflex action and reagent loss

Figure 2: Trendelenburg position.

Animals feed with 8% sucrose water in 0.22% saline to avoid dehydration, Weight monitored to sign weight loss.

Tissue sampling

After the various periods of the experiment 2 and 4 weeks of treatment, and one week of treatment withdrawal, the rats were weighed, anesthetized with diethyl ether (Alpha Chemika, India), and sacrificed. The abdomen was opened, and distal 8 cm of each rat's colon was removed and opened longitudinally and cleaned from their luminal contents with normal saline and observed for macroscopic evaluation for colitis then were cut into two pieces. One piece fixed for histological staining was immersed in 10% formalin. The second piece was froze for assessment of oxidative stress biomarkers

Macroscopic and microscopic assessments of colitis:

Macroscopic assessment scoring is carried out through the examination of colonic samples by the naked eye. The macroscopic damage scored on a 0- to 6-point scale based on the system according (Fornai et al., 2006)**.**

Microscopic assessment scoring: The microscopic damage and inflammation were assessed by light microscopy on hematoxylin/eosin-stained histological sections obtained from colon specimens. Histological criteria according (Fornai et al., 2006)**.**

Assessment of oxidative stress biomarkers

Evaluation of tissue Myeloperoxidase tissue assay. (Bioassay Technology Laboratory/ Rat myeloperoxidase ELISA Kit/ Cat. No E0574Ra) (Bioassay Technology Laboratory mpo, n.d.)**.**Myeloperoxidase (MPO) levels in

colonic tissues determined and assumed as a quantitative index to estimate the degree of mucosal infiltration by polymorph nuclear cells.

Evaluation of tissue Malondialdehyde. (Bioassay Technology Laboratory/ Rat Malondialchehyche ELISA Kit/ Cat. No E0156Ra) (Bioassay Technology Laboratory, 2017)**.**

Malondialdehyde (MDA) concentration in colonic specimens evaluated to obtain a quantitative estimation of membrane lipid peroxidation.

Statistical Analysis

Two-way ANOVA was used to analyze the data using SPSS version 26 (SPSS Inc) software. The LSD option was used to determine group differences, and significance was announced at $p < 0.05$. The results were presented as mean ± standard error (mean ± SE).

3. RESULTS

Assessment of the effect of Black pomegranate peel Ethanoic extract, on ulcerative colitis

Clinical signs observation:

Diarrhea and bloody stools (figure 3) were observed immediately following rectal administration of DNBS ون treated groups the appearance of stools changing to liquid pellets, then partially formed pellets to pasty at 2 weeks of treatment, and finally to normal after 3 weeks of treatment. Dehydration, abdominal discomfort, back arching, lethargy, anorexia, consuming major quantities of water, rough hair coat, dullness, calmness, move little and sat hunched over were other clinical indications reported in all experimental groups. These clinical symptoms persisted in the control positive group until the end of the study, while treatment groups improved gradually. Recovery assessed by end of diarrhea and hematochezia, weight stability, and return to normal activity levels. After 4 weeks of therapy, there were no differences in rat weight changes or stool consistency between the control, negative, and treated groups (Sulfasalazine, BPPE 100,200 mg/kg B.W).

Figure 3: Diarrhea after DNBS administration.

Macroscopic scoring of colonic tissue of the experimental animals:

The result of macroscopic observation of the colon illustrated in (figure 5), exhibited there were a significant $P \leq 0.05$ decrease in the treated groups (sulfasalazine, BPPE 100mg/kg B.W, BPPE 200 mg/kg B.W) in comparison with control positive. and there were no significant $P \leq 0.05$ differences between treated groups (sulfasalazine, BPPE 100mg/kg B.W, BPPE 200 mg/kg B.W) when compared with the control negative group especially after 4 weeks of treatment. All treated groups (sulfasalazine, BPPE 100mg/kg B.W, BPPE 200 mg/kg B.W) showed a significant $P \leq 0.05$ decrease in macroscopic score after 4 weeks of

treatment and 1 week of treatment withdrawal when compared with the result after 2 weeks of treatment.

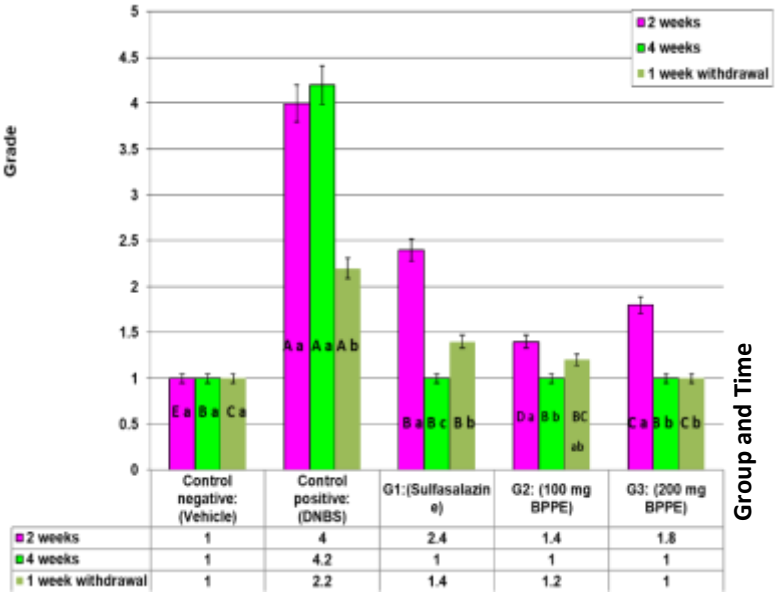


Figure 5: macroscopic of experimental animals(Rats) treated by vehicle, DNBS, sulfasalazine and 100 ,200 mg/Kg.BW of Black pomegranate peels ethanolic extract orally for four weeks and one week of treatment withdrawal.

[A-E] Significant differences at level p≤0.05 between groups.
[a-c] Significant differences at level p≤0.05 within groups.
Data as M±SE
LSD = 0.30

Gross appearance of colorectal tissue of rats of the experimental groups

Animals that received a single dose of DNBS demonstrated extensive mucosal damage characterized by extensive locally diffuse thick black-green pseudomembranous necrotic lesions (ulcerative colitis) (Figure 6). Whereas colon of the animals that received DNBS and were treated with sulfasalazine had marked thick multifocal to coalescing black-green foci of ulcerated and hemorrhagic mucosa (Figure 6). Interestingly, the colon of the DNBS-injected rats treated with BPPE of both doses 100 and 200 mg/Kg B.W showed moderate thick multifocal ulcerated and diffuse hyperemic mucosa (Figure 6). Four weeks post-treatment, the positive control group showed thick multifocal colored-necrotic foci as seen at 2 weeks (Figure 7). While the gross changes of sulfasalazine & BPPE-treated rats (200mg/kg) were less severe compared with lesions of 2 weeks of treatment and characterized by mild thickness with separated small foci of ulcerative and hyperemic mucosa (Figure 7). In the current study unexpected significant result appeared in rats treated with 100mg/kg of BPPE and these significant results showed, the mucosal thickness of this group was similar to the mucosal thickness of the negative control group regarding thickness and no obvious gross foci of mucosal damage (Figure 7). At the end of the experiment post 1 week of treatment withdrawal, the results of the positive control group showed fewer changes compared to 2 and 4 weeks, lesions characterized by moderate thickness with multifocal necrotic foci and hyperemic mucosa (Figure 8). While sulfasalazine & BPPE-treated rats showed

no significant gross changes as seen in the negative control (Figure 8).

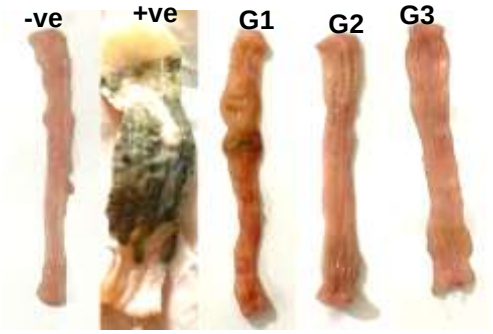


Figure 6: Representative gross image of the colon 2 weeks of treatment post-induction of ulcerative colitis in rats. Negative control (-ve), positive control (DNBS injection at 15mg/kg/intrarectally) (+ve), sulfasalazine treated group (25 mg/kg B.W/orally) (G1), BPPE treated group (100mg/kg/ orally) (G2), BPPE treated group (200mg/kg/ orally) (G3).

Figure 7: Representative gross image of colon 4 weeks of treatment post-induction of ulcerative colitis in rats. Negative control (-ve), positive control (DNBS injection at 15mg/kg/intrarectally) (+ve), sulfasalazine treated group (25 mg/kg B.W/orally) (G1), BPPE treated group (100mg/kg/ orally) (G2), BPPE treated group (200mg/kg/ orally) (G3).

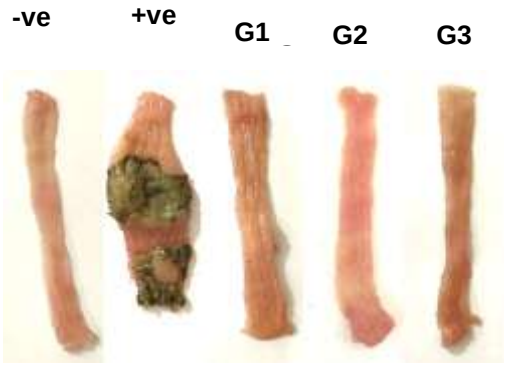


Figure 8: Representative gross image of colon 4 weeks of treatment + 1 week of withdrawal post-induction of ulcerative colitis in rats. Negative control (-ve), positive control (DNBS injection at 15mg/kg/intrarectally) (+ve), sulfasalazine treated group (25 mg/kg B.W/orally) (G1), BPPE treated group (100mg/kg/ orally) (G2), BPPE treated group (200mg/kg/ orally) (G3).

Microscopic scores in the colonic tissue of the experimental animals:

The results of microscopic scores in the colonic tissue illustrated in (Figure 9), showed that all treated groups (sulfasalazine, BPPE 100mg/kg B.W, BPPE 200 mg/kg B.W) showed a significant $P \leq 0.05$ decrease when compared with the control positive. It's worth mentioning the group 2 (BPPE 100 mg/kg B.W) was superior to another treated groups (Sulfasalazine and BPPE 200 mg/kg B.W) after 2 weeks and 4 weeks of treatment.

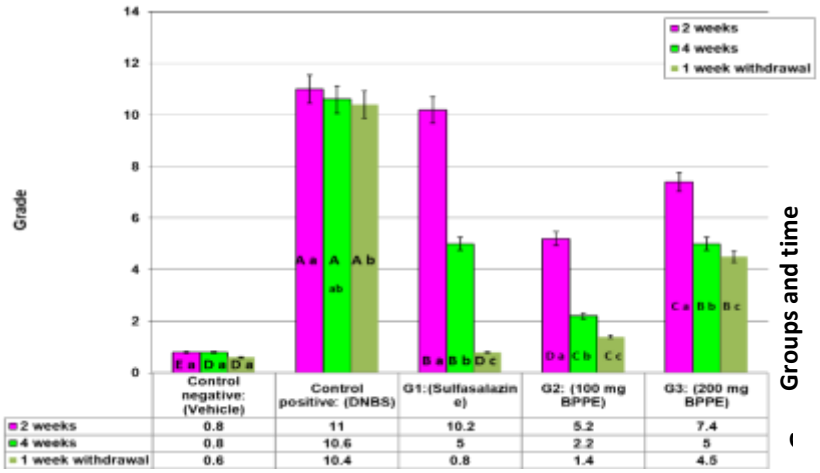


Figure 9: microscopic finding (histopathology) of experimental animals (Rats) treated by vehicle, DNBS, sulfasalazine, 100, and 200 mg/Kg.BW of Black pomegranate peels ethanolic extract orally for four weeks and one week of treatment withdrawal.

$^{A-E}$ Significant differences at level $p \leq 0.05$ between groups.

$^{a-c}$ Significant differences at level $p \leq 0.05$ within groups.

Data as M±SE

LSD = 0.47

Light Microscopy Findings

Histopathological Finding was represented by various histopathological lesions in the colon tissue of experimental groups such as severe loss of entire mucosa and crypts (ulcer). Some sections revealed the mucosa was replaced by areas of hemorrhage and necrosis in the control positive group (figure 10). Sulfasalazine treated group colonic lesions characterized by area of mucosal loss and coagulative necrosis area of mucosal loss after two weeks of treatment (figure 10). While groups treated with BPPE (100 and 200 mg/kg B.W) revealed mostly wide areas of colonic re-epithelization, organized crypts, and goblet cells architecture was marked, some sections showed mild mononuclear cell infiltration within the lamina propria and submucosa, crypt abscess, and/or cystic dilation (figure 10). Later, four and five weeks post-DNBS control positive group showed the same lesions as seen previously at 2 weeks (figure 11 and 12), Histopathological examination of group treated with Sulfasalazine 25 mg/kg B.W after 4 weeks of treatment showed colonic columnar re-epithelization, organized crypts and goblet cells architecture was marked with mild mononuclear cells infiltrate (figure 11). After one week of sulfasalazine treatment withdrawal, the same colonic histological changes as 4 weeks post-treatment were seen but with no signs of necrosis or sloughed mucosa (figure 12). While in-group treated with BPPE (100 mg/kg, B.W) the healing

process was significantly marked after 4 weeks of treatment and 1 week of treatment withdrawal which showed most of the simple columnar epithelium and crypts were completely restored with few inflammatory cell infiltrations (figure 11 and 12).

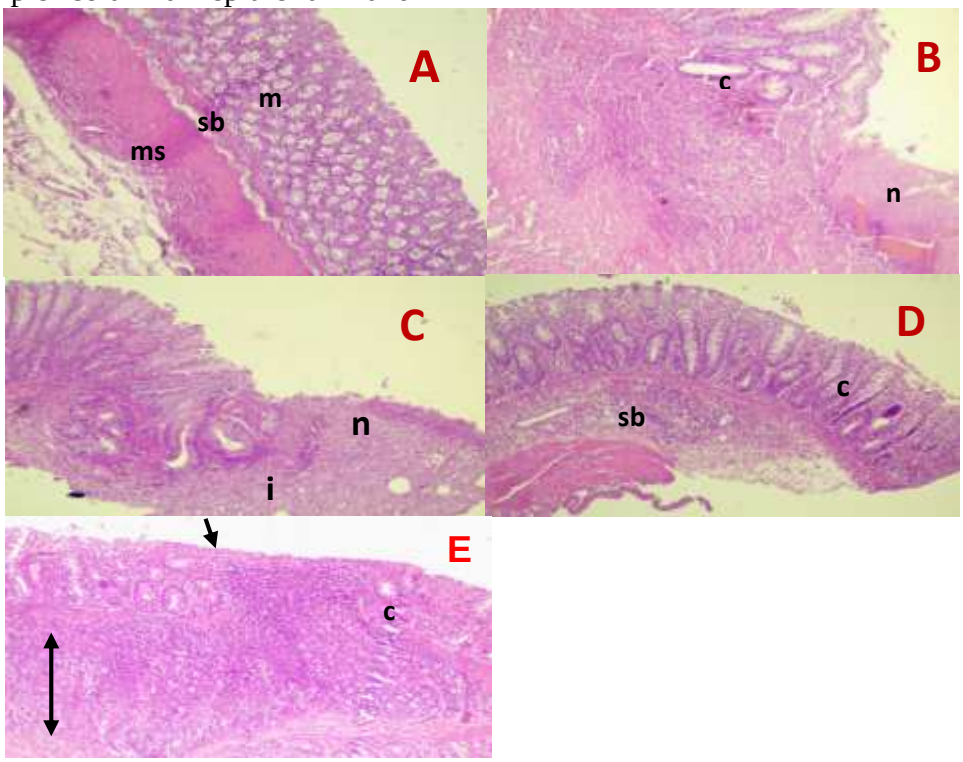


Figure 10: Histopathological finding after 2 weeks of treatment.

A: Representative section of the colon of rats 2 weeks post administrated 0.25 ml of 50% of ethanol intrarectally (negative control) shows identical histological features of the colon that composed of mucosa (m), submucosa (sb), and muscularis (ms). The simple columnar epithelium is lining the surface of the mucosa with no significant changes in crypts and goblet cells architecture. Mild mononuclear cells infiltration in the lamina propria is also seen filling the areas between the simple tubular straight glands. The Muscularis layer showed longitudinal and circular muscle bundles with no evident lesions (H&E, 10X).

B: Representative histopathological section of the colon of rats 2 weeks post-induction of ulcerative colitis with 2, 4-dinitrobenzene sulfonic acid (15mg/kg B.W intrarectally) (positive control) shows severe multifocal to coalescing areas of mucosal and crypts loss, areas of hemorrhage and necrosis (n). Submucosa and muscularis are markedly thickened and disorganized (double headed arrow) due to massive inflammatory cells infiltration mainly neutrophils. The edges of the ulcer show disrupted crypts (c) with decreased number of goblet cells. Some crypts collapsed and some are dilated with desquamated cellular debris in their lumens. (H&E, 10X).

C: Representative histopathological section of the colon of rats post-induction of ulcerative colitis with 2, 4-dinitrobenzene sulfonic acid (15mg/kg B.W intrarectally), and treated for 2 weeks with 25 mg/kg B.W of sulfasalazine, shows a wide area of mucosal loss and necrosis (n) are evident where crypt architecture is destroyed with thickened submucosa due to the presence of massive

inflammatory cells infiltration (i) (H&E, 10X).

D: Representative histopathological section of the colon of rats post-induction of ulcerative colitis with 2, 4-dinitrobenzene sulfonic acid (15mg/kg B.W intrarectally) and treated for 2 weeks with 100 mg/kg B.W of black pomegranate peel ethanolic extract, Shows mostly wide areas of mucosal re-epithelization (arrow) with mild necrotic and cellular debris on the surface, organized crypts and goblet cells architecture are marked (c). Moderate mononuclear cell infiltration within the lamina propria and submucosa (sb), crypt abscess and/or cystic (blue arrow) are seen due to moderate cellular infiltration (H&E, 10X).

E: Representative histopathological section of the colon of rats post-induction of ulcerative colitis with 2, 4- dinitrobenzene sulfonic acid (15mg/kg B.W intrarectally) and treated for 2 weeks with 200 mg/kg B.W of black pomegranate peel ethanolic extract Show some sort of re-epithelization with multifocal areas of mucosal loss that are replaced by moderate of necrotic and cellular debris on the surface (arrow), and crypts architecture (c) markedly disappeared. Massive mononuclear cell infiltration within the lamina propria, submucosa, and muscular (double-headed arrow) layer is obvious (H&E, 10X).

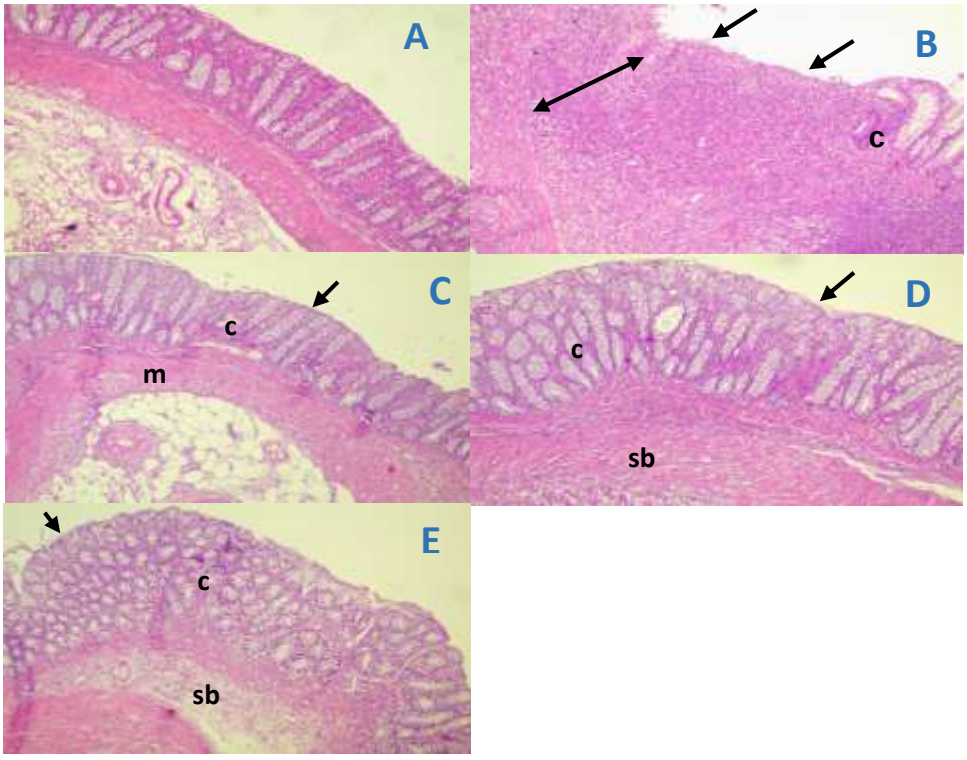


Figure 11: Histopathological finding after 4 weeks of treatment.

A: Representative section of the colon of rats 4 weeks post administrated 0.25 ml of 50% of ethanol intrarectally (negative control).

B: Representative histopathological section of the colon of rats, 4 weeks post-induction of ulcerative colitis with 2, 4-dinitrobenzene sulfonic acid (15mg/kg B.W intrarectally) (positive control) shows same lesions as seen previously at 2 weeks. Notice variable degrees of mucosal loss (arrow), thickened of submucosa and muscularis with massive submucosal granulation tissue formation (double-headed arrow). On the right side of the

section, crypts architecture is disorganized (c) (H&E, 10X)

C: Representative histopathological section of the colon of rats post-induction of ulcerative colitis with 2, 4-dinitrobenzene sulfonic acid (15mg/kg B.W intrarectally) and treated for 4 weeks with 25 mg/kg B.W of sulfasalazine shows mostly diffusely re-epithelization (arrow) is marked with mild necrotic and cellular debris covering the mucosal surface, organized crypts (c) and goblet cells architecture is marked with mild mononuclear cells infiltrate within the lamina propria and submucosa, with no evidence of submucosal and muscular (m) layer expansion (H&E, 10X).

D: Representative histopathological section of the colon of rats post-induction of ulcerative colitis with 2, 4-dinitrobenzene sulfonic acid (15mg/kg B.W intrarectally) and treated for 4 weeks with 100 mg/kg B.W of black pomegranate peel ethanolic extract shows recovered columnar epithelium (arrow) and most crypts (c) are completely restored with mild inflammatory cell infiltrations in the lamina propria and submucosa (sb) (H&E, 10X).

E: Representative histopathological section of the colon of rats post-induction of ulcerative colitis with 2, 4- dinitrobenzene sulfonic acid (15mg/kg B.W intrarectally) and treated for

weeks with 200 mg/kg B.W of black pomegranate peel ethanolic extract Show marked mucosal re-epithelization (arrow) and major crypts architecture are obviously restored (c) with moderate mononuclear cell infiltration within the lamina propria and submucosa (sb) (H&E, 10X).

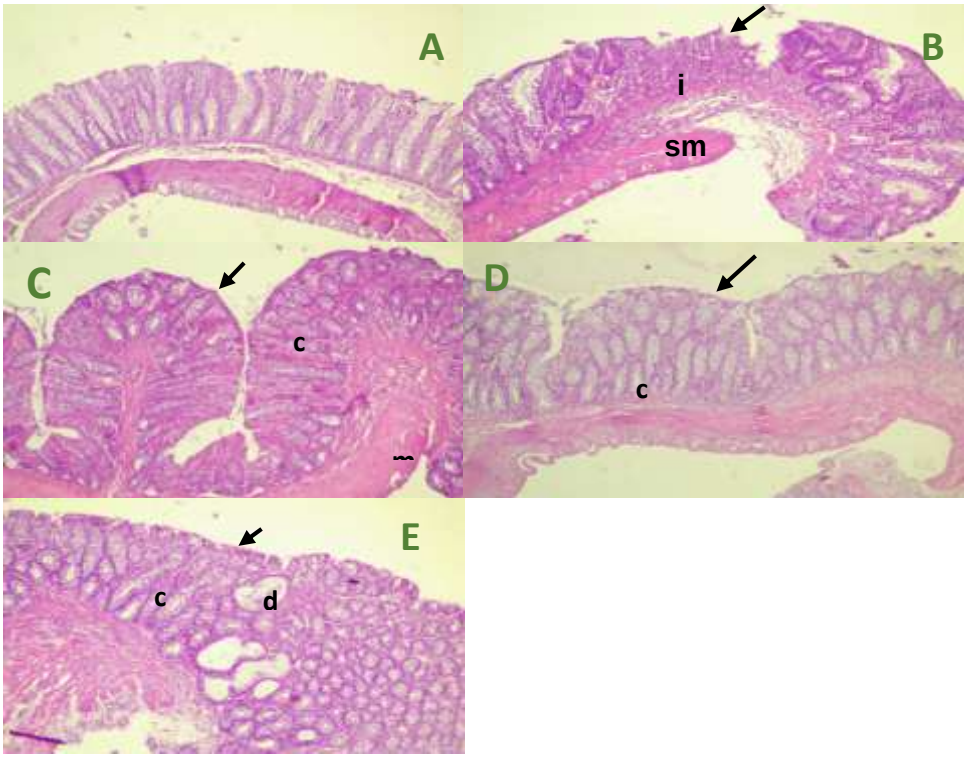


Figure 12: Histopathological finding after 4 weeks of treatment and 1-week withdrawal.

A: Representative section of the colon of rats 4 weeks post administrated 0.25 ml of 50% of

ethanol intrarectally (negative control).

B: Representative histopathological section of the colon of rats, 5 weeks post-induction of ulcerative colitis with 2, 4-dinitrobenzene sulfonic acid (15mg/kg B.W Intrarectally) shows marked mucosal desquamation (arrow) that replaced by inflammatory cells (i) and extravasated red blood cells (hemorrhage). Crypts architecture is disorganized and crypt dilated as well as are invaded by inflammatory cells (cryptitis) (c). Mild thickening of the submucosal layer (sm) is evident. (H&E, 10X).

C: Representative histopathological section of the colon of rat post-induction of ulcerative colitis with 2, 4-dinitrobenzene sulfonic acid (15mg/kg B.W intrarectally) and treated for 4 weeks and 1-week withdrawal with 25 mg/kg B.W of sulfasalazine shows diffusely, columnar re-epithelization (arrow) is marked with moderate necrotic and cellular debris covering the mucosal surface. organized crypts (c) and goblet cells architecture is marked with mild mononuclear cells infiltrate within the lamina propria and submucosa with no evidence of submucosal and muscular (m) layer expansion (H&E, 10X).

D: Representative histopathological section of the colon of rat post-induction of ulcerative colitis with 2, 4-dinitrobenzene sulfonic acid (15mg/kg B.W intrarectally) and treated for 4 weeks and 1-week withdrawal with 100 mg/kg B.W of black pomegranate peel ethanolic extract shows simple columnar epithelium (arrow) and crypts (c) is completely restored with mild inflammatory cell infiltrations in the lamina propria (H&E, 10X).

E: Representative histopathological section of the colon of rat post-induction of ulcerative colitis with 2, 4- dinitrobenzene sulfonic acid (15mg/kg B.W intrarectally) and treated for 4 weeks and 1-week withdrawal with 200 mg/kg B.W of black pomegranate peel ethanolic extract shows marked recovery of the epithelial lining of colonic mucosa (arrow) and crypts (c) with mild mononuclear cell infiltration within the lamina propria, However, some crypts are cystic and contain cellular debris (d) (H&E, 10X).

The concentration of Malondialdehyde (MDA) and Myeloperoxidase (MPO) in the colonic tissue of the experimental animals:

The result of MDA and MPO concentration in the colonic tissue illustrated in (figure 13 and 14), showed there was a significant $P \leq 0.05$ increase MDA and MPO level in experimental groups (control positive, sulfasalazine, BPPE 100mg/kg B.W, BPPE 200 mg/kg B.W) in comparison with control negative group along the period of the experiment. except group 2 (100 mg BPPE) after 4 weeks of treatment and 1 week of treatment withdrawal showed no significant $P \leq 0.05$ differences when compared with the control negative group. All treated groups (sulfasalazine, BPPE 100mg, and 200mg /kg. B.W) showed a significant decrease in MDA and MPO level in comparison with the control positive group. Both groups treated with BPPE (100,200 mg/kg B.W) showed no significant difference in MPO concentration when compared with control negative after one week of treatment withdrawal. While G2 (100 mg/kg B.W) show no significant difference in MDA concentration when compared with control negative group after four weeks of treatment and one week of treatment withdrawal.

Interestingly, the animals of group 2 (BPPE 100 mg /kg B.W) showed a significant $P \leq 0.05$ decrease in MDA level when compared with (sulfasalazine) and (BPPE 200mg/kg B.W) treated groups. Also group 2 (BPPE 100mg/kg B.W) showed a significant $P \leq 0.05$ decrease in MPO level after 4 week of

treatment and 1 week of treatment withdrawal in comparison with group 1 (sulfasalazine). While the BPPE 200mg/kg B.W treated group showed no significant $P \leq 0.05$ change in comparison with the sulfasalazine treated group after 2 and 4 weeks of treatment but exhibited a significant $P \leq 0.05$ decrease after one-week withdrawal of treatment.

The treated group 2 (BPPE 100mg/kg B.W) showed a significant $P \leq 0.05$ decrease in MDA and MPO concentration after 4 weeks and 1 week of treatment withdrawal in comparison with MDA and MPO concentration after 2 weeks of treatment. The treated group 3 (BPPE 200mg/kg B.W) showed a significant $P \leq 0.05$ decrease in MDA and MPO concentration after 1 week of treatment withdrawal in comparison with MDA and MPO concentration after 2 and 4 weeks of treatment. Obviously, the treated group 1 (Sulfasalazine) showed no significant $P \leq 0.05$ changes in MDA concentration between 2 weeks,4 weeks, and 1 week of withdrawal of treatment.

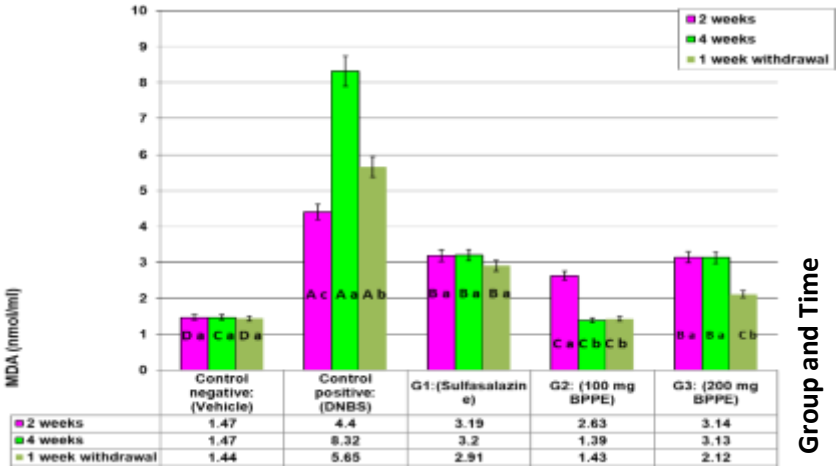
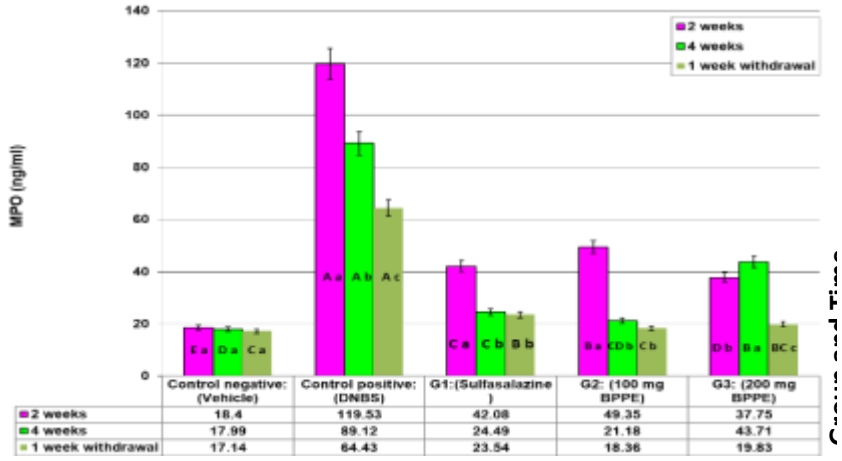


Figure 13 : Malondialdehyde (nmol/ml) of experimental animals (Rats) treated by vehicle, DNBS, sulfasalazine and 100, 200 mg/Kg.BW of Black pomegranate peels ethanolic extract orally for four weeks and one week of treatment withdrawal.

$^{A-D}$ Significant differences at level $p \leq 0.05$ between groups.
$^{a-c}$ Significant differences at level $p \leq 0.05$ within groups.
Data M±SE
LSD = 0.32



	Control negative: (Vehicle)	Control positive: (DNBS)	G1:(Sulfasalazine)	G2: (100 mg BPPE)	G3: (200 mg BPPE)
2 weeks	18.4	119.53	42.08	49.35	37.75
4 weeks	17.99	89.12	24.49	21.18	43.71
1 week withdrawal	17.14	84.43	23.54	18.36	19.83

Figure 14: Myeloperoxidase MPO (ng/ml) of control and experimental animals (Rats) treated by vehicle, sulfasalazine and 100, 200 mg/Kg.BW of Black pomegranate peel ethanolic extract orally for four weeks and one week of treatment withdrawal.

Data M±SE
LSD = 3.77

[A-E] Significant differences at level $p \leq 0.05$ between groups.
[a-c] Significant differences at level $p \leq 0.05$ within groups.

4. DISCUSSION

Ulcerative colitis is incurable, relapsing, and remitting intestinal disease that often requires lifelong treatment usage to maintain remission (Lee, 2020). UC is also accompanied with a high economic burden (Fumery et al., 2018). Containing costs in UC has become challenging due to the considerable burden of disease. It has been estimated that the current population affected with UC will cost the healthcare system about $377 billion over the years that they live (Lichtenstein et al., 2020).

There are currently several therapeutic strategies for treating IBD, but they are associated with several adverse effects, In addition, some patients do not respond to treatment at all or lose response to the drug over time. therefore; there is an ongoing search for new therapies that may be beneficial in treating these chronic diseases (Lichtenstein et al., 2009),

In this sense, interest in the use of plant products for treating IBD has increased (H Farzaei et al., 2015; Zorrilla et al., 2014). Particularly those rich in phenolic compounds, which have a variety of bioactivities including antioxidant activity, being able to act on different targets of the inflammatory process, and protect the tissue microenvironment from oxidative stress (Da Silva et al., 2018).

Antioxidants play an important role in the oxidation of oxidative stress induced by free radicals in biological cells (Lazeeza, 2021). The antioxidant and antibacterial activities of medicinal plant extracts may be related to their high phenolic content (Hussein et al., 2020). Phenolic chemicals are very powerful antioxidants that may neutralize free radicals and their cytotoxic effects, and thus play a significant role in health (Salimi et al., 2011).

In this study, black pomegranate was highlighted as a potential treatment option for inflammatory bowel diseases. Pomegranate has gained popularity due to the abundant bioactive chemicals and the various secondary metabolites it contains. It has promising biological properties that have been linked to the numerous elements that are found in edible and non-edible parts of the fruit, including antibacterial, antifungal, anti-inflammatory, antioxidant, anticancer, Antimicrobial and wound-

healing activities (Al-Dhaher, 2013; Alkhatib et al., 2022; Hadab & Dakheel, 2022; Hassan, 2004; Salim et al., 2022).

These finding is inconsistent with what that have been found in GCMS analysis of BPPE in our previous experemnt(in press), when there are several phytconsistents possessing anti-oxidant ability like Phenols, alkaloids, furans. The antioxidant activity of red pomegranate peel, white pomegranate peel, and black pomegranate peel is potent (Chasanah, 2020). There is a strong correlation between the phenolic compound content and total antioxidant capacity (Hooks et al., 2021). Black pomegranate is known to be a good source of phenolic compounds that are rich in antioxidants, so its antioxidant activity is very strong which is linked to potential health benefits (Vanani et al., 2020). A scientific research studies among different P. granatum L. varieties (Black, White, and Red skin fruit) showed that black skin fruit has the highest amount of phenolic compounds, total anthocyanin, and antioxidant properties (Bayati & Asadi-Gharneh, 2019). In addition, Black pomegranate peel extract contains active compounds, including flavonoids, saponins, and tannins (Achmad et al., 2019).This suggests that the most extracts of pomegranate peel are both anti-inflammatory and anti-ulcerogenic. Taken together, the results can provide an extra income and may contribute to have good nutritional values of this product (Ghazaleh et al., 2013).

Fortunately, these studies confirmed the presence of antioxidant compounds in black pomegranate peels extract especially since these substances (phenolic compounds and flavonoids) have proven their antioxidant effect, which confirms the ability of pomegranate peel extract to treat colitis. and this corresponds to the data of the therapeutic doses of pomegranate peel extract, that were used in the current experiment in terms of the disappearance of ulcers in the colorectal tissue and its return to its normal appearance, as well as a decrease in parameters indicative of oxidative stress.

The experimental rodent model of DNBS-induced colitis is well documented previously (Morris et al., 1989). The DNBS-induced colitis is a model of bowel inflammation characterized by body weight loss, diarrhea, ulceration, bleeding, depletion of goblet cells, and formation of granulomas within the gut wall (Jurjus et al., 2004). In this model, oxidative damage is also known to play a significant role in the development of tissue injury occurring within a few days from DNBS administration (Elson et al., 1995).

Therefore, after inducing colitis by DNBS, the parameters that can be analyzed include changes clinical symptoms, changes in colonic cytoarchitecture, and MPO and MDA concentrations (Cuzzocrea et al., 2001; Joshi et al., 2011; Martín et al., 2017; Wirtz et al., 2007). This confirms the validity of our choice of the model of the current experiment.

The estimation is of significant in confirmation the role of oxidative stress in arising and progression of IBD. since Studies have now confirmed an elevated level of MDA (a lipid peroxidation product, is a naturally occurring immune adjuvant implicated in promoting autoimmunity and inflammation) in patients with IBD (Barbosa et al., 2003; Chiarpotto et al., 1997; Forrest et al., 2003; Kruidenier et al., 2003; Levy et al., 2000).

Although studies have shown that MDA concentrations can be elevated in both, the patients with UC and CD compared with normal (Bouzid et al., 2013), MDA was found to be increased in rats treated with DNBS (Cuzzocrea et al., 2001). This is consistent with the finding of presence study when there is a prominent elevation in MDA in the colon tissue of rat that have induce IBD by DNBS.

Oxidative damage to intestinal mucosal cells and aggravation of inflammatory reaction have been proposed as two

significant mechanisms of oxidative stress involved in the pathophysiology of IBD (Sartor, 2006).

MDA levels increased in patients with UC. This would suggest that lipid peroxidation could have an important role in the pathogenesis of UC (Baskol et al., 2006).

Colonic inflammatory status is closely related to forming reactive compounds from activated neutrophils and phagocytes, and by generating oxidative stress; this may be a mechanism underlying the pathophysiology of IBD (Alzoghaibi, 2013). The attack of reactive oxygen species on polyunsaturated fatty acids from cellular phospholipid membranes can progress with the formation of peroxyl radicals, which attack the membrane and lead to the destruction of its structure. Secondary products are formed during this process, such as MDA, a dialdehyde that can be detected in biological samples and is widely used to assess oxidative stress (Gaschler & Stockwell, 2017; Hussain et al., 2003).

Thus, decreased MDA levels at tested doses of the BPP extract demonstrate its antioxidant potential, since there is a significant improvement of oxidative stress status in the experimental animals that have exposed to various dosage rate of BPPE along the period of this study. The supplementation with BPPE reduces MDA levels, and the phenolic compounds of BPPE may play a protective role by binding to lipid peroxidase.

Vanani et al, (2020) suggested that antioxidant-rich BPPE shows a protective effect against oxidative hepatotoxicity induced by t-BHP in Wistar rats. The BPPE demonstrates potency to reduce the level of liver marker enzymes, prevent lipid peroxidation, and adjust changes in antioxidant enzyme activity (Vanani et al., 2020).

Pomegranate tannins play a protective role against gastric ulcers. Its antiulcer effect is related to the increased secretion of adherent mucus and free mucus from the stomach wall. This may inhibit the generation of oxygen-derived free radicals, decrease the consumption of glutathione peroxidase and superoxide dismutase, and maintain the content of nitric oxide at a normal level (Lai et al., 2009). The antiulcer effect of BPEE was evident through the improvement of the macroscopic and microscopic appearance of colon tissue and disappearance of ulcers and necrotic lesions, as well as the re-epithelialization tissue in the animals that were treated with the BPEE compared to the animals that were not treated, as well as the animals that were dosed with sulfasalazine treatment.

Myeloperoxidase (MPO) levels are often used to quantify neutrophil functions and activity in inflamed tissues (Laroui et al., 2012; Tran et al., 2007).

Neutrophil infiltration is an indicator of oxidative stress that could be assessed by myeloperoxidase (MPO) activity, is one of the most abundant enzymes in azurophilic granule of neutrophils and monocytes, determination (Liu et al., 2011). In colitis, the activity of MPO in colonic tissue is a marker of neutrophil infiltration that damage the mucosal macromolecules and increase the mucosal disruption and ulceration (El-Abhar et al., 2008).

Tissue MPO activity, which is directly related to the number and activity of myeloid cell infiltrates in the inflamed tissue, was assayed to monitor the degree of inflammation (Bradley et al., 1982; J. W. Smith & Castro, 1978). An increase in leukocyte adhesion and accumulation in colon tissues as well as an increase in MPO activity is typically observed during IBD and in experimental animal models as DNBS-induced colitis.

Treatment with BPPE significantly reduced MPO levels in DNBS rats when compared to the DNBS group. Therefore, in this study, the anti-inflammatory effects of Black pomegranate presents bioactive compounds such as phenolic compounds, flavonoids, saponins, and tannins and it

was described as presenting pharmacological properties such as antioxidant, anti-carcinogenic, and anti-inflammatory activities.

The results found in this study demonstrate the therapeutic effect of BPPE in the acute colitis model induced by DNBS. Administration of BPPE caused a marked reduction in macroscopic colonic damage, with a decrease in inflammation along the colonic tissue being evidenced in the histological study, reduced colonic tissue MDA and MPO levels.

The intestinal anti-inflammatory activity of the BPPE at dosage tested 100 and 200 mg/kg B.W. can be attributed to its rich phenolic content.

Toxicity tests are needed to assess the safety of the drug, or ingredients used as supplements or food (Makiyah & Tresnayanti, 2017). Range finding study that has carried out in the present study confirmed the safety of BPEE, since there are no morbid and mortal animals have been observed.

Black pomegranate peel extract rich with bioactive compounds like phenolic compounds, flavonoids, saponins, and tannins (Achmad et al., 2019; Chasanah, 2020; Vanani et al., 2020).Because the antioxidant capacity of pomegranate peel extract is 10 times higher than the pulp extract (Ghazaleh et al., 2013). All waste parts of the pomegranate fruit, such as the peel can be processed into value-added products with industrial, medicinal, and cosmetic value (Dhumal et al., 2014).

Differences in the content of total phenols, tannins and flavonoids could be explained by different extraction conditions (type of the solvents, extraction method, temperature, duration of the extraction).

To our knowledge, no study has been conducted to evaluate this therapeutic effect of BPPE in UC. In previous study, Moghaddam et al., (2014) evaluated antiulcerogenic effects of black pomegranate peel methanol extract on ulceration induced by 80% ethanol, they confirmed the pretreatment with all three peel extracts (25, 50 and 100 mg/kg) for 15 days protected the gastric mucosa against the damage induced by 80 % ethanol. The treatment by black peel pomegranate 50 and 100 mg/kg group were protected from intraluminal bleeding. It was evident that 50 mg/kg dosage of black peel cultivar markedly inhibited the peptic ulcer, compared with ethanol-induced gastric ulcer. There was an apparent decrease in the infiltration of polymorph nuclear leukocytes and hemorrhage after administration of black peel extracts (50 mg/kg) (Moghaddam et al., 2014).

In another study, the aqueous extract of pomegranate peel showed a gastro protective effect in rats with ethanol-induced gastric lesions (Colombo et al., 2013), The simultaneous administration of ethanol and pomegranate peel significantly decreased gastric lesions and ulcer index. In addition, Pomegranate juice protects the liver from oxidative damage, according to Ali and Al-Okaily, (Ali & Al-Okaily, 2016).

Recently, others have also reported that pomegranate and other fruit-derived polyphenols were effective at reducing inflammation and pathology in chemically-induced colitis (A. D. Smith et al., 2020). In addition, Nayak et al., (2013) have shown that animals treated with pomegranate extract epithelialize faster than their controls and that the wound area was 95% smaller than in the controls area (Nayak et al., 2013). It could be concluded that Black pomegranate peel ethanolic extract administration for 30 days has a potential role to overcome ulcerative colitis, specifically, 100 mg/Kg.BW is superior to 200 mg/Kg.BW. It could be concluded that both 100 mg/Kg.BW and 200 mg/Kg.BW of BPPE taken orally for 30 days have the ability to treat ulcerative colitis, however 100 mg/Kg.BW is the superior dosage rate.

5. REFERENCES

Achmad, R. S., Aditya, L. A., & Cahyariza, N. I. (2019). Acute Toxicity Test of Black Pomegranate Peel Extract (Granati Fructus Cortex) Against Larvae of Shrimp (Artemia salina Leach). Medical Laboratory Technology Journal, 5(2), 62–69.

Akhtar, S., Ismail, T., Fraternale, D., & Sestili, P. (2015). Pomegranate peel and peel extracts: Chemistry and food features. Food Chemistry, 174, 417–425.

Al-Dhaher, Z. A. (2013). Evaluation of Antibacterial Activity of Aqueous Extracts of Pomegranate Peels, Green Tea Leaves and Bay Leaves against Vibrio cholera. The Iraqi Journal of Veterinary Medicine, 37(1), 90–95.

Alatab, S., Sepanlou, S. G., Ikuta, K., Vahedi, H., Bisignano, C., Safiri, S., Sadeghi, A., Nixon, M. R., Abdoli, A., & Abolhassani, H. (2020). The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet Gastroenterology & Hepatology, 5(1), 17–30.

Ali, E. H., & Al-Okaily, B. N. (2016). The protective role of Pomegranate seed oil (Pometone) on serum protein in sodium fluoride treated female rats. Part I). IJVM, 39(2), 61–68.

Alkhatib, M., Fayad, C., Badran, A., Hamade, K., Daou, A., Baydoun, E., & Hijazi, A. (2022). Preventive and Therapeutic Effects of Punica granatum (Pomegranate) in Respiratory and Digestive Diseases: A Review. Applied Sciences, 12(23), 12326.

Alzoghaibi, M. A. (2013). Concepts of oxidative stress and antioxidant defense in Crohn's disease. World Journal of Gastroenterology: WJG, 19(39), 6540.

Balli, D., Cecchi, L., Khatib, M., Bellumori, M., Cairone, F., Carradori, S., Zengin, G., Cesa, S., Innocenti, M., & Mulinacci, N. (2020). Characterization of arils juice and peel decoction of fifteen varieties of Punica granatum L.: a focus on anthocyanins, ellagitannins and polysaccharides. Antioxidants, 9(3), 238.

Balli, D., Tozzi, F., Khatib, M., Adessi, A., Melgarejo, P., Masciandaro, G., Giordani, E., Innocenti, M., & Mulinacci, N. (2020). Purple Queen® fruits of Punica granatum L.: Nutraceutical properties and unconventional growing substrates. Journal of Berry Research, 10(4), 637–650.

Barbara, G., Xing, Z., Hogaboam, C. M., Gauldie, J., & Collins, S. M. (2000). Interleukin 10 gene transfer prevents experimental colitis in rats. Gut, 46(3), 344–349.

Barbosa, D. S., Cecchini, R., El Kadri, M. Z., Rodríguez, M. A. M., Burini, R. C., & Dichi, I. (2003). Decreased oxidative stress in patients with ulcerative colitis supplemented with fish oil ω-3 fatty acids. Nutrition, 19(10), 837–842.

Baskol, G., Baskol, M., Yurci, A., Ozbakir, O., & Yucesoy, M. (2006). Serum paraoxonase 1 activity and malondialdehyde levels in patients with ulcerative colitis. Cell Biochemistry and Function: Cellular Biochemistry and Its Modulation by Active Agents or Disease, 24(3), 283–286.

Bayati, R., & Asadi-Gharneh, H. A. (2019). Study of Biochemical Compounds from Extract of Peel, Seed and Fruit Juice of some Pomegranate Cultivars (Punica granatum L.). Journal of Medicinal Plants and By-Product, 8(2), 133–141.

Benchagra, L., Berrougui, H., Islam, M.

O., Ramchoun, M., Boulbaroud, S., Hajjaji, A., Fulop, T., Ferretti, G., & Khalil, A. (2021). Antioxidant effect of moroccan pomegranate (Punica granatum L. sefri variety) extracts rich in punicalagin against the oxidative stress process. Foods, 10(9), 2219.

Binienda, A., Fichna, J., & Salaga, M. (2020). Recent advances in inflammatory bowel disease therapy. European Journal of Pharmaceutical Sciences, 155, 105550.

Bioassay Technology Laboratory. (2017). Rat Malondialchehyche ELISA. 1–8.

Bioassay Technology Laboratory mpo. (n.d.). Rat myeloperoxidase ELISA Kit. 1–8.

Bouzid, D., Gargouri, B., Mansour, R. Ben, Amouri, A., Tahri, N., Lassoued, S., & Masmoudi, H. (2013). Oxidative stress markers in intestinal mucosa of Tunisian inflammatory bowel disease patients. Saudi Journal of Gastroenterology: Official Journal of the Saudi Gastroenterology Association, 19(3), 131.

Bradley, P. P., Priebat, D. A., Christensen, R. D., & Rothstein, G. (1982). Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. Journal of Investigative Dermatology, 78(3), 206–209.

Chasanah, U. (2020). Studies on antioxidant activity of red, white, and black pomegranate (Punica granatum L.) peel extract using DPPH radical scavenging method. Farmasains: Jurnal Farmasi Dan Ilmu Kesehatan, 5(2), 51–55.

Chiarpotto, E., Scavazza, A., Leonarduzzi, G., Camandola, S., Biasi, F., Teggia, P. M., Garavoglia, M., Robecchi, A., Roncari, A., & Poli, G. (1997). Oxidative damage and transforming growth factor β1 expression in pretumoral and tumoral lesions of human intestine. Free Radical Biology and Medicine, 22(5), 889–894.

Colombo, E., Sangiovanni, E., & Dell'Agli, M. (2013). A review on the anti-inflammatory activity of pomegranate in the gastrointestinal tract. Evidence-Based Complementary and Alternative Medicine, 2013.

Cuzzocrea, S., McDonald, M. C., Mazzon, E., Mota-Filipe, H., Centorrino, T., Terranova, M. L., Ciccolo, A., Britti, D., Caputi, A. P., & Thiemermann, C. (2001). Calpain inhibitor I reduces colon injury caused by dinitrobenzene sulphonic acid in the rat. Gut, 48(4), 478–488.

Da Silva, V. C., De Araújo, A. A., Araújo, D. F. de S., Lima, M. C. J. S., Vasconcelos, R. C., de Araújo Júnior, R. F., Langasnner, S. M. Z., Pedrosa, M. de F. F., De Medeiros, C. A. C. X., & Guerra, G. C. B. (2018). Intestinal anti-inflammatory activity of the aqueous extract from Ipomoea asarifolia in DNBS-induced colitis in rats. International Journal of Molecular Sciences, 19(12), 4016.

Dhumal, S. S., Karale, A. R., Jadhav, S. B., & Kad, V. P. (2014). Recent advances and the developments in the pomegranate processing and utilization: a review. Journal of Agriculture and Crop Science, 1(1), 1–17.

El-Abhar, H. S., Hammad, L. N. A., & Gawad, H. S. A. (2008). Modulating effect of ginger extract on rats with ulcerative colitis. Journal of Ethnopharmacology, 118(3), 367–372.

Elson, C. O., Sartor, R. B., Tennyson, G. S., & Riddell, R. H. (1995). Experimental models of inflammatory bowel disease. Gastroenterology, 109(4), 1344–1367.

Fang, H., Fu, L., & Wang, J. (2018). Protocol for fecal microbiota

transplantation in inflammatory bowel disease: a systematic review and meta-analysis. BioMed Research International, 2018.

Fornai, M., Blandizzi, C., Antonioli, L., Colucci, R., Bernardini, N., Segnani, C., De Ponti, F., & Del Tacca, M. (2006). Differential role of cyclooxygenase 1 and 2 isoforms in the modulation of colonic neuromuscular function in experimental inflammation. Journal of Pharmacology and Experimental Therapeutics, 317(3), 938–945.

Forrest, C. M., Gould, S. R., Darlington, L. G., & Stone, T. W. (2003). Levels of purine, kynurenine and lipid peroxidation products in patients with inflammatory bowel disease. Developments in Tryptophan and Serotonin Metabolism, 395–400.

Fumery, M., Singh, S., Dulai, P. S., Gower-Rousseau, C., Peyrin-Biroulet, L., & Sandborn, W. J. (2018). Natural history of adult ulcerative colitis in population-based cohorts: a systematic review. Clinical Gastroenterology and Hepatology, 16(3), 343–356.

Gaschler, M. M., & Stockwell, B. R. (2017). Lipid peroxidation in cell death. Biochemical and Biophysical Research Communications, 482(3), 419–425.

Ghazaleh, M., Mohammad, S., Gholamreza, H., Mahnaz, K., & Mannan, H. (2013). Anti-ulcerogenic activity of the pomegranate peel (Punica granatum) methanol extract. Food and Nutrition Sciences, 2013.

H Farzaei, M., Rahimi, R., & Abdollahi, M. (2015). The role of dietary polyphenols in the management of inflammatory bowel disease. Current Pharmaceutical Biotechnology, 16(3), 196–210.

Hadab, N. S., & Dakheel, M. M. (2022). Application of pomegranate pomace as a natural antibacterial and antioxidant preservative in beef. Iraqi Journal of Veterinary Sciences, 36, 211–216.

Hadji, H., & Bouchemal, K. (2022). Advances in the treatment of inflammatory bowel disease: Focus on polysaccharide nanoparticulate drug delivery systems. Advanced Drug Delivery Reviews, 114101.

Hassan, W. A. (2004). Effect of Pomegranate barks solution (Punica granatum L) On some Pathogenic bacteria in vitro. The Iraqi Journal of Veterinary Medicine, 28(1), 257–263.

Hooks, T., Niu, G., Masabni, J., Sun, Y., & Ganjegunte, G. (2021). Performance and phytochemical content of 22 pomegranate (Punica granatum) varieties. HortScience, 56(2), 217–225.

Hou, C., Zhang, W., Li, J., Du, L., Lv, O., Zhao, S., & Li, J. (2019). Beneficial effects of pomegranate on lipid metabolism in metabolic disorders. Molecular Nutrition & Food Research, 63(16), 1800773.

Hussain, S. P., Hofseth, L. J., & Harris, C. C. (2003). Radical causes of cancer. Nature Reviews Cancer, 3(4), 276–285.

Hussein, S. I., Kaluf, A. F., Ahmed, Y., Ahmed, B., & Iyad, A. (2020). Determination of inhibition activity of α-Amylase enzyme, antioxidant activity, antibacterial activity and phenolic compounds by using some medical plants. The Iraqi Journal of Agricultural Science, 51(1), 411–421.

Hussen, W. M. (2014). Protective Role of Pomegranate Peel Extract on Testis in Adult Male Rabbits Treated with Carbon Tetrachloride. The Iraqi Journal of Veterinary Medicine, 38(1), 74–82.

Jairath, V., & Feagan, B. G. (2020). Global burden of inflammatory bowel disease. The Lancet Gastroenterology & Hepatology, 5(1), 2–3.

Joshi, S. V, Vyas, B. A., Shah, P. D.,

Shah, D. R., Shah, S. A., & Gandhi, T. R. (2011). Protective effect of aqueous extract of Oroxylum indicum Linn.(root bark) against DNBS-induced colitis in rats. Indian Journal of Pharmacology, 43(6), 656.

Jurjus, A. R., Khoury, N. N., & Reimund, J.-M. (2004). Animal models of inflammatory bowel disease. Journal of Pharmacological and Toxicological Methods, 50(2), 81–92.

Kaplan, G. G., Bernstein, C. N., Coward, S., Bitton, A., Murthy, S. K., Nguyen, G. C., Lee, K., Cooke-Lauder, J., & Benchimol, E. I. (2019). The impact of inflammatory bowel disease in Canada 2018: epidemiology. Journal of the Canadian Association of Gastroenterology, 2(Supplement_1), S6–S16.

Khorrami, S., Zarepour, A., & Zarrabi, A. (2019). Green synthesis of silver nanoparticles at low temperature in a fast pace with unique DPPH radical scavenging and selective cytotoxicity against MCF-7 and BT-20 tumor cell lines. Biotechnology Reports, 24, e00393.

Kohgo, Y. (2000). 2. Recent Advances in Therapy for Patients with Inflammatory Bowel Disease. Internal Medicine, 39(4), 342–345.

Konaté, K., Hilou, A., Mavoungou, J. F., Lepengué, A. N., Souza, A., Barro, N., Datté, J. Y., M'batchi, B., & Nacoulma, O. G. (2012). Antimicrobial activity of polyphenol-rich fractions from Sida alba L.(Malvaceae) against co-trimoxazol-resistant bacteria strains. Annals of Clinical Microbiology and Antimicrobials, 11, 1–6.

Kruidenier, L., Kuiper, I., Lamers, C. B. H. W., & Verspaget, H. W. (2003). Intestinal oxidative damage in inflammatory bowel disease: semi-quantification, localization, and association with mucosal antioxidants. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 201(1), 28–36.

Kusmardi, K., Khalilah, R. Y., Zuraidah, E., Estuningtyas, A., & Tedjo, A. (2022). The Effect of Pomegranate Peel Ethanol Extract to TNF-α Expression of Mice Colonic Epithelial Cells Induced Using Dextran Sodium Sulfate (DSS). Pharmacognosy Journal, 14(3).

Lai, S., Zhou, Q., Zhang, Y., Shang, J., & Yu, T. (2009). Effects of pomegranate tannins on experimental gastric damages. Zhongguo Zhong Yao Za Zhi= Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica, 34(10), 1290–1294.

Laroui, H., Sitaraman, S. V, & Merlin, D. (2012). Gastrointestinal delivery of anti-inflammatory nanoparticles. In Methods in enzymology (Vol. 509, pp. 101–125). Elsevier.

Larussa, T., Basile, A., Palleria, C., Iannelli, C., Vero, A., Giubilei, L., De Sarro, C., Suraci, E., Marasco, R., & Imeneo, M. (2021). Real-life burden of adverse reactions to biological therapy in inflammatory bowel disease: a single-centre prospective case series. Medicine and Pharmacy Reports, 94(3), 289.

Lazeeza, S. O. (2021). ANTIOXIDANT ACTIVITY OF POMEGRANATE. Iraqi Journal of Agricultural Sciences, 52(1), 196–203.

Lee, S. (2020). Ulcerative Colitis, An Issue of Gastroenterology Clinics of North America, E-Book (Vol. 49, Issue 4). Elsevier Health Sciences.

Levy, E., Rizwan, Y., Thibault, L., Lepage, G., Brunet, S., Bouthillier, L., & Seidman, E. (2000). Altered lipid profile, lipoprotein composition, and oxidant and antioxidant status in pediatric Crohn disease. The American Journal of Clinical Nutrition, 71(3), 807–815.

Lichtenstein, G. R., Hanauer, S. B.,

Sandborn, W. J., & Gastroenterology, P. P. C. of the A. C. of. (2009). Management of Crohn's disease in adults. Official Journal of the American College of Gastroenterology| ACG, 104(2), 465–483.

Lichtenstein, G. R., Shahabi, A., Seabury, S. A., Lakdawalla, D. N., Espinosa, O. D., Green, S., Brauer, M., & Baldassano, R. N. (2020). Lifetime economic burden of Crohn's disease and ulcerative colitis by age at diagnosis. Clinical Gastroenterology and Hepatology, 18(4), 889–897.

Liu, Y.-W., Su, Y.-W., Ong, W.-K., Cheng, T.-H., & Tsai, Y.-C. (2011). Oral administration of Lactobacillus plantarum K68 ameliorates DSS-induced ulcerative colitis in BALB/c mice via the anti-inflammatory and immunomodulatory activities. International Immunopharmacology, 11(12), 2159–2166.

Lu, J., Zhou, J., Wang, L., Zhong, C., Chen, X., & Jia, B. (2020). Efficacy and safety evaluation of acupoint embedding for patients with ulcerative colitis: a protocol of systematic review and meta-analysis. Medicine, 99(34).

Makiyah, A., & Tresnayanti, S. (2017). Uji toksisitas akut yang diukur dengan penentuan ld50 ekstrak etanol umbi iles-iles (Amorphophallus variabilis Bl.) pada tikus putih strain wistar. Majalah Kedokteran Bandung, 49(3), 145–155.

Malewska, K., Rychlik, A., Nieradka, R., & Kander, M. (2011). Treatment of inflammatory bowel disease (IBD) in dogs and cats. Polish Journal of Veterinary Sciences.

Martín, R., Chain, F., Miquel, S., Motta, J.-P., Vergnolle, N., Sokol, H., & Langella, P. (2017). Using murine colitis models to analyze probiotics–host interactions. FEMS Microbiology Reviews, 41(Supp_1), S49–S70.

Melgarejo, P., Núñez-Gómez, D., Legua, P., Martínez-Nicolás, J. J., & Almansa, M. S. (2020). Pomegranate (Punica granatum L.) a dry pericarp fruit with fleshy seeds. Trends in Food Science & Technology, 102, 232–236.

Moghaddam, G., Sharifzadeh, M., Hassanzadeh, G., Khanavi, M., Dolatshahi, F., Sadeghi, N., Oveisi, M. R., & Hajimahmoodi, M. (2014). Anti-ulcerative potential of Punica granatum L (Lythraceae) hydroalcohol fruit peel extract. Tropical Journal of Pharmaceutical Research, 13(7), 1093–1097.

Morris, G. P., Beck, P. L., Herridge, M. S., Depew, W. T., Szewczuk, M. R., & Wallace, J. L. (1989). Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology, 96(2), 795–803.

Morton, J. F. (1987). Fruits of warm climates. Creative Resource Systems. Inc. Winterville, USA, 505.

Mousavinejad, G., Emam-Djomeh, Z., Rezaei, K., & Khodaparast, M. H. H. (2009). Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. Food Chemistry, 115(4), 1274–1278.

Nakase, H., Uchino, M., Shinzaki, S., Matsuura, M., Matsuoka, K., Kobayashi, T., Saruta, M., Hirai, F., Hata, K., & Hiraoka, S. (2021). Evidence-based clinical practice guidelines for inflammatory bowel disease 2020. Journal of Gastroenterology, 56(6), 489–526.

Nayak, S. B., Rodrigues, V., Maharaj, S., & Bhogadi, V. S. (2013). Wound healing activity of the fruit skin of Punica granatum. Journal of Medicinal Food, 16(9), 857–861.

Nishida, A., Nishino, K., Sakai, K.,

Owaki, Y., Noda, Y., & Imaeda, H. (2021). Can control of gut microbiota be a future therapeutic option for inflammatory bowel disease? World Journal of Gastroenterology, 27(23), 3317.

Park, A., Kim, S., Jung, I. H., & Byun, J. H. (2020). An immune therapy model for effective treatment on inflammatory bowel disease. Plos One, 15(9), e0238918.

Pereira, P. H. F., Oliveira, T. Í. S., Rosa, M. F., Cavalcante, F. L., Moates, G. K., Wellner, N., Waldron, K. W., & Azeredo, H. M. C. (2016). Pectin extraction from pomegranate peels with citric acid. International Journal of Biological Macromolecules, 88, 373–379.

Pérez-Vicente, A., Gil-Izquierdo, A., & García-Viguera, C. (2002). In vitro gastrointestinal digestion study of pomegranate juice phenolic compounds, anthocyanins, and vitamin C. Journal of Agricultural and Food Chemistry, 50(8), 2308–2312.

Rana, T. S., Narzary, D., & Ranade, S. A. (2010). Systematics and taxonomic disposition of the genus Punica L. Pomegranate. Fruit Veg. Cereal Sci. Biotechnol, 4(2), 19–25.

Roda, G., Chien Ng, S., Kotze, P. G., Argollo, M., Panaccione, R., Spinelli, A., Kaser, A., Peyrin-Biroulet, L., & Danese, S. (2020). Crohn's disease. Nature Reviews Disease Primers, 6(1), 22.

Rozenberg, O., Howell, A., & Aviram, M. (2006). Pomegranate juice sugar fraction reduces macrophage oxidative state, whereas white grape juice sugar fraction increases it. Atherosclerosis, 188(1), 68–76.

Rubin, D. T., Ananthakrishnan, A. N., Siegel, C. A., Sauer, B. G., & Long, M. D. (2019). ACG clinical guideline: ulcerative colitis in adults. Official Journal of the American College of Gastroenterology| ACG, 114(3), 384–413.

Salim, F. D., Ibrahim, K. M., & Yousif, W. H. (2022). The effectiveness of extract the seed of pomegranate in healing the wound induced inrabbits skin. IRAQI JOURNAL OF AGRICULTURAL SCIENCES, 53(2), 265–271.

Salimi, F., Shafaghat, A., Sahebalzamani, H., & Alizadeh, M. M. (2011). α-Pinene from Pistacia atlantica Desf. Subsp. Kurdica (Zohary) Rech. F. Der Chemica Sinica, 2(3), 1–3.

Sartor, R. B. (2006). Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nature Clinical Practice Gastroenterology & Hepatology, 3(7), 390–407.

Setiawati, R. M. (2014). Pengaruh variasi komposisi tanaman delima (Punica granatum linn) terhadap sifat fisis membran komposit untuk menangkap radikal bebas asap rokok. Universitas Islam Negeri Maulana Malik Ibrahim.

Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2018). Phenolic compounds as beneficial phytochemicals in pomegranate (Punica granatum L.) peel: A review. Food Chemistry, 261, 75–86.

Smith, A. D., George, N. S., Cheung, L., Bhagavathy, G. V, Luthria, D. L., John, K. M., & Bhagwat, A. A. (2020). Pomegranate peel extract reduced colonic damage and bacterial translocation in a mouse model of infectious colitis induced by Citrobacter rodentium. Nutrition Research, 73, 27–37.

Smith, J. W., & Castro, G. A. (1978). Relation of peroxidase activity in gut mucosa to inflammation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 234(1), R72–R79.

Sorrenti, V., Randazzo, C. L., Caggia, C., Ballistreri, G., Romeo, F. V., Fabroni, S., Timpanaro, N., Raffaele, M., &

Vanella, L. (2019). Beneficial effects of pomegranate peel extract and probiotics on pre-adipocyte differentiation. Frontiers in Microbiology, 10, 660.

Taku, K., Britta, S., Chen, W. S., Ferrante, M., Shen, B., Bernstein, C. N., Silvio, D., Laurent, P.-B., & Toshifumi, H. (2020). Ulcerative colitis (primer). Nature Reviews: Disease Primers, 6(1).

Tran, C. D., Ball, J. M., Sundar, S., Coyle, P., & Howarth, G. S. (2007). The role of zinc and metallothionein in the dextran sulfate sodium-induced colitis mouse model. Digestive Diseases and Sciences, 52, 2113–2121.

Triantafyllidi, A., Xanthos, T., Papalois, A., & Triantafillidis, J. K. (2015). Herbal and plant therapy in patients with inflammatory bowel disease. Annals of Gastroenterology: Quarterly Publication of the Hellenic Society of Gastroenterology, 28(2), 210.

Vanani, A. R., Heidari, A., Kalantari, H., Mansouri, E., & Mahdavinia, M. (2020). Hepatoprotective Effects of Black Pomegranate (Punica granatum L.) Peel Extract on Tert-Butyl Hydroperoxide Induced Oxidative Stress in Rats. Jundishapur Journal of Natural Pharmaceutical Products, 15(4).

Vučić, V., Grabež, M., Trchounian, A., & Arsić, A. (2019). Composition and potential health benefits of pomegranate: a review. Current Pharmaceutical Design, 25(16), 1817–1827.

Wirtz, S., Neufert, C., Weigmann, B., & Neurath, M. F. (2007). Chemically induced mouse models of intestinal inflammation. Nature Protocols, 2(3), 541–546.

Zorrilla, P., Rodriguez-Nogales, A., Algieri, F., Garrido-Mesa, N., Olivares, M., Rondón, D., Zarzuelo, A., Utrilla, M. P., Galvez, J., & Rodriguez-Cabezas, M. E. (2014). Intestinal anti-inflammatory activity of the polyphenolic-enriched extract Amanda® in the trinitrobenzenesulphonic acid model of rat colitis. Journal of Functional Foods, 11, 449–459.