Effects of Imipramine and Fluvoxamine on Acetic Acid – Induced Ulcerative Colitis in Rats

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ABSTRACT

Ulcerative colitis (UC) is a chronic inflammatory disorder with unknown etiology. Depression sometimes is accompanied with ulcerative colitis in certain patients. The capability of using antidepressant drugs such as imipramine & fluvoxamine for treatment of UC in patients with or without depression is still controversy. Induction of ulcerative colitis resulted in macroscopic changes in the form of mucosal congestion, extensive disruption, linear and deep hemorrhage ulceration. The incidence of an inflammatory response was assessed by elevation of myeloperoxidase (MPO), an indicator of neutrophil infiltration, tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukine-10 (IL-10) and interferon-γ (IFN-γ) in colonic mucosa while reduced glutathione (GSH) was decreased. Administration of imipramine & fluvoxamine at a dose of 30 mg kg^{-1} body weight for 2 weeks before induction of colitis and for 2 weeks after induction of colitis significantly decreased the levels of MPO, TNF-α, IFN-γ, IL-1β, IL-2, IL-6 and increased GSH concentration. Moreover both drugs (mainly with fluvoxamine) attenuated the macroscopic colonic damage-induced by acetic acid. These results suggest that imipramine or fluvoxamine may be effective in the treatment of UC through their effect on cytokines production and scavenging oxygen-derived free radicals.

The objective of this study is to evaluate the effects of imipramine and fluvoxamine on the extent and severity of UC caused by intra colonic administration of acetic acid 3% in rats.

INTRODUCTION

Ulcerative colitis (UC) is a relapsing disease of unknown origin characterized by chronic idiopathic inflammation of the colon and bloody diarrhea. The complexity of symptoms is correlated with the severity of the disease and tends to differ according to its extent *(1)*. Inadequate understanding of the etiology of these disorders has hampered effective treatment. The activation of intestinal inflammatory processes is mediated by proinflammatory cytokines and chemokines *(2)*.

Cytokines have autocrine, paracrine, and endocrine activities that mediate the local and systemic manifestations of intestinal inflammation. These molecules regulate and amplify the immune response, induce tissue damage and mediate complications of the inflammatory response, such as diarrhea and fibrosis. They exert their responses either directly or indirectly by stimulating production of other effector molecules *(2,3)*.

Interleukin-1 (IL-1) and (IL-2) are key immunoregulatory cytokines that amplify the inflammatory response by activating a cascade of immune cells. IL-1α, IL-1β, and tumor necrosis factor alpha (TNF-α), secreted by activated macrophages stimulate the production of other cytokines *(4)*. In several different diseases the proinflammatory cytokine TNF-α forms a necessary element in the chain of pathological events and is responsible for so many of the inflammatory pathways activated in ulcerative colitis *(5)*. It is now well recognized that reactive oxygen species (ROS) are produced in excess by the inflamed mucosa in ulcerative colitis and may be pathogenic *(6)*.

Depression and anxiety may predispose people to inflammatory bowel diseases (IBD), both of them share a common environmental, behavioral, or genetic etiology *(7)*. It has been

reported that nearly 50% of patients with irritable bowel syndrome responded symptomatically to regimens including antidepressant therapy *(8)*. Recently, there is a great suggestion that psychiatric disorders could be one of the etiological factors in some patients with UC *(7)*. The evidence that major depression in particular is accompanied by activation of the inflammatory system and that proinflammatory cytokines may play a role in the etiology of depression *(1)*.

Kubera et al. *(9)* reported that systemic administration of proinflammatory cytokines such as IL-1β & IFN-γ induced changes in the endocrine and central monoamine systems which are reminiscent of those observed in depression. In recent years, the role of antidepressant drugs in the management of different pain syndromes has elicited considerable interest. Experimental and clinical evidence suggested a decrease in intensity of pain perception following tricyclic antidepressants (TCAs). Drugs such as amitriptyline and maprotiline were reported to be effective in the treatment of polyneuropathy pain in both diabetic and nondiabetic patients *(10)*. In animal models of inflammatory or neuropathic pain TCAs exhibited anti-nociceptive properties *(11)*.

Imipramine is a commonly prescribed TCAs because it increases the production of IL-6 (12), IL-10 *(13)* and decreases the level of INF- γ *(13)*. Also, after chronic administration of imipramine, a marked decrease in the expression of corticotropin–releasing hormone messenger RNA in the hypothalamus was observed, an action which has been proposed as contributing to its anti-inflammatory effect *(14)*. The selective serotonin reuptake inhibitors (SSRIs), fluvoxamine is widely used in clinical practice for the treatment of depression. In addition, fluvoxamine might be of benefit in the management of pain of neuropathic origin *(15)*. In experimental models of inflammation, fluoxetine has been shown to exert anti-inflammatory and pain relieving effects *(16)*. The mechanism by which SSRIs alleviate inflammation is still unclear *(17, 18)*.

Therefore the current study was undertaken to determine whether imipramie or fluvoxamine has a protective or therapeutic effects on one of the inflammatory bowel syndrome (ulcerative colitis) induced experimentally by acetic acid in rats. Also, to study the possible underlying mechanism(s) of action by which the antidepressant drugs can emerge their actions.

MATERIALS AND METHODS

1.Chemicals

Imipramine hydrochloride was purchased from the Sigma Chemical CO. (St Louis, MO, USA). Fluvoxamine hydrochloride was obtained from the Lilly Pharmaceutical Co. (Geneva, Switzerland). Reagents for reduced glutathione (GSH), Myeloperoxidase (MPO), Malondialdehyde (MDA), and other ELISA kits were purchased from R&D systems Inc. (Minneapolis, MN, USA). All other chemicals used were of analytical grade.

2.Animals

Forty eight adult male Wistar Albino rats (150-200 g) were used throughout the experiment. The animals were obtained from the animal house, Medical College at King Saud University. The animals were maintained in a room under standard conditions of light, feeding, and temperature in accordance with the standard established guide lines for the care of laboratory animals in the department of pharmacology. The animals were allocated randomly to 6 groups (n=8) as the following:

1. Group 1: Normal control which had received saline i.p. 0.5 ml kg^{-1}.
2. Group 2: Acetic acid control group which were given 2 ml acetic acid rectally /rat.
3. Group 3: Rats were injected with imipramine i.p.30 mg kg^{-1}, daily for 2 weeks before induction of colitis.
4. Group 4: Rats were injected with imipramine i.p.30 mg kg^{-1} daily, daily for 2 weeks after induction of colitis.
5. Group 5: Rats which had received fluvoxamine in a dose of 30 mg kg^{-1} i.p, daily for 2 weeks before induction of colitis.

6. Group 6: Rats which had received fluvoxamine 30 mg kg^{-1} i.p., daily for 2 weeks after induction of colitis.

2.2.Induction of experimental colitis in rats

Acetic acid–induced colitis was performed using a modification of the described method ***(19)***. The rats were fasted for 24h with access to water *ad libitum* before induction of colitis. Each rat was sedated by an intraperitoneal injection of phenobarbitone (35 mg kg^{-1}). An infant feeding tube (Pennine Health Care FT-1608/40) with an outside diameter of 2mm was inserted into the colon to 8cm distance. 2ml of acetic acid (3%v/v in 0.9% saline) was infused into the colon. The acetic acid was retained in the colon for 30 sec. after which fluid was withdrawn. The rats in groups 2, 3, 5 were killed at 24 h after induction of colitis. The rats in groups 4, 6 were killed after 2 weeks of treatment. The rats were killed by CO_2 asphyxiation. Colonic biopsies were taken for macroscopic scoring, histopathological and biochemical studies.

2.3. Assessment of colitis by macroscopic scoring

At post-mortem laparotomy, 6 cm of colon extending proximally for 2 cm above the anal orifice was removed, then immediately transferred into Krebs buffer (pH 7.5), which was gently bubbled with O_2 and 5% CO_2. The colon was incised along its mesenteric border, gently washed and placed (its mucosal surface upwards) on a glass dish containing the buffer for macroscopic evaluation (colonic damage score). The severity of inflammation was evaluated by three experienced observers unaware of the treatments, using a visual analogue scale ranging from 0 to 4 ***(6)*** as follows:

(0) No macroscopic change.
(1) Mucosal erythema.
(2) Mild mucosal oedema, slight bleeding or small erosion.
(3) Moderate oedema, bleeding ulcers or erosion.
(4) Severe ulceration, erosions, oedema and tissue necrosis.

2.4. Biochemical studies

Colonic samples were stored immediately at –20°C till analysis .Tissue samples were homogenized in 1ml of 10mmol / l Tris-HCL buffer (pH 7.1) and the homogenates were used for the measurement of the following biochemical parameters.

2.5.Determination of myeloperoxidase (MPO) activity

MPO activity was used as an index of leukocyte adhesion and accumulation in several tissues including the intestine. The principle of the method depends on release of MPO enzyme in the homogenate of colonic tissue used. Its level was detected using 0.3 mmol of H_2O_2 as a substrate for MPO. A unit of MPO activity was defined as that converting /µmol of H_2O_2 to water in 1 min at 25°C. In brief, segments of the distal colon (0.5g) were homogenized in 10 volumes of 50 mm sodium phosphate buffer (pH 7.4) in an ice bath using polytron homogenizer (50 mg tissue/ml). The pellet (contained 95% of the total tissue MPO activity) was resuspended in an equal volume of potassium phosphate buffer (pH 6). Another centrifugation step for a period of 20 min at 16,000 xg was done. The resultant supernatant was used for MPO assay using tetramethylbenzidine (TMB), and the activity of MPO was measured using Jenway 6505 UV-Vi spectrophotometer at 655 nm ***(20)***.

2.6.Determination of reduced glutathione (GSH)

GSH was determined using the method previously described ***(21)*** which is based on the reaction of 5,5 dithiobis-(2-nitrobenzoic acid) with GSH present and the absorbance was measured at 412 nm in a Shimadzu double beam spectrophotometer (UV200 S). The amount of glutathione present in the sample was calculated using a standard solution of GSH containing 1mg of GSH /ml 3% metaphosphoric acid. The increase in the extinction at 412 nm was proportional to the amount of GSH present.

2.7.Determination of malondialdehyde (MDA)

MDA is the end product of lipid peroxidation and is well–known parameter for determining the increased free radical formation in colonic tissues. In our study, MDA levels were assessed *(22).* The essence of the method is based on measuring the concentration of the pink chromogen compound that forms when MDA couples to thiobarbituric acid (TBA). MDA was used as the standard, and the calibration was performed using a standard curve obtained from the MDA –TBA reaction. The protein content of homogenates was determined according to the previous procedure *(23)* and the values were expressed as nanomoles of MDA /mg of protein (nmol MDA/mg protein). All analysis was performed in duplicate.

2.8.Determination of serum tumor necrosis factor alpha (TNF-α) and interferon-γ (IFN-γ)

Concentrations of TNF-α and IFN-γ were measured using commercially available rat ELISA kits (R&D systems; Inc. Minneapolis, MN, USA). The results were expressed as pg of TNF-α or IFN-γ ml^{-1} of serum *(24).*

2.9.Determination of serum interleukin- 1β (I L- 1β), IL-6 and IL-10

Concentrations of IL-1β, Il-2, Il-6, and IL-10 were measured using commercially available rat ELISA kits (R&D systems; Inc. Minneapolis, MN, USA). The results were expressed as pg IL ml^{-1} of serum *(25).*

3.Statistical analysis

Data were entered and analysed on a personal computer using SPSS version 10.0 (statistical software). All values were expressed as mean ± SEM. The significance of the data obtained was evaluated using analysis of variance (ANOVA). Differences between means were analyzed using the post-ANOVA Tukey-Kramer multiple comparison test; P-values <0.05 were considered significant.

RESULTS

4.1.Body weight of rats

As shown in Table 1, there was evidence of weight loss in untreated acetic acid group. Prior or post administration of imipramine or fluvoxamine at a dose of 30 mg kg^{-1} for 2 weeks, attenuated the degree of haemorrhagic diarrhoea and weight loss particularly with fluvoxamine groups.

4.2. Macroscopic findings of the colon

Twenty four hours after administration of 3% acetic acid into the colon, macroscopic inspection showed evidence of damage, in the form of mucosal congestion, extensive disruption, linear and deep hemorrhage ulceration. Treatment of rats with either imipramine or fluvoxamine significantly attenuated the extent and severity of the colonic injury and reduced the score of the macroscopic damage (**Table 2 & Fig. 1 A, B).**

4.3. Biochemical results

4.3.1. Myeloperoxidase activity

The levels of MPO activity in the tissues showed a statistically significant difference among the six tested groups (P<0.05). By performing pair-wise comparisons among these six groups, we can infer that the mean value of acetic acid control group was significantly increases (P<0.05) as compared with the normal control group. On the other hand, after the use of imipramine or fluvoxamine before or after induction of UC, a significant reduction in MPO activity was observed, as compared with the acetic acid control group (P<0.05) (Table 3).

4.3.2. Malondialdehyde concentrations

The MDA levels of the study groups are shown in Table 3. In the untreated group, MDA level was significantly increased as compared with the normal control (P<0.05).After the use of imipramine or fluvoxamine as prophylaxis or as treatment, MDA levels were significantly decreased as compared with acetic acid group (P<0.05).

4.3.3. Reduced glutathione concentrations

The tissue levels of GSH were significantly decreased after induction of colitis as compared with the normal control. After the administration of imipramine or fluvoxamine as prophylaxis or treatment, there was a significant increase in the GSH concentrations (Table 3).

4.3.4.Tumor necrosis factor-α concentrations

The serum levels of TNF-α were significantly increased in the inflamed colon 24 hours after administration of acetic acid as compared with the normal control group. Administration of either imipramine or fluvoxamine before or after induction of colitis with acetic acid significantly decreased the serum levels of TNF-α as compared with the acetic acid control group (Table 4).

4.3.5. IFN-γ concentrations

The serum levels of TNF-γ showed a significant reduction as compared with acetic acid group $p<0.05$ (Table 4).

4.3.6. Interleukin-1β concentrations

The serum levels of IL-1β were significantly increased in the acetic acid control group as compared with the normal control group ($p<0.05$). Administration of imipramine or fluvoxamine before or after induction of experimental ulcerative colitis was significantly decreased the levels of IL-1β as compared with the acetic acid control group (Table 4).

4.3.7. Interleukin-2 concentrations

Table 5 showed a significant increase in serum IL-2 in acetic acid group as compared with the normal control group and a significant reduction in the prophylaxis or the treated groups.

4.3.8. Interleukin-6 concentrations

A significant increase in serum IL-6 was noticed in the prophylaxis & treated groups as compared with acetic acid control group ($p<0.05$) (Table 5).

4.3.9. Interleukin-10 concentrations

There was a non significant change in the serum levels of IL-10 in the prophylaxis or treated groups as compared with the control acetic acid group (Table 5).

Table 1: Effects of imipramine and fluvoxamine in a dose of 30 mg/kg B. wt, i.p. on the body weight two weeks, before and after acetic acid-induced colitis in rats

Group	Treatment	Body weight
1	Saline Control	255.17 ± 04.80
2	3% Acetic Acid	232 21 ±09.11 * (a)
3	Imipramine (2weeks before UC)	247.71 ± 09.41 * (a)
4	Imipramine (2weeks after UC)	246.57 ± 09.40 * (a)
5	Fluvoxamine (2weeks before UC)	248.14 ± 13.50 * (a)
6	Fluvoxamine (2weeks after UC)	250.14 ± 13.20 ** (b)

Results are expressed as mean ± S.E.M. of eight observations.
Vehicle (saline) or drugs were administered intraperitoneally once daily for 2 weeks before or after induction of UC.
(a) * $p<0.05$ as compared with control saline group.
(b) ** $p<0.05$ as compared with acetic acid control group .

Table 2. Effects of imipramine and fluvoxamine in a dose of 30 mg/kg wt, i.p. on gross lesion score and protection % (two weeks) before and after acetic acid-induced colitis in rats

Group	Treatment	Gross lesion score	protection %
1	Saline Control	0.0 ± 0.0	-
2	3% Acetic Acid	4.0 ± 0.0	---*
3	Imipramine (2 weeks before UC)	1.43 ± 0.31	65**
4	Imipramine (2 weeks after UC)	1.2 ± 0.2	70**
5	Fluvoxamine(2 weeks before UC)	0.28 ± 0.1	93**
6	Fluvoxamine (2 weeks after UC)	0.27 ± 0.1	93**

Results are expressed as mean ± S.E.M. of eight observations.
The vehicle (saline) or drugs were administered intraperitoneally once daily 2 weeks before or after induction of UC.
* p<0.05 as compared to saline control group.
** p<0.05 as compared to acetic acid control group.

Table 3. Effects of imipramine and fluvoxamine in a dose of 30 mg/kg wt, i.p. on colonic myeloperoxidase (MPO), malondialdehyde (MDA) and reduced glutathione (GSH) concentrations (two weeks) before and after acetic acid-induced colitis in rats

Group	MPO (Units mg^{-1} tissue)	MDA (nmol g^{-1} tissue)	GSH (nmol g^{-1} tissue)
Saline Control	0.59 ± 0.01	0.257 ± 0.021	1141.16 ± 06.14
3% Acetic Acid	1.06 ± 0.04*	0.540 ± 0.034*	836.65 ± 11.32*
Imipramine (2 weeks before UC)	0.68±. 0.02**	0.411 ± 0.03**	1008.32 ±08.80**
Imipramine (2 weeks after UC)	0.57 ± 0.01**	0.342 ± 0.022 **	1101.76 ± 03.50**
Fluvoxamine (2 weeks before UC)	0.65 ± 0.02**	0.330 ± 0.031**	1120.78 ± 04.80**
Fluvoxamine (2 weeks after UC)	0.62 ± 0.02**	0.345 ± 0.021**	1118.33 ± 04.90**

Results are expressed as mean ± S.E.M. of eight observations.
The vehicle (saline) or drugs were administered intraperitoneally once daily for 2 weeks before or after induction of UC.
* P<0.001 as compared to saline control group.
** P<0.001 as compared to acetic acid control group.

Table 4. Effects of imipramine and fluvoxamine in a dose of 30 mg/kg wt, i.p. on serum tumor necrosis factor- α (TNF-α), interferon- γ (IFN-γ) and interleukin-1β (IL-1β) concentrations (two weeks) before and after acetic acid-induced colitis in rats

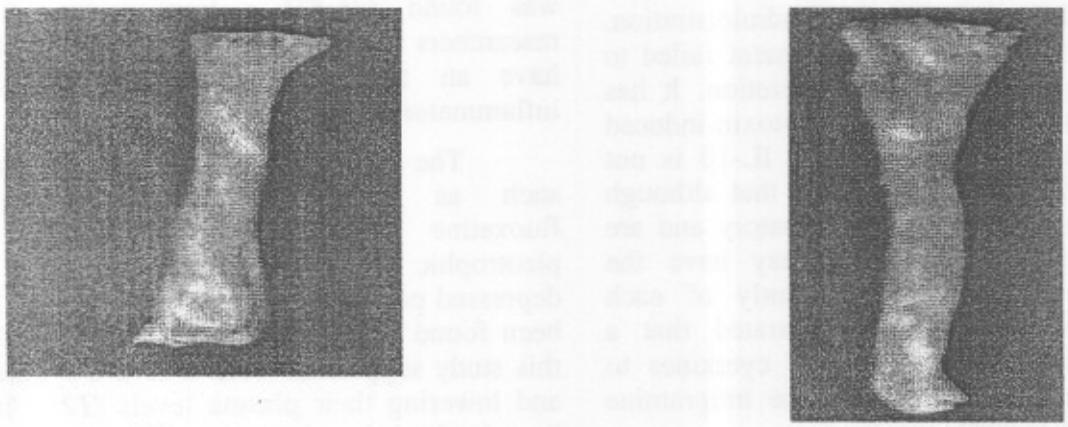
Group	TNF-α (pg ml^{-1})	IFN-γ(pg ml^{-1})	IL-1β (pg ml^{-1})
Saline Control	01.47 ± 0.021	35.00 ± 2.09	50.54 ± 0.66
3% Acetic Acid	13.92 ± 0.28*	63.40 ± 1.018*	577.13 ± 5.85*
Imipramine (2 weeks before UC)	09.32 ± 0.14**	56.10 ± 2.06**	493.23 ± 1.83**
Imipramine (2 weeks after UC)	07.71 ± 0.18**	56.30 ± 1.48**	422.12 ± 5.17**
Fluvoxamine (2 weeks before UC)	06.63 ± 0.16**	59.70 ± 0.64**	371.36 ± 7.44**
Fluvoxamine (2 weeks after UC)	03.73 ± 0.17**	58.70 ± 1.33**	275.03 ± 15.05**

Results are expressed as mean ± S.E.M. of eight observations.
The vehicle (saline) or drugs were administered intraperitoneally once daily for 2 weeks before or after induction of UC.
* P<0.001 as compared to saline control group.
** P<0.001 as compared to acetic acid control group.

Table 5. Effects of imipramine and fluvoxamine in a dose of 30 mg/kg wt, i.p. on serum interleukin-2 (IL-2), Il-6 and IL-10 concentrations (two weeks) before and after acetic acid-induced colitis in rats

Group	IL-2 (pg ml $^{-1}$)	IL-6(pg ml $^{-1}$)	IL-10 (pg ml $^{-1}$)
Saline Control	76.23 ± 0.366	253.5 ± 3.930	40.61 ± 1.037
3% Acetic Acid	102.0 ± 2.214*	156.0 ± 6.549*	40.17 ± 2.852*
Imipramine (2 weeks before UC)	91.5 ± 3.67**	386.5 ± 26.80**	36.46 ± 1.23**
Imipramine (2 weeks after UC)	89.1 ± 1.1**	241.0 ± 14.40**	39.62 ± 3.231**
Fluvoxamine (2 weeks before UC)	113.0 ± 1.61**	154.0 ± 18.01**	39.90 ± 3.13**
Fluvoxamine (2 weeks after UC)	105.0 ± 3.04**	153.0 ± 17.90**	37.70 ± 1.66**

Results are expressed as mean ± S.E.M. of eight observations.
The vehicle (saline) or drugs were administered intraperitoneally once daily for 2 weeks before or after induction of UC.
* P<0.001 as compared to saline control group.
** P<0.001 as compared to acetic acid control group.



(A) (B)

Fig. 1. (A) Macroscopic pictures for normal control colon. (B) Macroscopic pictures for colon after induction of colitis using acetic acid which showed multiple ulceration and haemorrhagic areas with signs of inflammation.

DISCUSSION

The present results showed that administration of imipramine or fluvoxamine attenuated the colonic tissue damage induced experimentally by acetic acid in rats. These results were verified by macroscopic and biochemical studies. The data from animal models suggested that defects in both innate and adaptive immunity can contribute to the development of UC *(26)*. The histopathologic picture of UC also supported an immune-mediated mechanism of disease pathogenesis *(27-29)*.

A body of evidence indicates that the therapeutic activity of antidepressants is connected with their modulatory effect on the inflammatory response system and cell-mediated immunity. Imipramine and fluvoxamine are commonly used antidepressant drugs in the treatment of depression & other forms of psychiatric illness. Some reports have been found regarding the suppressive effects of these drugs on immune function. However, information is still limited concerning their effects on the immune system. In addition to their antidepressant effects, they have

neuromodulatory, and analgesic properties. Because of their inhibitory effect on bowel function, they may be particularly beneficial in patients with IBD who have well-defined depression or panic disorders *(30)*.

Biochemical studies revealed that either imipramine or fluvoxamine produced significant reduction in the levels of the pro-inflammatory cytokines IL-1β and TNF-α in colonic tissues. It has been demonstrated that a lipopolysaccharide (LPS) challenge in rats resulted in an increased circulating concentrations of the pro-inflammatory cytokines TNF-α and IL-1β. Pretreatment with a single high dose of imipramine inhibited LPS induced secretion of TNF-α after 3 and 6 hours following administration. In contrast imipramine pretreatment failed to alter LPS stimulated IL-1β secretion. It has been demonstrated that endotoxin-induced increases in leucocytes-derived IL-1β is not mediated by TNF-α suggesting that although both cytokines are pro-inflammatory and are produced by macrophages, they have the ability to fluctuate independently of each other and this study demonstrated that a differential sensitivity of these cytokines to the suppressive effects of acute imipramine administration *(32)*.

A significant suppression of IFN-γ, IL-1β, IL-2 and TNF-α secretion is mediated by activated T cells or monocytes preincubated with tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) or heterocyclic antidepressants *(33)*. It has been suggested that various types of antidepressive drugs may have immunoregulatory effect that was markedly observed with TCAs and SSRIs group of drugs *(33)*. Prolonged treatment of depressed patients with antidepressants, including fluoxetine was accompanied by a normalization of the initially increased production of IL-6, IFN- γ, TNF-α, and positive acute phase proteins *(34)*. These results showed that antidepressants may have *in vivo* anti-inflammatory effects.

Plasma levels of IL-1β, IL-6 and TNF-α was measured in women with anorexia nervosa or bulimia nervosa showed a significant increase in the concentrations of these cytokines with a marked reduction in their levels after treatment with fluoxetine. It was attributed the changes in the levels of cytokines to the anti-inflammatory effect of antidepressant drugs used *(35)*. The authors reported that SSRIs have a well-recognized effect on depression or and anxiety which may co-exist in patients with IBS. Study on the effect of paroxetine in the treatment of patients with irritable bowel syndrome showed that it improves the symptoms and has been attributed to the action on the central nervous system and the enteric nervous system where 95% of the body's serotonin was found *(36,37)*. Furthermore, other researchers have also proposed that SSRIs have an antinociceptive and an anti-inflammatory effects *(38)*.

The effects of antidepressant agents, such as imipramine, venlafaxine and fluoxetine on the production of the pleotrophic cytokines TNF-α and IL-6 in depressed patients has been studied and it has been found that antidepressant drugs used in this study suppressed TNF-α & Il-6 synthesis and lowering their plasma levels *(12)*. It is hypothesized that the increased production of some cytokines may play a role in the etiology of depression. Systemic administration of these cytokines may induce changes in the neuroendocrine and central monoamine systems, which are reminiscent of those observed in depression. In experimental animals the injection of lipopolysaccharide (inductor of production of pro-inflammatory cytokines and IL-6) induces a state of anhedonia–one of the most essential features of major depressive episodes *(9)*.

In another study, fluoxetine, but not desipramine, had suppressive effect on some local and systemic changes occurring in adjuvant–induced arthritis. The above results point to particular effectiveness of fluoxetine in suppressing inflammatory process *(39)*. Imipramine significantly reduced acute local inflammatory responses induced by carrageenan in rats *(40)*. This indicated that

imipramine may ameliorate the inflammatory reactions. The effect of fluoxetine on the carrageenan induced paw inflammation in the rat had been studied ***(18)***. Fluxoteine reduced the oedema response in post–carrageenan induced inflammation. Fluoxetine has anti-inflammatory effect through reduced the production of pro-inflammatory cytokines (TNF-α, IL-1β & IL-2). Also, it has been claimed that fluoxetine is benefit in the setting of neuropathic or inflammatory pain conditions. In another study ***(17)***, which showed the anti-inflammatory and anti-nociceptive effects of imipramine, amitriptyline and fluoxetine on paw oedema induced by carrageenan in rats. Data in this study confirmed anti-inflammatory and anti-nociceptive effect of some antidepressant drugs ***(17)***.

Foley et al. (41), studies showed that Mucosal serotonin (5-HT) transporter (SERT) expression is decreased in animal models of colitis and irritable bowel syndrome after administration of TNF-α & IFN- γ. ***(41)*** . The changes in gut motility, secretion and sensation seen in these inflammatory gut disorders was attributed to the decreased SERT function or expression caused by these cytokines. Also the role of TNF-α in the pathogenesis of IBD. Was a significant rise of the serum level of TNF-α in patients with ulcerative colitis ***(42)***. Similarly it has been TNF-α forms a necessary element in the chain of pathophysiological events leading to inflammation.

Oxygen free radicals produced by neutrophils are important in the pathogenesis of mucosal damage in ulcerative colitis. Depletion of intracellular GSH also promotes formation of free radicals, loss of dopamine storage and neurodegeneration ***(43, 44)***. GSH is a major intracellular non protein sulfhydryl compound. It has many biological functions including maintenance of membrane protein sulfhydryl groups in the reduced form, the oxidation of which can otherwise cause altered cellular structure and function ***(45)***. Glutathione is also cofactor for many enzymes that catalyze detoxification of intracellular peroxides. Thus maintenance of glutathione levels is proved for cellular integrity ***(46)***. Among the types of organ damage due to glutathione deficiency, extensive intestinal damage has been identified in a mouse model of UC ***(47)***.

Furthermore, it has been shown that glutathione depletion was present in colonic tissues from patients with inflammatory bowel disease ***(48,49)***. In the present study, acetic acid–induced colonic injuries are associated with significant reduction in GSH levels. This depletion may be due to consumption of glutathione by the ODFRs liberated during experimental colitis because GSH is known to be a major low molecular weight scavenger ***(49)***. The restoration of normal values of GSH after administration of either imipramine or fluvoxamine may be due to inhibition of generation as well as scavenging of superoxide and hydroxyl radicals, thus sparing consumption of GSH by the generated ODFRs. These findings are consistent with the previous reports that showed fluoxetine caused marked elevation of GSH levels in rat models of inflammation ***(50,51)***.

Membrane lipids are considered as a great target for free radical-induced damage, and measuring lipid peroxides in tissues provides an index of free radical activity. Increased lipid peroxidation has been reported in colo-rectal cancer and ulcerative colitis in humans ***(52)***. In this study a significant increase in MDA concentrations ($p<0.05$) in rectal biopsies of acetic acid control animals compared with the normal control group, suggesting free radical liberation. Pretreatment & treatment of rats with either imipramine or fluvoxamine protected colonic mucosa from this oxidative cell damage as shown by the significant reduction in MDA formation.

There is good evidence that an enhanced formation of reactive oxygen species contributes to the pathophysiology of inflammatory bowel disease ***(53)***. Quantitatively, the principal free radical in tissues is superoxide anion (O^{-2}), which is converted to H_2O_2 by superoxide dismutase.

Superoxide anion (O^{-2}) can be produced by activated neutrophils through NADPH oxidase, which reduces molecular oxygen to the O^{-2} radical through the enzyme myeloperoxidase. In our present study, the increase in myeloperoxidase activity in the colon was reduced by imipramine or fluvoxamine. This finding is in agreement with the results previously reported by other investigators *(18,40),* which showed that antidepressants such as imipramine or fluoxetine reduced cellular infiltration in inflammatory conditions. Additionally, further support to our finding comes from the results which showed that pretreatment with either amitriptylline or fluoxetine was associated with increased superoxide dismutase in cultured rat cells *(54).* Several studies have reported the partial efficacy of antioxidant enzymes superoxide dismutase (SOD) and catalase in attenuating experimental colitis in animal models *(55).*

The results of the present study indicated that antidepressant drugs afford protective and ameliorative effects on UC induced experimentally in rats. This effect may be through the prevention of depletion of the antioxidant enzymes namely GSH and catalase also through restoration of lipid peroxides to normal levels. It may also be through their influence on the production of some cytokines which are involved in the inflammatory process. This study will open a novel potential therapeutic approach for the treatment of UC.

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الملخص العربى

تأثير اميبرامين والفلوفكسامين على التهاب القولون المحدث باستخدام حمض الأستيك في الجرذان

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يعتبر التهاب القولون من الأمراض المزمنة غير معلومة السبب ، يصاحب الاكتئاب حدوث التهاب للقولون في بعض المرضى. إن قدرة مضادات الاكتئاب على علاج المرضى المصابين بالتهاب القولون والمصاحب لوجود الاكتئاب غير مؤكدة٠ وعلى ذلك فإن هذا البحث يهدف إلى دراسة تأثيرات اميبرامين والفلوفكسامين على شدة ومدى التهاب القولون المحدث باستخدام حمض الأستيك في تركيز ٣% والذي يتم حقنه في القولون في الجرذان٠

أدى إحداث التهاب القولون إلى وجود رد فعل التهابي وقد تم تقييمه بارتفاع ميلوبيروكسيداز كمؤشر لتدخل خلايا الثيوتروفيل ، عامل التنكزر- ألفا، انترلوكين –ب١ ، انترلوكين – ٢ ، انترلوكين – ٦ ، انترلوكين -١٠ ، انترفيرون – جاما في القولون بينما انخفض مستوى الجلوتاثيون المختزل٠ وقد أوضحت الدراسات الهستوباثولوجية عن حدوث تنكرز٠ أدى تناول دواء إميبرامين وفلوفوكسامين في جرعة مقدارها (٣٠مجم/كجم) لمدة أسبوعين قبل إحداث التهاب القولون إلى التقليل من مستويات كل من ميلوبيروكسيداز ، عامل التنكرز – ألفا ، انترلوكين – ب ١ ، انترلوكين -٢ ، انترلوكين – ٦ كما زاد تركيز الجلوتاثيون المختزل٠ بالإضافة إلى ذلك فقد اضعف كلاً من العقارين وخصوصاً الفلوفوكسامين من التغيرات الهستوباثولوجية المحدثة بحمض الأسيتيك٠ وتشير هذه النتائج أن الأميبرامين والفلوفوكسامين من الممكن أن يكونا ذو فاعلية في علاج التهاب القولون من خلال تأثيرهم على إنتاج السيتوكينز وكسح شوارد الأكسجين الحرة٠