Araştırma Makalesi (Research Article)

Intestinal Anti-Inflammatory and Anti-Oxidant Activity of The Aqueous Extract From Arum Dioscoridis in Acetic Acid Induced Colitis in Rats

Ülseratif Kolit Oluşturulan Hayvan Modelinde Arum Dioscoridis Bitki Ekstraktı Uygulamasının Antioksidan ve Anti-inflamatuvar Etkisi

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Özet

Amaç: Arum dioscoridis ülkemizde yetişen oksidasyon, immün sistem, inflamasyon üzerine birçok biyolojik aktiviteyi düzenlediği gösterilmiş bir bitkidir. Bizde çalışmamızda farelerde asetik asit ile oluşturulan deneysel kolitte arum dioscoridisin etkilerini araştırmayı amaçladık.

Gereç ve Yöntemler: Ağırlıkları 180-210 gr arasında değişen 6 haftalık 56 adet wistar albino cinsi dişi sıçanlar her grupta 8 hayvan olacak şekilde 7 eşit gruba ayrıldı.Grup 1; kontrol grubu. Grup 2 deneysel kolit oluşturulan grup. Grup 3 profilaktik oral arum dioscoridis tedavisi verilerek deneysel kolit oluşturulan grup. Grup 4 deneysel kolit oluşturulan ve etanollü çözücüyle oral arum dioscoridis tedavisi verilen grup. Grup 5 deneysel kolit oluşturulan ve DMSO (Dimetil sülfoksit)'lu çözücüyle oral tedavi verilen grup. Grup 6 deneysel kolit oluşturulan ve DMSO'lu çözücüyle rektal tedavi verilen grup. Grup 7 deneysel kolit oluşturulan ve 2 kat oral tedavi verilen grup. Farelerden alınan doku örneklerinde arum dioscoridisin etkileri makroskopik, histopatolojik ve biyokimyasal olarak değerlendirildi.

Bulgular: Kolit grubunda tedavi gruplarına göre önemli kilo kaybı meydana geldi ($p<0.001$). Arum dioscoridis 2.5 ml oral etanolik solvent tedavisi alan grupta ve 2.5 ml oral profilaksi alan grupta makroskopik ve mikroskobik analizde istatistiksel olarak anlamlı iyileşme bulundu ($p<0.001$). Asetik asit ile oluşturulan kolit modelinde toplam oksidan durum, tiyol/disülfid, malondialdehit, miyeloperoksidaz seviyelerinde artış ve toplam antioksidan kapasitede azalma tespit edildi.

Sonuç: Bu çalışma, arum dioscoridis'in ratlarda oluşturulan kolit modelinde olumlu etkileri olduğunu ve özellikle etanol ile eritilerek verilen ve profilaksi amaçlı kullanılan gruplarda barsak iltihabını önemli ölçüde iyileştirdiğini göstermiştir.

Anahtar kelimeler: Arum dioscoridis, Deneysel kolit, Malondialdehit, Oksidatif stres, Total oksidan durum,

Abstract

Objective: Arum dioscoridis is a plant that has been shown to regulate biological activity on oxidation, immune system and inflammation grown in our country. We aimed to investigate the effects of arum dioscoridis in experimental colitis related to acetic acid in rats.

Material and Methods: In this study 56 Wistar Albino female rats, 6 weeks old, weighing 180- 210 g, were seperated into 7 equal groups with 8 animals in each group. Group 1 is the control one without any administration. An experimental colitis has been created on Group 2. Group 3 is treated with prophylactic oral arum dioscoridis before creating experimental colitis. The rats belongs to Group 4 has been received oral arum dioscoridis treatment with ethanolic solvent after creating experimental colitis. Group 5 was given oral treatment with solvent with DMSO (Dimethyl sulfoxide) after experimental colitis was created. Group 6 rectal treatment with solvent with DMSO after experimental colitis has been created. Group 7 was given a 2-fold oral treatment after experimental colitis. In tissue samples taken from mice, the effects of arum dioscoridis were evaluated macroscopically, histopathologically and biochemically.

Results: In the colitis group, significant weight loss occurred compared to the treatment groups ($p<0.001$). Statistically significant improvement was found in macroscopic and microscopic analysis with in the group receiving arum dioscoridis 2.5 ml oral ethanolic solvent treatment and in the group receiving 2.5 ml oral prophylaxis ($p<0.001$). In the colitis model which is created with acetic acid, total oxidant status, thiol/disulfide, malondialdehyde, myeloperoxidase levels increased and a decrease in total antioxidant capacity was detected.

Conclusion: This study showed that arum dioscoridis has positive effects on the colitis model created in rats and it significantly improves intestinal inflammation especially in groups given by dissolving with ethanol and used for prophylaxis purposes.

Key words: Arum dioscoridis, Experimental colitis, Malondialdehyde, Oxidative stress, Total oxidant status

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Geliş Tarihi: 07.01.2022

Kabul Tarihi: 17.01.2022

DOI: 10.17517/ksutfd.1054545

INTRODUCTION

Ulcerative colitis (UC) is a chronic, recurrent inflammatory bowel disease (IBD). The incidence of UC varies by countries. The highest incidences of inflammatory bowel disease are seen in Europe, England and North America. Its prevalence is 286-505 per 100000 people (1). Disease incidence increases in regions where UC is quite rare. For example, in the last 2 decades, the incidence of UC has increased six-fold in Hong Kong. UC peaks between the ages of 15-30 and 60-80 years (2). Mortality is highest in the first year of the disease. The risk of colorectal cancer for any patient with UC is known to be elevated. UC disrupts the quality of life and work performance dramatically. Therefore, UC should be effectively treated and kept in remission.

In the treatment of UC, 5-aminosalicylates, corticosteroids, immunomodulators and biological drugs are used. With these treatments, patients may experience nausea, vomiting, weakness, headache, hepatitis, and infertility problems. According to some recent studies, induction therapy fails in approximately 20-30% of patients (3). Therefore, alternative therapies are being investigated in the treatment of UC.

The result of sustained excessive or false antigenic stimulation by enteric bacteria which leads to pathogenesis of UC. Polymorphonuclear neutrophil infiltration is associated with the primary lesion of colitis and followed by loss of the epithelium, goblet cells and crypt damage. It was shown in recent studies that increased interleukin-1ß, IL-6, tumor necrosis factor-α (TNF-α), IL-12 and interferon gamma (IFNγ) due to excessive oxidative stress in IBD are correlated with severity of mucosal inflammation. Nuclear Factor kappa B is important in the release of these cytokines and activation of UC. Biochemically and histopathologically, it has been shown that oxidative stress increases and antioxidant activity decreases in colitis models (4). Based on these findings, recently in experimental colitis models; the beneficial effects of antioxidant substances such as Arthrocen, alstonia boonei, phloretin have been shown (5-7). Arum dioscoridis; it is a wild plant that grows in the Eastern Mediterranean, Southwestern Anatolia and Cyprus. Arum dioscoridis is an antioxidant and anti-inflammatory agent. Arum dioscoridis has been shown to regulate immunity, inflammation and neoplastic transformation (8). This study aimed to investigate the effects of arum dioscoridis in acetic acid-induced experimental colitis in rats.

MATERIALS AND METHODS

Animals

Female wistar albino rats (Sutcu Imam University School of Medicine Experimental Research Laboratory, Kahramanmaras, Turkey) weighing between 180-210 g, were used. The rats were housed at least a week to adapt to the new environment with a temperature of 22-25° C and a relative humidity of under a 12 h light/dark cycle **(Figure 1A).** This study was conducted at the Kahramanmaras Sutcu Imam University Experimental and Clinical Research Center following the approval by the Ethics Committee for Experimental Animals of Kahramanmaras Sutcu Imam University (date:2018/12–decision number: 01). Additionally, this study has been funded by the Scientific Research Projects Unit of Kahramanmaras Sutcu Imam University. This study was conducted in accordance with the ethical standards of the Declaration of Helsinki.

Figure 1A. Rats and cages

Induction of colitis and evaluation

Before the induction of colitis, animals were anesthetized with ketamine. Colitis was induced by intrarectal administration of 1 ml of 4% acetic acid aqueous solution through a 6F pediatric catheter (9), which end was inserted into the bowel 6 cm deep from the anal canal and rats were maintained in a trendelenburg position for 45 seconds to limit the expulsion of the solution **(Figure 1B).**

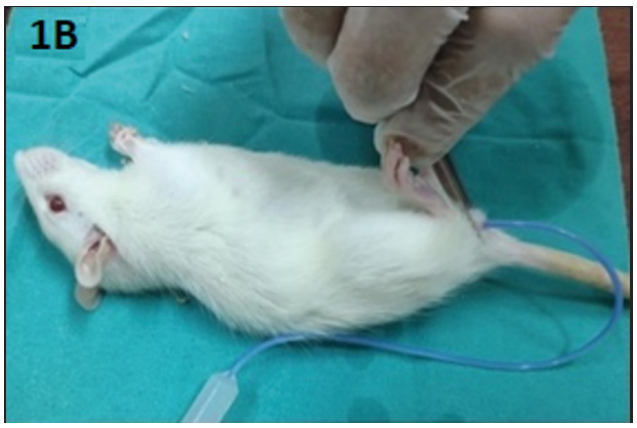

Figure 1B. Colitis induced by intrarectal administration

Rats were randomly divided into seven groups, each group consisting of 8 rats. Rats in the group I (control group) received only distilled water. The rats in group II (colitis group) received 1 ml of 4% acetic acid by intrarectal and then received only distilled water. Following 4 days of acetic acid administration, rats in groups IV (2.5 ml arum dioscoridis extract with ethanol), V (2.5 ml arum dioscoridis extract

with dimethyl sulfoxide (DMSO)), VII (5 ml arum dioscoridis extract with DMSO) received arum dioscoridis orally. At the same time, rats in the group VI were treated with arum dioscoridis rectally (2.5 ml arum dioscoridis extract with DMSO). Rats in group III received 1 day pretreatment orally (2.5 ml arum dioscoridis extract with DMSO) and colitis was induced by intrarectal administration on the 2nd day **(Figure 1C).**

Figure 1C. Administration of oral therapy and prophylaxis

Preparation of arum dioscoridis extracts

Arum dioscoridis plant used in this study; It is a wild plant that grows spontaneously in the gardens and mountains in and around Kahramanmaras. Its leaves and stems are consumed by the local people as a soup. Plants collected from the countryside of Goksun were confirmed as species at the faculty of agricultural and brought to the laboratory will be left to dry at room temperature. Drying samples were pulverized with a mechanical blender in accordance with aseptic conditions and 5 g cartridges were prepared for extraction in Soxhlet device. The prepared cartridges will be extracted with 100 ml of solvent for 12 hours. In the extraction, 4 groups of DMSO and 1 group of ethanol will be used as solvent. The extracts obtained will be evaporated in rotary evaporator until they remain 1 ml. The extracts prepared in this way will be stored at +4°C until the time of use.

Assessment of the colonic damage:

Rats were sacrificed under general anesthesia with ketamine by cervical decapitation after 4 days of follow-up. Sterilization was not performed in operations. A 2-3 cm incision line was made and the abdomen was opened. The distal 6 cm colon was excised from the rectum to proximal. Resected colon materials opened by longitudinal incision. One of both parts was put into 10% formaldehyde for histopathological examination. Other parts were placed in 2 ml eppendorf tubes for biochemical evaluation and stored at +4°C. The colon materials were scored from 0 to 4 according to the scale **(Table 1)** organized by Morris et al (10).

Table 1. Criteria for scoring of gross morphologic damage

Score	Gross morphology
0	No damage
1	Localized hyperemia, but no ulcers.
2	Linear ulcers with no significant inflammation.
3	Linear ulcer with inflammation at one site.
4	Two or more sites of ulceration and/or inflammation.

Histopathological examination

Distal colon samples were collected and fixed distal colon samples with neutral buffered formalin embedded in paraffin and stained with hematoxylin-eosin (HE) for histopathology examination. The severity of epithelial inflammatory cell infiltration and intestinal architecture were evaluated. Microscopic changes were scored between 0-3 values **(Table 2)** (11).

Biochemical examination

Tissues taken from rats and stored at -80°C are homogenized after dissolution. It was planned to determine the levels of total oxidant status (TOS), total antioxidant status (TAS), thiol/disulfide, malondialdehyde (MDA), myeloperoxidase (MPO), glutathione peroxidase (GPx). MDA level were analyzed using the manual beugea method (12). MPO activity

Table 2. Scoring scheme for chemically-induced colonic inflammation

Inflammatory cell infiltrate		***Score***	**Intestinal architecture**	***Score***
Severity	Extent		Epithelial changes - Mucosal architecture	
Mild	Mucosa	1	Focal erosions	1
Moderate	Mucosa and submucosa	2	Erosions	2
Marked	Transmural	3	Extended ulcerations±granulation tissue±pseudopolyps	3
Sum of scores 1 and 2: 0-6				

was measured by a modification of the method described by Bradley (13). Protein determination was done by the Lowry method (14). TAS, TOS and thiol/disulfide analysis were using commercial kits. The brand of the commercial kits were Rel Assay diagnostics kits.

Statistical analysis

Data were analyzed using SPSS (Version 20.0). Descriptive statistics were presented as mean±standard deviation and median (min-max) values. The distribution of the groups was evaluated using the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) was used to compare the averages of the groups found to be suitable for normal distribution, and the Tukey test was used to determine the differences between the groups. Kruskal-Wallis test was used for non-parametric and non-normal distribution data and Mann-Whitney test for multiple comparisons. P<0.05 value was considered statistically significant in the tests.

RESULTS

In our study, inflammation was classified pathologically according to macroscopic and microscopic features **(Table 3)**.

Table 3. Macroscopic and Histological Scoring under arum dioscoridis treatment and prophlaxis

Groups/Score	Mean Macroscopic Score	Mean Histological Score
Control	1.00±0.00	0.50±0.53
Colitis	3.50±0.53	5.37±0.74
Oral 2.5 ml prophylaxis **	1.25±0.46*	1.50±1.41*
Oral 2.5 ml treatment ***	1.75±0.88*	1.50±0.92*
Oral 2.5 ml treatment **	2.37±1.30	3.87±1.64
Rectal 2.5 ml treatment **	3.00±0.75	4.00±1.30
Oral 5 ml treatment **	2.75±0.88	4.25±1.58

* p<0.001 (statistically significant compared to the control group), ** DMSO + AD(Arum dioscoridis), ***Etanol +AD

In both macroscopic and microscopic evaluation (**Figure 2**), inflammation was found to be significantly decreased in the group given oral arum dioscoridis prophylaxis and the group treated with ethanol as the solvent (p<0.001). In macroscopic evaluation of inflammation, the best response was observed in the group receiving 2.5 ml oral prophylaxis (p<0.001), while the best response in microscopic evaluation was observed in the group receiving 2.5 ml oral ethanolic solvent (p<0.001).

In all treatment and prophylaxis groups, arum dioscoridis treatment decreased MPO, MDA, tissue TOS and tissue oxidative stress index levels compared to colitis group but the difference in this decrease was not statistically significant (p>0.05). Glutathione levels increased in all treatment and prophylaxis groups compared to colitis group, but the difference in this decrease was not statistically significant (p>0.05) **(Figure 3)**.

In prophylaxis and ethanol treatment groups, arum dioscoridis treatment increased tissue oxidized, natural and total thiol, tissue disulfide and TAS compared to colitis group, but the difference in this increase was not statistically significant (p>0.05) **(Figure 4)**.

Especially in prophylaxis and ethanol treatment groups, it was observed that the results of MDA and tissue oxidative stress index decreased statistically with arum dioscoridis treatment.

It was observed that the weight level in the colitis group was statistically significantly decreased compared to the control group (p<0.001). When the prophylaxis and ethanolic solvent treatment groups were compared with the colitis group (**Figure 5**), the weight levels of arum dioscoridis increased and the difference in this increase was found to be statistically significant (p<0.05). In the other treatment groups, an increase was observed in weight levels compared to the colitis group. But the difference in this increase was not statistically significant (p>0.05).

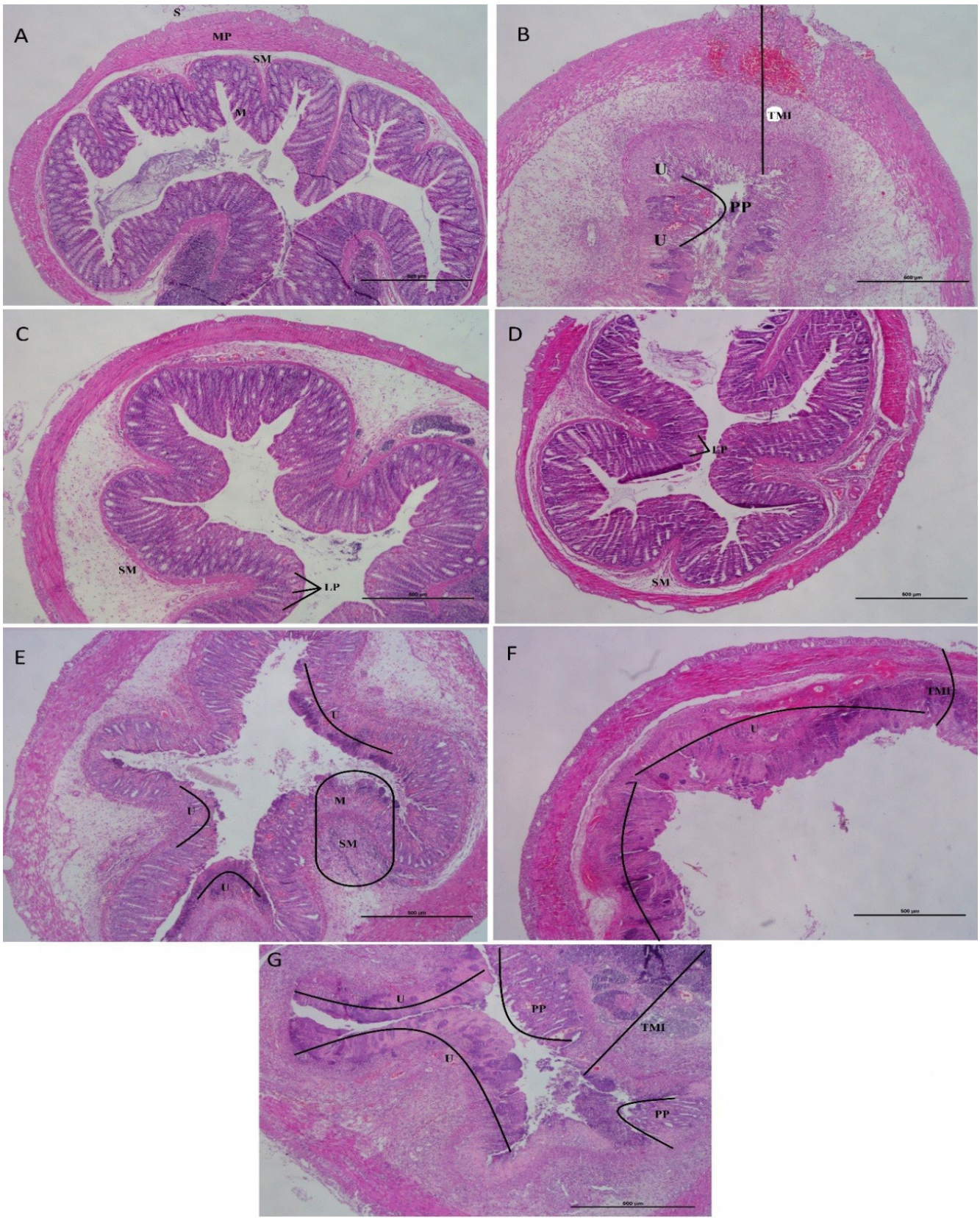

Figure 2. A-G. Microscopic view of the colon (H&E). A: Control group. Full-thickness colon in normal histology. B: Colitis group. In addition to inflammation starting from mucosa to serosa (vertical line), there is mucosal ulceration and pseudopolyp appearance (curve). C: Prophylaxis group. Mild inflammation and submucosal edema in lamina propia. D: Ethanol solvent treatment group. Mild inflammation in lamina propia, with complete tissue integrity. E: Oral 2.5 ml treatment group with solvent with DMSO. Intense inflammatory cell accumulation in the mucosa and submucosa, patched mucosal ulcerations (curves) with regional intact areas. F: Oral 5 ml treatment group with solvent with DMSO. Widespread mucosal ulceration (horizontal curves) with transmural intense inflammatory cell accumulation. G: Rectal treatment group. Transmural severe inflammatory cell accumulation (vertical line) and mucosal ulceration (horizontal curves) and pseudopolyp formations.

Abbreviations: M: Mucosa, SM: Submucosa, MP: Muscularis Propria, S: Serosa, LP: Lamina Propria, TMI: Transmural Inflammation, U: Ulceration, PP: Pseudopolyp. Hematoxylin and eosin stain, 4x objective, Scale bar: 500 μm (micrometer)

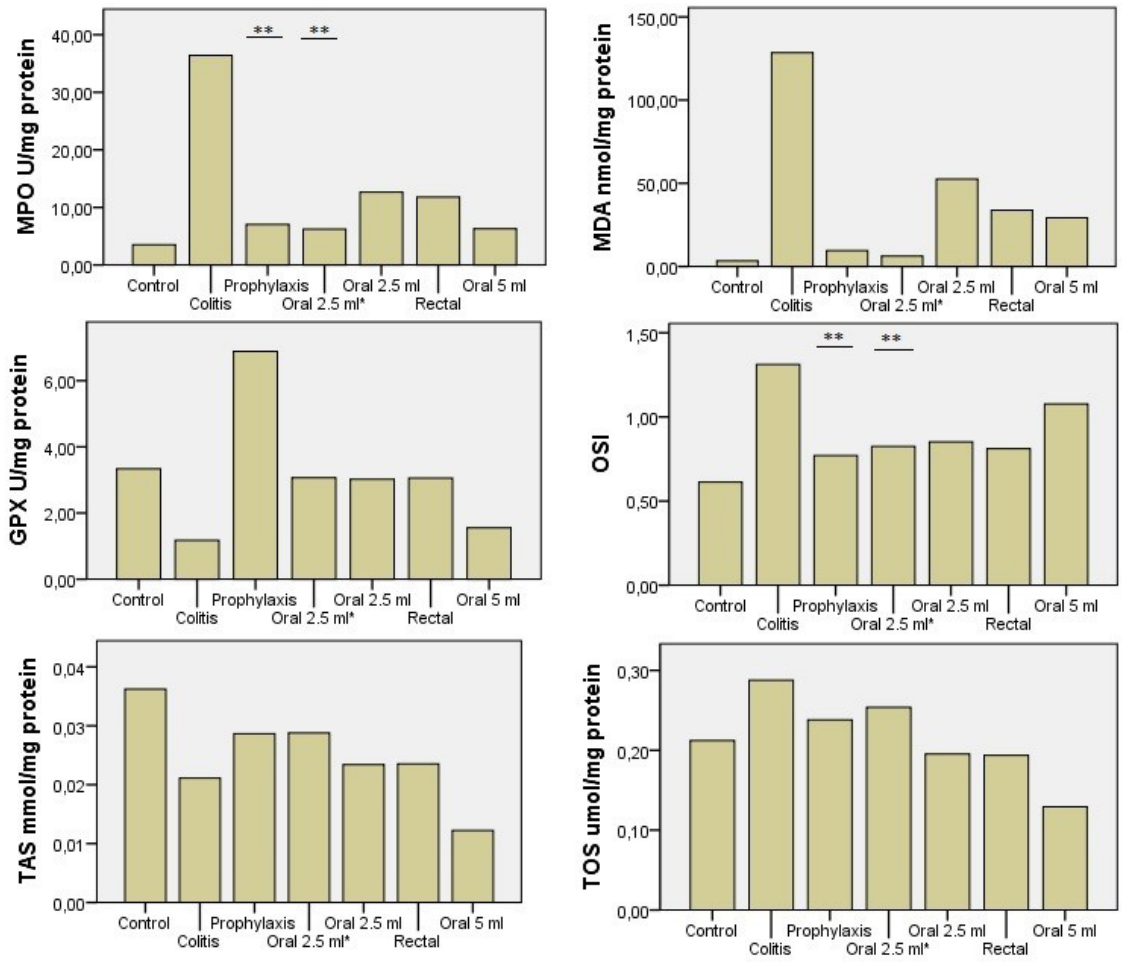

Figure 3. Levels of myeloperoxidase, malonic dialdehyde, glutathione peroxidase, oxidative stress index, total antioxidant status, total oxidant status in gut for rats experienced acute colitis and treated with arum dioscoridis. Data are presented as the means ±SEM (n:8 per group).The results were analyzed using one-way ANOVA. **$p < 0.05$ compared with the colit group. *extract with etanol

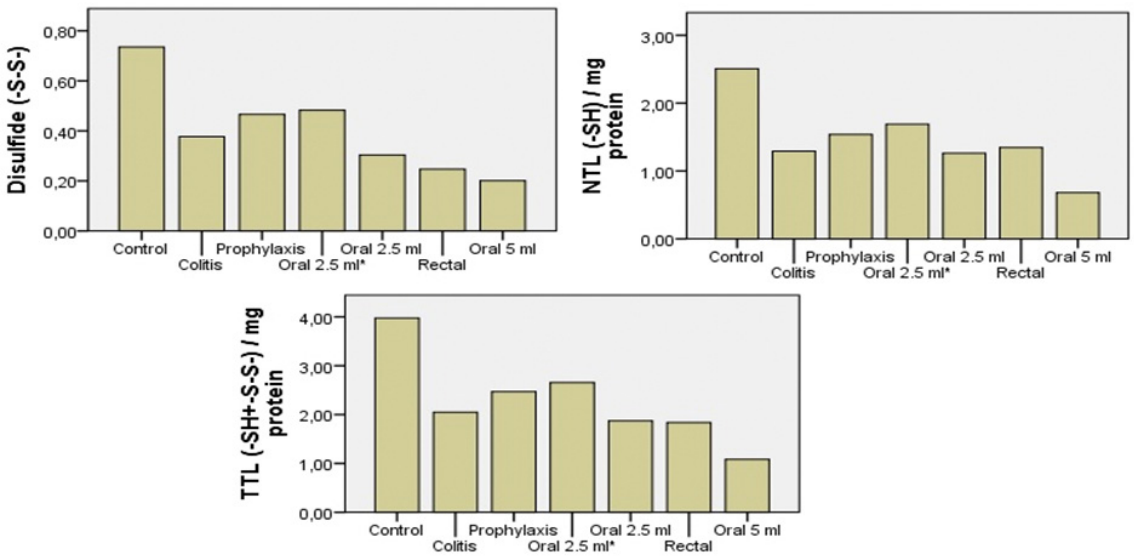

Figure 4. Levels of disulfide, natural thiol and total thiol in gut for rats experienced acute colitis and treated with arum dioscoridis. *extract with etanol

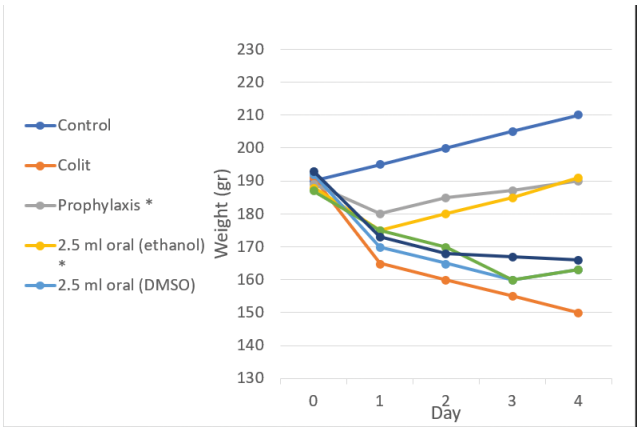

Figure 5. Daily course of weight change in rats with ulcerative colitis under arum dioscoridis treatment and prophlaxis *p < 0.05 compared with the colit group.

DISCUSSION

The main features of UC are defined as ulcers in the colon, loss of epithelium, loss of goblet cells and inflammatory cell infiltration of the colon mucosa. However, there is no any specific treatment for UC, our study has led to the new researches for other therapeutic targets on the treatment of UC (15).

Ischemia is induced in the colonic mucosa and mucosal permeability increases with the damage of the colonic mucosa from chemicals such as acetic acid. This mechanism triggers the system and causes the release of the proinflammatory cytokines. The inflammatory process in the intestinal mucosa is characterized by an increase in proinflammatory cytokines such as IL-1β, IL-6 and TNF-α associated with neutrophil and macrophage infiltration. Proinflammatory cytokines are important for host defense, but overproduction leads to uncontrolled inflammation and damages colon tissue. These proinflammatory cytokines have an important role in pathological angiogenesis formation. The newly formed blood vessels feed the inflamed tissue with oxygen and nutrients. Thus, pathological angiogenesis causes chronic intestinal inflammation to persist (16). Fabia et al. investigated the experimental colitis model that most histopathologically resembles human UC. He stated that creating toxicity with 4% acetic acid in rats is the most similar method to UC in humans, and it can be used to create experimental UC (17). Yamada et al. compared the model of trinitrobenzene sulfonic acid (TNBS) and acetic acid-induced colitis and showed that even the colitis model induced by TNBS preparations from the same vendor had varying degrees of damage and inflammation (18). For this reason, in our study, the colitis model induced by rectally administered acetic acid was used for colitis induction. The occurrence of IBD has been proven by macroscopic determination of hyperemia, linear ulcers in the colon mucosa and histopathologically loss of mucosal structure, cellular infiltration, decrease in goblet cells and crypt abscess. In particular, a significant increase in the density of inflammatory cells such as eosinophils and neutrophils has been identified. Similar to the study by Rezayat et al. macroscopic grading score was found to be the highest in the colitis group with the induction of UC with acetic acid (19). According to a recent study, a decrease in the tissue damage in the colon is expected in the antioxidant treatment groups compared to the colitis group (20). In our study, tissue damage in the colon was found to be reduced macroscopically in all treatment groups. However, the difference in this decrease was not statistically significant. Additionally, significant weight loss was found due to the inflammation and diarrhea that developed in the colitis group. These results are similar to the study by Farooq et al. on weight loss observed in patients with UC (21). In the colitis model created with acetic acid in rats, a statistically significant weight loss was observed compared to the control group. It was observed that weight loss was less in all treatment groups compared to the colitis group. It was observed that weight loss was statistically significantly less in the treatment groups given prophylaxis and using ethanolic solvent. This was thought to be caused by increased inflammation and suppression of appetite due to cytokines, diarrhea, and bleeding (22). Weight loss in the groups correlated with microscopically and biochemically confirmed inflammation (23).

Our histopathological studies were performed in accordance with the histomorphological evaluation of Erben et al. on rats with experimental colitis (11). Our histopathological studies correlated with our macroscopic findings. Inflammatory changes such as edema, bleeding, crypt damage, epithelial cell loss, inflammation degree, inflammation severity and necrosis were shown to decrease significantly after treatment. Similar histopathological results were obtained with the study conducted by Johnson et al. demonstrating that phyllanthus nivosus leaf reduces inflammation in rats induced with UC (24). Arum dioscoridis administration significantly reduced leukocyte infiltration in the inflamed colon. Although oral 2.5 mg, oral 5 mg and rectal 2.5 mg treatments using DMSO as solvent were not statistically effective, 2.5 mg prophylaxis dose dissolved with DMSO and 2.5 mg treatment dose using ethanol as solvent showed maximum anti-inflammatory and anti-colitis effects. While arum dioscoridis extract dissolved with DMSO was found to be effective in prophylactic use despite giving one-day prophylaxis, it was statistically insignificant in oral and rectal treatments.

A recent study has shown that arum dioscoridis inhibits the production of inflammatory cytokines in various in vitro and in vivo models (25-28). Excessively secreted inflammatory cytokines play a key role in the pathogenesis of UC (16). Therefore, we evaluated proinflammatory species levels (TOS, oxidized thiol, MDA, and MPO) in colon tissue in the acetic acid-induced colitis model (29,30). In addition, it has been shown that the treatment and prophylaxis of arum dioscoridis in the colon tissue reduces the level of inflammation. In the research of Ozturk et al. investigating the antimetastatic effect of arum dioscoridis, it has been shown that it has an

anti-migrative effect (28). This effect suggests that it may also reduce neutrophil migration and have a role in suppressing inflammation.

Oxidative stress plays an important role in the pathogenesis of UC. It has been reported that there is an increase in free oxygen radicals, oxidative stress and a decrease in antioxidant defense in patients with IBD (20). Oxidative damage with free oxygen radicals triggers the formation of lipid peroxide radicals (31). Thus, inhibition of lipid peroxidation or elimination of free oxygen radicals makes an important contribution to the therapeutic treatment of UC and prevention of disease. Enzymes such as GPx and substances such as thiol and disulfide are part of the natural cell defense system against damage, including oxidative stress (32). Experimental studies have shown an increase in GPx levels in cases recovering from oxidative stress (33). In our study, we found that tissue GPx levels in the colitis group were lower than the treatment groups. This result indicates that cellular defense is initiated against acetic acid induced colon damage.

MDA is the end product of lipid peroxidation showing cellular damage. Increased MDA is considered a sign of damaged tissue generated by free radicals in the inflammatory process (34). In this study, MDA levels increased in the colitis group. Administration of arum dioscoridis to colitis groups has been found to decrease tissue MDA level. Moreover, this decrease was statistically significant in the prophylaxis and ethanolic solvent treatment groups. These results support that arum dioscoridis reduces lipid peroxidation by preserving cell integrity in damaged colonic mucosa. In the study of Guvenc et al (35), investigating the protection of tyrosol in rats with UC, it was shown that MDA increased in the colitis group and these values decreased with treatment. Similar results were found in our study.

MPO shows neutrophil infiltration in tissues in experimental studies, is a direct indicator of inflammation, and gives a rapid response, especially in the acute phase (36). High MPO activity indicates excessive neutrophil infiltration and tissue damage. In our study, a decrease was achieved in tissue MPO levels in the treatment and prophylaxis groups, although it was not statistically significant.

Physiologically, natural thiol, disulfide and total thiol are in an equilibrium. This balance is affected by various conditions such as oxidation and reduction reactions. Reversible disulfide bonds are formed through oxidation reactions of thiol. The disulfide bonds formed are converted by reducing again to thiol groups (37). In our study, the reduction rates of natural thiol, disulfide and total thiol and oxidized thiol were measured. In the study by Neselioglu et al. these values were shown to decrease in active phases of UC and approach the healthy population again in remission periods (37). In our study, it was found to be increased in prophylaxis and oral 2.5 ml treatment groups. These values decreased in other groups.

Plasma concentrations of different oxidant and antioxidant species can be measured in laboratories separately, but the measurements are labor-intensive, time-consuming and costly. Since the measurement of different oxidant and antioxidant molecules separately is not practical and their oxidant and antioxidant effects are additive, the total antioxidant and oxidant capacity of a sample is measured, and these are called TAS and TOS. As a result of the study, it was observed that TOS values increased in the colitis group. A decrease in this value was observed in the treatment groups. This result is similar to the observations of Karimi et al. study of the effect of two different vitamin D regimens on oxidant and antioxidant status in UC (38). TAS values were decreased in colitis groups compared to the control group. TAS levels were higher in the prophylaxis and ethanolic solvent treatment groups than in the colitis group. TOS values were significantly higher in the colitis group compared to the control group. There was a statistically significant decrease in the prophylaxis and ethanolic solvent groups after treatment. These findings are consistent with other experimental colitis studies.

The exact mechanism of the anti-inflammatory and anti-colitis effects of arum dioscoridis cannot be determined simply, but some hypotheses can be developed. 15 types of antioxidant substances could be analyzed in arum dioscoridis extracts **(Table 4)** (29). As a result of these analyzes, arum dioscoridis with known flavonoid and phenolic content may have shown anti-colitis and anti-inflammatory effects. Since OH- groups of flavonoids and phenolic substances have higher polarity, they are more soluble in solvents with higher polarity (26,27). Ethanol has higher polarity than DMSO. Therefore, ethanol dissolved arum dioscoridis treatment may have been found to be statistically significant compared to other treatment applications. In addition, the antibacterial activity of the arum dioscoridis plant has been observed (27), so it can be suggested that there is a reduction in bacterial inflammation in the colon. Arum dioscoridis also has anti-cancer activity (28). Thus, it can be effective and protective against UC-related cancer.

This study is the first to examine the effects of arum dioscoridis in an animal model with colitis induced by acetic acid. It is known that the balance between inflammatory and anti-inflammatory cytokines is impaired in UC. Colon biopsies of rats with UC have been reported to have increased inflammatory cytokine levels and decreased anti-inflammatory cytokine levels. It has been observed that arum dioscoridis given with ethanolic solvent for treatment and arum dioscoridis given with solvent with DMSO for prophylaxis has significantly reduced colon inflammation. The findings were provided biochemically and histopathologically. Arum dioscoridis has anti-cancer activity, antimigrative, antibacterial and antiinflammatory effect (25-28). Therefore, arum dioscoridis may play protective role against ulcerative colon injury.

As a conclusion in our study, biochemical, microscopic and macroscopic improvement was achieved in the oral treatment (ethanolic solvent) and prophylaxis group. Our findings suggest the potential application of arum dioscoridis for effective, inexpensive and alternative treatment of UC. In or-

Table 4. Arum dioscoridis plant extracts HPLC analysis			
Content	**Methanolic extract**	**Acetone extract**	**Hexane extract**
Gallic acid	-	-	-
Catechin hydrate	-	-	-
Caffeic acid	-	-	-
Epicatechin	-	-	-
p-coumaric acid	5.4±0.8	5.6±0.4	-
Ferulic acid	325.5±2.5	255.9±1.9	-
Vitexin	1125.0±13.4	-	23.4±2.4
Rutin	-	-	-
Naringin	98.4±5.5	19.4±3.2	-
Hesperidin	-	-	-
Rosmarinic acid	-	-	-
Eriodictyol	43.7±3.1	25.0±4.2	5.2±0.7
Quercetin	-	-	-
Naringenin	-	-	-
Carvacrol	-	-	-
Σ Total	1598.0	305.9	34.2

HPLC: Yüksek performanslı sıvı kromatografi

der for arum dioscoridis to be used in UC patients, it should be supported by human-based studies and arum dioscoridis may create an alternative to current treatments for UC.

Acknowledgment: I thank and appreciate the Scientific Research Projects Unit of Kahramanmaras Sutcu Imam University for their financial support for this research project number (2019/4-19D).

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Alpproval: This study was conducted at the Kahramanmaras Sutcu Imam University Experimental and Clinical Research Center following the approval by the Ethics Committee for Experimental Animals of Kahramanmaras Sutcu Imam University (date:2018/12–decision number: 01).

Author contribution statement: Author Contributions: Concept – ABA, KG; Design -ABA, KG; Supervision –BK, MI; Resources –ABA,FIT, AYB; Materials – ABA,KG; Data Collection and/or Processing –ABA, AYB,FIT; Analysis and/or Interpretation – ABA, KG, MI; Literature Search –ABA, KG; Writing Manuscript –ABA, KG; Critical Review –BK,ABA,KG

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