PHARMACOLOGIA

Impact of the Dopaminergic System on Mucosal Integrity in Indomethacin-induced Gastric Ulcers in Rats: Possible Modulation by Ranitidine or L-carnitine

[2]Sally A. El-Awdan, [1]Hala F. Zaki, [2]Omar M. Abdel Salam, [2]Wafaa I. El-Iraqy and [1]Sanaa A. Kenawy

[1]Department of Pharmacology, Faculty of Pharmacy, Cairo University, Egypt
[2]Department of Pharmacology, National Research Centre, Dokki, Cairo, Egypt

ABSTRACT

The present study aim to explore the impact of bromocriptine, or ziprasidone on rats mucosal integrity and the possible modulation by L-carnitine or ranitidine. Adult male Wistar rats were divided into 10 groups: the 1st and 2nd groups received 1% Tween 80 and served as normal and control groups, respectively. The remaining groups were treated as follows: 3-6 received bromocriptine (2.5 mg kg^{-1}), L-carnitine (50 mg kg^{-1}), ranitidine (50 mg kg^{-1}) or ziprasidone (3 mg kg^{-1}), respectively. Groups 7-10 received combinations of bromocriptine or ziprasidone with either L-carnitine or ranitidine. Drugs were daily administered (p.o.) for two weeks then all groups were subjected to pyloric ligation. Indomethacin (30 mg kg^{-1}; p.o.) was immediately administered to all groups except the normal one. Rats were euthanized 4 h thereafter and stomachs were opened to evaluate the number and severity of the lesions. Gastric volume, acid output, peptic activity as well as mucin and gastric mucus concentrations were determined. Moreover, stomach content of lipid peroxides, reduced glutathione, nitric oxide metabolites and tumor necrosis factor were also estimated. Indomethacin administration resulted in increased ulcer index and severity that was coupled by increased titratable acidity, acid output and disturbance in antioxidant status. Such effects were intensified by ziprasidone and ameliorated by bromocriptine, L-carnitine or ranitidine. Moreover, administration of L-carnitine or ranitidine with ziprasidone attenuated its damaging effect. In conclusion, dopamine agonists play a significant role in maintenance of gastric mucosal integrity; meanwhile, dopamine antagonists complicate indomethacin-induced ulcers, an effect that could be mitigated by combined treatment with L-carnitine or ranitidine.

Key words: Ziprasidone, L-carnitine, ranitidine, indomethacin-induced ulcer, oxidative stress

INTRODUCTION

Peptic ulcer diseases affect a large portion of the population and are induced by several factors, including stress, smoking, nutritional deficiencies and administration of non-steroidal anti-inflammatory drugs (NSAIDs) (Mafertheiner *et al.*, 2009).

The pathophysiology of peptic ulcer diseases has centred on an imbalance between aggressive and protective factors. Two main approaches for treatment of peptic ulcer exist; the first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection (Hoogerwerf and Pasricha, 2001).

Indomethacin, a representative of NSAIDs, causes gastric ulcers through various processes, including generation of Reactive Oxygen Species (ROS), initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis and inhibition of prostaglandin and nitric oxide (NO) synthesis (Vananenn *et al.*, 1991; Beck *et al.*, 2000).

Ziprasidone is an atypical antipsychotic drug with a benzisothiazolyl structure that acts by blocking dopamine receptors (Nemeroff *et al.*, 2005). Current literature suggests a modulating role for dopamine (DA) agonists and antagonists on peptic ulcer (Rashid *et al.*, 2010). Administration of DA antagonists caused aggravation of gastric mucosal lesions whereas a strong protection was observed after the administration of DA agonists as bromocriptine (Thorner, 1975).

Consequently, it seemed of importance to study the effect of DA related drugs on experimentally-induced gastric ulcer and to compare such effects with ranitidine as a standard by measuring gastric acidity and gastric

Corresponding Author: Sally Wanees El-Awdan, Department of Pharmacology, National Research Center, El-Bohoth St., Dokki, PO: 12622, Cairo, Egypt Tel: +202-3335963(NRC), +202-0106235761

mucin, mucus content and peptic activity. In addition, as oxidative stress plays a role in a wide variety of pathological conditions, particularly peptic ulcer, it seemed of importance to study the role of oxidative stress in the present model as well and to compare the effects of ziprasidone and bromocriptine with that of L-carnitine, a known potent antioxidant.

MATERIALS AND METHODS

Animals: Adult male albino Wistar rats, weighing 120-150 g, were used in the present study. They were obtained from the animal house colony of the National Research Center (Giza, Egypt). Standard food pellets and water were supplied *ad libitum*. All animals' procedures were performed in accordance with the recommendations for the proper care and use of laboratory animals. The study was approved by the ethics committee of Faculty of Pharmacy, Cairo University.

Drugs: Ziprasidone was purchased from Pfizer (USA), bromocriptine from Delta Pharma (Egypt), L-carnitine from Mepaco (Egypt) and ranitidine from Sigma (USA). All test drugs were freshly prepared in 1% Tween 80 and orally administered. Doses of the drugs were selected from preliminary studies.

Chemicals: Alcian blue, bovine serum albumin, Ellman's reagent and N-(1-naphthyl) ethylendiamine dihyrochloride were purchased from Sigma (USA). Kits for total antioxidant capacity and tumor necrosis factor (TNF-α) were purchased from Biosource kits, (USA) and Invitrogen Corporation (USA), respectively.

Experimental design: Animals were classified such that the 1st and 2nd groups received 1% Tween 80 and served as normal and control groups respectively; whereas the remaining groups were treated as follows: groups 3-6 received bromocriptine (2.5 mg kg^{-1}), L-carnitine (50 mg kg^{-1}), ranitidine (50 mg kg^{-1}) or ziprasidone (3 mg kg^{-1}), respectively. Groups 7-10 received combinations of bromocriptine or ziprasidone with either L-carnitine or ranitidine.

Methods

Induction of gastric ulcer: Pyloric ligation was carried out according to the method described by Shay *et al.* (1945). Indomethacin (30 mg kg^{-1}) was orally administered immediately after pyloric ligation. Four hours later, the animals were euthanized by cervical dislocation under ether anesthesia. The abdominal cavity was opened and the stomach was removed. An opening was then made along the greater curvature and the gastric juice was collected. Stomach mucosa was examined for mucosal necrotic lesions, red streaks and red erosions

(Mozsik *et al.*, 1982). Total lesions number was counted and the severity of lesions was determined based on the following scores:

0 = No ulcer
1 = Lesion size ≤than 1 mm
2 = Lesion of size 1-2 mm
3 = Lesion of size 2-3 mm
4 = Lesion of size 3-4 mm
5 = Lesion of size > 4 mm

Determination of peptic ulcer biomarkers and mucin content: Titratable acidity (mEq L^{-1}) was determined according to the method described by Grossman (1963). A fixed amount of the supernatant of gastric secretion was titrated with 0.01 N sodium hydroxide using phenolphthalein as indicator.

Acid output was calculated as μEq/4 h according to the method described by Brodie and Hooke (1971) by multiplying the volume of the gastric secretion by the titratable acidity in mEq L^{-1}.

Peptic activity of gastric juice was determined according to the method described by Sanyal *et al.* (1971). The method depends on the fact that peptic activity is a major factor involved in the proteolytic activity of the gastric secretion. Mucus concentration was determined according to the method described by Corne *et al.* (1974). Briefly, the glandular portion of the stomach was excised, weighed and immersed for 2 h in 10 mL of 0.1% w/v Alcian blue dissolved in 0.16 mol L^{-1} sucrose solution buffered to pH 5.8 with 0.05 mol L^{-1} sodium acetate and HCL. The excess dye was removed by two successive rinses, 15 min each, in 0.25 mol L^{-1} sucrose.

The mucus-bound dye was extracted by immersing the gastric tissue in 0.5 mol L^{-1} MgCl$_2$ solution, which was intermittently shaken for 1 min every 30 min intervals for 2 h. The obtained blue extract was shaken with equal volume of diethyl ether for 10 min and the resulting emulsion was centrifuged at 3600 rpm for 10 min.

Mucin was determined according to the methods described by Winzler (1955). The method is based on the determination of the hexose component of mucin. It depends on the reaction of carbohydrate in concentrated sulphuric acid with orcinol (5-methyl resorcinol) to give a colored product, which can be measured colorimetrically at 425 nm.

Determination of oxidative stress and inflammatory markers: Lipid peroxides were determined according to the method described by Uchiyama and Mishara (1978) and expressed as nmol g^{-1} wet tissue. Lipid peroxidation products were estimated by the determination of the level of TBARS that were

measured as malondialdehyde (MDA). The latter is the decomposition product of the process of lipid peroxidation and is used as an indicator of this process. The principle of the assay depends on the colorimetric determination of a pink pigment product, resulting from the reaction of TBARS with thiobarbituric acid (TBA) in an acidic medium, at high temperature. Reduced glutathione (GSH) content was determined in stomach homogenates according to the method of Beutler *et al.* (1963) and expressed as mg g^{-1} wet tissue. The method depends on the fact that both protein and non-protein thiol (SH-) groups (mainly GSH) react with Ellman's reagent [5,5'-dithiobis (2-nitrobenzoic acid)] to form a stable yellow color of 5-mercapto-2-nitrobenzoic acid, which can be measured colorimetrically at 412 nm. Stomach NO metabolites were determined according to the method described by Miranda *et al.* (2001) and expressed as M g^{-1} wet tissue. The assay determines total NOx content based on the reduction of any nitrate to nitrite by vanadium followed by the detection of total nitrite (intrinsic + nitrite obtained from reduction of nitrate) by Griess reagent. The Griess reaction leads to the formation of a chromophore from the diazotization of sulfanilamide by acidic nitrite followed by coupling with bicyclic amines such as N-(1-naphthyl) ethylenediamine. The chromophoric azo derivative can be measured colorimetrically at 540 nm.

Stomach total antioxidant capacity was determined according to the method described by Koracevic *et al.* (2001) and expressed as mM g^{-1} wet tissue. TNF-α was estimated using rat specific immunoassay kit (biosource rat TNF-α invitrogen Elisa kit, USA) according to the method of Tian *et al.* (2005) and expressed as pg g^{-1} wet tissue.

Preparation of samples for histological examination: The dissected stomachs were washed with saline and preserved in 10% formalin for histopathological studies. The prepared sections were stained with hematoxylin and eosin and used to assess the severity of histopathological changes such as congestion, edema and hemorrhage.

Statistical analysis: Values were expressed as Means±SE. Results of ulcer number and severity were analyzed using Kruskal-Wallis non-parametric one way analysis of variance (ANOVA) followed by Mann Whitney multiple comparisons test. The results of the remaining experiments were analyzed using one way ANOVA followed by Least Significant Difference (LSD) multiple comparisons test. p<0.05 was accepted as being significant in all types of statistical tests. Statistical analysis of results, were done using software SPSS 17.

RESULTS

Ulcer number and severity were significantly increased in the indomethacin-induced ulcer model and were further elevated by ziprasidone administration. Bromocriptine, L-carnitine and ranitidine significantly decreased the ulcer number and severity. Moreover, the combination of ziprasidone with either L-carnitine or ranitidine decreased the ulcer number and severity when compared to control and ziprasidone treated groups (Fig. 1, 2).

Administration of ziprasidone resulted in a significant increase in gastric acid volume, titratable acidity and acid output by 79.40, 32.28 and 129.41%, respectively when compared to control group. Such effects were not prevented by co-administration of either L-carnitine or ranitidine (Table 1).

Administration of ziprasidone significantly decreased gastric mucus content by 52.56% when compared to the normal group; an effect that was reversed by co-administration of L-carnitine or ranitidine (Fig. 3).

Administration of ziprasidone significantly increased gastric peptic activity when compared to the normal group and the control ulcer groups; an effect that was reversed by co-administration of L-carnitine or ranitidine (Fig. 4).

Similarly, co-administration of L-carnitine or ranitidine with ziprasidone prevented its induced depletion of mucin content (Fig. 5).

Gastric MDA content was increased in control and ziprasidone treated rats by 62.26 and 78.8%, respectively as compared to the normal group. Administration of bromocriptine, L-carnitine or ranitidine significantly reduced the elevated MDA content (Fig. 6).

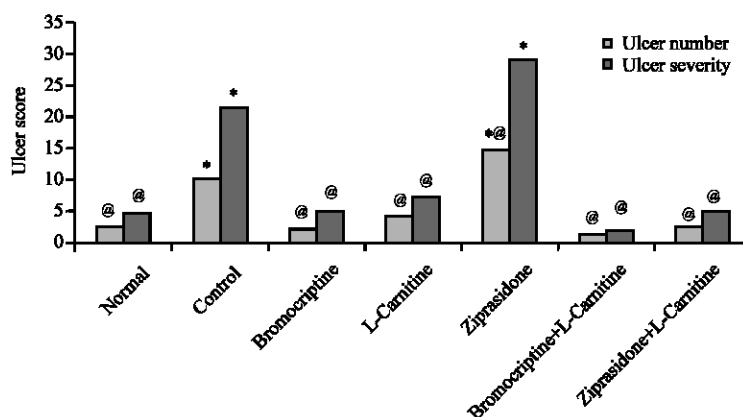Table 1: Effect of two weeks daily treatment with ziprasidone (3 mg kg^{-1}) or bromocriptine (2.5 mg kg^{-1}) alone or combined with L-carnitine (50 mg kg^{-1}) or ranitidine (50 mg kg^{-1}) on gastric juice volume, titratable acidity and acid output in indomethacin-induced gastric ulcer in pyloric-ligated rats

Groups	Gastric juice volume (mL)	Titratable acidity (mEq L^{-1})	Acid output (µEq/4 h)
Normal	3.37±0.24	78.16±6.04	258.56±34.39
Control	4.03±0.52	80.00±9.03	328.91±60.23
Bromocriptine	4.60±0.90	91.66±7.87	352.00±82.02
L-Carnitine	3.9 5±0.36	80.33±10.12	306.51±35.07
Ranitidine	3.13±0.48	78.83±4.77	259.83±57.41
Ziprasidone	7.23±0.53*	110.62±2.06*@	800.68±63.49*@
Bromocriptine+ L-Carnitine	4.96±1.07	87.88±9.86	366.66±70.30
Bromocriptine + Ranitidine	2.92±0.74	97.14±14.63	299.14±96.83
Ziprasidone + L-Carnitine	6.20±0.75*	99.28±2.58	680.20±79.68*@
Ziprasidone + Ranitidine	5.73±0.80*	99.00±8.26	634.63±112.40*@

Values are Means±SE of 6 rats. *p<0.05 vs normal group; @p<0.05 vs control group

Fig. 1: Effect of two weeks daily treatment with ziprasidone (3 mg kg^{-1}) or bromocriptine (2.5 mg kg^{-1}) alone or combined with L-carnitine (50 mg kg^{-1}) on indomethacin-induced gastric ulcer in pyloric-ligated rats. Values are Means±SE of 6 rats. *p<0.05 vs normal group; @ p<0.05 vs control group
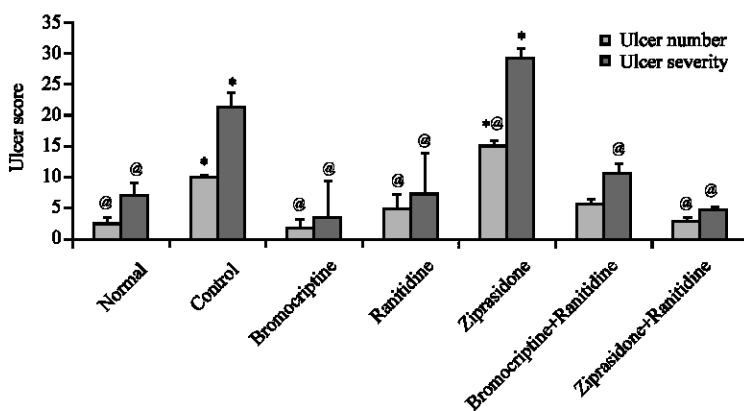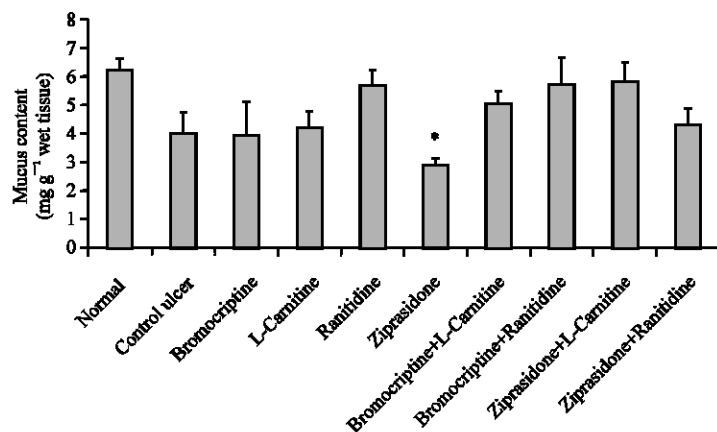

Fig. 2: Effect of two weeks daily treatment with ziprasidone (3 mg kg^{-1}) or bromocriptine (2.5 mg kg^{-1}) alone or combined with ranitidine (50 mg kg^{-1}) on indomethacin-induced gastric ulcer in pyloric-ligated rats. Values are Means±SE of 6 rats. *p<0.05 vs normal group; @p<0.05 vs control group

Fig. 3: Effect of two weeks daily treatment with ziprasidone (3 mg kg^{-1}) or bromocriptine (2.5 mg kg^{-1}) alone or combined with L-carnitine (50 mg kg^{-1}) or ranitidine (50 mg kg^{-1}) on gastric mucus content in indomethacin-induced gastric ulcer in pyloric-ligated rats. Values are Means±SE of 6 rats. *p<0.05 vs normal group

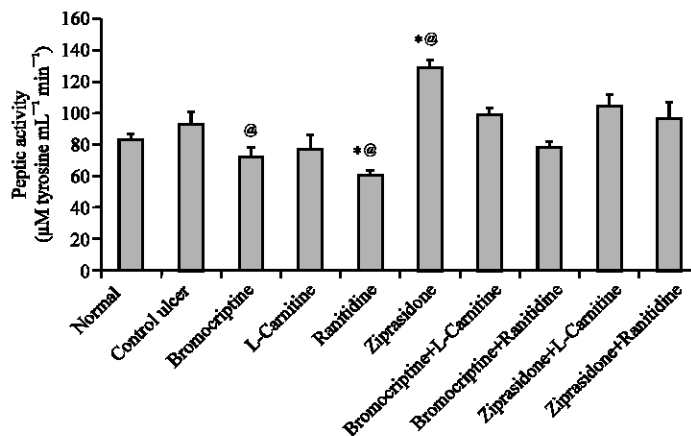Fig. 4: Effect of two weeks daily treatment with ziprasidone (3 mg kg^{-1}) or bromocriptine (2.5 mg kg^{-}) alone or combined with L-carnitine (50 mg kg^{-1}) or ranitidine (50 mg kg^{-1}) on gastric peptic activity in indomethacin-induced gastric ulcer in pyloric-ligated rats. Values are Means±SE of 6 rats. *$p < 0.05$ vs normal group; $^{@}p < 0.05$ vs control group
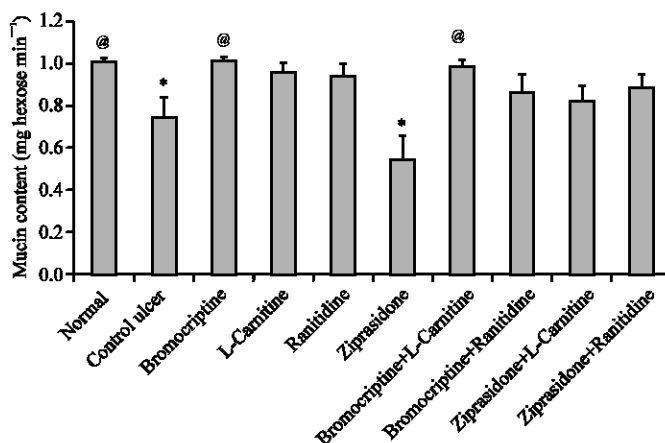

Fig. 5: Effect of two weeks daily treatment with ziprasidone (3 mg kg^{-1}) or bromocriptine (2.5 mg kg^{-1}) alone or combined with L-carnitine (50 mg kg^{-1}) or ranitidine (50 mg kg^{-1}) on gastric mucin content in indomethacin-induced gastric ulcer in pyloric-ligated rats. Values are Means±SE of 6 rats. *$p < 0.05$ vs normal group; $^{@}p < 0.05$ vs control group

Fig. 6: Effect of two weeks daily treatment with ziprasidone (3 mg kg^{-1}) or bromocriptine (2.5 mg kg^{-1}) alone or combined with L-carnitine (50 mg kg^{-1}) or ranitidine (50 mg kg^{-1}) on gastric mucosal malondialdehyde (MDA) content in indomethacin-induced gastric ulcer in pyloric-ligated rats. Values are Means±SE of 6 rats. *$p < 0.05$ vs normal group; $^{@}p < 0.05$ vs control group
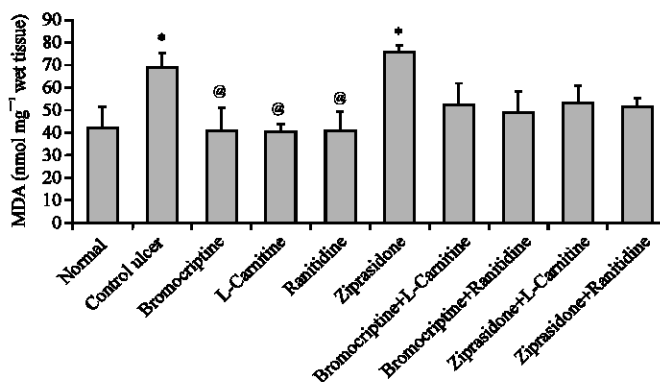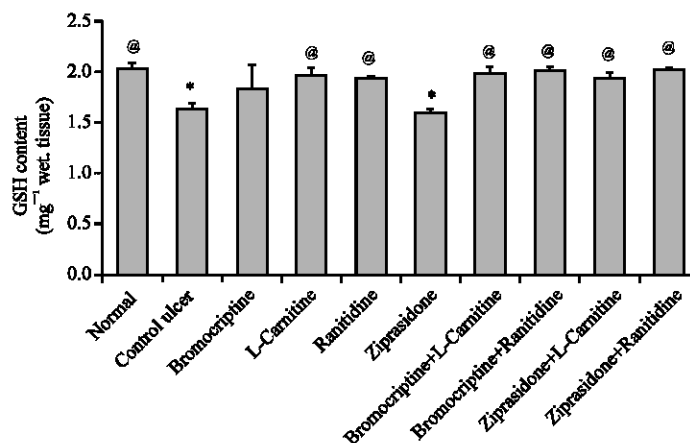
Fig. 7: Effect of two weeks daily treatment with ziprasidone (3 mg kg^{-1}) or bromocriptine (2.5 mg kg$^-$) alone or combined with L-carnitine (50 mg kg^{-1}) or ranitidine (50 mg kg^{-1}) on gastric reduced glutathione (GSH) content in indomethacin-induced gastric ulcer in pyloric-ligated rats. Values are Means±SE of 6 rats. *p < 0.05 vs normal group; @p < 0.05 vs control group

Fig. 8: Effect of two weeks daily treatment with ziprasidone (3 mg kg^{-1}) or bromocriptine (2.5 mg kg^{-1}) alone or combined with L-carnitine (50 mg kg^{-1}) or ranitidine (50 mg kg^{-1}) on gastric total nitrate/nitrite (NO$_x$) content in indomethacin-induced gastric ulcer in pyloric-ligated rats. Values are Means±SE of 6 rats. *p < 0.05 vs normal group; @p < 0.05 vs control group

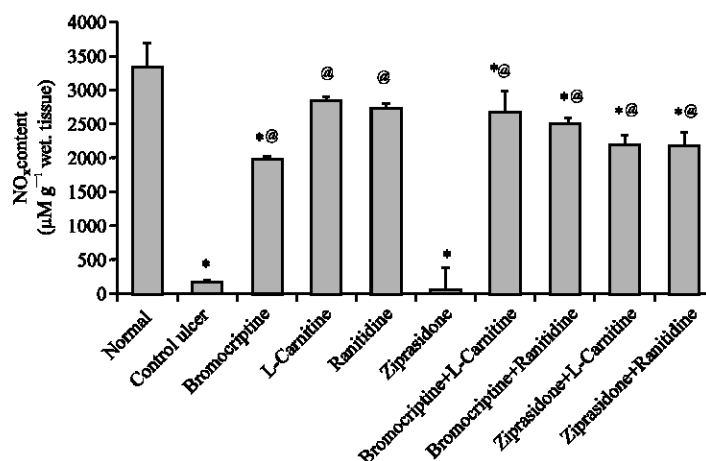Parallel to the observed increase in lipid peroxidation, GSH content was significantly depleted in both control and ziprasidone treated rats; such effect was reversed by co-administration of either L-carnitine or ranitidine (Fig. 7).

Similarly, induction of indomethacin ulcer significantly reduced gastric NOx content by 94.11%. This effect was prevented by pretreatment with bromocriptine, L-carnitine or ranitidine. Ziprasidone administration also reduced gastric NOx content, an effect that was prevented by co-administration of L-carnitine or ranitidine (Fig. 8).

Indomethacin-induced ulcer was accompanied by a significant decrease in the gastric total antioxidant capacity by 7.4% when compared to the normal group. Pretreatment with bromocriptine, L-carnitine or ranitidine or the combination of bromocriptine with L-carnitine or ranitidine prevented such effect (Fig. 9).

Pretreatment with ziprasidone resulted also in a significant decrease in gastric total antioxidant capacity which was not reversed by co-administration of L-carnitine or ranitidine (Fig. 9).

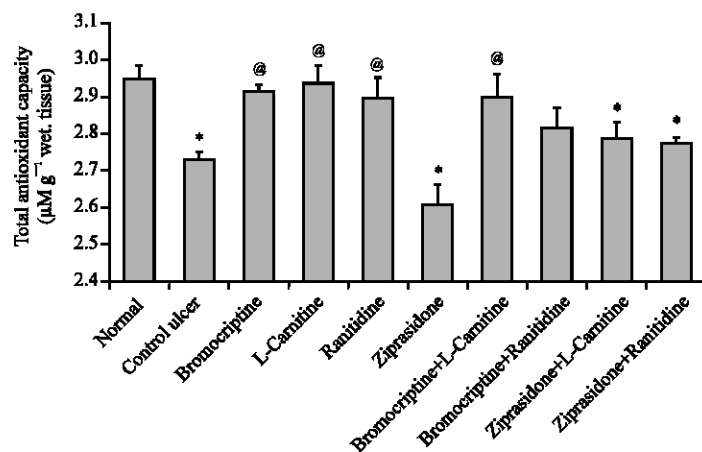Administration of ziprasidone resulted in a significant increase in the gastric TNF-α by 763.18% as

Fig. 9: Effect of two weeks daily treatment with ziprasidone (3 mg kg⁻¹) or bromocriptine (2.5 mg kg⁻¹) alone or combined with L-carnitine (50 mg kg⁻¹) or ranitidine (50 mg kg⁻¹) on gastric total antioxidant capacity in indomethacin-induced gastric ulcer in pyloric-ligated rats. Values are Means$\pm$SE of 6 rats. *$p < 0.05$ vs normal group; @$p < 0.05$ vs control group

Fig. 10: Effect of two weeks daily treatment with ziprasidone (3 mg kg⁻¹) or bromocriptine (2.5 mg kg⁻¹) alone or combined with L-carnitine (50 mg kg⁻¹) or ranitidine (50 mg kg⁻¹) on gastric tumor necrosis factor alpha (TNF-α) content in indomethacin-induced gastric ulcer in pyloric-ligated rats. Values are Means$\pm$SE of 6 rats. *$p < 0.05$ vs normal group; @$p < 0.05$ vs control group
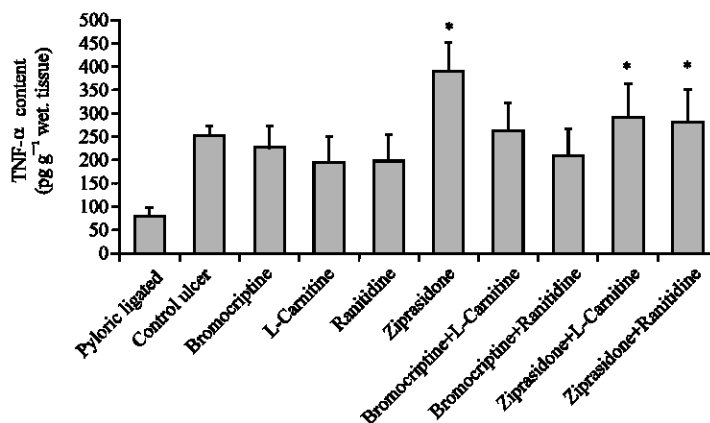
compared to the normal group, an effect that was not changed even by its combination with ranitidine or L-carnitine (Fig. 10).

Examination of gastric mucosa of normal and pyloric ligated rats showed normal stomachs structure (Fig. 11a, b). Gastric mucosa of a rat subjected to indomethacin showed a large gap in the gastric mucosa resulting from complete degeneration and shedding of the upper 2/3 of the gastric glands at this area. This was accompanied by observable thickening and dilatation of blood vessels of muscularis mucosa (Fig. 11c).

Gastric mucosa of a rat treated with bromocriptine then subjected to indomethacin showed regeneration of the gastric mucosa tissue at the site of ulceration, although small gaps at the site of regeneration were still observed (Fig. 11d). Moreover, L-carnitine or ranitidine reversed the damaging effects of indomethacin (Fig. 11e,f).

Gastric mucosa of a rat subjected to indomethacin with ziprasidone resulted in severe damage and atrophy of gastric glands and cells (Fig. 11g).

Using a combination of bromocriptine with either L-carnitine or ranitidine revealed their marked good
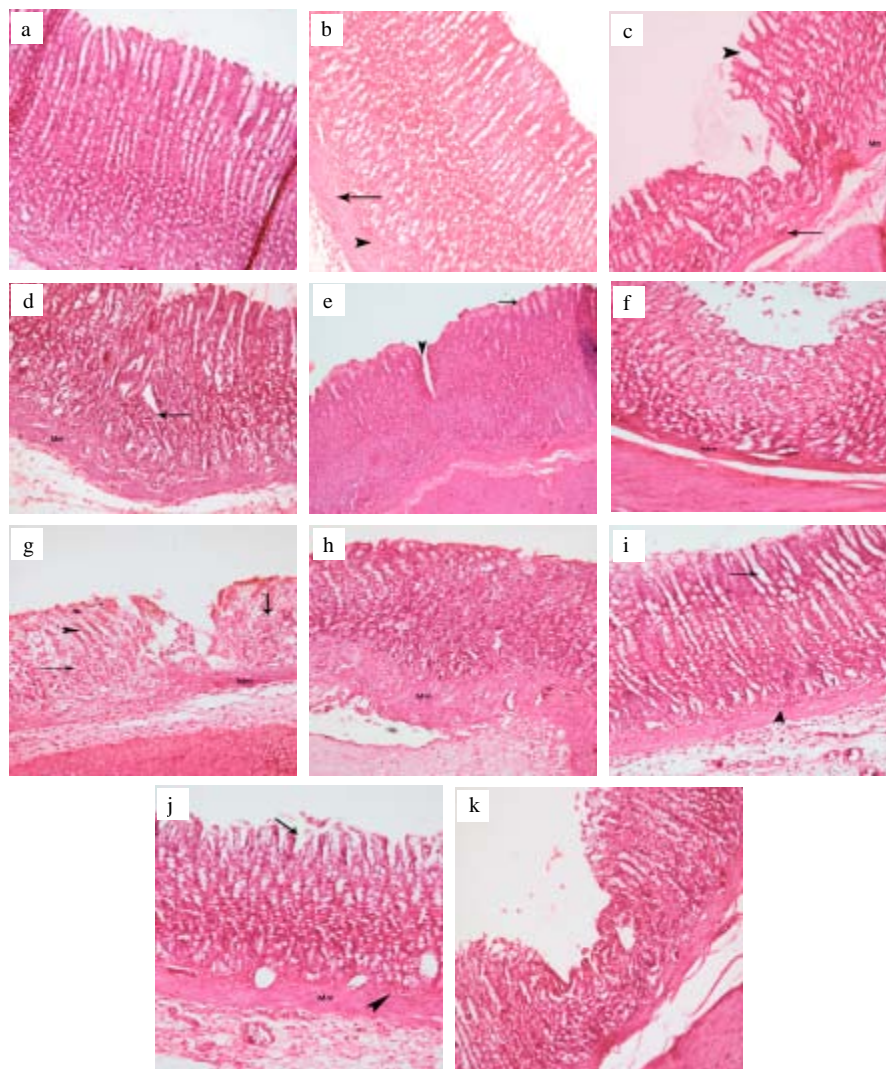
Fig. 11: A photomicrograph of a section of gastric mucosa showing normal rat without pyloric ligation (a), pyloric ligated rat (normal) (b), indomethacin (c), bromocriptine + indomethacin (d), L-carnitine + indomethacin (e), ranitidine + indomethacin (f), ziprasidone (g), ziprasidone + indomethacin (h), bromocriptine + L-carnitine + indomethacin (i), bromocriptine + ranitidine + indomethacin (j), ziprasidone + L-carnitine + indomethacin (k), ziprasidone + ranitidine + indomethacin (l) (Hx. and E. X 100)

regenerative effects (Fig. 11h, i). In addition, the combination of ziprasidone with either of the two drugs reversed its deleterious effects on the stomach (Fig. 11j, k).

DISCUSSION

In the present model, pylorus ligation resulted in acid-pepsin accumulation due to pylorus obstruction and subsequent mucosal damage by indomethaicn (Kaithwas and Majumdar, 2010).

Current administration of indomethacin as a single dose caused ulceration in the glandular area of the stomach manifested by increased ulcer number and severity. Similar results were obtained by other investigators (Heeba et al., 2009; Albayrak et al., 2010).

Two main mechanisms are implicated in explaining the role of indomethacin-induced gastric mucosal damage, a direct topical effect that results in disruption of the mucosal barrier and systemic inhibition of gastric mucosal protection through inhibition of the cyclooxygenase activity (Heeba et al., 2009).

Furthermore, administration of indomethacin in the present study resulted in increased titratable acidity and acid output as well as reduced gastric mucin content as compared to the normal group. Similar results were obtained by other investigators (Devi *et al.*, 2007; Heeba *et al.*, 2009).

Indomethacin-induced ulcers in the present investigation were coupled by increased gastric mucosal lipid peroxides and reduction in GSH content. The present results are in harmony with those of Heeba *et al.* (2009) and Chattopadhyay *et al.* (2006).

Depletion of reduced GSH by indomethacin weakens gastric mucosal defenses making tissues more susceptible to free radical induced lipid peroxidation (Altinkaynak *et al.*, 2003).

The present investigation revealed that indomethacin resulted in decreased production of gastric NO metabolites and decreased total antioxidant capacity of the gastric mucosa. These results are in accordance with the findings of Banerjee *et al.* (2008). This could be explained by the decrease in NO biosynthesis consequent to decreased nitric oxide synthase (NOS) activity associated with increased mucosal damage (Tripp and Tepperman, 1995). Endogenous NO plays an important role in the protection of gastric mucosa possibly through maintenance of mucosal blood (Calatayud *et al.*, 1999).

In the present study, ziprasidone augmented indomethacin-induced ulcer in pyloric ligated rats manifested by the significant increase in ulcer number and severity when compared with the control ulcer group. On the other hand, using bromocriptine, a dopamine agonist, did not affect the gastric acid output.

The present findings find support in the results of Caldara and Barbieri (1987) as well as Glavin and Szabo (1990).

Administration of L-carnitine in the current study significantly decreased indomethacin-induced increase in gastric ulcer number and severity and reversed the ulcerogenic effects of ziprasidone. These findings are in agreement with the results of Arafa and Sayed-Ahmed (2003) and Erkin *et al.* (2006) who reported that L-carnitine reduced ulcer number and severity induced by ethanol and indomethacin, respectively. The observed gastroprotective effect of L-carnitine may be attributed to its well-known antioxidant effect (Erkin *et al.*, 2006).

In a similar fashion, administration of ranitidine in the current study decreased indomethacin- and ziprasidone- induced increase in gastric ulcer number and severity. These findings are in agreement with the results of Alam *et al.* (2009) who reported similar protective effects of ranitidine in chemically- and stress-induced gastric ulcer, by virtue of its antisecretory properties.

The present results showed that ziprasidone reduced the gastric mucus wall content, an effect that was abolished by its concomitant administration with L-carnitine or ranitidine. Gastric mucus is an important protective factor for the entire gastrointestinal mucosa (Penissi and Piezzi, 1999). The decrease in the mucus content by ziprasidone further confirms its harmful effects on the gastric mucosa and correlates with results of ulcer number and severity (De La Lastra *et al.*, 1994).

The observed protective effect of L-carnitine on gastric mucus was previously documented (Izgut-Uysal *et al.*, 2007; Nunes *et al.*, 2009).

Administration of bromocriptine in the current study protected the gastric mucosa by preventing mucus depletion that resulted from indomethacin. The increased mucus secretion can prevent gastric ulceration by several mechanisms, including lessening of stomach wall friction during gastric contractions and improving the buffering of acid gastric juice (Devi *et al.*, 2007).

In a similar fashion, ziprasidone increased peptic activity and reduced mucin content. Such effects were decreased by the simultaneous administration of L-carnitine or ranitidine. On the other hand, bromocriptine alone or in combination with L-carnitine reversed indomethacin-induced decrease in mucin content, further confirming its reported gastroprotective effects (Izgut-Uysal *et al.*, 2001).

Parallel to the observed effects on peptic ulcer markers, both ziprasidone and indomethacin increased stomach lipid peroxidation as evident from elevated MDA content. In addition, L-carnitine or ranitidine improved the deleterious effects induced by either indomethacin alone or in combination with ziprasidone.

Increased MDA levels cause deleterious effects on the stomach tissue resulting in depletion of stomach antioxidative self-defense system (Muthuraman and Sood, 2010). L-carnitine and ranitidine antioxidative effects were previously shown (Izgut-Uysal *et al.*, 2001, 2007; Dursun *et al.*, 2009). L-carnitine has the ability to scavenge H_2O_2 and hydroxyl radical and may inhibit hydroxyl radical production in the Fenton reaction system (Gulcin, 2006). Moreover, L-carnitine acts as a metal chelator (Muthuswamy *et al.*, 2006) which further adds to its antioxidant mechanism.

Ziprasidone-induced lipid peroxidation, in the present study was coupled by marked reduction in gastric mucosal GSH content, an effect that was prevented by co-administration with L-carnitine or ranitidine.

GSH is present in the stomach at high concentrations as it plays an important role in maintaining the integrity of gastric mucosa by protecting the present thiol groups (Altinkaynak *et al.*, 2003).

Elevation of GSH content by L-carnitine therapy may be a result of increased NADPH generation through

increased activity of G_6PDH as NADPH is used by GSH reductase to reduce oxidized glutathione (GSSG) to GSH (Kumaran *et al.*, 2003).

The observed protective effects of ranitidine against indomethacin-induced GSH depletion are in agreement with the results of other investigators (Odabasoglu *et al.*, 2006; Dengiz *et al.*, 2007).

In the present study, application of indomethacin alone or combined with ziprasidone resulted in a significant decrease in gastric NO_x content. This effect was prevented the concomitant administration of L-carnitine or ranitidine. NO serves various important functions in the GIT including promotion of prostaglandins synthesis increasing mucosal blood flow and mucus secretion. Moreover, NO participates in the gastric defense mechanisms (Gurbuz and Yesilada, 2007).

The current elevation of NO by ranitidine is in harmony with the results of Kim and Kim (2001) and Odabasoglu *et al.* (2006). Moreover, the observed increased gastric mucosal NO by bromocriptine is in accordance with Samini *et al.* (2002).

Results of the present study revealed that rats treated with indomethacin alone or combined with ziprasidone showed marked reduction in stomach total antioxidant capacity. Concomitant administration of L-carnitine or ranitidine prevented the effects of indomethacin alone but not that of indomethacin and ziprasidone.

According to the present results, administration of ziprasidone elevated stomach TNF-a content which was significantly improved by the concomitant administration of L-carnitine or ranitidine when compared to the normal pylorus ligated group.

TNF-α, a cytokine produced during gastric mucosal injury, seems to be a key factor to many forms of gastric mucosal injury. It stimulates caspase-3 in epithelial and endothelial cells of gastric mucosa and thus contributes to apoptosis and subsequent damage (Baraka *et al.*, 2010).

Hence, the pathological effects of ziprasidone could be explained by its induced increase in TNF-α resulting in neutrophils infiltration and activation leading to generation of ROS and initiation of apoptosis (Souza *et al.*, 2004). The histopathological results are in accordance with the data described before.

In conclusion, the present study rings a bell to keep an eye on the commonly used antipsychotic drugs if therapeutically used for long periods of time. Their ulcerogenic effects may be very dangerous especially in people who suffer from peptic ulcers or are susceptible to gastrointestinal abnormalities.

ACKNOWLEDGMENTS

REFERENCES

Alam, S., M. Asad, S.M.B. Asdaq and V.S. Prasad, 2009. Antiulcer activity of methanolic extract of *Momordica charantia* L. in rats. J. Ethnopharmacol., 123: 464-469.

Albayrak, A., B. Polat, E. Cadirci, A. Hacimuftuoglu, Z. Halici and M. Gulapoglu *et al.*, 2010. Gastric anti-ulcerative and anti-inflammatory activity of metyrosine in rats. Pharmacol. Rep., 62: 113-119.

Altinkaynak, K., H. Suleyman and F. Akcay, 2003. Effect of nimesulide, rofecoxib and celecoxib on gastric tissue glutathione level in rats with indomethacin-induced gastric ulcerations. Polish J. Pharmacol., 55: 645-648.

Arafa, H.M.M. and M.M. Sayed-Ahmed, 2003. Protective role of carnitine esters against Alcohol-induced gastric lesions in rats. Pharmacol. Res., 48: 285-290.

Banerjee, D., A.K. Bauri, R.K. Guha, S.K. Bandyopadhya and S. Chattopadhyay, 2008. Healing properties of malabaricone B and malabaricone C, against indomethacin-induced gastric ulceration and mechanism of action. Eur. J. Pharmacol., 578: 300-312.

Baraka, A.M., A. Guemei and H.A. Gawad, 2010. Role of modulation of vascular endothelial growth factor and tumor necrosis factor-α in gastric ulcer healing in diabetic rats. Biochem. Pharmacol., 79: 1634-1639.

Beck, P.L., R. Xavier, N. Lu, N.N. Nanda, M. Dinauer, D.K. Podolsky and B. Seed, 2000. Mechanism of NSAID-induced gastrointestinal injury defined using mutant mice. Gastroenterology, 119: 699-705.

Beutler, E., O. Duron and B.M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

Brodie, D.A. and K.F. Hooke, 1971. The effect of vasoactive agents on stress-induced gastric haemorrhage in the rat. Digestion, 4: 193-204.

Calatayud, S., M.J. Sanz, A. Canet, R. Bello, F.D. de Rojas and J.V. Esplugues, 1999. Mechanisms of gastroprotection by transdermal nitroglycerin in the rat. Br. J. Pharmacol., 127: 1111-1118.

Caldara, R. and C. Barbieri, 1987. The dopaminergic System in the Regulation of Gastric Acid Secretion and Gastrin Release: Experimental Data and Therapeutic Implications. In: New Pharmacology of Ulcer Disease, Szabo, S. and G. Mozsik (Eds.). Elsevier, New York, USA., pp: 232-242.

Chattopadhyay, I., U. Bandyopadhyay, K. Biswas, P. Maity and R.K. Banerjee, 2006. Indomethacin inactivates gastric peroxidase to induce reactive-oxygen-mediated gastric mucosal injury and curcuminprotects it by preventing peroxidase inactivation and scavenging reactive oxygen. Free Radical Biol. Med., 40: 1397-1408.

Corne, S.J., S.M. Morrissey and R.J. Woods, 1974. A method for the quantitative estimation of gastric barrier mucus. J. Physiol., 242: 116-117.

De La Lastra, A., M.J. Martin and V. Motilva, 1994. Antiulcer and gastroprotectective effect of quercetin, a gross and histologic study. Pharmacology, 48: 56-62.

Dengiz, G.O., F. Odabasoglu, Z. Halici, H. Suleyman, E. Cadirci and Y. Bayir, 2007. Gastroprotective and antioxidant effects of amiodarone on indomethacin-induced gastric ulcers in rats. Arch. Pharm. Res., 30: 1426-1434.

Devi, R.S., S. Narayan, G. Vani and C.S. Shyamala-Devi, 2007. Gastroprotective effect of Terminalia arjuna bark on diclofenac sodium induced gastric ulcer. Chem. Biol. Interact., 167: 71-83.

Dursun, H., M. Bilici, F. Albayrak, C. Ozturk, M. Saglam, H.H. Alp and H. Suleyman, 2009. Antiulcer activity of fluvoxamine in rats and its effect on oxidant and antioxidant parameters in stomach tissue. BMC Gastroenterol., Vol. 9. 10.1186/1471-230X-9-36

Erkin, B., D. Dokmeci, S. Altaner and F.N. Turan, 2006. Gastroprotective effect of L-carnitine on indomethacin-induced gastric mucosal injury in rats: A preliminary study. Folia Med., 48: 86-89.

Glavin, G.B. and S. Szabo, 1990. Dopamine in gastrointestinal disease. Dig. Dis. Sci., 35: 1153-1161.

Grossman, M.I., 1963. Physiology for physician. Am. Physiol. Soc., 1: 1-5.

Gulcin, I., 2006. Antioxidant and antiradical activities of L-carnitine. Life Sci., 78: 803-811.

Gurbuz, I. and E. Yesilada, 2007. Evaluation of the anti-ulcerogenic effect of sesquiterpene lactones from Centaurea solstitialis L. ssp. solstitialis by using various In vivo and biochemical techniques. J. Ethnopharmacol., 112: 284-291.

Heeba, G.H., M.K.A. Hassan and R.S. Amin, 2009. Gastroprotective effect of simvastatin against indomethacin-induced gastric ulcer in rats: Role of nitric oxide and prostaglandins. Eur. J. Pharmacol., 607: 188-193.

Hoogerwerf, W.A. and P.J. Pasricha, 2001. Pharmacologic therapy in treating achalasia. Gastrointest. Endosc. Clin. N. Am., 11: 311-324.

Izgut-Uysal, V.N., A. Agac and N. Derin, 2001. Effect of carnitine on stress-induced lipid peroxidation in rat gastric mucosa. J. Gastroenterol., 36: 231-236.

Izgut-Uysal, V.N., M. Bulbul, R. Tan, N. Derin, I. Ustunel, A. Agar and P. Yargicoglu, 2007. Effect of chronic stress and l-carnitine on rat stomach. J. Physiol. Sci., 57: 187-192.

Kaithwas, G. and D.K. Majumdar, 2010. Evaluation of antiulcer and antisecretory potential of Linum usitatissimum fixed oil and possible mechanism of action. Inflammopharmacology, 18: 137-145.

Kim, H. and K.H. Kim, 2001. Role of nitric oxide and mucus in ischemia/reperfusion-induced gastric mucosal injury in rats. Pharmacology, 62: 200-207.

Koracevic, D., G. Koracevic, V. Djordjevic, S. Andrejevic and V. Cosic, 2001. Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol., 57: 356-361.

Kumaran, S., B. Deepak, B. Naveen, B. Naveen and C. Panneerselvam, 2003. Effects of levocarnitine on mitochondrial antioxidant systems and oxidative stress in aged rats. Drugs, 4: 141-147.

Mafertheiner, P., F. Chan and K. Mccoll, 2009. Peptic ulcer diseases. Lancet, 374: 1449-1461.

Miranda, K.M., M.G. Espey, K. Yamada, M. Krishna and N. Ludwick et al., 2001. Unique oxidative mechanisms for the reactive nitrogen oxide species, nitroxyl anion. J. Biol. Chem., 276: 1720-1727.

Mozsik, G., F. Moron and T. Javor, 1982. Cellular mechanisms of the development of gastric mucosal damage and of gastrocytoprotection induced by prostacyclin in rats. A pharmacological study. Prostagland Leukot Med., 9: 71-84.

Muthuraman, A. and S. Sood, 2010. Antisecretory, antioxidative and antiapoptotic effects of montelukast on pyloric ligation and water immersion stress induced peptic ulcer in rat. Prostaglandins Leukot. Essent. Fatty Acids, 83: 55-60.

Muthuswamy, A.D., K. Vedagiri, M. Ganesan and P. Chinnakannu, 2006. Oxidative stress-mediated macromolecular damage and dwindle in antioxidant status in aged rat brain regions: Role of L-carnitine and DL-α-lipoic acid. Clin. Chim. Acta., 368: 84-92.

Nemeroff, C.B., Lieberman J.A., Weiden P.J., Harvey P.D and J.W. Newcomer et al., 2005. From clinical research to clinical practice: A 4-year review of ziprasidone. CNS Spectr., 10: 1-20.

Nunes, P.H., P.M. Cavalcanti, S.M. Galvao and M.C. Martins, 2009. Antiulcerogenic activity of Combretum leprosum. Pharmazie, 64: 58-62.

Odabasoglu, F., A. Cakir, H. Suleyman, A. Aslan, Y. Bayir, M. Halici and C. Kazaz, 2006. Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. J. Ethnopharmacol., 103: 59-65.

Penissi, A.B. and R.S. Piezzi, 1999. Effect of dehydroleucodine on mucus production. A quantitative study. Digestion Dis. Sci., 44: 708-712.

Rashid, N., A. Ahmad, N. Singh, P. Singh and V. Mishra et al., 2010. Differential response of A 68930 and sulpiride in stress-induced gastric ulcers in rats. Eur. J. Pharmacol., 643: 121-128.

Samini, M., L. Moezi, N. Jabarizadeh, B. Tavakolifar, H. Shafaroodi and A.R. Dehpour, 2002. Tavakolifar B. Evidences for involvement of nitric oxide in the gastroprotective effect of bromocriptine and cyclosporin on water immersion stress-induced gastric lesions. Pharmacol. Res., 46: 519-523.

Sanyal, A., O. Denath, S. Bhattacharya and K. Gode, 1971. The Effect of Cyproheptadine on Gastric Acidity. In: Peptic Ulcer, Pfeiffer, C. (Ed.). Scandinavian University Books, Munksgoard, pp: 312-318.

Shay, H., S.A. Komarov, S.S. Fels, D. Meraze, M. Gruenstein and H. Siplet, 1945. A simple method for uniform production of gastric ulceration in rat. Gastroenterology, 4: 43-61.

Souza, M.H.L.P., H.P. Lemos, R.B. Oliveira and F.Q. Cunha, 2004. Gastric damage and granulocyte infiltration induced by indomethacin in tumor necrosis factor receptor 1 (TNFR-1) or inducible nitric oxide synthase (iNOS) deficient mice. Gut, 53: 791-796.

Thorner, M.O., 1975. Dopamine is an important neurotransmitter in the autonomic nervous system. Lancet, 1: 662-665.

Tian, B., D.E. Nowak and A.A. Brasier, 2005. A TNF-induced gene expression program under oscillatory NF-kappaB control. BMC Genomics, Vol. 6

Tripp, M.A. and B.L. Tepperman, 1995. Effect of nitric oxide on integrity, blood flow and cyclic GMP levels in the rat gastric mucosa: Modulation by sialoadenectomy. Br. J. Pharmacol., 115: 344-348.

Uchiyama, M. and M. Mishara, 1978. Determination of malonaldehyde precursor in tissue by thiobarbituric acid test. Ann. Biochem., 86: 271-278.

Vananenn, P.M., J.B. Medding and J.L. Wallace, 1991. Role of oxygen-derived free radicals in indomethacin-induced gastric injury. Am. J. Physiol., 261: G470-G475.

Winzler, R.J., 1955. Determination of serum glycoproteins. Methods Biochem. Anal., 2: 279-311.