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Investigations on gastroprotective effect of citalopram, an antidepressant drug against stress and pyloric ligation induced ulcers

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Abstract:
The present study investigates the gastroprotective effect of citalopram, an antidepressant drug. Gastroprotective activity of citalopram (5, 10 and 20 mg/kg, *bid, po*) was evaluated both by single and 14 days repeated pretreatment in the cold restraint stress (CRS) model and 14 days repeated pretreatment in pyloric ligation (PL) model. In addition to ulcer scoring and its histological assessment, levels of corticosterone, hexosamine, nitrite, PGE_2, lipid peroxide and microvascular permeability were also estimated. Mechanism underlying gastroprotective activity was further explored by investigating the involvement of nitric oxide (NO), sulfhydryl (SH) compunds, ATP-sensitive K^+ channels (K_{ATP} channels) and prostaglandins (PGs). Results show that against CRS model, repeated pretreatment with citalopram exhibit a significant gastroprotective effect while single pretreatment was ineffective. In CRS model, citalopram repeated pretreatment, in contrast to its single pretreatment, attenuates the corticosterone level and also mitigates the stress-induced increase in nitrite level, lipid peroxidation and microvascular permeability. Additionally, the repeated pretreatment increases the hexosamine and PGE_2 level in CRS model. This gastroprotective effect of citalopram was found to be decreased with L-NAME, NEM, glibenclamide and indomethacin pretreatment. Thus, gastroprotective activity of citalopram appears to be mediated by endogenous NO, SH, PGs and K_{ATP} channel opening. In contrast to CRS model, repeated pretreatment with citalopram was ineffective in reducing ulcer formation in PL model.

Key words:
citalopram, antidepressant, gastroprotective, prostaglandin, K_{ATP} channel, nitric oxide

Introduction

In the current state of affairs, gastric ulcers are identified as a very common chronic disease in working-age adults. Approximately 4 million individuals are suffering from gastric ulcers in USA. Around 350,000 new cases are diagnosed; about 100,000 patients are hospitalized and at least 3,000 patients die as a result of peptic ulcer every year [12]. Gastric ulcer is a multifactorial etiological disease. Several factors which play a significant role in gastric ulcerogenesis include stress, trauma, sepsis, hemorrhagic shock, burns, *Helicobacter pylori*, steroidal and non-steroidal drugs etc. [15, 23]. Regardless of great advances in the field of biological science and understanding of the peptic ulcer illness, gastric ulcers etiology is still not completely comprehensible. The most important factor responsible for the genesis of gastric ulcers is the imbalance between the defensive factors, such as secretion of mucus and bicarbonates, and offensive factors, such as increased secretion of acid and pepsin [46]. Thus, gastric ulcer is a benign lesion of the gastric mucosa, which occurs at the site where the mucosal epithelium is continuously exposed to acid and pepsin [2].

Gastric ulcer may also occur due to ischemia. Ischemia is a state of oxygen deprivation of tissue caused by less blood supply to the tissue. However, the blood flow to the ischemic tissue is restored by reperfusion. Despite the unequivocal benefit of reperfusion of blood to an ischemic tissue, reperfusion itself can elicit a cascade of adverse reactions at the site of injury like free radical generation and inflammatory reactions. Free radical generation [4] and inflammatory mechanisms [8] play a major role in the pathogenesis of ischemic reperfusion injury. Several endogenous factors which are also related to the pathophysiology of gastroprotection include prostaglandin (PGs), ATP-sensitive K^+ (K_{ATP}) channels, nitric oxide (NO) and sulfhydryl (SH) compounds.

H_2 receptor antagonists and proton-pump inhibitors are currently used anti-ulcer drugs. Despite increasing ulcer cure rate, prolonged use of these medications provokes serious side effects such as hypergastrinemia. Thus, the success of pharmacologic treatment of gastric lesion also depends on augmentation of the defensive factors of the gastric mucosa in addition to the blockade of acid secretion.

Patients with gastrointestinal tract (GIT) diseases have also been diagnosed with depression [20]. Many antidepressant drugs like maprotilin, mianserin, trimipramine [16], fluoxetine, bupropion [17], imipramine [22], amitriptyline [48], dothiepin [49], doxepin [50] etc., have been found to exhibit antiulcer activity. It is also reported that serotonin reuptake inhibitors prevent duodenal ulcer [29]. In contrast to this, there are a few antidepressant drugs like paroxetine which aggravates the formation of gastric ulcer [54]. Citalopram is a selective serotonin reuptake inhibitor (SSRI) and a well-known antidepressant drug. However, till now citalopram has not been explored for its gastroprotective activity. Therefore, the present work evaluates the gastroprotective effect of citalopram against the cold restraint stress (CRS) and pyloric ligation (PL) induced gastric ulcer models. In addition to ulcer scoring and its histological examination, the present study also includes estimation of corticosterone, hexosamine, PGE_2, nitrite, microvascular permeability and lipid peroxidation. For further elucidating the mechanism of action underlying gastroprotective activity of citalopram, the involvement of NO, SH, PGs and K_{ATP} channels are also evaluated.

Materials and Methods

Animals

Experiments were carried out on adult male albino Wistar rats (body weight 180–220 g). Rats were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University (B.H.U.). Animals were housed in polypropylene cages at constant temperature of 25 ± 1°C and relative humidity of 45–55%, with a 12:12 h light/dark cycle. The animals had free access to commercial rat feed and water *ad libitum*. Experiments were carried out between 09:00 and 14:00 h. All procedures were approved by Institutional Ethical Committee and conducted according to the guidelines "Principles of laboratory animal care" (NIH publication number 85–23, revised 1985).

Drugs, reagents and solvents

Glibenclamide, indomethacin, L-(G)-nitro-L arginine methyl ester (L-NAME) and N-ethylmaleimide (NEM) were procured from Sigma-Aldrich (St. Louis, MO, USA). All the other reagents and solvents were of analytical grade and used as received. The dose (10 mg/kg), frequency of dosing (twice a day) and route of administration (oral) of citalopram were in accordance with Zahorodna and Hess [55]. However, lower (5 mg/kg) and higher (20 mg/kg) doses were selected in geometric progression to study dose related effects.

Effect of single and repeated pretreatment with citalopram on CRS-induced ulcer model

The gastroprotective effect of single citalopram administration against stress-induced ulcer was estimated by distributing rats into five groups with six animals each. Control and stress control (CRS) groups received the vehicle (distilled water) (DW, 3 ml/kg). The other three groups, that are ACIT-5, ACIT-10 and ACIT-20 were given single dose of 5, 10 and 20 mg/kg of citalopram, respectively. After 1 h of vehicle/citalopram administration, all the groups were subjected to CRS (4–7°C) for 2 h. Blood was collected from all the animals for the estimation of corticosterone. Thereafter, the animals were sacrificed and their stomachs were taken out for ulcer scoring as described by Sairam et al. [47].

The gastroprotective effect of repeated citalopram administration was investigated by dividing rats into five groups (one control, one CRS and three treatment groups) with six animals each. Control and CRS groups received the vehicle (DW, 3 ml/kg) for 14 days. Other three groups, RCIT-5, RCIT-10 and RCIT-20, received repeated oral doses (twice a day) of 5, 10 and 20 mg/kg citalopram, respectively, for 14 consecutive days. All groups except the control group were then subjected to 2 h of CRS after 1 h of vehicle/citalopram administration on the 14th day. Blood was collected from all the animals for estimation of corticosterone. Animals were sacrificed after the experiment and their stomachs were taken out for ulcer scoring and estimations of PGE_2, hexosamine, lipid peroxidase (LPO) in terms of malondialdehyde (MDA) and nitric oxide in terms of nitrite.

Effect of repeated citalopram pretreatment in PL model

For evaluating the effect of repeated citalopram pretreatment in PL method, rats were divided into four groups each comprising six animals. In the first group (control), oral administration of vehicle (DW, 3 ml/kg) was done. Other three groups, RCIT-5, RCIT-10 and RCIT-20, received repeated oral doses (twice a day) of 5, 10 and 20 mg/kg citalopram, respectively, for 14 consecutive days. On 14th day, after 1 h of vehicle/citalopram administration, animals were anesthetized using pentobarbital (35 mg/kg, *ip*) followed by opening of the abdomen. Pylorus ligation was carried out avoiding any damage to blood supply. After careful replacement of stomach, the abdomen wall was closed with interrupted sutures. After 4 h, stomach was taken out and ulcer scoring was done as described by Sairam et al. [47]. Finally, the stomach contents were collected for the estimation of acidity and volume of gastric content following the procedure of Debnath et al. [13].

Histological studies

The stomach tissues were excised, washed with ice-cold 0.9% saline solution and fixed for 24 h in 10% v/v solution of formalin. The tissues were washed with running tap water overnight, to remove any additional fixative. The tissues were finally cleaned with methyl benzoate and embedded in paraffin wax after dehydrating through a graded series of alcohol. Sections of 5 μm thickness were cut and stained with eosin as well as hematoxylin. Thereafter, the sections were mounted and observed under a light microscope.

Determination of plasma corticosterone level

Plasma was separated by cold (4°C) centrifugation (5 min, 5000 rpm). Level of corticosterone in plasma was estimated by HPLC/PDA system (Waters, USA) as per Woodward and Emery method [53]. Dexamethasone was used as an internal standard. Briefly, 500 μl of plasma containing a known quantity of dexamethasone was extracted with 5 ml of dichloromethane. The dichloromethane extract was evaporated and dissolved in 100 μl of mobile phase. Twenty microliters of extract was injected into HPLC system for quantification. Mobile phase consisted of methanol : water (70:30, v/v) at a flow rate of 1.0 ml/min and corticosterone was detected at a wavelength of 250 nm using PDA detector (Model 2998, Waters, USA). Water Spherisorb® C18 (250 × 4.6 mm, 5 μm) was used as analytical column. The chromatogram was recorded and analyzed with Empower software.

Lipid peroxidation (LPO), nitric oxide and PGE_2 estimation in CRS model

The stomach mucosa was cut, weighed and minced. Ten percent homogenate was prepared at 4°C in 20 mM Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was termed as post mitochondrial supernatant (PMS) and it was used for further experiments. The level of lipid peroxide in gastric mucosa was determined by estimating MDA concentrations using the thiobarbituric acid test [44, 52]. It is expressed as nmoles per min per mg protein. Nitrite in the PMS was estimated by the method of Green et al. [19]. The samples were incubated with Griess reagent (1% naphthylethylenediamine dihydrochloride and 1% sulfanilamide in 5% phosphoric acid) for 10 min at room temperature. The optical density was observed at the wavelength of 546 nm. The standard curve was made by using 10 mM sodium nitrite. Results were expressed as nmoles of nitrite per mg protein. PGE_2 level was determined with an ELISA kit from R&D Systems (USA). Protein estimation was done with Lowry method [34].

Determination of gastric hexosamine level

Hexosamine is a marker of gastric adherent mucus. Therefore, the effect of citalopram on gastric mucus was studied in terms of hexosamine. For this, gastric mucosa was scraped, and after homogenizing it in ice-cold saline, the concentration (mg per g of tissue) of hexosamine was determined by the method described by Dische and Borentrend [14].

Evaluation of microvascular permeability

For the microvascular permeability studies, control and stress control group (CRS) were given vehicle (DW) for 14 days while the other three groups (RCIT-5, RCIT-10 and RCIT-20) received repeated oral administration of citalopram (5, 10 and 20 mg/kg, *bid*) for 14 days. Animals of all the groups (Control, CRS, RCIT-5, RCIT-10 and RCIT-20) were injected intravenously with Evans blue (10 mg/kg) under the light anesthesia, 30 min before exposing to CRS for 2 h and finally the animals were killed by cervical dislocation. Dye extraction was conducted as per the report of Katayama et al. [28]. The absorbance of the extracted dye was monitored at the wavelength of 620 nm on a Hitachi single beam spectrophotometer and the amount of the dye recovered from the gastric content was expressed as μg per mg of tissue.

Determination of the role of NO metabolic pathway in the gastroprotective effect of citalopram

L-NAME is a known inhibitor of NO synthetase and therefore, for the present investigation, it was used in order to explore the participation of endogenous nitric oxide (NO) in the gastroprotective activity of citalopram. Dose (25 mg/kg) and dosing frequency of L-NAME were kept the same as reported by Chandranath et al. [9]. Rats were divided into nine groups each containing six animals. For 14 consecutive days, one group (CRS) received the vehicle (DW, 3 ml/kg) orally and three groups that are RCIT-5, RCIT-10 and RCIT-20 were treated with 5, 10 and 20 mg/kg of citalopram, *bid*, respectively. Other four groups were treated as illustrated above with additional intraperitoneal L-NAME pretreatment on the 14^{th} day, 15 min prior to vehicle/citalopram administration. On 14^{th} day, all the groups were exposed to CRS for 2 h after 1 h of vehicle/citalopram administration. Only vehicle was administered to the control group without CRS exposure. Finally, animals were killed by cervical dislocation and their stomachs were taken out for ulcer scoring.

Determination of effect of sulfhydryl (SH) compounds on gastroprotective activity of citalopram

Role of SH in the gastroprotective activity of citalopram was examined by employing NEM (10 mg/kg), a blocker of SH compounds [5]. Rats were divided into nine groups each with six animals. For 14 consecutive days, one group (CRS) received the vehicle (DW, 3 ml/kg) orally and three groups that are RCIT-5, RCIT-10 and RCIT-20 were treated with 5, 10 and 20 mg/kg of citalopram, *bid*, respectively. Other four groups were treated as illustrated above with additional intraperitoneal NEM pretreatment on the 14^{th} day, 15 min prior to vehicle/citalopram administration. On 14^{th} day, all the groups were exposed to CRS for 2 h after 1 h of administration of vehicle/citalopram. In the control group, only vehicle was administered without CRS exposure. Finally, the rats were killed by cervical dislocation and their stomachs were taken out for ulcer scoring.

Determination of participation of prostaglandins in the gastroprotective effect of citalopram

The possible involvement of endogenous PGs in the gastroprotective activity of citalopram in CRS-induced gastric lesions was determined by using indomethacin. Indomethacin was used at a dose (10 mg/kg, dissolved in 0.5% CMC and diluted with distilled water, *po*) that inhibits the synthesis of PGs but does not induce gastric ulceration [1]. Animals were divided into nine groups each with six animals. One group (CRS) received vehicle (DW, 3 ml/kg) orally and three groups, RCIT-5, RCIT-10 and RCIT-20 were treated with 5, 10 and 20 mg/kg of citalopram, respectively, twice daily for 14 consecutive days. Other four groups were treated as explained above with an additional oral indomethacin (Indo) pretreatment on the 14^{th} day prior to vehicle/citalopram administration. On 14^{th} day, all the groups were exposed to CRS for 2 h. Indomethacin was administered 2 h and citalopram 30 min prior to CRS [40]. In the control group, only vehicle was administered without CRS exposure. Finally, animals were killed by cervical dislocation and their stomachs were taken out for ulcer scoring.

Determination of role of K_{ATP} channels in gastroprotecive effect of citalopram in glibenclamide pretreated rats

Glibenclamide is a K_{ATP} channels antagonist. It was used in the present study to determine the role that the opening of K_{ATP} channels play in the gastroprotective activity of citalopram. Rats were divided into nine groups each with six animals. Dose (10 mg/kg) of glibenclamide administration was selected as reported by Peskar et al. [43]. Glibenclamide was prepared with 4% glucose to minimize hypoglycemia [43]. One group (CRS) received the vehicle (DW, 3 ml/kg) orally and three groups that are RCIT-5, RCIT-10 and RCIT-20 were treated with 5, 10 and 20 mg/kg of citalopram, respectively, twice daily for 14 consecutive days. Other four groups were also treated in the same manner as illustrated above with an additional oral glibenclamide (Glib) pretreatment on the 14th day, 30 min prior to vehicle/citalopram administration. On 14th day, after 1 h of vehicle/citalopram administration, all the groups were exposed to CRS for 2 h. In the control group, only vehicle was administered without CRS exposure. Finally, animals were killed by cervical dislocation and their stomachs were taken out for ulcer scoring.

Statistical analysis

Results were expressed as the mean ± SEM (n = 6). The data were analyzed with GraphPad Prism 4 (San Diego, CA). Statistical analysis of data was carried out by one-way ANOVA, followed by Tukey's test; $p < 0.05$ was considered statistically significant.

Results

Effect of single and repeated citalopram pretreatment on ulcer index in CRS model

Table 1 illustrates the effect of single pretreatment with citalopram on the ulcer index in CRS model. Results were analyzed with one-way ANOVA which indicates significant differences in ulcer index among the different groups [$F(4,29) = 60.40$, $p < 0.0001$]. In CRS exposed rats, ulcer index increased significantly compared to the normal control group. Single pretreatment with citalopram did not attenuate stress-induced increase in the ulcer index. Although the percentage of gastroprotection was 9.33, 12.22 and 4.16 with single 5, 10 and 20 mg/kg dose of citalopram, respectively, but these were not statistically significant. These results demonstrate that single citalopram administration showed no beneficial effect on stress-induced ulcer score in the CRS model.

Tab. 1. Effect of single treatment with citalopram (5, 10 and 20 mg/kg) (ACIT-5, ACIT-10 and ACIT-20) on ulcer index and % gastroprotection in the rat CRS model

Groups	Dose (mg/kg)	Ulcer index	% Gastroprotection
Control	vehicle	0	–
CRS	vehicle	32.17 ± 2.06*	–
ACIT-5	5	29.17 ± 1.76*	9.33
ACIT-10	10	28.24 ± 2.47*	12.22
ACIT-20	20	30.83 ± 1.33*	4.16

Results in each column are expressed as the mean ± SEM (n = 6). * $p < 0.05$ compared to control (one way ANOVA followed by Tukey's test)

The results of the study on the effect of repeated oral pretreatment with citalopram on the ulcer index in CRS model are summarized in Table 2. One-way ANOVA indicated significant differences in ulcer index of various groups [$F(4,29) = 53.34$, $p < 0.0001$]. This study showed that gastric injury significantly increased in the stress group. Repeated pretreatment with 5, 10 and 20 mg/kg of citalopram resulted in 21.87, 62.29 and 37.15% gastroprotection, respectively. Thus, repeated citalopram administration showed gastroprotective effect at all doses.

Tab. 2. Effect of repeated treatment with citalopram (5, 10 and 20 mg/kg, *po*) (RCIT-5, RCIT-10 and RCIT-20) on ulcer index and % gastroprotection in the CRS model

Groups	Dose (mg/kg)	Ulcer index	% Gastroprotection
Control	vehicle	0	–
CRS	vehicle	30.50 ± 1.63*	–
RCIT-5	5	23.83 ± 2.36*@	21.87
RCIT-10	10	11.50 ± 1.28*@a	62.29
RCIT-20	20	19.17 ± 1.76*@	37.15

Results in each column are expressed as the mean ± SEM (n = 6). * $p < 0.05$, @ $p < 0.05$ and a $p < 0.05$ compared to control, stress group and CIT-5, respectively (one way ANOVA followed by Tukey's test)

Tab. 3. Effect of oral repeated treatment with citalopram at a dose of 5, 10 and 20 mg/kg (RCIT-5, RCIT-10 and RCIT-20) on ulcer index, gastric acidity and gastric volume of gastric content in the PL model

Groups	Ulcer index	Acidity (μEq/ml)	Gastric volume (ml/100g)
PL control	21.50 ± 1.31	29.32 ± 1.74	1.38 ± 0.19
RCIT-5	22.33 ± 1.50	33.17 ± 1.58	2.30 ± 0.20*
RCIT-10	22.83 ± 1.76	34.13 ± 1.47	2.39 ± 0.28*
RCIT-20	24.33 ± 1.09	39.7 ± 2.09*	2.31 ± 0.20*

Results in each column are expressed as the mean ± SEM (n = 6). * $p < 0.05$ compared to pyloric control (one way ANOVA followed by Tukey's test)

site can be observed in the gastric mucosa of rats exposed to CRS (Fig. 1B). Animals pretreated with a dose of 5 mg/kg citalopram followed by CRS (Fig. 1C) showed mucosal ulceration that was less severe than the CRS alone. However, rats administered with citalopram at a dose of 10 mg/kg showed least epithelial cell loss and ruptured mucosal layer at ulcer site (Fig. 1D). The rats treated with 20 mg/kg dose of citalopram (Fig. 1E) showed less epithelial cell loss and ruptured gastric mucosal layer in comparison to CRS. However, the injury was greater than that found in the group pretreated with 10 mg/kg dose of citalopram.

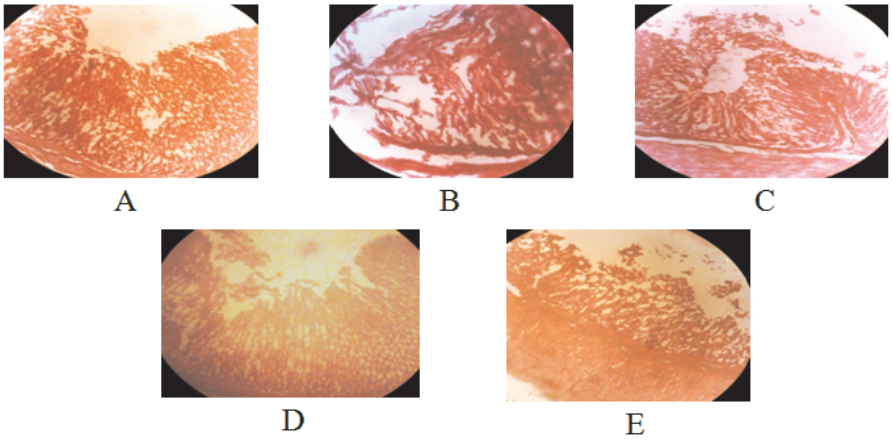

Fig. 1. Histological examination of stomach of control and experimental group of rats (magnification 200×, hematoxylin and eosin staining). (**A**) Control rats showed normal gastric mucosa; (**B**) CRS rats showed mucosal ulceration, epithelial cell loss and ruptured gastric mucosal layer in ulcer site; (**C**) CRS + RCIT-5 rats showed mucosal ulceration with epithelial cell loss and ruptured gastric mucosal layer in ulcer site though less than CRS alone (**D**) CRS + RCIT-10 rats showed normal gastric mucosal architecture while (**E**) CRS + RCIT-20 showed ruptured gastric mucosal layer at ulcer site lesser than CRS alone while greater than lower dose of 10 mg/kg

Effect of repeated administration of citalopram in PL model

Table 3 depicts the effect of repeated pretreatment with citalopram on ulcer index, acidity and volume of gastric content in PL model. No significant difference was observed in the ulcer index among the various groups in PL model. However, repeated pretreatment with citalopram significantly increased the acidity and volume of gastric content.

Histopathological study

The histopathological examinations of stomach of control and experimental group of rats are shown in Figure 1. As evident from Figure 1A, the gastric mucosa of control group revealed a normal mucosal architecture. In contrast, mucosal ulceration, epithelial cell loss and ruptured gastric mucosal layer at ulcer

Effect of single and repeated citalopram pretreatment on plasma corticosterone level in CRS model

The results of single oral pretreatment with citalopram on the plasma corticosterone level in CRS model are shown in Figure 2A and analyzed by one-way ANOVA [$F(4,29) = 72.44$, $p < 0.0001$]. It was observed from the results that the plasma corticosterone level significantly increased in stress. This was further increased with single citalopram administration.

Figure 2B aptly demonstrates the effect of repeated oral pretreatment with citalopram on the plasma corticosterone level in CRS model. One-way ANOVA [$F(4,29) = 93.34$, $p < 0.0001$] revealed that the plasma corticosterone level significantly increased in stress as compared to the control group. Repeated citalopram pretreatment decreased the CRS-induced enhancement in corticosterone level.

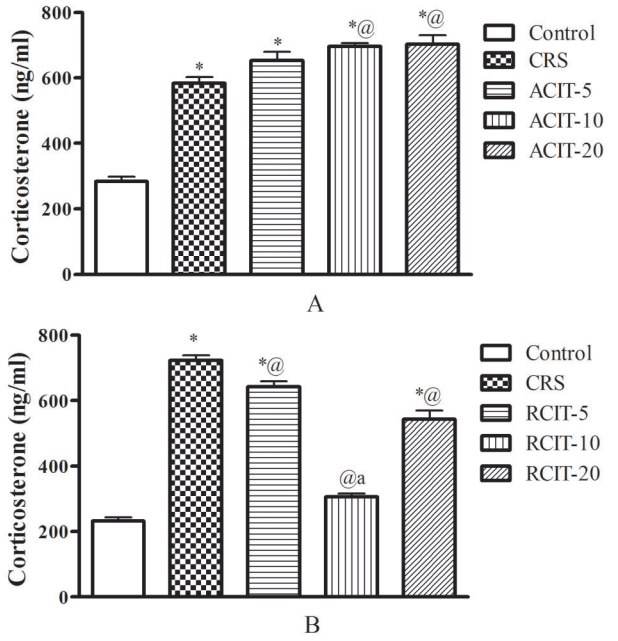

Fig. 2. Effect of single (**A**) and repeated (**B**) pretreatment with citalopram (5, 10 and 20 mg/kg) on plasma corticosterone level (ng/ml) in CRS model. Results in each column are expressed as the mean ± SEM (n = 6). * $p < 0.05$ and @ $p < 0.05$ compared to control and stress group, respectively (one way ANOVA followed by Tukey's test)

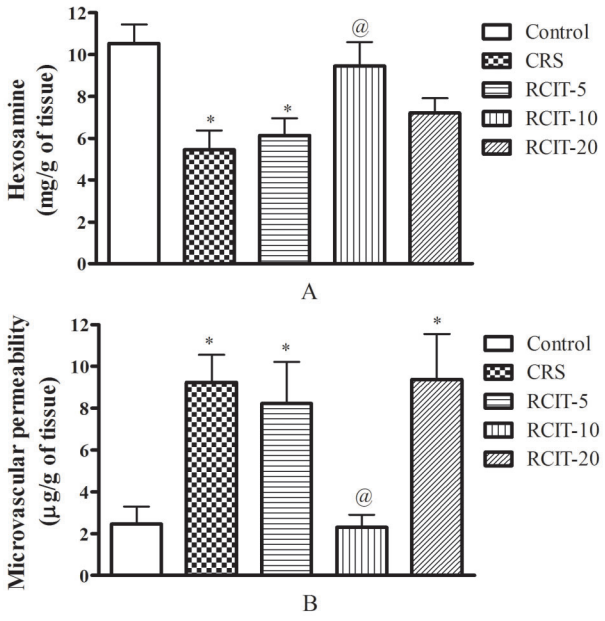

Fig. 3. Effect of repeated pretreatment with citalopram (5, 10 and 20 mg/kg) on hexosamine content (mucus marker) (**A**) and microvascular permeability (**B**) in CRS model. The results are expressed in each column as the mean ± SEM (n = 6). * $p < 0.05$ and @ $p < 0.05$ compared to control and stress group, respectively (one way ANOVA followed by Tukey's test)

Effect of repeated pretreatment with citalopram on hexosamine level in CRS model

The results of repeated oral pretreatment with citalopram on gastric hexosamine concentration in CRS model were analyzed by one-way ANOVA [$F(4,29) = 5.753$, $p < 0.005$] (Fig. 3A). In the present study, significant drop in the mucosal hexosamine content was observed in stress in contrast to the control group. Repeated pretreatment with citalopram reversed stress-induced changes in hexosamine level only at a dose of 10 mg/kg while other doses were found ineffective.

Effect of repeated pretreatment with citalopram on microvascular permeability in CRS model

Figure 3B represents the effect of repeated oral citalopram pretreatment on the microvascular permeability in CRS model. Significant differences in microvascular permeability were found among various groups as illustrated by one-way ANOVA [$F(4,29) = 5.785$, $p < 0.001$]. The amount of Evans blue extracted from gastric mucosa was found to be significantly higher in the stress group than obtained from the control and repeatedly citalopram (10 mg/kg) pretreated group (RCIT-10). Thus, stress-induced increase in microvascular permeability was significantly attenuated by pretreatment with 10 mg/kg citalopram while other doses were found ineffective.

Effect of L-NAME on gastroprotection offered by repeated citalopram administration on CRS-induced gastric mucosal lesions

The effect of L-NAME pretreatment on the gastroprotective effect of citalopram was studied and the results are summarized in Figure 4A. Significant differences in ulcer index among various group were observed from one-way ANOVA [$F(8,53) = 53.90$; $p < 0.0001$]. Citalopram at doses of 5, 10 and 20 mg/kg, *po* significantly attenuated the CRS-induced mucosal lesions. Pretreatment with L-NAME (25 mg/kg, *ip*) reduced the gastric mucosal protective effect of citalopram at all the doses. The extent of gastric injury in rat group treated with L-NAME followed by CRS was greater than the group exposed to CRS alone.

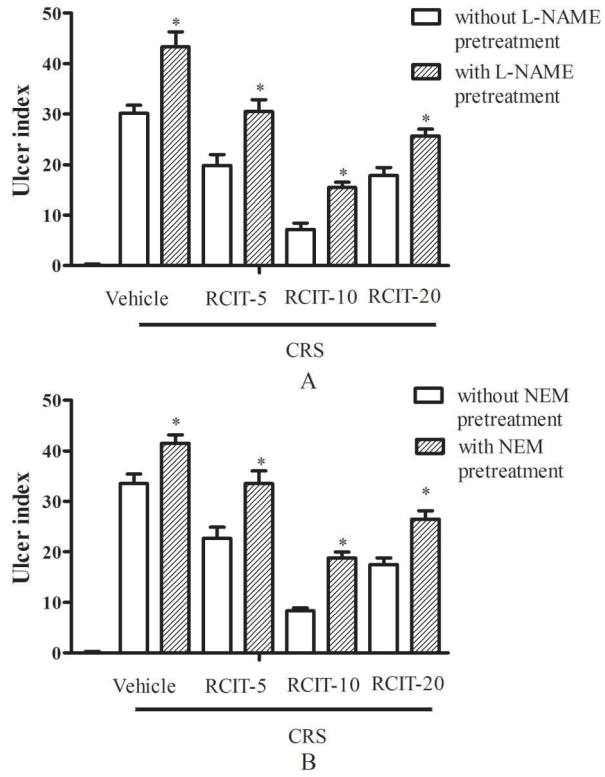

Fig. 4. Effect of pretreatment with L-NAME (**A**) and NEM (**B**) on the gastroprotection produced by citalopram in rats (n = 6) subjected to CRS-induced gastric ulceration. The results are presented as the mean ± SEM. * $p < 0.05$ compared to corresponding vehicle/citalopram (RCIT-5, RCIT-10, RCIT-20) treated group (one way ANOVA followed by Tukey's test)

Effect of repeated pretreatment with citalopram on nitrite in CRS model

Table 4 represents the effect of repeated citalopram pretreatment on nitrite level. One-way ANOVA revealed that there were significant differences in nitrite level among various groups [$F(4,29) = 34.82$; $p < 0.0001$]. It was observed that stress had increased the nitrite level significantly and citalopram pretreatment attenuated the increased nitrite level at all the doses.

Effect of NEM on gastroprotection offered by repeated citalopram administration on CRS-induced gastric mucosal lesions

Figure 4B elucidates the effect of NEM pretreatment on the gastroprotective effect obtained by repeated citalopram administration against CRS-induced gastric mucosal lesions. The results were analyzed by one-way ANOVA [$F(8,53) = 64.05$; $p < 0.0001$]. Significantly, attenuation of CRS-induced mucosal lesions was found by repeated citalopram administration at doses of 5, 10 and 20 mg/kg. Pretreatment with NEM (10 mg/kg, *po*) was found to reduce the gastric mucosal protective effect of citalopram at all the doses. Group pretreated with NEM followed by CRS was showing more injury as compared to that of CRS alone.

Tab. 4. Effect of repeated treatment with citalopram (5, 10 and 20 mg/kg, *po*) (RCIT-5, RCIT-10 and RCIT-20) on LPO and nitrite in the CRS model

Groups	Dose (mg/kg)	LPO (nmole/mg of protein)	Nitrite (nmole/mg of protein)
Control	vehicle	54.27 ± 3.36	11.05 ± 0.87
CRS	vehicle	91.91 ± 3.14*	32.64 ± 2.44*
RCIT-5	5	76.02 ± 3.05*@	22.10 ± 1.08*@
RCIT-10	10	57.31 ± 3.06@a	17.23 ± 0.90*@
RCIT-20	20	70.03 ± 2.89*@	18.11 ± 0.68*@

Results in each column are expressed as the mean ± SEM (n = 6). * $p < 0.05$, @ $p < 0.05$ and a $p < 0.05$ compared to control, stress group and CIT-5, respectively (one way ANOVA followed by Tukey's test)

Effect of repeated pretreatment with citalopram on gastric lipid peroxide level in CRS model

Table 4 demonstrates the effect of repeated citalopram pretreatment on lipid peroxide level in terms of MDA. One-way ANOVA revealed significant differences in MDA level among various groups [$F(4,29) = 23.98$; $p < 0.0001$]. Stress did increase lipid peroxide level in stomach significantly while citalopram treatment effectively attenuated the CRS-induced increase in lipid peroxidation at all the doses.

Effect of glibenclamide on gastroprotection offered by repeated citalopram administration on CRS-induced gastric mucosal lesions

Figure 5A depicts the effect of repeated administration of citalopram on CRS-induced lesions in rats pretreated with glibenclamide. The results were analyzed with one-way ANOVA which demonstrated significant differences in gastric ulcer among various groups [$F(8,53) = 61.85$; $p < 0.0001$]. Pretreatment with K_{ATP} channel blocker, glibenclamide (10 mg/kg, *ip*), significantly reduced the gastroprotection produced by

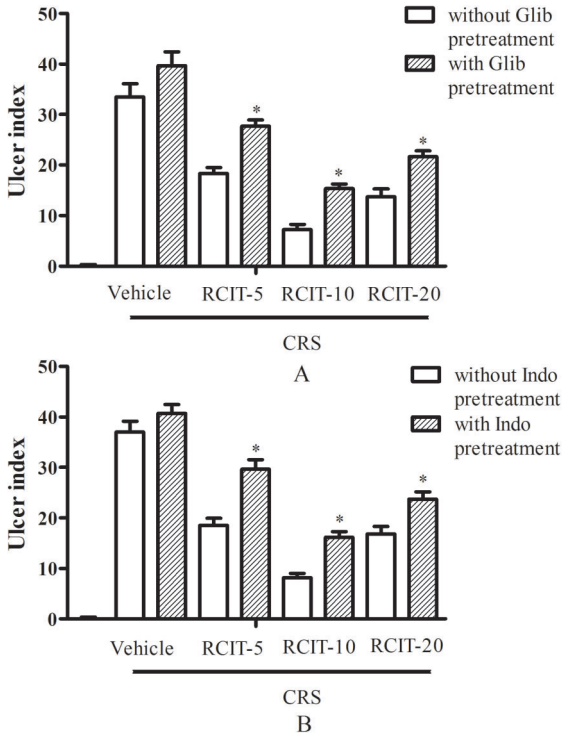

Fig. 5. Effect of pretreatment with glibenclamide (Glib) (**A**) and indomethacin (Indo) (**B**) on the gastroprotection produced by citalopram in rats (n = 6) subjected to CRS-induced gastric ulceration. The results are presented as the mean ± SEM. * $p < 0.05$ compared to corresponding vehicle/citalopram (RCIT-5, RCIT-10, RCIT-20) treated group (one way ANOVA followed by Tukey's test)

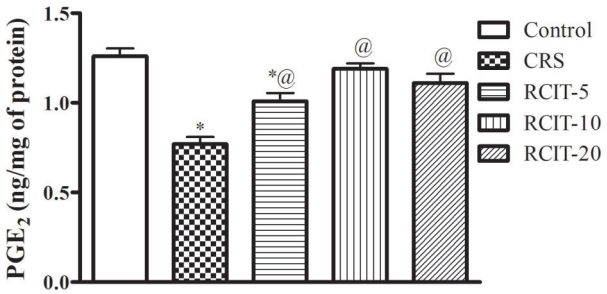

Fig. 6. Effect of repeated pretreatment with citalopram (5, 10 and 20 mg/kg) on PGE_2 level in CRS model. The results are expressed in each column as the mean ± SEM (n = 6). * $p < 0.05$ and @ $p < 0.05$ compared to control and stress group, respectively (one way ANOVA followed by Tukey's test)

citalopram. On the contrary, no significant difference in gastric damage has been observed between rats pretreated with glibenclamide followed by CRS and the CRS control animals.

Effect of repeated citalopram administration on gastric lesions induced by CRS in rats pretreated with indomethacin

The effect of pretreatment with indomethacin on the gastroprotective effect of citalopram was studied and the results were analyzed by one-way ANOVA (Fig. 5B). Significant differences were observed in ulcer index among the groups [$F(8,53) = 78.26$; $p < 0.0001$]. Citalopram at doses of 5, 10 and 20 mg/kg, significantly attenuated the CRS-induced mucosal lesions. Pretreatment with indomethacin (10 mg/kg, *po*) reduced the gastric mucosal protective effect of citalopram at all the doses. The gastric injury shown in the group pretreated with indomethacine followed by CRS and CRS group was insignificantly different.

Effect of repeated pretreatment with citalopram on gastric PGE_2 in CRS model

Figure 6 illustrates the effect of repeated oral pretreatment with citalopram on the concentration of gastric mucosal PGE_2 in CRS model. One-way ANOVA [$F(4,29) = 19.56$, $p < 0.001$] showed that the mucosal PGE_2 content was decreased in CRS group. The level of PGE_2 in gastric mucus was found to be increased by repeated pretreatment with citalopram at all the doses.

Discussion

The present investigation deals with the evaluation of gastroprotective effect of citalopram against CRS and PL models in rats. This study also explored the mechanism underlying gastroprotective activity of citalopram by evaluating corticosterone, NO, GSH, PGs, K_{ATP} channels, hexosamine, microvascular permeability and lipid peroxidation.

In CRS model, the single citalopram treatment was found to be ineffective. Therefore, preliminary antiulcer activity of 7, 14, 21 and 28 days repeated citalopram administration was conducted in CRS model. It was found that the repeated treatment for 14 days was most effective. However, treatment for 21 and 28 days did not lead to any further increase in the gastroprotective activity significantly (unpublished data). Therefore, 14 days treatment schedule was followed for the experiments in the present study.

Citalopram showed bell shaped dose independent anti-ulcer effect against CRS, i.e., an increase in dose from 5 to 10 mg/kg enhanced the gastroprotective activity while further increase in the dose from 10 mg/kg to 20 mg/kg decreased the gastroprotective activity. Thus, citalopram proves to be maximally effective for stress ulcer at the dose of 10 mg/kg. The results obtained in histopathological analysis had suggested that CRS caused gastric mucosal injuries characterized by epithelial cell loss and ruptured gastric mucosal layer. Pretreatment with citalopram (10 mg/kg) was able to inhibit such alterations up to the control, while other doses (5 and 20 mg/kg) mitigated the alteration due to stress but not up to the control.

Citalopram did not decreased ulcer index but it increased the gastric acidity and volume in PL model. PL model is mainly used for evaluating anti-ulcer activity of drugs which are effective in decreasing the local acid and pepsin secretion. There are certain reports which show that SSRI like fluoxetine also increases the acid secretion through vagal stimulation [41]. However, fluoxetine has been reported to produce gastroprotective action against stress ulcer in spite of its acid secretion stimulating effect [17]. Similarly, in spite of increased gastric acidity and volume, citalopram did not aggravate the ulcer formation in PL model instead produced gastroprotective action against stress ulcer. Thus, it can be postulated that this increase in acid secretion is not sufficient enough to cause significant gastric erosion.

Stress causes allostasis in which there is continuous effort of the body to maintain bodily functions. However, too much stress or insufficient management of allostasis leads to allostatic load [25]. Gastric erosion is one of the outcomes of allostatic load. CRS is commonly used model for evaluating anti-ulcer activity of drugs which act by virtue of their central anti-stress effect [11]. CRS model creates an acute stress with the activation of hypothalamic-pituitary-adrenocortical (HPA) axis. Activation of HPA axis, in addition to the production of gastric injury, also results in secretion of corticosterone through adrenal gland. The results of present study also show that CRS had produced gastric injury and increased plasma corticosterone level.

Central serotonin has a putative role in the coordination of HPA axis activity under basal and stressed conditions [10, 26]. Serotonergic dysfunction is associated with hypercortisolemia, i.e., altered basal HPA axis activity [37, 21]. SSRI treatment can alter the activity of the HPA axis [24]. Present results showed that single pretreatment with citalopram augmented the CRS-induced increase in corticosterone level and 14 days repeated pretreatment with citalopram mitigated the CRS-induced increase in corticosterone level. These results are in agreement with the reported results [24]. Thus, it can be postulated that the single administration of citalopram aggravated the stress-induced hyperactive HPA axis while repeated administration of citalopram attenuated the stress-induced hyperactive HPA axis. The attenuation of stress-induced hyperactive HPA axis by repeated pretreatment with citalopram might be due to re-establishment of the feedback control. This is in consonant with the report of Maes et al. [36]. SSRIs re-establish the feedback control by increasing the mineralocorticoid receptor levels principally in the hippocampus to normalize the hyperactive HPA axis [42]. The repeated citalopram pretreatment showed bell shaped dose independent effect on corticosterone level. This pattern is similar to that of antiulcer effect of citalopram. Therefore, it is concluded from the results that gastroprotective activity of citalopram is due to its central anti-stress effect.

Gastric mucosa can enhance resistance to injury after exposure to repeated insults of noxious agents, such as aspirin, alcohol, stress or *H. pylori*-related gastrotoxins. This phenomenon is called gastric adaptation [30] or allostatic systems. Excessive stress may bring about a number of cellular adaptations in which an altered steady state is achieved. Further, there is a report that suggests that the hypersensitivity of the 5-HT system may be related to the neuronal mechanism of stress adaptation [39]. Additionally, exaggerated feedback control over raphe-hippocampal serotonin neurotransmission in restrained rats results in behavioral deficits [27]. Therefore, it can be postulated that the 14 days citalopram pretreatment promotes adaptation to stress.

HPA has an important role in the maintenance of homeostasis and serves as a neuroendocrine stress response system [7]. The HPA axis is regulated at the central level by the paraventricular nucleus (PVN). In addition to corticosterone secretion, stress-induced stimulation of PVN decreases gastric mucosal blood flow. This results in ischemia which leads to free radical generation. The resulting free radicals cause oxidative damage and ultimately results in ulcer formation [4]. Thus, the primary factor in CRS-induced gastric lesions is generation of free radicals. In the present study, the lipid peroxidation increased after

stress exposure. This is in agreement with earlier report [4]. Repeated pretreatment with citalopram decreased the lipid peroxidation at all the doses. Therefore, repeated citalopram pretreatment decreased the CRS-induced free radical generation.

SH plays many important roles in protecting the cell. Firstly, it limits the production of free radicals and thus protects the cell against stress exposure [31]. Secondly, it protects the mucus to unite its subunits by disulfide bridges. If these bridges are reduced, the mucus becomes more soluble and is more prone to harmful agents [6]. On that basis, the animals were pretreated with NEM, an SH inhibitor, to evaluate the interference of this protection mechanism in the citalopram action. The results showed that the gastroprotective activity of citalopram is reduced by NEM pretreatment. This study demonstrated that the SH inhibition decomposed the mucus structure and that without mucus, the gastroprotective effect of citalopram decreased.

Several studies have suggested that vasodilation results in enhancement of the blood flow. Therefore, vasodilation is important in the maintenance of gastric integrity and removing irritant to prevent the activation of inflammatory factors [51]. NO, a potent vasodilator agent, is produced by NO synthase (NOS). NO appears to maintain the integrity of the gastric epithelium by accelerating the gastric ulcer healing. It is also involved in regulating the gastric mucosal blood flow and stimulating synthesis as well as secretion of mucus [32]. In the present investigation, the role of NO in the gastroprotective activity of citalopram was evaluated by using L-NAME. L-NAME is a nonspecific NOS inhibitor. L-NAME pretreatment decreased the gastroprotective activity of citalopram against CRS. It can be predicted from the results that the NO production by NOS was decreased in stress group. This decrease in NO level caused ulcer formation. Citalopram restored the stress induced decrease in NO level and therefore, offered gastroprotective activity against stress. L-NAME pretreatment inhibited the restoration of stress-induced decrease in NO level by citalopram. Therefore, it reduced the gastroprotective activity of citalopram. Thus, it can be concluded that NO plays a significant role in the gastroprotective activity of citalopram.

There are two types of NOS inhibitors that are cNOS (constitutive) and iNOS (inducible). Pharmacological studies proposed that NO produced by Ca^{2+}-dependent cNOS is cytoprotective, whereas NO produced by Ca^{2+}-independent iNOS is cytotoxic. In normal gastric physiology cNOS is mainly active. Therefore, L-NAME pretreatment before stress exposure aggravated the ulcer formation due to inhibition of cytoprotective NO produced by cNOS. Stress results in drastic increase of iNOS activity. iNOS activation results in increased production of cytotoxic NO. This increased cytotoxic NO level leads to ulcer formation. This describes the increased gastric NO level in terms of nitrite in stress on direct measurement. Citalopram repeated pretreatment attenuated the stress-induced increase in nitrite level. Thus, above findings explain the contradictory results found in L-NAME pretreatment and direct gastric nitrite level measurement studies. Similar kind of work with L-arginine, a precursor for NO formation, was earlier reported by Nishida et al. [38].

Vagal and humoral stimulation results in the generation of prostaglandins (PG) of E and I series throughout the gastrointestinal tract and are released into the gut lumen. Endogenous PGs are involved in the maintenance of mucosal integrity and blood flow. PGs also protect mucosa against potentially noxious agents. Moreover, PGs seem to stimulate the secretion of bicarbonate and mucus, inhibit the acid secretion, as well as regulate the mucosal cell turnover and repair in the stomach mucosa [45]. Gastric mucosa of ulcer patients tends to generate smaller amounts of PGs of E and I series. This suggests that the insufficient production of protective PGs may play a primary role in the pathogenesis of gastric ulcer [30]. Therefore, in order to investigate the role of PGs in the gastroprotection offered by citalopram, rats were pretreated with indomethacin and then subjected to CRS. Indomethacin is a non-selective cyclooxygenase (COX) inhibitor. The results showed that pretreatment with indomethacin attenuated the protection afforded by citalopram against CRS. The results obtained in this experiment suggest that PGs possibly participate in the gastroprotective effect of citalopram. The involvement of PG in the antiulcer activity of citalopram was further confirmed by direct measurement of the gastric PGE_2 level in control, CRS and citalopram pretreated CRS groups. The results showed that CRS decreased the PGE_2 level in gastric mucosa to that of control, whereas citalopram repeated pretreatment increased the PGE_2 level at all the doses.

Endogenous PG acts as an activator of K_{ATP} channels [43] which are a class of ligand-gated proteins. K_{ATP} channels play important roles in variety of physi-

ologic functions of the stomach, such as regulation of gastric blood flow, acid secretion and stomach contractility [18]. The results of present investigation suggest that the gastroprotection mechanism of citalopram was K_{ATP} channel-dependent, since citalopram gastroprotective effects were reverted by pretreatment with glibenclamide which is a potent antagonist of these channels. Thus, K_{ATP} channels play a major role in the gastroprotective effects of citalopram.

It is known that the mucus layer protects the gastric lining from the continuous gastric juice secreted from the parietal cell. Stress can cause the weakening of this defensive mucosal barrier by decreasing the mucus content, which may also lead to the exposure of stomach to assault of ulcerogenesis and the development of gastric ulcers. Hexosamine is a marker of mucus content. In the present study, stress was found to decrease the hexosamine level. Repeated citalopram (10 mg/kg) treatment increased the mucus secretion and thus strengthened the mucosal defense. Thus, citalopram reverses the imbalance of offensive and defensive factors by promoting mucus secretion. Histopathological study also revealed that mucosal barrier was disrupted in the case of CRS. Pretreatment with citalopram (10 mg/kg) restored the intactness of mucosal barrier up to the control while other two doses were found to be ineffective. This may be because 5 and 20 mg/kg do not have enough gastroprotective effect to restore the CRS-induced decrease in mucus content.

Ischemia is a major determinant of stress ulcer [35]. The injury process is aggravated further by reperfusion of ischemic tissues causing release of pro-inflammatory mediators and chemotaxis of inflammatory cells [8]. This leads to mucosal inflammation which increases the microvascular permeability. The same has been found in the present study. The microvascular permeability in stomach was significantly increased by CRS suggesting mucosal inflammation. Microvascular permeability was found to be significantly decreased with citalopram in 10 mg/kg repeated dose. Thus, by reducing inflammation at the ulcer site, the aggravation of tissue injury can be prevented and ulcers can be healed by citalopram. Gastric mucosal injuries caused by CRS were further demonstrated by histopathological studies characterized by epithelial cell loss and ruptured gastric mucosal layer. CRS induced alterations in gastric physiology was inhibited near to control by pretreatment with citalopram (10 mg/kg). However, the lower dose (5 mg/kg) and higher dose (20 mg/kg) mitigated the alteration due to stress but not up to the control. Similar to the hexosamine, doses of 5 and 20 mg/kg of citalopram do not have enough gastroprotective effect to restore the gastric physiology to that of the control. Citalopram gastroprotective activity involves PGE_2 which are known to increase mucus content and mucosal blood flow. An increase in blood flow decreases the ischemic reperfusion injury, which in turn causes decrease in the microvascular permeability. Hence, citalopram *via* PGE_2 significantly mitigates CRS-induced increase in microvascular permeability and decrease in hexosamine level.

SSRI drugs lead to the impairment in the platelet hemostasis by blocking the uptake of serotonin into platelets. This can increase the risk of abnormal bleeding [3]. The clinical use of citalopram in depressive patients is associated with gastrointestinal bleeding and perforations [3]. However, it has also been found that bleeding will not develop immediately upon the initiation of SSRI therapy even if SSRIs do increase the gastric acidity [3]. Further, bleeding will not occur until primary pathology develops. Upper GI bleeding occurs after 25 weeks of administration of SSRIs [33]. Moreover, in light of clinically observed side effects, prolonged (upto 4 weeks) treatments with citalopram without CRS exposure were carried out. No significant change in gastric physiology occurred after 4 weeks (unpublished data). Thus, citalopram did not increase the risk of abnormal bleeding after 4 weeks of administration.

Conclusion

The results of the present study demonstrate that CRS stimulates the HPA axis, which in turn increases corticosterone release in blood through adrenal gland. HPA has an important role in the maintenance of homeostatsis. The HPA axis is regulated at the central level by PVN. In addition to corticosterone secretion, stimulation of PVN by stress causes a decrease in gastric mucosal blood flow. This results in ischemia which leads to free radicals generation. The resulting free radicals cause oxidative damage and ultimately result in ulcer formation. CRS also causes the gastric damage by inhibiting cytoprotective SH, PG, NO syn-

thesis and altering the opening of K_{ATP} channels. In CRS, the defensive mucus secretion attenuated while microvascular permeability increased due to inflammation. Repeated administration of citalopram for 14 days normalizes the basal hyperactive HPA axis to stress and thus promotes adaptation to stress. This may be *via* re-establishment of the feedback control. The gastric adaptation to stress appears to be mediated by endogenous SH, PGE_2, K_{ATP} channel opening and NO synthesis. Moreover, citalopram decreases free radicals generation which decreases lipid peroxidation. Citalopram, by decreasing stress-induced increase in microvascular permeability, can mitigate inflammation-induced exacerbation of gastric ulcers. Citalopram also augments the mucus, a defensive barrier. This shows that citalopram interferes at several levels of stress-induced ulcer pathogenesis pathways and therefore, can be a potential drug for the treatment of stress ulcer.

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