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The anti-inflammatory effects of antidepressants on colitis

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ABSTRACT

Aim: Clomipramine (tricyclic antidepressant), Risperidone (a non-typical antidepressant), and Escitalopram (selective serotonin reuptake inhibitor antidepressant) might be good candidates for investigating the anti-colitis activity.

Background: The incidence of depression with ulcerative colitis in patients has led to the use of antidepressants in their treatment. In addition to the antidepressant effect of these drugs, anti-inflammatory effects have also been reported.

Methods: In this study, 36 rats were used 2 ml of 3% acetic acid solution rectally to show the colitis. Then, Clomipramine (25 mg/kg), Escitalopram (10 mg/kg), Prednisolone (5 mg/kg), Risperidone (2 mg/kg), and normal saline as the control was administered orally for six days. The levels of Tumor Necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6), and myeloperoxidase (MPO) were measured by Enzyme-linked immune sorbent assay (ELISA), and changes in the tissue pathology were investigated.

Results: IL-6 level was significantly reduced after the administration of clomipramine and Prednisolone (p=0.025). Risperidone has significantly reduced MPO activity in colonic tissue (P=0.006). We did find no statistical decrease in MPO activity and TNF-α and IL-6 levels after consumption of Escitalopram (p>0.05).

Conclusion: Clomipramine showed the best anti-inflammatory effect compared to Escitalopram and Risperidone. Therefore, clomipramine showed the best relieving effect on inflammation of ulcerative colitis in rats.

Keywords: Risperidone, Escitalopram, Clomipramine, Inflammatory bowel diseases, Colitis, Antidepressive agents, Selective serotonin reuptake inhibitors

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Introduction

Ulcerative colitis is a life-long gastrointestinal disease that many patients are concerned about the treatment (1). Depression and diminished quality of life play an important role in this issue (2). The inflammatory bowel disease (IBD) might disrupt work, family, and the patient's feelings, and consequently, anxious and depressed moods were observed (1). Ulcerative colitis is usually accompanied by depressive disorders, and antidepressants can reduce its complications by controlling the disease activity and lengthening remission (3). The anti-colitis effect of antidepressants was established, but their mechanism of action is not clear completely (3).

Several researchers reported that levels of pro-inflammatory cytokines were raised in depressed patients, mainly Interleukin-1(IL-1), Interferon-gamma (IFN-γ), Interleukin-6 (IL-6), and Tumor Necrosis factor-alpha (TNF-α) (4-6).

Dahl et al. also suggested that inflammation had a main role in ongoing depression (3) and depression can affect gut inflammation and increase the mucosal pro-inflammatory cytokines.

Many in vitro and in vivo studies reported that some antidepressants had anti-inflammatory activities (7-10).

Selective serotonin reuptake inhibitors (SSRIs) and tricyclic anti-depressant drugs (TCAs) are the main anti-depressants that have anti-inflammatory effects; in addition to treating depression, which could be used in patients with IBD (11). The roles of the sympathetic nervous system

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(SNS), histamine, serotonin, and dopamine in the pathogenesis of colitis suggested that adjuvant therapy with an antidepressant drug might help to control ulcerative colitis (12). In several studies, the anti-inflammatory activities and anti-colitis effects of Fluoxetine (7), and Doxepin (12), were revealed. There are many different antidepressants with different mechanisms of action. The anti-inflammatory effects of antidepressants are reported by different mechanisms of action.

Escitalopram is a relatively common SSRI antidepressant (13). Risperidone is an antipsychotic medication that is used for mood disorders. It has an affinity for D2, 5-HT, alpha 1, alpha 2, and H1 receptors (14). Clomipramine, a tricyclic antidepressant, is a noradrenaline or serotonin reuptake inhibitor (15).

The beneficial effects of these three antidepressants above (Clomipramine, Escitalopram, and Risperidone) with three different mechanisms of action were studied on intestinal damage of an inflammatory bowel disease experimental model and measured inflammatory markers like Interleukin 6 (IL-6), and Tumor necrosis factor-α (TNF-α) levels and Myeloperoxidase (MPO) activity.

Methods

Animals

This study was approved by the local Ethics Committee of Shiraz University of Medical Sciences (SUMS) by the no. IR.SUMS.REC.1398.184.

Forty-two male Sprague Dawley rats weighing 200-250 gr were studied. The rats were housed in rooms with standard temperature (20-23°C), humidity (50-60%) and free access to standard food and tap water. The animals were kept and handled according to the local guidelines of care and work with laboratory animals at SUMS.

Experimental design

All rats were kept fasting for 24 hours before the induction of colitis by acetic acid (3% v/v) that was described previously (16). The process of anesthesia and confirmation of the induced UC model showed the exact symptoms, e.g., loss of appetite, bloody defecation, and weight loss. The rats were randomly assigned to 6 groups including 7 rats each; sham (without induction of colitis), colitis group, and test groups. Sham and colitis groups received normal saline (2 ml/kg, intra-colonic); four test groups received three desired antidepressants and prednisolone (as a reference) for evaluating the anti-inflammatory effect. Test groups received Clomiperamine (25 mg/kg), Escitalopram (10 mg/kg), Risperidone (2 mg/kg), and prednisolone (5 mg/kg) and were given once daily for the next six days.

Effect of antidepressants on macroscopic parameters

Twenty-four hours after the last treatment, animals were sacrificed by the cervical dislocation method. Then, the abdomen was opened, and the 8 cm distal colon was processed for macroscopic, histological, and biochemical assessment. To assess the severity of colitis macroscopically, the photographs were analyzed by an expert pathologist. The macroscopic morphological damage score was calculated based on clinical features of the colon using an arbitrary scale ranging from 0-5 as follows: 0 (no macroscopic damage), 1 (localized hyperemia but no ulcers or erosions), 2 (ulcers or erosions with no significant inflammation), 3 (ulcers or erosions with inflammation at one site), 4 (two or more sites of ulceration and/or inflammation), 5 (Two or more major sites of inflammation and ulceration or one major site of inflammation and ulceration extending >1 cm along the length of the colon) (17). Then, the tissues were used for histopathology study and measuring myeloperoxidase (MPO) enzyme activity and cytokine (TNFα, IL6) levels kept at -70°C freezer until further analysis.

The effects of antidepressants on histopathological features

The colon pieces fixed in formalin (10%) solution were dried, paraffin zed, and cut into slices with 5μm thickness. Then, the slices were analyzed using the hematoxylin and eosin staining (H&E) method with slight modifications (18). Total colitis index was calculated by summing the grades: inflammation severity (0-3), inflammation extent (0-3), and crypt damage (0-4) (17).

Determination of the cytokines levels and MPO activity in the colon tissue

After thawing sample in the laboratory environment, they were chopped into small pieces and homogenized in 0.01 M Phosphate-buffered saline (BPS) (pH=7.4) containing 8.5 g NaCl, 1.4 g Na2HPO4, and 0.2 g NaH2PO4 to 1000 ml distilled

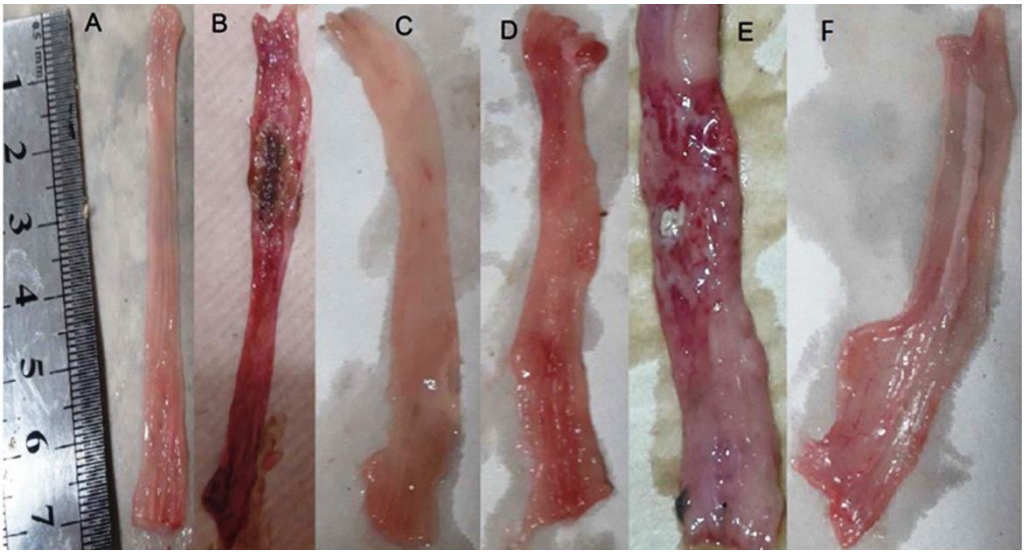

Figure 1. Macroscopic image of rat colons in treatment groups, normal rats treated with normal saline (A), colitis treated with normal saline with edema, erythema, ulcer, necrosis and thickening of tissue (B). Prednisolone (C), Clomipramine (D), Risperidone (E), and Escitalopram (F) treated colons showed healing of ulcers.

water. One ml PBS per 1 g tissue was used to prepare the supernatant. TNF-α, IL-6, and MPO activity levels in the colon tissues were evaluated by enzyme-linked immunosorbent assay (ELISA; Bioassay Company, China).

Chemicals

Escitalopram (SSRI) (Tehran Darue), Clomipramine (TCAs), and Risperidone (A non-typical antidepressant) (Darupakhsh Company, Tehran, Iran) were dissolved in isotonic saline. Prednisolone (Tehran Daru, Iran) was dissolved in 0.9% saline solution, and an emulsion was prepared at 37°C in bathwater. Ketamine and xylazine vials (Darupakhsh Company, Tehran, Iran) were used to induce anesthesia in rats. Formalin solution 37% w/w, PBS, diethyl ether, and acetic acid were supplied by Merck (Darmstadt, Germany).

Statistical analyses

All statistical analyses were done using SPSS software (version 22). We used the Kolmogorov–Smirnov test for the normality of data distribution, which was analyzed by Kruskal-Wallis test. Mann-Whitney U test was used to compare each group and the colitis group without treatment. Data were expressed as mean ± S.E, and $P<0.05$ was considered statistically significant.

Results

Effect of antidepressants on macroscopic parameters

Colitis was induced in rats using acetic acid and showed symptoms within 24 hours after induction. The colons of the sham group showed intact epithelium with no damage, while the colons of the colitis group revealed severe inflammation, ulceration, wall thickening, edema, and sometimes necrosis (Figure 1). The summation of the macroscopic scores was significantly decreased between clomipramine (20 mg/kg) and Prednisolone (5 mg/kg) compared to the colitis groups. (Figure 2; P=0.02).

Effects of antidepressants on histopathological features

Treatment with clomipramine reduced the microscopic scores compared to the colitis group (Table 1). Figure 3 showed the total colitis index that reduced, but the clomipramine (P=0.03) and prednisolone (P=0.04) showed a significant difference. Figure 4a showed the sham group with no histological damage, and Figure 4b showed mild transmural acute inflammation with basal crypt damage, and complete regeneration was seen only in the clomipramine group. Figure 4 shows the crypt damage decreased in the Risperidone group (c) compared with the colitis group (d).

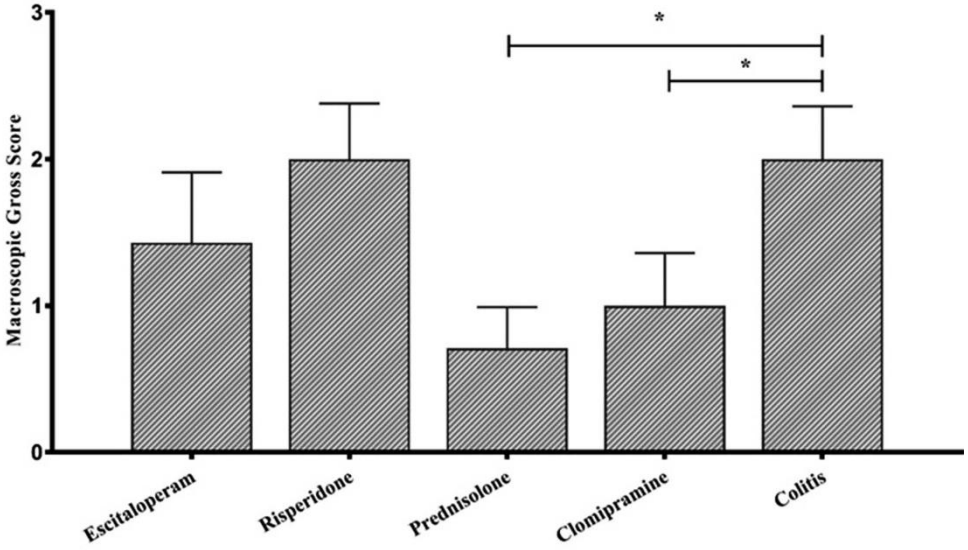

Figure 2. The comparison of the macroscopic score among different treatment groups and colitis groups (* P<0.05).

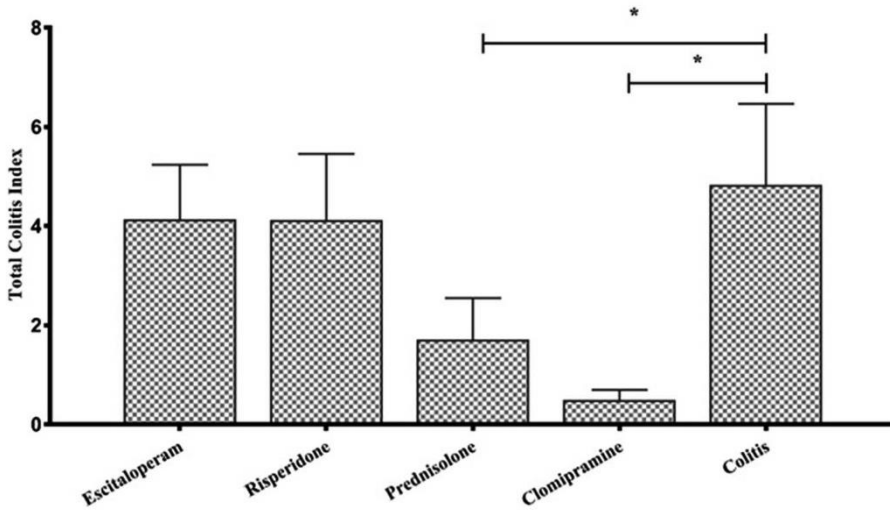

Figure 3. The comparison of the total colitis index scores among different treatment groups and colitis groups (*P<0.05).

Table 1. The comparison of the microscopic score (inflammation, extent, regeneration, and crypt damage) among different treatment groups (* P<0.05) (N=7).

Groups	Inflammation $_{Mean±SE}$	Extent $_{Mean±SE}$	Regeneration $_{Mean±SE}$	Crypt damage $_{Mean±SE}$
Escitaloperam	1.43±0.3	2.00±0.31	0.86±0.59	0.71±0.56
Risperidone	1.25±0.36	1.62±0.46	1.00±0.53	1.25±0.56
Clomipramine	0.17±0.17	0.17±0.17	0.00±0.00	0.17±0.17
Prednisolone	0.71±0.36	0.43±0.20	0.14±0.14	0.57±0.30
Control free	1.50±0.50	1.67±0.56	1.33±0.49	1.67±0.61
Normal Sham	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
p-value	0.038*	0.002*	0.208	0.238

Effect of antidepressants on myeloperoxidase (MPO) activity, TNF-α, and IL-6 levels

Risperidone has significantly reduced MPO activity in the colonic tissue compared to the colitis group (Figure 5; P=0.006). Decreased activity of the MPO was significantly observed in the Risperidone group, as compared with the prednisolone group (p=0.028; Figure 5).

Prednisolone and Clomipramine decreased the MPO activity and TNF- α level compared to the colitis group, but the difference was insignificant (p>0.05, Figure 6).

The tissue levels of IL-6, TNF-α, and MPO increased in the colitis group compared to the Sham group; we showed a significant increase in TNF-α levels (P=0.033). Also, the levels of TNF-α showed decreased in prednisolone and clomipramine groups, but it was statistically non-significant (Figure 6).

Prednisolone and Clomipramine significantly decreased IL-6 levels in colon compared to the colitis group (p=0.025, Figure 7). No significant differences in IL-6 levels were detected in colonic tissue after using Risperidone (Figure 7).

There was no statistical difference between Prednisolone and clomipramine in MPO activity and TNF-α and IL-6 levels (p>0.05). We did find no statistical decrease in MPO activity and TNF-α and IL-6 levels after consumption of Escitalopram in comparison with the colitis group (p>0.05; Figures 5, 6, 7).

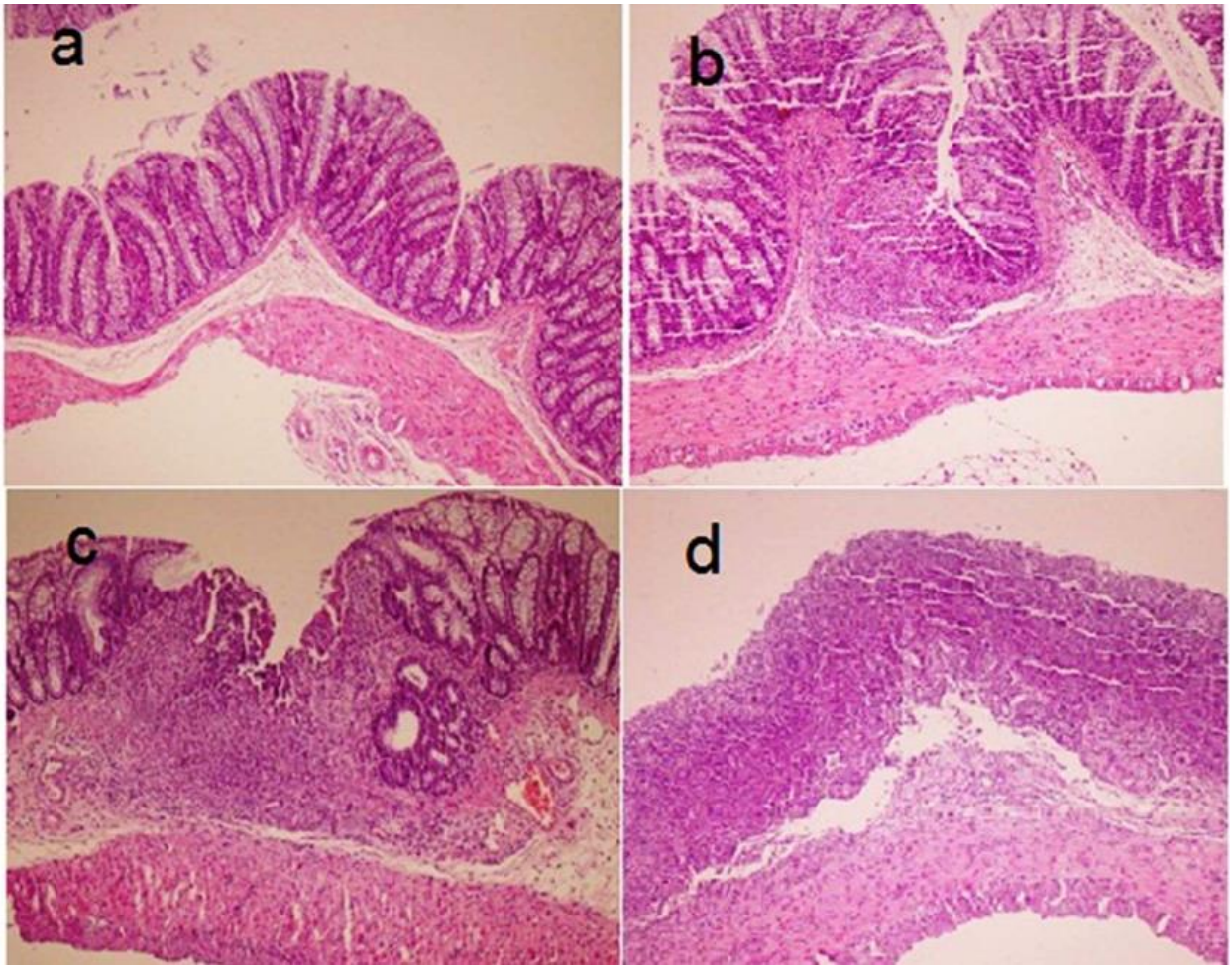

Figure 4. Microscopic section shows (a) normal colonic wall with no inflammation, (b) moderate acute inflammation with basal crypt damage and almost complete regeneration, (c) severe acute inflammation with regeneration and crypt damage, and (d) Severe acute inflammation with entire crypt and epithelium lost and no tissue repair. (Hematoxylin and Eosin, 100×)

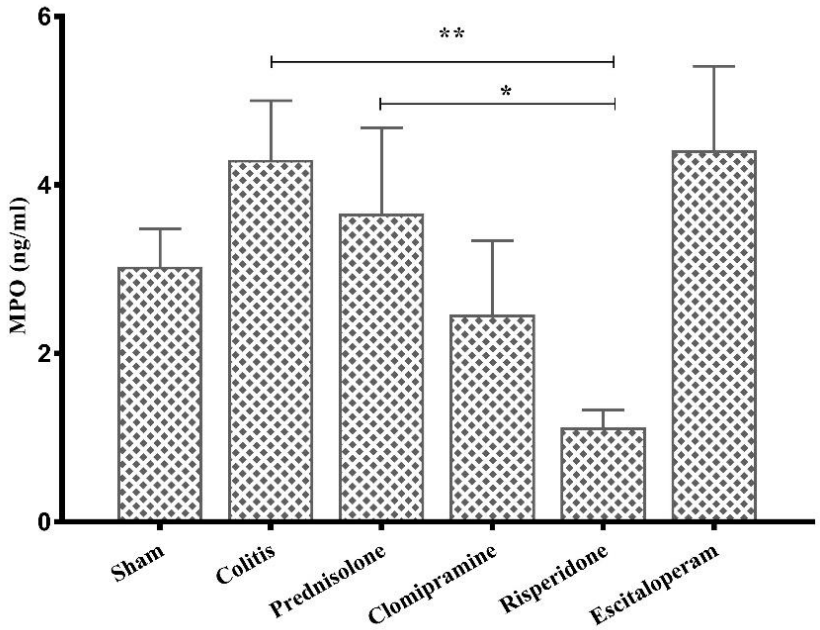

Figure 5. Evaluation of myeloperoxidase (ng/ml) activity in colitis and different treatment groups (* P<0.05, **P<0.01).

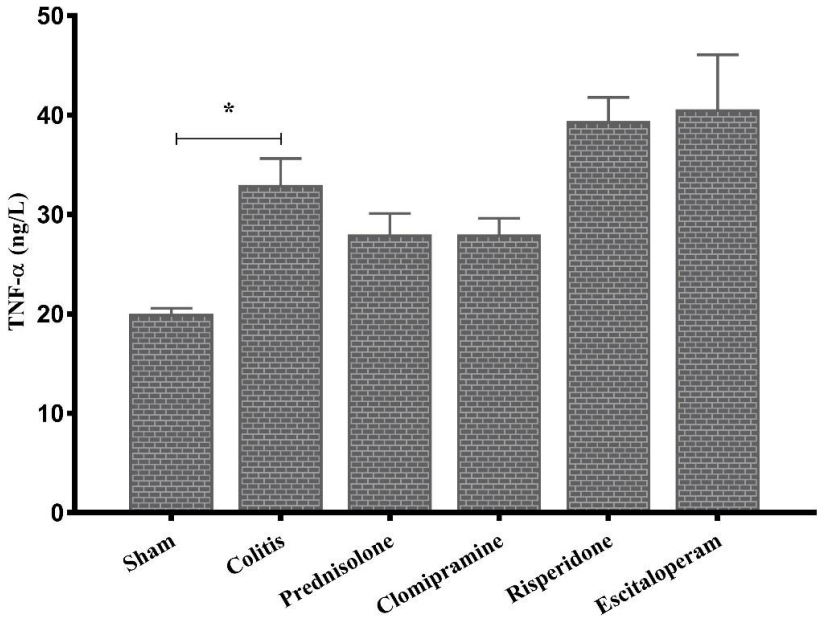

Figure 6. Evaluation of TNF-α (ng/L) levels in colitis and different treatment groups.

Discussion

The current treatment for ulcerative colitis is corticosteroids, NSAIDs (non-steroidal anti-inflammatory drugs), and TNF-α inhibitors consumption (19) that has many side effects, and the recurrence of symptoms is not avoided. On the other hand, some researches are done on new drugs that show different results.

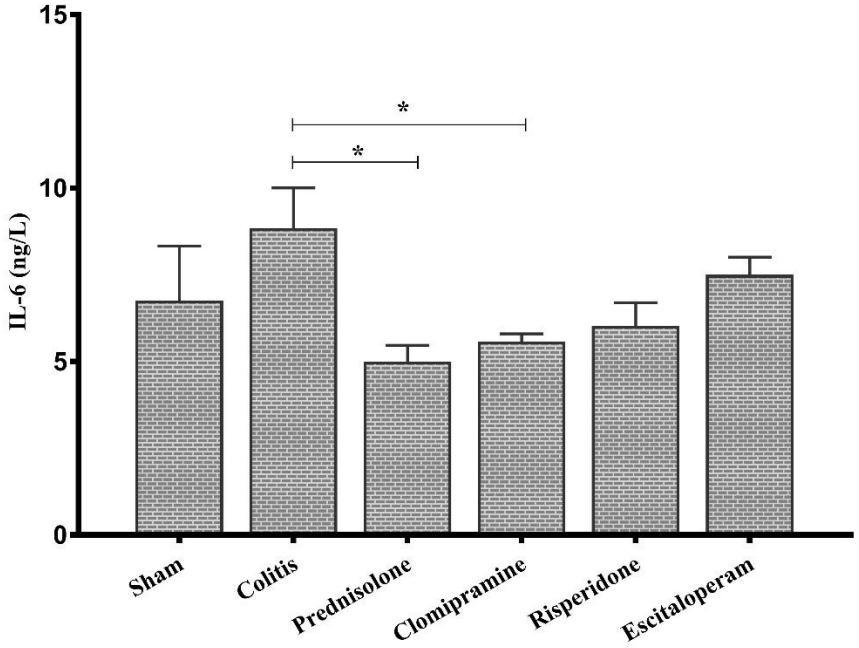

Figure 7. Evaluation of IL-6 levels (ng/L) in colitis and different treatment groups (*P<0.05).

In our study, treatment with some the antidepressants reduced microscopic scores compared to the colitis group. Also, histological amelioration of colitis and significant differences in total colitis index occurred after treatment that the modulation of immune cells (20) could cause.

As an oxidative stress biomarker, Myeloperoxidase is often overexpressed in ulcerative colitis and could be assessed in this problem (21). A previous study demonstrated that antidepressant consumption could reduce MPO activity in ulcerative colitis (1). Antioxidant capacities of the colonic tissues are restored by MPO activity reduction after amitriptyline treatment as a TCA (1). Our results indicated that the MPO level was significantly reduced after 7 days by Risperidone (p=0.025).

Certain pro-inflammatory cytokines, such as TNF-α and IL-6, are modulating factors of the mucosal immune system in ulcerative colitis patients (22). Similarly, we also found that the colitis group is associated with an increase in the TNF- α and IL-6 levels and MPO activity.

Janssen DG et al. showed that antidepressants could reduce the stimulated release of pro-inflammatory cytokines such as IL-1β, IL6, TNF-α, and IFN-γ in vitro study (2). In another study, the potent anti-inflammatory effect of doxepin in colitis parameters was reported (12). We found that Prednisolone or Clomipramine caused significantly decreased IL-6 levels after treatment.

It seems that the antidepressants had their anti-inflammatory mechanism by inhibiting interferon-gamma (IFN-γ) production by T-helper1 in colitis tissues and suppressing mitogenic-stimulated T-cell proliferation (20). On the other hand, most of the immune cells express at least one serotonin component. Serotonin has 7 known receptor subtype classes (15 known subtypes) in humans (23). Studies reported that serotonin and its receptors had an important role in activating the immune responses and inflammation (9). However, in this study, escitalopram, our treatment, did not show any anti-inflammatory effect in colitis as an SSRI. Still, risperidone and clomipramine had anti-inflammatory effects by dose-dependent trait. Regarding the antagonistic effects of risperidone and clomipramine on 5HT receptors, their anti-colitis effects could be related to their antihistaminic activity. The monitoring of patients with ulcerative colitis and depressive disorders is supportive to prevention of the complications with an antidepressant.

Limitation

Limited samples in each group were our limiting practical point because the animal studies should be done under ethical considerations.

Conclusion

Our study showed the role of antidepressants against inflammation in rats with colitis. Clomipramine showed the best anti-inflammatory effect in comparison with Escitalopram and Risperidone. We suggested that clomipramine might be a good candidate in patients with IBD for relieving the comorbidities related to inflammation. Further studies are required to confirm the effectiveness of clomipramine as a safe drug in the prevention and/or treatment of IBD.

Ethical statement

This study was animal study and according to the standards, it was approved by the no. IR.SUMS.REC.1398.184.

Acknowledgment

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Conflict of interests

The authors declare no conflict of interest.

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