Al-Azhar University Journal
for Virus Research and Studies

The Potential Anti-Inflammatory Effect on a Model of Ulcerative Colitis in Rats for the Anti-Depressant Venlafaxine

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Abstract

Recently anti-inflammatory effects of antidepressants have been demonstrated. Venlafaxine belongs to antidepressants with serotonin norepinephrine reuptake inhibition property. The anti-inflammatory effect of venlafaxine has been studied in different studies and different models including animals and humans. The present study was designed to evaluate the anti-inflammatory effects of venlafaxine on ulcerative colitis model in rats. We examined the anti-inflammatory effects of venlafaxine in the rat model of acetic acid induced colitis. Colitis was induced in rats by intra rectal injection of 2ml acetic acid 4%. After induction of colitis there were six groups; group1control, group 2 actic acid, group 3 dexamethasone, groups 4,5 and 6 treated with 10, 20, 40 mg/kg venlafaxine i.p. 2 h after the induction of colitis and daily for 4 successive days. Dexamethasone (1 mg/kg, i.p.) was used as reference drug. Colonic inflammation was evaluated using macroscopic assessment, pathological examination and myeloperoxidase activity measurements. Results showed that, after induction of colitis acetic acid group showed sever inflammation, ulceration, bleeding and necrosis, while venlafaxine at doses of 20 and 40 mg/kg as well as dexamethasone significantly improved colitis parameters in colonic tissue of animals. Compared to acetic acid and control, venlafaxine reduced signs of inflammation; it lowerd elevated myeloperoxidase and improved pathological picture both micro and macro in this animal model of induced colitis. Venlafaxine was found to have anti-inflammatory activity in the animal model of ulcerative colitis, as it decreased myeloperoxidase level and improved the histo-pathological profile in the colonic tissue of rats.

Keywords: Depression, induced colitis, IBD, Venlafaxine.

1. Introduction

Major depression is not just a simple mental disorder or brain disease, but also a systemic disease. Patients often suffer from various disorders simultaneously, such as brain dysfunction and periphery dysfunction. Depressed patients often have gut brain dysfunction, mainly includes gastrointestinal disorders such as appetite disturbances, metabolic disturbances, functional gastrointestinal disorders, and

gut microbiota abnormalities [1]. The bidirectional communication between the gut and the brain is organized at four distinct levels, namely neuroanatomic, neuroendocrine, immunologic, and one that consists of neurotransmitters, neuropeptides, and microbial derived products [2]. This complex and multifaceted system of gut-brain communication not only ensures proper maintenance and coordination of gastrointestinal functions to support behavior and physiological processes, but also permits feedback from the gut to exert profound effects on mood, motivated behavior, and higher cognitive functions [3]. As a result of this communication, disorders of the gut brain axis are associated with depression, anxiety, irritable bowel syndrome, inflammatory bowel disease, and other CNS diseases [4]. In gastrointestinal tract (GIT), the stress response system is made up of the hypothalamic pituitary adrenal axis (HPA) and the autonomic system of the brain gut axis. Stress stimulates the hypothalamus, triggering the activation of the sympathetic nervous system, to immediately deal with the stressor. If the threat continues to be perceived as stressful, the HPA axis is activated, which release cortisol in order to regulates various functions in the gastrointestinal tract. These include intestinal motility, secretion, and intestinal permeability and represent direct mechanisms by which stressors can impact GIT functions [5]. In GIT, cortisol regulates the activity of intestinal immune cell and releases cytokine, which affects intestinal permeability and barrier functions, and changes the structure of the intestine. Cortisol also affects the vague output. Thus, both neural and hormonal lines of communication combine to allow brain to influence the activities of intestinal functional effector cells, such as immune cells, epithelial cells, enteric neurons, smooth muscle cells, and interstitial cells of Cajal and enterochromaffin cells [6]. Inflammatory bowel disease (IBD) is a group of chronic inflammatory autoimmune diseases that primarily affect the gastrointestinal tract. The main symptoms of IBD include abdominal pain, diarrhea, bloody stools, and mucus stools. In severe cases, malnutrition and intestinal perforation may occur. IBD primarily includes two different classifications of ulcerative colitis (UC) and Crohn's disease (CD), which affect approximately 5 million people worldwide [7]. Because IBD is a lifelong condition, with bidirectional communication via the gut–brain axis, patients with IBD might have psychological illness, including symptoms of common mental disorders and somatization. The high prevalence of symptoms of anxiety and depression, in patients with IBD has been used to support the premise that a patient's mental health might play a role in both the development and clinical course of IBD [8] . The etiology of depression in IBD includes both external and somatic factors. At the level of external factors, the condition of IBD has been suggested to enhance insecurity of patients and alter their psychological attachment style, thus creating chronic stress and leading to an impact on their mental health [9] . At the somatic level, the pathological effects of IBD may disrupt the neurological function of the patients by themselves. With deepening research on gut-brain interactions, diseases with obvious features of gut-brain dysfunction, such as irritable bowel syndrome and IBD, have recently been proposed to be called disorders of "gut-brain interaction. The pathology of these diseases generally consists of both central nervous disorders and gut dysfunction [10]. On the other hand, depressive symptoms may be mechanistically linked to excess inflammation and dysregulation of the gut-brain axis. Given the close link between the intestinal microbiota and host immune responses, excess inflammation is associated with brain changes e.g., depressive symptoms, fatigue, sleep

difficulties and worsening gastrointestinal symptoms, which are exacerbated by psychological distress. Equally, treatments for both depressive symptoms and IBD provide opportunities to break this cycle by reducing the causes and effects of inflammation [11]. Therefore, if psychological stress is indeed a pathogenic factor in IBD, then stress reduction therapy may have therapeutic benefit. Many researchers hypothesized that the course of IBD is influenced by psychological factors such as depression as it is influenced by somatic factors; so psychiatric treatment of patients improves both mental and somatic status of IBD patients [12]. Antidepressant treatment, including medication and psychotherapy, may have an ameliorative effect on depression in IBD. Based on epidemiological investigations, drugs such as selective serotonin reuptake inhibitors and tricyclic antidepressants have been shown to be protective against IBD. A meta-analysis evaluated 14 psychological treatments for IBD, found that patients with IBD in remission showed significant improvements in depression scores and quality of life, but this improvement was not sustained over time [7]. Venlafaxine was the first serotonin norepinephrine reuptake inhibitors (SNRIs) antidepressant introduced for the treatment of depression and anxiety disorders, being one of the antidepressant agents most commonly prescribed [13]. Our study was conducted to evaluate the potential anti-inflammatory effect of venlafaxine on induced colitis in rats.

2. Materials and Methods

2.1 Drugs and Chemicals

Venlafaxine (Idixor): The drug is provided as tablets containing venlafaxine base as the hydrochloride salt.

Normal Saline 0.9 % w/v Sodium Chloride (Nacl): Supplied as 500 ml aquas solution. Otuska pharmaceutical Company, Egypt.

Dexamethasone Sodium Phosphate was supplied as ampoules containing (8mg/2 ml). Amriya pharm, Egypt.

Glacial Acetic Acid was supplied as a bottle of 1-liter concentrated aquas solution and diluted by 0.9 % w/v Nacl saline. Biotech for laboratory chemicals, Egypt.

Formalin Solution: Biotech for laboratory chemicals, Egypt: supplied as a bottle of 1-liter concentrated aquas solution and diluted by distilled water to 10 % solution, used for pathological samples.

ELISA Kits: Rat myeloperoxidase ELISA kits MBS046496, 48 well from My BioSource Company (USA).

Doses: Doses of venlafaxine and other drugs were according to Minaiyan et al [12].

2.2 Animals Used

Male rats with an average body weight of 250-300 gm were used for induction of colitis. All animal experiments were approved by the Ethics Committee of Al-Azhar University- faculty of medicine.

2.3 Assessment of Anti- Inflammatory Effect of Venlafaxine

Adult male albino rats with average weight 250 – 300 gm were kept for a week prior to study to be adapted to the animal room conditions. The animal room was maintained at 25±2°C and a lighting regimen of 12 h light/12 h dark. Rats were given standard pelleted chaw and having free access to water.

2.3 Induction of Experimental Colitis

All rats were fasted for 24 h before the induction of colitis but were allowed free access to water. All groups proceeded for induction of colitis except the control group. Colitis was induced according to the procedure described by MacPherson and Pfeiffer [14]. Animals were lightly anesthetized by ether, and colon was catheterized intra rectally, such that the tip

advanced 8 cm proximal to the anus, two ml of acetic acid (4% v/v in 0.9% saline) was slowly infused into the colon. Animals were then maintained in a head down position for 30 s to limit expulsion of the solution and return.

2.4 Animal Grouping

Thirty rats were randomly divided into six groups each group consist of five animals:
Group 1 (Control group): Rats were received 2 ml saline rectal in the catheter instead of acetic acid.
Group 2 (Acetic Acid): Rats were received 2 ml aceti acid 4% intra rectal for induction of colitis.
Group 3 (Dexamethasone group): Rats were received dexamethasone (1 mg/kg,i.p) 2 h after induction of colitis as a reference drug, then injected daily for four consecutive days.
Group 4 (venlafaxine10 mg/kg): Rats were received venlafaxine (10 mg/kg, i.p) 2 h after induction of colitis, then injected daily for four consecutive days.
Group 5 (venlafaxine 20 mg/kg): Rats were received venlafaxine (20 mg/kg, i.p)2 h after induction of colitis, then injected daily for four consecutive days.
Group 6 (venlafaxine 40 mg/kg): Rats were received venlafaxine (40mg/kg, i.p) 2 h after induction of colitis, then injected daily for four consecutive days. All drugs were administered in a volume equivalent to 1 ml /kg. All drug doses were calculated as mg/kg, dissolved in normal saline and prepared freshly each morning. On the fifth day all rats were weighed first, then sacrificed by cervical dislocation, the abdomen was longitudinally dissected, then the distal colon was removed and processed for the macroscopic assessment, histopathological examination and myeloperoxidase (MPO) measurement.

2.5 Effect of Venlafaxine on Acetic Acid Induced Colitis in Rats

2.5.1 Body Weight Measurement

All rats in each group were weighed using a sensitive balance daily during the period of the experiment to assess the effect of each one of the following: induction of colitis, treatment with dexamethasone (1mg/kg i.p), and venlafaxine (10, 20 and 40 mg/kg i.p) on rat's body weight.

2.5.2 Macroscopic assessment:

Immediately after scarification of rats and dissection of the abdomen, the distal colon (about 10cm) was removed and cut longitudinally, slightly cleaned in physiological saline to remove fecal residues. Then, tissue was fixed on a white plastic sheet and a photo was taken using an appropriately camera (Nicon model 2018).
Colon mucosal damage (CMD) was measured as macroscopic damage scores which were determined by an independent observer according to the following criteria [15]:

0: no macroscopic changes.
1: mucosal erythema only.
2: mild mucosal edema, slight bleeding, or slight erosion.
3: moderate edema, bleeding ulcers, or erosions.
4: severe ulceration, erosions, edema, and tissue necrosis.

2.5.3 Histopathological Examination

After macroscopic examination the tissues were cut into two pieces, one piece for histopathology assessment and the other for measuring myeloperoxidase (MPO) enzyme activity. Colon tissues were individually fixed in 10% formalin, dehydrated, paraffin embedded, processed, sectioned in 4 μm thick slices, deparaffinized with xylene, hydrated and stained with hematoxylin and eosin (H&E) respectively.
Inflammation severity and extent as well as crypt damage were evaluated on H&E-stained and coded using a validated scoring system [16] as shown in Table [1].

2.5.4 Myeloperoxidase Measurement

Myeloperoxidase levels were measured and used as an approximation of leukocyte count and therefore degree of inflammation.

Principle:

Myeloperoxidase concentrations in rat colon is determined by using Purified rat MPO antibody to coat Micro Elisa Strip plate wells to make solid-phase antibody, then add MPO and MPO antibody which has been labeled with HRP to wells, then the reactants become antibody-antigen-antibody-enzyme complex, after washing completely, add TMB substrate solution, TMB substrate becomes blue color under HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wave length of 450 nm [17]

Assay procedure:

- All reagents and samples were brought to room temperature (18°C-25°C) naturally for 30min before starting assay procedures.
- Standard wells, Sample wells and Blank/Control wells, were settled, Standard 50µl was added to each Standard well and Sample 50µl was added to each Sample well, Sample Diluent 50µl was added to each Blank/Control well.
- 100µl of HRP-conjugate reagent was added to each well, covered with a Closure Plate Membrane and was incubated for 60 minutes at 37°C, all wells were aspirated, then plates were washed four times using Wash Buffer
- 50µl Stop Solution (sulphoric acid) was added to each well. The color in the wells was changed from blue to yellow, color change is measured spectrophotometrically at a wavelength of 450 nm.

2.6 Calculation

The concentration of MPO in the samples is then determined by comparing the optic density (O.D) of the samples to the standard curve.

2.7 Statistical Analysis

The results were expressed as the mean of individual response reported as mean ± SE. Differences among groups were compared using one-way analysis of variance (ANOVA). Tukey test was used as post hoc test for comparison among groups. Statistical significance was set as p value lower than 0.05.

3. Results

Induction of colitis by acetic acid produced sever inflammation, bleeding, necrosis and ulceration. This was very obvious in acetic acid group, but there was clear improvement in other treated groups, dexamethasone and venlafaxine.

3.1 Body Weight Measurement (gm)

3.1.1 Effect of acetic acid on rat body weights

Induction of colitis by acetic acid reduced the mean body weights of acetic acid group from **275 ± 4.81** to **242.4 ± 5.57**, the mean % reduction was 11.88**± 0.68** and it was highly significant ($p<0.01$) as compared with control group and other groups.

3.1.2 Effect of Treatment with Dexamethasone after Induction of Colitis

Treatment with dexamethasone (1mg/kg) after induction of colitis decreased the mean body weight from **270.2 ± 3.76 to 259 ± 1.41**, the mean % reduction was 4.08 **±1.26** and it was highly significant ($p<0.01$) in comparison with control, acetic acid and venlafaxine 40, while significant ($p<0.05$) as compared with venlafaxine 10,

but insignificant in comparison with venlafaxine 20.

3.1.3 Effect of Treatment with Venlafaxine 10mg/kg after Induction of Colitis

Treatment with venlafaxine 10mg/kg after induction of colitis decreased the mean body weight from **271.8 ± 3.54 to 250.6 ± 3.12**, the mean % reduction was 7.79 ± 0.72 and it was highly significant in comparison with control, acetic acid, venlafaxine 20 and venlafaxine 40 groups, while significant in comparing by dexa group.

3.1.4 Effect of Treatment with Venlafaxine 20mg/kg after Induction of Colitis

Treatment with venlafaxine 20mg/kg after induction of colitis decreased the mean body weight from **268.4 ± 5.39 to 259 ± 5.63**, the mean % reduction was 3.51**± 0.54** and it was highly significant in comparing by control, acetic acid, venlafaxine 20 and venlafaxine 40, while insignificant in comparing by dexamethasone group.

3.1.5 Effect of Treatment with Venlafaxine 40mg/kg after Induction of Colitis

Treatment with venlafaxine 40mg/kg after induction of colitis increased the mean body weight from **269.6 ± 4.61 to 280.6 ± 4.63**, the mean % increase was 4.09 **± 0.56** and it was highly significant in comparison with control, acetic acid, dexamethasone, venlafaxine 10 and venlafaxine 20.

3.2 Macroscopic Assessment

3.2.1 Effect of Acetic Acid on Rat Colonic Tissue

In comparing the control group by acetic acid group, it was found that the later had sever erythema, bleeding ulceration and necrosis. The macroscopic ulcer score (Ma US) ranged from 3 to 4, the mean was 3.80 ± 0.20 and the difference was highly significant in comparison with other groups.

3.2.2 Effect of Treatment with Dexamethasone after Induction of Colitis

In comparing the group which was treated with dexamethasone (1mg/kg) by control group it was found that dexamethasone group had slight bleeding and erythema, but in comparison with acetic acid group there was marked improvement which was noticed**.** Ma US ranged from 1 to 2, the mean was 1.80 ± 0.20 and the difference was highly significant in comparison with control group and acetic acid group, significant with group treated with venlafaxine 10 mg/kg, while it was non-significant in comparison with groups treated with venlafaxine 20 and 40 mg/kg.

3.2.3 Effect of Treatment with Venlafaxine 10mg/kg after Induction of Colitis

In comparing the group which was treated with venlafaxine 10 mg/kg by control group it was found that group venlafaxine 10 had edema, bleeding and erythema, but in comparison with acetic acid group there was slight improvement**.** Ma US ranged from 2 to 4, the mean was 3.00 ± 0.31and the difference was highly significant in comparison with control group and group received venlafaxine 40 mg/kg, significant with dexamethasone group, while it was non-significant in comparison with acetic acid group and group treated with venlafaxine 20 mg/kg.

3.2.4 Effect of Treatment with Venlafaxine 20mg/kg after Induction of Colitis

In comparing the group which treated with venlafaxine 20mg/kg by control group it was found that venlafaxine 20 group had

spotting bleeding and mild erythema, but in comparison with acetic acid group there was improvement and healing. Ma US ranged from1to3, the mean was 2.20 ± 0.37 and the difference was highly significant in comparison with control group and acetic acid group, but non-significant in comparison with group treated with dexamethasone and groups treated with venlafaxine 10, 40 mg/kg.

3.2.5 Effect of Treatment with Venlafaxine 40mg/kg after Induction of Colitis

In comparing the group which was treated with venlafaxine 40mg/kg by control group it was found that treatment with venlafaxine 40 mg/kg completely relived ulceration**.** Ma US ranged from1to2, the mean was 1.40 ± 0.24 and the difference was highly significant in comparison with acetic acid group, significant in comparison with control group and group treated with venlafaxine 10 mg/kg, while the difference was non-significant in comparison with dexamethasone group and group treated with venlafaxine 20 mg/kg.

3.3 Histopathological Examination

3.3.1 Effect of Acetic Acid on Rat Colonic Tissue

In comparing the control group, which show normal architecture with intact epithelium in rat colonic mucosa and normal crypts, with acetic acid group it was found that intra-colonic administration of acetic acid resulted in loss of mucosal architecture with ulceration, necrosis and acute inflammatory cell infiltration involved mucosa and sub-mucosal layers. The total microscopic ulcer score (t Mi US) ranged from **7 to 10** and the mean was **8.80 ± 0.49,** the difference was highly significant in comparison with other groups $p < 0.001$.

3.3.2 Effect of Treatment with Dexamethasone after Induction of Colitis

Treatment with dexamethasone (1mg/kg) after induction of colitis decreased the inflammation severity and extent in rat's colonic tissue. There was mild inflammation, the inflammatory infiltrate markedly decreased and limited to mucosa only, but there was still crypt damage with superficial ulceration. The t Mi US ranged from 3 to 4 and the mean was 3.60 ± 0.24, the difference was highly significant in comparison with control, AA and venlafaxine 10, while significant in comparison with venlafaxine 20 and non-significant in comparison with venlafaxine 40.

3.3.3 Effect of Treatment with Venlafaxine 10mg/kg after Induction of Colitis

Treatment with venlafaxine 10mg/kg after induction of colitis decreased the inflammation severity and extent in rat colon tissue, there was moderate inflammation involved mucosa and submucosa, mixed chronic and acute inflammatory infiltrate extended to submucosa, with crypt damage**.** The t Mi US ranged from **4 to 9** and the mean was **5.80 ± 0.86,** the difference was highly significant in comparison with control, AA and venlafaxine 40, while significant in comparison with Dexamethasone group, but in comparison with venlafaxine 20 the difference was non-significant.

3.3.4 Effect of Treatment with Venlafaxine 20mg/kg after Induction of Colitis

Treatment with venlafaxine (20mg/kg) after induction of colitis decreased inflammation severity, there was moderate inflammation involved mucosa and submucosa, but there was healing of surface epithelium, and crypt regeneration.

The t Mi US ranged from **5 to 6** and the mean was **5.20 ± 0.20,** the difference was highly significant in comparison with control, acetic acid and venlafaxine 40, but non-significant in comparison with Dexamethasone and venlafaxine 10 groups.

3.3.5 Effect of Treatment with Venlafaxine 40mg/kg after Induction of Colitis

Treatment with venlafaxine (40mg/kg) significantly decreased the inflammatory parameters in rat colon, there was mild inflammation, the inflammatory infiltrate markedly decreased, healing of surface epithelium with regenerated crypts. The t Mi US ranged from **2 to 3** and the mean was **2.60 ± 0.24,** the difference was highly significant in comparison with control, AA, venlafaxine 20, but non-significant in comparison with Dexamethasone group.

3.4 Myeloperoxidase Measurement

3.4.1 Effect of Acetic Acid on Myeloperoxidase Level

In comparing the control group in which MPO level ranged from **16.4 to 19.2** by acetic acid group it was found that induction of colitis by acetic acid significantly elevated the inflammatory marker MPO, it was ranged from **45.2 to 81.5**, the mean was **65.38 ± 5.88**, the mean % elevation was 266.60 **± 37.35** and it was highly significant ($p<0.01$).

3.4.2 Effect of Treatment with Dexamethasone after Induction of Colitis

In comparing group which was treated with dexamethasone (1mg/kg) after induction of colitis by acetic acid, it was found that dexamethasone reduced MPO level significantly, it was ranged from **21.9 to 32.7**, the mean was **27.10 ± 1.79**, the mean % elevation from control was **50.73 ± 8.27,** and it was non-significant. In comparison with acetic acid group the difference was highly significant ($p<0.01$).

3.4.3 Effect of Treatment with Venlafaxine 10mg/kg after Induction of Colitis

Administration of venlafaxine 10mg/kg, after induction of colitis, lowered MPO level significantly in comparison with acetic acid group, it was ranged from **36.5 to 46.2**, the mean was **40.59 ± 1.62**, the mean % elevation from control was **126.92 ± 12.18** and it was significant, but in comparison with dexamethasone group the difference was non-significant $p > 0.05$.

3.4.4 Effect of Treatment with Venlafaxine 20mg/kg after Induction of Colitis

Administration of venlafaxine 20mg/kg after induction of colitis, lowered MPO level significantly in comparison with acetic acid group, it was ranged from **26.2 to 45.6**, the mean was **33.76 ± 3.61**, the mean % elevation from control was **89.74 ± 24.28** and it was non-significant. In comparison with dexamethasone group the difference was non-significant.

3.4.5 Effect of Treatment with Venlafaxine 40mg/kg after Induction of Colitis

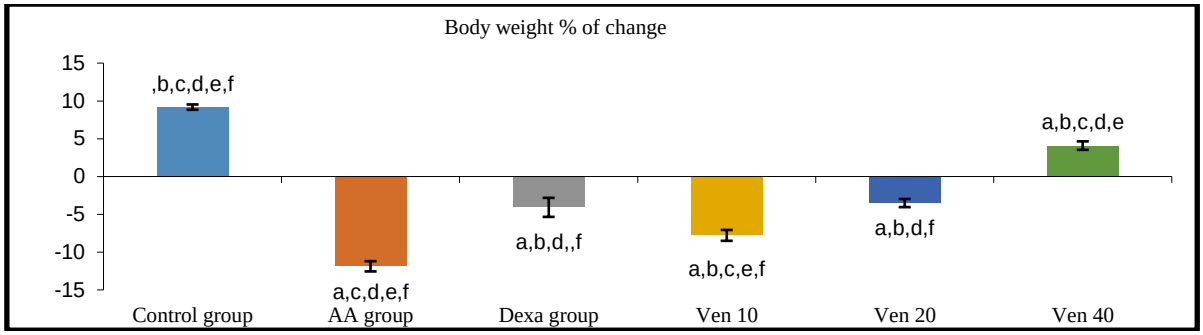
In comparing group which was treated with venlafaxine 40mg/kg after induction of colitis, by acetic acid group it was found that treatment with venlafaxine 40 mg/kg lowered MPO level, it was ranged from **18.5 to 42.1,** the mean was **31.98 ± 4.45,** this reduction was highly significant, in comparing by acetic acid group.

Table 1: Total colitis index was measured by summing three sub scores of inflammation severity, inflammation extent, and crypt damage (Motavallian et al., 2012).

Scoring parameter		Score definition
1	**Inflammation severity**	**0 (none)** **1 (mild)** **2 (moderate)** **3 (severe**
2	**Inflammation extent**	**0 (none)** **1 (mucosa)** **2 (submucosa)** **3 (transmural)**
3	**Crypt damage**	**0 (none)** **1 (basal1/3 damaged)** **2 (basal 2/3 damaged)** **3 (crypts lost and surface epithelium present)** **4 (crypts lost and surface epithelium lost)**

Table 2 and Figure 1: Effect of treatment with dexamethasone (1mg/kg i.p) and venlafaxine (10, 20, 40mg/kg i.p) on mean body weight (gm) of rats and the mean % change after acetic acid induced colitis

	Body weight 1 st day	Body weight 5th day	% Change
	Mean ± SE	Mean ± SE	
Control group	263.2 ± 2.89	287.4 ± 3.94	9.18 ± 0.35 **b,c,d,e,f**
AA group	275 ± 4.81	242.4 ± 5.57	-11.88 ± 0.68 **a,c,d,e,f**
Dexa group	270.2 ± 3.76	259 ± 1.41	- 4.08 ± 1.26 **a,b,d,f**
Ven 10	271.8 ± 3.54	250.6 ± 3.12	-7.79 ± 0.72 **a,b,c,e,f**
Ven 20	268.4 ± 5.39	259 ± 5.63	-3.51 ± 0.54 **a,b,d,f**
Ven 40	269.6 ± 4.61	280.6 ± 4.63	4.09 ± 0.56 **a,b,c,d,e**



- Induction of colitis by acetic acid followed by treatment for four days.
- Data was expressed as mean ±SE

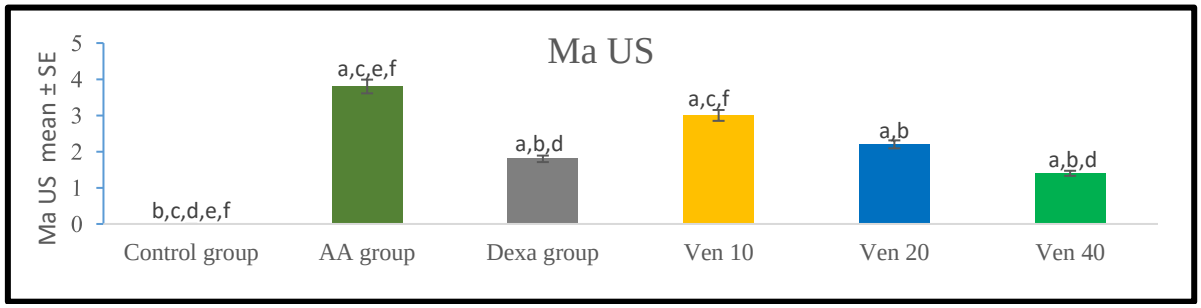
P-value > 0.05: Non-significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant

a: Difference was significant in comparison with control group.
b: Difference was significant in comparison with AA group.
c: Difference was significant in comparison with dexamethasone group.
d: Difference was significant in comparison with venlafaxine 10mg group.
e: Difference was significant in comparison with venlafaxine 20mg group.
f: Difference was significant in comparison with venlafaxine 40mg group

- One way analysis of variance **(ANOVA)** followed by post hoc Tuckey test for multiple comparison.

Table 3 and Figure 2: Effect of treatment with dexamethasone (1mg/kg i.p) and venlafaxine (10, 20 and 40mg/kg i.p) on macroscopic ulcer score after acetic acid induced colitis in rats.

	Ma US	
	Range	**Mean ± SE**
Control group	0 – 0	0.00 ± .00 b**, c, d, e, f**
AA group	3 – 4	3.80 ± 0.20 **a, c, e, f**
Dexa group	1 – 2	1.80 ± 0.20 a**, b, d**
Ven 10	2 - 4	3.00 ± 0.31 a**, c, f**
Ven 20	1 – 3	2.20 ± 0.37 a**, b**
Ven 40	1 – 2	1.40 ± 0.24 **a, b, d**



-Induction of colitis by acetic acid followed by treatment for four days.
- Data was expressed as mean ±SE

P-value > 0.05: Non-significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant

a: Difference was significant in comparison with control group.
b: Difference was significant in comparison with AA group.
c: Difference was significant in comparison with dexamethasone group.
d: Difference was significant in comparison with venlafaxine 10mg group.
e: Difference was significant in comparison with venlafaxine 20mg group.
f: Difference was significant in comparison with venlafaxine 40mg group

-One way analysis of variance **(ANOVA)** followed by post hoc Tuckey test for multiple comparison.

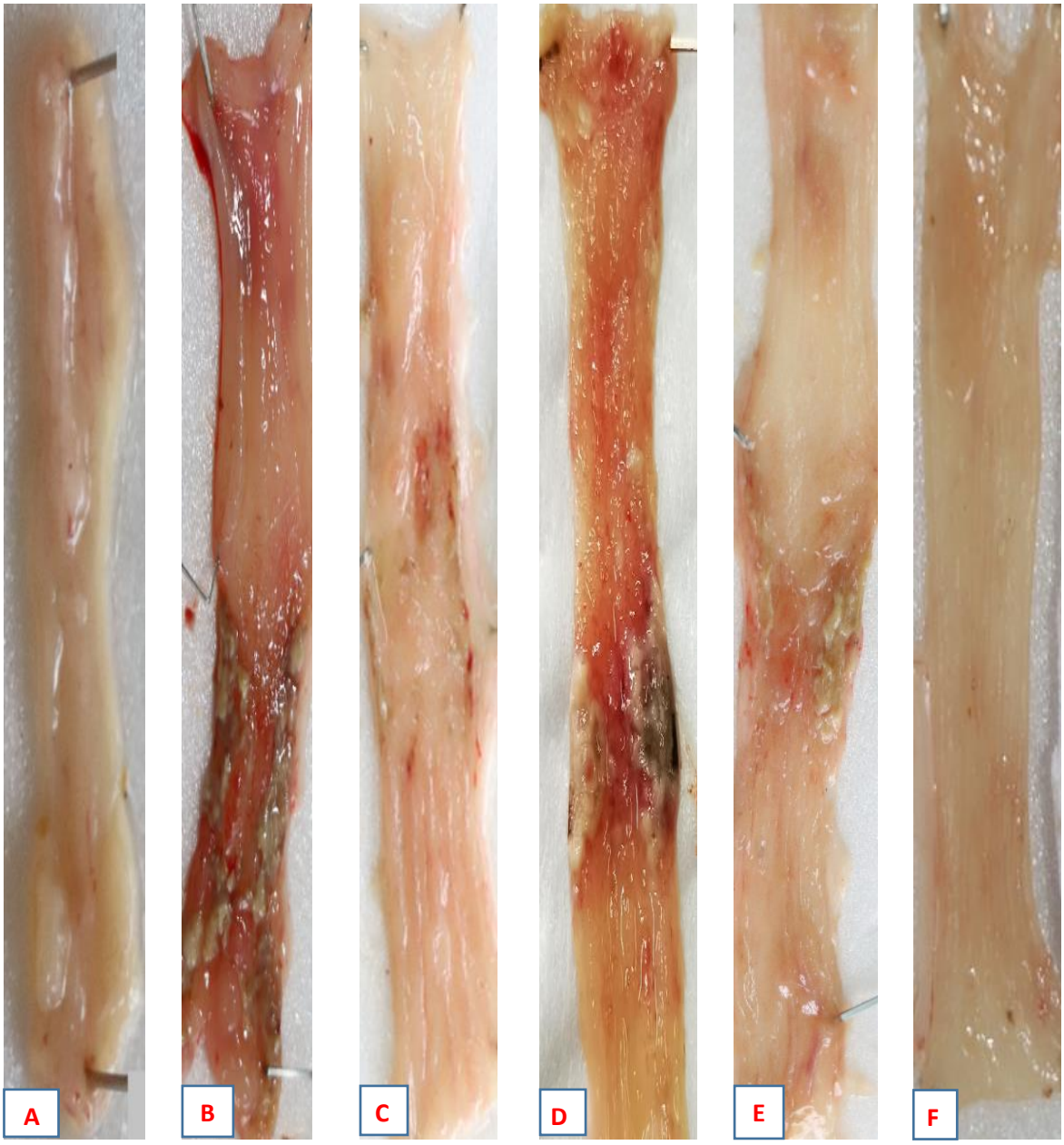
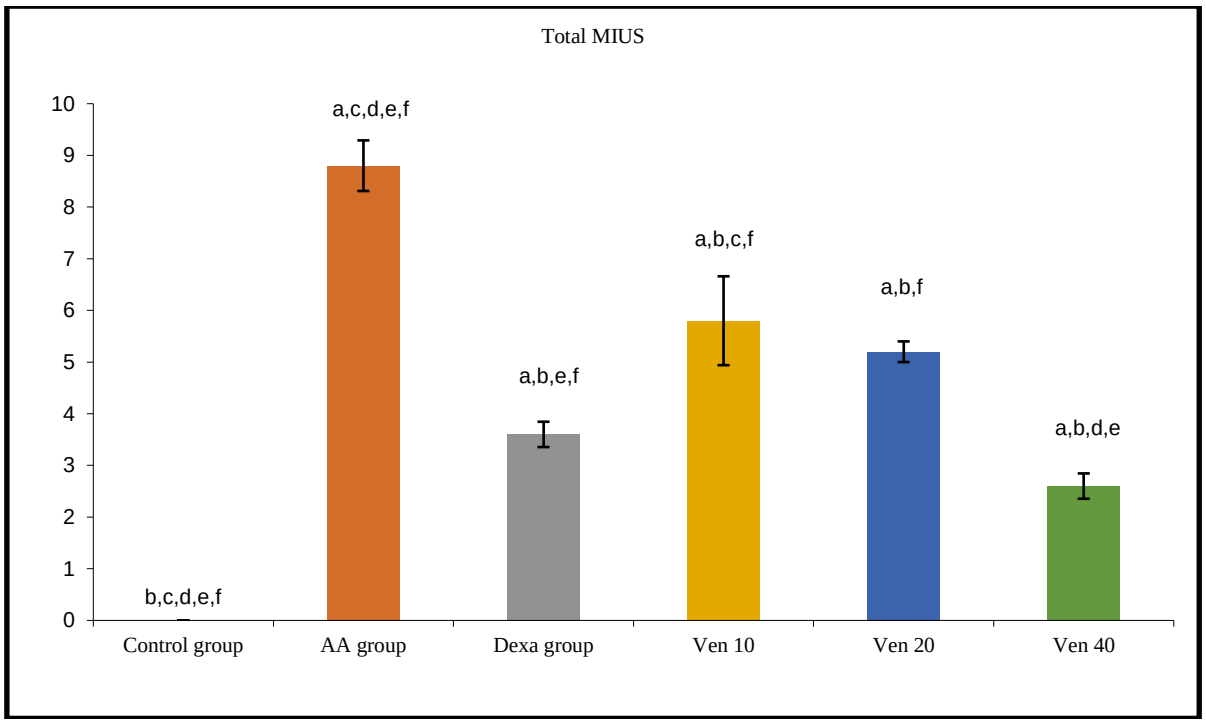


Figure (3): Macroscopic assessment of rat colonic tissue; four days after induction of colitis by acetic acid and treatment with dexamethasone (1mg/kg i.p) and venlafaxine (10, 20, 40 mg/kg i.p).

A: Normal rat colon tissue (score: 0).
B: Rat colon after induction of colitis by acetic acid 4% without treatment, there was sever erythema, bleeding ulceration and necrosis (score, 4).
C: Rat colon after induction of colitis and treatment with dexamethasone (1mg/kg), there was slight bleeding and erythema (score, 2).
D: Rat colon after induction of colitis and treatment with venlafaxine 10mg/kg, in comparison with control there was bleeding, ulceration and erythema (score, 3).
E: Rat colon after induction of colitis and treatment with venlafaxine 20mg/kg, in comparison with control their mild edema and erythema (score: 3).
F: Rat colon after induction of colitis and treatment with venlafaxine 40mg/kg, in comparison with control there was marked improvement and healing just slight erythema, the tissue was close to the control group (score, 1).

Table 4 and Figure 4: Effect of treatment with dexamethasone (1mg/kg i.p) and venlafaxine (10, 20, 40mg/kg i.p) on total microscopic ulcer score after acetic acid induced

	Inflammation severity		Inflammation extent		Crypt damage		Total MiUS	
	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE
Control group	0 – 0	0.00 ± 0.00	0 – 0	0.00 ± 0.00	0 – 0	0.00 ± 0.00	0 – 0	0 ± 0 **b,c,d,e,f**
AA group	2 – 3	2.80 ± 0.20	2 – 3	2.80 ± 0.20	2 – 4	3.20 ± 0.37	7 – 10	8.80 ± 0.49 **a,c,d,e,f**
Dexa group	1 – 1	1.00 ± 0.00	1 – 1	1.00 ± 0.00	1 – 2	1.60 ± 0.25	3 – 4	3.60 ± 0.24 **a,b,e,f**
Ven 10	1 – 3	2.00 ± 0.32	1 – 3	2.00 ± 0.32	1 – 3	1.80 ± 0.37	4 – 9	5.80 ± 0.86 **a,b,c,f**
Ven 20	1 – 2	1.80 ± 0.20	1 – 2	1.60 ± 0.25	1 – 3	1.80 ± 0.37	5 – 6	5.20 ± 0.20 **a,b,f**
Ven 40	1 – 1	1.00 ± 0.00	0 – 1	0.60 ± 0.25	0 – 2	1.00 ± 0.32	2 – 3	2.60 ± 0.24 **a,b,d,e**



-Induction of colitis by acetic acid followed by treatment for four days.
- Data was expressed as mean ±SE
P-value > 0.05: Non-significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
a: Difference was significant in comparison with control group.
b: Difference was significant in comparison with AA group.
c: Difference was significant in comparison with dexamethasone group.
d: Difference was significant in comparison with venlafaxine 10mg group.
e: Difference was significant in comparison with venlafaxine 20mg group.
f: Difference was significant in comparison with venlafaxine 40mg group
-One way analysis of variance **(ANOVA)** followed by post hoc Tuckey test for multiple comparison.

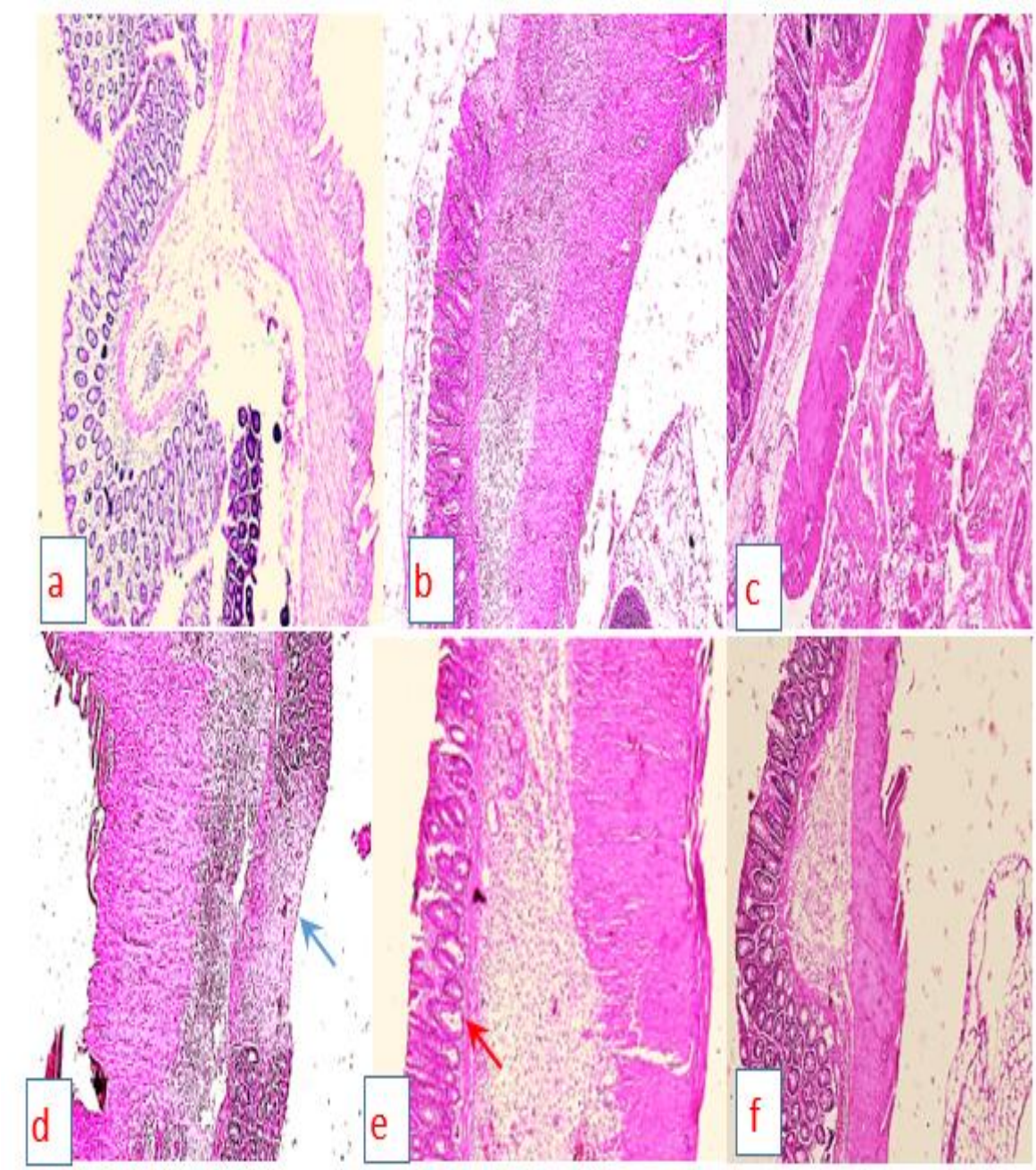


Figure 5: Histopathological presentation for rat colon after induction of colitis by acetic acid and treatment with dexamethasone (1mg/kg i.p) and venlafaxine (10, 20, 40 mg/kg i.p). H&E staining (M.P ×40).

a- Normal colonic mucosa has crypts with underlying submucosa, and small nodules of lymphoid tissue. **(Score, 0).**

b- After induction of colitis, sever inflammation involve all layers of the colon and shedding of the surface epithelium **(Score,8)**

c- After treatment with dexamethasone, mild inflammation, the inflammatory infiltrate markedly decreased and limited to mucosa only, but still crypt damage **(Score, 3).**

d- After treatment with venlafaxine 10mg/kg, moderate inflammation with superficial erosion **(Score, 9).** The arrow in **d** point to erosion of surface epithelium.

e- After treatment with venlafaxine 20mg/kg, moderate inflammation involves mucosa and submucosa, with healing of surface epithelium and regeneration of crypts **(Score, 6).** The arrow in **e** point to regenerated crypts**.**

f- After treatment with venlafaxine 40mg/kg, mild inflammation, the inflammatory infiltrate markedly decreased, healing of surface epithelium with regenerated crypts **(Score, 3).**

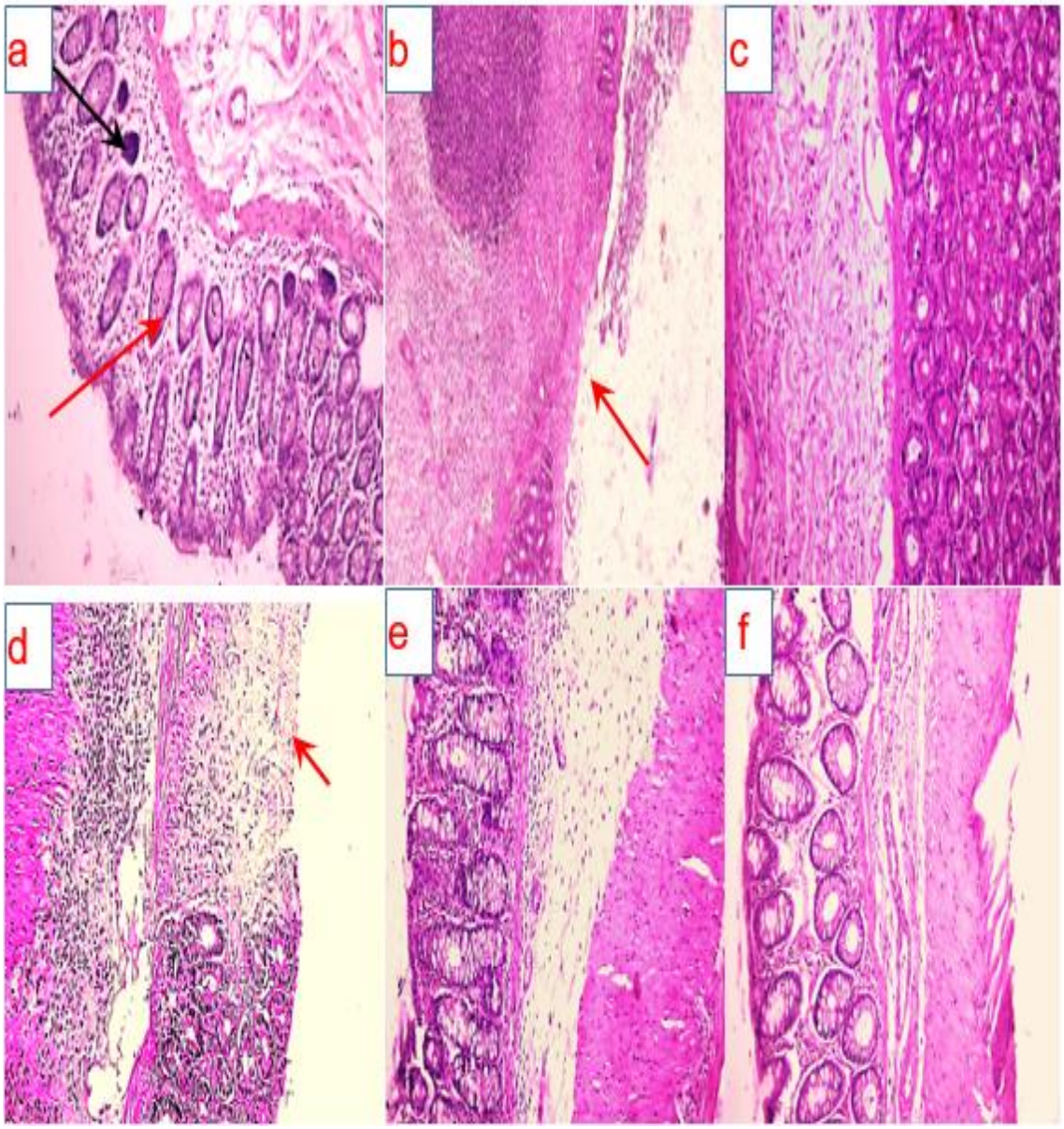
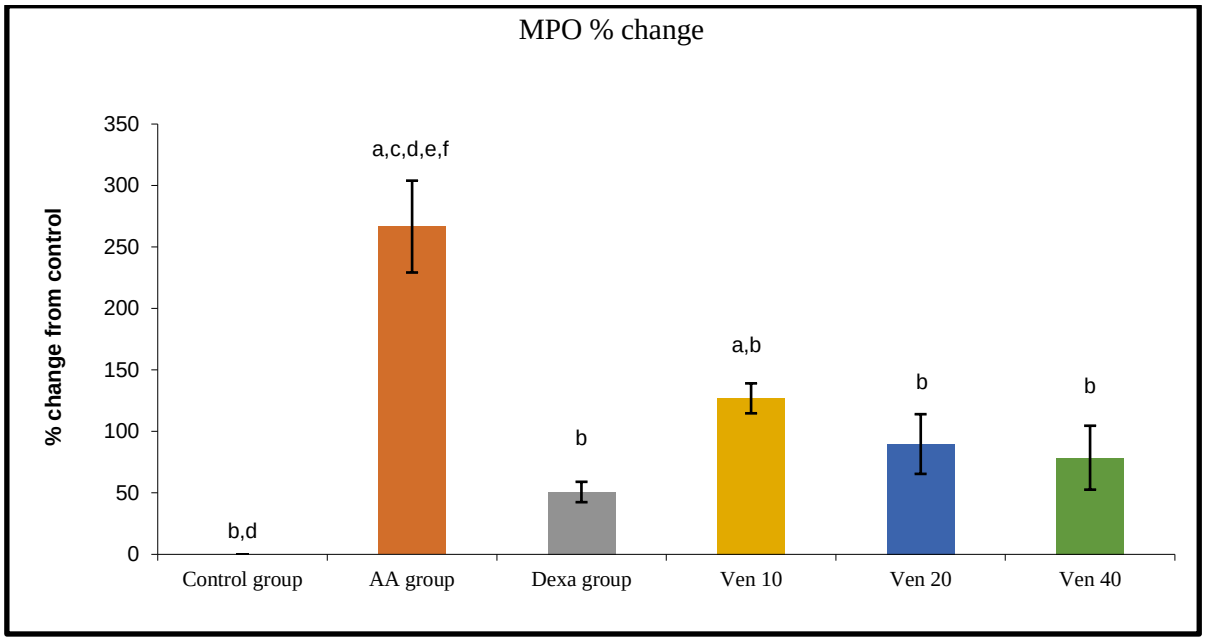


Figure 6: Histopathological presentation for rat colon after induction of colitis by acetic acid and treatment with dexamethasone (1mg/kg i.p) and venlafaxine (10, 20, 40 mg/kg i.p). H&E staining (M.P ×100).

a- Normal colonic mucosa has crypts with underlying submucosa, and small nodules of lymphoid tissue. **(Score, 0).** Red arrow point to crypts filled with mucin, black arrow point to lymphoid nodules.
b- After induction of colitis, sever inflammation involve all layers of the colon and shedding of the surface epithelium and ulceration **(Score, 8)**. Arrow point to shedding of surface epithelium.
c- After treatment with dexamethasone, mild inflammation, the inflammatory infiltrate markedly decreased and limited to mucosa only, but still crypt damage **(Score, 3).**
d- After treatment with venlafaxine 10mg/kg, moderate inflammation with superficial erosion **(Score, 9).** The arrow in **d** point to erosion of surface epithelium.
e- After treatment with venlafaxine 20mg/kg, moderate inflammation involves mucosa and submucosa, with healing of surface epithelium and regeneration of crypts **(Score, 6).**
f- After treatment with venlafaxine 40mg/kg, mild inflammation, the inflammatory infiltrate markedly decreased, healing of surface epithelium with regenerated crypts **(Score, 3).**

Table 5 and Figure 7: Effect of treatment with dexamethasone (1mg/kg i.p), venlafaxine (10, 20, 40mg/kg i.p) on mean MPO level and the mean % change from control in acetic acid induced colitis in rats.

	MPO		
	Range	**Mean ± SE**	**% Change**
Control group	16.4 – 19.2	17.96 ± 0.49	0 ± 0 **b,d**
AA group	45.2 – 81.5	65.38 ± 5.88	266.60 ± 37.35 **a,c,d,e,f**
Dexa group	21.9 – 32.7	27.10 ± 1.79	50.73 ± 8.27 **b**
Ven 10	36.5 – 46.2	40.59 ± 1.62	126.92 ± 12.18 **a,b**
Ven 20	26.2 – 45.6	33.76 ± 3.61	89.74 ± 24.28 **b**
Ven 40	18.5 – 42.1	31.98 ± 4.45	78.62 ± 25.99 **b**



-Induction of colitis by acetic acid followed by treatment for four days.
- Data was expressed as mean ±SE
P-value > 0.05: Non-significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
a: Difference was significant in comparison with control group.
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c: Difference was significant in comparison with dexamethasone group.
d: Difference was significant in comparison with venlafaxine 10mg group.
e: Difference was significant in comparison with venlafaxine 20mg group.
f: Difference was significant in comparison with venlafaxine 40mg group
-One way analysis of variance **(ANOVA)** followed by post hoc tuckey test for multiple comparison.

MPO: myeloperoxidase**.**

4. Discussion

Inflammatory Bowel Disease (IBD) is a chronic, heterogeneous, relapsing and remitting condition primarily as a consequence of inflammation within the bowel lumen. IBD includes Crohn's disease (CD) and ulcerative colitis (UC), and is a lifelong disease marked by episodes of remission and relapse [18] Because IBD is a lifelong condition with chronic symptoms, reduced quality of life and social functioning, with bidirectional communication via the gut–brain axis, patients with IBD might have psychological illness, including symptoms of common mental disorders and somatization [8].

The prevalence of anxiety and depression in IBD was at least twice that of the general population. Up to 34.7% of IBD patients in active stage were found to have comorbid depression compared with 19.9% of inactive IBD patients. Patients with history of depression were more likely to develop IBD, which could be selectively protected by certain antidepressant treatments [19]. The involvement of a cortical influence in the brain-gut axis as well as the interaction of the hypothalamic-pituitary-adrenal axis and the peripheral nervous system provide an initial explanation of the psychological symptoms associated with IBD [20].

These psychological disorders may impact on the clinical course of disease, have the potential to exacerbate disease activity and may disturb the mainstream treatment of IBD disease [21].

The recent demonstration of a tonic counter inflammatory influence mediated by the vague nerve in experimental colitis provides a potential link between behavior and gut inflammation. Experimental conditions that induced depressive-like behaviors in mice increased susceptibility to intestinal inflammation by interfering with the tonic vagal inhibition of pro-inflammatory macrophages [22]. Adjuvant therapy with an antidepressant drug which has anti-inflammatory effect, may exert favorable effects in the control of the disease [23].

Anti-inflammatory activity has been reported for a few of antidepressant drugs and suggested that these drugs may be useful in the management of some inflammatory syndromes and also for patients who suffer from both inflammatory and psychological disorders. [24]. Antidepressants targeting 5-HT and NE transporters seem to suppress inflammatory processes in vitro [25].

Acetic acid-induced colitis is a rapid and reproducible model of colitis for screening of drugs with anti-colitic activity. This model has similarity to pathological and clinical features of the human ulcerative colitis [26].

In the current study, we found that treatment with venlafaxine exhibited anti-inflammatory effect in rat model of acetic acid induced colitis .

In agreement with our result, Minaiyan et al., [12]; showed that anti-inflammatory effect of the anti-depressant, venlafaxine in acetic acid-induced colitis and a coherent communication exist between depression and the course of IBD probably through the brain-gut axis.

Also, it was confirmed by, Hajhashemi et al., [27], in his research on the anti-inflammatory effects of venlafaxine in the rat model of carrageenan –induced paw edema, that venlafaxine has potent anti-inflammatory effect which is related to the peripheral and central effects of this drug .

Additionally, anti-nociceptive and anti-inflammatory effects of venlafaxine were evaluated by Aricioglu and his colleagues in a rat model of inflammation [28] .

It was concluded by Chugh et al., [29]; that a significant anti-inflammatory activity of fluoxetine and venlafaxine 40 mg/kg was observed in rat paw oedema model, but this activity was blunted, when prazosin was added to venlafaxine and fluoxetine. Addition of prazosin resulting in loss of anti-inflammatory activity of fluoxetine and venlafaxine is indicative of probable

role of noradrenergic/serotonergic pathway in inflammation.

It was mentioned by Maes et al., [30]; those studies have shown that noradrenaline suppresses the production of IFN-α and enhances the production of IL-10, and so has significant negative immune regulatory effects.

It is well known that venlafaxine causes alterations in central norepinephrine and serotonin neurotransmitters [31]. It could be suggested that enhancement of norepinephrine concentrations in the locus coeruleus, which is one of the most important nuclei involved in depression, pain, and inflammation via venlafaxine, can lead to over activation of α2-adrenoceptors and suppression of locus coeruleus electrical activity [32] .

In accordance, Vollmar et al., [25] reported that data clearly indicate anti-inflammatory properties of venlafaxine which might be a result of monoamine-mediated immunomodulation, supporting the thesis of a noradrenergic- and serotonergic-mediated immunoregulation.

Piletz et al [33]; suggested that venlafaxine treatment of depressed patients may lower pro-inflammatory biomarkers, and regarding venlafaxine's ability to normalize pro-inflammatory biomarkers especially in the lower serotonergic dose range is in agreement with several previous studies which show that pure SSRIs can down-regulate these biomarkers .

In contrary, Tynan et al [34] in comparison between anti-inflammatory effects of SSRI and SNRI, found that SSRIs but not the SNRI venlafaxine, potently inhibit lipopolysaccharide-induced microglial TNF-αand NO production.

Venlafaxine has been safe and effective in animal models, healthy human volunteers, and patients for treatment of various pain syndromes. Possesses important peripheral anti-nociceptive effect that may contribute to its effectiveness as an anti-inflammatory drug [28] .

In the current work, following induction of colitis, infiltration of neutrophils and other immune cells increased in the inflamed tissues that caused an increase in MPO activity enzyme level in the tissue, venlafaxine corrected the elevated amount of biochemical marker MPO.

In agreement, Minaiyan et al [12] concluded that after induction of colitis, venlafaxine lowerd the elevated MPO owing that to its anti- inflammatory effect.

It was explained by Hajhashemi et al [27] that the effect of venlafaxine on central nervous system to alter neuroimmune interactions and/or sympathetic nervous system activity that affect the function of the immune system could be considered as one of the possible mechanisms of venlafaxine responsible in attenuation of inflammation. The anti-inflammatory effect of venlafaxine is mediated mostly through the inhibition of IL-1β and tumor necrosis factor (TNF)-α production and decreases myeloperoxidase MPO activity in the site of inflammation.

5. Conclusion

Venlafaxine was found to have anti-inflammatory activity in the animal model of ulcerative colitis, as it decreased MPO level and improved the histo-pathological profile in the colonic tissue of rats.

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