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Evaluation of anti-colitic effect of fluvoxamine against acetic acid-induced colitis in normal and reserpinized depressed rats

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ABSTRACT

High prevalence of psychological comorbidities such as depression and anxiety in patients with inflammatory bowel disease (IBD) supports the premise that adding an anti-depressant drug with known anti-inflammatory effect to the medical treatment have beneficial effect in the course of the underlying disease. Colitis was induced by intracolonic instillation of 2 ml of 4% v/v acetic acid solution in rats. Anti-colitic effect of fluvoxamine was evaluated in two categories: A: normal rats, B: reserpinized (6 mg/kg, i.p.) depressed rats. In group A, fluvoxamine (2.5, 5, 10 mg/kg, i.p.) was administered 2 h after induction of colitis and in group B: reserpine (6 mg/kg, i.p.) was administered 1 h prior to colitis induction and then fluvoxamine (2.5, 5, 10 mg/kg, i.p.) was administered 2 h after colitis induction. Dexamethasone (1 mg/kg) was used as reference drug. All the treatments continued daily for five days. The effect was assessed on the basis of macroscopic score, biochemical (myeloperoxidase) changes and histopathological studies. Results showed that fluvoxamine (2.5 and 5 mg/kg) and dexamethasone treatment markedly reduced disease severity in both reserpinized and non-reserpinized rats as indicated by reduction in macroscopic and microscopic colonic damages while reserpine adversely exacerbated the colitis damage. Myeloperoxidase activity which was increased following colitis induction was also decreased. The findings of this study elucidate the anti-colitic and anti-inflammatory properties of fluvoxamine and so introduced it as a good candidate to treat depressive symptoms in people comorbid to IBD.

1. Introduction

Inflammatory bowel disease (IBD), which include Crohn's disease (CD) and ulcerative colitis (UC) is characterized by episodes of exacerbations and remissions (Bernstein et al., 2009). Patients frequently suffer from abdominal pain, diarrhea along with blood and/or mucus, fever, weight loss, fatigue, inflammation, ulceration and edema (Thoreson and Cullen, 2007). The chronic course of the disease can cause a wide range of psychological and interpersonal concerns to patients. Indeed, symptoms, such as fecal incontinence or soiling and lack of bowel control, can lead to a loss of self-unworthiness or cause stigmatization in patients (Sajadinejad et al., 2012). There have been many reports over the years that prevalence of psychiatric illness in particular anxiety and depressive disorders are significantly more common in patients with IBD compared to the general population (Kurina et al., 2001) and the symptoms of these conditions are more severe during periods of active disease (Graff et al., 2009). Studies show a 30% rate of depression during remission, with 80% and 55% of patients reporting anxiety and depression, respectively, during relapse (Mikocka-Walus et al., 2012a). There is increasing evidence that depressive mood exerts negative effects on the course of several chronic diseases (Turner and Kelly, 2000). As IBD is a chronic and relapsing gastrointestinal disorder, it is not separate from this rule. A recent pilot study in patients with ulcerative colitis and a conserved colon demonstrated that depression was a risk factor of relapse (Häuser et al., 2011). So, questions of quality of life and of coping strategies with the disease are particularly important. Gastroenterologists reported that treating psychological co-morbidities with antidepressants was successful in reducing pain, gut irritability, urgency of defecation and to control disease activity and lengthen remission (Mikocka-Walus et al., 2006, 2012b). Furthermore anti-inflammatory and analgesic effects of some anti-depressant drugs such as amitriptyline (Sadeghi et al., 2011), maprotiline (Hajhashemi et al., 2010a), venlafaxine (Aricioğlu et al., 2005) and fluoxetine (Abdel-Salam et al.,

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2004) have been evaluated. Systematic reviews by Mikocka-Walus et al. (2007, 2008, 2012a) indicate that antidepressants appeared not only to help certain individual patients with IBD to cope with their emotional problems, but also improved their quality of life. The published observations also hold out the intriguing possibility that anti-depressant therapy may have specifically influenced the course of their inflammatory disease. Due to lower rate of side effect in comparison to other anti-depressant drugs, SSRIs are the most wide group which is used for treating depressive symptoms (Filipovic and Filipovic, 2014). Following administration of SSRIs (citalopram, sertraline) in patients with IBD, they felt that psychological problems responded well to this treatment however these drugs have no beneficial effect on somatic aspects of the disease (Mikocka-Walus et al., 2007). Furthermore, fluoxetine was found to protect against colitis in a randomized controlled trial (Itatsu et al., 2011). So the present study aims at evaluating the effect of fluvoxamine as a potent SSRI drug with known anti-inflammatory properties, on experimental colitis in normal and reserpine induced depressed rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–250 g) obtained from the laboratory animal house of the School of Pharmacy, Isfahan University of Medical Sciences, Iran, were used in this experiment. Animals were kept at controlled environmental conditions where the temperature of the experimental room was maintained at 20–23 °C, relative humidity at 50–60% with a 12:12 h light/dark cycle. All animals were given access to a standard pellet diet and water. Animals were housed individually in standard cages and were acclimatized for 7 days before initiation of the treatment. The animal study was approved by the guideline of the ethical committee of Isfahan University of Medical Sciences.

2.2. Chemicals

Fluvoxamine maleate was a gift from Abidi Pharmaceutical Company (Tehran, Iran). Dexamethasone was also a gift from Raha Pharmaceutical Company (Isfahan, Iran). Reserpine, hexadecyl trimethyl-ammonium bromide (HTAB) and O-dianisidine dihydrochloride were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Formalin solution 35% w/w, glacial acetic acid and diethyl ether oxide were purchased form Merck (Darmstadt, Germany). All other solvents and chemicals were of analytical grade.

2.3. Behavioral tests

2.3.1. Determination of anti-depressant dose of fluvoxamine in reserpinized depressed rats

This part of experiment was designed to determine the optimum dose of fluvoxamine which has anti-depressant effect in reserpinized depressed animals to be used in colitis part of the experiment. So rats were randomly assigned to six groups of rats comprising six rats per group as follows: Sham group received normal saline (2 ml/kg, i.p.) daily for four days. Control group received a single dose of reserpine (6 mg/kg, i.p.) and was treated with normal saline 3 h after reserpine injection and then daily for four days. Test groups received a single dose of reserpine (6 mg/kg, i.p.) and then were treated with fluvoxamine (1.25, 2.5, 5, and 10 mg/kg, i.p.) 3 h after reserpine injection and daily for four days. Then animals were subjected to forced swimming test. So at the third day, the rats were individually placed in a cylinder containing water 15 cm in height at 25 °C for 15 min (pre-test). On the following day (fourth day) the rats were again immersed in water and total duration of immobility was measured for 5 min. The immobility time was regarded as the time that the rat spent floating in the water without struggling and making only those movements necessary to keep its head above water (Porsolt et al., 1978).

2.4. Body weight measurement

Every morning at the start of the experiment and daily thereafter, animals were individually weighed by a digital scale (ACCU-LAB V-3000) and the body weight was recorded subsequently in order to measure body weight change and also to calculate all drug doses as mg/kg base (Niu et al., 2013).

2.5. Induction of experimental colitis

Acute colitis was induced by acetic acid using a technique introduced by MacPherson and Pfeiffer (1978). Briefly, rats were fasted for 24 h before induction of colitis in stainless steel cages with free access to water. The rats were lightly anesthetized with ether and a flexible plastic catheter with an outside diameter of 2 mm was inserted 8 cm into the colon via the anus. Two milliliter 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 returned.

2.6. Animal grouping

The rats were randomly divided into the following groups of six rats in each: Sham group: received normal saline (2 ml/kg, i.p.) without induction of colitis; control group: received normal saline (2 ml/kg, i.p.) following induction of colitis; dexamethasone group: dexamethasone (1 mg/kg, i.p.) was given 2 h following induction of colitis. Test groups include non-reserpine treated groups which received fluvoxamine (2.5, 5, 10 mg/kg, i.p.) 2 h following induction of colitis and reserpine treated groups which received reserpine (6 mg/kg, i.p.) 1 h prior to induction of colitis and then treated with fluvoxamine (2.5, 5, 10 mg/kg, i.p.) 2 h following induction of colitis. Administration of medications was performed for the following four days. All the drug doses were prepared freshly each morning.

2.7. Assessment of colon macroscopic damage

The rats were killed 24 h after the last treatment (Day 5) by an overdose of ether inhalation. The colons were dissected, slightly rinsed with normal saline and the length and weight were measured (Minaiyan et al., 2011). Then segments of colon were used for the assessment of macroscopic and histopathology damage and measurement of tissue myeloperoxidase activity.

Macroscopic damage scores were assigned by an independent observer according to the following criteria: 0=no macroscopic changes, 1=mucosal erythema only, 2=mild mucosal edema, slight bleeding, or slight erosion, 3=moderate edema, bleeding ulcers, or erosions, and 4=severe ulceration, erosions, edema, and tissue necrosis (Deshmukh et al., 2010). Then, tissue was fixed on a white plastic sheet and a photo was taken using an appropriately adjusted Nikon camera (Coolpix p100) to calculate the ulcer area. Pieces were cut into two pieces, one piece for histopathology assessment (maintained in 5 ml formalin 10% as fixator) and one piece for measuring myeloperoxidase (MPO) enzyme activity. The pieces for measuring the myeloperoxidase (MPO) enzyme activity were frozen in liquid nitrogen and kept at freezer (−85 °C) (Minaiyan et al., 2013).

Furthermore, ulcer area was measured by Fiji-win 32 software, an image processing and analysis software inspired by NIH Image for the Macintosh (Ghosh et al., 2004). For each specimen ulcer

index was calculated using the following equation as described by Varshosaz et al. (2010).

$$\text{Ulcer index} = \text{Ulcer area}(\text{cm}^2) + \text{Macroscopic score}$$

2.8. Assay for myeloperoxidase (MPO) activity

Tissue MPO activity was carried out to measure neutrophil accumulation. According to the technique described by Bradley et al. (1982) with some modification, segments of the colon (0.1 g) were chopped to small pieces and homogenized in 1 ml of 50 mM potassium phosphate (pH=6) with 0.5% HTAB in an ice bath using polytron homogenizer. More buffers were added to obtain a concentration equivalent to 5 ml per 0.1 g of colon tissue. The resultant homogenate was sonicated in an ice bath for 10 s, then subjected to a sequence of freezing and thawing 3 times, and sonicated again for 10 s and centrifuged for 15 min at 15,000 rpm at 4 °C. A 0.1 ml of the supernatant was mixed with 2.9 ml of 50 mM phosphate buffer (pH=6) containing 0.167 mg/ml O-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured using a UV/VIS spectrophotometer (LSI Model Alfa-1502).

2.9. Histopathological evaluation of colon damage

Sections of colon specimens were fixed in formalin solution (10%), dehydrated, embedded in paraffin, sliced into 5 μm-thick sections, deparaffinized with xylene, hydrated and stained with hematoxylin and eosin (H&E) respectively and then scored according to the criteria previously described by Dieleman et al. (1998) (Table 1). Total colitis index was measured by summing the scores of inflammation severity, inflammation extent and crypt damage (Minaiyan et al., 2011).

2.10. Statistical analysis

Results are expressed as mean ± S.E.M for parametric data and median (range) for non-parametric data. The data was analyzed by one-way ANOVA followed by TUKEY post hoc test for multiple comparisons. For histopathological data, a non-parametric test (Kruskal–Wallis test) with Mann–Whitney *U* test was employed. All statistical analyses were assessed using Graph Pad Prism (ver. 5.04) software. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ (in the figures).

3. Results

3.1. Assessment of anti-depressant dose of fluvoxamine using forced swimming test

Induction of depression by single administration of reserpine (6 mg/kg, i.p.) in Res group significantly increased immobility time compared to Sham group ($P<0.01$). As illustrated in Fig. 1, fluvoxamine at doses of 2.5, 5, and 10 mg/kg reduced this immobility in reserpinized rats ($P<0.001$). Having distinguished their anti-depressant effects in FST following four days treatment, these three doses of fluvoxamine were selected for evaluating in the colitis part of this study.

3.2. Change in animals' body weight

As shown in Fig. 2, induction of experimental colitis caused loss of body weight during five days experiment in control group. Administration of reserpine (6 mg/kg, i.p.) in Res group caused the same body weight loss as in the control group (Fig. 1B). Rats treated with dexamethasone (1 mg/kg, i.p.) as a reference drug and fluvoxamine (2.5, 5 mg/kg, i.p.) showed a significant

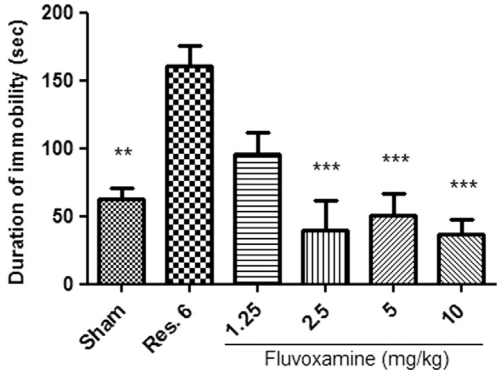

Fig. 1. Effect of fluvoxamine (1.25, 2.5, 5, and 10 mg/kg, i.p.) on duration of immobility (seconds) during forced swimming test in reserpinized (6 mg/kg, i.p.) rats. Res=reserpine (6 mg/kg), i.p.=intraperitoneally. Results are presented as mean ± S.E.M ($n=6$). $^{**}P<0.01$ and $^{***}P<0.001$ compared to Res; one-way ANOVA followed by Tukey test.

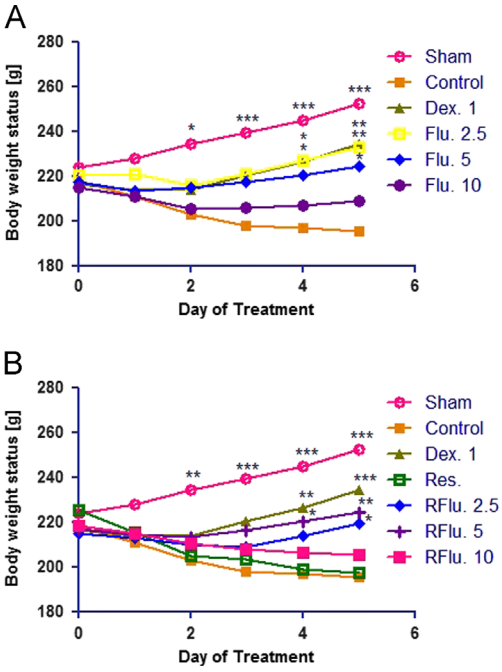

Fig. 2. Effect of fluvoxamine (2.5, 5, and 10 mg/kg, i.p.) on body weight change (g) in rats with acetic acid colitis. Treatments were administered 2 h after acetic acid instillation and daily thereafter for 4 consecutive days. A: normal rats, B: reserpinized (6 mg/kg, i.p.) depressed rats; i.p.=intraperitoneally, Dex. 1=dexamethasone (1 mg/kg), Res=reserpine (6 mg/kg). Results are expressed as mean ± S.E.M ($n=6$). $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to control, one-way ANOVA followed by Tukey test.

improvement of the wasting disease in day 4 and/or day 5 compared with control group. However following five days experiment, rats in Sham group gained body weight.

3.3. Effect of fluvoxamine on macroscopic features

Following induction of colitis, the colons of control group showed severe inflammation, ulceration, wall thickening and edema and also necrosis. These colonic damages were more intense in reserpine treated group (Res, 6 mg/kg, i.p.); whereas colons of Sham group showed intact epithelium with no damage. As illustrated in Figs. 3 and 4, weight of distal colon and ulcer index (summation of ulcer area and macroscopic score) was significantly increased in control and Res group five days after colitis induction ($P<0.001$). Moreover, treatment with dexamethasone (1 mg/kg, i.p.) as a reference drug, reduced both weight

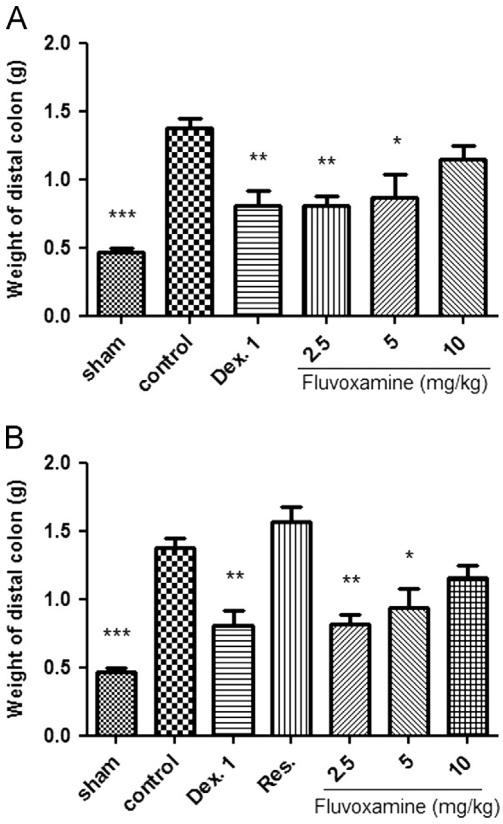

Fig. 3. Effect of fluvoxamine (2.5, 5, and 10 mg/kg, i.p.) on weight of distal colon. A: normal rats, B: reserpinized (6 mg/kg, i.p.) depressed rats; i.p.=intraperitoneally, Dex. 1=dexamethasone (1 mg/kg), Res=reserpine (6 mg/kg). Results are presented as mean ± S.E.M ($n=6$). $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to control, one-way ANOVA followed by Tukey test.

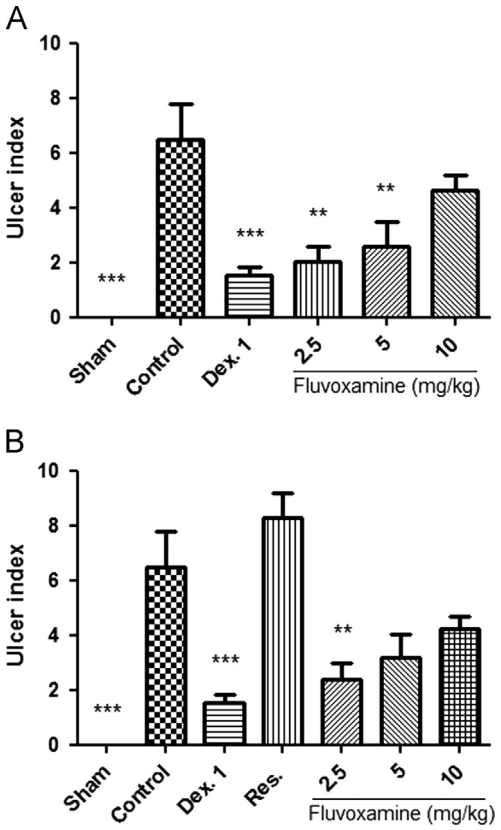

Fig. 4. Effect of fluvoxamine (2.5, 5, and 10 mg/kg, i.p.) on ulcer index. A: normal rats, B: reserpinized (6 mg/kg, i.p.) depressed rats; i.p.=intraperitoneally, Dex. 1=dexamethasone (1 mg/kg), Res=reserpine (6 mg/kg). Results are presented as mean ± S.E.M ($n=6$). $^{**}P<0.01$ and $^{***}P<0.001$ compared to control, one-way ANOVA followed by Tukey test.

of colon ($P<0.01$) and ulcer index ($P<0.001$) in Dex. 1 group compared with the acetic acid-control group. Fluvoxamine produced a significant reduction of weight of colon in both reserpinized and non-reserpinized rats at doses of 2.5 and 5 mg/kg ($P<0.01$, $P<0.05$, respectively) (Fig. 3). Compared with control group, fluvoxamine at dose of 2.5 mg/kg showed a significant reduction in ulcer index ($P<0.01$) in both reserpinized and non-reserpinized animals whereas fluvoxamine at dose of 5 mg/kg just in non-reserpinized rats reduced ulcer index significantly ($P<0.05$) but not in reserpinized depressed rats Fig. 5.

3.4. Effect of fluvoxamine on histopathological features

In Sham group, no histological damage was observed and colonic mucosa has an intact epithelium. In contrast, control group included transmural necrosis, edema and diffuse inflammatory cell infiltration in the mucosa, desquamated areas and loss of epithelium. An infiltrate consisting of mixed inflammatory cells was observed (Fig. 6). Administration of reserpine (6 mg/kg, i.p.) in Res group exacerbated histopathological damages additionally (Fig. 6C). In rats treated with dexamethasone (1 mg/kg, i.p.) histopathological scores including inflammation severity, inflammation extent, crypt damage and total colitis index were significantly decreased ($P<0.01$). In non-reserpinized groups, treatment of rats with fluvoxamine (2.5, 5 mg/kg, i.p.) reduced colonic damage as evaluated in histopathological score reduction. Also, total colitis index in comparison with control group, was significantly decreased ($P<0.01$) (Table 1).

In reserpinized depressed groups, fluvoxamine at doses of 2.5, 5 mg/kg also attenuated the histological scores (Fig. 6G and H). As compared to control, these treatments brought about a decline in total colitis index in injurious colons. However fluvoxamine at a dose of 10 mg/kg could not reduce the colonic damage while evaluating histopathological features.

3.5. Effect of fluvoxamine on myeloperoxidase (MPO) activity

The results of MPO activity correlated closely with other parameters studied including macroscopic and pathologic. This result confirmed the histological assessment indicating increased leukocyte infiltration to the control and Res groups and MPO activity in the intestinal tissue of the control and Res groups was significantly increased ($P<0.01$) as compared to Sham group. In contrast, MPO activity in dexamethasone treated rats ($P<0.01$) and fluvoxamine (2.5, 5 mg/kg, i.p.) treated groups ($P<0.05$) decreased in depressed and non-depressed rats as compared with control group Fig. 7.

4. Discussion

At first, in a pilot study we showed that single administration of reserpine at the specified dose (6 mg/kg, i.p.) significantly ($P<0.01$) increased duration of immobility in the FST four days later, so it is interpreted that reserpine can induce depression during the time we need to evaluate anti-colitic effects of fluvoxamine; although, it has been demonstrated that this anti-depressant drug, at selected doses (2.5, 5, and 10 mg/kg, i.p) reduced the immobility time four days after single administration of reserpine, and so has anti-depressive effect (Fig. 1). This model of depression is a suitable and discriminative model for evaluating depressive or anti-depressive effects of

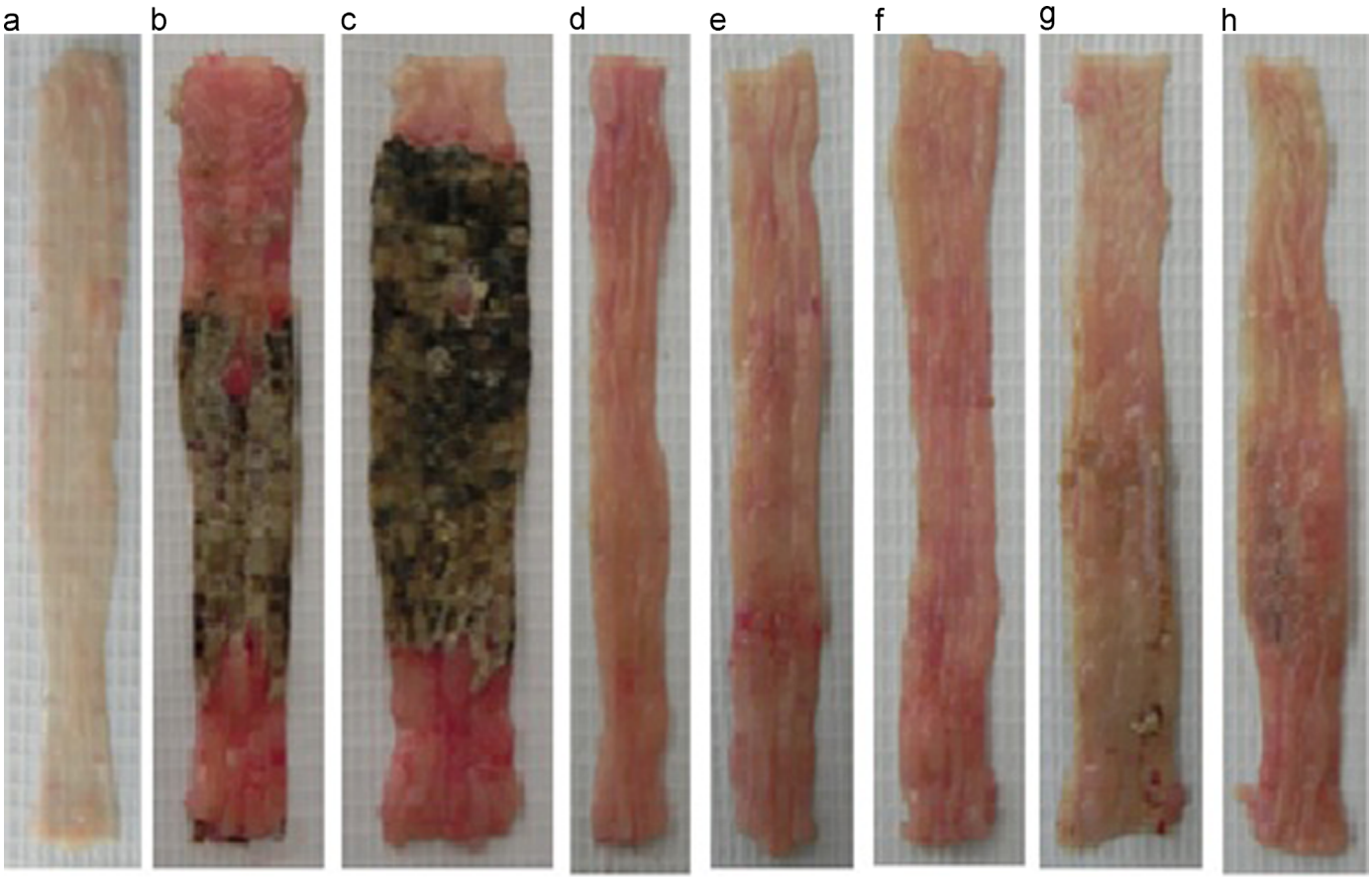

Fig. 5. Photographs of colonic tissue five day after colitis induction in rats. (a) Normal colon; (b) acetic acid-control rat; (c) reserpinized (6 mg/kg, i.p.) colitic rat; (d) treatment with dexamethasone(1 mg/kg, i.p.) in colitic rat; (e, f) treatment with fluvoxamine (2.5, 5 mg/kg, i.p.) in colitic rats; (g,h) treatment with fluvoxamine (2.5, 5 mg/kg, i.p.) in reserpinized (6 mg/kg, i.p.) colitic rats.

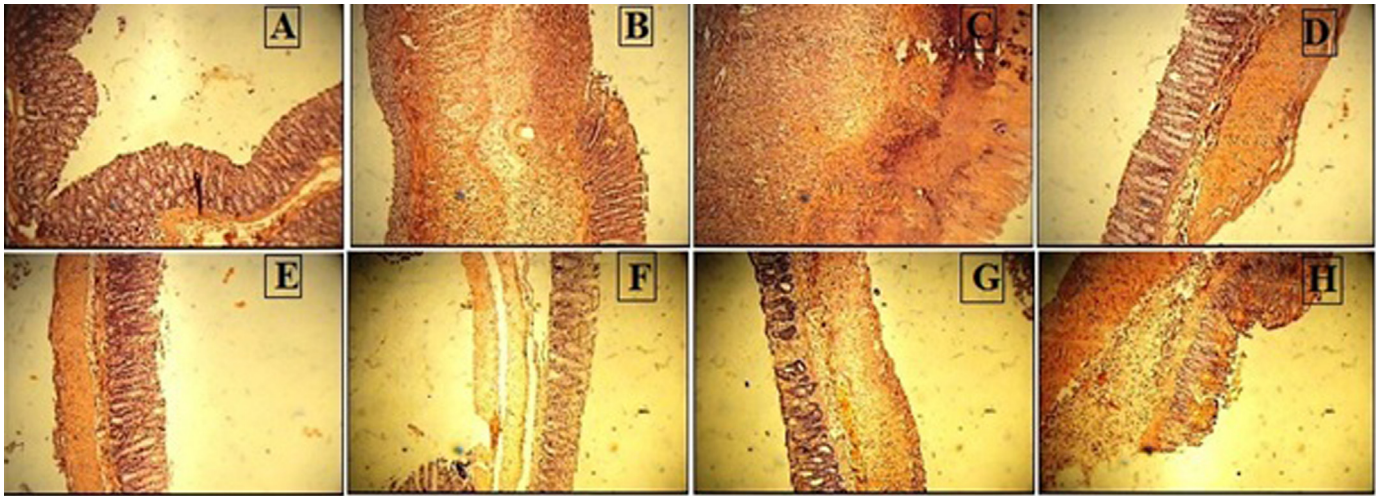

Fig. 6. Effect of fluvoxamine treatment on experimental colitis in rat. (A) Appearance of a normal rat colon. (B) Appearance of a control colitic rat colon: associated with mucosal layers destruction with inflammatory cell infiltration and crypt damage. (C) Appearance of an acetic acid-treated rat colon in reserpinized (6 mg/kg, i.p.) depressed rat: associated with great mucosal layer destruction with more inflammatory cell infiltration and cryptic damage. (D–F) Appearance of an acetic acid rat colon treated with dexamethasone (1 mg/kg, i.p.), fluvoxamine (2.5 mg/kg, i.p.), fluvoxamine (5 mg/kg, i.p.) in non-depressed rats. (G, H) Colitis tissue treated with fluvoxamine (2.5 mg/kg, i.p.), fluvoxamine (5 mg/kg, i.p.) in reserpinized (6 mg/kg, i.p.) depressed rats: associated with attenuating the extent and severity of the histological cell damage; i.p.= intraperitoneally, H&E staining, Original magnification × 10.

reserpineor fluvoxamine respectively. In a recent study, depression was induced by injection of reserpine (6 mg/kg, i.p.) and then the anti-depressive effect was evaluated by FST (Bolandghamat et al., 2011). Clinically effective SSRIs are active in the forced swimming test (Kuśmider et al., 2007).

In colitic part of the experiment, acetic acid-induced colitis was used. It is a well-characterized model, which resembles to human colitis (MacPherson and Pfeiffer, 1978). It was shown that colonic inflammation is characterized by increased neutrophils infiltration into the intestinal tissue, edema, ulceration and necrosis. Our

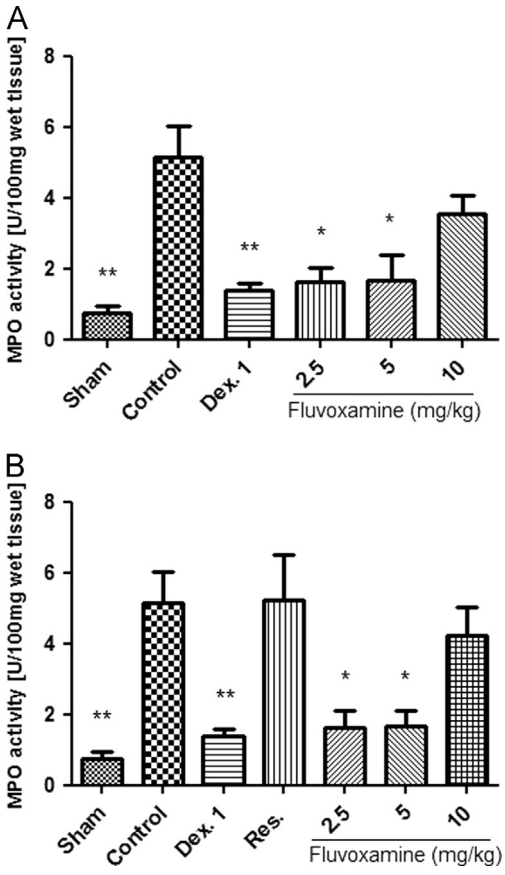

Fig. 7. Effect of fluvoxamine (2.5, 5, and 10 mg/kg, i.p.) on myeloperoxidase (MPO) enzyme activity in the colonic tissue. A: normal rats, B: reserpinized (6 mg/kg, i.p.) depressed rats; i.p.=intraperitoneally, Dex. 1=dexamethasone (1 mg/kg), Res=reserpine (6 mg/kg). Results are presented as mean ± S.E.M ($n=6$). *$P<0.05$ and **$P<0.01$ compared to control, one-way ANOVA followed by Tukey test.

findings showed that treatment with fluvoxamine at lower doses (2.5 or 5 mg/kg) markedly attenuated the colonic damage in acetic acid-colitis in reserpinized depressed rats and non-resepinized rats. Decreased body weight loss and colonic weight/length ratio, reduced colonic macroscopic and microscopic damage scores and inhibited MPO activity were the criteria for improvement. Furthermore, induction of depression by reserpine (6 mg/kg, i.p.) exacerbated the colitis damage in reserpine-treated group. The results of this study are consistent with findings of recent studies indicating that depressive-like behavior is associated with an exaggerated response to inflammatory stimuli in the gut, using 2 models of depression induction of maternally separated (MS) (Varghese et al., 2006) and i.c.v. administration of reserpine (Ghia et al., 2008, 2009). Both maternally separated and reserpine (1 μg/d, i.c.v) induced depressed mice showed evidence of depressive like behavior in tail suspension test and enhanced intestinal permeability after colitis induction by DSS or DNBS (dinitrobenzene sulfonic acid). Impaired parasympathetic function and reduction in acetylcholine level was mentioned as a basis for the vulnerability of reserpinized mice to colitis. Treatment with the anti-depressant drug, desmethylimipramine reversed the behavioral change and protected against this vulnerability to colitis in depressed mice. In the current study fluvoxamine at doses of 2.5 and 5 mg/kg improved the colitic status in both depressed and non-depressed animals; while in the mentioned experiment, the anti-depressant did not influence inflammation in the absence of depression. Combination of pharmacological models (reserpine-induced depression, forced swimming test and acetic acid-induced colitis)

Table 1
Effect of fluvoxamine (Flu, 2.5, 5, and 10 mg/kg, i.p.) on pathologic parameters of colitis induced by acetic acid in normal and reserpinized (6 mg/kg, i.p.) depressed (RFlu, 2.5, 5, and 10) rats.

Groups	Inflammation severity (0–3)	Inflammation extent (0–3)	Crypt damage (0–4)	Total colitis index (0–10)
Sham	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Control	3 (2–3)[a]	3 (2–3)[a]	4 (1–4)[a]	9.5 (6–10)[a]
Dex. 1	0.5 (0–1)[c]	0 (0–2)[c]	0 (0–2)[c]	1 (0–5)[c]
Flu. 2.5	1 (0–2)[c]	1 (0–2)[c]	1 (0–2)[b]	3 (1–6)[c]
Flu. 5	0.5 (0–3)[b]	0.5 (0–2)[c]	1 (0–2)[b]	2.5 (1–6)[c]
Flu. 10	2 (1–3)	2 (1–3)	2.5 (1–4)	5.5 (5–10)
Res	3 (1–3)	3 (1–3)	4 (1–4)	10 (4–10)
RFlu. 2.5	1 (0–2)[c]	1 (0–2)[c]	0.5 (0–3)[b]	2.5 (1–4)[c]
RFlu. 5	1.5 (1–2)[b]	1.5 (1–3)[b]	2 (0–2)[b]	4.5 (2–7)[b]
RFlu. 10	2.5 (1–3)	2 (1–3)	2 (0–4)	5.5 (5–10)

Dex. 1=dexamethasone (1 mg/kg), Res=reserpine (6 mg/kg), Flu=Fluvoxmine, RFlu=reserpinized (6 mg/kg, i.p.) colitic rats treated with fluvoxamine, i.p.=intraperitoneally. Values are presented as median (range) ($n=6$).

[a] $P<0.01$ compared to Sham.
[b] $P<0.05$.
[c] $P<0.01$ compared to control, Mann–Whitney U test.

in our study, were used in order to show the depression impact on clinical course of colitis and also to show the salutary effect of anti-depressant therapy in IBD patients. In another study the anti-inflammatory effect of fluoxetine and desipramine at two doses (10 and 20 mg/kg/day) were reported in acetic-acid induced colitis in rats. Fluoxetine was shown to have anti-inflammatory effect at lower dose (10 mg/kg) only in normal rats (Guemei et al., 2008) while our study evaluated the effect of fluvoxamine in both normal and depressed animals to discriminate the effect of depression on colitis additionally.

Rajesh et al. (2013) showed that following treatment with *Tribulus terrestriss* extract at a dose of 160 mg/kg, body weight loss showed significant protection (57%) against DSS-induced colitis. The results of the current study also showed that fluvoxamine at lower dose (2.5 mg /kg) reduced the body weight loss in both normal and depressed animals (Fig. 2).

Psycho–neuro–endocrine–immune modulation through the brain–gut axis likely has a key role in the pathogenesis of inflammatory bowel disease (IBD) (Prins, 2011). Qiu et al. (1999) demonstrated that colitis induction in mice by DNBS can subsequently be reactivated by environmental stresses which are due to reduction of colonic mucin and increased colon permeability. This vulnerability to environmental stress is perceived initially by the CNS, which responds to environmental stimuli by modulating inflammatory or immune response through a complex network of signals that communicate with the intestine (Hollander, 2003). This theory confirmed why depression induction following reserpine injection exacerbates colitis and why treatment with fluvoxamine improved intestinal inflammation.

Selective serotonin reuptake inhibitors (SSRIs), which are prescribed for anxiety, have also been used for treating inflammatory, chronic and neuropathic pain. Acute SSRI administration increases brain extracellular serotonin and the anti-nociceptive effect of SSRI was considered to be induced via serotonin (5-HT) receptor activities (Hayashi et al., 2009). We conclude that increasing brain serotonin level following fluvoxamine treatment may have direct anti-inflammatory effects to reduce inflammatory conditions in the gut.

It has been reported that numbers and content of mast cells and other immune cells increase in the inflamed colonic tissue (He, 2004). Anti-depressants following increasing monoamine levels and activating monoamine receptors inhibit LPS or

IFN-gamma evoked inflammatory transactivations through the up-regulation of cAMP/PKA pathway in intestinal immune cells (Hashioka et al., 2009). Inhibition of NF-κB signaling causes to inhibit activation of gene expression of iNOS (inducible nitric oxide synthase) and various pro-inflammatory cytokine. It is one of the plausible mechanisms with which fluvoxamine may decrease inflammatory mediator from intestinal immune cells. Koh et al. (2011) showed that preventive and therapeutic administration of fluoxetine, another SSRI anti-depressant can ameliorate DSS-induced colitis in mice. The mentioned mechanism in the study was that fluoxetine directly inhibits NF-κB signaling in intestinal epithelial cells (IEC) and ameliorates experimental colitis. Although fluvoxamine is a SSRI drug with similar properties as fluoxetine, it is conferred from the study that beneficial effect of fluvoxamine in the acetic acid colitis in both normal and depressed rats is to some extent mediated through inhibition of NF-kB signaling in IEC. Modulation of 5-HT release from IEC is critical to normal and perhaps abnormal gastrointestinal function (Galligan, 2004). Recent studies declared that, inhibition of $5HT_3$ receptor by ondansetron (Motavallian et al., 2012), granisetron (Fakhfouri et al., 2010) or tropisetron (Motavallian et al., 2013) has salutary effect on experimental colitis in rat. It is justified that serotoninergic receptors and in particular, $5HT_3$ receptor were found in immune cells including macrophages and play an important role in infiltration and activation of macrophages in intestinal inflammation. However the results of our study have no controversies to the result of the mentioned studies because of diversity of serotonergic receptors and their roles in the gastrointestinal system. The reason for ineffectiveness of fluvoxamine at higher dose (10 mg/kg) may be due to overcoming of serotonergic drive and then activating the mentioned receptor in macrophage. Similar study with Guemei et al. (2008) also showed that fluoxetine only at lower dose (10 mg/kg) has beneficial effect in acetic acid colitis whereas a dose of 20 mg/kg had also been evaluated.

Administration of a SSRI drug in a short period of time stimulates the hypothalamo–pituitary–adrenal (HPA) axis. The increase in synaptic 5-HT concentration and activation of post-synaptic 5-HT receptors is responsible for the immediate increase in hormonal output via the HPA axis after SSRI treatment. This action is mediated through postsynaptic $5\text{-}HT_{1A}$ and $5\text{-}HT_2$ receptors which lead to corticosterone release, a well-known endogenous substance with strong anti-inflammatory property, from the adrenal cortex (Mikkelsen et al., 2004). Anti-inflammatory effect of fluvoxamine in this model of colitis may be to some extent thorough this mechanism. In addition, anti-inflammatory effect of fluvoxamine in a rat model of inflammation was demonstrated. Hajhashemi et al. (2010b) had shown that following i.p. administration of fluvoxamine before or after subplantar injection of carrageenan considerably inhibited paw edema response at 4 h post-carrageenan ($P<0.001$), and this anti-inflammatory effect was mediated through HPA axis and glucocorticoid receptor which mifepristone inhibited the effect.

In this regard, we have speculated that the beneficial effects of fluvoxamine on ulcerative colitis are mainly mediated through these mechanisms including stimulating the HPA axis and corticosterone release, inhibition of NF-κB signaling pathways in the inflammatory cell and reducing pro-inflammatory cytokine and NO levels and modulating brain–gut axis through increasing neural serotonin level.

In conclusion, the results of this study have clinical importance. Our findings support the need for identification and management of depressive mood in IBD patients instead of merely attributing depressed mood to the severity of the disease. It is suggested that routine screening of IBD patients for depressive disorders, especially at the time of first diagnosis and during disease flares is helpful to add an anti-depressive drug to the patient drug regimen. Also based on our findings fluvoxamine can be a good candidate to treat depression comorbidities in people with IBD.

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