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Original Article

Antisecretory and antioxidative effects of the antidepressants fluvoxamine and mirtazapine on water immersion stress and pyloric ligation-induced gastric ulcer in rats

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ABSTRACT

Objectives: Although there are numerous drugs available for the treatment of gastric ulcers (GU), these drugs are not always effective. Antidepressant medications have been used for a variety of non-psychiatric indications, including antiulcer activity in various ulcer models. The purpose of this study was to compare the antiulcer effects of fluvoxamine and mirtazapine in two rat GU experimental models and to determine their relationship to antioxidant and antisecretory mechanisms.

Materials and Methods: The antiulcer activities of various doses of fluvoxamine and mirtazapine on water immersion restraint stress (WIRS) and pyloric ligation-induced GU in rats have been studied against the positive control antiulcer drug famotidine. Various oxidative stress markers were evaluated.

Results: Fluvoxamine and mirtazapine significantly protected against WIRS and pyloric ligation-induced gastric lesions, as evidenced by a dose-dependent decrease in ulcer index, myeloperoxidase (MPO) activity, lipid peroxidation, and an increase in prostaglandin E2, nitric oxide (NO), and reduced glutathione levels, as well as increased antioxidant enzyme activity. In the pyloric ligation model, fluvoxamine and mirtazapine improved GU more than famotidine. Furthermore, a 30 mg/kg dose of mirtazapine significantly improves both NO levels and MPO activity compared to famotidine.

Conclusions: The results highlighted the relationship in correlating the antiulcer effect of drugs from different antidepressant classes across two animal GU models, implying that antidepressants that affected both norepinephrine and serotonin levels (mirtazapine) had a more potent antiulcer effect in WIRS-induced gastric model than drugs that only affected serotonin levels (fluvoxamine).

Keywords: Fluvoxamine, gastric ulcer, mirtazapine, oxidative stress, pyloric ligation

Introduction

Gastric ulcer (GU) is the most common gastrointestinal tract disorder with a higher morbidity and mortality rate.[1] It is considered a medical–social problem of global economic importance.[2] Although there are many drugs for the treatment of gastroduodenal ulcers, these drugs sometimes cannot succeed because the disease etiology is affected by various aggressive and defensive factors such as acid-pepsin secretion,[3] mucosal barrier, mucus secretion, blood flow, cellular regeneration, and endogenous protective agents, for example, prostaglandins (PG) and epidermal growth factors.[4]

Most patients with GUs have depression, which is accompanied by psychotic and somatic symptoms.[5] The neuronal pathogenic pathways involved in ulcer genesis and depression appear to have a high degree of overlap. As a result, it is not surprising that antidepressants have a powerful protective effect against GUs.[6] In experimental animals, an increased vulnerability to depression[7] and anxiety[8] is associated with ulcer development, and the same is true in humans.

Since the 1950s, antidepressants have been used for a variety of non-psychiatric indications. In the field of gastroenterology, they have been used for a variety of conditions.[9] Moreover, classic antidepressants can significantly reduce stress ulcer formation,[10] possibly to a greater extent than traditional anti-ulcer therapies.[11] The treatment of peptic ulcer disease with tricyclic antidepressants was the first reported use of antidepressants for gastrointestinal disease.[5]

Water immersion restraint stress (WIRS) is a clinically relevant experimental model for acute GU.[12] The pathological basis for the development of this lesion is thought to be a combination of factors including increased gastric acid secretion,[13] inhibition of gastric mucosal PG synthesis, disruption of gastric mucosal barrier,[14,15] release of myeloperoxidase (MPO) enzymes and various inflammatory mediators, and the development of oxidative stress.[16]

Pylorus ligation (PL)-induced ulcer is one of the most extensively used methods for testing the effect of drugs on gastric secretion. The ligation of the stomach's pyloric end causes an accumulation of gastric acid in the stomach, which leads to the development of GUs.[17]

To date, there is no information available regarding the antiulcer effects of fluvoxamine and mirtazapine in acute stress and pyloric ligation GU models. Fluvoxamine is a selective serotonin reuptake inhibitor that specifically inhibits the serotonin transporter,[18] whereas mirtazapine, a noradrenergic and serotonergic antidepressant, is characterized by a potent antagonism of presynaptic α_2-adrenergic receptor on both norepinephrine and serotonin neurons, as well as a potent antagonism of postsynaptic serotonin 5-HT_2 and 5-HT_3 receptors.[19]

The current study aimed to assess the antiulcer effects of fluvoxamine and mirtazapine in two acute experimental GU models in rats, as well as their association with oxidative stress and antisecretory mechanisms.

Materials and Methods

Animals

One hundred and twelve adult male albino Wistar rats weighing 180–200 g were purchased from the Egyptian Organization for Biological Products and Vaccines. The animal chow diet and water were provided *ad libitum*. Throughout the experiment, rats were kept on a normal light-dark cycle and at a temperature of 25 ± 3°C. An adaptation period of 1 week was allowed before beginning the experiment. Experimental animals were kept and used in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). All experimental protocols were approved by the Ethics Committee at the Faculty of pharmacy, Suez Canal University (Ismailia, Egypt) (code # 201907RA1).

Drugs and chemicals

Fluvoxamine hydrochloride Lilly S.A. (Indiana, USA), mirtazapine (ORGANON, Co. Turkey), and famotidine were purchased from Sigma-Aldrich, while thiopental sodium was purchased from EPICO, Cairo, Egypt. All other chemicals and solvents were of analytical grades. Famotidine is a widely used antiulcer medication. It was used in this study as a positive control antiulcer drug.

WIRS induced GU model

The rats were fasted for 24 h before the experiment. Rats were exposed to WIRS where restrained in the stainless steel cages (16 cm long and 4 cm × 4 cm bottom) and immersed up to their xiphoid in a water bath kept at 21 ± 0.5°C for 6 h.[14] The animals were then sacrificed; their stomachs were removed and opened along the greater curvature, and then washed with physiological saline solution (0.9% w/v of sodium chloride). Gastric tissue samples were collected for the measurement of ulcerative index and biochemical parameters.

PL induced GU model

The pyloric ligation process was used in rats to study ulcers, as described by Shay *et al*.[20] Animals were fasted for 24 h before PL with water *ad libitum*. Under light, rats were deeply anaesthetized with thiopental sodium (40 mg/kg, i.p.)[21] and the abdomen was opened by midline incision below the xiphoid process. The pyloric portion of the stomach was slightly lifted out and ligated, avoiding damage to its blood supply. The stomach was placed back carefully and the abdominal muscular and skin layers were closed with sutures. Throughout the surgical procedure, the animal's heart rate, respiration, and body temperature were all kept stable. Six hours after PL, animals were sacrificed.[22] The rat stomach was removed and opened along the greater curvature, and then washed with saline solution (0.9% w/v of sodium chloride). Gastric volume, total and free acidity, PH, and MPO activity were all measured in the gastric content. Furthermore, isolated tissue was used to calculate the ulcerative index and perform biochemical analysis.

Experimental design

A total of 56 rats were used (for each model), divided randomly into seven groups of eight animals each. Famotidine (50 mg/kg),[23] fluvoxamine (25, 50 mg/kg),[23] and mirtazapine (15, 30 mg/kg)[9] were dissolved in distilled water and administered by oral gavage to 24 h fasted rat groups 60 min before WIRS or PL-induced GU in a total volume of 2 mL/kg.

Groups of WIRS -induced GU model

Group 1 (Vehicle control), normal rats received distilled water orally as a vehicle in a total volume of 2 mL/kg. Group 2 (WIRS model), rats exposed to WIRS and sacrificed 6 h after that. Group 3 (Famotidine group), rats received famotidine (50 mg/kg) 60 min before WIRS. Groups 4, 5 (Fluvoxamine groups), rats received fluvoxamine (25 and 50 mg/kg, respectively) 60 min before WIRS. Groups 6, 7 (Mirtazapine groups), rats received mirtazapine (15 and 30 mg/kg, respectively) 60 min before WIRS.

Groups of pyloric ligation-induced GU model

Group 1 (Vehicle control), normal rats received distilled water orally as a vehicle. Group 2, (pyloric ligation model), rats exposed to pyloric ligation and sacrificed 6 h after

that. Group 3 (Famotidine group), rats received famotidine (50 mg/kg) 60 min before pyloric ligation. Groups 4, 5 (Fluvoxamine groups), rats received fluvoxamine (25 and 50 mg/kg, respectively) 60 min before pyloric ligation. Groups 6, 7 (Mirtazapine groups), rats received mirtazapine (15 and 30 mg/kg respectively) 60 min before pyloric ligation.

Collection and biochemical analysis of gastric juice in pyloric ligation model

Determination of volume, pH, total, and free acidity of gastric juice

The gastric juice was collected and its volume was measured, and then centrifuged at 2000× *g* for 20 min. The clear supernatant volume was analyzed for pH as well as total and free acidity using the method of Kulkarni.[24] Briefly, 1 mL of gastric juice supernatant was diluted to 10 mL using distilled water. The solution was titrated against 0.01 N sodium hydroxide using Topfer's reagent as an indicator till the color became orange. The volume of NaOH corresponds to free acidity. Then 2–3 drops of phenolphethaline solution were added, and titration was continued until a definite red ting reappears. Again, the total volume of alkali added was noted as it corresponded to the total acidity. Acidity was calculated using the formula:

Acidity = Volume of NaOH × concentration of NaOH/volume of sample.

Determination of pepsin activity

One mL of diluted gastric juice was mixed with 2% hemoglobin solution in 0.06 M HCl and incubated for 20 min. 0.6 M ice cold trichloroacetic acid was then added to it. Later, the solution was centrifuged and the supernatant fluid was mixed with alkaline copper sulfate solution reagent and diluted Folin reagent, and the optical density was measured at 610 nm against a blank of distilled water.[25]

Assessment of gastric mucosa in WIRS and pyloric ligation models

Ulcer index was measured by the methods of Shay and Hano *et al*.[20,26] Any macroscopically visible lesions were measured to calculate the gastric damage score. For this purpose, the ulcerous stomach was ingrained on a planar surface with small pins. Then the total areas of the stomach and ulcerous areas were drawn on a cellophane sheet. The cellophane sheet was placed on a millimeter paper and the sum of ulcerous areas and total stomach area were calculated and expressed as mm^2. Ulcerative index was estimated from the formula UI = [Ulcerated area (mm^2)/total stomach area (mm^2)] × 10.

The antiulcer activities of the drugs were assessed by comparing the results obtained from the control model group and the drug-treated groups using this formula: Antiulcer effect (% protection) = ulcer index of (control model group - drug treated group)/control model group × 100.

Biochemical analysis of stomach tissue in WIRS and pyloric ligation models

Determination of PGE_2

Gastric mucosa was scrapped, homogenized in 2 mL normal saline containing 0.1 M dithiothreitol, and centrifuged at 2000× *g* for 10 min at room temperature. The supernatant was used to determine PGE_2 levels by enzyme-linked immunosorbent assay using PGE_2 immunoassay kit (R and D Systems, USA, Catalog No. KGE004B).[27]

Determination of nitric oxide (NO) level

Tissue gastric NO levels were calculated as total nitrite + nitrate levels using the Griess reagent, as described previously by Moshage *et al*.[28] The method is based on a-two-step process. The first step is the conversion of nitrate into nitrite using a nitrate reductase. The second step is the addition of the Griess reagent, which converts nitrite into a deep purple azo compound; photometric measurement of absorbance at 540 nm is possible because this azo chromophore accurately determines nitrite concentration. NO levels were expressed as μmol/g.

Estimation of MPO activity

MPO activity was measured according to the modified method of Bradley *et al*.[29] The homogenized samples were frozen and thawed 3 times, and centrifuged at 1500× *g* for 10 min at 4°C. MPO activity was determined by adding 100 mL of the supernatant to 1.9 mL of 10 mmol/L phosphate buffer (pH 6.0) and 1 mL of 1.5 mmol/L o-dianisidine hydrochloride containing 0.0005% (w/v) hydrogen peroxide (H_2O_2). The changes in absorbance at 450 nm of each sample were recorded on a ultraviolet (UV)-visible spectrophotometer (UV-1601PC, Shimadzu, Japan). MPO activity in gastric tissues was expressed as micromoles per minute per milligram tissue (μmol/min/mg tissue).

Determination of gastric oxidative stress parameter and antioxidant markers

A part of the stomach (0.25 g) was ice-cooled, homogenized in 2.5 mL phosphate buffer saline (pH 7.4), and then centrifuged at 3000× *g* for 15 min at 4°C. The supernatant was collected and stored at −80°C until the analysis of oxidative stress parameter, malondialdehyde (MDA),[30] and antioxidant markers, reduced glutathione (GSH),[31] as well as superoxide dismutase (SOD) and catalase (CATA)[32,33] activities using a UV-visible spectrophotometer (UV-1601PC, Shimadzu, Japan).

Statistical analysis

Results were collected and expressed as mean ± SE. Results were analyzed using The Statistical Package for the Social Sciences, version 20 (SPSS Software, SPSS Inc., Chicago, USA). One-way analysis of variance followed by Bonferroni's *post hoc* test was used to test the significance of the difference between quantitative variables. $P < 0.05$ was considered to be statistically significant.

Results

Water-immersion plus restraint stress induced GU in rats

Effects of fluvoxamine and mirtazapine on GU index

As shown in Table 1, 6 h water restrain stress (21 ± 0.5°C) produced ulcers that had been dispersed to all stomach surfaces with different forms and sizes. There was remarkable hyperemia in the ulcerative stomachs. The mean GU index in WIRS group was (24.22 ± 2.31). Famotidine significantly decreased GU index by 48.21%. Fluvoxamine (25 and 50 mg/kg) significantly reduced GU index to (24.12 and 33.11%, respectively) compared to WIRS and famotidine treated rats, ($P < 0.05$). In mirtazapine (15 and 30 mg/kg) treated rats, GU index was also decreased significantly to (45.79 and 48.81%, respectively) compared to WIRS group. Notably, mirtazapine (30 mg/kg) can normalize GU index to a level that was significantly better than both doses of fluvoxamine. In addition, a 30 mg/kg dose of mirtazapine had approximately the same effect as a 50 mg/kg dose of famotidine in maintaining ulcer inhibition in WIRS-induced GU model.

Effects of fluvoxamine and mirtazapine on PGE_2, NO concentrations, and MPO activity

Table 1 showed that, WIRS group exhibited significant reduction in PGE_2 (20.11 ± 2.01) and NO (20.21 ± 1.20) concentrations, as well as significant increase in MPO (12.40 ± 0. 80) activity in comparison to normal control group. Famotidine treatment reversed all of these findings, as evidenced by a significant increase in PGE2 and NO concentrations and a significant decrease in MPO activity compared to WIRS group. While treatment with fluvoxamine dose dependently and significantly restored these levels compared to the non-treated group, mirtazapine normalized these levels. In addition, mirtazapine (30 mg/kg) increased PGE_2 (56.33 ± 3.91) and NO (28.33 ± 2.20) concentrations, as well as decreased MPO (3.11 ± 0.11) activity significantly more than both doses of fluvoxamine ($P < 0.05$). Notably, a 30 mg/kg dose of mirtazapine was more effective than famotidine in normalizing PGE2, NO, and MPO levels.

Effects of fluvoxamine and mirtazapine on oxidative stress parameter and antioxidant markers

Figure 1 showed that, WIRS exposure induced oxidative stress in stomach homogenates in the form of significant increase ($P < 0.05$, [Figure 1a]) of MDA (90.70 ± 6.91) associated with significant reduction ($P < 0.05$, [Figure 1b-d]) in GSH (25.11 ± 1.14) concentrations, as well as SOD (21.11 ± 2.90) and CATA (4.91 ± 0.21) activities in comparison with the normal control group. These deleterious effects associated with WIRS exposure were improved by treatment with famotidine, fluvoxamine (25 and 50 mg/kg), and mirtazapine (15 and 30 mg/kg) in comparison with WIRS group ($P < 0.05$). It was obvious that, mirtazapine administration was associated with significant effect on these markers (reduce oxidative parameter and elevate antioxidant activity) in comparison to those afforded by fluvoxamine treatment ($P < 0.05$); indicating that mirtazapine offered more protective effects than fluvoxamine. Furthermore, it was noticed that a 30 mg/kg dose of mirtazapine was more effective than famotidine in decreasing MDA levels, and that both doses of mirtazapine were more effective in increasing GSH, SOD, and CATA.

PL induced GU model

Effects of fluvoxamine and mirtazapine on the gastric juice analysis

PL caused the accumulation of gastric secretions (8.83 ± 0.81 mL/100 g rat) and hence, intense lesions in the stomach in model control rats [Table 2]. Total acidity was 81.31 ± 7.60 mEq/L, free acidity was 60.13 ± 5.80 mEq/L, and pepsin activity was 7.94 ± 0.06 µg/ml, while PH was 2.30 ± 0.16. A significant decrease ($P < 0.05$, [Table 2]) in gastric volume, total acidity, free acidity, and pepsin activity was observed upon pretreatment with famotidine, fluvoxamine, or mirtazapine compared to pyloric ligation group. The gastric juice analysis revealed no significant differences between the famotidine, fluvoxamine, and mirtazapine groups.

Effects of fluvoxamine and mirtazapine on GU index

As shown in Table 3, 6 h pyloric ligation produced widespread ulcers to all stomach surfaces. There was

Table 1: Effect of fluvoxamine and mirtazapine on PGE2 and NO concentrations, MPO activity and ulcer index in WIRS-induced GU in rats

Groups	PGE2 (pg/ml)	NO (µM/g)	MPO (µmol/min/mg tissue)	Ulcer index	Ulcer inhibition (%)
Control	65.23±10.40	30.92±2.55	2.61±0.11		
WIRS	20.11±2.01*	20.21±1.20*	12.40±0.80*	24.22±2.31	00.00
Famotidine	53.17±4.14$^{\#}$	25.11±1.17*$^{\#}$	4.1±0.3$^{\#}$	12.63±1.11$^{\#}$	48.21
Fluvoxamine 25 mg/kg	33.65±2.33*$^{\#\bullet}$	21.23±0.91*$^{\#}\bullet$	7.9±0.3*$^{\#\bullet}$	18.50±1.54$^{\#\bullet}$	24.12
Fluvoxamine 50 mg/kg	41.11±2.75*$^{\#\bullet}$	23.1±2.88*$^{\#}$	6.70±0.31*$^{\#\bullet}$	16.25±1.32$^{\#}\bullet$	33.11
Mirtazapine 15 mg/kg	50 0.74±4.37*$^{\#\dagger}$	26.12±2.88$^{\#\dagger}$	4.52±0.23$^{\#\dagger}$	13.13±0.81$^{\#\dagger}$	45.79
Mirtazapine 30 mg/kg	56.33±3.91$^{\#\dagger\ddagger}$	28.33±2.20$^{\#\dagger\ddagger}$	3.11 ± 0.11$^{\#\dagger\ddagger}$	12.53±0.91$^{\#\dagger\ddagger}$	48.81

WIRS: Water immersion restrain stress, PGE2: Prostaglandin E2, NO: Nitric oxide, MPO: Myeloperoxidase, GU: Gastric ulcer. Data were expressed as mean±SE and analyzed using one-way ANOVA followed by Bonferroni's *post hoc* test. n=8. *P<0.05 compared to normal control group, $^{\#}P$<0.05 compared to WIRS group, •P<0.05 compared to famotidine group, $^{\dagger}P$<0.05 compared to fluvoxamine (25 mg/kg) group, $^{\ddagger}P$<compared to fluvoxamine (50 mg/kg) group, ANOVA: Analysis of variance

remarkable hyperemia in the ulcerative stomachs. The mean GU index in pyloric ligation group was (20.51 ± 2.31). Compared with the PL rats, famotidine significantly decreased GU index to 10.39 ± 0.72. Fluvoxamine (25 and 50 mg/kg) significantly reduced GU index to (7.15 ± 0.55 and 8.00 ± 0.71, respectively) compared to PL rats, improvement rates of GU index were 65.14 and 61% ($P < 0.05$). In mirtazapine (15 and 30 mg/kg) treated rats, significant improvement rates of GU index were 61.82% and 53.53% compared to PL rats ($P < 0.05$). As a result, treatment with fluvoxamine (25 and 50 mg/kg) or mirtazapine (15 and 30 mg/kg) resulted in a higher rate of GU improvement than treatment with famotidine.

Effects of fluvoxamine and mirtazapine on PGE_2, NO concentrations, and MPO activity

Table 3 showed that pyloric ligation resulted in a significant decrease in gastric mucosal PGE_2 (25.16 ± 2.11 vs. 50.33 ± 4.40), NO (21.22 ± 1.29 vs. 35.72 ± 3.51) associated with significant increase in MPO activity (14.60 ± 0. 90 vs. 4.11 ± 0.22) as compared to normal control group. Obviously, famotidine, fluvoxamine, and mirtazapine normalized PGE_2 level. NO concentration was also increased significantly in all treatment groups when compared to pyloric ligation group ($P < 0.05$). The larger dose of mirtazapine can ameliorate both NO level and MPO activity significantly better than famotidine ($P < 0.05$).

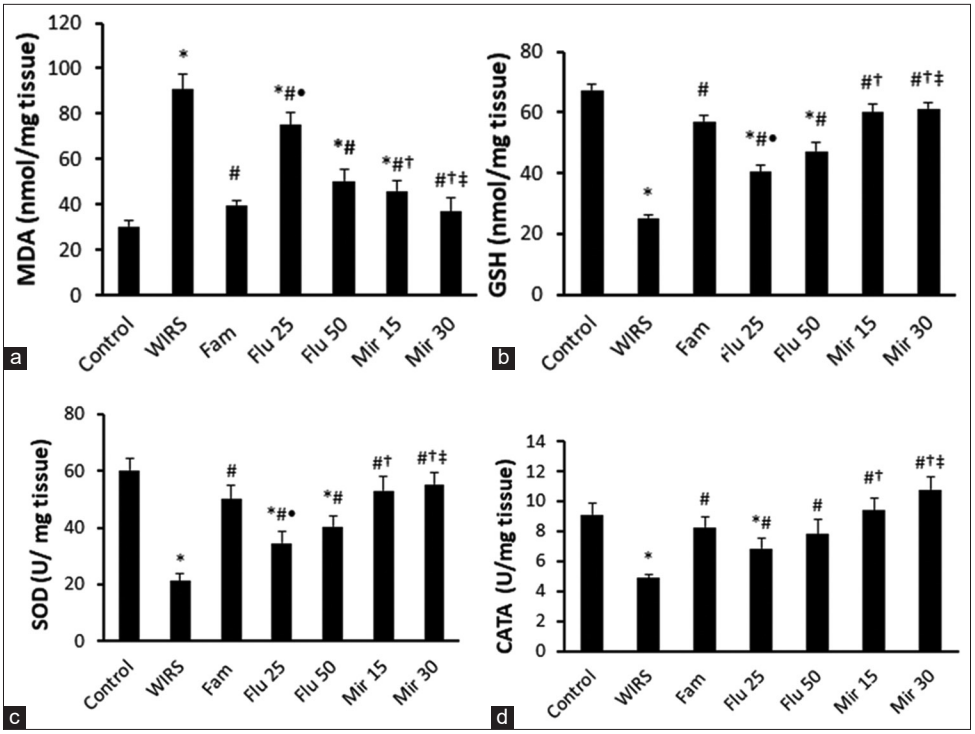

Figure 1: Effects of famotidine (Fam), fluvoxamine (Flu) (25 and 50 mg/kg) and mirtazapine (Mir) (15 and 30 mg/kg) on oxidative stress parameter; malondialdehyde and antioxidant markers; reduced glutathione, superoxide dismutase and catalase (a-d, respectively) in water-immersion plus restraint stress (WIRS)-induced gastric ulcer in rats. Treatment groups were exposed to vehicle or drugs 60 min before WIRS that was induced for 6 h. Data were expressed as mean ± SE and analyzed using one-way Analysis of variance followed by Bonferroni's *post hoc* test. n=8. *P<0.05 compared to normal control group, #P<0.05 compared to WIRS group, •P<0.05 compared to famotidine group, †P<0.05 compared to fluvoxamine (25 mg/kg) group, ‡ P<0.05 compared to fluvoxamine (50 mg/kg) group

Table 2: Effect of fluvoxamine and mirtazapine on gastric volume, pH, total acidity, free acidity, and pepsin activity in pyloric ligation-induced GU in rats

Groups	Gastric volume (ml/100 g rat)	PH	Total acidity (mEq/L)	Free acidity (mEq/L)	Pepsin activity (μg/ml)
Pyloric ligation-control	8.83±0.81	2.30±0.16	81.31±7.60	60.13±5.80	7.94±0.06
Famotidine	4.81±0.30#	1.11±0.12#	51.3±5.6#	35.3±3.6#	4.3±0.03#
Fluvoxamine 25 mg/kg	4.13±0.43#	1.19±0.11#	59.7±5.3#	41.3±3.9#	4.1±0.02#
Fluvoxamine 50 mg/kg	3.85±0.21#	1.16±0.08#	51.2±4.6#	45.1±2.9#	3.8±0.03#
Mirtazapine 15 mg/kg	4.21±0.34#	0.98±0.08#	61.1±5.1#	39.4±3.8#	4.5±0.04#
Mirtazapine 30 mg/kg	3.89±0.41#	1.13±0.14#	58.4±4.1#	38.2±2.7#	3.9±0.01#

Data were expressed as mean±SE and analyzed using one-way ANOVA followed by Bonferroni's *post hoc* test. n=8. #P<0.05 compared to pyloric ligation control group, ANOVA: Analysis of variance, GU: Gastric ulcer

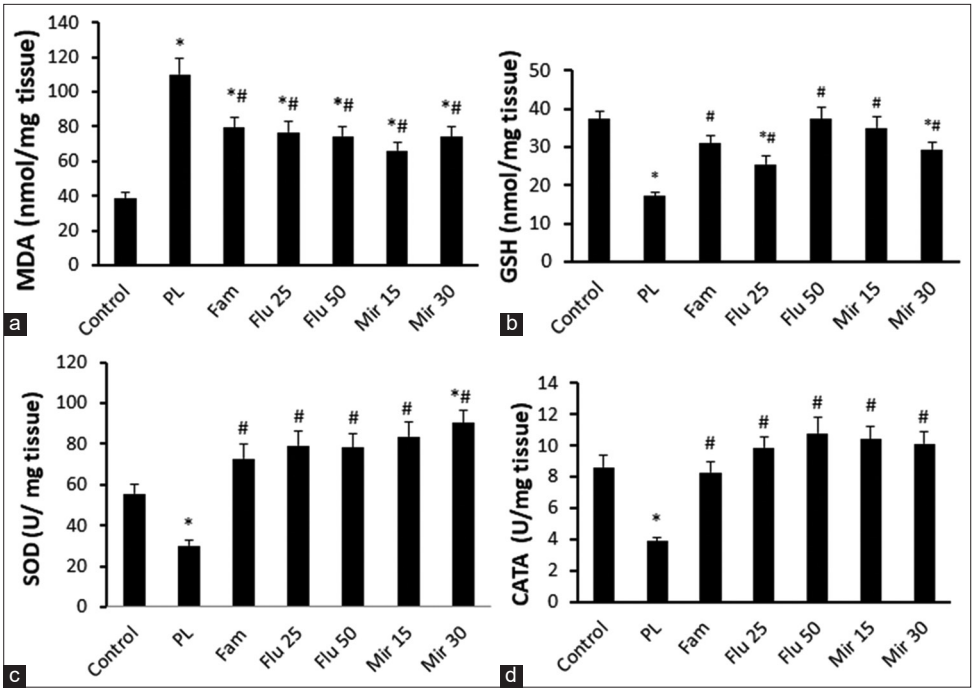

Figure 2: Effects of famotidine (Fam), fluvoxamine (Flu) (25 and 50 mg/kg) and mirtazapine (Mir) (15 and 30 mg/kg) on oxidative stress parameter; malondialdehyde and antioxidant markers; reduced glutathione, superoxide dismutase and catalase (a-d, respectively) in pyloric ligation (PL) induced gastric ulcer in rats. Treatment groups were exposed to vehicle or drugs 60 min before PL that was induced for 6 h. Data were expressed as mean ± SE and analyzed using one-way analysis of variance followed by Bonferroni's *post-hoc* test. *n*=8. **P*<0.05 compared to normal control group, #*P*<0.05 compared to PL control group

Table 3: Effect of fluvoxamine and mirtazapine on PGE2 and NO concentrations, MPO activity and ulcer index in pyloric ligation-induced GU in rats

Groups	PGE2 (pg/ml)	NO (μM/g)	MPO (μmol/min/mg tissue)	Ulcer index	Ulcer inhibition (%)
Control	50.33±4.40	35.72±3.51	4.11±0.22		
Pyloric ligation	25.16±2.11*	21.22±1.29*	14.60±0.90*	20.51±2.31	00.00
Famotidine	44.16±4.18#	26.11±1.17*#	10.21±0.41*#	10.39±0.72#	49.34
Fluvoxamine 25 mg/kg	45.55±3.63#	27.20±0.11*#	9.91±0.30*#	7.15±0.55#•	65.14
Fluvoxamine 50 mg/kg	46.19±3.77#	29.1±2.38*#	8.16±0.32*#	8.00±0.71#•	61
Mirtazapine 15 mg/kg	46 0.74±4.37#	26.12±2.88*#	7.41±0.61#	7.83±0.81#	61.82
Mirtazapine 30 mg/kg	44.53±4.31#	32.31±3.21#•	6.98±0.44#•	9.53±0.91#	53.53

PGE2: Prostaglandin E2, NO: Nitric oxide, MPO: Myeloperoxidase, GU: Gastric ulcer. Data were expressed as mean±SE and analyzed using one-way ANOVA followed by Bonferroni's *post hoc* test. *n*=8. **P*<0.05 compared to normal control group, #*P*<0.05 compared to PL group, •*P*<0.05 compared to famotidine group

Effects of fluvoxamine and mirtazapine on oxidative stress parameter and antioxidant markers

Pyloric ligation significantly increased gastric mucosal MDA content (110.22 ± 8.81) compared to normal control value (38.71 ± 3.11). Pre-treatment with famotidine, fluvoxamine (25 and 50 mg/kg), as well as mirtazapine (15 and 30 mg/kg) significantly reduced gastric mucosal MDA content to 72.14, 69.48, 67.40, 59.93, and 67.14%, respectively, compared to the ulcer control value ($P < 0.05$, [Figure 2a]). Pyloric ligation induced also a significant decrease in GSH content (17.12 ± 1.14) in rat gastric mucosa compared to normal control value (38.00 ± 2.13). Administration of famotidine, fluvoxamine (25 and 50 mg/kg), as well as mirtazapine (15 and 30 mg/kg) significantly increased GSH content to 31.0 ± 2.11, 25.52 ± 2.19, 37.31 ± 2.19, 35.22 ± 2.11, and 29.30 ± 2.21, respectively, compared to the ulcer control value ($P < 0.05$, [Figure 2b]). Regarding SOD and CATA activities, pyloric ligation significantly decreased them to (30.13 ± 2.9) and (3.91 ± 0.40), respectively. Famotidine, fluvoxamine (25 and 50 mg/kg), as well as mirtazapine (15 and 30 mg/kg) significantly increased SOD activity by 142.83, 163.27, 159.76, 177.13, and 195.99%, respectively, and CATA by 112.13, 153.22, 179.56, 167.10, and 185.59%, respectively, as compared to the pyloric ligation control group ($P < 0.05$, [Figure 2c and d]). Clearly, a 30 mg/kg dose of mirtazapine outperformed famotidine and both fluvoxamine doses in increasing SOD activity. Furthermore, fluvoxamine and mirtazapine were both more effective than famotidine in increasing CATA activity.

Discussion

Because of the serious consequences of illness, the importance of stress erosions, which have been reported worldwide, has stimulated considerable research.[34] Stress-related gastric mucosal lesions occur as a typical stress induced organ injury.[35] The mechanisms of acute gastric mucosal injury are not yet fully understood, its origin may be multifactorial due to the imbalance between protective factors, for example, adequate mucosal blood flow, mucosal bicarbonate barrier,[36] endothelial cell regeneration, and PGs and damage factors, for example, gastric acid, pepsin, bile, and oxygen free radicals.[4] Therefore, the efficacy of drug treatment is dependent not only on the reduction of damage factors, but also on the increase of gastric mucosal protective factors to maintain the gastric integrity of endothelial cells.[37]

In this study, we used two experimental rat models (WIRS and pyloric ligation–induced GU) to assess the antiulcer activity of two different antidepressant mechanisms (fluvoxamine and mirtazapine) and compare their action to that of a commonly used antiulcer drug (famotidine 50 mg/kg).

Antidepressants have been shown in numerous studies to have antiulcer effects by reducing histamine secretion from mast cells, inhibiting gastric acid secretion, repairing the mucus-bicarbonate barrier, and blocking leukotriene (LTC4, D4, and E4) receptors.[38] They increased the expression of antioxidant markers such as GSH and SOD, while decreasing the expression of oxidative markers such as H_2O_2, MDA, and MPO.[37] Apart from these factors, brain monoamines and their modulation during brain-gut axis have previously been reported.[39]

In the present study, rats exposed to 6 h of WIRS or pyloric ligation, in addition to the developed hemorrhagic lesions in the stomach assessed by the ulcer index; they developed a significant increase in oxidative stress and MPO activity associated with a significant decrease in antioxidant activity, PGE_2, and NO levels.

The WIRS model, which mimics clinical GU caused by trauma, surgery, and sepsis, is widely used in animal experiments to study stress-induced acute gastric mucosal injury.[40] Stress-induced rat GU is accompanied by arteriolar spasm, venous congestion, perivascular edema, reduction of gastric mucosal blood flow, and micro-hemorrhages.[41] It also promotes neutrophil infiltration and H^+ back diffusion, and it plays an important role in the development of GU.[42,43] In addition, stress is likely to be accompanied by mast cell degranulation and the release of histamine, which increases gastric secretion while decreasing mucous production. Vagal activity has been proposed as the primary factor in stress-induced ulceration, as it stimulates hydrochloric acid in the stomach through the action of acetyl choline. It was concluded that acid stasis caused by stress, as well as increased volume of gastric acid production, are important factors in ulcer formation.[44]

Administration of famotidine, fluvoxamine, or mirtazapine significantly reduce the GU index in different degrees and showed potential anti-ulcer activity [Table 1] Ulcerogenesis in WIRS model was modulated by serotonergic as well as noradrenergic input.[45,46] Because famotidine is a strong H_2 receptor antagonist, we used it as a positive control antiulcer group in this study and compared its antiulcer effect to that of fluvoxamine and mirtazapine.

Fluvoxamine is a selective serotonin reuptake inhibitor that causes an increase in brain serotonin levels by blocking serotonin reuptake.[47] It is also regarded as an antioxidant because it inhibits the CYP 1A2 enzyme, which is known to generate ROS.[48] Selective serotonin reuptake inhibitors could increase cortical gamma-aminobutyric acid (GABA) levels in stressed rats,[49] and gastroprotective activity of GABA appears to be mediated by increasing the gastric mucosal blood flow that was depended on sensory neuron and NO systems.[50] Unlike most selective serotonin reuptake inhibitors, which enhance upper GIT bleeding, fluvoxamine is postulated to be beneficial in the management of peptic ulcer disease.[51] On the other hand, mirtazapine enhances noradrenergic neurotransmission via blocking of presynaptic α2-adrenoreceptors in the central nervous system and/or stimulating postsynaptic α2- receptors.[52] Blockage of 5-HT2 and 5-HT3 receptors may also be responsible for mirtazapine's antiulcer effects.[19] A more plausible mechanism was suggested that antidepressants could protect gastric mucosa through interactions with H_2 receptors in the brain.[53]

It was reported that central injection of norepinephrine into central amygdalar nucleus and intracerebro-ventricular injection of serotonin produced dose-related attenuations of WIRS induced GU formation in rats.[39,43] Postsynaptic α2-adrenoreceptors also have been shown to mediate the antiulcer effects of adrenalin.[54] Thus, the enhanced both norepinephrine and serotonin neurotransmission in brain may account for the preferential antiulcer effect of mirtazapine over fluvoxamine, which enhances serotonin neurotransmission only.

Reactive oxygen species (ROS)-mediated lipid peroxidation is an important primary factor in stress and pyloric ligation-induced GU.[55] Hence, the effects of fluvoxamine and mirtazapine were evaluated on MDA, GSH, SOD, CATA, PGE_2, NO, and MPO levels in order to at least partially explain their antiulcer effect mechanism (s), rather than their effects on neurotransmitters.

It has been showed that the oxidant marker; MDA level was decreased accompanied by significant increase in the endogenous antioxidant GSH levels in the stomach tissue of treated rats compared to those in the control model (s) groups [Figures 1a and b, 2a and b]. Lipid peroxidation is a major

cause of cell membrane damage; MDA is the end product of lipid peroxidation and is used to determine lipid peroxidation levels.[56] In stomach tissue, increasing GSH levels produce a gastroprotective effect as it reacts with H_2O_2 and ROS to protect cells against injury. It also keeps-SH groups of proteins in reduced form and protects them from oxidation.[57] A strong relationship between GSH levels and the levels of ulcer severity has been reported.[58] The decrease in GSH level is a sign of increased tissue oxidative stress.[59]

Regarding the antioxidant (SOD and CATA) enzymes activities, they are also increased upon treatment when compared to the model (s) control groups [Figures 1c and d, 2c and d] indicating the drugs' antioxidant properties. SOD catalyzes the dismutation of superoxide anion radicals to H_2O_2, and the CATA degrades H_2O_2 into an oxygen and water molecule.[60] Lowered SOD and CATA activities, as observed in the current study on control model (s) groups, resulted in the accumulation of these highly reactive free radicals, which cause detrimental effects in various tissues due to an imbalance between ROS generation and the antioxidant system. The previous studies have reported that decreased SOD activity causes gastric damage in stomach tissue.[61]

In stomach tissue damaged by WIRS or pyloric ligation, NO levels have been shown to be reduced as reported previously.[62] NO is known to prevent membrane lipid peroxidation, modulate acid levels, gastric mucus secretion,[63] and blood flow in gastric tissues.[62] In accordance, the previous studies reported that the NO synthase inhibitors aggravated ulcer formation.[64] In the current study, all doses of fluvoxamine and mirtazapine, which exerted a significant antiulcer effect, also increased gastric NO levels significantly when compared to the model control (s) [Tables 1 and 3]. A parallel between the decreased antioxidant of NO levels and severity of gastric damage was also noted. Our results are in line with those reported by Dengiz *et al*.[65]

Water immersion restrain stress as well as pyloric ligation have both been shown to cause damage by increasing mucosal MPO levels in gastric tissue.[66] All doses of fluvoxamine and mirtazapine decreased the MPO activity significantly when compared to the model (s) control groups [Tables 1 and 3]. MPO is highly concentrated in polymorph nuclear leukocyte cells; the activation of neutrophils causes excessive release of radicals such as O_2^-, H_2O_2, and OH^-. As a result of the reaction between these radicals and MPO, products such as hypochlorous acid and *N*-chloramine, which cause oxidative tissue damage and lipid peroxidation arise.[67]

Although several mechanisms are thought to play a role in the pathogenesis of pyloric ligation-induced peptic ulcer, gastric acid secretion and accumulation are thought to be the most important. Moreover, pyloric ligation is one of the major factors of mucosal damage by interfering with gastric mucosal resistance and alters the level of cytoprotective PGs, cytokines,[68] membrane lipid peroxidation, and endogenous GSH.[16] In parallel with the previous results, fluvoxamine and mirtazapine significantly suppressed GU index, gastric acid secretion, and acidic content in the PL model, which was indicated by a decrease in gastric volume as well as total and free acidity [Table 2]; we considered that the protective effect of the treatments may be partially mediated by antisecretory effect.[69]

Another important factor responsible for gastric injury is the weakening of defensive mucosal barrier against the offensive assault of acid-pepsin, is the quantity of gastric mucus secretion.[70] PGE_2 is a well-established mediator in gastric mucosal defense and repair as it maintains the integrity of the gastric mucosa by stimulating secretion of the mucus and bicarbonate, modulating mucosal blood flow, and inhibiting the neutrophil-mediated free radicals generation.[27,44,71] In the present study concurrent with the previous observations, WIRS and pyloric ligation-induced GU were associated with inhibition of defensive gastric mucosal PGE_2 synthesis.[72]

Conclusions

The findings of this study revealed a link between the antiulcer effect of drugs from different antidepressant classes across two animal GU models, implying that antidepressants that affected both norepinephrine and serotonin levels (mirtazapine) had a more potent antiulcer effect in WIRS-induced GU model than drugs that only affected serotonin levels (fluvoxamine). Antidepressants' effectiveness in ulcer prevention may be based on their ability to stimulate protective factors such as antioxidant enzymes, NO, and PGE2 while suppressing destructive factors such as increased MPO and pepsin activity, as well as lipid peroxidation and acidity, which is exactly what was observed. We can use antidepressants for the development of new drugs to protect against GU in depressive patient, if full experimental and clinical data is available.

Authors' Declaration Statements

Ethics approval and consent to participate

All experimental protocols were approved by the Ethics Committee at the Faculty of Pharmacy, Suez Canal University (Ismailia, Egypt) (code # 201907RA1).

Availability of data and material

The data used in this study are available and will be provided by the corresponding author on a reasonable request.

Competing interests

The authors declare that there are no conflicts of interest.

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Authors' Contributions

All authors contributed in the design of the study**.** All authors participated in data collection and analysis, data interpretation, and manuscript writing.

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