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**Research Article**

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# Significant reduction in colonic damage by *Chamomilla recutita* L. aqueous extract in acetic acid-induced colitis in rats

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**ABSTRACT**

*Chamomilla recutita L (Cr) has analgesic and anti-inflammatory effects. In Iran dried flower heads of Cr, is used traditionally for several medicinal purposes such as nervous diarrhea, spasms, colitis, gastritis, and hemorrhoids as well as restlessness, insomnia, dysmenorrhea and mastitis. Because of Anti-inflammatory, antioxidant and also spasmolytic properties of Cr we suggested that this plant may have beneficial effects on inflammatory bowel diseases so the present study was carried out. Colonic inflammation was induced with injection of 4% acetic acid (pH 2.3) into the rectal lumen of male Wistar rats. After colitis induction, different doses of extract (10, 20 and 30 mg/kg i.g) were administrated during 6 consecutive days. The macroscopic and histopathologic indices of colon injury, myeloperoxidase (MPO) activity and malondialdehyde (MDA) levels were assessed. The data showed that 20 and 30 mg/kg of extract could significantly reduce body weight loss and wet weight of distal colon as well as severe injury indices. In addition, the extract significantly decreased MPO activity and MDA content of inflamed colon. It is concluded that orally administration of Cr extract was effective to treat acute colitis in acetic acid model and its effects are due to decreasing of inflammatory indices in injured colon.*

**Key words**: Acute colitis, *Chamomilla recutita* extract**,** Rat.

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## INTRODUCTION

Inflammatory bowel disease (IBD) is the general term used to describe two different nonspecific disorders of the gastrointestinal tract i.e., ulcerative colitis (UC) and Crohn's disease (CD). Both diseases are more common in western populations and in urban rather than rural areas and UC is most prevalent in North America, Northern Europe and Australia [1]. Psychological stress is usually participating in the pathogenesis of UC however the etiology of this disease is unknown [2, 3]. The current leading hypotheses for the etiology of the IBD emphasize genetic predispositions to dysregulation of the gastrointestinal immune system [4]. Different factors have been postulated as possible etiologic agents for IBD. They are genetic factors, infective agents, immunological basis, smoking, medications and pathological factors. None of the above stated factors can be considered as primary cause of the disease, but it is clear that they do influence its development [5]. Current approaches to the treatment of this

disease are based on nonspecific suppression of the immune system, assuming that uncontrolled activity may be the direct cause for tissue damage [3]. The treatment of IBD, based on the anti-inflammatory agents, sulfasalazine and steroids, is still nonspecific and the results of the treatment in some patients remain unsatisfactory [6]. The drugs currently used are of varying efficacy, and inevitably show side effects and are also expensive [3]. Until today, there is no permanent cure available for the disease. Consequently, there is a need for alternative agents that may be equally or more effective, besides being less expensive. Recent studies have shown that Mitogen-activated protein kinases (MAPKs) inhibitors or nuclear factor-kappa B (NF-κB) inhibitors could be used for the treatment of UC [3]. The mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species (ROS) from activated neutrophils and macrophages. This over production leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes [7]. In addition; ROS propagate inflammation by stimulating release of cytokines, such as interleukin-1, tumour necrosis factor-α, and interferon-γ, which stimulate recruitment of additional neutrophils and macrophages [8]. The cellular components of this inflammation are capable of producing reactive oxygen species, hydrogen peroxide ($H_2O_2$), superoxide anions ($O_2^-$), and nitric oxide (NO) [9].Prostaglandin and nitric oxide biosynthesis is involved in inflammation, and inducible isoforms of nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) are responsible for the production of large amounts of these pro-inflammatory mediators. Myeloperoxidase (MPO) is an enzyme found in neutrophils and in much lower concentrations in monocytes and macrophages. This enzyme catalyzes the oxidation of electron donors (e.g., halides) by hydrogen peroxides. In case of inflammatory conditions like IBD, the levels of neutrophils in inflamed tissues, and consequently MPO enzyme, increase [1]. Free radicals are important mediators that provoke or sustain inflammatory processes and, consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation. In the search for sources of natural antioxidants, in recent years some medicinal plants have been extensively studied for their antioxidant activity and radical-scavenging activity [10]. *Matricaria recutita* (*Chamomilla recutita*) traditionally used as carminative, sedative and tonic. It is the most popular herbs for the treatment of gastrointestinal spasms indigestion, cramps and inflammatory diseases of the gastrointestinal tract [11, 12].It can also be administered as a compress for skin and mucous membrane inflammations and bacterial skin disease [12]. In Iran, dried flower heads are used traditionally for several medicinal purposes as nervous diarrhea, spasms, colitis, gastritis, and hemorrhoids as well as restlessness, insomnia, dysmenorrhea, mastitis [13]. Chamomile contains quercetin, apigenin, coumarins, and the essential oils matricin, chamazulene, alpha bisaboloid and bisaboloid oxides [14]. It also contains sesquiterpenes (α-bisabolol, bisabolol-oxides A and B, farnesene), sesquiterpenelactones (chamazulene, with intense blue colour) and acetylene-derivatives (spiroethers) which are responsible for the anti-inflammatory, anti-bacterial and anti-fungal actions of chamomile [15].

From these reported activities for this plant and regarding above-mentioned points about UC, we evaluate the effect of*Chamomilla recutita* extract on acetic acid-induced colitis in rats. We attempted to demonstrate the status of oxidative stress by investigating colon MDA and MPO activity accompanied by colon macroscopic and microscopic findings in acetic acid-induced colitis in rat.

## EXPERIMENTAL SECTION

*Animals*

Adult male Wistar rats weighting 230-280 g were obtained from the Shahid Bahonar University Animal House. The animals were housed at 22 ± 2°C under a 12 h light/dark cycle. For acclimation, standard diet (Javaneh khorasan, Mashhad, Iran) and water were provided ad libitum for at least 7 days. Each animal was used for one experiment. Rats were divided randomly into 5 different groups of seven animals: In groups 1, 2, 3 and 4 the animals were given orally 10, 20 and 30 mg/kg of extract and vehicle respectively for 6 consecutive days after colitis induction. Control groups (intact) did not receive the treatment. The study and the experimental protocols were approved by the Research and Animal Care Committees at the shahid Bahonar University of Kerman.

*Preparation of extract*

Dry *Chamomilla recutita* L. flowers were weighed and crushed to powder and a 5% w/v suspension was prepared in a flask by adding hot boiled water. The flask was then placed on a shaker (200 rpm) for 4 h and the temperature was maintained at 37°C. After shaking, the flask was brought to room temperature and the suspension was filtered through a series of Whatman filters and finally the filtered aqueous extract was freeze-dried and stored at −20°C until use.

*Induction of colitis and treatment*

Colonic inflammation was induced in rats using the technique that previously described [16]. Rats were fasted for 36 h and anaesthetized with ether. A rubber cannula (8 cm long) was inserted into the colon, via the anus and 2 ml solution of 4% acetic acid (pH 2.3) was instilled into the rectal lumen .The rats were thereafter maintained in a vertical position for 3 min. The control rats were treated with the same volume of physiological saline. All the rats were checked daily for body weights and clinical symptoms of UC such as diarrhea and/or bloody stools. In all animals, the daily weight, daily hemoccult or presence of gross blood, and daily stool consistency were examined in order to determine the disease activity index during the extract treatment periods.

*Macroscopic assessment*

On day 7, posttreatment of colitis, the rats were anaesthetized. The abdomen was opened along the median line, and a 7 cm segment of distal colon which contain the major gross pathologic changes was removed. The colon was opened by a longitudinal incision and rinsed with isotonic saline, pinned out on a wax platform, and macroscopically visible damage was assessed according to the method of Morris et al.[17]. After rapid removal of the colon, the specimen was flushed with ice cold PBS, cut open, and photographed. The photographs of the colonic specimens were then scored by two observers who were unaware of the treatment. The scores were assessed by the following damage scoring system [17, 18].We used Satoh's criteria to determine the severity of colonic inflammation which was visually assessed at the end of the experiments using a scale of 0–3; briefly, a grade of zero was given to normal mucosa, 1 to mucosal erosion, 2 to a moderate lesion and 3 to a deep lesion or ulcer. Immediately after scoring, a 4 cm segment with the major gross pathologic changes was removed from the 7 cm segment and stripped longitudinally into two strips for measurement of MDA, MPO activity and histologic slides.

*Histological examination*

For histological analysis, the tissues were fixed in 10% buffered paraformaldehyde, dehydrated, and embedded in paraffin. Thereafter, sections of the tissue were cut on rotary microtome, mounted on clean glass slides and dried overnight at 37°C. The sections were cleaned, hydrated, and stained with hematoxylin and eosin (H&E), using the standard methods for histological evaluation of colonic damage.The histological damage was scored using the criteria described by Videla et al.[19], which include ulceration (0: none, 1: small ulcers <3 mm, 2: large ulcers >3 mm); inflammation (0: none, 1: mild, 2: moderate, 3: severe); depth of the lesion (0: none, 1: submucosa, 2: muscularis propria, 3: serosa); fibrosis (0: none, 1: mild, 2: severe). Examination of sections was made by a pathologist who was blinded to the experimental protocols. Above criteria were used to assess each tissue sample.

*Biochemical Analysis*

Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte accumulation, was determined by a previously described method [20].To measure MPO activity, colonic samples were minced on ice and were homogenized in a solution containing 0.5% hexadecyl trimethyl ammonium bromide (HETAB) dissolved in 50 mM potassium phosphate buffer (pH 6.0), before sonication in an ice bath for 10 s. The homogenates were freeze–thawed three times, repeating the sonication after which they were centrifuged for 15 min at 20,000 g. The level of MPO activity was measured spectrophotometrically: 0.1 ml of supernatant was mixed with 2.9 ml of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg/ml *o*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was then measured for 5 min using a Beckman spectrophotometer (Beckman DU 640B). One unit of MPO activity was defined as the change in absorbance per minute at 25°C and was expressed in units per gram (U/g) of wet tissue. MPO activity (U/g) = X/weight of the piece of tissue taken, where X = 10 × change in absorbance per minute/volume of supernatant taken in the final reaction.

Malondialdehyde (MDA) levels in the colon tissue were assessed by the method of Satoh (38)[21]. MDA is the main end product of the oxidation of polyunsaturated fatty acids and its concentration in the medium is an established measure of lipid peroxidation extent. In this test, the reaction of TBA with lipid peroxide products makes a complex which is determined spectrophotometrically and lipid peroxidation in samples are assessed in terms of TBARS produced. Briefly, the colonic samples were homogenized in buffered saline (1:5) and then 800 µl of TCA (28% w/v) was added to 400 µl of this mixture and centrifuged in 3000 g for 30 min. Then, 600 µl of the supernatant was added to 150 ml of TBA (1% w/v). Then the mixture was incubated for 15 min in a boiling water bath and then 4 ml *n*-butanol was added. After that, the solution was centrifuged and cooled and absorption of the supernatant was recorded in 532 nm by Beckman DU 640B spectrophotometer. MDA was expressed as µmol $g^{-1}$ of colon.

*Statistical analysis*

Data were expressed as mean ± SEM. The statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Tukey post hoc test. T-Test was also used for comparing the sham and control groups. *P* values less than 0.05 were considered statistically significant.

## RESULTS

- *Effect of Matricaria recutita L aqueous extract on macroscopic injury in acetic acid-induced colitis*.

Intracolonic administration of 4% acetic acid resulted in acute UC in rats. The control (acetic acid-treated) animals developed colonic macroscopic damage such as diffuse hyperaemia and ulcerations. Daily oral treatment with *Matricaria recutita* L aqueous extract by gavage for six constitutive days at a dose of 10, 20 and 30 mg/kg significantly reduced the colitis-induction indices such as macroscopic injury weight lose, hayperemia and inflammatory agent, MDA and MPO activity. Intracolonic administration of 2 ml of acetic acid caused macroscopic damage of the distal colon. After 24h macroscopic and microscopic studies showed colitise induction. Body weight lose, wet colon weight, weight/length ratio of colon, area of severe and mild injury, index of ulcer and severity of inflammation, inflammation extend and percent of involved area significantly change between the control and sham group (Table 1).

**Table 1.Effect of intragastrically doses of Matricaria recutita aqueous extracts (consecutive for 6 days) on the macroscopic & microscopic factors in rat**

| Groups | Body weight (g) | wet weight(g) | Mild ulcerative area($mm^2$) | Severe ulcerative area($mm^2$) | w/L(g/cm) | Ulcer Index | Involvement | Inflammation severity | Inflammation extent | Percent | Gain or loose |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control (normal) | 42.44 ± 2.25 | 0.560 ± 0.26 | 0 | 0 | 0.080±0.003 | 0 | 0 | 0 | 0 | | |
| Sham (vehicle) | -58.55 ± 3.153*** | 1.88 ± 0.094*** | 502.28 ± 140.45 | 274.57 ± 79.64* | 0.26±0.014*** | 776.8±114.65* | 2.85±0.142*** | 2.85±0.142*** | 3.71±0.285*** | | |

*Each value represents mean of macroscopic scores ± SEM of seven animals in each group, (n=7). T-Test; *p<0.05 : ***p<0.001 versus control*

*Matricaria recutita* extract at dose of 20 and 30 mg/kg significantly reduced body weight loss (p<0.01) and wet weight (p<0.05) and (p<0.001) of distal colon (Figure 1 and Figure 2).

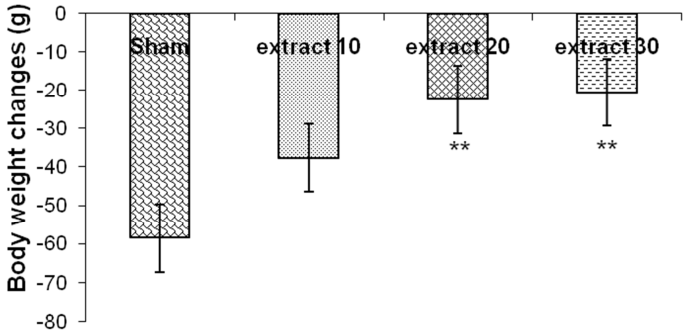


**Fig. 1. Effects of *Matricaria recutita* extract on body weight changes in acetic acid-induced colitis**

*Data are expressed as mean ±SEM from 7 rats. **p<0.01 versus sham.*

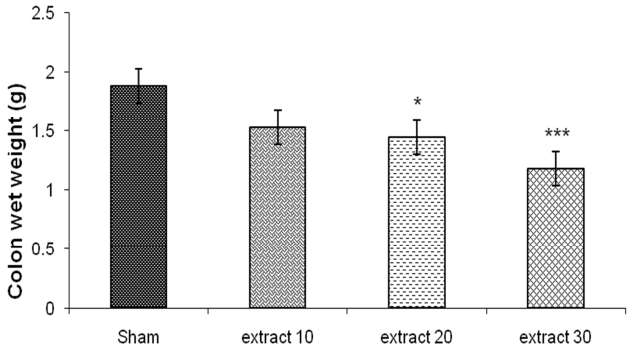


**Fig. 2. Effects of *Matricaria recutita* extract on colon wet weight in acetic acid-induced colitis**
*Data are expressed as mean ±SEM from 7 rats. *p<0.05 : ***p<0.001 versus sham.*

Administration of all doses of *Matricaria recutita* L aqueous extract significantly reduced the aria of severe injury ($p<0.05$, $p<0.01$) but not mild injury (Figure 3 and Figure 4).

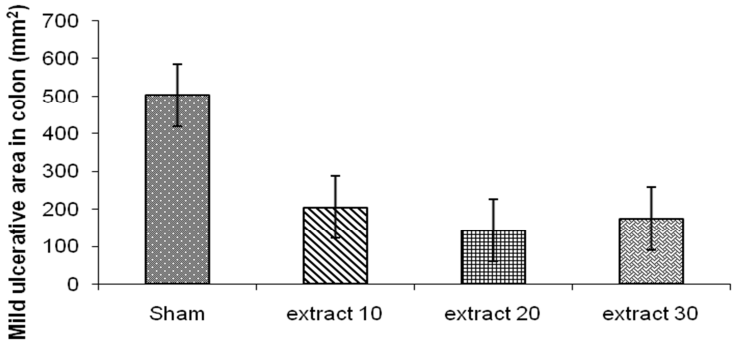


**Fig. 3. Effects of Matricaria recutita extract on mild ulcerative area in acetic acid-induced colitis**
*Data are expressed as mean ±SEM from 7 rats.*

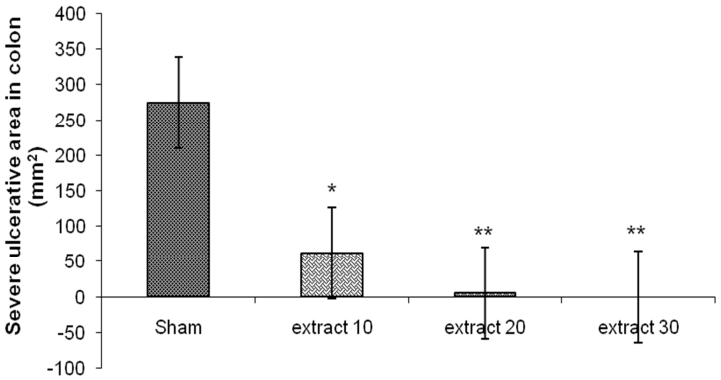


**Fig. 4. Effects of Matricaria recutita extract on severe ulcerative area in acetic acid-induced colitis**
*Data are expressed as mean ±SEM (n=7). *p<0.05 : **p<0.01 versus sham.*

*- Effect of Matricaria recutita L. aqueous extract on MPO activity and MDA in acetic acid-induced colitis.*
The myeloperoxidase assay showed significant increase in MPO activity of sham group compared to normal un-treated group. The extract treated group showed significant reduction in MPO activity com- pared to the sham animals (Figure 5).

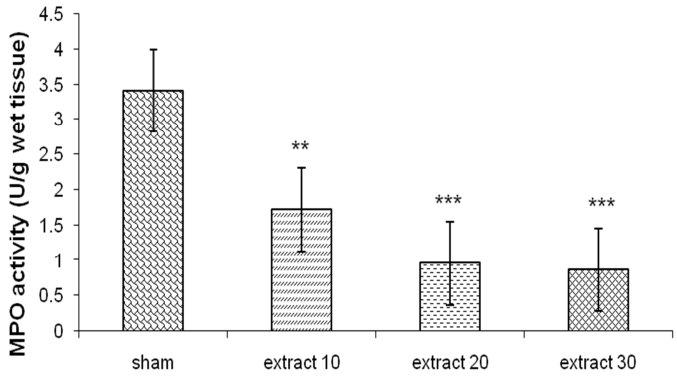


**Fig. 5. Effects of Matricaria recutita extract on MPO activity in acetic acid-induced colitis**
*Data are expressed as mean ±SEM from 7 rats. **p<0.01, ***p<0.001 versus sham.*

Figure 6 depicts that MDA content of colon significantly decreased in extract-treated groups at doses of 20 and 30 mg/kg ($p<0.001$).

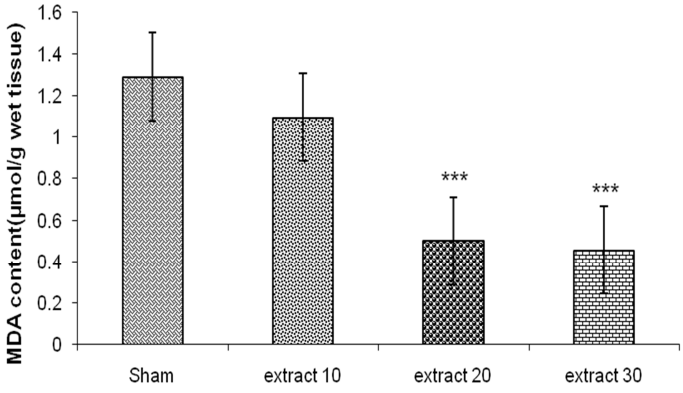


**Fig. 6.Effects of *Matricaria recutita* extract on MDA content in acetic acid-induced colitis**
*Data are expressed as mean ±SEM (n=7). ***p<0.001 versus sham.*

## DISCUSSION

Evidence provided by our experimental study revealed the potential therapeutic effect of *Matricaria recutita* L. aqueous extract in rat model of UC. In last related study we have shown that aqueous of *M. recutita* reduced weight/length ratio, ulcer index, inflammation severity and extend. In addition, histopathological studies on colon sections showed a significant curing or treating effect of extract [22]. The wet weight of the inflamed colonic tissue is considered a reliable and sensitive indicator of the severity and extent of the inflammatory response [23]. *M. recutita* L. aqueous extract significantly reduced the wet weight of distal colon segments compared with the sham in dose of 20 and 30 mg/kg. In addition, extract-treated rats showed a significant reduction of macroscopic lesions such as area of injury indicating reduced oedema and tissue injury. A significant reduction of the body weight was also observed in animals treated with extract at the doses of 20 and 30 mg/kg. It is documented that diarrhea, occult blood, melena, mucosal inflammatory cell infiltration, crypt abscess formation, and mucosal erosion bloody diarrhea and abdominal pain are the main characteristics of UC [24, 25]. Our previous and also the current studies showed that *M. recutita* extract could reduced some of this characteristics.

In animal experimental model of colitis the cascade of free-radical production consumes the cellular antioxidant capability and also increases the level of leukotriene-$B_4$ (LTB4) which may play an important role in colitis [23].

Cyclooxygenase-1, a constitutive enzyme, has crucial role for mucosal integrity and produces cytoprotective and anti-inflammatory prostaglandins. Cyclooxygenase-2 is the inducible form of the enzyme and is significantly increased at inflammation sites [26].

The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs. In the country the dried flower heads of the *M. recutita* L have been widely used in traditional and herbal medicine for centuries because of its anti-inflammatory, spasmolytic, antipeptic, sedative, antibacterial analgesic and antifungal properties [13].

Over-production of free radical provokes or sustains inflammatory processes and, consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation [8]. In oxidative stress, situation, reactive oxygen species (ROS), such as superoxide ($O_2^{-\cdot}$, $OOH^{\cdot}$), hydroxyl ($OH^{\cdot}$) and peroxyl ($ROO^{\cdot}$) radicals, are generated [27]. Ulcerative colitis (UC) is a chronic inflammatory disease that produces ROS and increase the risk of colorectal cancer [28].Several constituents of chamomile including apigenin 7-*O*-glucoside, luteolin, terpene compounds, chamazulene, and (α)-alpha-bisabolol, patuletin, quercetin, myricetin and rutin have been studied with respect to their anti-inflammatory activities [29].It also contains chamazulene, alpha bisabolol, bisabol oxides, Spiro ethers apigenin, coumarins, matricin, chamazulene, alpha bisaboloid, and bisaboloid oxides[30, 14].

Flavonoids, kaempferol-3,7-*O*-α-dirhamnoside and quercetin-3,7-*O*-α-dirhamnoside, showed antinociceptive and anti-inflammatory effects in vivo models in mice [31].

Quercetin and kaempferol have potent anti-inflammatory activity through blocking both the cyclooxygenase and lipoxygenase pathways [32]. It is also reported that quercetin is an effective inhibitor of phospholipase A2 which catalyses the hydrolysis of phospholipids to release arachidonic acid as the precursor of the inflammatory response [33]. Chamazulene, alpha bisabolol, bisabol oxides, Spiro ethers have anti-inflammatory, antibacterial, and antifungal properties [30]. In vitro study showed that apigenin which belongs to the subclass flavones, significantly inhibited LPS-induced IL-6, but not TNF-α production [34]. This flavonoid could inhibit the inflammatory mediators nitric oxide and prostaglandin $E_2$ ($PGE_2$) [35,36]and has inhibitory effects on adhesion molecule expression[37], $PGE_2$, [36, 37]cyclooxygenase (COX)-2 [36] production and the proinflammatory cytokine interleukin (IL)-6 [37] in cell culture models.

It has been reported that nitric oxide (NO) and superoxide anions produced by activated macrophages and neutrophils during chronic inflammation are related to deterioration in UC and development of carcinoma[38].

Inhibition of iNOS by quercetin may be one of the mechanisms responsible for the anti-inflammatory effects of extract. Flavonoids have a variety of biological effects, including antioxidant, antitumor, anti-microbial, anti-allergic, anti-angiogenic and anti-edematous properties [39]. Related to these abilities, it could suppress the production of pro-inflammatory cytokines and mediators[40], inactivate 5-lipoxygenase and cyclooxygenase [41], synthesis and release of nitric oxide [42], decrease blood vessels permeability and tissue congestion [43]. Luteolin, a plant flavonoid, has potent anti-inflammatory properties both in vitro and in vivo conditions [44, 3]. The flavonols, quercetin, isoquercitrin and rutin, suppressed the release of inflammatory mediators including TNF-α, RANTES, macrophage inflammatory protein-2 (MIP-2) and $PGE_2$ [45]. TNFα, IL-1β and IL-6 are pro-inflammatory cytokines and are important in the inflammatory stages of several chronic inflammatory diseases [35]. It has been demonstrated that they may inhibit several enzymes that are activated in certain inflammatory conditions [46, 47].

A recent study has reported that quercetin suppresses TNF-α-mediated stimulation of IL-8 and MCP-1 expression by inhibiting the activation of NF-κB in the cultured human synovial cells[48]. Chamomilla contains flavonoids, which exert benzodiazepine-like activity [49].In the pathogenesis of UC, psychological stress and stress hormones, e.g., epinephrine, norepinephrine, and ACTH are important [2] so in addition to antioxidant and antiinflammatory capability of extract the benzodiazepine-like activity also can be considered as curing effect of extract on UC.

Since the *Matricariarecutita* L. aqueous extract has a wide range of anti-inflammatory, antioxidant and ani-stress agents it seems logical that the UC symptom can be attenuated by the extract in a rat model of colitis.

In conclusion, the result suggested that the *Matricariarecutita* L. aqueous extract has potential efficacy against acetic acid-induced colitis,anti-inflammatory effect of extract appear to be involve in curing colitis. However the exact mechanism(s) for UC curing effect of extract needs to be clarified by further studies.

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