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ORIGINAL ARTICLE

Cotinus coggygria Scop. Attenuates Acetic Acid-Induced Colitis in Rats by Regulation of Inflammatory Mediators

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Abstract

In traditional medicine, many medicinal plants are used in the treatment of various diseases caused by inflammation. The objective of the present study is to elucidate for the first time the effects of *Cotinus coggygria* (CC) ethanol extract (CCE) on colonic structure and inflammation of acetic acid-induced ulcerative colitis in rats. Colonic damage was assessed using disease activity index score, enzyme-linked immunosorbent assay, and hematoxylin–eosin staining. Also, in vitro antioxidant activity of CCE was investigated by ABTS methods. Total phytochemical content of CCE was measured spectroscopically. Acetic acid caused colonic damage according to disease activity index and macroscopic scoring. CCE significantly reversed these damages. While the levels of proinflammatory cytokines TNF-alpha, IL-1beta, IL-6, and TGF-1beta increased in tissue with UC, IL-10 level decreased. CCE increased inflammatory cytokine levels to values close to the sham group. At the same time, while markers indicating disease severity such as VEGF, COX-2, PGE2, and 8-OHdG indicated the disease in the colitis group, these values returned to normal with CCE. Histological research results support biochemical analysis. CCE exhibited significant antioxidant against ABTS radical. Also, CCE was found to have a high content of total polyphenolic compounds. These findings provide evidence that CCE might be benefit as a promising novel therapy in the treatment of UC in humans due to high polyphenol content and justify the use of CC in folkloric medicine for treatment of inflamed diseases.

Keywords Anti-inflammatory activity · Antioxidant activity · *Cotinus coggygria* ethanol extract · Ulcerative colitis · Total phytochemical content

Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disease of two types, ulcerative colitis (UC) and Crohn's disease characterized by an imbalance between inflammation and regulatory immune responses in the intestinal mucosa [1]. UC has a multifaceted pathology characterized by inflammation, mucosal tissue damage, and impaired immune

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response [2]. UC is usually manifested by symptoms such as recurrent and persistent abdominal pain and bloody or purulent diarrhea that adversely affect the patient's quality of life and require effective treatment options [3]. In the treatment of UC, anti-inflammatory drugs, immunosuppressants and immunomodulators or surgery is preferred for necessary conditions.

Inflammation plays an active role in the initiation, exacerbation, and maintenance of UC [4]. In tissue, proinflammatory cytokines, including TNF-α, IL-6, and IL-1β, perform various functions, including production of inflammatory mediators, platelet activating factor, leukotrienes, prostaglandin, and suppression of the apoptotic pathway. This situation reveals results that may mediate mucosal inflammation and injury, which may lead to organ damage [5]. In addition, oxidative damage, epithelial cell apoptosis, and disintegration of the epithelial barrier are closely associated with colonic tissue damage and immunological abnormality caused by UC [5].

Although pharmaceuticals such as steroids, immunomodulators, and antibodies used for the prevention and treatment of IBD today have somewhat improved the quality of life of patients with IBD, they have not been effective at all stages of the disease [6]. Long-term use of these drugs poses a problem due to serious side effects such as infection, fever, diarrhea, and high recurrence rates [7]. Therefore, there is a need for novel therapeutic strategies that can effectively cure mucosal inflammation with fewer side effects. Since ancient times, medicinal plants have been the remedy for the treatment of some diseases due to their accessibility and cost, as well as their effective pharmacological structures. Nowadays, many drugs that are currently used have been developed based on the traditional use of natural resources [8]. The anti-inflammatory and antioxidant effects of natural compounds indicate their potential as drugs in the treatment of chronic inflammatory diseases [9].

The genus *Cotinus*, a member of the Anacardiaceae family, is represented by a single species in Turkey [10]. This species is *Cotinus coggygria* (CC) and the leaves of this species are used directly by the people as fresh, or in the form of infusion or decoction. Leaves of CC have been used in the treatment of stomach pain, gastric, ulcer, cuts, burns, wounds, eczema, fractures, diabetes, urinary diseases, cardiac diseases, kidney stones, nephritis, cancer, cough, abdominal pain, arm numbness, asthma, hemorrhoids, enteritis, and anthrax in Turkey. It is also used as antifungal, antihypertensive, and vasodilator [11]. Total flavonoids, total tannins, and total phenols are the major group of biologically active constituents of CC leaves [12]. Previous studies on phytochemical analysis of CC indicated the presence flavonoids (fisetin, fustin, sulfuretin, myricetin, quercetin), anthocyanins (leucodelfinidine, leucocyanidin, delfinidin 3-galactoside, cyanidin 3-galactoside, petunidin 3-glucoside, delphinidin 7-glucoside, cyanidin 3-glucoside-7-rhamnoside), essential oil (limonene, (Z)-β-ocimene, (E)-β-ocimene), gallic tannin (gallic acid), methyl gallate, pentagalloylglucose, and biauron [13–19].

Studies indicate that the secondary metabolites of *Cotinus coggygria* mediate pharmacological effects such as antioxidant and anti-inflammatory [20]. In the present study, we hypothesize that the leaves of *C. coggygria* can be effective in the treatment of colitis thanks to the ethnomedical use reports and previously reported antioxidant and anti-inflammatory activities. Therefore, the current study was performed to elucidate, for the first time, the potential antioxidant and anti-inflammatory effects of *Cotinus coggygria* leaves, in comparison with sulfasalazine, against acetic acid-induced UC and to investigate the molecular mechanisms underlying the protective action of *Cotinus coggygria* leaves.

Material and Methods

Plant Material

Cotinus coggygria leaves were collected in the flowering periods from the Kirklareli province of Turkey and identified by Dr. Sukran Kultur, a botanist of the Faculty of Pharmacy, University of Istanbul. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE No: 22260).

Extraction

Cotinus coggygria leaves were dried in the shade and powdered by a mechanical grinder. A total of 100 g of powdered material was extracted with ethanol (96%) using a Soxhlet device, and extraction was continued until the solution became colorless (up to 24 h). The obtained ethanol extract was dried under a vacuum at 40 °C. The powder ethanol extract obtained from the extraction of 100 g of powdered leaves was 38.94 g. This powder extract was used for activity tests and stored in the dark and refrigerated (4 °C) until use.

In Vitro Antioxidant Activity of *Cotinus coggygria* Extract

ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity of the extract was measured according to Zou et al. [21].

Determination of the Total Phenolic, Flavonoid, and Tannin Content of *Cotinus coggygria* Extract

The total phenolic content of the extract was measured as described by Gao et al. with slight modifications described by Yıldırım et al. [22, 23]. Total flavonoid content was determined following a method by Zhang et al. with slight modifications described by Yıldırım et al. [23, 24]. Total tannin content was tested using insoluble polyvinylpolypyrrolidone (PVPP) according to Singh et al. [25].

Animals

All animal experiments were conducted strictly according to the Marmara University Animal Care and Use Committee under protocol number 093.2016.mar. Adult male/female Sprague–Dawley (SD) rats (220–240 g) were obtained from Marmara University Animal Center (DEHAMER) and maintained at 22 ± 2 °C, with 65–70% humidity and a 12-h light/dark cycle. The animals were fed a standard rat chow and drinking tap water available ad libitum. All conditions were checked before the beginning of the study and any adverse conditions that could affect the results were removed and a week was given for the rats to adapt to the environment.

Induction of Ulcerative Colitis

According to the procedure [26] we preferred in our previous studies, the ulcerative colitis (UC) model was formed in animals. Briefly, after fasting overnight, rats were ketamine/xylazine anesthetized. One milliliter of 5% acetic acid (AA) (diluted in saline pH: 2.3) was slowly instilled into the column with the help of an 8-cm medical-grade polyurethane catheter. Rats were held in an upright position for 30 s to ensure AA distribution throughout the colon. The same amount of saline solution was administered to the sham group animals intrarectally.

Grouping and Treatment

Afterward induction of colitis, rats arbitrarily were allocated into the following groups with 8 rats in each group:

- Group 1 (sham group): received 2 mL/kg distilled water orally for 7 days without UC induction
- Group 2 (colitis group): received 2 mL/kg distilled water orally for 7 days induction of UC
- Group 3 (CC50): received a dose of 50 mg/kg CC orally for 7 days following induction of UC
- Group 4 (CC100): received a dose of 100 mg/kg CC orally for 7 days following induction of UC
- Group 5 (CC200): received a dose of 200 mg/kg CC orally for 7 days following induction of UC
- Group 6 (sulphasalazine): received a dose of 100 mg/kg sulphasalazine (reference drug) orally for 7 days following induction of UC

All treatments started simultaneously and continued daily for consecutive 7 days after colitis was induced. On the seventh day, the rats were sacrificed by cervical dislocation and then the colon specimens were collected. An 8-cm segment of the colon was excised, freed of adherent adipose tissue, rinsed with cold normal saline, and used for macroscopic scoring. The colon/body weight index ratio was calculated using body weights and colon weights. Colon samples were stored at − 80 °C for use for measurements biochemical parameters. Other portions of colon tissues were fixed in 10% formalin for histopathological evaluation.

Macroscopic Assessment of Colonic Damage

Assistance was obtained from two observers independent of the study to evaluate the severity of colitis macroscopically in colon tissues [27]. The cleaned wet tissue was weighed, and mucosal lesions were measured macroscopically in the colon. Macroscopic injuries to the colonic mucosa were evaluated according to criteria ranging from zero to four, as shown in Table 1.

Table 1 Macroscopic score table of colonic damage

Colon damage	Score
None	0
Hyperemia	1
Hyperemia and wall thickness	2
Solid ulceration	3
Two or more ulcerated /inflammatory areas	4
Severe inflammation or ulceration (longer than 1 cm)	5
Severe inflammation or ulceration (longer than 2 cm) and 1 more point for every 1 cm	6–10

Analysis of the Disease Activity Index (DAI)

The DAI score is based on scoring body weight loss, changes in stool consistency, and detection of rectal bleeding to assess the severity of UC (Table 2). DAI = (combined score of weight loss, stool consistency, and bleeding) / 3 [28].

Enzyme-Linked Immunosorbent Assay (ELISA)

Tumor necrosis factor alpha (TNF-α), transforming growth factor (TGF)-1β, interleukin 6 (IL-6), IL-1β, IL-10, VGEF, PGE2, and COX-2 levels were quantified with enzyme immunoassay (ELISA) kits in colonic homogenates according to the manufacturer's protocols. The absorbance was measured at 450 nm.

Histological Evaluation

For light microscopic evaluations, colonic tissues were fixed in 10% neutral-buffered formalin for at least 48 h. After fixation, tissue samples were dehydrated in graded ethanol series (70, 90, 96, and 100%), cleared in toluene, embedded in paraffin, and sliced into 4-μm-thick sections by a rotary microtome. Sections were stained with hematoxylin and eosin stain to assess colon injury and inflammation. Semi-quantitative scores were given from 0 to 3 (0, none; 1, mild; 2, moderate; 3, severe) for damage of the surface and crypt epithelium, submucosal edema, and inflammatory cell infiltration with a maximum score of 9. Photographs were taken finally using a digital camera (Olympus DP72, Tokyo, Japan) attached to a photomicroscope (Olympus BX51, Tokyo, Japan).

Table 2 The standard of DAI score

Score	Decline in body weight (%)	Stool	Hematochezia
0	Naught	Normal	Negative
1	1–5	-	-
2	5–10	Semi-loose	BLD +
3	10–15	-	-
4	15	Loose	Bloody

Statistical Analysis

Statistical analysis was carried out by using GraphPad Prism 8.0 statistics program (GraphPad Software, San Diego, CA, USA). The data was shown as means ± SEM and statistical difference criterion is $p < 0.05$. One-way analysis of variance (ANOVA) was used to determine statistically significant differences followed by the Tukey's multiple comparison tests.

Results

In Vitro Antioxidant Activity of CCE

CCE with an IC_{50} value of 5.24 µg/mL exhibited an antioxidant activity as potent as the standard (IC_{50} for Trolox: 3.17 µg/mL) against the ABTS radical (Table 3).

Total Phenolic, Flavonoid, and Tannin Content of CCE

The total phenolic and tannin content of the CCE were found to be 540.4 and 197.3 mg/g as mg of gallic acid equivalents per gram of extract while its total flavonoid content was 176.5 mg/g as milligrams of catechin equivalents per g of dried extract (Table 3).

Effects of *Cotinus coggygria* on Colonic Damage and Disease Activity of Ulcerative Colitis Rats

It was observed that in the colitis group, significant weight loss and reduction in colon weight were observed in rats compared to the sham group (Fig. 1b). As shown in Fig. 1c, the mean macroscopic scoring values obtained from the colon tissues were found to be lower in the treatment groups with CC (2.4) than in the colitis group (7.25). As confirmed by the macroscopic score, different doses of CC extract showed that the treatment of colon tissue of acetic acid-induced colitis model significantly improved the damage to the colon tissues at different rates. The DAI results showed that the DAI score increased in the AA-administered colitis group compared to the sham group. In these rats, significant weight loss, decrease in daily activity, fecal consistency, slurry,

Table 3 Antioxidant activity and total compound content of CCE

Assays	CCE	Trolox
ABTS activity (IC_{50}, µg/mL)	5.24 ± 0.33^{a}	3.17 ± 0.03^{a}
Total phenolic content (mg GAE/g extract)*	540.4 ± 0.69	
Total flavonoid content (mg CE/g extract)**	176.5 ± 9.61	
Total tannin content (mg GAE/g extract)*	197.3 ± 0.35	

CCE, ethanol extract of *Cotinus coggygria* leaves.

* Total phenolic content and total tannin content were expressed as gallic acid equivalent (GAE).

** Total flavonoid content was expressed as catechin equivalent (CE).

Each value in the table is represented as mean ± SD ($n = 3$). Different letter superscripts in the same line indicate significant differences ($p < 0.05$).

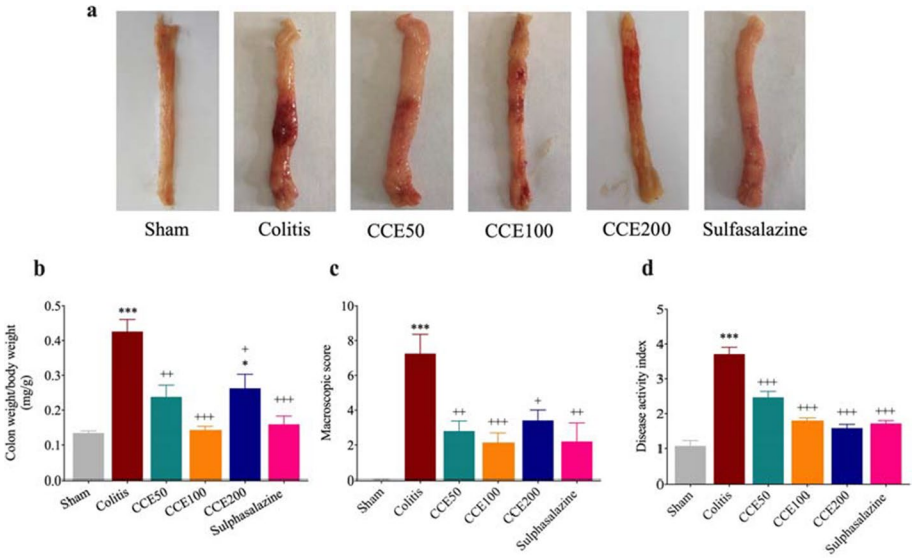

Fig. 1 **a** Macroscopic appearance of colonic tissues of all groups. **b** Colon weights/animal weights index. **c** Macroscopic scoring all groups. **d** Disease activity index score in all groups. One asterisk (*) $p < 0.05$, three asterisks (***) $p < 0.001$ versus sham group; one plus sign ($^{+}$) $p < 0.05$, two plus signs ($^{++}$) $p < 0.01$, three plus signs ($^{+++}$) $p < 0.001$ versus colitis group

and significant blood loss were observed throughout the study. Compared to the colitis group, these markers were mild in the treatment groups. CC administration significantly reduced the high DAI caused by AA-induced colitis. These results suggested that AA could significantly attenuate colonic damage and disease activity of colitis rats (Fig. 1d).

Effect of *Cotinus coggygria* on Cytokine Levels in Ulcerative Colitis Rats

The effect of different doses of CC treatment on pro/anti-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-10 and TGF-1β in AA administration colon tissue was evaluated with the ELISA kit. As depicted in Fig. 2, there was a significant increase in colonic TNF-α, IL-1β, and IL-6 levels in the AA-treated colitis group, and a significant decrease in IL-10 levels compared to the sham group. Treatment with CC significantly reduces colonic inflammatory levels and increases IL-10 level. Furthermore, 100 mg/kg CC treatment converted inflammatory cytokine levels to values close to the values of sulphasalazine treatment (Fig. 2a–d). TGF-1β is defined as a profibrogenic cytokine that promotes the migration of inflammatory structures and fibroblasts to the damaged tissue. In parallel with these findings, intracolonic AA administration results in about 1.6-fold increase in TGF-1β in comparison with sham group (Fig. 2e). In 50 mg/kg and 100 mg/kg CC treatment, the colon TGF-1β content has significantly decreased by 87.93% and 72.41%, respectively, compared to the colitis group. However, no significant change was observed in the 200 mg/kg CC treatment.

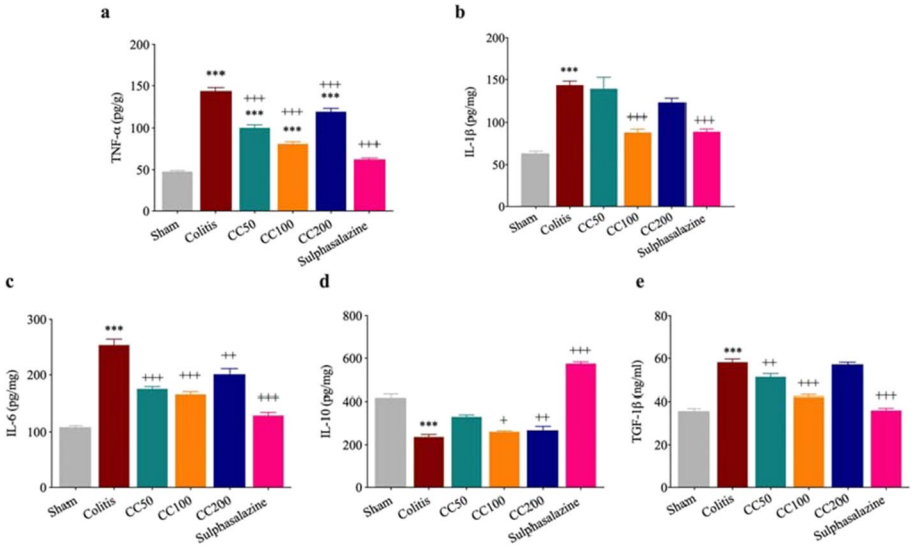

Fig. 2 Effect of CC on inflammation and proinflammatory cytokines in acetic acid-induced colitis. **a** TNF-α, **b** IL-1β, **c** IL-6, **d** IL-10, **e** TGF-1β. One asterisk (*) $p < 0.05$, three asterisks (***) $p < 0.001$ vs sham group, two plus signs (++) $p < 0.01$, three plus signs (+++) $p < 0.001$ vs colitis group

Effect of *Cotinus coggygria* on Levels of VEGF, COX-2, 8-OHdG, and PGE_2 in Ulcerative Colitis Rats

Compared with the sham group, rats with AA-induced ulcerative colitis group showed a significant decrement in the level of VEGF by about 2.71-fold. The administration of CC at 50 mg/kg, 100 mg/kg, and 200 mg/kg doses increments in the VEGF levels by 1.72, 1.99, and 2.04-fold, respectively, compared to the colitis group (Fig. 3a). Levels of COX-2 were significantly high ($p < 0.001$) in the colon with colitis group compared to sham group rats. The CC administration at 50 and 100 mg/kg caused a significant decrease ($p < 0.05$ and $p < 0.001$) in the COX-2 level in the colon compared to the colitis control (Fig. 3b). In the present study, it was observed that there was a significant ($p < 0.001$) increase in 8-OHdG levels in colonic tissues of rats with colitis when compared to the sham group. Compared to acetic acid-induced ulcerative colitis model, the treatment with 100 mg/kg CC was observed to a significant ($p < 0.001$) decrease in 8-OHdG levels (Fig. 3c). PGE2 levels in the colon tissues of the rats with acetic acid were significantly increased compared to the sham group ($p < 0.001$). Compared to colitis group, 50 mg/kg and 100 mg/kg CC treatment showed a significant decrease in PGE2 levels in rat colon tissues (Fig. 3d). However, 200 mg/kg CC treatment had no difference level of PGE2 level in colon compared to colitis group.

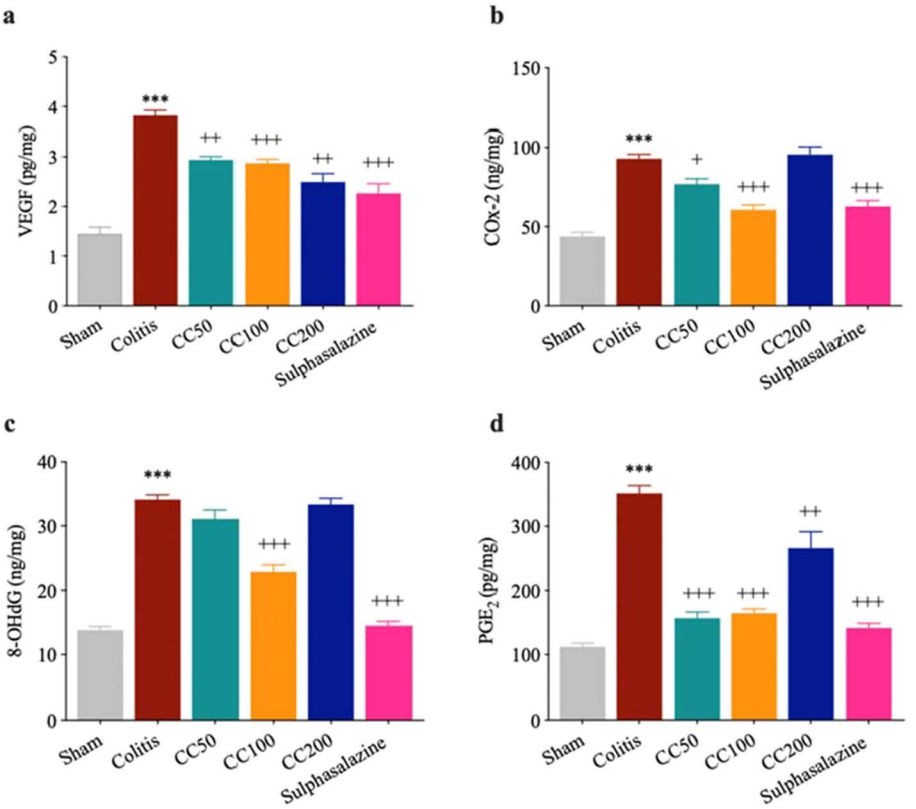

Fig. 3 Effect of CC treatment of **a** VEGF, **b** COX-2, **c** 8-OHdG, and **d** PGE2 levels in acetic acid-induced colitis. Three asterisks (***) $p < 0.001$ vs sham group and on plus sign (+) $p < 0.05$, two plus signs (++) $p < 0.01$, three plus signs (+++) $p < 0.001$ vs colitis group

Effect of *Cotinus coggygria* on Histopathological Changes in Hematoxylin and Eosin (H&E)-Stained Colon Specimen

To investigate the effects of CC on histological changes in the colonic tissues of rats, the specimens were evaluated by H&E staining. In the control group, the mucosa was observed normally with a regular structure of Lieberkuhn crypts and submucosa. Massive surface and Lieberkuhn crypt epithelial loss with hemorrhage, submucosal edema, and inflammatory cell infiltration were observed in the colitis group. However, after CC (50, 100, and 200 mg/kg) administration, colonic mucosa was relatively intact, a quite regular surface and glandular epithelium were observed, and submucosal edema and inflammatory cell infiltration were decreased regarding colitis group rats (Fig. 4) (Table 4).

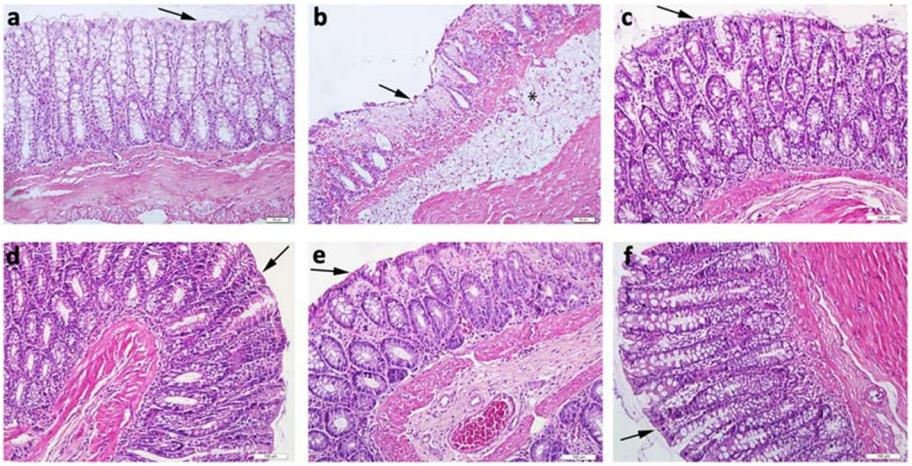

Fig. 4 Micrographs illustrating the histological appearances of colonic tissues in different experimental groups. **a** Sham group, **b** colitis group, **c** *Cotinus coggygria* 50 mg/kg group, **d** *Cotinus coggygria* 100 mg/kg group, **e** *Cotinus coggygria* 200 mg/kg group, **f** sulphasalazine group. Regular mucosa and submucosa structure in the sham group. Destruction of the mucosal structure with epithelial loss and hemorrhage, submucosal edema (asterisk (*)) and inflammatory cell infiltration in the colitis group. Restored mucosal structure evident in the sulphasalazine and *Cotinus coggygria* 100 and 200 mg/kg groups. Arrows show surface epithelial lining. H&E staining. Scale bars: 50 μm (**a**–**b**) and 100 μm (**c**–**f**)

Table 4 Histopathological examination of the colon tissue

Groups	Mean ± SEM		
	Inflammatory cell infiltration	Epithelial damage	Submucosal edema
Sham group	0.0 ± 0	0.0 ± 0	0.0 ± 0
Colitis group	$2.40 \pm 0.24^{***}$	$2.70 \pm 0.2^{***}$	$2.80 \pm 0.2^{***}$
CC50	$1.60 \pm 0.20^{+}$	$1.0 \pm 0.40^{++}$	$1.4 \pm 0.37^{++}$
CC100	$1.33 \pm 0.13^{++}$	$0.66 \pm 0.21^{+++}$	$1.0 \pm 0.15^{+++}$
CC200	1.80 ± 0.20	$1.2 \pm 0.33^{+}$	$1.40 \pm 0.20^{++}$
Sulfasalazine	$0.8 \pm 0.20^{+++}$	$0.8 \pm 0.15^{+++}$	$0.60 \pm 0.10^{+++}$

CC, ethanol extract of *Cotinus coggygria* leaves.

$^{***}p < 0.001$ vs sham group.

$^{+}p < 0.05$, $^{++}p < 0.01$, and $^{+++}p < 0.001$ vs colitis group.

Discussion

Ulcerative colitis (UC) is an inflammatory bowel disease characterized by recurrent inflammation and ulceration of the colon [29]. Current clinical treatments are mainly performed with drugs with anti-inflammatory and immunomodulatory effects, and easy relapse and serious adverse reactions have been reported in the treatment with these drugs [30]. The presence of biologically active components of plants used in traditional folk medicine explains their potential evaluation in drug research. It is in this context

that we set out to evaluate the effects of ethanolic extract of *Cotinus coggygria* acetic acid-induced ulcerative colitis rats.

In this study, AA administration caused bleeding in the feces of the animal and liquefaction in the stool consistency, decrease in body weight, and ulceration and hyperemia in the colon tissue. In addition, reductions in macroscopic deterioration, colon weight, and animal weight were also prominent in rats with UC. These results are consistent with the preference for intracolonic placement of AA as an experimental ulcerative colitis model in studies to date [31].

Cotinus coggygria is used in traditional medicine against abdominal pain and enteritis (11). Childers et al. (2015) reported that colitis and enteritis are common causes of abdominal pain. (32). Therefore, we have investigated the *Cotinus coggygria* Scop. extract in a rat model of colitis.

Matic et al. examined the genotoxic properties of the *Cotinus coggygria* extract with comet analysis and reported a low genotoxicity at a dose of 1000–2000 mg/kg and no toxicity at a dose of 500 mg/kg (12). Pavlov et al. showed that aqueous infusions (1%, 2%, and 4%) from *C. coggygria* did not cause subchronic toxicity on the liver and kidney (33). Administration of three different doses of *Cotinus coggygria* ethanolic extract determined based on this information significantly reduced the number of diarrheal stools and body weight loss. Significant improvement was observed in disease markers such as weight loss, colon weight, and macroscopic deterioration at three different doses (50 mg/kg, 100 mg/kg, 200 mg/kg) administered in *Cotinus coggygria* treatment. In addition, the DAI score as a clinical parameter of UC was clearly increased in the colitis group. In our study, CC administration markedly reduced the high DAI induced by AA, which remarks that AA effectively alleviates disease activity of UC rats. The mentioned results encourage us to examine the mechanisms that mediate the therapeutic effect of CC.

Proinflammatory cytokines such as IL-6, TNF-α, and IL-1β were increased in the colon tissues of AA administered rats [34]. In the inflammatory process, inflammatory cells localize to the damaged tissue and cause an increase in cytokines [35]. These results can be interpreted as aggravating colonic damage through the release of inflammatory factors in tissue penetrating with AA. Accumulating evidence showed that weight loss, which is a clinical marker of ulcerative colitis, is mediated by the release of appetite suppressing neuropeptides by TNF-α and IL-6 [36]. The decrease in these cytokine levels after treatment with CC suggests that it is due to the anti-inflammatory effect of the extract. TGF-1β mediates both inflammatory and fibrotic events by acting as a chemoattractant for macrophages and by automatically inducing its production [36]. According to the results presented, the increase in TGF-1β level in colonic tissue following acetic acid administration may be associated with oxidative damage and inflammation process. In addition, the TGF-1β level after CC treatment at 50 mg/kg and 100 mg/kg doses, being close to the sham group, suggests that it may be an effective treatment in the treatment of colitis. Compared with those of the sham group, the level of IL-10 in the colonic tissues was significantly decreased in the group of rats with induced UC. IL-10 is an important anti-inflammatory cytokine that maintains intestinal homeostasis by keeping the immune system in balance [5]. Experimental data showed that IL-10 prevents intestinal tissue damage by inhibiting the release of proinflammatory cytokines such as TNF-α, IL-6, IL-1β, and IL-12, controlling the differentiation and proliferation of macrophages and T/B cells, and preventing excessive immune reaction [37]. The improvement in the increase in IL-10 level caused by AA after treatment with CC extract can be interpreted as that this extract has anti-inflammatory properties and protects it against tissue damage.

Accumulating evidence has shown that VEGF, an ischemic factor, is upregulated in the colon tissues of patients with UC, mediated by angiogenesis [38]. The increase in blood vessel density in tissue is mediated by the secretion of immune cells and proinflammatory mediators, and an increase in microvascular and epithelial permeability. Inhibiting disease activity by targeting attenuation of VEGF signaling in UC appears to be an effective treatment strategy [39]. Consistent with previous results, the level of VEGF was decreased in tissue in a dose-dependent manner following treatment with CC. As is well known, COX-2 has a role in cellular mechanisms such as angiogenesis and apoptosis. In addition, studies have revealed that COX-2 creates a proinflammatory early response to cytokines such as TNF-α [40]. The increase in TNF-α, IL-1, IL-8, and COX-2 in intestinal epithelial cells leads to oxidative damage that causes tissue damage [38]. In accordance with the present results, the increased COX-2 level with AA administration after CC treatment was significantly reduced. These results can be interpreted as CC treatment can prevent tissue damage by reducing the inflammatory response. On the other hand, increased oxidative DNA damage in the colon accompanying UC is also an important factor in tissue damage. The active hydroxyl radical 8-OHdG is a marker in oxidative DNA damage, and this marker is expected to decrease to cope with tissue damage in UC. According to our results, the increased 8-OHdG level caused by AA administration returned to values close to the sham group after 100 mg/kg CC treatment. Clinical and experimental studies have clearly demonstrated that PGE2 has inhibitory capacity to interfere with the immune response with its role in growth, differentiation, and death of immune cells [41]. Increased PGE2 level in colonic tissue with UC catalyzes oxidative stress-induced inflammatory mediator release [42]. The data of our study revealed that the increase in PGE2 level accompanied by UC in the colon tissue decreased with the anti-inflammatory effect of CC treatment. Also, in vitro anti-inflammatory effects of CCE against 5-lipoxygenase enzyme were demonstrated in our previous study [43]. In addition, while our previous study found that CCE showed a significant in vitro antioxidant activity against the DPPH radical [43], in our current study, it also showed a significant in vitro antioxidant activity against the ABTS radical. Dziąbowska-Grabias et al. (2021) suggested that natural products or compounds with antioxidant and anti-inflammatory properties may be useful in the treatment of IBD due to promising research results [44]. Thus, the curative effect of CC against colitis may be due to its antioxidant and anti-inflammatory effects.

The current study showed that the ethanol extract of *Cotinus coggygria* had high total phenol, flavonoid, and tannin content. These groups of substances include polyphenolic compounds and these compounds have been reported to have therapeutic effects against colitis [45]. Also, LC–MS/MS analysis of *Cotinus coggygria* ethanol extract in our previous study showed that the extract contained polyphenolic compounds such as quinic acid, gallic acid, protocatechuic acid, methyl gallate, myricetin glucoside, myricetin rhamnoside, quercetin rhamnoside, ethyl gallate, ethyl ester of digallic acid, and myricetin with pentagalloylglucose being the major compound [43]. Previous studies have shown that pentagalloylglucose had antioxidant and anti-inflammatory activity [46, 47]. Therefore, pentagalloylglucose along with other polyphenolic compounds in extract could be responsible for the curative effect of the extract against colitis.

Histological research has revealed results that confirm the biochemical findings that acetic acid causes acute inflammation after epithelial damage, causing widespread bleeding and increasing the release of proinflammatory cytokines that play a role in increasing the permeability of the intestinal epithelial barrier [48]. Overproduction of proinflammatory mediators such as IL-1β and TNF-α causes epithelial cell necrosis, edema, and leukocyte infiltration in the colon in the pathogenesis of UC. In this study, CC administration impressively reduced inflammatory cell infiltration, hyperemia, edema, and ulceration. This result indicated that CC could be protective against colonic damage of UC.

Conclusion

The potential therapeutic effect of CC in the treatment of colitis was evaluated with the support of antioxidant and anti-inflammatory activity studies. This study showed that CCE treatment could remarkably alleviate colonic injury and inhibit the inflammatory response in UC rats. While our findings show that it can be a natural and effective alternative in UC, more studies are needed on the mechanism of action and effective dose of CC. At the same time, these results confirm the use of the plant in traditional medicine for inflammatory diseases. Although our study showed the beneficial effect of the extract on colitis, the lack of similar activity studies on the major compound responsible for the activity of the extract limits the study.

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Data Availability All data is included in the article.

Declarations

Ethical Approval All procedures for experimental protocols of the present study involving animals were performed in accordance with the ethical standards of the institution of practice at which the studies were conducted. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University Marmara (093.2016.mar).

Conflict of Interest The authors declare no competing interests.

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