Original Research

Evaluating the Protective Effect of *Ailanthus altissima* (Mill.) Swingle in a Rat Model of Acetic Acid-Induced Ulcerative Colitis

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Abstract

Inflammatory bowel disease (IBD) has two subtypes called ulcerative colitis (UC) and Crohn's disease (CD). They have the same tissue damage processes; however, differ in the initial procedures and immune regulatory aberrations. Tumor necrosis factor-alpha (TNF-α) appears to have an important function and a key pathogenic role in CD and UC. In this paper, we evaluated the protective impact of the extract of *Ailanthus altissima* (Mill.) Swingle (tree of heaven), Simaroubaceae, in acetic acid-induced UC. Ferric-reducing capacity of plant extract, total phenols, and total flavonoid contents were measured in *A. altissima* ethanolic extract. Thirty male rats were randomly divided into six groups including control, normal, mesalazine 300 mg/kg (positive control) and 3 treatment groups with doses of 50, 100, and 200 mg/kg for 7 days. Except for the normal group, all animals received 2 mL of 4% acetic acid infusion into the large intestine. Upon 48 h after the last gavage, the animals were euthanized. Acetic acid intracolonic administration resulted in a severe acute inflammation in the colonic tissue, which was improved by *A. altissima* extract at all examined doses in both microscopic and macroscopic aspects. In comparison to the control, TNF-α was considerably lower in the mesalazine and 200 mg/kg *A. altissima* extract groups. Conclusively, the valuable effects of the extract in the treatment of UC were confirmed by TNF-α levels, histological data, and macroscopic observations, especially at 200 mg/kg dosage. Future studies are recommended to further clarify the action mechanisms of this plant.

Keywords: *Ailanthus altissima*; Acetic acid; Colitis; IBD

Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory condition in the gastrointestinal tract which is divided into two main subtypes, namely ulcerative colitis (UC) and Crohn's disease [1]. UC is a chronic illness with recurrent symptoms and high morbidity. The disease affects roughly 6-8 persons per one hundred thousand people in the USA, with a rate of 70-150 people per 100,000 [2]. UC always affects the rectum, but it can also affect the descending colon, the sigmoid colon, or the entire colon in a continuous pattern. UC strikes most people among the ages of 15 and 40, with a second surge in occurrence among the ages of 50 and 80 [3]. Though the specific etiology of UC is unidentified, the disease is likely due to a mix of environmental and genetic factors [1]. Bloody diar-

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rhea with or without mucus, tenesmus, rectal urgency, and varying degrees of stomach pain that is typically eased via defecation are common symptoms of UC. Clinical presentation, histology, endoscopic findings, and the absence of other diagnoses are used to diagnose UC. In addition to verifying the diagnosis of UC, it is important to determine the severity and degree of inflammation, which helps physicians choose the right treatment and anticipate the patient's prognosis [4]. CD and UC are likely to share susceptibility genes and non-specific inflammatory mediator profiles. These disorders are caused via various factors and respond to several stimuli. They have comparable tissue damage processes but differ in their immunoregulatory aberrations and beginning events [5]. CD is immunologically linked to interferon-gamma, T helper (Th) type 1 and 17, interleukin (IL)-12, IL-17, IL-23, and pro-inflammatory cytokines (e.g., IL-1, IL-17A, IL-17F, IL-18, IL-2, IL-21, IL-22, IL-26). UC, on the other hand, is believed to be caused via a Th17 and a changed Th2 response (IL-5, IL-9, and IL-13). Th1 and Th2 cells both generate tumor necrosis factor-alpha (TNF-α) and IL-6 [6]. TNF-α appears to have an important function in UC, in both in vivo and *in vitro* studies [7]. It is a pro-inflammatory cytokine that has a pathogenic role in both CD and UC. It is responsible for tissue injury due to its capacity to disrupt the epithelial barrier in colonic epithelial cells [8,9]. TNF-α levels are higher in IBD patients' serum, feces, and irritated intestinal mucosa [10]. The treatment of UC entails immediate treatment of all inflammatory symptoms, followed by maintaining remission. The symptoms severity and the degree of intestinal involvement dictate the therapeutic method in general [3]. In a non-research setting, gastroenterologists use a limited number of pharmacological therapies to manage UC. Corticosteroids (prednisolone and hydrocortisone), aminosalicylates or 5-ASA-based medications (such as olsalazine, sulfasalazine, balsalazide, and mesalazine), ciclosporin, and azathioprine and its active ingredient mercaptopurine are some examples of these pharmacological therapies [11]. While conventional medicines could be useful in establishing relapse and shortening the duration of active disease, they are not without negative impacts, and a great number of patients are unable to response, even to the strongest medications [1]. Nowadays, the tendency for using herbal antioxidants has dramatically enhanced due to a safe, powerful, and affordable nature of these compounds [12]. *Ailanthus altissima* (Mill.) Swingle (tree of heaven), Simaroubaceae, is an invasive early successional tree that has spread over Europe and all other continents except for Antarctica [13]. One advantage of *A. altissima's* widespread occurrence is its possible use as a source of pharmaceutically active chemicals, primarily ailanthone (quassinoids) [14]. *A. altissima*

extracts were used in ancient Chinese medicine for treating a wide range of illnesses, including epilepsy, asthma, scabies, and seborrhea, dysentery, diarrhea, as well as an astringent, antispasmodic, anthelmintic, antiparasitic, and narcotic [13,15,16]. Additionally, a decoction of *A. altissima* was indicated to decrease the inflammatory cytokines production like TNF-α [15]. To our knowledge, no study has been conducted to investigate the impact of this plant extract on UC in vivo. One of the animal models for the evaluation of anticolitis medicines is induction of colon inflammation using rectal acetic acid (AA) [17]. The mechanism of AA-induced colitis is unknown; however, reactive oxygen species are thought to have an important role. AA induction enhanced oxidative damage; while suppressing antioxidant systems. Histopathological investigations indicated that AA produces tissue damage and apoptosis [18]. The aim of the present study was to investigate the impact of *A. altissima* extract on UC on the animal model of AA-induced colitis.

Methods

Chemicals
All chemicals were supplied from Sigma-Aldrich (GmbH, Munich, Germany). Rat-specific enzyme-linked immunosorbent assay (ELISA) kits of TNF-α were supplied from Enzo Life Sciences (Lorrach, Germany).

Plant material
Ailanthus altissima (Mill.) Swingle bark was collected in October 2021 from Kermanshah and botanically identified by Herbarium, Faculty of Pharmacy, Kermanshah university medical science. A voucher specimen was deposited with identification number 273 002 001.

A. altissima ethanolic Extract
A. altissima barks were washed with distilled water and dried. Then weighed and macerated in 70% ethanol and stirred for 72 h. The extract was filtered using vacuum filtration by a Büchner funnel and repeated 2 times in a row, then the solvent was evaporated via a rotary evaporator device.

Ferric Reducing Antioxidant Potential Assay (FRAP)
The FRAP test was used to evaluate the ferric-reducing capacity of plant extracts. This approach relies on the Fe^{3+}-tripyridyltriazine (TPTZ) reduction (colorless) to a Fe^{2+}-tripyridyltriazine (blue) caused by the action of antioxidants that donate electrons. The 20 mM ferric chloride and TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM hydrochloric acid were combined with ten volumes of 300 mM acetate buffer, pH 3.6, to create the

FRAP solution [19, 20].

The volume of 0.4 mL of distilled water and 3.6 mL of FRAP solution were combined and incubated at 37°C for 5 min. This solution was then combined with a specific amount of plant extract and incubated for 10 min at 37°C. At 593 nm, the absorbance of reaction mixture was evaluated. Five concentrations of $FeSO_4$, $7H_2O$ (0, 0.2, 0.4, 0.6, 0.8, 1 mM) were used to generate the calibration curve, and the absorbance values were determined for sample solutions [21].

Evaluation of total phenolic and flavonoid content of *A. altissima* ethanolic extract

The extract was dissolved in methanol (5 mL), subjected to 45 min of sonication at 40°C, and then centrifuged at 1,000 ×g for 10 min. For analysis, the clear supernatant was gathered and kept in an amber glass bottle [22].

Total phenolic content

The Folin-Ciocalteu reagent was used to evaluate the total phenolic content of the extracts. A spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian) was employed for measuring the sample and the standard at 765 nm in comparison to the reagent blank.

Folin-Ciocalteu reagent (0.2 mL) was added to 0.2 mL of the test sample along with water (0.6 mL) (1:1). Following the addition of saturated sodium carbonate solution (1 mL) (8% w/v in water) and making up to 3 mL with distilled water (after 5 min). After 30 min of dark storage, the reaction solution was centrifuged, and the blue color absorbance from several samples was assessed at 765 nm. Using the equation derived from a standard calibration curve of gallic acid, the total phenolic content was calculated. A duplicate of each determination was made [22].

Total flavonoids content

The total flavonoid amount in the sample was evaluated via the aluminum chloride colorimetric technique. Quercetin was utilized to create the standard calibration curve for the evaluation of total flavonoid content. The stock quercetin solution was produced via solubilizing quercetin (5 mg) in methanol (1 mL), and the standard quercetin solutions were produced via sequential dilutions using 5-200 μg/mL of methanol. Furthermore, 0.6 mL of 2% aluminum chloride was combined with diluted standard quercetin solutions or extracts (0.6 mL). The mixture was incubated at room temperature for 60 min. Using a Varian UV-Vis spectrophotometer, the reaction solutions absorbance was assessed toward a blank at 420 nm (Cary 50 Bio UV-Vis Spectrophotometer, Varian). Total flavonoid content concentration was calculated using the standard calibration curve and a duplicate of each determi-

nation was made [22].

Animals

Thirty adult male Wistar rats from 8 to 9 weeks old (weighing 180-220 g) were held in the animal house of the Faculty of Pharmacy Kermanshah university medical science. They were kept in standard conditions (free access to tap water and standard food, 12h light/dark cycle, 22 ± 0.1°C, and 50-55% humidity) (five rats in each cage). Kermanshah University of Medical Sciences' Ethics Committee approved the animal procedures (IR.REC.1400.266)

Induction of colitis

AA-induced colitis is an animal model that closely look a lot like some of the acute inflammatory responses seen in UC [23]. Rats were fasted for 24 h before any intracolonic studies and were only permitted to drink water. An intraperitoneal injection of xylazine (1 mg/100 g) and ketamine (4 mg/100 g) mixture was employed to anesthetize the rats, and for enteral feeding a medical-grade polyurethane cannula (2 mm of external diameter) was inserted into the anus, with the tip advanced eight cm proximal to the anus verge. Except for the normal group, all animals received a 2 mL of 4% acetic acid infusion into the colon. As the acute colitis rat model was induced by the acid solution, animal welfare has been carefully monitored.

Experimental design

All treatment groups received the interventions by gavage for 7 days, on the eighth day, UC was induced by acetic acid and they received the interventions again for three days. Animals were given different concentrations of *A. altissima* ethanolic extract and mesalazine, 24 h after colitis was induced. *A. altissima* was dissolved in water. Our pilot study determined the dosage of *A. altissima*. Rats were divided into six groups based on a random classification system.

1. Normal group: rats were administrated 1 mL saline via gavage, once a day

2. Control group: AA-induced colitis, rats were treated with saline, once a day.

3. Mesalazine /Mes group: AA-induced colitis, rats were administrated 300 mg/kg mesalazine through gavage, once a day

4. 50 mg/kg *A. altissima* extract group: AA-induced colitis, rats were administrated 50 mg/kg extract through gavage, once a day.

5. 100 mg/kg *A. altissima* extract group: AA-induced colitis, rats were administrated 100 mg/kg extract through gavage, once a day.

6. 200 mg/kg *A. altissima* extract group: AA-induced colitis, rats were administrated 200 mg/kg extract through gavage, once a day.

Forty-eight hours after the last gavage, the animals

were euthanized. The protruding portion of the colon (the last 8 cm) was cut and lightly cleansed with normal saline then fixed on a clean, white surface, and macroscopic observations were made. Then colonic segments were allocated into 2 sections, one for histopathologic analysis (kept in 10% formalin) and the other for biochemical analysis (kept at -80 °C).

Macroscopic assessment of colonic damage

The macroscopic damage was evaluated using the criteria defined by Abdolghaffari et al. (2010). Table 1 presents the criteria [24].

Table 1. Macroscopic colonic damage scoring criteria

Score	Criteria of scoring of IBD morphological damage
0	Normal appearance with no damage
1	Localized hyperemia without ulceration
2	Linear ulceration without significant inflammation
3	Linear ulceration with inflammation at one site
4	Two or more sites of ulceration extending more than 1 cm along the length of the colon
5-8	Damage extending more than 2 cm long, the length of the colon, and the score is enhanced by 1 for each increased cm of involvement

Microscopic assessment of colonic damage

To conduct microscopic assessment, the fixed segments in formalin (10%) were embedded in paraffin and stained with eosin and hematoxylin. The scoring was conducted by a blind pathologist. The microscopic scoring was evaluated based on the following scale (Table 2).

Measurement of TNF-α

Levels of TNF-α in colon tissues were measured with an ELISA kit according to the producer's instruction. The absorbance of the final solution was assessed at 620 nm as the reference wavelength and 450 nm as the primary wavelength.

Statistical analysis

All data in this work were expressed as mean ± standard deviation (M±SD). Data analyses were conducted using SPSS software and one-way ANOVA, followed by Newman-Keul's post hoc test for multiple comparisons.

Results

Total phenolic and total flavonoid content

Table 2. Microscopic colonic damage scoring criteria

0	Very bad condition
+1	Bad condition
+2	Weak
+3	Moderate
+4	Good condition
+5	Excellent/Normal

Total phenol and flavonoid contents were calculated using calibration curve (Figure1 and Figure 2 respectively), the phenolic content of the extract was calculated as 880 mg gallic acid equivalent/mL (OD=1.66); and the flavonoid content was calculated as 221 mg quercetin equivalent/mL (OD= 0.056).

FRAP analysis

The FRAP assay was employed to calculate the ferric reduction power of *A. altissima* extract. Based on the calibration curve, 0.787 mg/L of $FeSO_4.7H_2O$ was the determined concentration.

Macroscopic evaluation of colonic damage

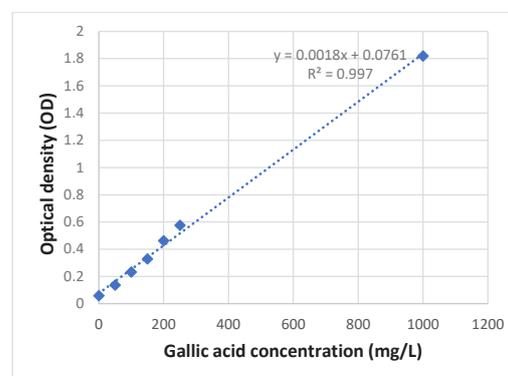The results of the macroscopic score are indicated in table 2. According to the table, the control group

Figure 1. Standard calibration curve of gallic acid (0-1000 mg/L) for the determination of total phenolic content
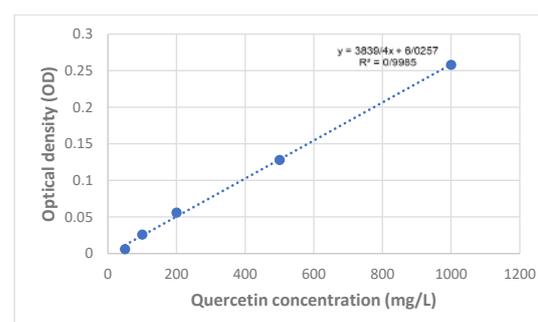

Figure 2. Standard calibration curve of quercetin (0-1000 mg/L) for the determination of total flavonoid content
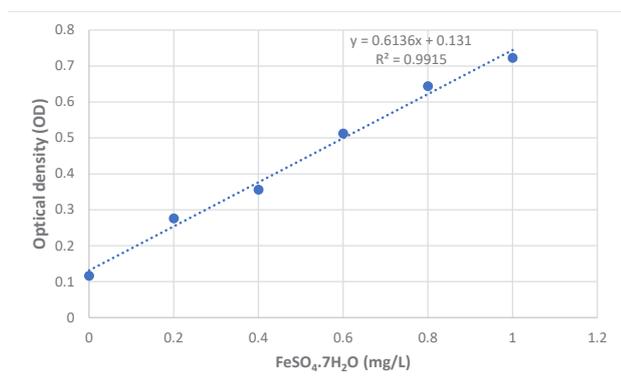
Figure 3. Standard calibration curve for the determination of FRAP

has the highest macroscopic scores. Compared to the normal group, intra-rectal administration of AA led to ulceration, adhesion, wall thickening, and severe inflammation in the control group. The 200 mg/kg *A. altissima* extract had the best healing activity among the treated groups. Furthermore, there was no noticeable difference in the efficacy of mesalazine and any of the extract treatment groups.

Microscopic evaluation of colonic damage

Histological manifestations and condition of tissues are exhibited in figures 5-10 and the condition of the mucosa, submucosa, muscular and glandular epithelium, active bleeding, and the process of tissue repair and angiogenesis are interpreted.

Table 3. The extent of colonic injury in rats

Group	Macroscopic score	
	Mean±SD	Median (min-max)
Normal	0±0	0 (0-0)
Control	6.75±0.95	6.5 (6-8)
Mesalazine (300mg/kg)	2.87±0.47	2.75 (2.5-3.5)
50 mg/kg *A. altissima* extract	4.25±1.89#	3 (3-7)
100 mg/kg *A. altissima* extract	4±1.41#	3.5 (3-6)
200 mg/kg *A. altissima* extract	3±0.81###	3 (2-4)

Data are presented as mean ± SD; SD: standard deviation; #: $P < 0.05$, ##: $P < 0.01$, and ###: $P < 0.001$ compared with control group

Colonic TNF-α

In comparison to the control, TNF-α was considerably lower in the mesalazine and 200 mg/kg *A. altissima* extract groups; and between the extract groups, the lowest level was observed in 200 mg/kg dosage of extract (24.13±2.40), followed by the 100 mg/kg *A. altissima* extract group (28.98±3.91). TNF-α level in the control group was considerably higher ($p<0.001$) than in the normal group (Table 5).
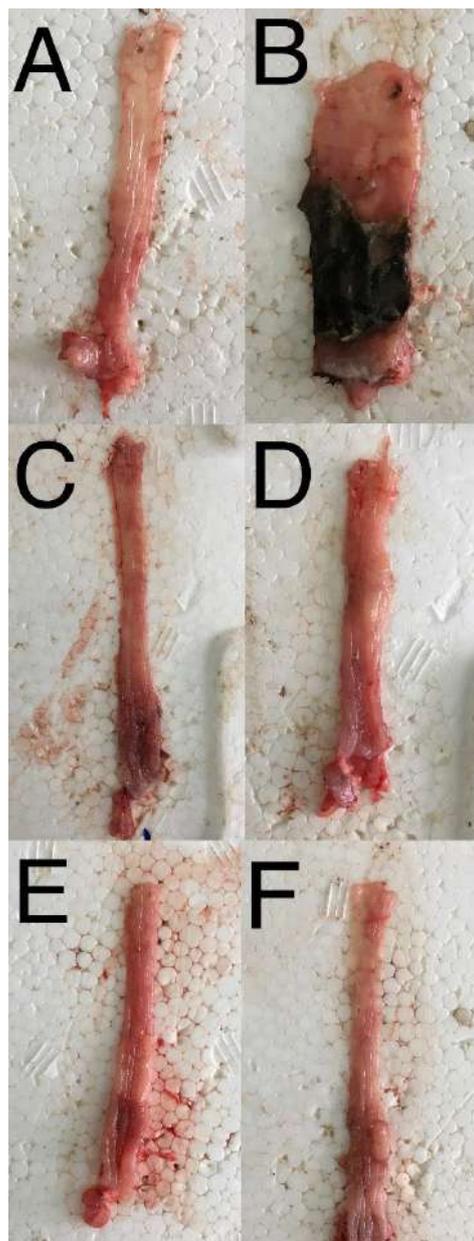

Figure 4. Macroscopic presentation of rat colons. Normal (A) which shows an intact colon. Control (B) shows the highest level of tissue injuries like edema, erythema, ulcer, necrosis, and thickening of tissue. Mesalazine (C) 50 mg/kg (D) 100 mg/kg (E) 200 mg/kg (F) of *A. altissima* extract which represents the healing of ulcers and obvious improvement in tissue injuries
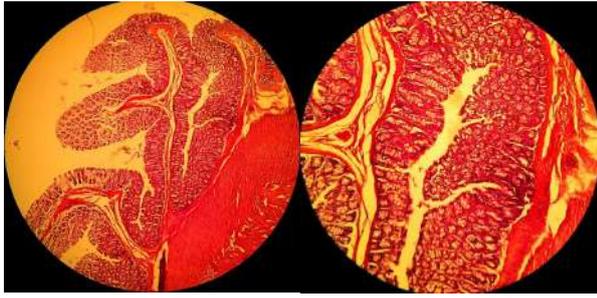
Figure 5. Normal (A): In healthy colonic tissue, the appearance of the mucosal epithelium (glandular epithelium) is normal. There is no sign of edema between the mucosal muscle and the epithelium, where the lamina propria is located. The accumulation of lymph cells is normal (Lymphoid follicles). Lymphocyte and inflammatory cell proliferation due to immune and inflammatory reactions are not observed. The number of goblet cells and the morphological appearance of the glands and muscle layers are normal and free of edema. In the serous membrane, adipose tissue is normal. Blood vessels in all layers (mucous membranes, submucosal, muscular, and serous) are normal and do not have any inflammatory and congestive manifestations. There are no signs of fibrosis and stimulation of collagen secretion in connective tissues
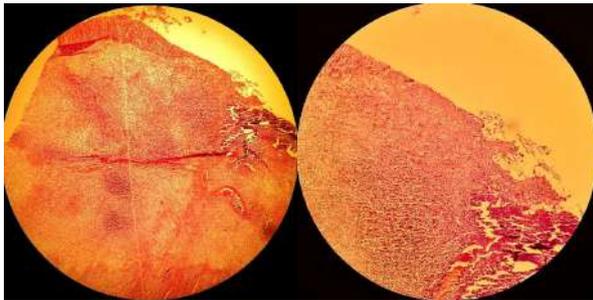

Figure 6. Control (B): Ulceration and removal of the gastrointestinal epithelium are quite evident. All layers have severe inflammation, severe edema, hyperemia, bleeding, severe lymph accumulation, and stimulation of lymph nodes. All vessels are dilated and their number and diameter are immoderate. Initiation of connective tissue cell invasion: Fibroblasts, macrophages, secretion of delicate collagen fibers, and reactive stimulation of connective tissue are evident. Traces of lamina propria, mucosal and submucosal muscles are not clearly visible and no specific category called layers are found at the site of the wound
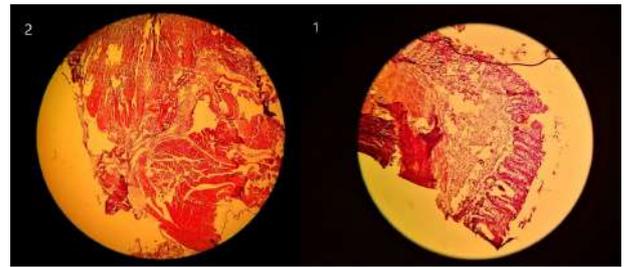

Figure 7. 50 mg/kg (D) of A. altissima extract: Figure 7.1 shows that the intestinal glandular epithelial tissue has recently been repaired in some areas and has a basic structure. Severe bleeding and edema are seen in all layers (Lamia propria, submucosa). Severe proliferation of inflammatory cells is seen; edema is evident in all layers. Very bloody vessels and the beginning of the fibrosis process are seen in connective tissues. In many places (7.2) repair has not been done on the damaged tissues and the wound is still not visible
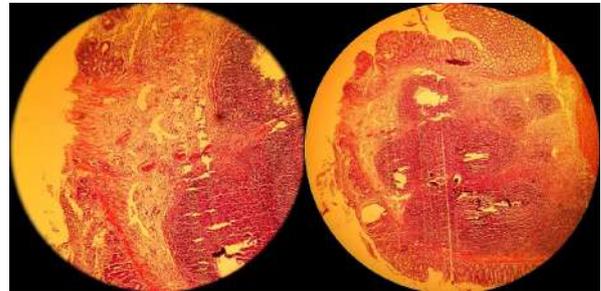

Figure 8. 100 mg/kg of A. altissima extract (E): The wound is closed by the deposition of collagen fibers and proliferation of the muscularis mucosa system. Lymphocyte proliferation is observed in the lamina propria. Blood vessels are found between the mucosal and Submucosal layers, and are full of blood. No active bleeding is noticed (release of red blood cells outside the vascular space). In the mucosal and muscular layer, a wide spread of inflammatory cells, especially lymphocytes, is detected

Figure 9. 200 mg/kg (F) of A. altissima extract: Wound edges have begun to contract, and Epithelial tissue is closing the wound. There is no evidence of bleeding in the lamina propria. Among the colonic glands, very small arteries with a natural wall are evident (angiogenesis process). In lamina propria, the presence of fibroblasts, lymphocytes, and macrophages with a clear and recognizable population is evident. The mucosal muscle is repaired in all wound positions or is in a normal state and is seen with normal morphology. In the submucosa, connective tissue edema is observed. In the same layer, inflammatory cells involved in the repair of connective tissues such as fibroblasts and macrophages are not seen. There is also no bleeding or red blood cell diffusion. Collagen deposition is clearly seen in small clusters along the arteries. Collagen deposition, stronger and more pronounced, is easily visible at the site of the wound repaired in the space of the Lamia propria. In other parts, even the glandular epithelium is formed in the mucous layer and is visible.
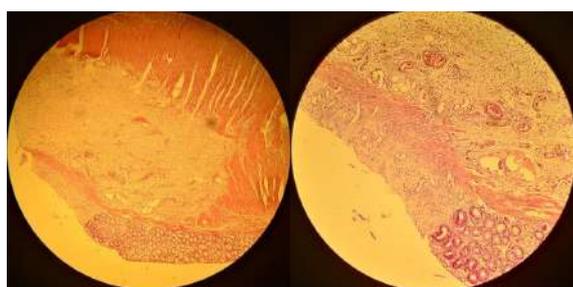
Figure 10. Mesalazine (C): Examination of the wound tissue and adjacent areas reveals that the underlying layer of the repaired mucosa has expanded and the muscular part has weakened. Due to the effect of the drug, the damaged epithelium is replaced with connective tissue and collagen deposition and angiogenesis (granulation) process and no trace of open blood vessels or active bleeding is seen (lamina propria and glandular epithelium replaced with this connective tissue). In submucosa layer, a large amount of inflammatory and lymphocyte secretion is observed. Significant edema is not seen in different tissues and layers. There are many vessels with a medium diameter and blood in the submucosa and there is no sign of bleeding. There is no sign of intestinal epithelial tissue regeneration at the damaged site

Discussion

IBD refers to a group of chronic diseases (Crohn's disease and ulcerative colitis) that have a critical impact on the quality of life. The prevalence of IBD has progressively increased over the years [25]. The intestinal mucosa is the main target of UC. The etiology of colitis may be influenced by a variety of factors, such as infections, the environment, and immunological abnormalities [26].

There is substantial proof that the inflammatory immunoregulation of intestinal mucosa is marked by elevated levels of pro-inflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF-α [27-32]. It is widely acknowledged that TNF-α may play a key role in colitis in both initiating and maintaining intestinal inflammation and that its expression is significantly increased during the inflammatory phase of colitis. TNF-α has been shown to stimulate the production of IL-6, IL-1 and IL-8. These cytokines modulate the expression of several NF-κB inducible genes that regulate the generation of pro-inflammatory mediators, cell adhe-

Table 4. Microscopic score of histopathological sections of colonic tissue

Groups	Mucus Condition	Epithelium	lamina propria	Mucosal muscle	Submucosa condition	Edema	Inflammatory cells	Collagen	Angiogenesis	Muscles condition	Serous membrane condition	Connective tissue	Fat
Normal	+++++	+++++	+++++	+++++	+++++	0	+	+++++	+++++	+++++	+++++	+++++	+++++
Control	+	+	+	+	+	0	0	+	+	+++	+	++	+++
Mes	++	+	+	++++	+++	+++	++++	+++	++++	+++	++++	+++	+++
AA50	+++	+++	++	+++	+++	++	++	++	++	+++	+++	+++	+++
AA100	+++	+++	++++	++++	++++	+++	++++	+++	+++	++++	++++	++++	++++
AA200	++++	++++	+++++	+++++	++++	+++++	++++	++++	+++++	+++++	+++++	++++	+++++

mes: Mesalazine (300 mg/kg); AA50: 50 mg/kg *A. altissima* extract; AA100: 100 mg/kg *A. altissima* extract; AA200: 200 mg/kg *A. altissima* extract 1

Table 5. TNF-α levels in colon tissue (pg/mL protein) in rats with acetic acid-induced colitis

	TNF-α	
Group	Mean±SD	Median (min-max)
Normal	12.03±0.41	12.03 (11.74-12.32)
Control	38.87±2.90	38.44 (36.21-41.98)
Mesalazine (300 mg/kg)	21.00±3.44	21.00 (18.57-23.44)
50 mg/kg A. altissima extract	29.74±2.17#	31.28 (28.21-32.01)
100 mg/kg A. altissima extract	28.98±3.91#	28.51 (25.33-33.11)
200 mg/kg A. altissima extract	24.13±2.40##	24.79 (21.47-26.14)

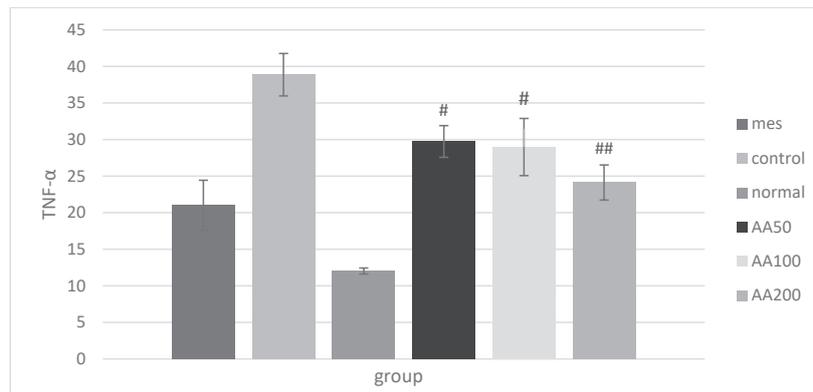#: $P < 0.05$; ##: $P < 0.01$; ###: $P < 0.001$ compared with control group 1

Figure 11. TNF-α levels in colon tissue (pg/mL protein) in rats with acetic acid-induced colitis. Results are expressed as means ± SD; (n = 6 rats/group). mes: Mesalazine (300 mg/kg); AA50: 50 mg/kg *A. altissima* extract; AA100: 100 mg/kg *A. altissima* extract; AA200: 200 mg/kg A. altissima extract; #: $P < 0.05$; ##: $P < 0.01$; ###: $P < 0.001$ compared with Control group.

AA intracolonic administration resulted in a severe, acute, inflammation in the colonic tissue; while *A. altissima* extract at all examined doses improved the symptoms in both microscopic and macroscopic aspects. The reduction in macroscopic and microscopic scores was observed after administration of different *A. altissima* doses (50,100, and 200 mg/kg).

sion molecules, and other cytokines [33,34]. Increased amounts of pro-inflammatory cytokines trigger the synthesis of excessive amounts of matrix-degrading enzymes by colon fibroblasts, a loss of mucosal integrity, and ulceration in the mucosa [34,35].

There are several IBD models that have been used in various studies. We used AA to induce colitis because it causes inflammation and ulceration, increases intestinal mucosal vascular permeability, activates kinin, and interrupts blood coagulation, so it is useful for studying IBD pathogenesis and new treatment options [36-38].

A. altissima has previously documented antimalarial, antioxidant, antituberculosis, antiviral, antifungal, insecticidal, antiproliferative, anti-inflammatory, cytotoxic, antiasthmatic, and phosphodiesterase inhibitory effects [12,39-43]. Recent pharmacological investigations have demonstrated that *A. altissima* is a strong therapeutic agent with the capability to treat a variety of illnesses, particularly cancer and gastrointestinal inflammation [44].

The antioxidant activities of *A. altissima* are reported in the study by Albouchi and colleagues. From a pharmacological perspective, the reported antioxidant activity represents the test extracts' capacity to provide hydrogen atoms or electrons to neutralize radical species [45].

A. altissima has been shown to reduce inflammatory cytokines like TNF-α, IL-6, and IL-8. TNF-α stimulates the synthesis of cytokines, which is regarded to be the beginning of cytokine-related inflammatory states that lead to inflammation, leukocyte infiltration, and tissue fibrosis [46,47].

The findings show that *A. altissima* suppresses TNF-α, IL-6, and IL-8 production via reducing NF-κB activation [47].

The studies came to the conclusion that *A. altissima* had the ability to treat inflammatory disorders by reducing mast cell activation [47].

TNF-α is a conductor cytokine which elevated expression promotes the pathogenic progression of numerous inflammatory disorders [48]. The purpose of this work was to investigate the protective impacts of *A. altissima* extract on AA-induced colitis in rats; in which, the beneficial impacts of the extract were confirmed by TNF-α levels, histological data, and macroscopic observations. Generally, we discovered that *A. altissima* extract, especially at 200 mg/kg daily dosage, considerably decreased TNF-α levels.

Several studies have indicated that in pro-inflammatory situations, some cytokines, such as TNF- α, are produced in large quantities [49] and lead to an aggravation of the inflammatory response, which causes cells to produce other cytokines, leading to intestinal necrosis and edema of the tissue [50,51]. Blocking inflammatory mediators has been shown to decrease the severity of inflammatory disorders. *A. altissima* extract has been indicated to have anti-inflammatory and inhibitory impacts on inflammatory cytokines in cellular and animal studies [44,52]. Some alkaloids of this plant showed a significant decrease in TNF-α expression [52].

In conclusion, our investigation demonstrated that *A. altissima* extract had therapeutic effects on animal model of colitis by lowering serum TNF-α levels, a key pro-inflammatory cytokine, and reducing colonic

inflammation-related damage. These findings imply that *A. altissima* can effectively reduce the inflammation cascade in colitis.

Conflict of Interests

None.

Acknowledgements

None.

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