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Salsola imbricata Forssk. ameliorates acetic acid-induced inflammatory bowel disease by modulating dysregulated antioxidant enzyme system and cytokine signaling pathways in mice

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ABSTRACT

Objective: To explore the protective effect of the crude extract of *Salsola imbricata* against acetic acid-induced inflammatory bowel disease in mice and its mechanism of action.

Methods: Ethanolic crude extract of *Salsola imbricata* was characterized by HPLC. *Salsola imbricata* extract at different doses was administered and ulcerative colitis was induced by 200 μL, 7.5% acetic acid and macroscopic parameters were evaluated to assess the homeostatic condition of intestinal mucosa along with hematological and biochemical assays. The levels of malondialdehyde, glutathione peroxidase 1, superoxide dismutase, and catalase were determined in colon tissues. Proinflammatory cytokines including interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) were quantified by ELISA. The extent of tissue damage was assessed by histological analysis.

Results: Phytochemical analysis confirmed the presence of phytochemicals including quercetin, gallic acid, syringic acid, benzoic acid and chlorogenic acid in the crude extract. The crude extract of *Salsola imbricata* (300 and 500 mg/kg) markedly decreased malondialdehyde and nitric oxide ($P<0.01$) and increased antioxidant activities of glutathione peroxidase 1 ($P<0.001$) and superoxide dismutase ($P<0.001$). Moreover, it decreased the levels of IL-1β, IL-6 and TNF-α significantly ($P<0.001$) and reduced the damage to the colon mucosa, promoting tissue healing and regeneration.

Conclusions: *Salsola imbricata* extract restores the colonic epithelial layers by maintaining mucosal homeostasis and cell integrity by modulating antioxidant defense system and inflammatory cytokine signaling in ulcerative colitis mice.

KEYWORDS: *Salsola imbricata*; Acetic acid; Antioxidant enzymes; Cytokines; Inflammation; Reactive oxygen species

1. Introduction

Inflammatory bowel disease (IBD) comprising of ulcerative colitis (UC), Crohn's disease, and IBD unspecified, is an idiopathic chronic gastrointestinal inflammatory disorder, characterized by relapsing inflammatory episodes with extensive destruction of colonic mucosal layer[1]. At present, new epidemiological findings proposed that the rate of IBD might be quickly expanding in Eastern Europe, South America, Asia, and Africa, but the exact number of the affected individuals is, yet, unsure[2]. IBD, especially UC, presents many clinical indications, including abdominal pain with cramps, fever, weight loss, fatigue, diarrhea, and blood from the rectum, which may start progressively, or all at once[3]. IBD pathogenesis is multifactorial and is still imcompletely understood. It is assumed that an unpredictable connection between hereditary (genetic factors), natural (environmental and life style modifictaions), and/or microbial (intestinal flora) components may prompt upgraded unsusceptible reaction, involving the proliferation and activation of T cells along with provocative cytokines [interleukins (IL-1β and IL-6)], and tumor necrosis factor (TNF-α) that contribute in the development and progression of the disease. Dysfunction or imbalance in the immune balance of the intestine results in inflammatory cell infiltration

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and release of proinflammatory cytokines, which leads towards the tissue damage and exacerbates the condition of UC. Currently, 5-aminosalicylic acid, immunomodulators, corticosteroids, calcineurin inhibitors and TNF-α monoclonal antibodies are mostly used in the management of UC, but long-term practice of these therapies are associated with serious adverse reactions; *e.g.* immune suppression. Therefore, seeking assistance from folkloric and traditional therapies is of great interest[5]. Since ancient times, plants have been used as a source of medication to treat various diseases. Currently, the utilization of medicinal plants for wellbeing purposes and for the counteraction and treatment of illness and diseases has expanded quickly. In such circumstances, it is necessary to scientifically evaluate the impacts of plant resources, especially the ones that have ethnomedical utilization. Undoubtedly, an enormous number of currently used medications have been developed based on folkloric use of medicinal plants[6].

Salsola imbricata (*S. imbricata*) Forssk., a small shrub, commonly known as lani, lana, and haram, belongs to the family Amaranthaceae. The motherland of *S. imbricata* is saline sandy places of the desert belt of Africa, Iran, Pakistan, Afghanistan, and India. In Pakistan, it grows in desert areas of deep Cholistan and is commonly used as traditional medicine[7]. This wildly growing shrub is used as camel food in Egypt[8]. Traditionally, *S. imbricata* is used in gastrointestinal disorders including poor digestion, vomiting, piles, dyspepsia, and abdominal distention[9]. It is also used in the management and treatment of headaches, migraine, vertigo, scabies, eruption, and wounds[10]. In scientific literature, the crude extract of *S. imbricata* has been reported for its tyrosine inhibitory potential[11], antioxidant and spasmolytic action, butyrylcholinesterase[12], vermicidal[13], antibacterial[14], male contraceptive[15], diuretic[16], antispasmodic and broncho-relaxant[7], analgesic, anti-inflammatory, and antipyretic effects and secondary metabolites that produce these effects were alkaloids, flavonoids, saponins, tannins and anthraquinones[11]. Hence, in view of the reported traditional and pharmacological activities and phytochemicals discussed for *S. imbricata* with no scientific evidence reported on IBD, the present study was designed to evaluate the intestinal anti-inflammatory potential along with possible mechanism of action in Swiss albino mice to provide scientific ground for its folkloric use in the traditional system of medicine.

2. Materials and methods

2.1. Plant material collection and extraction

S. imbricata aerial parts were collected from Deengarh, district of Bahawalpur, Pakistan, and plant specimen was identified and deposited in the Herbarium and herbarium number was issued for future reference (Herbarium no: Fl.P.225-9). Shade dried aerial parts (1 kg) were grounded into coarse powder and soaked in 30:70 aqueous ethanolic mixture for 3 d and the combination was blended with a stirrer. Soaked material was strained by using a muslin cloth and filter paper to collect filtrate and residues were again soaked for 3 d in an aqueous ethanolic mixture. The above-explained procedure was repeated thrice and joined filtrates were concentrated with a rotary evaporator at a temperature not surpassing 40℃. The dried semisolid mixture; *i.e.* the crude extract of *S. imbricata*, was weighed to calculate percent yield, labeled and stored at −20℃ for future use.

2.2. Drugs and reagents

Drugs and reagents used were of analytical grade including acetic acid (Fluka Analyticals, US), formalin (Riedel-de Haen, Germany), sulphasalazine (Ferozsons Laboratories, Pakistan), potassium dihydrogen phosphate (BDH England), 0.9% sodium chloride solution (Unisa Pharmaceuticals, Pakistan), potassium chloride (Merck, Germany), disodium hydrogen phosphate (Merck, Germany), xylazine (Prix Pharmaceutical, Pakistan), ketamine (Global Pharmaceutical, Pakistan).

2.3. Preliminary phytochemical analysis

The crude extract of *S. imbricata* was analyzed for the detection of different phytoconstituents; *e.g.* flavonoids, alkaloids, phenols, saponins, tannins, *etc*[17].

2.4. High-performance liquid chromatography (HPLC) analysis

HPLC investigations were performed by following the procedure adopted by Javed *et al*[18]. Stock solutions of various standards were prepared by using ethanol to make a final concentration of 50 μg/mL. Ethanol was used as dissolvable for polyphenol at the concentration of 10 mg/mL. All samples were freshly prepared before evaluation and SIL-20A autosampler and C-18 column were used for analysis. Solution system with linear gradient consisted of acetic acid with water and ethanol with 1 mL/min flow rate and absorbance was measured at 280 nm and components of crude extract were identified after comparison with the standard solutions.

2.5. Experimental animals

Swiss albino mice (25-40 g) were kept in the animal house of research laboratory of Department of Pharmacology, Faculty of Pharmacy, the Islamia University of Bahawalpur, Pakistan. All the mice used in the study were kept in the polycarbonate cages (47 cm^3 × 34 cm^3 × 18 cm^3) with a maximum of 6 animals per cage. The standard housing conditions of humidity (50%-55%) and temperature [(25±2)℃] along with the exposure to 12 h:12 h light-dark cycle, were maintained during the study. Animals were fed with standard diet and allowed to drink water *ad libitum*.

2.6. Acute toxicity analysis

Acute toxicity analysis of the crude extract of *S. imbricata* was performed by following the Organisation for Economic Co-operation and Development guidelines. Swiss albino mice (25-30 g) were separated into different groups (n=5). They were acclimatized to laboratory conditions and provided food and water *ad libitum* before the start of the toxicity assay. Normal saline (10 mL/kg, *p.o.*) was given to the control group, while the crude extract of *S. imbricata* was given at the doses of 300, 1 000, 3 000, and 5 000 mg/kg, *p.o.*, to other mice groups after 12 h of fasting. Mice were observed at 1 h interval on the 1st day and then daily for 14 d. Animals were observed for mortality or any behavioral changes like tremors, sweating, convulsions, somatomotor activity, and behavior pattern[18].

2.7. Induction of ulcerative colitis

The procedure of Wang *et al*. was adopted with minor modifications[19]. Mice were randomly divided into five groups (n=6). The normal control group and the intoxicated group received 0.9% normal saline (10 mL/kg, *p.o.*). The standard group received sulphasalazine (500 mg/kg, *p.o.*), while treatment groups received the crude extract of *S. imbricata* at the doses of 300 and 500 mg/kg, *p.o.* All the treatment substances were administered by gastric gavage once a day, and consecutively for 7 d. On the 8th day, after 24 h of fasting, colitis was induced by 7.5% of acetic acid at the dose of 200 μL *via* intra-rectal administration. First, mice were lightly anesthetized by intraperitoneal injection of ketamine (50 mg/kg) in combination with xylazine (5 mg/kg) in a 10∶1 ratio at the dose of 0.2 mL/100 g of animal body weight. Acetic acid (200 μL, 7.5%) was administered into the rectum through Teflon catheters (0.8 mm diameter) inserted 5-6 cm into the anus, while the control group received a similar procedure with an equal volume of saline. Then, the mice were kept for 30 s in a supine and Trendelenburg posture to prevent the outflow of instilled solution and for even distribution of acetic acid within the colon. After 24 h, animals were sacrificed and blood and tissue specimens from each animal were collected for macroscopic, hematological, and biochemical analysis, and colorectal tissue specimens were preserved in 10% formalin for histopathological studies.

2.7.1. Determination of macroscopic colon damage

The extent of macroscopic damage by acetic acid was estimated by determining disease activity index (DAI) through adopting the procedure of Niu *et al*. and colonic mucosal damage index (CMDI) through following the protocol of Jagtap *et al*[20,21].

Spleen and colonic tissues were separated, washed, and weighed, and the length was measured for assessment of the severity of inflammation[22].

2.7.2. Determination of hematological parameters

Mice were anesthetized at the end of the experimental procedure and blood was collected in EDTA-covered test tubes for examination of hematological parameters [white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb), hematocrit (HCT), platelets count (PLT)][23].

2.7.3. Determination of antioxidant parameters

Colon tissues were removed, cut into sections, homogenized in ice-cold phosphate buffer saline to prepare tissue homogenate (10% *w*/*v*), and centrifuged at 3 000 rpm at 4 ℃ controlled temperature for 30 min to obtain clear supernatant, which was used for biochemical assays. Superoxide dismutase (SOD), catalase (CAT), and nitric oxide (NO) were determined by colorimetric method, while glutathione peroxidase-1 (GPX-1) and malondialdehyde (MDA) levels were estimated by following instructions of Elab science, biotechnology, ELISA kit method[2,23].

2.7.4. Determination of inflammatory parameters

Levels of cytokines (IL-1β, IL-6, and TNF-α) were estimated in the colon tissue by ELISA kit method. The level of IL-1β was estimated by following protocols of Bioassay technology ELISA kit method, IL-6 by international immunodiagnostic kit method, while TNF-α level was determined by following the instructions of Elab science biotechnology ELISA kit.

2.7.5. Determination of microscopic histological parameters

Colon tissue was removed, cleaned by using saline, sliced in thick paraffin-embedded sections, and stained by hematoxylin & eosin to assess colon damage. Photomicrographs were taken at a magnification of ×10[19].

2.8. Statistical analysis

Results were presented as mean±SEM and data were analyzed using GraphPad Prism 8.0 software (GraphPad, SanDiego, CA). Data analysis was done using one-way analysis of variance (ANOVA) followed by Tukey Kramer's multiple comparison test and P<0.05 was considered statistically significant.

2.9. Ethical statement

Studies were conducted according to the guidelines approved by the Pharmacy Animal Ethics Committee under registration no. 11-2020/PAEC dated 04-03-2020.

3. Results

3.1. Preliminary phytochemical analysis

Preliminary analysis of the crude extract of *S. imbricata* (percent

yield 8.90%) confirmed the presence of alkaloids, steroids, tannin, phenols, flavonoids, coumarins, and saponins.

3.2. HPLC analysis

HPLC fingerprinting of the crude extract of *S. imbricata* revealed the presence of quercetin [retention time (RT): 2.70], gallic acid (RT: 3.72), syringic acid (RT: 6.43), benzoic acid (RT: 6.81), and chlorogenic acid (RT: 16.89) (Figure 1).

3.3. Acute toxicity analysis

No mortality or behavioural changes were observed in all the tested doses. Therefore, the crude extract of *S. imbricata* was found safe up to the highest dose of 5000 mg/kg.

3.4. Effect of the crude extract of S. imbricata on macroscopic colon damage

The colitis control group showed markedly increased DAI compared with the normal control group. Treatment with sulphasalazine and the crude extract of *S. imbricata* significantly lowered the increased DAI with percent protection of 81.40%, 57.14% and 72.57%, respectively. CMDI assessment including gross lesion score and percent area affected was performed. Damage scores of sulphasalazine and the crude extract of *S. imbricata* (300 and 500 mg/kg, *p.o.*) treated groups were decreased with percent protection of 53.65%, 19.0% and 33.0%, respectively, compared with the colitis control group. In parallel with these findings, pre-treatment with the crude extract of *S. imbricata* significantly decreased the splenic enlargement and colon weight to length ratio (P<0.001) (Table 1).

3.5. Effect of the crude extract of S. imbricata on hematological parameters

WBC, RBC, PLT, Hb, HCT levels were decreased significantly in the colitis control group compared with the normal control group (P<0.001). The crude extract of *S. imbricata* and sulphasalazine pronouncedly reversed the acetic acid-induced changes (P<0.05) (Table 2).

3.6. Effect of the crude extract of S. imbricata on antioxidant parameters

In the colitis control group, the levels of antioxidant enzymes including SOD, CAT, and GPX-1 were decreased significantly (P<0.001). Pre-treatment with the crude extract of *S. imbricata* markedly augmented the SOD and GPX-1 levels except for CAT (P<0.05). Sulphasalazine also showed a significant effect on elevating the levels of antioxidant parameters (Figures 2A-C). Excess of free radicals caused an increase in the MDA and NO levels in the colitis control group, which was decreased by treatment with the crude extract of *S. imbricata* and sulphasalazine (Figures 2D and 2E).

3.7. Effect of the crude extract of S. imbricata on inflammatory parameters

In the colitis control group, significantly increased levels of IL-1β, IL-6, and TNF-α were observed (P<0.001). Pre-treatment

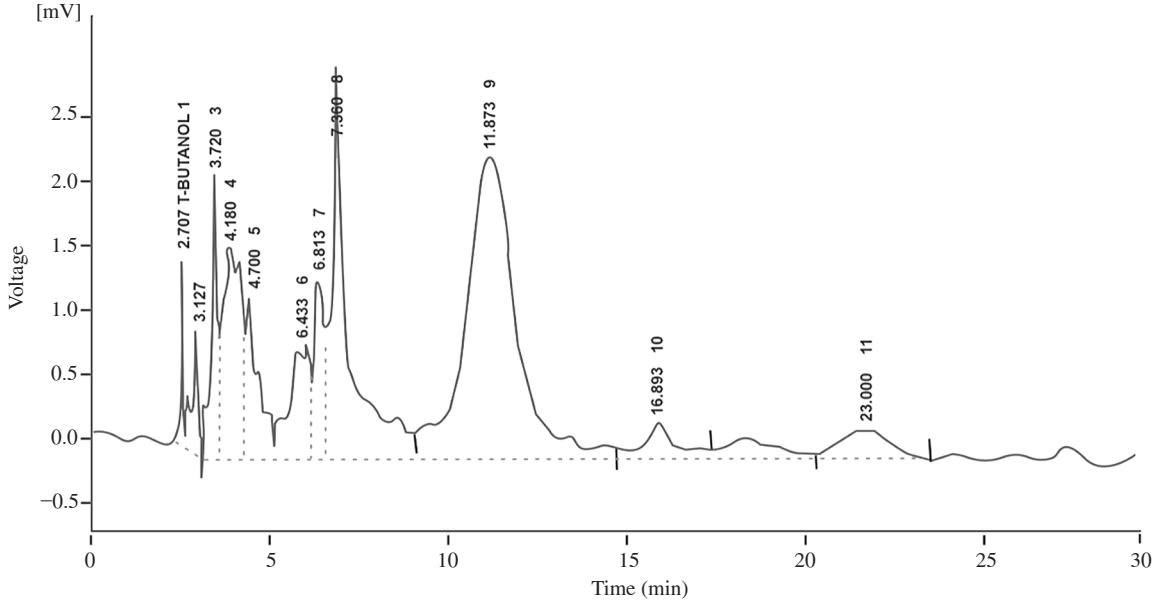

Figure 1. HPLC chromatogram of the crude extract of *Salsola imbricata*.

Table 1. Effects of the crude extract of *Salsola imbricata* on macroscopic parameters in mice with acetic acid-induced ulcerative colitis.

Treatment	Disease activity index	Colonic ulcer score	Wet colon weight/length (mg/cm)	Spleen weight (mg)
Normal control	0	0	73.97±1.62	215.00±0.02
Colitis control	3.50±0.50$^{###}$	6.30±0.70$^{###}$	206.40±6.58$^{###}$	410.00±0.02$^{###}$
Sulphasalazine (500 mg/kg, *p.o.*)	0.65±0.18***	2.92±0.10**	94.49±2.13***	254.00±0.01***
Salsola imbricata (300 mg/kg, *p.o.*)	1.50±0.25*	5.10±0.41	168.90±1.44***	351.00±0.05***
Salsola imbricata (500 mg/kg, *p.o.*)	0.96±0.14**	4.25±0.25*	142.60±1.31***	325.00±0.01***

Values are given as mean±SEM of 6 animals in each group and analyzed by one-way ANOVA followed by Tukey-Kramers. $^{###}P$<0.001 between the normal control and the colitis control groups, $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 between the colitis control and the treatment groups.

Table 2. Effects of the crude extract of *Salsola imbricata* on hematological parameters in mice with acetic acid-induced ulcerative colitis.

Treatment	WBC (×10^3/μL)	RBC (×10^6/μL)	Hb (g/dL)	PLT (×10^5/μL)	HCT (%)
Normal control	16.75±0.16	16.10±0.21	15.35±0.28	10.95±0.22	45.33±1.54
Colitis control	5.82±0.19$^{###}$	7.00±0.16$^{###}$	9.78±0.24$^{###}$	4.87±0.18$^{###}$	30.87±0.46$^{###}$
Sulphasalazine (500 mg/kg, *p.o.*)	15.42±0.16***	14.25±0.14***	13.80±0.31***	9.93±0.10***	42.02±1.40***
Salsola imbricata (300 mg/kg, *p.o.*)	13.10±0.18***	11.95±0.20***	11.03±0.26*	8.65±0.10***	36.00±1.27
Salsola imbricata (500 mg/kg, *p.o.*)	14.17±0.19***	13.72±0.22***	12.28±0.22***	9.32±0.10***	38.00±1.13**

Values are given as mean±SEM of 6 animals in each group and analyzed by one-way ANOVA followed by Tukey-Kramers. $^{###}P$<0.001 between the normal control and the colitis control groups, $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 between the colitis control and the treatment groups. WBC: white blood cell count, RBC: red blood cell count, Hb: hemoglobin (Hb), PLT: platelet count. HCT: hematocrit.

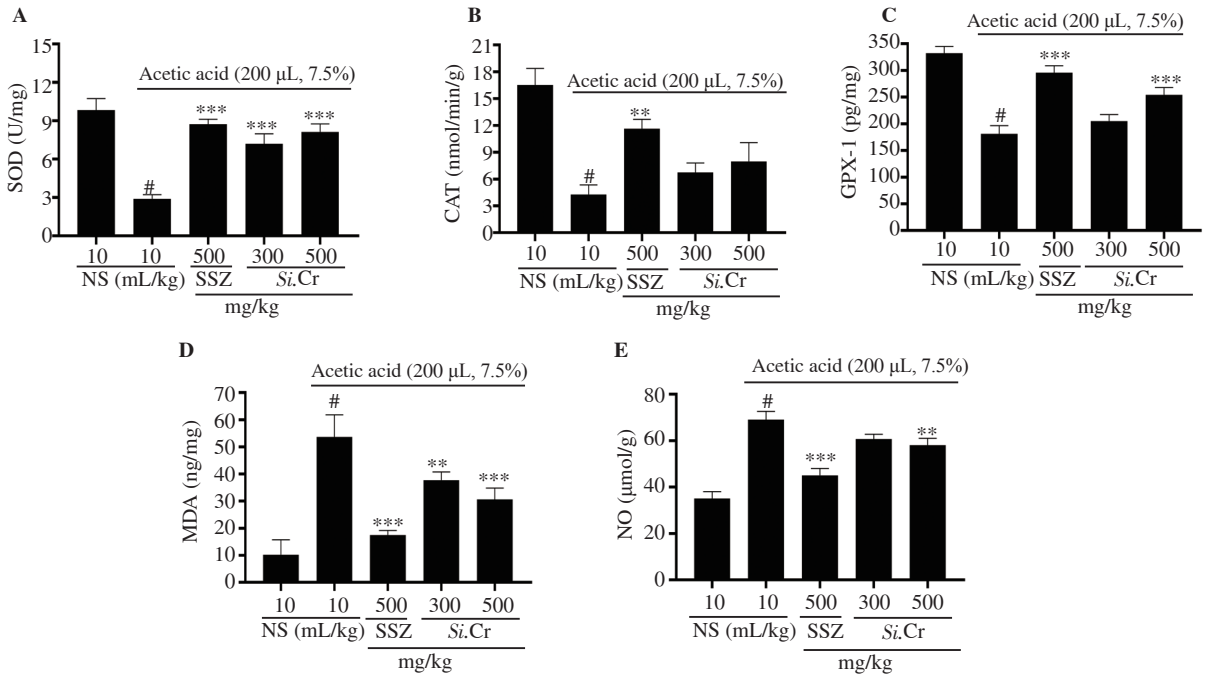

Figure 2. Effects of the crude extract of *Salsola imbricata* (300 and 500 mg/kg, *p.o.*) and sulphasalazine (500 mg/kg, *p.o.*) on antioxidant parameters. A: superoxide dismutase (SOD), B: catalase (CAT), C: glutathione peroxidase-1 (GPX-1), D: malondialdehyde (MDA), E: nitric oxide (NO). Data are expressed as mean ± SEM (n=6) and analyzed by one-way ANOVA followed by Tukey-Kramers; $^{#}P$<0.001 *vs*. the normal control group; $^{**}P$<0.01 and $^{***}P$<0.001 *vs*. the colitis control group. NS: normal saline (*p.o.*); SSZ: sulphasalazine (*p.o.*); *Si*.Cr: crude extract of *S. imbricata* (*p.o.*).

with sulphasalazine and the crude extract of *S. imbricata* notably diminished the levels of inflammatory cytokines (IL-1β, IL-6, and TNF-α) compared with the colitis control group (Figures 3A-C).

3.8. Effect of the crude extract of S. imbricata on microscopic histological parameter

Histological examination of colon tissues showed necrotic damage to the epithelial layer. Specimen from the colitis control group showed acetic acid-induced immense damages that disrupted muscularis and sub-mucosa and distorted epithelial tissues in comparison with the specimen of the normal control group (Figures 4A and 4B). Pre-treatment with sulphasalazine and the crude extract of *S. imbricata* (300 and 500 mg/kg, *p.o.*) alleviated the acetic acid-induced damages and demonstrated the protective effects (Figures 4C-4E).

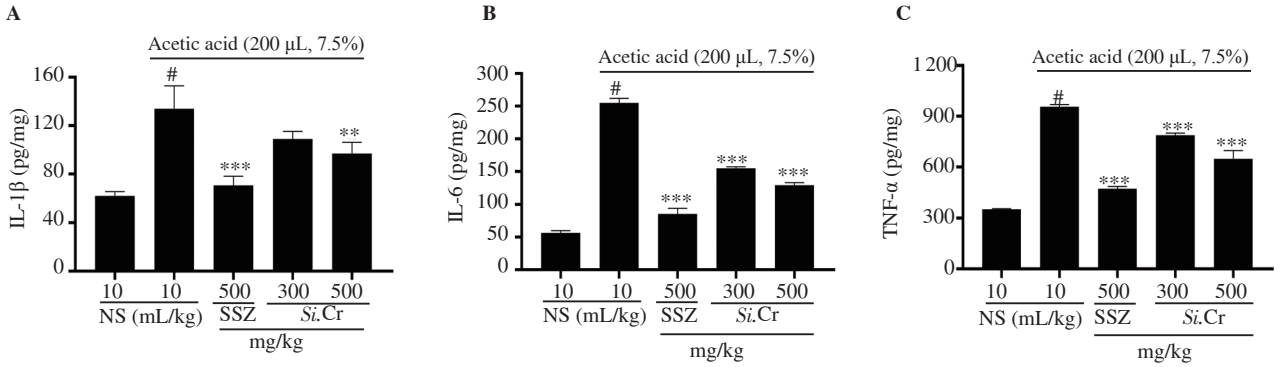

Figure 3. Effects of the crude extract of *Salsola imbricata* (300 and 500 mg/kg, *p.o.*) and sulphasalazine (500 mg/kg, *p.o.*) on inflammatory parameters. A: IL-1β, B: IL-6 and C: TNF-α. Data are expressed as mean ± SEM (n=6) and analyzed by one-way ANOVA followed by Tukey-Kramers; $^{\#}P$<0.001 *vs*. the normal control group; $^{**}P$<0.01 and $^{***}P$<0.001 *vs*. the colitis control group.

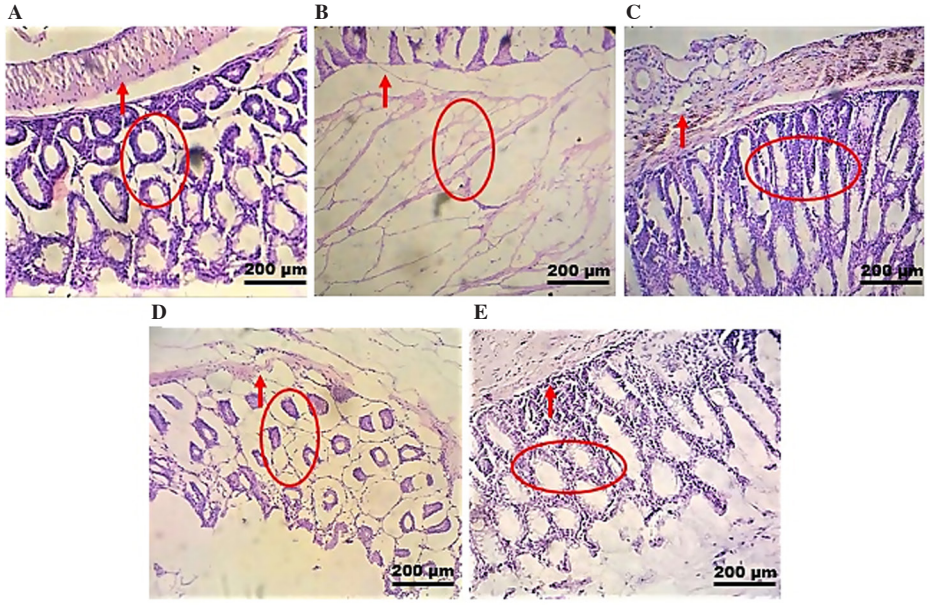

Figure 4. Effects of the crude extract of *Salsola imbricata* (300 and 500 mg/kg, *p.o.*) and sulphasalazine (500 mg/kg, *p.o.*) on histological alterations in colon tissues. A: The tissue specimen of the normal control group shows no epithelial mucosal abnormalities and changes in mucosal cell architecture. B: The colitis control (acetic acid, 200 µL of 7.5%) shows ulcerated mucosa, rupture of epithelial layers and immense damage to the normal cell architecture. C: The sulphasalazine-treated group, (500 mg/kg, *p.o.*), the standard aminosalicylate drug, shows remarkable anti-inflammatory effects as observed by normalization of structural integrity of intestinal mucosa. D: *Salsola imbricata* (300 mg/kg, *p.o.*) and E: *Salsola imbricata* (500 mg/kg, *p.o*). Pre-treatment with the crude extract of *Salsola imbricata* results in the normalization of the structural integrity of cells and reduction in the damage to the epithelial mucosa. Red circles and arrows describe the cell architecture and structural changes of the intestinal mucosa. Colon tissues were stained with hematoxylin & eosin and visualized under a light microscope at ×10 magnification. Scale bar: 200 µm.

4. Discussion

This study was designed to explore the folkloric claim of *S. imbricata* as an anti-inflammatory and gastroprotective remedy and this potential was assessed against the ulcerative colitis model induced by acetic acid in Swiss albino mice. This model is widely accepted for pre-clinical testing of different agents because it resembles ulcerative colitis in humans. Acetic acid is reported to produce intestinal inflammation *via* acidification mechanism and liberating protons from acid that results in epithelial injury. On exposure with acid, mild injury initiated with increased permeability, necrotic damage to epithelium that further got severe depending on the exposure, duration, and concentration of acid[19]. Damage to mucosal epithelium resulted in macrophage and neutrophil infiltration along with the production of reactive oxygen and nitrogen species and cytokines that lead towards cellular dysfunction and intestinal tissue damage[24]. In this study, acetic acid-induced ulcerative colitis was associated with macro and microscopic as well as hematological and biochemical parameters. Pre-treatment with the crude extract of *S. imbricata* significantly mitigated macroscopic parameters *i.e*. DAI, CMDI, spleen weight, colon weight to length ratio, when compared with the colitis control group. Macroscopic parameters provide details about the homeostatic conditions of intestinal flora[20]. Pre-treatment with the crude extract of *S.*

imbricata significantly decreased rectal bleeding, diarrhea, ulceration, and percent area affected and improved intestinal homeostasis by decreasing DAI and CMDI. Hematological studies provide indications about the characteristics of ulcerative colitis. Low Hb and RBC levels indicate the conditions of anemia, which is the characteristic feature of chronic inflammatory diseases[25]. Decrease in RBC and Hb and other hematological parameters may be due to impaired iron absorption and chronic intestinal bleeding[26]. The crude extract of *S. imbricata* stabilized the hematological parameters in a significant manner compared with the intoxicated group. Biochemical analysis portrayed the extent of tissue damage produced by reactive metabolites and inflammatory mediators[27]. The levels of antioxidant enzymes (SOD, CAT, and GPX-1) were decreased, and the NO and MDA levels were increased in the colon tissues of the colitis control group. The colitis control group also showed a significant increase in the cytokine (IL-1β, IL-6, and TNF-α) levels. SOD is an enzyme that needs a metal ion cofactor to catalyze $O_2^{\bullet}$ to O_2 and H_2O_2. The obtained byproduct H_2O_2 is converted to H_2O by CAT and GPX-1. These defense enzymes work in a balanced and coordinated way to maintain the ROS steady state. During ulcerative colitis, these endogenous antioxidants reduce the oxidative stress in the intestinal mucosa, but inflammatory reactions increase the need for these antioxidants that create an imbalance between antioxidants and pro-oxidants resulting in damage to the intestinal epithelial mucosa. Interleukins (IL-1β, IL-6) and TNF-α alleviate the severity of inflammation by increasing ROS production[2]. The crude extract of *S. imbricata* could produce an increase in the antioxidant enzymes and a decrease in the inflammatory cytokine level that showed the mucosal defensive action of the extract. These results were further supported by microscopic histopathological studies. Specimen from the colitis control group showed neutrophil infiltration, loss of goblet cells, and disruption of the epithelial layer as compared to the specimen of the normal control group. Sulphasalazine and the crude extract of *S. imbricata* treated group alleviated epithelial damage. Phytochemical and HPLC analysis revealed the presence of quercetin, gallic acid, syringic acid, benzoic acid, and chlorogenic acid. Quercetin and gallic acid have been reported to have strong antioxidant and anti-inflammatory properties[18]. Chlorogenic acid has also been reported to have potent anti-inflammatory action *via* suppression of reactive oxygen metabolites. Syringic acid is found to have potent anti-inflammatory activity against dextran sulphate sodium-induced ulcerative colitis in mice[28]. Mao *et al*. reported that benzoic acid helps to maintain gut function and also regulates microbiota, immunity, redox status, and enzyme activity[29].

The previous investigations have found that these phytoconstituents have potent anti-inflammatory properties. Despite this, the study had some limitations. First, the trial was brief in duration, and a longer pre-treatment duration of up to two or three weeks might be beneficial, especially for determining the efficacy of the aqueous extract. Second, hematological indicators such as C-reactive protein concentrations and biochemical NF-κB analysis were not assessed in the study, which may have confirmed our macroscopic and microscopic findings. Therefore, further research is needed to determine the exact mechanisms of the (isolated) constituent(s) behind its anti-inflammatory and gastroprotective effects.

Results of our study revealed that the crude extract of *S. imbricata* possessed gastroprotective and anti-inflammatory properties and these actions were mediated by supporting the antioxidant enzyme system, decreasing inflammatory mediators, and promoting the repair of epithelial layers. Therefore, *S. imbricata* is a promising folkloric remedy to treat IBD.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

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Authors' contributions

FJ contributed in conceptualization, methodology, investigation, formal analysis and writing of the original draft. QJ was responsible for supervision, article review and editing.

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