Int J Clin Exp Med 2014;7(8):2031-2044
www.ijcem.com /ISSN:1940-5901/IJCEM0001282

Original Article

Aripiprazole an atypical antipsychotic protects against ethanol induced gastric ulcers in rats

Abdulrahman Al Asmari[1], Mohammed Arshaduddin[1], Ibrahim Elfaki[1], Saeed Kadasah[2], Abdulrahman Al Robayan[3], Saeed Al Asmary[4]

[1]Research Center, Departments of [2]Psychiatry, [3]Gastroenterology, [4]Family and Community Medicine, Prince Sultan Military Medical City, Riyadh, Saudi Arabia

Received July 2, 2014; Accepted July 31, 2014; Epub August 15, 2014; Published August 30, 2014

Abstract: The present investigation was undertaken, to study the gastro-protective potential of aripiprazole (ARI) an atypical antipsychotic drug in ethanol induced gastric ulcers in rats. ARI (10, 30, 100 mg/kg) was tested for gastric secretion and antiulcer activity in different groups of male Sprague Dawley rats. Gastric secretion and acidity studies were performed in pylorus ligated rats while indices of gastric ulcers were measured in ethanol (1 ml-100%) induced gastric ulcers. Histological changes and the levels of gastric wall mucus, malondialdehyde (MDA), non-protein sulfhydryls (NP-SH), myeloperoxidase (MPO), and serotonin were used to assess ethanol induced gastric mucosal injuries. Exposure of rats to ethanol resulted in gastric mucosal injury and a high index of ulcer. Pretreatment with ARI significantly ($P < 0.001$), reduced the gastric lesions induced by ethanol and also resulted in a significant decrease in the gastric secretion, and total acidity in pylorus ligated rats. ARI also significantly attenuated the ethanol induced reduction in the levels of gastric wall mucus, and NP-SH ($P < 0.001$). The histological changes and the increased MDA and MPO activity were also significantly ($P < 0.001$) inhibited by ARI. Ethanol induced depletion in the levels of serotonin in the gastric tissue were also significantly restored by pretreatment with ARI ($p < 0.001$). ARI showed significant antiulcer and gastroprotective activity against ethanol induced gastric ulcers. The gastroprotective effects of ARI may be due to its anti-secretory, antioxidant and anti-inflammatory action and also due to the restoration of the depleted gastric serotonin levels.

Keywords: Gastric ulcer, ethanol, aripiprazole, oxidative stress, serotonin, dopamine, myeloperoxidase

Introduction

Gastric ulcer is one of the common diseases of the digestive system among adults. Around 5-10% of the people are affected by this disease during their life time [1] resulting in considerable morbidity and high financial burden. It is a multi-etiologic disease and commonly associated with nutritional deficiencies, helicobacter pylori infection, use of alcohol, and anti-inflammatory drugs and exposure to severe stress [2-6]. Some of the common mechanisms involved in the pathogenesis of gastric mucosal damage include an increase in acidity and gastric secretions, ischemia, the production of free radicals, a decrease in antioxidants and blood flow of the gastric mucosa, neutrophil accumulation and production of inflammatory cytokines [7]. Monoamines, dopamine and serotonin which are extensively found in the gastrointestinal (GI) tract also play important role in its physiological functioning including acid and mucus secretions, mucus formation, gastric motility, mucosal blood flow and fluid secretion. Changes in their levels have a profound modulatory effect on the mucosal defense of the GI tract and severity of ulcerations [8-12].

The two most commonly used approaches for the management of gastric ulcers is based on the reduction of gastric secretion and strengthening of the gastric mucosal defense [13]. Gastric secretion and gastric mucosal characteristics are regulated through the brain-gut axis. Dopamine regulates gastric function through its modulatory effects on the gastrointestinal tract [14], while modulation of different serotonin receptors has been observed to play a vital role in attenuation of stress induced gastric ulcers [5, 6, 10, 12, 15, 16].

Aripiprazole (ARI) an atypical antipsychotic is used for the treatment of schizophrenia, and acute manic or mixed episodes associated with bipolar disorder and irritability in children with autism [17-19]. Besides the modulation of the monoaminergic system, the protective action of aripiprazole has been assigned to its pleiotropic activities including scavenging of free radicals [20], inhibition of glutamate-induced neurotoxicity associated with reactive oxygen species formation [21], and reduction of inflammatory reactions [22]. Recent reports show a significant gastroprotective effects of antipsychotic drugs in cold restraint stress induced gastric lesions [5, 14, 15]. Risperidone an atypical antipsychotic has been reported to have significant gastroprotective effects in cold restraint stress induced gastric lesions. The protective effect was suggested to be mediated through activation of enzymatic endogenous nitric oxide, sulfhydryl group prostaglandins and opening of KATP channels [5]. Though risperidone and aripiprazole are both atypical antipsychotics they show a variation in their comparative efficacy due to the differences in their pharmacokinetics and adverse effects [23]. At the same time aripiprazole is unlike other psychotropics and its dopamine agonist and antagonist properties hinge on regional monoamine concentrations [24]. Therefore keeping in view the monoamine modulatory and other pleiotropic effects of ARI, this investigation was undertaken to study the gastroprotective potential of aripiprazole in ethanol induced gastric ulcers in rats.

Methods

Male adult Sprague Dawley rats, weighing 200-250 g, fed on a standard chow diet were maintained in a temperature and humidity controlled room at 12 h light/dark cycles. The animals were divided into experimental groups of six animals each. The distribution of animals into groups and the treatment allotted to each group were randomized. The protocol of animal study was approved by Research and Ethical Committee of Prince Sultan Military Medical City, Riyadh, Saudi Arabia, and the guidelines of animal care were strictly adhered during animal maintenance and experimentation. All the experiments were started between 8 and 10 am in the morning.

Aripiprazole was freshly prepared in doses of 10, 30 and 100 mg/kg body weight before administration and given by gavage (i.g.) before pylorus ligation and administration of ethanol. The animals were sacrificed under deep anesthesia. The stomachs were removed and opened along the greater curvature.

Gastric secretions

Pylorus ligated (shay) rats: The animals were fasted for 36 h with access to water ad libitum before the pylorus was ligated under anesthesia, care being taken not to cause bleeding or to occlude blood vessels [25]. Aripiprazole in doses of 10 mg, 30 mg and 100 mg/kg was administered by gavage 30 minutes before pylorus ligation. The animals were sacrificed at 6 h after the pylorus ligation. The stomachs were removed, contents collected, volume measured, centrifuged and subjected to analysis for titratable acidity against 0.01 N NaOH to pH 7 using a pH meter and total acid output was calculated.

Gastric lesions induced by ethanol (cytoprotection studies): The animals were administered (i.g.) with 1 ml of absolute ethanol [26]. Different doses of Aripiprazole were given (i.g.) 30 min before the administration of ethanol. One hour after the administration of ethanol the animals were sacrificed and examined for the lesions in stomachs. The scoring of lesions was done as follows: Patched lesions of the stomach induced by 100% ethanol were scored according to the method described by Schiantarelli et al. [27] using the following scale; 0 = normal mucosa; 1 = hyperemic mucosa or up to three small patches; 2 = four-10 small patches; 3 = more than 10 small or up to three medium-sized patches; 4 = four-six medium-sized patches; 5 = more than six medium-sized or up to three large patches; 6 = four-six large patches; 7 = seven-10 large patches; 8 = more than 10 large patches or extensive necrotic zones. 'Small' was defined as up to 2 mm across (maximum diameter), 'medium-sized' as between 2 and 4 mm across, and 'large' as more than 4 mm across.

Separate batches of ethanol treated rats were used for biochemical and histological studies. Aripiprazole (10 mg, 30 mg and 100 mg/kg), was administered 30 minutes before ethanol administration. The animals were killed 1 h after administering ethanol. The stomachs were collected and the levels of gastric mucus,

MDA, non-protein sulfhydryls group (NP-SH), and myeloperoxidase (MPO) and serotonin were determined.

Determination of gastric wall mucus: Gastric wall mucus was determined according to the modified procedure of Corne et al. [28]. The glandular segment of the stomach was separated from the lumen of the stomach, weighed, and transferred immediately to 10 ml of 0.1% w/v Alcian blue solution (in 0.16 M sucrose solution buffered with 0.05 ml sodium acetate at pH 5). Tissue was stained for two hours in Alcian blue, excess dye was removed by two successive rinses with 10 ml of 0.25 M sucrose, first for 15 min and then for 45 min. Dye complexed with the gastric wall mucus was extracted with 10 ml of 0.5 M magnesium chloride which was intermittently shaken for 1 min at 30 min intervals for 2 hrs. Four milliliters of blue extract were then vigorously shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 rpm for 10 min and the absorbance of aqueous layer was recorded at 580 nm. The quantity of alcian blue extracted per gram of wet glandular tissue was then calculated.

Determination of malondialdehyde

Gastric mucosal malondialdehyde designated as an index of lipid peroxidation was measured according to the method of Ohkawa et al. [29]. Pre weighed gastric mucosa was homogenized in 1.15% KCL. For the assay of MDA, 0.1 ml of homogenate was supplemented with 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 0.8% aqueous solution of thiobarbituric acid and the mixture was kept in boiling water bath at 95°C for 1 h and cooled at room temperature for 15 minutes. After cooling 1.0 ml of distilled water and 5.0 ml of the mixture of *n* - butanol and pyridine (15:1, v/v) were added. After centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer (upper layer was measured at 532 nm against TBA as a blank. Results were calculated as nmol TBARS formed/h/gm tissue using an extinction coefficient of 1.56×10^6.

Determination of nonprotein sulfhydryl groups (NP-SH)

NP-SH levels in the gastric mucosa were determined by following the method described by Sedlak and Lindsay [30]. Homogenates of the isolated glandular part of the stomach were prepared in ice-cold 0.02 M mmol/L EDTA. Aliquots of the of the homogenates (5 ml) were mixed with distilled water (4 ml) and 50% trichloroacetic acid (1 ml) in a test tube and the mixture was centrifuged at 3000 g after shaking intermittently for 15 minutes. The supernatant was collected and 2 ml of the supernatant were added to 4 ml of 0.4 M Tris buffer (pH 8.9) and 0.1 ml of DTNB [5, 5'-dithio-bis-(2-nitrobenzoic acid)]. The mixture was shaken, and the absorbance was read at 412 nm against a reagent blank with no homogenate. Care was taken to record the absorbance in less than 5 minutes after adding DTNB.

Determination of myeloperoxidase (MPO)

The method as described by Bradley et al. [31] was used to determine the gastric mucosal MPO activity. The homogenates were subjected to three cycles of freezing and thawing and sonicated in an ice bath for 20 seconds. The supernatant collected after centrifugation of samples was assayed for MPO activity.

Determination of serotonin

The level of serotonin in the stomach tissue was determined by the method of Patrick et al. [32]. The stomachs were weighed and homogenized for 10 s in 0.1 M perchloric acid containing 0.05% EDTA using Teflon homogenizer. The homogenates were immediately centrifuged at 17,000 rpm at 4°C for 10 min. The supernatants were filtered using 0.45 μm pore filters and analyzed by high performance liquid chromatography (HPLC). The HPLC system consisted of an electrochemical detector (Model 2465), and an auto injector (Model 2707,), a solvent delivery pump (Waters Model 1525 Waters Associate Inc., Melford, MA, USA). The mobile phase consisted of a mixture of 0.1 M citric acid monohydrate, 0.1 M sodium acetate, 7% methanol, 100 mM EDTA and 0.01% sodium octane sulfonic acid and the column was C-18 m Bondapak (3.9 mm × 150 mm). The flow rate was maintained at 1.5 ml/min and the injection volume was 20 μl.

Histology of ethanol-induced gastric lesions

Separate batch of rats were used for histological studies. Rats were treated with aripiprazole and ethanol as mentioned above and the ani-

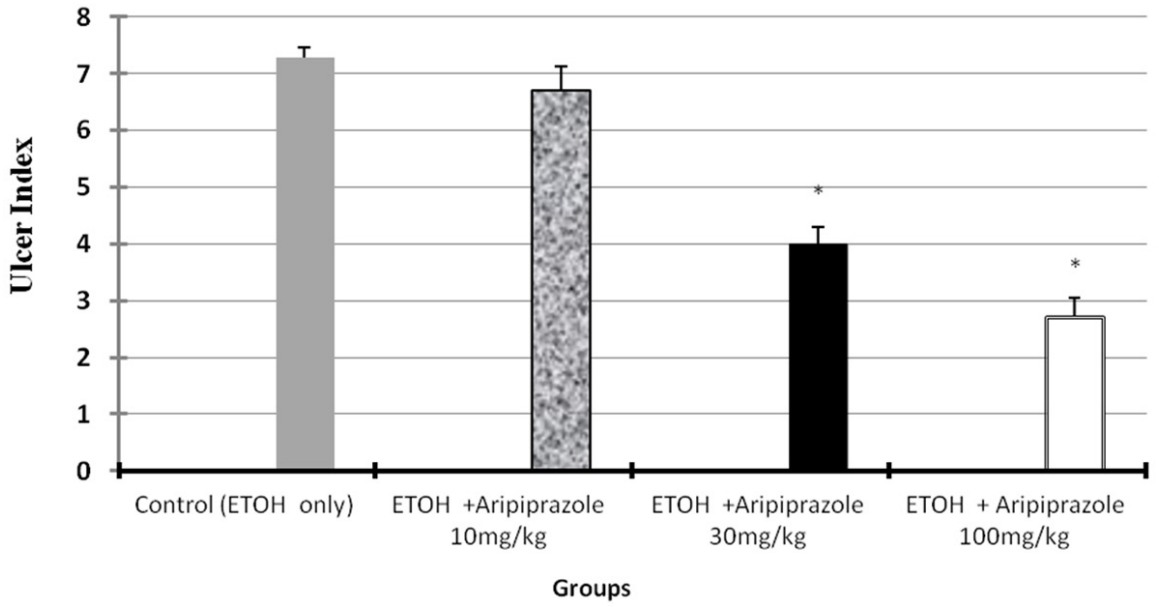

Figure 1. Effect of Aripiprazole on ethanol-induced gastric lesions in rats. Values are represented as mean ± SEM from 6 rats per group. Animals in control group were killed 1 h after the oral administration of ethanol. In the test group, Aripiprazole was given by gavage 30 min before the administration of ethanol. *P < 0.001 as compared with control group using ANOVA followed by Dunnett's multiple comparison test.

mals were killed under deep anesthesia. Stomach was opened along the greater curvature, washed with saline and fixed in 10% neutral buffered formalin for 24 h. The specimens were then processed overnight for dehydration and clearing steps, using an automatic tissue processor (Shandon Southern 2L Processor MKÏ; Runcorn. Cheshire, UK). The specimens were embedded in paraffin blocks and sections of 5 µm thickness were cut and stained with hematoxylin-eosin. The slides were examined for the histological changes under a light microscope.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. Differences with a *P* value less than 0.05 were considered statistically significant. The statistical program SPSS (Version 15) was used to analyze the data.

Results

Effect of ARI on ethanol-induced gastric lesions

Administration of absolute ethanol produced extensive gastric lesions in the glandular mucosa of the stomach in 100% of the control animals. The ulcer index was 7.28 ± 0.18 in control rats 1 h after ethanol administration. Pretreatment of rats with ARI at the doses of 30 mg/kg (4.0 ± 0.3) and 100 mg/kg (2.71 ± 0.35) significantly inhibited the formation of gastric lesions and their severity (ANOVA, F = 43.67, P < 0.001) (**Figures 1**, **2**). Although ARI in low dose (10 mg/kg) reduced gastric lesions the difference was not statistically significant.

Effect of ARI on gastric secretion and acidity in 6 h pylorus-ligated shay rats

Our results show that ARI possesses significant anti gastric secretory activity. In control rats' pylorus ligation for 6 h resulted in the accumulation of 7.0 ± 0.24 ml of gastric secretion and total acid output of 1054.1 ± 58.25 mEq (**Table 1**). The volume of gastric secretion in the rats treated with 10, 30 and 100 mg/kg of ARI was significantly (ANOVA, F = 14.06, P < 0.001) reduced to 4.0 ± 0.49 ml, 2.7 ± 0.32 ml and 2.1 ± 0.14 ml respectively (**Table 1**). Treatment with ARI showed a significant and dose dependent reduction in the total acid output in the rats treated with 10 mg/kg (636.6 ± 131.5 mEq), 30 mg/kg (273.3 ± 43.33 mEq), and 100 mg/kg (196.0 ± 18.1 mEq) of ARI (ANOVA, F = 29.31 P < 0.001).

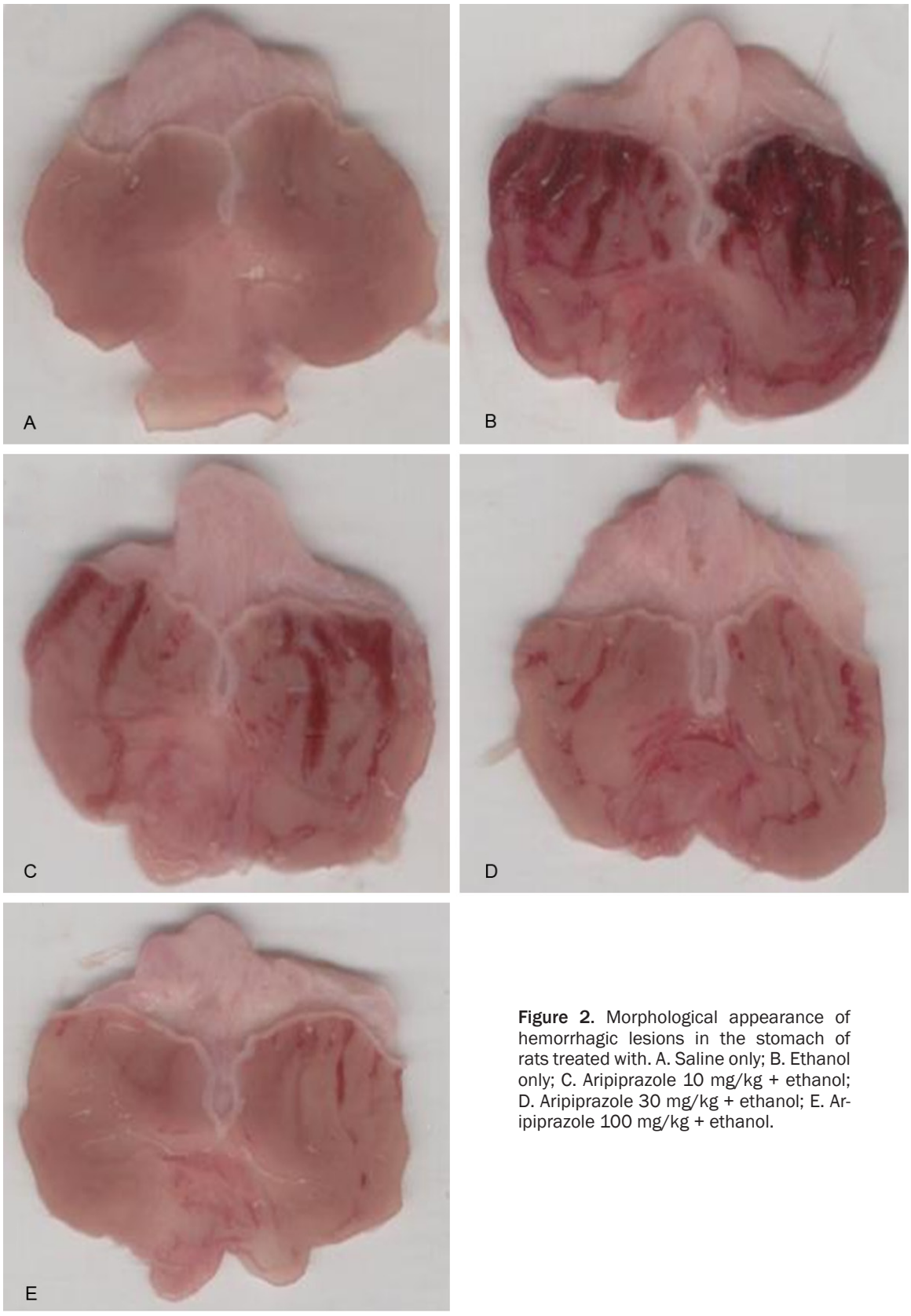

Figure 2. Morphological appearance of hemorrhagic lesions in the stomach of rats treated with. A. Saline only; B. Ethanol only; C. Aripiprazole 10 mg/kg + ethanol; D. Aripiprazole 30 mg/kg + ethanol; E. Aripiprazole 100 mg/kg + ethanol.

Table 1. Effect of Aripiprazole (ARI) on gastric secretions and total acid output in pylorus ligated shay rats

Treatment	Volume of gastric secretion (ml)	Total acid output (mEq)
Pylorus ligation alone	7.0 ± 0.24	1054 ± 58.25
Ligation + ARI 10 mg/kg	4.0 ± 0.49*	636.6 ± 131.5*
Ligation + ARI 30 mg/kg	2.7 ± 0.32**	273.3 ± 43.33**
Ligation + ARI 100 mg/kg	2.1 ± 0.14**	196.0 ± 18.1**

Values are mean ± standard error of means (SEM). *P < 0.05 and **P < 0.0001 as compared with pylorus ligation alone using ANOVA followed by Dunnett's multiple comparison test.

Table 2. Effect of Aripiprazole (ARI) on ethanol induced changes in Alcian blue binding capacity (gastric wall mucus) in gastric mucosa of rats

Treatment	Alcian blue binding (µg/g tissue)
Normal	4877 ± 25.74
Ethanol alone (EtOH)	978.2 ± 20.28#
ARI 10 mg + EtOH	1218 ± 132.63
ARI 30 mg + EtOH	1478 ± 204.79*
ARI 100 mg + EtOH	2423 ± 171.72##

Values are mean ± SEM. *P < 0.001 as compared with control group and #P < 0.05, ##P < 0.001 as compared with Ethanol (ulcer only) group using Dunnett's multiple comparison test.

Effect of ARI on ethanol-induced changes in gastric wall mucus

Alcian blue binding capacity of gastric wall mucus was significantly reduced in the rats exposed to ethanol treatment (978.16 ± 20.28 µg/g of tissue) as compared to control rats (4877.5 ± 25.74 µg. Pretreatment of rats with ARI at the doses of 10 mg/kg (1218.3 ± 132.6 µg/g), 30 mg/kg (1478.6 ± 204.79 µg/g) and 100 mg/kg (2423 ± 171.7 µg/g) significantly enhanced alcian blue binding capacity of gastric mucosa (ANOVA F = 159.34, P < 0.001) (**Table 2**).

Histological changes

Administration of ethanol to rats resulted in a severe ulcerative injury to the glandular part of gastric mucosa. The gastric mucosal injury was characterized by a loss of glandular cells, numerous hemorrhagic red bands (patches) of various sizes distributed in the glandular stomach (**Figure 2**). Histological examination of gastric mucosa showed the appearance of these lesions in the form of hemorrhagic gastric pits with inflammation, and disruption of the surface epithelium; epithelial cells appeared to be vacuolated and the microvessels were elongated (**Figure 3**). Pretreatment with ARI reduced the size and severity of ethanol induced damage to the gastric mucosal architecture (**Figures 2** and **3C-E**).

Effect of ARI on ethanol-induced changes in the levels of malondialdehyde (MDA) in the gastric mucosa

The effect of ARI on ethanol induced changes in the gastric mucosa MDA levels is given in **Table 3**. Administration of ethanol resulted in a significant increase in the levels of MDA in the gastric mucosa (168.58 ± 4.05 nmol TBA/g tissue) as compared to the levels in control animals (139.04 ± 5.2 nmols/g). Pretreatment with ARI at 30 mg/kg (148.07 ± 3.69 nmols/g) and 100 mg/kg (141.8 ± 6.47 nmols/g) resulted in a significant inhibition of the ethanol induced increase of MDA (ANOVA F = 5.084, P < 0.05).

Effect of ARI on ethanol-induced changes in gastric mucosa levels of non-protein sulfhydryls (NP-SH)

Administration of ethanol resulted in a significant reduction in the levels of NP-SH in the gastric mucosa of ethanol treated rats (1.64 ± 0.61 µmol/g tissue) as compared to normal animals (2.56 ± 0.13 µmol/g tissue). Pretreatment of rats with ARI at 30 mg/kg (2.16 ± 0.57 µmol/g tissue) and 100 mg/kg (2.17 ± 0.87 µmol/g tissue) significantly increased the gastric mucosal NP-SH levels (ANOVA F = 11.33 P < 0.001). ARI in low dose (10 mg/kg) though increased the NP-SH levels (1.92 ± 0.90), as compared to ethanol alone treated rats, the change was statistically insignificant (**Table 3**).

Effect of ARI on ethanol-induced changes in the levels myeloperoxidase (MPO) activity of gastric mucosa

Changes in gastric accumulation of neutrophils following ethanol induced gastric lesions were assessed by measurement of MPO activity. Administration of ethanol resulted in a significant increase in the levels of MPO (7.28 ± 0.75 Δ/g tissue) as compared to the levels in the control animals (1.6 ± 0.29 Δ/g tissue). Pretreatment with ARI at 30 mg/kg (4.66 ± 0.64 Δ/g tissue) and 100 mg/kg (4.09 ± 0.79 Δ/g tissue) significantly attenuated ethanol induced increase in gastric MPO activity (ANOVA F = 12.79 P < 0.001). However the gastric MPO

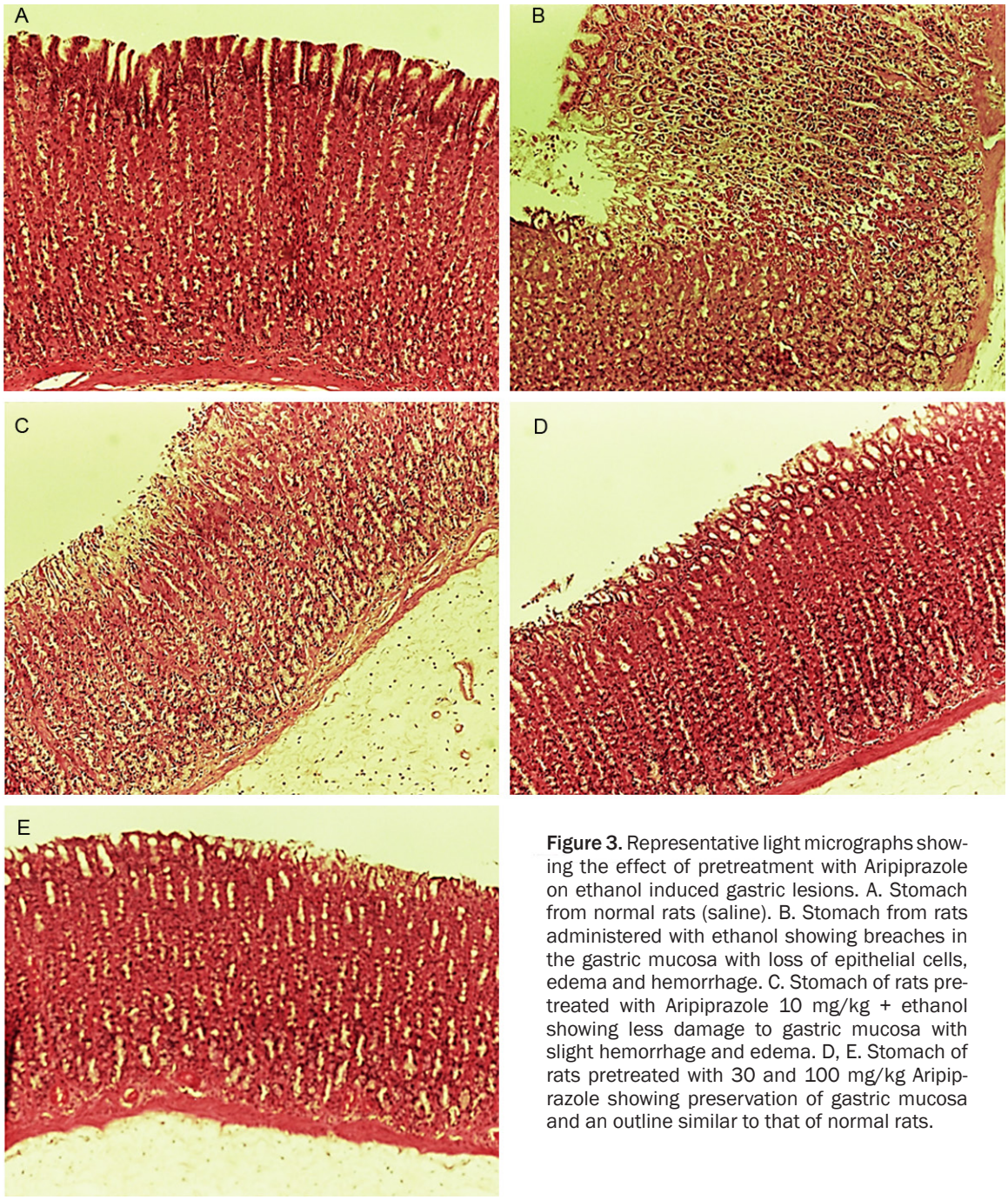

Figure 3. Representative light micrographs showing the effect of pretreatment with Aripiprazole on ethanol induced gastric lesions. A. Stomach from normal rats (saline). B. Stomach from rats administered with ethanol showing breaches in the gastric mucosa with loss of epithelial cells, edema and hemorrhage. C. Stomach of rats pretreated with Aripiprazole 10 mg/kg + ethanol showing less damage to gastric mucosa with slight hemorrhage and edema. D, E. Stomach of rats pretreated with 30 and 100 mg/kg Aripiprazole showing preservation of gastric mucosa and an outline similar to that of normal rats.

activity in the low dose ARI treated rats (10 mg/kg) was less than ethanol treated rats but the levels were not statistically significant (**Table 3**).

Effect of ARI on ethanol-induced changes in the gastric serotonin levels

The effect of ARI on ethanol induced changes in the gastric serotonin levels is given in **Figure 4**. Administration of ethanol resulted in a significant decrease in the gastric serotonin levels (0.133 ± 0.04 µg/g tissue) as compared to the levels in control animals (1.430 ± 0.16 µg/g tissue). Pretreatment with ARI at 30 mg/kg (0.98 ± 0.12 µg/g tissue) and 100 mg/kg (1.38 ± 0.12 µg/g tissue) resulted in a significant inhibition of the ethanol induced decrease of serotonin levels (ANOVA $F = 8.96$ $P < 0.001$). However ARI at low doses did not produce a statistically significant change in the levels of

Table 3. Effect of Aripiprazole (ARI) on ethanol induced changes in Myeloperoxidase, NP-Sulfhydryl groups and Malondialdehyde levels in gastric mucosa of rats

Treatment	MPO A/g tissue Δ	NP-SH µmol/g tissue	MDA Nano mol/g tissue
Normal	1.60 ± 0.29	2.56 ± 0.13	139.04 ± 524
Ethanol alone (Etoh)	7.28 ± 0.75#	1.64 ± 0.61#	168.58 ± 4.05#
ARI 10 mg + EtOH	6.93 ± 1.01	1.92 ± 0.90	154.60 ± 1.97
ARI 30 mg + EtOH	4.66 ± 0.64*	2.16 ± 0.57*	148.07 ± 3.69**
ARI 100 mg + EtOH	4.09 ± 0.79**	2.17 ± 0.88**	141.80 ± 6.47**

Values are means ± standard error of mean #P < 0.001 as compared with control group and *P < 0.05 **P < 0.009 as compared with Ethanol (ulcer only) group using Dunnett's multiple comparison test.

gastric serotonin levels as compared to ethanol alone treated animals (**Figure 4**).

Discussion

The present investigation was undertaken to assess the gastro protective potential of aripiprazole a novel antipsychotic drug against ethanol induced gastric ulceration in rats. The results of this study suggest that ARI has significant protective action against alcohol induced gastric ulcers. The gastroprotective effect of ARI was associated with the regulation of gastric secretion and protection of gastric mucosa against ethanol induced oxidative stress and suppression of inflammation by inhibiting the activation of neutrophils. The protective action of aripiprazole may also be due to the attenuation in the levels of ethanol induced decrease in the serotonin levels of the stomach.

Pretreatment with ARI significantly reduced the gastric secretion and acidity in pylorus ligated shay rats (**Table 1**). Our results differ from earlier results of Saxena et al. [5] who failed to observe any change in the gastric secretion in pylorus ligated and stress induced ulcers upon treatment with another atypical antipsychotic drug risperidone. While another recent report [33] showed ziprasidone also an atypical antipsychotic and a dopamine receptor antagonist to enhance indomethacin induced ulcers in pylorus ligated rats by increasing the gastric secretion, and peptic activity. On the contrary bromocriptine a dopamine agonist did not affect the gastric output in pylorus ligated rats [16] The observed reduction in gastric secretion and acidity in our study may be assigned to the distinct characteristics of aripiprazole which acts as a partial dopamine D2 and 5HT1A agonist and 5HT 2 A receptor antagonist [23]. Dopamine D2 receptors play a protective role in the stomach, and activation of these receptors results in a long-term inhibitory effect on induced gastric secretion. This long lasting effect on gastric secretion may be due to the extensive distribution of dopamine D2 receptors in the central nervous system and/or in the gut, as well as in the enteric nervous system and non-neuronal membranes. On the contrary dopamine D1 receptors are considered as a prerequisite for a reduction in stress induced gastric lesions, while D2 receptors agonists are inactive in stress induced gastric ulcerogenesis [34].

Regulation of serotonin levels in the gastric tissue is another possible mechanism through which gastric secretion is influenced. Serotonin is extensively distributed in the enterochromaffin cells of the gastric mucosa and enteric neurons of the gastrointestinal tract [35]. It is an important neurotransmitter in the gastrointestinal system and helps in the regulation of mucus secreting process [36], gastric acid secretion [11, 37], motility and blood flow to the mucosa [38]. Serotonin levels in the gastrointestinal tissue are decreased in ulcerated conditions. The severity of gastric ulceration was observed to increase with a decrease in the level of gastric serotonin levels and enterochromaffin cell density in different models of ulcers [10, 36, 39, 40]. Serotonin inhibits gastric acidity by increasing the gastric mucus secretion [36]. Agents that enhance gastric serotonin levels help in the reduction of gastric secretion and pepsin output in rat stomach [9, 11]. The inhibitory action of ARI on gastric secretion in our study may be assigned to its stabilization action on serotonin and also due to its potential to stabilize dopamine serotonin balance [41, 42], which is reflected in the restoration of the ethanol induced depletion of gastric serotonin (**Figure 4**) and dopamine levels in the cortex of the ethanol treated rats (data not shown).

Dopamine regulates gastric function through the brain gut axis, and in ulcer pathogenesis dopamine appears to act through both periph-

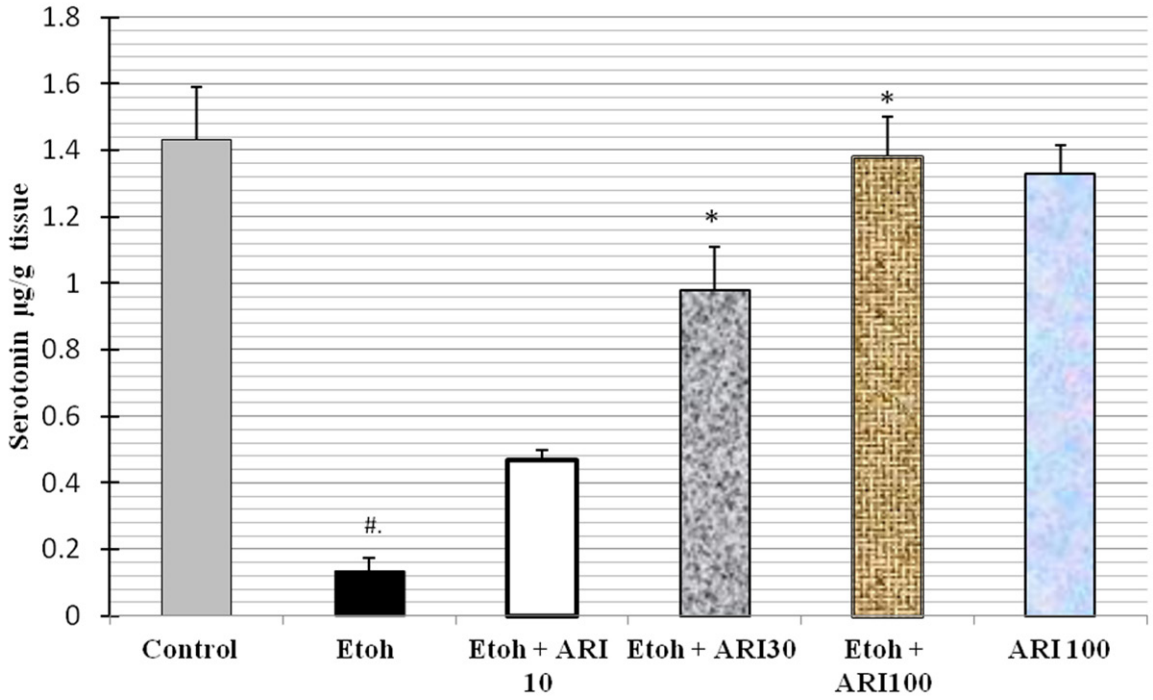

Figure 4. Effect of Aripiprazole (ARI) on ethanol induced changes in serotonin levels of the glandular stomach of rats. Values are means ± standard error of mean. $^{\#}P < 0.001$ as compared with control group and $^{*}P < 0.001$ as compared with Ethanol (ulcer only) group using Dunnett's multiple comparison test. Control-Normal rats; Etoh-Ethanol alone; Etoh + Ari 10, 30, 100 - Ethanol + Aripiprazole 10 mg, 30 mg, and 100 mg/kg.

eral and central components [43]. Dopamine receptors are extensively distributed in the gastro intestinal tract signifying the importance of dopamine in the gastrointestinal function [44, 45]. Endogenous dopamine produced in normal stomach tissue is responsible for control of a wide range of physiological functions including reducing gastric acid secretion and stimulating bicarbonate and mucus secretion [46-48]. These actions are mediated through dopamine receptors. Dopamine D2 receptors have been suggested to have a protective role in gastric cancer [49], as well as in the regulation of gastric acid secretion [14]. Carbachol, pentagastrin and histamine induced gastric secretion were inhibited by a selective D2 receptor agonist quinpirole [14], confirming the strong role of dopamine D2 receptors in the regulation of gastric secretion. Chemically induced ulcers were also dose and time dependently prevented by dopamine D2 agonists [50]. Aripiprazole possesses unique pharmacological characteristics. It acts as a partial agonist as opposed to an antagonist or inverse agonist at the dopamine D2 receptor [51]. ARI's partial agonism stabilizes dopamine D2 receptor mediated neurotransmission: in hyper dopaminergic states ARI behaves more like an antagonist blocking the effects of increased dopamine levels while in hypo dopaminergic state it produces agonist effects [52, 53]. Therefore the gastroprotective action of ARI may also be assigned to its regulatory effect on gastric secretion through its unique pharmacological properties.

Oral administration of ethanol resulted in ulcerative gastric lesion formation in the glandular part of mucosa and a significant reduction in the levels of gastric wall mucus (**Figures 1**, **2**; **Table 2**). Exposure to ulcerogens such as aspirin, alcohol, and non-steroidal inflammatory substances (NSAIDs), results in the thinning of the protective mucus gel and the phospholipids layer, leading to acid back diffusion and mucosal injury [54, 55]. Any breach of the gastric mucosal defense may lead to ulceration even at the normal rate of acid secretion [56]. On the contrary, agents that restore the mucosal defense capacity exert a protective effect against ulcerogens induced gastrointestinal lesions [3, 4, 57, 58]. ARI pretreatment not only

reduced gastric secretion and acidity but also shielded the gastric mucosa against ethanol induced gastric lesions by restoring the reduced levels of gastric wall mucus (**Table 2**). Increased secretion of mucus by gastric mucosal cells helps to prevent gastric ulcer by better buffering of the acidic gastric secretions, by reducing stomach wall friction during peristalsis and gastric contraction [54], and by assisting in the repair of the damaged gastric epithelium [59]. Epithelial cells of the gastric mucosa secrete mucus gel produced by mucin (MUC) genes [56]. Mucin and Zone Occludes -1 (Zo-1) a trans-membrane protein that preserves the tight junctions' integrity are the indicators of mucosal epithelial integrity [57]. A decrease in their levels has been associated with mucosal damage in an animal model of inflammatory bowel disease (IBD) [60].

ARI was effective in augmenting the protective mechanism of the gastric mucosa and the restoration of the damaged histological structure (**Table 2**; **Figure 3**). This may be due to its effect on 5HT and dopamine levels. Penissi et al. [9] have demonstrated that dehydroleucodine, a sesquiterpene lactone prevents ethanol induced damage to the gastric mucosa by preventing the depletion of gastric dopamine and 5 HT levels. Dehydroleucodine was suggested to act as a cell stabilizer by inhibiting the degranulation of monoamines containing cells and help in the secretion of abundant mucoid blanket that acts as a diffusion barrier against ethanol induced damage of gastric mucosa [9]. ARI has also been confirmed as a dopamine system stabilizer [61]; in addition to its potential to stabilize dopamine serotonin balance [41]. Therefore, the cytoprotective effect of ARI against ethanol induced gastric lesions may be ascribed in part to its stabilization action. This is further supported by the results of histological studies (**Figure 3**). Ethanol induced changes in the gastric mucosa such as loss of glandular cells, interruption of epithelium and edema were all reduced in ARI treated groups and showed a better histological architecture with a more compact mucosa, less inflammation and hemorrhage (**Figure 3C-E**). ARI and other atypical antipsychotics such as clozapine and risperidone have anti-inflammatory action and reduce serum levels of IL-2, IL-6 and tumor necrosis factor (TNFα), all of which play important roles in the mucosal damage [62]. Therefore, the gastro protective effect of ARI against ethanol induced gastric damage may be due to the replenishment of gastric mucosal levels and its anti-inflammatory action.

The specific mechanism of gastric ulceration is not very clear, but the gastric mucosal injury induced by ethanol has been associated with enhanced oxidative stress and depletion of antioxidants [2, 3, 63-65]. MDA is a major oxidative marker for lipid peroxidation, while sulfhydryls are considered the first line of defense in cytoprotection against free radicals induced damage. Our observation of a significant increase in the MDA levels following ethanol administration (**Table 3**) is in agreement with earlier studies [2, 3] and suggests a massive release of reactive oxygen species (ROS). The release of ROS plays a significant role in ethanol induced gastric mucosal damage [2, 64, 66]. On the other hand, ARI is a potent antioxidant. Both in vitro and in vivo studies have shown ARI to possess potent antioxidant properties. The protective effect of ARI in animal models of depression and neurodegenerative diseases has been linked with decrease of oxidative stress and an increase in the level of antioxidants [20, 67], which are evident from the results of this study. Treatment of rats with ARI resulted in a significant depletion of the ethanol induced increased MDA levels and restoration of the decreased NP-SH levels of the gastric tissue (**Table 3**). Non-protein sulfhydryls are endogenous anti-oxidants and protect the cells by different mechanisms, primarily by limiting the production of free radicals [68, 69]. Secondarily, they protect the gastric mucous by strengthening the disulfide bridges of its subunits. A decrease in these bridges reduces mucus thickness and makes it more vulnerable to harmful agents [70]. Changes in the SH levels may be crucial for ulcer formation, given that they help in the preservation of mucosal integrity, and their reduced levels in the gastric mucosa result in the formation of gastric ulcers. Moreover, depletion in glutathione levels results in a significant increase of ulcerogens induced gastric mucosal damage [2, 5], while compounds that augment the mucosal NP-SH levels have gastro protective effects [2, 63]. The cytoprotective effects of ARI against ethanol induced gastric lesions may therefore be attributed to the direct antioxidant and free radical scavenging activity of ARI and also to the augmentation of the NP-SH levels (**Table 3**).

Besides generation of free radicals, infiltration of inflammatory cells (neutrophils, macrophages) also plays a major role in ethanol induced gastric ulcers. Accumulation of neutrophils may lead to microcirculatory disturbances and induce ischemia by occluding microvessels [71]. Activated neutrophils also release many injurious factors that include free radicals, metabolites and proteolytic enzymes [72]. MPO, which is expressed in neutrophils, is considered as a reliable indicator for neutrophil infiltration and inflammation in the gastric mucosa [2, 73]. We observed a significant increase in gastric mucosal MPO activity after treatment with ethanol, indicating an increase in the infiltration of activated neutrophils. On the other hand, treatment with ARI had a significant attenuating effect on ethanol induced increase in MPO activity (**Table 3**). A decrease in the MPO activity along with the reduction in the gastric lesions in ARI pretreated rats suggest that the anti-ulcerogenic effect of ARI may also be due to its anti-inflammatory action [22].

In conclusion, the results of the present study suggest that acute ARI has significant gastro protective effects against ethanol induced gastric ulcers. The protective effects were associated with reduced gastric secretion, oxidative stress, neutrophil activity and gastric mucosal breakdown. These protective actions may also be ascribed to the modulating and stabilizing effect of ARI on the serotonin and dopamine levels. Our study is limited in the use of a single dose of ARI, whereas ARI is generally used for a long term. Further studies are warranted to determine the effects of chronic use of ARI in gastric ulcers.

Acknowledgements

The authors are thankful to the Prince Sultan Military Medical City and the Medical Services Department, Ministry of Defense, Saudi Arabia for their encouragement and support. The authors also thank Ms. Biju Prasad and Mr. Dhaya Sankar, for their technical assistance.

Disclosure of conflict of interest

None.

Address correspondence to: Abdulrahman Al Asmari, Research Center, Prince Sultan Military Medical City, P.O. Box 7897 (S- 775), Riyadh 11159, Saudi Arabia. Tel: 966-11-4786601; Fax: 966-11-4786601; E-mail: abdulrahman.alasmari@gmail.com

References

[1] Sumbul S, Ahmad MA, Mohd A, Mohd A. Role of phenolic compounds in peptic ulcer: An overview. J Pharm Bioallied Sci 2011 Jul; 3: 361-7.

[2] Al Asmari AK, Al Omani S, Elfaki I, Tariq M, Al Malki A, Al Asmari S. Gastric antisecretory and antiulcer activity of bovine hemoglobin. World J Gastroenterol 2013; 21: 3291-3299.

[3] Asmari AA, Omani SA, Otaibi MA, Abdulaaly AA, Elfaki I, Yahya KA, Arshaduddin M. Gastroprotective effect of minocycline in experimentally induced gastric ulcers in rats. Int J Clin Exp Med 2014 Mar 15; 7: 586-96.

[4] Ghosh P, Bodhankar S. Application of polymerase chain reaction for determination of prevalence of Helicobacter pylori in salivary samples of asymptomatic subjects. Asian J Pharm Clin Res 2011; 4: 21-24.

[5] Saxena B, Krishnamurthy S, Singh S. Gastroprotective potential of risperidone, an atypical antipsychotic, against stress and pyloric ligation induced gastric lesions. Chem Biol Interact 2011; 190: 155-164.

[6] Abdel-Sater KA, Abdel-Daiem WM, Sayyed Bakheet M. The gender difference of selective serotonin reuptake inhibitor, fluoxetine in adult rats with stress-induced gastric ulcer, Eur J Pharmacol 2012; 688: 42-8.

[7] Yeomans ND. The ulcer sleuths: The search for the cause of peptic ulcers. J Astroenterol Hepatol 2011 Jan; 26 Suppl 1: 35-41.

[8] Kaise M, Echizen H, Umeda N, Ishizaki T. Catecholamine concentration in biopsied gastroduodenal tissue specimens of patients with duodenal ulcer. Dig Dis Sci 1993 Oct; 38: 1866-73.

[9] Penissi AB, Fogal TH, Guzman JA, Piezzi RS. Gastroduodenal mucosal protection induced by dehydroleucodine: mucus secretion and role of monoamines. Dig Dis Sci 1998 Apr; 43: 791-8.

[10] Debnath S, Guha D. Role of Moringa oleifera on enterochromaffin cell count and serotonin content of experimental ulcer model. Indian J Exp Bio 2007; 45: 726-731.

[11] Lai YC, Ho Y, Huang KH, Tsai LH. Effects of erotonin on acid secretion in isolated rat stomach: the role of $5HT_3$ receptors. Chinese J Physiol 2009; 52 Suppl: 395-405.

[12] Ji CX, Fan DS, Li W, Guo L, Liang ZL, Xu RM, Zhang JJ. Evaluation of the anti-ulcerogenic activity of the antidepressants duloxetine, ami-

triptyline, fluoxetine and mirtazapine in different models of experimental gastric ulcer in rats. Eur J Pharmacol 2012 Sep 15; 691: 46-51.
[13] Hoogerwerf WA, Pasricha PJ. Pharmacotherapy of gastric acidity, peptic ulcers and gastroesophageal reflux disease. In: Brunton L editor. Goodman and Gilman's the Pharmacological Basis of Therapeutics. New York: Mc Graw-Hill; 2006. pp. 967-981.
[14] Eliassi A, Aleali F and Ghasemi T. Peripheral Dopamine D2-Like Receptors have a Regulatory Effect on Carbachol-, Histamine- and Pentagastrin-Stimulated Gastric Acid Secretion. Clin Exp Pharmacol Physiol 2008; 35: 1065-1070.
[15] Dursun H, Bilici M, Albayrak F, Ozturk C, Saglam MB, Alp H, Suleyman H. Antiulcer activity of fluvoxamine in rats and its effect on oxidant and antioxidant parameters in stomach tissue. BMC Gastroenterol 2009; 9: 36.
[16] Popovic M, Janicijevic-Hudomal S, Kaurinovic B, Rasic J, Trivic S. Antioxidant Effects of Some Drugs on Ethanol-induced Ulcers. Molecules 2009; 14: 816-826.
[17] Keck PE Jr, McElroy SL. Aripiprazole: a partial dopamine D2 Receptor agonist antipsychotic. Expert Opin Invest Drugs 2003; 12: 655-662.
[18] Fountoulakis KN, Vieta E, Schmidt F. Aripiprazole monotherapy in the treatment of bipolar disorder: a meta-analysis. J Affect Disorder 2011; 133: 361-370.
[19] Yildiz A, Vieta E, Leucht S, Baldessarini RJ. Efficacy of antimanic treatments: meta-analysis of randomized, controlled trials. Neuropsychopharmacology 2011; 36: 375-389.
[20] Eren I, Naziroglu M, Demirdas A. Protective Effects of Lamotrigine, Aripiprazole and Escitalopram on Depression-induced Oxidative Stress in Rat Brain. Neurochem Res 2007; 32: 1188-1195.
[21] Koprivica V, Regardie K, Wolff C, Fernalld R, Murphy JJ, Kambayashi J, Kikuchi T, Jordan S. Aripiprazole protects cortical neurons from glutamate toxicity. Eur J Pharmacol 2011 Jan 25; 651: 73-6.
[22] Kato T, Mizoguchi Y, Monji A, Horikawa H, Suzuki SO, Seki Y, Iwaki T, Hashioka S, Kanba S. Inhibitory effects of aripiprazole on interferon-gamma-induced microglial activation via intracellular Ca2+ regulation in vitro. J Neurochem 2008 Jul; 106: 815-25.
[23] Khanna P, Suo T, Komossa K, Ma H, Rummel-Kluge C, El-Sayeh HG, Leucht S, Xia J. Aripiprazole versus other atypical antipsychotics for schizophrenia. Cochrane Database Syst Rev 2014 Jan 2; 1: CD006569.
[24] Bhati MT. What makes aripiprazole the 'different' antipsychotic. Current Psychiatry 2005; 4: 51-60.
[25] Shay H, Kumarov SA, Fels SA, Meraze D, Gruenstein M, Siplet H. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology 1945; 5: 43-61.
[26] Natale G, Lazzeri G, Blandizzi C, Gherardi G, Lenzi P, Pellegrini A, Del Tacca M. Seriate histomorphometry of whole rat stomach: an accurate and reliable method of quantitative analysis of mucosal damage. Toxicol Appl Pharmacol 2001; 174: 17-26.
[27] Schiantarelli P, Cadel S, Folco GS. Gastroprotective effects of morniflumate, an esterified anti inflammatory drug. Arzneimittelforschung 1984; 34: 885-890.
[28] Corne SJ, Morrisey SM, Woods RJ. A method for the quantitative estimation of gastric barrier mucus. J Physiol 1974; 242: 116-17.
[29] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.
[30] Sedlak J, Lindsay RH. Estimation of total protein bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968; 25: 192-5.
[31] Bradley PP, Erdman SH, Christensen RD, Rothstein G. Supply and release of storage neutrophils, a developmental study. Biol Neonate 1984; 41: 132-7.
[32] Patrick SL, Thompson TL, Walker JM, Patrick RL. Concomitant sensitization of amphetamine-induced behavioral stimulation and in vivo dopamine release from rat caudate nucleus. Brain Res 1991 Jan 11; 538: 343-6.
[33] El-Awdan SA, Zaki HF, Abdel Salam OM, El-Iraqy WI and Kenawy SA. Impact of the Dopaminergic System on Mucosal Integrity in Indomethacin-induced Gastric Ulcers in Rats: Possible Modulation by Ranitidine or L-carnitine. Pharmacologia 2013; 4: 22-33.
[34] Glavin GB. Central dopamine involvement in experimental gastrointestinal injury. Prog Neuropsychopharmacol Biol Psychiatry 1992 Mar; 16: 217-21.
[35] Beattie DT and Smith JA. Serotonin pharmacology in the gastrointestinal tract: a review. Naunyn Schmiedebergs Arch Pharmacol 2008; 377: 181-203.
[36] Guha D, Ghosh A. Effect of cerebellar nodulation on gastro duodenal PGE_2 and 5-HT content of rat. Indian J Exp Biol 1995; 33: 361-4.
[37] Coleman NS, Marciani L, Blackshaw E, Wright J, Parker M, Yano T, Yamazaki S, Chan PQ, Wilde K, Gowland PA, Perkins AC and Spiller RC. Effect of a novel 5-HT_3 receptor agonist MKC-733 on upper gastrointestinal motility in humans. Aliment Pharmacol Ther 2003; 18: 1039-1048.
[38] Lepard KJ, Chi J, Mohammed JR, Gidener S and Stephens RL Jr. Gastric antisecretory effect of serotonin: quantitation of release and

site of action. Am J Physiol 1996; 271: E669-E677.
[39] Guha D, Maiti AK. Gastric pathology produced by posterior cerebellar lesion in rats. Indian J Physiol & Allied Sc 1978; 32: 25.
[40] Debnath S, Biswas D, Ray K, Guha D. Moringa oleifera induced potentiation of serotonin release by 5-HT_3 receptors in experimental ulcer model. Phytomedicine 2011; 18: 91-95.
[41] Burris KD, Molski TF, Xu C, Ryan E, Tottori K, Kijuchi T, Yocca FD, Molinoff PB. Aripiprazole, a novel antipsychotic, is a high-affinity partial agonist at human dopamine D2 receptors. J Pharmacol Exp Ther 2002; 302: 381-389.
[42] Shibasaki M, Kurokawa K, Mizuno K and Ohkuma S. Effect of Aripiprazole on Anxiety Associated with Ethanol Physical Dependence and on Ethanol-Induced Place Preference. J Pharmacol Sci 2012; 118: 215-224.
[43] Flemstrom G, Safsten B, Jedstedt G. Stimulation of mucosal alkaline secretion of rat doudedum by dopamine and dopaminergic compounds. Gastroenterology 1993 Mar; 104: 825-33.
[44] Vaughan CJ, Aherne AM, Lane E, Power O, Carey RM, O'Connell DP. Identification and regional distribution of the dopamine D (1A) receptor in the gastrointestinal tract. Am J Physiol Regul Integr Comp Psysiol 2000; 279: R599-609.
[45] Baglole CJ, Davison JS, Meddings JB. Epithelial distribution of neural receptors in the guinea pig small intestine. Can J Physiol Pharmacol 2005; 83: 389-9.
[46] Eisenhofer G, Aneman A, Friberg P, Hooper D, Fåndriks L, Lonroth H, Hunyady B, Mezey E. Substantial production of dopamine in human gastrointestinal tract. J Clin Endocrinol Metab 1997; 82: 3864-71.
[47] Missale C, Nash R, Robinson S, Jaber M, Caron M. Dopamine receptors: From structure to function. Physiol Rev 1998; 78: 189-225.
[48] Mezey E, Eisenhofer G, Hansson S, Hunyady B, Hoffman B. Dopamine produced by the stomach may act as a paracrine/autocrine hormone in the rat. Neuroendocrinology 1998; 67: 336-48.
[49] Basu S, Dasgupta PS. Decreased dopamine receptor expression and its second-messenger cAMP in malignant human colon tissue. Dig Dis Sci 1999 May; 44: 916-21.
[50] Szabo S. Dopamine disorder in duodenal ulceration. Lancet 1979; 2: 880-2.
[51] Kessler R. Aripiprazole: What is the role of dopamine D(2) receptor partial agonism? Am J Psychiatry 2007 Sep; 164: 1310-2.
[52] Davies MA, Sheffler DJ, Roth BL. Aripiprazole: A novel atypical antipsychotic drug with a uniquely robust pharmacology. CNS Drug Rev 2004 Winter; 10: 317-36.
[53] Natesan S, Reckless G, Nobrega J, Fletcher P, Kapur S. Dissociation between in vivo occupancy and functional antagonism of dopamine D_2 receptors: comparing aripiprazole to other antipsychotics in animal models. Neuropsychopharmacology 2006; 31: 1854-1863.
[54] Alqasoumi S, Al-Howiriny TA, Al-Yahya M, Rafatulla S. Gastroprotective effects of radish "raphanus sativus" L. on experimental gastric ulcer models in rats. Farmacia 2008; 46: 204-214.
[55] Allen A. Flemstrom G, Gastroduodenal Mucus bicarbonate barrier: protection against acid and pepsin. Am J Physiol Cell Physiol 2005; 288: C1-C19.
[56] Laine L, Weinstein WM. Histology of alcoholic haemorrhagic gastritis; a prospective evaluation. Gastroenterology 2008; 94: 1254-64.
[57] Wallace JL, Whittle BJ. Role of mucus in the repair of gastric epithelial damage in the rat. Inhibition of epithelial recovery by mucolytic agents. Gastroenterology 1986; 91: 603-611.
[58] Glavin GB, Hall AM. Central and peripheral dopamine D1/DA1 receptor modulation of gastric secretion and experimental gastric mucosal injury. Gen Pharmacol 1995; 6: 1277-9.
[59] Tripathi KD. Essentials of medical pharmacology. 6th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2008. pp. 597.
[60] Garrido-Mesa N, Utrilla P, Comalada M, Zorilla P, Garrido-Mesa J, Zarzuelo A, Rodríguez-Cabezas ME, Gálvez J. The association of minocycline and the probiotic Escherichia coli Nissle 1917 results in an additive beneficial effect in a DSS model of reactivated colitis in mice. Biochem Pharmacol 2011; 82: 1891-1900.
[61] Lawler CP, Priopleau C, lewis MM, Mak C, Jiang D, Schetz A, Gonzalex AM, Sibley DR, Mailman RB. Interactions of the novel antipsychotic aripiprazole (OPC-14597) with dopamine and serotonin receptor subtypes. Neuropsychopharmacology 1999; 20: 612-627.
[62] Wallace JL. Gastric ulceration: critical events at the neutrophil-endothelium interface. Can U Physiol Pharmacol 1992; 71: 98-102.
[63] Tariq M, Khan HA, Elfaki I, Arshaduddin M, Al Moutaery M, Al Rayes H, Al Swailam R. Gastric antisecretory and antiulcer effects of simvastatin in rats. J Gastroenterol Hepatol 2007 Dec; 22: 2316-23.
[64] Hsu DZ, Pei-Yi Chu, Ming-Yie Liu. Effect of sesame oil on acidified ethanol-induced gastric mucosal injury in rats. JPEN J Parenter Enteral Nutr 2009 Jul-Aug; 33: 423-7.
[65] Al Moutaery M, Al Rayes H, Al Swailam R, Elfaki I, Arshaduddin M, Tariq M. Protective effect of a cysteine prodrug and antioxidant, L-2-oxothiazolidine-4-carboxylate, against ethanol-in-

duced gastric lesions in rats. Exp Toxicol Pathol 2012; 64: 233-237.
[66] Mahmood AA, Al-Bayaty FH, Salmah I, Nor Syuhada AB, Harita H, Mughrabi FF. Enhancement of gastric ulcer by Areca catechu nut in ethanol-induced gastric mucosal injuries in rats. J Med Plant Res Vol 2011; 5: 2562-2569.
[67] Miljevic C, Nikolic-Kokic A, Nikolic M, Niketic V, Spasic MB, Lecic-Tosevski D, Blagojevic D. Effect of atypical antipsychotics on antioxidant enzyme activities in human erythrocytes (in vitro study). Hum Psychopharmacology 2013 Jan; 28: 1-6.
[68] Pihan C, Regillo C, Szabo S. Free radicals and lipid peroxidation in ethanol- or aspirin-induced gastric mucosal injury. Dig Sci 1987; 32: 1395-401.
[69] Wakulich CA, Tepperman BL. Role of glutathione in nitric oxide-mediated injury to rat gastric mucosal cells. Eur J Pharmacol 1997 Jan 29; 319: 333-41.
[70] La Casa C, Villegas I, Alarcon de la Lastra C, Motilva V, Martin Calero MJ. Evidence for protective and antioxidant properties of rutin, a natural flavones, against ethanol induced gastric lesions. J Ethnopharmacol 2000; 71: 45-53.
[71] Kitahora T, Guth PH. Effect of aspirin plus hydrochloric acid on the gastric mucosal microcirculation. Gastroenterology 1987; 93: 810-817.
[72] Kobayashi T, Ohta Y, Yoshino J, Nakazawa S. Teprenone promotes the healing of acetic acid-induced chronic gastric ulcers in rats by inhibiting neutrophil infiltration and lipid peroxidation in ulcerated gastric tissues. Pharmacol Res 2001 Jan; 43: 23-30.
[73] Seo CS, Lee MY, Shin IS, Jeon WY, Ha H, Huh JI, Seo CS, Shin HK. Protective effect of Bojungikki-tang, a traditional herbal formula, against alcohol-induced gastric injury in rats. J Ethnopharmacol 2012; 142: 346-353.