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Comparative histological and histochemical studies between ranitidine and nizatidine in treatment of peptic ulcer with evaluation of their adverse effects on male sex hormones

Ahmed S. Alazzouni[1*], Elsayed A. Abdel Aziz[2], Sameh Elnabtity[2] and Areej I. Salem[2]

Abstract

Background: Peptic ulcer is an excoriated area of stomach or intestinal mucosa. Two experimental designs were proceeded: the first aimed. on twenty adult male albino rats, used to study the protective effect of both ranitidine and nizatidine; on the second, including sixty adult male albino rats, was used to study the therapeutic effect of ranitidine and nizatidine after induction of ulcer and also to evaluate the adverse effects of therapeutic doses of H2-receptor antagonists on male hormonal profile. The study aims to assess the gastroprotective effects of nizatidine and ranitidine and on treating of non-steroidal anti-inflammatory drugs (NSAIDs) induced peptic ulcer and to evaluate its adverse effect on male sex hormones.

Result: The result revealed that ranitidine and nizatidine reduced incidence of ulceration. Histopathological findings showed a significant recovery of the alteration, and disturbance in male sex hormones.

Conclusion: Nizatidine is better than ranitidine in the management of NSAIDs induced peptic ulcer in rats.

Keywords: H2-receptor, Antagonists, Peptic ulcer, Male sex hormones, Nizatidine, Ranitidine, NSAIDs, Gastroprotective, H2 blocker

Introduction

Peptic ulcer is a suffering of the gastrointestinal tract (GIT), which includes both GIT and duodenal ulcers.

Ulcers are lesions penetrating through the entire thickness of GIT mucosa and muscularis mucosa (Amandeep et al., 2012) (Fig. 1). One of its major complications was the causes of morbidity and mortality (Sung et al., 2010).

Peptic ulcer develops when there is a disturbance between the "aggressive" and "protective" factors at the luminal surface epithelium. Aggressive factors include *Helicobacter pylori*, as half of the world's population reported to be colonized by *H. pylori*, that remains one of the most common causes of the peptic ulcer disease, hydrochloric acid (HCl), pepsins, NSAIDs, bile acids, alcohol, smoking and hypoxia (Siddique et al., 2018). On the other hand, protective factors, which affect a significant variety of individuals worldwide (Harold et al., 2007).

In general, NSAIDs are used for the analgesic, anti-inflammatory, and antipyretic properties and are usually prescribed for the treatment of assorted diseases like rheumatoid and osteoarthritis. As a side effect, gastric ulcer may develop in 35-60% of patients (Hawkey, 2000), and its action is mediated by inhibition of the synthesis of prostaglandins, cyclo-oxygenases (cox), and leukotriene (Yamada et al., 1993 and Gambero et al., 2005).

* Correspondence: drahmedalazzouni@gmail.com; drahmedalazzouni@science.helwan.edu.eg
[1]Zoology Department, Faculty of Science, Helwan University, Cairo, Egypt
Full list of author information is available at the end of the article

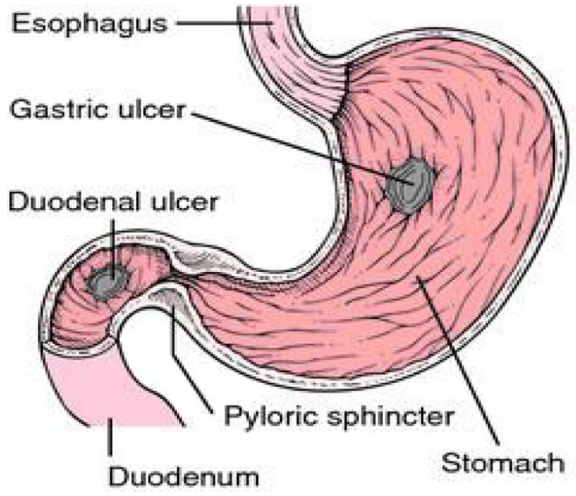


Fig. 1 Peptic ulcer, human stomach (Prajapati et al., 2014)

Ulcers associated with the NSAIDs remain a major problem, which has not been resolved through introduction of selective inhibitors of cyclo-oxygenase (COX) blocker (Wallace, 2005). Stomachic pain, mucosal erosion, and ulceration are created by most NSAIDs to varying extents, but relative stomachic toxicity is the major consideration (Werawatganon et al., 2014 & Fong et al., 2015).

The usual medical treatment for peptic ulceration is either by the inhibition of HCL acid secretion or by the neutralization of the acid. Many categories of pharmacological agents have well-tried to be effective within the management of the acid peptic disorders (Katzung, 2004 & Waller et al., 2005).

H2-receptor antagonists are most generally prescribed drugs for NSAIDs induced stomachic lesions (Patrignani et al., 2011). H2-receptor antagonists (nizatidine and ranitidine) are types of antihistaminic drugs (Panula et al., 2015), known as H2 blockers, because they form a category of medications that block the action of histamine at the histamine H2 receptors of the parietal or oxyntic cells in the stomach. This decreases the production of stomach acid. They are used in the treatment of peptic ulcer disease and gastroesophageal reflux disease (Eriksson et al., 1995).

In male rats, the administration of histamine dose and time dependently increased plasma hormones (GnRH, FSH, LH) and testosterone levels (Niaz et al., 2018).

LH and FSH are secreted under the hypothalamic control of gonadotropin-releasing hormone (GnRH). Leydig-cells are located between seminiferous tubules of the testis secrete testosterone under the control of LH, so they are called interstitial cell-stimulating hormones. Testosterone stimulates the development and maturation of germ cells in seminiferous tubules. FSH acts directly on the seminiferous tubules. Sertoli cells possess receptors for androgen and FSH. So, testosterone and FSH have trophic effects on gametogenesis that are mediated via somatic Sertoli cells. The testis and the hypothalamo-pituitary axis are regulated through hormones. Androgen inhibits the secretion of GnRH and gonadotropins. Inhibin has a negative feedback effect on the release of FSH from the pituitary gland (Nieschlag et al., 2008).

The present work aims to evaluate the gastroprotective effect of two antiulcer drugs (nizatidine and ranitidine as H2-receptor antagonists) and their ability to treat of NSAIDs-induced peptic ulcer in adult male albino rats. It also assesses the possible adverse effects of therapeutic doses on hormonal profiles of male albino rats (follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), total testosterone (T-TESTO), and free testosterone (F-TESTO)).

Experimental animals

Adult male albino rats weighing around 180-200 g, obtained from the Faculty of Veterinary Medicine-Zagazig

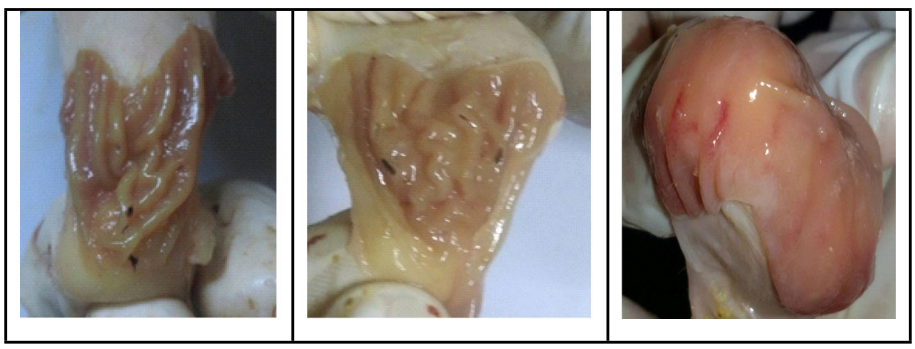
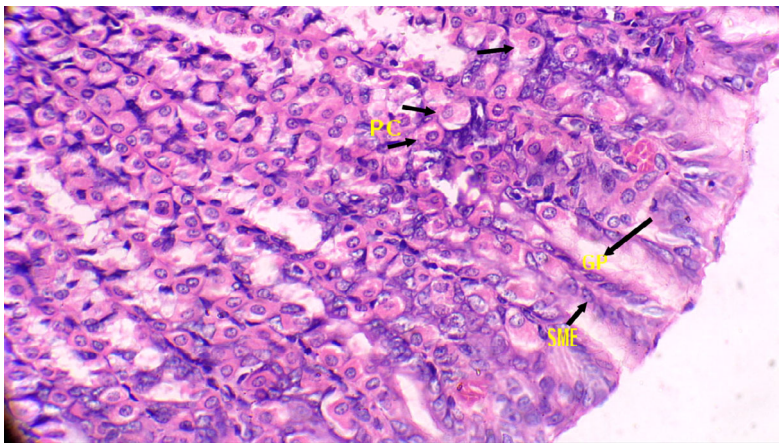
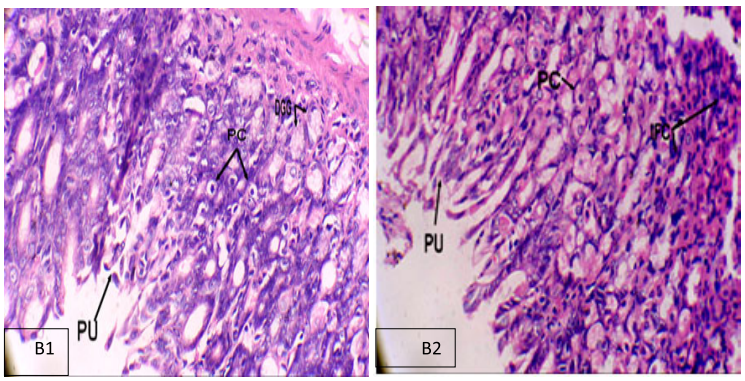


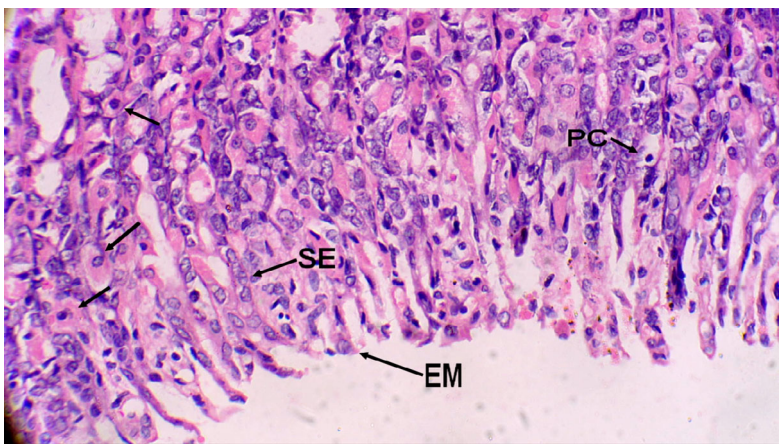
Fig. 2 Peptic ulcer induced in our study



a): Negative control showed normal parietal cells (PC) distribution with normal gastric pits (GP) and normal surface mucus epithelium (SME) E). **(H&E) X40**



b1,b2): NSAIDs induced peptic ulcer group showed marked increase of peptic cells (PC) with degenerated gastric glands (DGG) marked degeneration of surface mucus epith. With loss of histoarchtecture of gastric mucosa , submucosal layer showed marked infiltration with inflammatory cells (IFC). **(H&E) X10**



C): Nizatidine prophylactic group showed changes of parietal cells (PC) with mild degeneration of surface mucus epith. (SE) , the leumenal surface of gastric mucosa showed marked erosion of surface epith. (EM). **(H&E) X40**

Fig. 3 (See legend on next page.)

(See figure on previous page.)
Fig. 3 a Negative control showed normal parietal cells (PC) distribution with normal gastric pits (GP) and normal surface mucus epithelium (SME) (H&E, X40). **b** NSAIDs induced peptic ulcer group showed a marked increase of parietal cells (PC) with degenerated gastric glands (DGG) marked degeneration of surface mucus epithelium. With loss of histoarchitecture of gastric mucosa, the submucosal layer showed marked infiltration with inflammatory cells (IFC) (H&E, X10). **c** Nizatidine prophylactic group showed changes of parietal cells (PC) with mild degeneration of surface mucus epithelium (SE), erosion of mucus membrane (EM) (H&E, X40)

University, were used. Animals were adapted to laboratory conditions before experimental procedures at normal room temperature (27 °C) and humidity. All the animals were fed under standard roles. All the animals were weighed and numbered. Animals were kept in polypropylene cages.

Induction of gastric ulcer by NSAIDs was produced according to Konturek et al. (1982) by a single oral administration of piroxicam (5 mg/kg b. wt.) followed 60 min later by paracetamol (80 mg/kg b. wt.) as single dosage was given via an oro-gastric tube.

First experiment (gastroprotective effect of H2 blockers)

Twenty adult male albino rats were divided randomly into four equal groups each includes five animals as follow:

Group 1: Rats were left as a control group.
Group 2: Ulcer induced group, according to Konturek et al. (1982).
Group 3: After fasting overnight, animals received a daily dose of ranitidine (27 mg/kg b. wt.) for 1 week before the induction of ulcer on the last day.
Group 4: After fasting overnight, animals received a daily dose of nizatidine (27 mg/kg b. wt.) for 1 week before the induction of ulcer.

Second experiment

Sixty adult male albino rats were allocated into four equal groups as follows:

Group 1: (Negative control group) this group was maintained on a normal-pelleted diet throughout the duration of the experiment.
Group 2: (NSAIDs-induced peptic ulcer group, according to Konturek et al., 1982), this group was considered a positive control group.
Group 3: After fasting overnight, animals received a daily dosage of ranitidine (27 mg/kg b. wt.) for 4 weeks after the induction of ulcer.
Group 4: After fasting overnight, animal received a daily dosage of nizatidine (27 mg/kg b. wt.) for 4 weeks after the induction of ulcer.

Five rats from these groups were sacrificed throughout the second, the third, and the fourth week.

Animals of all groups were sacrificed under chloroform anesthesia and abdomens were opened by midline incisions in order to take blood samples from hearts.

Stomachs were removed, cut along the greater curvature, and washed with normal saline to remove gastric contents. The glandular parts were observed for the ulceration and ulcer index, kept in 10% neutral formalin, and processed for the histopathological examination.

The score of lesions was calculated according to the 1 to 5 scoring system devised by Wilhelmi and Gdynia (1972) as follows:

1 2 min, sporadic, punctuate lesion.
2 Several small lesions.
3 One extensive lesion or multiple moderate-sized lesions.
4 Several large lesions.
5 Several large lesions with perforation of the gastric or duodenal wall.

Ulcer index "U.I" (stomach ulceration) was determined according to Radwan and West (1971).

U.I means (X) of the ulcer score of animals similarly treated (X% of the ulcerated animals of the group).

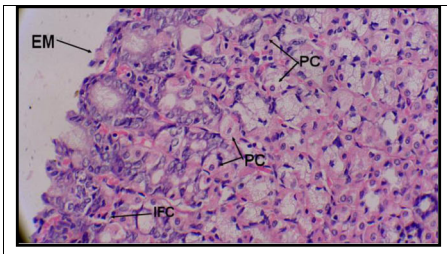
Preventive index "P.I." (the preventive effect of any anti-ulcer agent used against the severity of ulceration) was calculated according to the method of Hano et al. (1976).

$$P.I = \frac{\text{U.I control} - \text{U.I treated}}{\text{U.I control}} \times 100$$

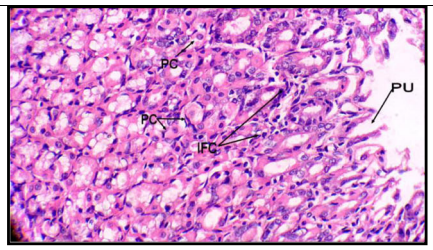
Blood sampling

Blood samples were collected in centrifuge tubes from the heart. Samples were left to clot, then centrifuged at 3000 r.p.m.

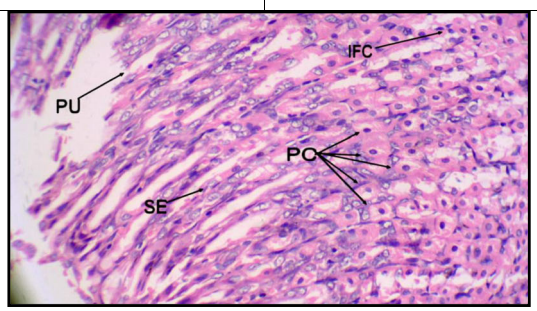
After centrifugation, clear sera were carefully separated, then transferred into a clean dry Eppendorf and kept frozen until used for the hormonal analysis of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), total testosterone, and free testosterone.



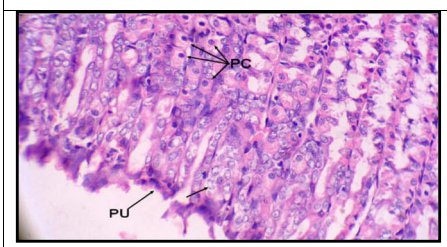
(A): Nizatidine treated group for 2 weeks showed changes in parietal cells with low esinophilic cytoplasm and low basophilic nucleus , with moderate infiltration of inflammatory cells and mild erosive luminal surface., gastric gland showed mild degenerative changes**(H&E,X40)**



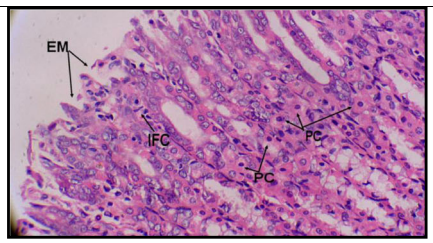
(B): Nizatidine group treated for 3 weeks showed changes of parietal cells with pale eosinophilc cytoplasm and pale basophilic and karyolyed nuclei , marked infiltration of inflammatory cells , leuminal surface showed marked ulcerative changes with surface epith. degeneration**(H&E,X40)**



(C): Rantidine prophylactic group showed normal distribution of parietal cells (PC) with eosinophilic cytoplasm and basophilic nucleI , degenerative changes of surface epith.(SE) and mild infiltration of inflammatory cells(IFC) , with ulcerative leuminal surface (PU)**(H&E,X40)**



(D): Rantidine group treated for 2 weeks showed changes of parietal cells with low eosinophilc cytoplasm and low basophilic nuclei , degenerative changes of surface epith. With karyolysis ,leumenal surface showed mild ulcerative and marked erosive changes**(H&E,X40)**



(E): Ranitidine treated group for 3 weeks showed changes of parietal cells with marked eosinophilic cytoplasm and marked basophilic nucleus , mild infiltration of inflammatory cells (IFC) with mild erosive changes at the leuminal surface , normal structure of gastric glands**(H&E,X40)**

Fig. 4 a Nizatidine treated group for 2 weeks showed changes in parietal cells with low eosinophilic cytoplasm and low basophilic nucleus, with moderate infiltration of inflammatory cells and mild erosive luminal surface, gastric gland showed mild degenerative changes (H&E,X40). **b** Nizatidine group treated for 3 weeks showed changes of parietal cells with pale eosinophilic cytoplasm and pale basophilic and karyolyed nuclei, marked infiltration of inflammatory cells, the luminal surface showed marked ulcerative changes with surface epithelium degeneration (H&E, X40). **c** Ranitidine prophylactic group showed normal distribution of parietal cells (PC) with eosinophilic cytoplasm and basophilic nuclei, degenerative changes of surface epithelium (SE) and mild infiltration of inflammatory cells (IFC), with ulcerative luminal surface (PU) (H&E, X40). **d** Ranitidine group treated for 2 weeks showed changes of parietal cells with low eosinophilic cytoplasm and low basophilic nuclei, degenerative changes of surface epithelium. With karyolysis, the luminal surface showed mild ulcerative and marked erosive changes (H&E, X40). **e** Ranitidine-treated group for 3 weeks showed changes of parietal cells with marked eosinophilic cytoplasm and marked basophilic nucleus, mild infiltration of inflammatory cells (IFC) with mild erosive changes at the luminal surface, normal structure of gastric glands (H&E, X40)

Statistical analysis

In order to assess the influence of nizatidine and ranitidine on some pituitary-gonadal hormone data and along with the post-treatment (2, 3, and 4 weeks), one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (Tukey's HSD) test as post hoc test, was used.

Analysis was done using Statistical Package for Social Sciences version 22.0 (IBM Corp., Armonk, NY, USA).

Results were recorded in means ± SEM (standard error of mean). The value of $P < 0.05$ was used to indicate statistical significance.

Results

Induction of ulcer

A single oral administration of piroxicam (5 mg/kg) followed, 60 min later, by paracetamol (80 mg/kg) as a single dosage via a gastric tube lead to extensive number of gastric mucosal lesions in the glandular segments of the stomach as shown in Fig. 2, and resulted in a high incidence of ulceration 100% mean ulcer score (4 ± 0.42) and a high ulcer index (400 ± 22.0) in the glandular segments of the stomach.

A gastroprotective effect of ranitidine, nizatidine (27 mg/kg b. wt.) against NSAIDs-induced ulcer group was observed (Figs. 3c and 4c). They reduced the incidence of ulceration, significantly decreased the mean ulcer score, highly lowered the ulcer index, and importantly improved the preventive index to 50% and 60% in comparison with the positive control group (NSAIDs-treated group) (Table 1 and Fig. 7).

Oral administration of ranitidine, nizatidine (27 mg /kg b. wt.) showed a great effect on the NSAIDs-induced ulcer group. They reduced the incidence of peptic ulceration, significantly decreased the mean ulcer score, reduced ulcer index, and greatly improved the preventive index in the NSAIDs treated (to 40%, 85%, and 90% for ranitidine and to 60%, 90%, and 95% for nizatidine during the second, the third, and the fourth week respectively) versus zero for the positive control group (Table 1 and Fig. 7).

Treatment with H2 blockers caused a significant progressive increase in the level of follicle-stimulating hormone, luteinizing hormone, and prolactin. It also showed a significant progressive decrease in the level of total serum testosterone and free serum testosterone in the group treated with ranitidine and nizatidine in comparison with the control group along the whole duration of the experiment (Table 2 and Fig. 8).

Table 1 Effects of oral administration of nizatidine and ranitidine (27 mg/kg b. wt.) on ulceration in rats administrated NSAIDs (piroxicam (5 mg/kg) followed 60 min later by paracetamol (80 mg/kg) as single dosage)

Parameters	Positive control group (n = 5)	Group received ranitidine (n = 5)	Group received nizatidine (n = 5)	p
Ulceration percent (%)				
• 1 week before	100	100	80	0.34
• 2 week after	100	80	80	0.56
• 3 weeks after	100	60	40	0.12
• 4 weeks after	100	40[a]	20[a,b]	0.03*
Mean ulcer score				
• 1 week before	4 ± 0.4	2 ± 0.2[a]	2 ± 0.4[a]	< 0.0001*
• 2 week after	4 ± 0.4	2 ± 0.2 [a]	2 ± 0.1[a]	< 0.0001*
• 3 weeks after	4 ± 0.4	1 ± 0.3 [a]	1 ± 0.3[a]	< 0.0001*
• 4 weeks after	4 ± 0.4	1 ± 0.3 [a]	1 ± 0.3[a]	< 0.0001*
Ulcer index				
• 1 week before	400 ± 22	200 ± 11.2[a]	160 ± 32.1[a,b]	< 0.0001*
• 2 week after	400 ± 22	140 ± 23.7[a]	160 ± 26.5[a]	< 0.0001*
• 3 weeks after	400 ± 22	60 ± 14.9[a]	40 ± 13.4[a]	< 0.0001*
• 4 weeks after	400 ± 22	40 ± 10.6[a]	20 ± 9.6[a,b]	< 0.0001*
Preventive index (%)				
• 1 week before	0	50[a]	60[a]	< 0.0001*
• 2 week after	0	40[a]	60[a,b]	< 0.0001*
• 3 weeks after	0	85[a]	90[a]	< 0.0001*
• 4 weeks after	0	90[a]	95[a]	< 0.0001*

Data presented as mean ± SEM or percentage
p one-way ANOVA significance
Tukey's honestly significant difference (Tukey's HSD) test
[a]Significant difference compared to positive control
[b]Significant difference compared to group received ranitidine
* means significant p<l0.05

Table 2 Effects of oral administration of nizatidine, ranitidine (27 mg/kg b. wt.) on serum hormones of male albino rats

Parameters	Positive control group (n = 5)	Group received ranitidine (n = 5)	Group received nizatidine (n = 5)	p
Serum FSH (ng/mL)				
• 2 week after	0.75 ± 0.07	1.38 ± 0.14[a]	1.8 ± 0.07[a,b]	< 0.0001*
• 3 weeks after	0.76 ± 0.07	2.83 ± 0.31[a]	2.08 ± 0.13[a,b]	< 0.0001*
• 4 weeks after	0.73 ± 0.07	3.18 ± 0.22[a]	3.5 ± 0.27[a]	< 0.0001*
Serum LH (pg/mL)				
• 2 week after	0.73 ± 0.06	1.11 ± 0.12[a]	1.34 ± 0.05[a,b]	< 0.0001*
• 3 weeks after	0.7 ± 0.06	1.71 ± 0.2[a]	1.38 ± 0.07[a]	< 0.0001*
• 4 weeks after	0.71 ± 0.06	2.12 ± 0.23[a]	2.34 ± 0.13[a]	< 0.0001*
Serum prolactin (ng/mL)				
• 2 week after	2.68 ± 0.12	2.88 ± 0.26	3.3 ± 0.25[a,b]	< 0.0001*
• 3 weeks after	2.53 ± 0.12	3.62 ± 0.33[a]	2.9 ± 0.2[a,b]	< 0.0001*
• 4 weeks after	2.34 ± 0.12	3.74 ± 0.22[a]	3.5 ± 0.18[a]	< 0.0001*
Serum total testosterone (ng/mL)				
• 2 week after	2.45 ± 0.11	1.32 ± 0.1[a]	1.25 ± 0.19[a]	< 0.0001*
• 3 weeks after	2.25 ± 0.11	1.06 ± 0.13[a]	1.28 ± 0.23[a]	< 0.0001*
• 4 weeks after	2.15 ± 0.11	0.76 ± 0.05[a]	0.92 ± 0.05[a,b]	< 0.0001*
Serum-free testosterone (pg/mL)				
• 2 week after	11.54 ± 0.71	9.08 ± 0.32[a]	9.06 ± 0.22[a]	< 0.0001*
• 3 weeks after	11.34 ± 0.71	8.86 ± 0.44[a]	8.84 ± 0.38[a]	< 0.0001*
• 4 weeks after	10.44 ± 0.71	6.84 ± 0.51[a]	7.42 ± 0.26[a]	< 0.0001*

Data presented as mean ± SEM
p one-way ANOVA significance
Tukey's honestly significant difference (Tukey's HSD) test
[a]Significant difference compared to positive control
[b]Significant difference compared to group received ranitidine

Histopathological findings

Morphological evaluation of isolated rats' gastric mucosa

Tissues of isolated rat gastric mucosa were examined by H&E and T.B. stains (Bancroft et al. 2008).

As for control rats, sections of the stomach showed a normal distribution of parietal cells with normal gastric pits and normal surface mucus epithelium (Fig. 3a) beside the normal structure of parietal cells (Fig. 5a).

Histological observation demonstrated a comprehensive damage to the gastric mucosa in the ulcerated control group. Furthermore, the ulcerated stomach in the control group had degenerated gastric glands, marked degeneration of surface mucus epithelium, with the loss of histoarchitecture of gastric mucosa. This showed extensive leucocyte infiltration and edema of the submucosal layer, as illustrated in Figs. 3b and 5b.

The key observation here is that the oral administration of nizatidine and ranitidine as prophylactic altered the secretory function concomitant to its structural degeneration of parietal cells at the first time of administration (Figs. 3c, 4c, 5c, and 6a) and up to 2 weeks as shown in (Figs. 4a, d and 5d).

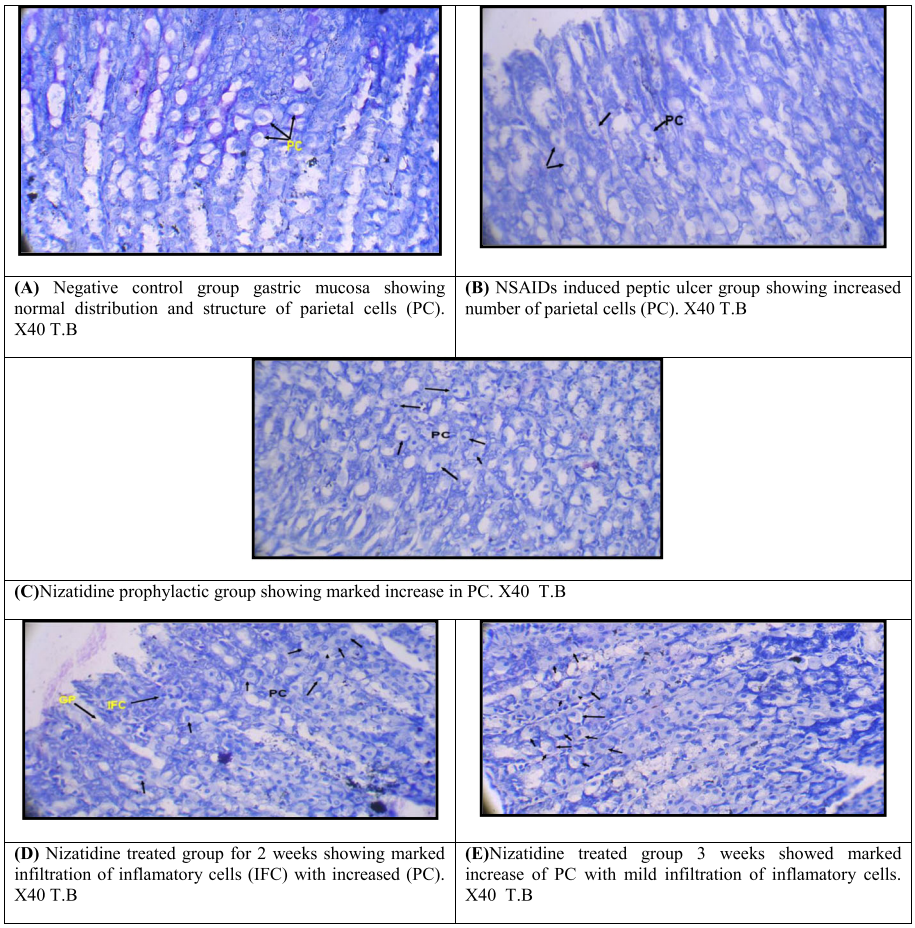
As for ranitidine, the parietal cells were recovered with normal basophilic nucleus and eosinophilic cytoplasm as indicated in Figs. 4e and 6b, c, but the cytoplasm and nucleus still pale for nizatidine after 3 weeks as shown in Figs. 4b and 5e.

Discussion

Gastric ulcers are common diseases constituting one of the main causes of morbidity and mortality for more than a century (Hoogerwerf & Pasricha, 2006). Peptic ulcer is a common disease worldwide defined as "a defect in the mucosa of stomach or duodenum that exceeds the layer of muscularis mucosa injuries as a result of imbalance between the defensive and the aggressive factors affecting the mucosal epithelium" (Asali et al., 2018).

The danger of vital gastrointestinal injury depends on the dose and it increase dramatically once over one anti-inflammatory is used (Feria, 2005).

NSAIDs act on by inhibiting the activity of one of the cyclo-oxygenase enzyme. Cox contains two associated enzymes with two different functions: cyclo-oxygenase activity, changing arachidonic acid liberated from the



(A) Negative control group gastric mucosa showing normal distribution and structure of parietal cells (PC). X40 T.B

(B) NSAIDs induced peptic ulcer group showing increased number of parietal cells (PC). X40 T.B

(C) Nizatidine prophylactic group showing marked increase in PC. X40 T.B

(D) Nizatidine treated group for 2 weeks showing marked infiltration of inflamatory cells (IFC) with increased (PC). X40 T.B

(E) Nizatidine treated group 3 weeks showed marked increase of PC with mild infiltration of inflamatory cells. X40 T.B

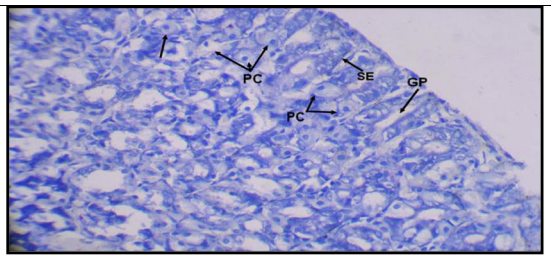
Fig. 5 a Negative control group gastric mucosa showing normal distribution and structure of parietal cells (PC), X40 T.B. **b** NSAIDs-induced peptic ulcer group showing an increased number of parietal cells (PC), X40 T.B. **c** Nizatidine prophylactic group showing a marked increase in PC, X40 T.B. **d** Nizatidine-treated group for 2 weeks showing marked infiltration of inflammatory cells (IFC) with increased (PC), X40 T.B. **e** Nizatidine-treated group 3 weeks showed marked increase of PC with mild infiltration of inflammatory cells, X40 T.B

phospholipid membrane by phospholipase to prostaglandin G2 (PGG2), then converting PGG2 into prostaglandin H2 (PGH2) by a peroxidase activity. PGH2 is then converted to a variety of prostaglandins according to the cell type-specific manner (Werawatganon et al., 2014).

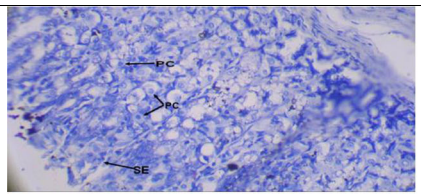
In experimental studies on gastric glands and in vivo, the presence of H2-receptor antagonists downregulates the gastrin stimulatory effect on the parietal cell, suggesting that the gastrin stimulation of acid secretion is regulated mainly by histamine released from the enterochromaffin-like ECL cell (Lindström, 2001). The histamine receptor is thought to be coupled only with adenylate cyclase and that elevation of cAMP is sufficient to stimulate the acid secretion in in vitro models (Chew et al., 1980). In addition, histamine is shown to elevate intracellular calcium in parietal cells, suggesting that this receptor has at least a dual system in the parietal cells (Chew & Brown, 1986). So, H2-receptor antagonists (ranitidine and nizatidine) have an obvious role in inhibiting the secretion of hydrochloric acid.

In the light of the results of the present study, it is noticed that nizatidine and ranitidine decreased the incidence of gastric ulceration, and significantly decreased the mean ulcer score and ulcer index in male albino rats (Figs. 7 and 8). The drug significantly increased the preventive index against NSAIDs induced ulcer. These results are in full agreement with those of Louise et al. (1993), they reported that pretreatment with nizatidine significantly reduces the incidence of ulceration formation in high-risk patients taking future NSAID medical care. It also relieves NSAIDs—associated dyspeptic symptoms in some patients.

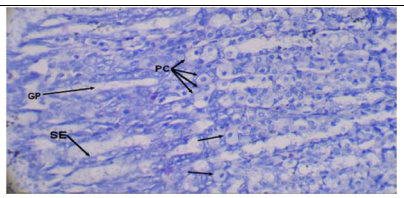
Yeomans et al. (2006) stated that there is a marked variation in the healing rates associated with ranitidine treatment among different studies. For instance, Goldstein et al. (2005) and Tildesley et al. (1993)



(A)Rantidine prophylactic group showed increased number of (PC). X40 T.B



(B) Ranitidine treated group 2 weeks showed increased number of parietal cells (PC) with degenrated surface epith.(SE) and mild infiltration of inflamatory cells. X40 T.B



(C) Ranitidine treated group 3 weeks showed marked increase of parietal cells (PC) and normal surface epith (SE) and gastric pit (GP). X 40 T.B

Fig. 6 a Ranitidine prophylactic group showed an increased number of (PC), X40 T.B. **b** Ranitidine-treated group 2 weeks showed increased number of parietal cells (PC) with degenerated surface epithelium (SE) and mild infiltration of inflamatory cells, X40 T. B. **c** Ranitidine-treated group 3 weeks showed marked increase of parietal cells (PC) and normal surface epithelium (SE) and gastric pit (GP), X 40 T.B

reporting gastric ulcer healing rates in patients continuing NSAID therapy for 4 weeks, but Campbell et al. (2002) and Agrawal et al. (2000) report a double duration. Additionally, the gastric ulcer healing rate of 8 weeks reported by Goldstein et al. (2005) was 20% greater than the rate associated with ranitidine in these two studies. The explanations for this are unclear because all of these studies used a similar ulceration definition (= 5 mm in diameter), but were most likely related to variations within the patient populations.

In the current study, a rapid recovery of the gastric epithelium was observed after using H2-receptors blockers. This recovery can be attributed to different main factors, including the ability of the gastric epithelial cells to restitute by extending lamillopodia to cover the denuded basement membrane (Lacy & Ito, 1984), to prevent hypergastrinemia which occurs due to inhibition of acid secretion (Ryberg et al., 1990) and to increase in the pre-parietal cell production which transformed into new parietal cells due to the cessation of treatment rapidly. The time required for the production of pre-parietal cells ranged from 1 to 2 days and within an additional day, anew parietal cell could be formed (Karam, 1993).

The present study showed a clear degeneration of parietal cells represented in low eosinophilic cytoplasm and low basophilic nuclei, which means that there is an alteration of the secretory function of parietal cells. These results are in agreement with those of Karam and Alexander (2001). They reported that the physiological degeneration of parietal cells is enhanced by inhibiting their secretion using a H2-receptor antagonist.

Based on the present histological results, the distinct observation is that nizatidine and ranitidine altered the secretory function of parietal cells due to the alteration of their histological criteria at the first time of administration and up to 2 weeks. For ranitidine, the parietal cells were recovered with a normal basophilic nucleus and eosinophilic cytoplasm, but in the case of nizatidine, the cytoplasm and nucleus remained pale after 3 weeks, which could lead to the increased tolerability rate of ranitidine more than nizatidine.

The results of the present study disclosed a significant increase in the level of serum FSH and LH and a significant decrease in the level of serum testosterone in H2 blockers-treated groups. These results are in agreement with those of Knigge et al. (1983). They reported that this increase may be attributed to H2-receptor antagonists which have anti-androgen effects. They block androgen receptors in the pituitary or hypothalamus, according to Sinha et al. (2006) who reported that H2-receptor blocker treatment over a period of 2 weeks can cause a significant reduction in epididymal tissue mast cell population and tissue histamine content in caput, corpus, and cauda regions in albino rats. There is also an extremely significant fall in the serum testosterone level.

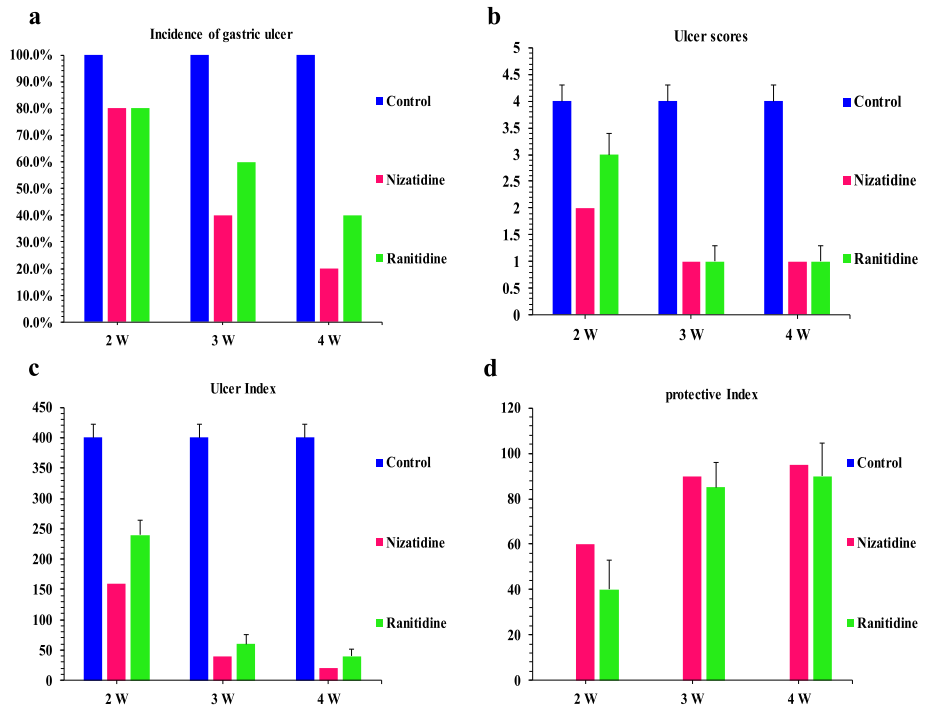


Fig. 7 **a** Effects of oral administration of nizatidine, ranitidine (27 mg/kg b. wt.) given once a day for 4 weeks on incidence of gastric ulceration in rats administrated NSAIDs (piroxicam (5 mg/kg) followed 60 min later by paracetamol (80 mg/kg) as single dosage). **b** Effects of oral administration of nizatidine and ranitidine (27 mg/kg b. wt.) given once a day for 4 weeks on mean ulcer score in rats administrated NSAIDs (piroxicam (5 mg/kg) followed 60 min later by paracetamol (80 mg/kg) as single dosage). **c** Effects of oral administration of nizatidine, ranitidine (27 mg/kg b. wt.) given once a day for 4 weeks on ulcer index in rats administrated NSAIDs (piroxicam (5 mg/kg) followed 60 min later by paracetamol (80 mg/kg) as single dosage). **d** Effects of oral administration of nizatidine, ranitidine (27 mg/kg b. wt.) given once a day for 4 weeks on preventive index (%) in rats administrated NSAIDs (piroxicam (5 mg/kg) followed 60 min later by paracetamol (80 mg/kg) as single dosage)

Serum LH and FSH levels are enhanced most likely due to suppression of feed-back inhibition of anterior pituitary glands (Fattahi et al., 2009).

In the testis, LH binds to receptors on the surface of Leydig cells and stimulates the production of androgenic hormone (Walker & Cheng, 2005). FSH enhances the production of androgen-binding protein by the Sertoli cells of the testes within binding to FSH receptors and this is critical for the initiation of gametogenesis (Boulpaep & Boron, 2005).

The present study recorded an observed increase in the FSH and LH levels along the time of the experiment, which was attributed by Bhale and Mahat (2013) to the stimulation of both the Sertoli and Leydig cells for a proportionate synthesis and secretion of testosterone thereby enhancing of spermatogenesis occurs. The high gonadotropin level (FSH and LH) exercise a negative feedback effect on the hypothalamo—pituitary—testicular axis and thus the plasma testosterone level becomes low.

On the other hand, the result of the present study is in disagreement with those of Aprioku et al. (2014). They reported that cimetidine may cause an alteration of the testicular function, while ranitidine may have no effect on the testis in rats, which use ranitidine, 8, and 16 mg/kg/day in 2 divided doses for 14 days. This difference may be due to the difference in doses or the exposure period of the experiment.

The present study showed a progressive increase in the level of prolactin. On the other hand, Molitch (2005) reviewed that several briefcase reports were published about patients experiencing symptoms related to hyperprolactinemia after the approval of histamine 2-receptor blockers such as cimetidine and ranitidine. However, in larger series, hyperprolactinemia has not been reported, and there has been reported a case of a woman treated with a twice-maximum dose of famotidine.

Conclusion

To sum up the observations of the present study, it could be concluded that nizatidine is much more effective than ranitidine in the management of NSAIDs induced peptic ulcer in rats.

As for histopathological findings, the present study showed that the protective effect of nizatidine surpasses than ranitidine. The effect of both H2-receptors

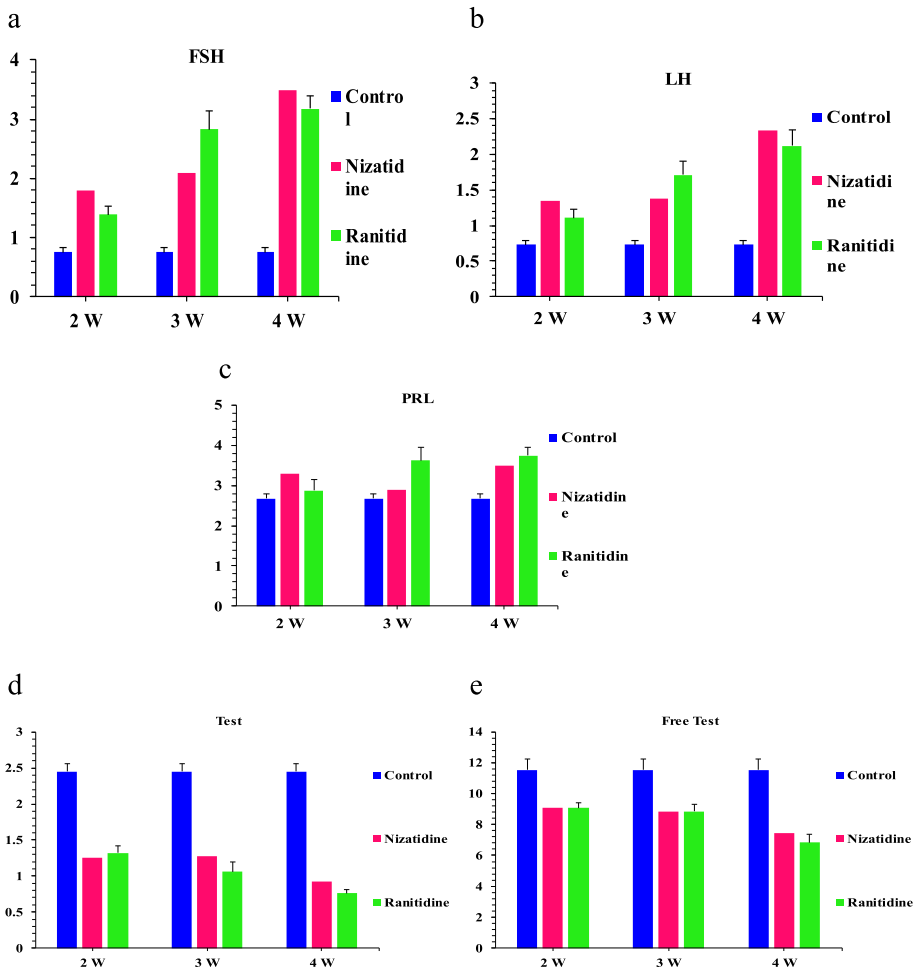


Fig. 8 **a** Effects of oral administration of nizatidine, ranitidine (27 mg/kg b. wt.) given once a day for 4 weeks on serum follicle-stimulating hormone of male albino rats. **b** Effects of oral administration of nizatidine, ranitidine (27 mg/kg b. wt.) given once a day for 4 weeks on serum luteinizing hormone of male albino rats. **c** Effects of oral administration of nizatidine, ranitidine (27 mg/kg b. wt.) given once a day for 4 weeks on serum prolactin hormone of male albino rats. **d** Effects of oral administration of nizatidine, ranitidine (27 mg/kg b. wt.) given once a day for 4 weeks on serum total serum testosterone hormone of male albino rats. **e** Effects of oral administration of nizatidine, ranitidine (27 mg/kg b. wt.) given once a day for 4 weeks on serum free testosterone hormone of male albino rats

antagonists is to alter the secretory function of parietal cells, but ranitidine was more successful than nizatidine in recovering (or removing) this effect. So, there is a tolerable effect for ranitidine. The present study indicated that oral administration of both drugs elicited a significant increase in FSH, LH, prolactin, and prompted a significant decrease in total and free testosterone in male albino rats.

Abbreviations

NSAIDs: Nonsteroidal anti-inflammatory drugs; H2: Blocker histamine blocker; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; PRL: Prolactin; T-TESTO: Total testosterone; F-TESTO: Free testosterone; GnRH: Gonadotropin-releasing hormone; GIT: Gastrointestinal tract; H&E: Hematoxyline and eosin; T.B.: Toluidine blue; ECL: Enterochromaffin-like; P.I.: Preventive index; U.I: Ulcer index; COX: Cyclo-oxygenase; *H. pylori*: *Helicobacter pylori*; HCl: Hydrochloric acid

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Guidelines followed for handling rats

HU-IACUC Institutional Animal Care and Use Committees in Helwan University

Authors' contributions

Experimental design: AA, SE .Data interpretation: EA, AA, SE, AS. Histological examination: AA. Writing and revision: AA, AS. All authors read and approved the final manuscript.

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Author details
[1]Zoology Department, Faculty of Science, Helwan University, Cairo, Egypt. [2]Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

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