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Protective effect of *Melissa officinalis* against acetic acid-induced ulcerative colitis in rat models: an experimental study

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Abstract

Background Inflammation and oxidative activities within the gut play major roles in the pathogenesis of ulcerative colitis (UC). We aimed to determine the effect of *Melissa officinalis*, an antioxidant and anti-inflammatory agent, on the colon histological characteristics in acetic acid (AA)-induced UC in rat models.

Methods Thirty-six male rats with AA-induced colitis were divided into 5 groups: no treatment (AA); daily treatment with 300 mg/kg *Melissa officinalis* orally (MO) and rectally (MR); and 100 mg/kg mesalamine orally (AO) and rectally (AR). Macroscopic and histopathological evaluation of the colon, along with a biochemical laboratory evaluation, were performed 10 days after UC induction.

Results All treatment groups demonstrated lower macroscopic grading scores compared to the AA group. After treatment with MO, 42.9% of cases demonstrated no macroscopic changes, while 14.3% demonstrated only mucosal erythema. In the MR group 28.6% of rats had no changes in their mucosal lining and 28.6% had only mucosal erythema. Following histopathological evaluation, the AO group had lower scores regarding the severity of ulcer, inflammation, destruction, crypt abscess, and disorganization compared to the MO group. (P=0.02) The MR group demonstrated lower microscopic scores compared to the MO group, and also lower macroscopic scores compared to the AR group, although not significantly (P>0.05).

Conclusions Both oral and topical administration of *Melissa officinalis* have satisfactory healing properties compared to mesalamine, with topical route having better results. Therefore, further studies are needed to establish the benefit of *Melissa officinalis* administration (both orally and topically) within a UC treatment protocol.

Keywords Anti-inflammatory effects, *Melissa officinalis*, mesalamine, treatment, ulcerative colitis

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Introduction

Ulcerative colitis (UC) is a form of chronic inflammatory bowel disease (IBD) that disrupts the mucosal barrier of the gastrointestinal tract, and is also known to increase the risk of colorectal cancer [1]. Furthermore, there has been a report of increased incidence and prevalence of the disease [2]. The incidence also varies among countries, with the annual incidence of UC ranging from 8.8-23.1 per 100,000 person-years in North America, 7.3-17.4 in Oceania, and 0.6-24.3 per 100,000 person-years in Europe [3,4].

The exact cause of UC has not yet been clarified; however, studies have established certain risk factors, such as stress,

smoking, familial history and prior appendectomy, that could play a role in its development [2]. The current standardized treatment of UC includes the administration of corticosteroids (e.g., prednisolone), aminosalicylates (e.g., 5-amino salicylic acid [5-ASA], sulfasalazine, mesalamine), immunomodulator agents and antibiotics. However, those medications have been shown to have several bothersome side effects, including headache, nausea, abdominal pain, lung infection, inflammation of the pancreas, and renal damage [5]. As it is hypothesized that the body's immune system, specifically its inflammatory and oxidative responses, contribute to the development of UC, many have attempted to study substances and agents that act on those responses and thus might help in the treatment of the disease, while mitigating side-effects [6]. Many efforts have been made to find more effective and benign treatments, focused on reducing the inflammatory and oxidative reactions in the process of UC development [7,8]. It is assumed that natural products with proven anti-inflammatory and antioxidative effects might have the potential to act as an alternative treatment for UC, with fewer side-effects [9,10].

Melissa officinalis (Melissa), also known as lemon balm, is a wild herb that is known to have special effects, such as relaxation, relief of nervousness, reduction in dizziness and headache, provision of energy and facilitation of digestion. The infusion and topical lotion from Melissa leaves extract are effective in relieving pain and healing wounds and injuries [11]. Melissa has also shown anti-inflammatory, antioxidant and antibacterial effects that may also be useful in treating diseases that are based on inflammatory and oxidative responses of the body, such as UC [11,12]. In this study, we aimed to evaluate the effects of Melissa on the pathological parameters and inflammatory markers of acetic acid (AA)-induced UC in rats.

Materials and methods

Plant extraction and drug preparation

Melisa leaves were supplied by the SIMR company, Shiraz, Iran (Voucher number: 31112) as a dried powder. The drug-extract ratio was 60:1, the dried extract corresponding to 1.63% of the primary raw plant's leaf material. The hydroalcoholic extract (65% v/v) was obtained by macerating 20 g of the herbal material for 1 week in the solution at 40°C in darkness. The solution was then decanted from the extracted leaf residues, filtered and stored at -4°C. The concentration was 100 mg/mL, with reference to the initial dried herbal material. The vehicle was prepared using carboxy-methylcellulose (CMC) 0.3% (v/w) solution, based on previous reports [13]. In accordance with a previously conducted study, the concentration of 300 mg/kg of Melissa extract was chosen for the main experiment, to be administered using a CMC vehicle both rectally and orally [14-16].

Before performing the main experiment, in a pilot study, oral and rectal administrations of the vehicle were evaluated and compared with an untreated group of rats with AA-induced colitis. No beneficial effect of the vehicle was seen in comparison with the rats that received no treatment.

Study design

The sample size was calibrated based on previous studies, and while assessing the risk of drop-out [17,18]. The Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran approved the experimental protocol, and all the criteria for taking care of laboratory animals outlined in the "Guide for the Care and Use of Laboratory Animals" were applied (Ethical code: IR.SUMS.REC.1394.s1101). All efforts were made to keep animal distress to a minimum and to use only the number of animals essential to attain reliable results.

The animals were maintained under standard conditions (12 h light/dark cycle; 24±3°C, 45-55% humidity) and free access to standard food and water *ad libitum*. They were acclimatized to laboratory conditions for a week prior to the experiment. The animal study was performed during the daylight portion between 09:00 and 12:00 am, to avoid possible circadian impacts. The health status and body weight of animals were monitored daily and a loss of more than 20% of body weight was considered the threshold for a humane endpoint (none of the subjects met this criterion).

Animal grouping

Thirty-six male Wistar rats (180±20 g) were obtained from the animal house of Shiraz university of medical sciences, Shiraz, Iran. The animals were divided into 5 groups: the control group (AA; n=4) consisted of rats with AA-induced colitis, which received no treatment; experimental groups MO (n=8) and MR (n=8), in which colitis was induced and which received 300 mg/kg Melissa solution daily orally and rectally, respectively; and the AO (n=8) and AR groups (n=8), which received a dose of 100 mg/kg mesalamine (5-ASA) orally and rectally, respectively.

Intervention

In accordance with previous experiments, all the treatments were started 4 days before induction of colitis, and on day 5 of the study colitis was induced [19]. One of the standardized

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experimental models of UC is through the induction of colitis by AA [20]. The oral treatments were given for 10 days using oral gavage; the rectal administration was performed using a 2-mm diameter polypropylene tube inserted into the colon to a distance of 5-8 cm, up to the limit where resistance was detected.

For induction of colitis, on the fourth day of the study the animals were fasted overnight with access to water *ad libitum*. On the fifth day, after 2 hours' administration of the treatments, the rats were anesthetized by ether inhalation and a polypropylene tube (2-mm diameter) was inserted through the rectum of the animal into the colon to a distance of 6-8 cm, depending on the body length. An AA (Sigma Aldrich, St. Louis, USA) solution (2 mL, 3% v/v) in 0.9% normal saline was instilled into the colon and the animal was maintained in a supine Trendelenburg position for about 30 sec to prevent leakage of the solution [19]. This method of AA-induction of colitis has also been examined in previous investigations [20-22].

Data collection

The treatments were continued until day 10 of the experiment, when the animals were anesthetized with ether inhalation and blood was collected by cardiac puncture for biochemical evaluations, including C-reactive protein (CRP), superoxide dismutase (SOD), white blood cell count (WBC), hemoglobin (HGB), and platelets. The enzymatic activities of SOD were based on the method developed by Misra and Fridovich [23]. Subsequently, we sacrificed the animals by decapitation, and their colons was dissected. A longitudinal incision was made to remove and open the distal part of the colon, approximately 8 cm. The mucosa was cleaned with saline solution, and mucosal injury was evaluated (macroscopically) in accordance with a previously described method by Millar *et al*. This uses an arbitrary scale with a 0-4 range to assign inflammation ratings based on the clinical characteristics of the colon: 0, no macroscopic alterations; 1, just mucosal erythema; 2, mild mucosal edema, slight bleeding, or minor erosions; 3, moderate edema, slight bleeding ulcers, or erosions; and 4, severe ulceration, edema, and tissue necrosis [24]. Additionally, samples were kept in 10% formalin for histological analysis.

Colonic samples were collected 2-4 cm from the anus. The tissue was then fixed in phosphate-buffered formaldehyde, embedded in paraffin, processed into 5-mm sections, stained with hematoxylin and eosin, examined under a light microscope, and eventually graded by an expert pathologist in a blinded fashion. The degree of the inflammatory reaction in the tissue was assessed using a histological grading system. Depending on the severity of changes, each parameter assessed was scored from 0-3 (0, no change; 1, mild; 2, moderate; 3, severe). The factors taken into consideration and subjectively graded included ulceration, inflammatory cell infiltration, mucosa damage, disarray and crypt abscess. Fig. 1 shows 2 colon samples from the MR group and Fig. 2 shows a sample of AA-induced colitis in different groups of the study.

Figure 1 Macroscopic evaluation of the excised distal part of the colon with no macroscopic change (top), compared with severe ulceration, edema and tissue necrosis in 2 subjects treated with rectal administration of *Melissa officinalis* (MR group)

Statistical analysis

SPSS software (v. 26) was used for the analysis. The data were checked for normal distribution and reported as mean±standard deviation or median and interquartile range. Data were analyzed using Fisher's exact test for descriptive data and using an independent sample t-test, Mann-Whitney *U* for 2-parameter evaluation, and one-way analysis of variance (ANOVA) or Kruskal-Wallis tests for multiple parameter evaluations. A *post hoc* test was used for intergroup comparison. A P-value <0.05 was considered as statistically significant.

Results

Out of a total of 36 rats, 7 died after the induction of AA: 1 in the MR group; 1 in the MO group; 2 in the AR group; and 3 in the AO group. There was no significant difference among all groups regarding their initial weight (P=0.71). Based on repeated measures ANOVA, all groups developed a significant decrease in weight after the administration of AA up to the eighth day (P<0.001) (Fig. 3); however, this change was not statistically significant (P=0.11).

The outcomes of the macroscopic evaluation are reported in Table 1. AO had significantly lower scores compared to all groups except the MO group (P=0.11). The MR group also demonstrated lower scores compared to the AR group, although the difference was not significant (P=0.87).

Based on microscopic evaluation, the AO group had significantly lower histopathological scores regarding the severity of ulcer, inflammation, crypt abscess, destruction and disorganization compared to the MO group. The AO group mainly demonstrated no or mild changes, while the MO group showed a level of mild to severe changes in each of the abovementioned parameters (Table 1) (P=0.02; P=0.01; P=0.004; P=0.01; P=0.01, respectively). Although the MR group demonstrated higher microscopic scores than the AR group in the histopathological evaluation of the abovementioned factors, this difference was not statistically significant (P=0.65; P=0.81; P=0.84; P=0.39; P=0.21, respectively).

Biochemical data revealed no significant differences either among all treated groups, or compared to the AA group. The

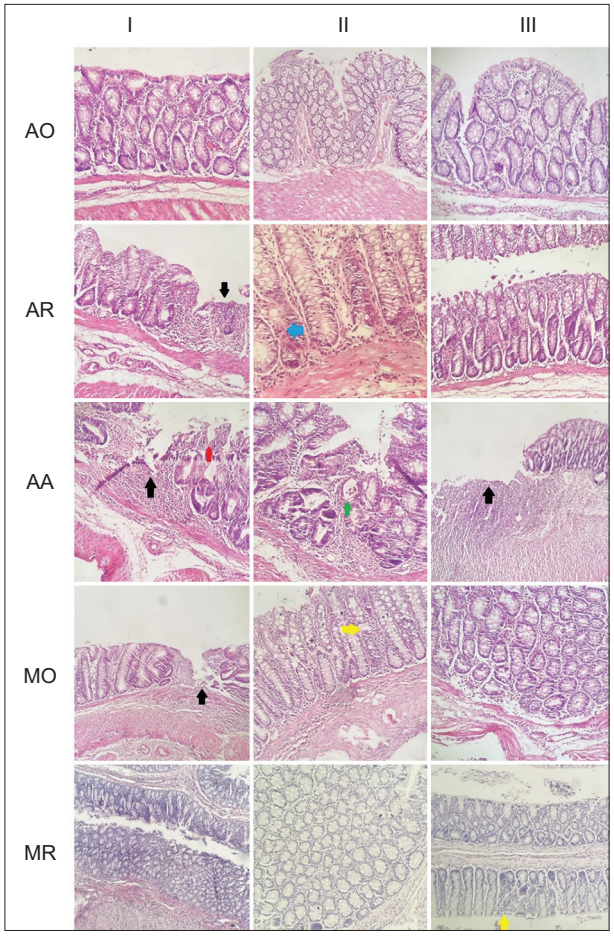

Figure 2 Hematoxylin and eosin staining of acetic acid-induced ulcerative colitis in the different groups. *AO*: orally administered mesalamine. I, II, III: no gland destruction no disorganization and no inflammation (normal mucosal and gland architecture). *AR*: rectally administered mesalamine. I: focal surface ulceration; II: mild active colitis (cryptitis); III: no destruction and gland disorganization. *AA*: without treatment. I: gland destruction and mucosal ulceration; II: crypt abscess and the overall slide demonstrates disorganization; III: surface ulceration. *MO*: orally administered *Melissa officinalis* (Melissa). I: ulceration and gland destruction; II: mucosal inflammation; III: mild disarray of gland architecture. *MR*: rectally administered Melissa. I: Mild disorganization of glands; II: no change in gland and mucosal architecture; III: mild mucosal inflammation. Black arrows show focal surface ulceration; yellow arrow shows mucosal inflammation; blue arrows show mild active colitis (cryptitis); green arrows show crypt abscess; red arrow shows gland destruction

outcomes of the biochemical laboratory examinations are shown in Table 1.

Discussion

The administration of Melissa, a substance known for its anti-inflammatory and antioxidative effects, showed significant results in reversing the damage to the intestinal

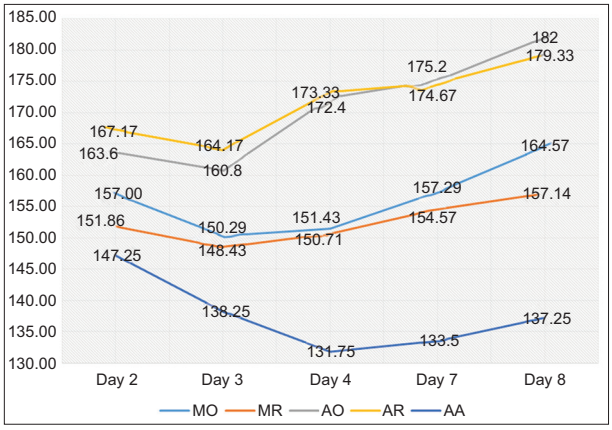

Figure 3 Comparison of the weight changes of rats with ulcerative colitis throughout the 5-day period of the study following treatment with *Melissa officinalis* and mesalamine in comparison to the control group (AA). The groups are as follows: *Melissa officinalis* Oral (MO), received 300 mg/kg oral treatment with *Melissa officinalis*; *Melissa officinalis* rectal (MR), received 300 mg/kg rectal treatment with *Melissa officinalis*; 5-aminosalicylic acid (5-ASA) oral (AO) received 100 mg/kg oral treatment with mesalamine; 5-ASA rectal (AR) received 100 mg/kg rectal treatment with mesalamine; acetic acid (AA) denotes untreated animals with induced colitis

mucosa due to AA. In addition, 42.9% of cases in the MO group demonstrated no macroscopic changes, while 14.3% demonstrated only mucosal erythema. The MO group had no significant differences from the reference group (AO) regarding macroscopic features. On the other hand, the rectal administration of Melissa (MR) was superior to rectal mesalamine (AR group); although these differences were non-significant. The similarities in the healing properties of these medications cannot be overlooked. Furthermore, our results confirmed that oral administration of both Melissa and mesalamine was superior to their topical equivalents in regard to the resulting macroscopic changes. Overall, we advise the administration of Melissa (both orally and topically) as an add-on or even an alternative treatment in UC.

In this study, the oral administration of mesalamine (AO group) demonstrated the most satisfactory results among all evaluated parameters, consistent with its administration as the reference drug in the management of UC [25]. Many studies have indicated that administering antioxidative and anti-inflammatory agents to the gut leads to improvements in the course of UC and reduces the disease's morbidity and mortality, in both experiments and human trials [19,26]. The application of herbal medicine in UC has been widely reported in the literature. Some of the administered treatments include Gegen Qinlian Decoction, fuzi-ganjiang, *Ramak* and *Cupressus sempervirens*, which are administered because of their antioxidant, immune boosting, anti-inflammatory and healing properties [27-31].

Although Melissa's therapeutic properties have been reported in the literature, studies supporting the administration of Melissa in the treatment of IBD are very limited [32,33]. Commercial capsules such as Melipass®, which is a flavonoid-rich phytotherapeutic agent based on

Table 1 The effect of *Melissa officinalis* extracts on acetic acid-induced colitis in rats

Factor	Group*					P-value**
	MO; n=7	MR; n=7	AO; n=5	AR; n=6	AA; n=4	
			Gross examination			
Macroscopic Grade ***						
Total	1.0 [2]	1.0 [4]	0 [0]	1.5 [2]	2.5 [3]	0.04
0	3 (42.9)	2 (28.6)	5 (100)	0 (0)	0 (0)	0.07
1	1 (14.3)	2 (28.6)	0 (0)	3 (50.0)	1 (25.0)	
2	2 (28.6)	1 (14.3)	0 (0)	1 (16.7)	1 (25.0)	
3	0 (0)	0 (0)	0 (0)	2 (33.3)	1 (25.0)	
4	1 (14.3)	2 (28.6)	0 (0)	0 (0)	1 (25.0)	
			Histological grade			
Ulcer						
Total	1.0 [2]	1.0 [1]	0 [0]	0 [1]	0.5 [2]	0.16
0: No Change	2 (28.6)	3 (42.9)	5 (100)	4 (66.7)	2 (50.0)	0.67
1: Mild	2 (28.6)	3 (42.9)	0 (0)	1 (16.7)	1 (25.0)	
2: Moderate	2 (28.6)	1 (14.3)	0 (0)	1 (16.7)	1 (25.0)	
3: Severe	1 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)	
Inflammation						
Total	2 [2]	1.0 [1]	0 [0]	1 [1]	1 [2]	0.13
1: Mild	2 (28.6)	5 (71.4)	5 (100)	4 (66.7)	3 (75.0)	0.28
2: Moderate	2 (28.6)	1 (14.3)	0 (0)	2 (33.3)	0 (0)	
3: Severe	3 (42.9)	1 (14.3)	0 (0)	0 (0)	1 (25.0)	
Destruction						
Total	2.0 [2]	1.0 [2]	0 [0]	0.5 [1]	0.5 [1]	0.10
No Change	2 (28.6)	3 (42.9)	5 (100)	3 (50.0)	2 (50.0)	0.11
Mild	1 (14.3)	2 (28.6)	0 (0)	3 (50.0)	2 (50.0)	
Moderate	4 (57.1)	2 (28.6)	0 (0)	0 (0)	0 (0)	
Disorganization						
Total	2.0 [2]	1.0 [2]	0 [0]	0 [1]	0.5 [1]	0.08
No Change	2 (28.6)	3 (42.9)	5 (100)	4 (66.7)	2 (50.0)	0.12
Mild	1 (14.3)	2 (28.6)	0 (0)	2 (33.3)	2 (50.0)	
Moderate	4 (57.1)	2 (28.6)	0 (0)	0 (0)	0 (0)	
Crypt abscess						
Total	1.0 [2]	1.0 [1]	0 [0]	0.5 [1]	0.5 [1]	0.11
No Change	2 (28.6)	3 (42.9)	5 (100)	3 (50.0)	2 (50.0)	0.12
Mild	2 (28.6)	4 (57.1)	0 (0)	3 (50.0)	2 (50.0)	
Moderate	3 (42.9)	0 (0)	0 (0)	0 (0)	0 (0)	
			Biochemical results			
CRP (mg/dL)	2.11±1.26	3.51±3.56	1.87±0.20	1.98±0.20	2.32±0.63	0.53
SOD (units/mL)	5.49±2.57	5.50±2.24	3.84±2.15	3.45±1.66	2.70±1.41	0.13
WBC ($\times 10^9$/L)	6.21±3.46	8.10±6.71	8.22±3.15	11.50±5.39	10.18±5.60	0.43
HGB (g/dL)	12.36±1.10	12.54±1.54	12.16±2.28	13.17±1.80	11.50±1.28	0.61
PLT ($\times 10^9$/L)	874.86±335.16	397.20±443.22	600.20±621.02	796.33±430.63	628.25±476.44	0.37

Data are presented either as frequency (percentage), median [interquartile range], or mean±standard deviation.

*The groups are described as: MO, received 300 mg/kg oral treatment with Melissa officinalis; MR, received 300 mg/kg rectal treatment with Melissa officinalis; AO received 100 mg/kg oral treatment with mesalamine; AR received 100 mg/kg rectal treatment with mesalamine; AA, the untreated animals with induced colitis

**Fishers' exact or one-way analysis of variance/Kruskal-Wallis test

***Scores are defined as 0, no macroscopic alterations; 1, just mucosal erythema; 2, mild mucosal edema, slight bleeding, or minor erosions; 3, moderate edema, slight bleeding ulcers, or erosions; 4, severe ulceration, edema, and tissue necrosis

CRP, C-reactive protein; HGB, hemoglobin; SOD, superoxide dismutase; WBC, white blood cells

127.5 mg of dried Melissa and 127.5 mg *Passiflora caerulea*, are used for the treatment of IBD, while also being effective in the treatment of other gastrointestinal disorders, insomnia and anxiety [34,35]. On a macroscopic field and paraclinical

evaluation, Melissa demonstrated similar properties to those of mesalamine, however, in our microscopic evaluation, Melissa, especially through the oral administration route, depicted poorer scores regarding ulceration, inflammation, destruction, disorganization, and crypt abscess, compared to the other groups. Protecting the colon structure from any pathologies caused by the inflammatory process, such as disorganization, adhesions, ulcerations, etc., is of the most important goals in the treatment of UC [26]. Therefore, based on the lack of studies regarding the effects of Melissa in the treatment of UC, and also our findings, further studies should be performed before the administration of these medications to human subjects.

Biochemical and laboratory changes were not significant in our study; however, we found lower levels of CRP and WBC, and higher SOD and HGB levels in the oral administration groups, compared to the control group. In the rectal administration groups, SOD and HGB levels improved. CRP improvement was only observed in the Melissa group, while WBC improvement was recorded in the mesalamine group. CRP, like other acute-phase reactive proteins, can have a negative effect on different phases of inflammation, which in our study was alleviated with the administration of Melissa. An increase in the SOD level improves colonic inflammation caused by UC [36], while our study showed that alleviation of bowel tract inflammation was achieved with the increase of SOD levels—a finding also supported by other studies on UC [37].

As a limitation of this study, some inflammatory mediators, such as colonic myeloperoxidase, colonic lipid peroxidation, colonic glutathione, and serum lactate dehydrogenase, which are sensitive markers for the inflammation of the bowel, were not evaluated [19]. Moreover, the net weight of the colonic specimen was not taken into account, and this is believed to be a sensitive and reliable marker for the extent and severity of the inflammatory response [38]. In addition, the stool consistency and degree of hematochezia were not documented; therefore, we are unable to add the disease activity index.

In conclusion, our study showed that *Melissa officinalis* has therapeutic effects against AA-induced UC in rats, particularly via topical administration. The advantages of these herbal remedies, given their lower reported adverse effects, should be taken into consideration. Therefore, further studies are needed to uncover the full potential and safety profile of the administration of this natural product as an alternative or complementary treatment in UC.

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Summary Box

What is already known:

- Ulcerative colitis (UC) is a form of chronic inflammatory bowel disease that affects the gastrointestinal tract and increases the chance of colorectal cancer
- Inflammation and oxidative activities within the gut play major roles in the pathogenesis of UC
- UC's current standard treatment has been shown to have several bothersome side-effect
- *Melissa officinalis,* an herb with anti-inflammatory, antioxidant and antibacterial effects, may be useful in diseases that are based on inflammatory and oxidative responses of the body, such as UC

What the new findings are:

- Administration of Melissa in an animal model showed significant results in reversing the damage to the intestinal mucosa due to acetic acid)
- In the group receiving oral administration of Melissa, 42.9% of animals demonstrated no macroscopic changes, while 14.3% demonstrated only mucosal erythema; there was no significant difference in the macroscopic features from the reference treatment group (mesalamine)
- Rectal administration of Melissa was superior to the reference group, although the difference was non-significant
- *Melissa officinalis* had therapeutic effects against acetic acid-induced UC in rats, particularly via topical administration

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