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Original article

Evaluating Anti-Inflammatory Effect of Hydroalcoholic Extracts of *Citrus medica* L. Pulp and Peel on Rat Model of Acute Colitis

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Abstract

Background and objectives: *Citrus medica* L. (citron) belongs to the Rutaceae family and contains several bioactive compounds including flavonoids, alkaloids, coumarins and essential oils with great antioxidant and anti-inflammatory properties. Since alleviating inflammation and ulcers have been suggested for these bio-compounds, this study was conducted in a model of experimental colitis. **Methods:** In order to standardize the extracts prepared by the maceration, total flavonoids were assayed. Colitis was induced by acetic acid in male Wistar rats. Rats received three doses (150, 300, and 600 mg/kg) of the citron's peel and pulp hydroalcoholic extracts for five days. Dexamethasone (1mg/kg) and sulfasalazine (150 mg/kg) were administered as reference medications. The macroscopic parameters including weight of colon, ulcerated area, the severity and indices of ulcers, as well as tissue microscopic features were assessed. In addition, levels of myeloperoxidase (MPO) activity and malondialdehyde (MDA) were measured. **Results:** Total flavonoid contents for peel and pulp extracts were obtained 6.25 and 37.5 mg/g equivalent to quercetin, respectively. Both citron extracts demonstrated great anti-inflammatory and antioxidant effects by decreasing MDA and MPO levels comparable to the reference drugs. Administration of the citron extracts also significantly reduced colon weight as well as ulcer index, score, and area compared to the control group. In addition, pathologic parameters such as inflammation, cryptal damage and leucocyte infiltration were considerably decreased in rats received citron extracts. **Conclusions:** Both citron extracts showed anti-inflammatory effects on experimental acute colitis. Further investigations are required to suggest these extracts for colitis treatment in clinical setting.

Keywords: *Citrus medica*; colitis; inflammation; plant extract; rats

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Introduction

Citrus medica L. (citron, "Balange" or "Otroj" in Persian), characterized by distinctive aroma and tastes, is a member of the Rutaceae family mainly grown in tropical areas [1]. Citron fruits are widely consumed around the world. Based on the regional distribution they are commercially classified into oranges (*Citrus sinensis*), lemons (*Citrus limon*), limes (*Citrus aurantiifolia*), mandarins (*Citrus reticulata*), pomeloes (*Citrus maxima*), and citrons (*Citrus medica*) [2]. Citrons

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are generally rich in bioactive constituents including flavonoids and flavonols (apigenin, hesperetin, and naringenin), neolignans, terpenoids (limonin and limonexin), alkaloids (N-methyltyramine and synephrine), coumarins (meranzin, scopoletin, andmarmin), and polysaccharides with various biological effects [3,4]. In Chinese traditional medicine, citron was primarily applied for healing of intestinal and stomach ailments, liver and pulmonary troubles, sea-sickness, poisoning, and for expelling phlegm [5]. Modern pharmacological investigations have identified beneficial properties of citron against inflammation, catarrh, cancer, hypertension, hyperglycemia, depression, and aging [6,7]. In addition, antimicrobial, antioxidant, expectorant, and cardio-protective effects of citron have been documented in literature [1]. The essential oil of citron are broadly utilized in the medicinal, pharmaceutical, food, and cosmetic industries. The citron peels contain coumarins, and umbelliferone as well as essential oils including citral (23.12%), so-limonene (39.37%), and limonene (21.78%) [1]. High concentrations of phenolic compounds in citron peel and pulp account for great antioxidant effects while citrus flavonoids promote diuretic, expectorant , and stomachic actions along with inflammation suppression [8]. The pulp of citron fruit contains essential oil components such as citral, limonene, and linalool and mucilage widely used to alleviate asthma, sore throat, cough, thirst, hiccough, vomiting, and earache [8,9]. Malleshappa et al. demonstrated the anti-inflammatory properties of citron peels by in vitro human red blood cell membrane stabilization assay and in vivo carrageenan-induced rat paw edema model. Accordingly, citron peels significantly decreased paw edema and exhibited anti-inflammatory effects in rat model [10]. Pathogenesis of colitis is associated with epithelial barrier defects, infections, oxidative stress, and formation of reactive oxygen species in the bowel [11]. Impaired immunity balance, infiltration and accumulation of neutrophils and secretion of pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) are key factors in the initiation of colitis [12]. Despite recent advances, the majority of therapeutic agents used for the treatment of colitis do not often lead to definitive treatment like amino-salicylic acid derivatives and corticosteroids. These agents require long-term administration leading to several side effects [13]. In spite of the beneficial effects of these agents, limited efficiency and a high rate of disease recurrence are among their disadvantages [14]. Therefore, the development of novel therapeutic agents with high efficiency and fewer side effects is of great importance. Herein, we investigated the anti-inflammatory effects of hydroalcoholic extracts of citron pulps and peels in a rat model of acetic acid-induced ulcerative colitis.

Material and Methods

Ethical consideration

All the animal procedures were carried out according to the national guidelines and recommendations represented by the Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.RESEARCH.REC.1400.137).

Chemicals

Dexamethasone (8 mg/2 mL; Chimidarou Co., Iran) was purchased from a community pharmacy. Sulfasalazine powder was gifted from Darou-Pakhsh, Iran. Ethanol and formaldehyde were acquired from Merck (Germany). The kit for malondialdehyde (MDA) assessment was purchased from NavandSalamat Corporation (Iran). O-dianisidine dihydrochloride (ODZ), quercetin and hexadecyl-trimethyl ammonium bromide (HDTAB) were obtained from Sigma-Aldrich (Germany).

Plant material

The fruits of the *Citrus medica* L. were purchased from a local market in Isfahan, Iran. The genus and variety of the fruits were authenticated by the Pharmacognosy Department of Isfahan School of Pharmacy and Pharmaceutical Sciences. The citron peels were removed and the fruits were manually flaked followed by rinsing with water for cleaning. The washed peels and pulps were then dried in an aerated room and the dried materials were ground using an electric mortar. The final powder (300 g) was immersed within ethanol/water (70/30%) for 2 days. Thereafter, the mixtures were filtered by the Büchner funnel. To get a semisolid viscous mass of each sample, the ethanol was vaporized using Rota-vaporizer [15].

Total flavonoids content determination

Total flavonoids of peels and pulps were

separately determined based on the colorimetric aluminum chloride method [16]. Accordingly, 0.5 mL of each extract was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The solutions were stored at room temperature for 30 min and the absorbance of each sample was measured at 420 nm using spectrophotometer (Perkin Elmer UV-Visible, USA). Total flavonoids were eventually estimated regarding the quercetin calibration curve (12.5-100 mg/mL) [17].

Animals

In the present study, 60 male Wistar rats weighing 180-220 g were supplied from the animal house of Isfahan School of Pharmacy, Isfahan, Iran. The rats were maintained in clean polypropylene cages (3 rats/cage) with a well-aerated standard stainless steel frame and wood mulch at the bottom of the cages. The animals had free access to chow pellets and water. The controlled condition for the maintenance of the rats was as followed: temperature; 22 ± 2°C, humidity; 55 ± 5%, and 12/12 h light/dark cycles.

Experimental groups

The rats were randomly divided into ten groups of 6 rats as follows:

Group 1 (normal): the rats without colitis received vehicle (normal saline/tween80 (0.1%), 5 mL/kg, p.o.) for 5 days.

Group 2 (control): the rats received vehicle (5 mL/kg, p.o.), 2 h before colitis induction, continued once daily for 5 days.

Groups 3, 4, and 5 (CPUE): the rats received citron pulp extract (150, 300, and 600 mg/kg, p.o.) 2 h before colitis induction, continued once daily for 5 days [18].

Groups 6, 7, and 8 (CPEE): the rats received citron peel extract (150, 300, and 600 mg/kg, p.o.) 2 h before colitis induction, continued once daily for 5 days [18].

Groups 9 and 10 (Dex. and Sulf.): the rats received dexamethasone (1 mg/kg, i.p.) or sulfasalazine (150 mg/kg, p.o.) as reference drugs, 2 h before colitis induction, continued once daily for 5 days.

All groups were euthanized 24 h after the last treatment through inhalation of CO_2 in a special chamber.

Induction of colitis in rats

After 24 h of fasting, the rats were anesthetized using ketamine/xylazine (60/10 mg/kg) and a thin and flexible catheter (2 mm inner diameter and 8 cm length) was placed into the anus and 2 mL acetic acid 3% was injected. Before taking the catheter out, the rats were held in head-down position to avoid expelling of acid [19].

Macroscopic assessment of colon

Colon sections were incised longitudinally and washed with normal saline and then weighted. Colon samples were fixed on a white sheet and imaged using Sony^R camera. Colitis lesions were graded based on a scale of 0 to 3. Zero (no macroscopic findings), 1 (inflammation, edema, thickness, and superficial erosions), 2 (hemorrhagic spots, bleeding, and deep ulcers), and 3 (necrosis and/or perforation) [20]. Ulcer area was also measured by Fiji-win 32 software. Ulcer index (UI) was finally calculated for each sample by using UI = US (ulcer score) + UA (mean of ulcer aria) equation [21].

Histopathologic assessment of colon

The incised colon tissues were fixed in 10% buffered formalin for an hour followed by washing with tap water, dehydration, and clearance in xylene. Colon samples were embedded in paraffin checked to be 5–6 microns thick and were stained with hematoxylin and eosin (H&E) stain for light microscopy [22]. The histopathological assessment of colon lesions was carried out by a scoring system based on the inflammatory severity and extent, crypt damage, and leukocyte infiltration as described by authors in previous works [23,24].

Myeloperoxidase (MPO) assessment of colonic tissue

MPO was determined using colonic tissue according to the following protocol. Colon samples were weighed (0.1 g) and homogenized in 10 mM potassium phosphate buffer (pH=7) containing 0.5% hexadecyl trimethyl-ammonium bromide (0.5% w/v) using a homogenizer. Homogenizing was conducted through 3 cycles of 45 seconds with 1 min intervals. The sample solutions were sonicated on the ice for 10 sec. and centrifuged at 20000 rpm for 30 min. Finally, H_2O_2 (0.1 mM) and o-dianisidine dihydrochloride (1.6 mM) were added to each sample. MPO activity was quantitatively estimated based on the absorbance of each sample at 450 nm at 0 and 3 min time intervals. MPO activity was calculated and reported as U/100 mg of colon tissue [25].

Malondialdehyde (MDA) assessment of colonic tissue

MDA was assessed using the specific assay kit (Navand-Salamat, Urmia). According to the manufacturer's instruction, potassium chloride solution (1.15 % w/v) was prepared and 1 mL was added to homogenized colon tissues (0.1 g). The homogenized samples were centrifuged for 10 min at 1200 rpm and the supernatant was picked up to measure MDA level; the absorbance was determined at 532nm [26].

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) with Tukey as post hoc test. For analysis of the weight changes, t-paired test was applied. Mann-Whitney U test was also used for scoring data using SPSS software (version16). The results were reported as mean ± SEM (standard error of mean) and the $p<0.05$ was considered statistically significant.

Results and Discussion

The weight of dried citron peel (CPEE) and pulp (CPUE) extracts were 94.1% and 93.4% respectively. Besides, the yield values obtained from CPEE and CPUE were 33.1% and 37.8%, respectively. Based on the colorimetric aluminum chloride method, the total content of flavonoids in CPEE and CPUE were 6.25 and 37.5 mg/g equivalent to quercetin, respectively. Ghasemi et al. clarified the total flavonoid contents of peels and tissues of 13 citrus species with the same method ranging from 0.3-31.1 mg/g in peels and 0.3-17.1 mg/g in tissues, which are close to the results obtained in this research [17]. In another study conducted by Wang et al., total phenolics content of four different parts of 35 varieties of *Citrus reticulate* including flavedo (peel), albedo (mesocarp), segment membrane and juice were studied. They found that the total phenolics content varied between the varieties and tissues of citrus fruits close to the results of this study [27]. In the current study, colitis was induced in rats by acetic acid instillation via rectum which closely mimics the pathogenesis and histological characteristics of the human colitis [28,29]. As shown in Table 1, weight loss caused by ulcerative colitis occurred in all groups, but it was significant only in the negative control, CPEE150 and dexamethasone treated groups. These findings indicate that citron extracts with different applied doses were effective in preventing weight loss; however, the lowest dose of peel extract (CPEE150) was not effective. The weight lowering effect of dexamethasone could possibly be attributed to the catabolic properties of glucocorticoids, which has been reported in this series of experiments [30]. By referring to Table 2, the results of the macroscopic examinations of the colon tissues in the experimental groups can be seen. In the normal group, none of the colitis features were visible. On the contrary, in the negative control group, the variables related to colitis (such as the area and severity of ulcer and inflammation) were at their highest level. Administration of citron hydroalcoholic extracts led to remarkable alterations of macroscopic parameters so that the best effect ($p<0.001$ vs control group) was obtained with CPEE 300 and CPUE 600 (Table 2).

Sulfasalazine and dexamethasone as reference drugs were also effective ($p<0.001$) in reducing these parameters as expected. Figure 1 illustrats the macroscopic features of colitis in different groups. It can be observed that the administration of CPEE300 and CPUE600 diminished the thickness of the colon, edema, hemorrhage and necrosis compared to the control group ($p<0.01$).

Table 1. Weight of whole body (g) in rats of experimental groups

Groups/doses (mg/kg)	Before treatment	After treatment	p-Value
Normal	201.0±18.1	206±20.1	NS
Control	168.8±17.2	152.0±15.7	<0.01
CPEE150	186.2±8.8	174.3±8.9	<0.01
CPEE300	197.7±16.2	192.3±15.4	NS
CPEE600	184.8±11.4	176.7±11.2	NS
CPUE150	201.0±7.2	195.0±5.3	NS
CPUE300	173.0±7.9	169.5±9.0	NS
CPUE600	170.5±14.7	171.0±15.4	NS
Dex.1	190.0±8.4	180.3±9.4	<0.01
Sulf.150	195.5±14.5	190.0±14.2	NS

Normal and control groups were treated with 5 mL/kg distilled water; CPEE: citron peel extract; CPUE: citron pulp extract; Dex: dexamethasone; Sulf: sulfasalazine; Data are shown as mean ± SEM. Student's t. paired test was used for analysis (n= 6); $p<0.05$ was considered as significant; NS: non-significant

Table 2. Effect of citron extracts on macroscopic parameters of experimental colitis

Groups/Doses (mg/kg)	Ulcer area (cm^2)	Ulcer score (0-3)	Ulcer index (0-8)	Colon weight (mg/8 cm)
Normal	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	365 ± 17
Control	2.78 ± 0.44$^{###}$	3.0 (2-3)$^{###}$	5.45 ± 0.60$^{###}$	1060.6 ± 53.1$^{###}$
CPEE150	2.17 ± 0.46	2.0 (2-3)	4.67 ± 0.77	961.3 ± 48.3
CPEE300	0.85 ± 0.18***	1.0 (1-2)***	2.82 ± 0.58***	662.7 ± 24.5***
CPEE600	1.48 ± 0.47*	2.0 (1-3)*	3.47 ± 0.78*	693.6 ± 27.6***
CPUE150	1.92 ± 0.31	1.5 (1-2)*	4.92 ± 0.54	930.4 ± 42.2
CPUE300	1.41 ± 0.47*	1.5 (0-3)**	3.41 ± 0.41**	740.1 ± 40.5**
CPUE600	1.08 ± 0.21**	1.0 (0-2)***	2.86 ± 0.16***	648.8 ± 35.5***
Dex.1	0.67 ± 0.14***	1.0 (0-2)***	2.11 ± 0.61***	585.6 ± 28.8***
Sulf.150	1.09 ± 0.23***	1.5 (1-3)***	3.12 ± 0.59**	614.4 ± 31.3***

Data are shown as mean ± SEM. For score of ulcers, data are shown as median (range). CPEE: citron peel extract (150, 300, 600 mg/Kg); CPUE: citron pulp extract (150, 300, 600 mg/Kg); Dex: dexamethasone; Sulf: sulfasalazine; $^{###}$ $p<0.01$: significant difference versus normal group; * $p<0.05$, ** $p<0.01$, *** $p<0.001$: significant difference versus control group; n=6

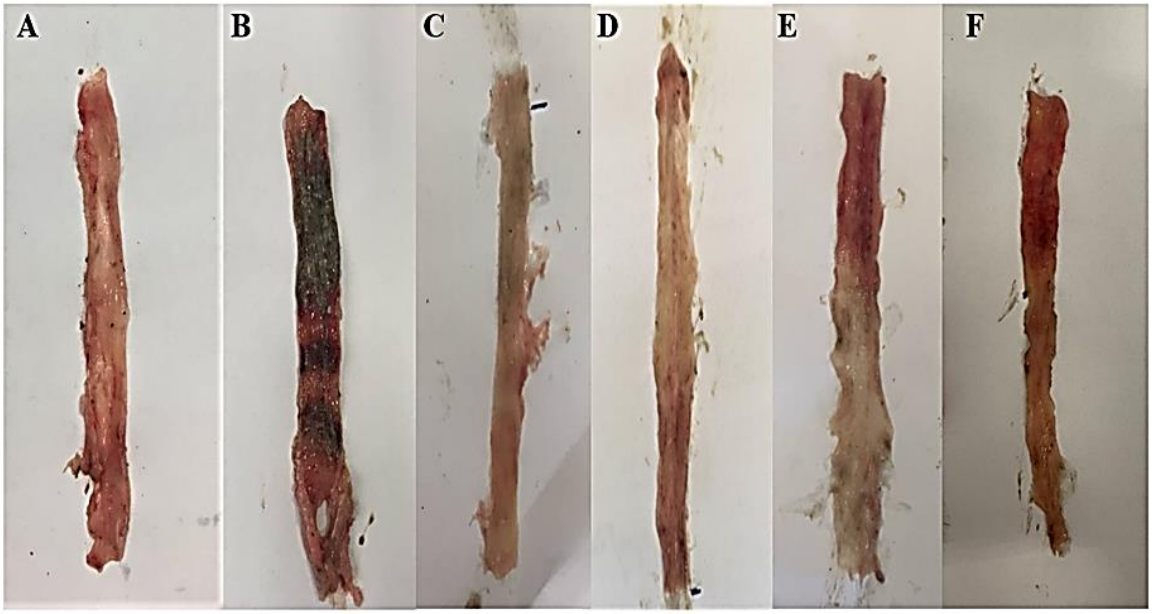

Figure 1. Macroscopic illustration of acetic acid-induced colitis in rats; normal (A), control (B), CPEE (citron peel extract 300 mg/kg, p.o.) (C), CPUE (citron pulp extract 600mg/kg, p.o.) (D), oral sulfasalazine (150 mg/kg, p.o.) (E), dexamethasone (1 mg/kg, i.p.) (F)

After evaluating pathological results, similar findings were obtained. No histological damages were observed in the colon tissue of normal group. On the contrary, the extent of inflammation, crypt damage, leukocyte infiltration, ulcer and necrosis were observed in the highest degree in the colon tissue of the control group (Figure 2). According to the results, the total colitis index was significantly reduced ($p<0.001$) after oral administration of CPEE (300 and 600mg/kg) and CPUE (300 and 600mg/kg) compared to the control group (Table 3). Moreover, the indices of the inflammation were significantly decreased after administration of dexamethasone and sulfasalazine in comparison to the control group ($p<0.001$). These findings showed that not only citron hydroalcoholic extracts were effective after oral administration, but also, this effect was dose-related. The effectiveness of extracts after oral intake can be likely due to the oral bioavailability of effective substances such as flavonoids, flavonols, coumarins, anthocyanides, essential oils, etc [31]. But the healing of colon tissue lesions can also be attributed to non-absorbable active substances such as tannins, pectin, insoluble polysaccharides and glycosylated flavonoids that reach the distal colon through the oral route and act locally [32]. Also, the better effectiveness of the CPUE at the lowest dose (150 mg/kg) can probably be considered due to its higher total flavonoid content compared to the peel of citron (CPEE) (37.5 vs 6.25%).

In a similar study carried out by Dodda et al., quercetin, a key flavonoid in citrus fruits decreased colon thickness and edema compared to the control group [33]. Several studies have confirmed the anti-inflammatory role of citrus extracts on experimental models of the colitis [34,35]. As reported by He et al. beneficial flavonoids of the *Citrus aurantium* L. like naringenin, hesperetin, and nobiletin effectively decreased the inflammation and diarrhea as well as jejunum contraction in rat model of tri-nitrobenzene sulfonic acid (TNBS)-induced colitis that was accompanied by weight loss. They also mentioned that *Citrus aurantium* extract halt weight loss during the period of therapy [36]. On the other hand, *Citrus junos*, also known as Yuzu, contains several phytochemicals that possess anti-inflammatory, antioxidant, and cardio-protective effects [37].

As shown in Table 4, activity levels of MPO and MDA in rats receiving CPEE and CPUE were significantly decreased in comparison to the control group ($p < 0.01$). Likewise, administration of the dexamethasone and sulfasalazine reference drugs lowered MPO and MDA levels significantly ($p<0.001$). Our findings were in accordance with the results of the study performed by Gholap et al. reporting decreased levels of the MDA and MPO in the mice models of acetic acid-induced colitis following the treatment with a combination of *Moringa oleifera* root and a peel extract of *Citrus sinensis* [38].

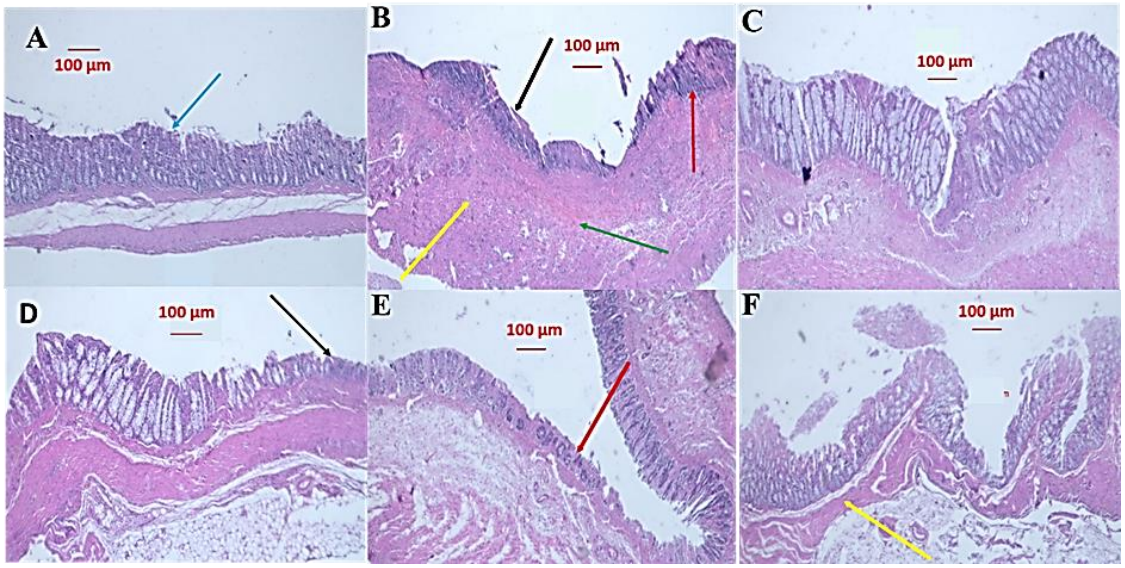

Figure 2. Microscopic presentation of acetic acid-induced colitis in rats; normal (A), blue arrows show normal crypts, control (B), black, red and green and yellow arrows show ulcerated area, crypt damage, leukocyte infiltration and extent of inflammation, respectively, CPEE (citron peel extract 300 mg/kg, p.o.) (C), CPUE (citron pulp extract 600 mg/kg, p.o.) (D), oral sulfasalazine (150 mg/kg, p.o.) (E), dexamethasone (1 mg/kg, i.p.) (F); H&E staining and X40 magnification

Table 3. Effect of citron extracts on histopathologic parameters of experimental colitis

Groups	Inflam. Score (0-3)	Inflam. extent (0-3)	Crypt damage (0-3)	Leu. Infil. (0-3)	TCI (0-12)
Normal	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Control	2.5 (2-3) $^{###}$	3 (2-3) $^{###}$	3 (3-3) $^{###}$	2.16 (2-3) $^{###}$	10.66 (9-12) $^{###}$
CPEE150	2.16 (2-3)	2.16 (2-3)	3.0 (3-3)	2.0 (2-3)	9.32 (9-12)
CPEE300	1.66 (1-3) ***	1.66 (1- 2)***	1.66 (1-3)	1.66 (2-3) ***	6.64 (5-11)**
CPEE600	2.0 (1-3) *	1.66 (1-3)**	1.5 (1-2) *	2.0 (2-3)*	7.16 (5-11)**
CPUE150	2.0 (1-3)	2.5 (1-3)	1.66 (1-3)*	2.66 (2-3)	8.82 (5-12)
CPUE300	1.66 (1-2)*	1.5 (0-3)**	1.66 (1-3) **	1.0 (0-2) **	5.82(2-10)***
CPUE600	1.16 (0-2) ***	1.66 (1- 2)***	1.66 (1-3)**	0.66 (0-2) ***	5.14 (2-9)***
Dex.1	0.66 (0-1) ***	0.66 (0-1)***	1.66 (1-2)***	1.66 (1-2)***	4.64 (2-6)***
Sulf1.50	1.5 (1-2)***	1.5 (1-2)***	1.5 (0-2)**	1.33 (0-2)**	5.83 (2-8)***

Data are shown as median (range); CPEE: citron peel extract (150, 300, 600 mg/Kg); CPUE: citron pulp extract (150, 300, 600 mg/Kg); Inflam: inflammation, Leu: leucocyte; TCI: total colitis index; Dex: dexamethasone; Sulf: sulfasalazine; $^{###}$ $p<0.001$: significant difference versus normal group; * $p<0.05$, ** $p<0.01$, *** $p<0.001$: significant difference versus control group; n=6

Likewise, the protective effects of quercetin as a key flavonoid of the citrus species were confirmed in the acetic acid-induced rat model of colitis by decreased levels of MDA and MPO along with increased contents of glutathione which is involved in tissue repair and immune system function [39]. Another research conducted by Kumar et al. showed that the administration of naringin as an abundant flavonoid of citrus genus could effectively inhibit acetic acid-induced colitis in animal models through suppression of the inflammatory responses. Pre-treatment of the animals with different doses of the naringin alleviated colon mucosal damage and restricted ulcer lesions along with decreased levels of MDA and MPO inflammation markers [40].

Table 4. Effects of citron extracts on myeloperoxidase (MPO) activity and malondialdehyde (MDA) levels of colon tissue

Groups (mg/kg)	MPO (U/100mg)	MDA (nmol/mL)
Normal	0.45±0.08	57.7±10.0
Control	1.51±0.25$^{###}$	171.1±19.1$^{###}$
CPEE150	0.93±0.13**	133.3±16.6**
CPEE300	0.61±0.15***	65.5±8.8***
CPEE600	0.76±0.20***	66.3±11.2***
CPUE150	0.71±0.11***	73.6±9.8***
CPUE300	0.67±0.08***	80.8±9.1***
CPUE600	0.55±0.08***	67.7±10.0***
Dex.1	0.50±0.12***	76.6±12.2***
Sulf.150	0.63±0.17***	71.9±10.4***

Data are shown as (mean ± SEM); CPEE: citron peel extract (150, 300, 600 mg/Kg); CPUE: citron pulp extract (150, 300, 600 mg/Kg); Dex: dexamethasone (1 mg/kg); Sulf: sulfasalazine (150 mg/kg); ### p<0.01: significant difference versus normal group; * p<0.05, ** p<0.01, *** p<0.001: significant difference versus control group, n=6

In a previous study, Khan et al. demonstrated the antioxidant and anti-inflammatory effects of *Citrus sinensis* L. and *Citrus paradisi* L. juices on rat models of TNBS-induced UC [41]. It was found that the administration of different doses the juices reduced MPO, glutathione activity (GSH), alkaline phosphatase (ALP) and C reactive peptide (CRP) compared to the control group. According to the results of the current study and base on the above mentioned research, it seems that *Citrus medica* juice will be probably effective in the treatment of ulcerative colitis and could be introduced as a suitable candidate for further studies [41]. By reviewing the studies that have been conducted on the anti-inflammatory effects of a number citrus species (*C. sinensis, C. paradise, C. aurantium*) in different models of colitis, it is concluded that the peel and/or pulp of these fruits have almost a similar efficacy within the range of doses used (100-600 mg/kg) in the current study. Therefore, it seems that there are similar active ingredients in these species, among which naringine, hesperidin and nobeltin are more important than others [37,40-41].

Conclusion

Taking together, our findings demonstrated the anti-inflammatory and anti-ulcerative effects of the hydroalcoholic extracts of the *Citron medica* L. peel and pulp extracts in a rat model of colitis. It is also concluded that CPEE300 and CPUE600 showed the best impact on both macroscopic and microscopic as well as biochemical parameters of colitis comparable to applied reference drugs. Many effective substances like flavonoids, flavonols, tannins and pectins that could be abundantly found in citron can be involved in the occurrence of these beneficial therapeutic effects. So more experiments are necessary for the exact identification of these effective substances and their mechanisms of action.

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Author contributions

Mohsen Minaiyan presented the idea of research, designed and supervised all of the parts related to interventions, induction of colitis and statistical analysis of data; Amir Hossein Keyvanara, executed all of the experiments and interventions under supervision of professors; Afsaneh Yegdaneh designed and supervised all of the experiments related to identification, preparation and evaluation of herbal materials and extracts; Ardeshir Talebi designed and supervised all of the experiments related to the sampling, preparing and evaluation of tissues for histopathologic assessment. All authors contributed in writing, revision and preparation of manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

CPEE: citron peel extract; CPUE: citron pulp extract; MPO: myeloperoxidase; MDA: malondealdehyde; TNBS: tri-nitrobenzene sulfonic acid