

Microscopic Studies on the Effects of Esomeprazole on the Gastric Mucosa: An Experimental Study in Albino Rat

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ABSTRACT

Background: Detailed histopathological and histomorphometric analysis of gastric mucosa after long term treatment or ingestion of esomeprazole specifically is lacking in literature. **Methods:** 24 male and female rats, 12 each were divided into control and experimental groups (6 each for both sexes). Rats of experimental group got 15 mg/kg body weight of esomeprazole by oral intubation for 130 days. After perfusion fixation by Karnovsky's solution, two pieces of gastric wall were procured from anterior aspect, 1 each from distal part of body and pyloric region respectively. Sections of 8 µm thickness, obtained by using wax embedding technique, were stained with haematoxylin and eosin. **Results:** In both the regions of stomach in two sexes of control rats, the mucosa showed 1/5th superficial layer of absorptive epithelial cells, 3/5th parietal and deepest 1/5th layer of pepsin secreting glands. Parietal and pepsin gland layers were involved in body of male and pyloric region of female. Marked oedema, hypertrophy and hyperplasia were uniformly observed in experimental animals. **Conclusion:** Atrophic gastritis observed in female pyloric region is an alarming observation because it is the precursor of gastric carcinoma.

Keywords: Esomeprazole, Gastric Mucosa.

INTRODUCTION

Protein pump inhibitors (PPIs) are one of the most widely used drug groups as antacids. These constitute the frontline medications for gastroesophageal reflux disease (GERD) and dyspepsia.^[1] PPIs remains priority to treatment of disorders leading to hyperacidity and are still unchallenged with regard to their efficacy and popularity among doctors and patients.^[2]

Esomeprazole, the S-isomer of omeprazole, is the first proton pump inhibitor to be developed as optical isomer. Esomeprazole has produced significantly greater healing effect in patients with erosive oesophagitis after long term therapy.^[3]

Concerns were first raised in 1996 by Kuipers et al of increased occurrence of atrophic gastritis,^[4] itself a precursor of gastric cancer on long term omeprazole therapy. At the same time the long term PPI therapy as gastric cancer risk has been denied.^[4]

Blom for the first time found an increase in gastric mucosal thickness after its use for 130 days in animal experimental model.^[5]

Oral ingestion of omeprazole by rats for 3 months led to hyperplasia of the oxyntic mucosal cells of stomach.^[6]

Unfortunately detailed histopathological and histomorphometric analysis of gastric mucosa after long term treatment or ingestion of esomeprazole specifically is lacking in literature. Several contradictory reports on gastric mucosa after PPIs intoxication, as mentioned earlier, forced us to undertake the study to find the changes at light microscopic level specifically for newer drug, esomeprazole, freely used recently at large scale. Moreover, we have considered body and pyloric region of stomach simultaneously because of their structural and functional differences.

MATERIALS AND METHODS

24 male and female Wistar Albino rats (*Rattus norvegicus*) 12 each were divided into control and experimental groups (6 each for both sexes). Rats received standard pellet laboratory diet (Lipton India Limited) and water ad-libitum. Rats of control group received 0.5 ml normal saline once a day by oral intubation for 130 days. Rats of experimental group got 15mg/kg body weight (40 µmol/kg) of

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esomeprazole (Astra Zenica Company, U.K. London) once a day on an empty stomach by oral intubation for 130 days.

Animals of both the experimental and control groups were anaesthetized by giving injection Nembutol 30 mg/kg body weight intraperitoneally on Day 131. Karnovsky's fixative was infused through left ventricle till the body showed signs of fixation. After exposing stomach by abdominal incision, two pieces of gastric wall, 3 mm each, were procured from anterior aspect. First piece was taken from distal part of body (glandular region) and second from pyloric region.

Sections of 8 µm thickness, obtained by using wax embedding technique, were stained with haematoxylin and eosin.

RESULTS

Gastric mucosal thickness of body of control rat in both sexes showed 1/5th superficial layer of absorptive epithelial cells, 3/5th parietal and deepest 1/5th layer of pepsin secreting glands [Table 1]. Gastric mucosa when observed under 40 x showed following features, 1. Surface epithelium of columnar cells with basal nuclei. Parietal cells were forming glands. Cells are polygonal with light eosinophilic granular cytoplasm and central nuclei. 2. Parietal cells and lots of HCl secreting glands were seen. 3. Pepsin secreting glands were lined by smaller cubical cells with dark basophilic granular cytoplasm and central nucleus with cartwheel appearance [Figure 1].

Photomicrograph of the body of stomach in male experimental showed hypertrophic gastric mucosa and hyperplasia in all the layers along with generalized oedema seen as spaces in between glands. Chronic inflammatory cells infiltrate were more marked in basal and middle layers. Disarray of glands was seen and size of parietal cells and pepsin secreting glands was also increased [Figure 2].

Body of stomach in female experimental rat under 4x [Figure 3] showed gross congestion with dilated blood vessels along with oedema. Under 10x, hypertrophic gastric mucosa of thickness 1.2 mm [Table 1] showed an increase in the number of cells in all the layers.

Photomicrograph of pylorus of stomach of both sexes in control rats [Table 2] showed gastric mucosal glands with superficial layer of absorptive columnar cells in the form of folded epithelium and middle layer of parietal cells as elongated glands. A deeper layer of smaller pepsin secreting glands, showed a thinner mucosa than body Mucosa showed 1/5th superficial layer of columnar cells, 3/5th deeper parietal cell layer and the deepest 1/5th layer of large pepsin secreting glands [Figure 4]. Mucosa was slightly folded with superficial epithelium of columnar cells and mostly parietal cells forming glands and chief cells.

Photomicrograph of pylorus of stomach in male experimental rat [Table 2] showed hypertrophic gastric mucosa. Superficial layer showed extensive necrosis on surface with necrotic material in lumen. The 3/5th parietal cell layer showed coagulative necrosis. The deep 1/5th of pepsin glands showed inflammatory cell infiltrates [Figure 5]. Odema fluid was present in submucosa and muscle layer. Mucosa showed degenerative changes. Swelling of columnar cells and nuclei, partial loss of morphology and haziness were also seen. Gastric mucosal hypertrophy showed larger number of parietal cells with crowding of cells [Figure 6].

Photomicrograph of pylorus of stomach in female experimental rat under 4 x [Table 2] showed gastric mucosa with marked edema separating and compressing all the glands. Marked edema of submucosa and muscle coat was separating the muscle bundles [Figure 7]. Under 40 x, gastric mucosa showed hypertrophic superficial epithelium as folds, with mild disarray, edema fluid in lamina propria distorting the folds and few inflammatory infiltrates. Gastric mucosa showed deeper layers made of a few parietal cells and more pepsin secreting glands. Cells were slightly enlarged [Figure 8].

Table 1: A Comparison Of Thickness Of Gastric Mucosa Of The Body Of Stomach And Relative Proportion Of Various Cell Layers.

	Total gastric wall thickness (in mm)	Thickness of gastric mucosa (mm)	Arbitrary comparative thickness showing proportion of various cell layers of Gastric mucosa		
			Absorptive columnar epithelial cell layer	Parietal cell layer	Pepsin gland layer
Male Control Body (MCB)	1.5mm	1mm	1/5th of mucosal thickness	3/5th of mucosal thickness	1/5th of mucosal thickness
Male Experimental (Body) (MEB)	2 mm	1.5 mm	1/5th of mucosal thickness	2.5/5th of mucosal thickness	1.5/5th of mucosal thickness
Female Control Body (FCB)	1.3 mm	0.8 mm	1/5th of mucosal thickness	3/5th of mucosal thickness	1/5th of mucosal thickness
Female experimental body (FEB)	2 mm	1.2 mm	1/5th of mucosal thickness	3/5th of mucosal thickness	1/5th of mucosal thickness

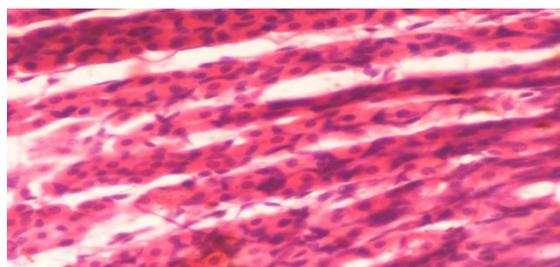


Figure 1: Photomicrograph showing body of stomach in male control rat x40 H&E Parietal cells and lots of HCl secreting glands are seen.

Table 2: A Comparison Of Thickness Of Gastric Mucosa Of The Pylorus Of Stomach And Relative Proportion Of Various Cell Layers.

	Total gastric thickness (in mm)	Thickness of gastric mucosa (mm)	Arbitrary comparative thickness showing proportion of various cell layers of Gastric mucosa		
			Absorptive columnar epithelial cell layer	Parietal cell layer	Pepsin gland layer
Male Control Pylorus (MCP)	1 mm	0.8 mm	1/5th	3/5th	1/5th
Male Experimental Pylorus (MEP)	1.8 mm	1.3 mm	1/5th	3/5th	1/5th
Female Control Pylorus (FCP)	1 mm	0.6 mm	1/5th	3/5th	1/5th
Female experimental Pylorus (FEP)	1.4 mm	1.0 mm	1/5th	2.5/5th	1.5/5th

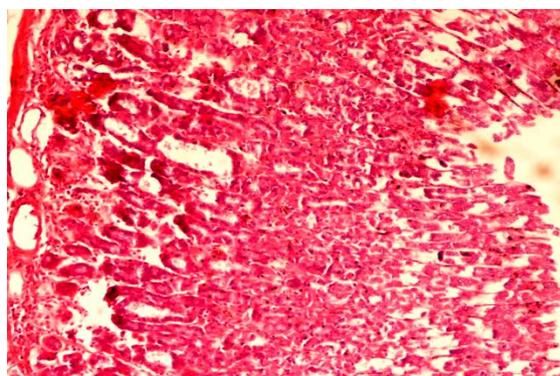


Figure 2: Photomicrograph showing body of stomach in male experimental rat x10 H&E Hypertrophic gastric mucosa 1.5 mm thick with hyperplasia in all the layers along with generalized oedema seen as spaces in between glands and chronic inflammatory cells infiltrate more marked in basal and middle layers. Disarray of glands is seen and size of parietal cells and pepsin secreting glands is also increased

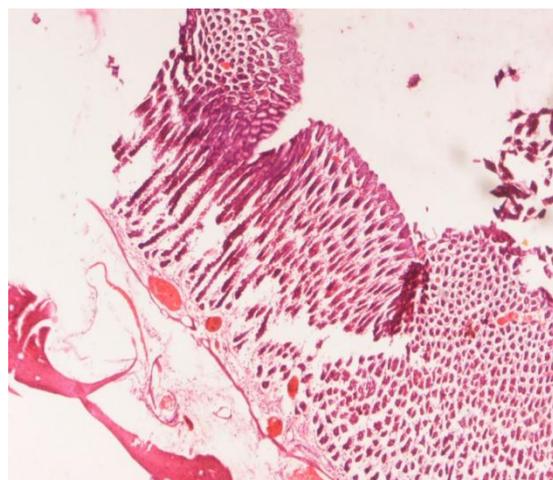


Figure 3: Photomicrograph showing body of stomach in female experimental rat x4 H&E Gross congestion with dilated blood vessels along with oedema.

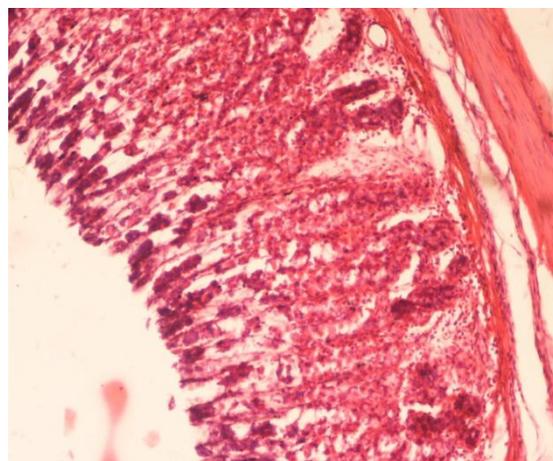


Figure 4: Photomicrograph showing pylorus of stomach in male control rat x10 H&E Gastric mucosal thickness 0.8 mm showing 1/5th superficial layer of columnar cells, 3/5th deeper parietal cell layer and deepest 1/5th layer of large pepsin secreting glands.

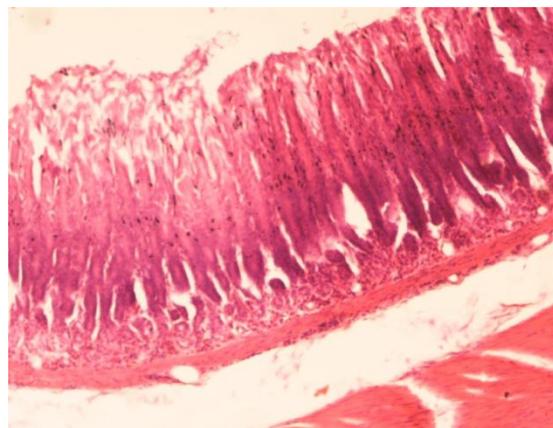


Figure 5: Photomicrograph showing pylorus of stomach in male experimental rat x10 H&E Gastric mucosa shows extensive coagulative necrosis with loss of cell morphology and nuclei. Only cell outlines are seen. Marked oedema seen as spaces between mucosa and muscle. Inflammatory cells are present.

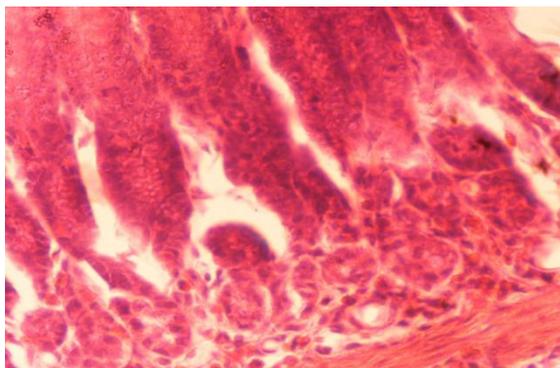


Figure 6: Photomicrograph showing pylorus of stomach in male experimental rat x40 H&E Gastric mucosal hypertrophy showing larger number of parietal cells with crowding of cells. Pepsin secreting glands show mild distortion of glands. Edema fluid is seen. Moderate grade lymphoplasmocytic infiltrate is seen in basal layer.



Figure 7: Photomicrograph showing pylorus of stomach in female experimental rat x4 H&E Gastric mucosa 1 mm thickness showing marked edema separating and compressing all the glands, marked edema of submucosa and muscle coat seen as separating the muscle bundles

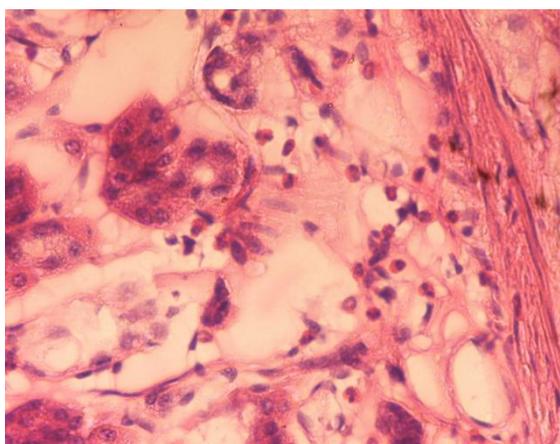


Figure 8: Photomicrograph showing pylorus of stomach in female experimental rat x40 H&E Gastric mucosa shows deeper layers made of a few parietal cells and more pepsin secreting glands. Cells are slightly enlarged. Edematous lamina propria showing loose connective tissue infiltrated by lymphocytes and plasma cells

DISCUSSION & CONCLUSION

The detailed histopathological analysis is the unique feature of present research. Esomeprazole is a newer proton pump inhibitor so least explored compared to omeprazole which has been maximally considered for research.^[7] Since the former is s-isomer of latter, its selection for the present study is greatly justified. Reported affinity of omeprazole for stomach has forced the authors to select the same for esomeprazole.^[5,6,8-12] The study has been made more fascinating by considering two physiologically different parts of stomach, i.e. body and pyloric region to find out any differential effects.

A feature uniformly noticed in gastric mucosa of esomeprazole treated rats was increase in its thickness. This effect involved both body and pyloric region in both sexes [Table 1 & 2]. Although thickness of wall in general and mucosa in particular are constant features, the sublayers of mucosa from superficial to deep i.e. absorptive cell layer, parietal cell layer and chief cell layer have very different responses. Absorptive cell layer is found to be most resistant to drug as its thickness is reduced only in pyloric region of female experimental rats [Table 2]. This change coincides with marked edema fluid in lamina having compressing effect on mucosal epithelial cells [Figure 7&8]. Parietal cell layers show preferential effect by the esomeprazole. In male rats this layer is affected in body while in female animals this layer is involved in pyloric region [Table 1 & 2, Figure 2,7&8]. Thickness in parietal cell layer of mucosa is reduced in the body of experimental male rats as well as in pyloric region of experimental female animals [Table 1 & 2]. The only explanation to these effects is the general mucosal oedema [Figure 2,7 & 8]. Chief cell layer shows similar preferential effects in both male and female experimental rats i.e., in male, body is involved while in female, pyloric region is affected. But no differential phenomenon is noticed i.e. the pepsin gland layer is increased in body of male as well as pyloric region of female [Table 1 & 2]. In both the cases increase in thickness seems to be due to increase in size and number of chief cells [Figure 2,7&8]. Supportive literature showing relative thicknesses of different layers of mucosa after PPIs toxicity, is lacking.

Changes in gastric mucosa of pyloric region in male rats are very alarming. Gastric mucosa was found to be hypertrophic with superficial layer showing extensive necrosis on the surface and necrotic material in lumen [Figure 5 & 6]. Loss of mucosal cell morphology and nuclei was noticed [Figure 5&6]. Marked edema and inflammatory cells were also observed. Crowding of parietal cell was also present. Such extensive degenerative changes were reported earlier in electron

microscopic studies performed in rats, by giving omeprazole orally. In one report, after 35 days, a significant decrease in tubulovesicles and microvilli on the membranes of secretory canaliculi in parietal cells were found.^[12] In another case, the observations were made after 3 months and a hyperplasia of oxyntic mucosal cells of stomach was noticed.^[6] In none of aforementioned studies the region of stomach was specified. Our research showing preferential effects in two sexes, is original.

Changes observed in female gastric pyloric mucosa in esomeprazole treated rats are very interesting. The generalized edema and inflammation is seen involving all the layers of stomach wall. There were few parietal cells and increase in pepsin secreting glands [Figure 7&8]. Loss of parietal cells associated with chronic inflammatory changes are the features of atrophic gastritis. Such alarming reports exist in literature for omeprazole.^[4,11] Atrophic gastritis is taken very seriously due to its being precursor of stomach cancer. Differential effects in male and female stomach are not reported earlier.

The study needs further elaboration at electron microscopic, histochemical and molecular levels to explore conclusive evidences.

month, randomized, double-blind, placebo-controlled study of efficacy and safety. *Aliment Pharmacol Ther* Jul 2001; 15(7): 927-35.)

8. Larsson H, Carlsson E, Ryberg B, Fryklund J, Wallmark B. Rat parietal cell function after prolonged inhibition of gastric acid secretion. *Am J Physiol* 1988 Jan; 254 (1 Pt 1) : G33-9.
9. Georgopoulos SD, Robotis JF. Gastric acid inhibitors; impact on gastric and colon cancer. *Annals of Gastroenterology* 2004, 17(2): 133-135.
10. De Souza IKF, de Silva AL, de Araujo AJ, Santos FC. Qualitative analysis of anatoopathological changes of gastric mucosa due to long term therapy with proton pump inhibitors: Experimental studies X clinical studies ABCD. *ARQ. BRAS. CIR. DIG* 2013; 26(4):
11. Genta RM . Acid suppression and gastric atrophy: Sifting fact from fiction Article in *gut*. August1998 DOI: 10.113/gut. 43. 2008.S35. PubMed Citations 33 Reads 151.
12. Furuhashi, M. Akira Nakahara; Hisayuki Fukutomi; Eiki Kominami; Yasuo Uchiyama, Changes in Subcellular Structures of Parietal Cells in the Rat Gastric gland after Omeprazole. *Archives of Histology and Cytology*; 1992; ISSN: 0914-9465; 55; (2): 191-20.

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REFERENCES

1. Mullin JM, Valenzano MC, Whitby M, Lurie D, Schmidt JD, Jain V, Tully O, Kearney K, Lazowick D, Mercogliano G and Thornton JJ. Esomeprazole induces upper gastrointestinal tract transmucosal permeability increase. *Alimentary Pharmacology and Therapeutics* 2008 Dec; 28 (11-12): 1317-1325.
2. Raghunath AS ,Morain CO, Mc loughlin RC, Review article: the long term use of proton pump inhibitors *Alimentary Pharmacology and Therapeutics* 2005; 22 (Suppl. 1); 55-63.
3. Vakil NB, Shaker R, Johnson DA, Kovacs T., Baerg RD, Hwang C., D. The new proton pump inhibitor esomeprazole is effective as a maintenance therapy in GERD patients with healed erosive oesophatitis: a 6-month, randomized, double-blind, placebo-controlled study of efficacy and safety. *Aliment Pharmacol Ther* 2001; 15: 927-935.
4. Kuipers EJ, Lundell L, Klinkenberg-Knol EC, Havu N, Festen HP, Bea L. Atrophic gastritis and *Helicobacter pylori* infection in patients with reflux esophagitis treated with omeprazole or fundoplication. *N Engl J Med* 1996; 334: 1018-22.
5. Blom H. Cimetidine and parietal cell regeneration in experimental wounds in rat gastric mucosa. A light and electron microscopic study. *Scand J Gastroenterol* 1938, 18, 853-857.
6. Istvan H, Laszlo H. Bela M, Zsolt T, Laszlo P, Long-term omeprazole and esomeprazole treatment does not significantly increase gastric epithelial cell proliferation and epithelial growth factor receptor expression and has no effect on apoptosis and p53 expression. *World J Gastroenterol* 2005; 11 (30): 4721-4726.
7. Vakil NB, Shaker R, Johnson DA, Kovacs T, Baerg RD, Hwang C, D'Amico D, Hamelin B. The new proton pump inhibitor esomeprazole is effective as a maintenance therapy in GERD patients with healed erosive oesophagitis: a 6-