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Research Article

A COMPARATIVE STUDY ON MONOTHERAPY AND COMBINATION THERAPY OF PANTOPRAZOLE WITH PREGABALIN AGAINST SURGICAL ESOPHAGITIS

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ABSTRACT

The objective of this study was to evaluate the effect of pantoprazole and pregabalin on experimental esophagitis in albino rats. The groups of rats fasted for 24 hours and were subjected to pylorus and forestomach ligation. Rats of different groups received normal saline (3 ml/kg, po; sham control), pantoprazole (30 mg/kg, po), pregabalin (30 mg/kg, po) and their combinations along with a parallel toxic control group. Animals were sacrificed after 8 h and evaluated for the gastric pH, total acidity, free acidity and esophagitis index. The morphological changes were scrutinized by digital microscopy. The beneficial effect of pantoprazole and pregabalin against GERD could be attributed to the anti-secretory action of pantoprazole and reduction in the tracheal lower esophageal sphincter release rate by pregabalin. Combination therapy of gamma-aminobutyric acid derivative promotes proton pump inhibitor based healing of reflux esophagitis in animal model.

Keywords: Esophagitis, Pantoprazole, Pregabalin, Pylorus ligation, Gamma-aminobutyric acid.

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INTRODUCTION

The occurrence of gastro esophageal reflux disease (GERD) in India ranges from 8 to 20% as stated by a recently conducted study.¹ Esophagitis is the inflammation of the lining of the esophagus, the tube that carries food from throat to the stomach. If left untreated, then this condition can become very uncomfortable causing troublesome of swallowing, ulcers, scarring of esophagus, esophageal cancer etc. When acid from the stomach leaks up into the gullet (esophagus), the condition is known as reflux. Therefore, gastro esophageal reflux is the return of stomach's contents back into the esophagus. GERD is a digestive disorder that affects the lower esophageal sphincter (LES), the ring of muscle between esophagus and stomach. In normal digestion, the LES opens to allow food to pass into the stomach and closes to prevent food and acidic stomach juices from flowing back into the esophagus. Gastro esophageal reflux occurs when

the LES is weak or relaxes inappropriately. Symptoms of GERD consist of difficulty in swallowing (dysphagia), pain during swallowing (odynophagia), acid reflux, heart burn, nausea & vomiting, abdominal pain, sore throat, hoarse voice, cough and sometimes muscle ache or fever.² Endoscopically GERD broadly classified into two groups; Erosive esophagitis or Barrett's esophagus (having esophageal mucosal damage) and non-erosive reflux disease, NERD (no mucosal damage).^{3,4} The pathophysiology of GERD is multifactorial, involving transient lower esophageal sphincter relaxation (TLESR), reduced LES pressure, poor esophageal clearance, impaired esophageal mucosal defense, visceral hypersensitivity, hiatal hernia and delayed gastric emptying. The enhanced acid secretion and reflux mechanism suggests playing a key role in the elementary pathogenesis of GERD.^{5,6} Irregularities in the gastro-esophageal junction, the stomach, the esophagus and the nervous system may all contribute to this disease state.⁷ Mechanism of gastro-

esophageal reflux befallen by three different mechanisms: transient complete relaxation of the lower esophageal sphincter, a transient increase in intra-abdominal pressure, or spontaneous free reflux associated with a low resting pressure of the lower esophageal sphincter.⁸ For all immune-compromised patients, the most frequently identified esophageal pathogen is *Helicobacter pylori*.⁹

Both genetic and environmental factors appear to influence the presence of GERD. Several studies have shown that obesity, weight gain, pregnancy, frequent vomiting and increasing body mass index (BMI) are associated with GERD.^{10,11} Research also indicates that some risk factors as smoking, excess alcohol consumption, irritable bowel syndrome and a family history of upper GI disease are responsible for GERD. Consuming certain foods and drinks in large quantities regularly such as tomato-based ones, citrus fruits, chocolates, garlics, onions, spicy foods and caffeine's can cause GERD. Factors also contribute to GERD are Hiatal hernia, Zollinger Ellison syndrome, NASIDS, anti-cholinergics, antidepressants.¹² GERD is a chronic disorder that often requires long-term maintenance therapy. Proton pump inhibitors (PPIs) are the most effective therapy.^{13,14} Pantoprazole is a PPI used in the treatment of dyspepsia, peptic ulcer disease, gastro-esophageal reflux disease and Zollinger–Ellison syndrome.^{15,16}

Pantoprazole suppress the final step in gastric acid production by forming a covalent bond to two sites of the (H⁺-K⁺) - ATPase enzyme system. This phenomenon occurs at the secretory surface of the gastric parietal cell. The maintenance therapy by PPIs alone is not very effective due to weak inhibitory activity in early phase and less effectiveness of the therapy within the initial hours of dosing.¹⁷ Pregabalin is a derivative of gamma-amino butyric acid (GABA) and is a potent ligand for the alpha-2-delta subunit of voltage-gated calcium channels in the central nervous system.¹⁸ A recent study has revealed that pregabalin reduces the tracheal lower

esophageal sphincter release rate (TLESR) and reflux episode by increasing esophageal sphincter basal pressure and accelerating gastric emptying.^{19,20}

The present study was put forward to evaluate the effect of monotherapy and combination therapy of pregabalin and pantoprazole on experimentally induced esophageal lesions in rat model.²¹

MATERIALS AND METHODS

Drugs and Chemicals

Pantoprazole was received as a gift sample from Alkem Laboratories Ltd Baddi, Himachal Pradesh 173205, and Pregabalin was procured from the local market. All other chemicals used were of analytical grade.

Animals

Wistar strain albino rats (80–120 g) were obtained from Breeding Centre BHU. Rats were kept in polypropylene cages under standard condition of temperature (37±1⁰C) and 40-45% relative humidity with 12 h light: 12 h dark cycle. Rats were provided with commercial pellet diet and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of UIP, Allahabad (Ref. number UIP/IAEC/2015/10).

Induction of Esophagitis

Animals were randomized and divided into five groups of six animals in each, fasted for 24 h, received, sham control (normal saline, 3 ml/kg, po), toxic control (pylorus and forestomach ligated; normal saline, 3 ml/kg, po), pantoprazole (pylorus and forestomach ligated; 30 mg/kg, po), pregabalin (pylorus and forestomach ligated; 30 mg/kg, po), and their combination (pylorus and forestomach ligated; 30 mg/kg+30 mg/kg, po). After 1 h, coeliotomy was accomplished and esophagitis was induced by ligating the forestomach and pylorus with 2-0 silk suture under pentobarbitone anesthesia (50 mg/kg, ip) (**Figure1**).²²

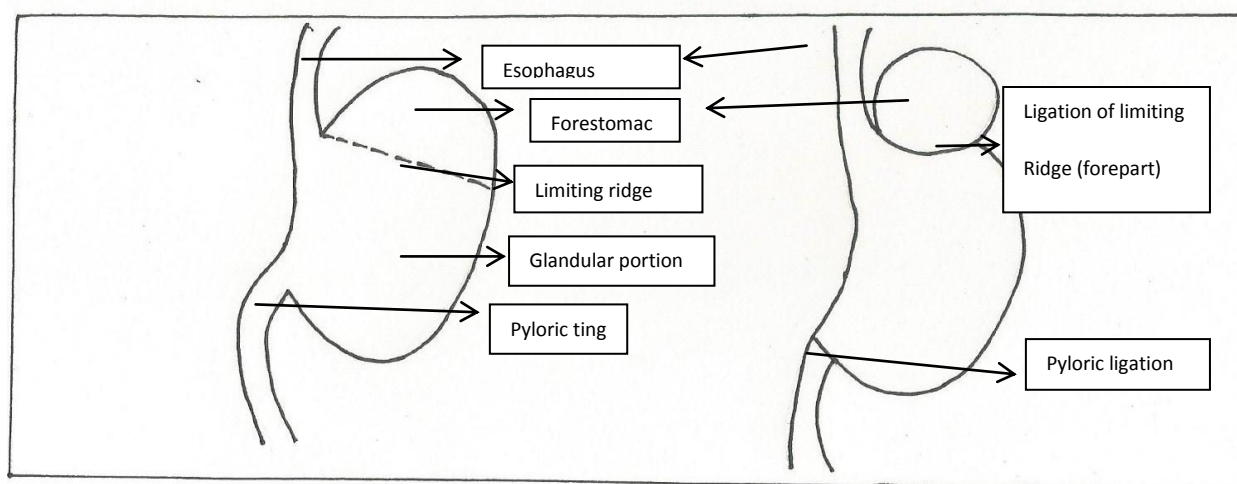


Figure 1: Demonstration of forestomach and pylorus ligation.

After 8 h animals were sacrificed by cervical dislocation and the abdomen was opened with a median incision and the esophagus & the stomach were removed. The tissue was washed with distilled water and examined for

lesion. The severity of the erosions was scored using **Table 1** and the esophagitis index was calculated by dividing the total score by ten.²³

Table 1: Scoring table of esophagitis index

Erosion(mm)	1 or less	1-2	2-3	>3
Score	1	2	3	4

The pH is measured by using a pH meter (Paras). The volume of gastric juices was dignified as described consequently under “gastric secretion in pylorus ligated rats”.²⁴

Estimation of free radical generation

Esophageal tissue was minced well, homogenized in ice-cold 0.01 M Tris-HCl buffer, pH 7.3 and exposed to the evaluations of thiobarbituric acid reactive substances (TBARS), tissue glutathione (GSH), catalase and superoxide dismutase (SOD).^{25,26,27,28}

Histological Evaluation

Digital Microscopy of esophageal tissue has been done for the study of the tissues before and after the therapy. The tissues for histological study were embedded in paraffin wax and sectioned on a rotary microtome at 6µm thickness followed by staining of the tissue with hematoxyline. The stained sections were observed under a Dewinter microscope and digital photomicrographs were taken.

Statistical Analysis

All the data were offered as mean ± SD and analyzed by one way ANOVA followed by Bonferroni test for the possible significance identification between the various groups. Statistical analysis was carried out using Graph pad software (3.2).

RESULTS

Treatment with combination therapy of pantoprazole and pregabalin significantly controlled the lesion formation in esophagus. The combination therapy of pantoprazole and pregabalin produced 73.42% inhibition of esophagitis index respectively. The amalgamation of pantoprazole and pregabalin, inhibited the esophagitis index, decreased the volume of gastric juices, total acidity and increased the gastric pH to a judgmental level (Table 2), suggesting the possible synergistic effect. Earlier studies have explained the role of free radicals in pathogenesis of the reflux esophagitis in experimental animals.²⁹ Reflux esophagitis has been testified to increase Malondialdehyde (MDA), a stable product of lipid peroxidation and a sensitive marker of membrane damage.^{30,31} The oxidative stress leads to deprivation of cellular membrane which produces MDA. It is a reactive species that reacts to form a color complex with thiobarbituric acid and we recorded a momentous upsurge in MDA augmentation in toxic control group.^{32,33} Associated administration of the pantoprazole and pregabalin as a monotherapy and combination therapy critically inhibited the lipid peroxidation expressed by decreased TBARS levels, that is, 2.87±0.01, 1.89±0.04, and 1.07±0.01 nmol of MDA/mg of protein, respectively (Table 3).

Table 2 Effect of pantoprazole and pregabalin on gastric content, pH, free acidity, total acidity and esophagitis index in albino rats.

Groups	Treatment(p.o)	pH	Volume of gastric juices(ml/100g)	Esophagitis index	Total acidity (mEq/l)	Free Acidity (mEq/l)
Group- I	Sham Control (3.0 ml/kg)	3.52±0.21***	1.53±0.23***	0.65±0.34***	28.22±1.06***	26.73±1.45***
Group- II	Toxic Control (Normal Saline 3 ml/kg)	2.78±0.32	5.43±0.19	1.66±0.12	80.48±3.16	56.33±2.41
Group-III	Pantoprazole (30 mg/kg)	3.29±0.11	1.51±0.33*	0.50±0.11***	42.78±1.05***	32.67±1.13***
Group- IV	Pregabalin (30 mg/kg)	3.42±0.26***	1.89±0.24***	0.91±0.26*	47.26±1.29***	37.15±1.33***
Group- V	Pantoprazole +Pregabalin (30mg/kg and 30 mg/kg)	3.30±0.12**	1.81±0.32**	0.72±0.18***	45.26±0.65***	33.74±1.57***

Each group contains six animals. Data is represented as Mean±SD. Statistical significance compared to toxic control using one-way ANOVA followed by Bonferroni test where *p<0.05, **p<0.01, ***p<0.001 were considered statistically significant.

Table 3 Effect of pantoprazole and pregabalin therapy on TBARS, protein carbonyl, GSH, Catalase and SOD in albino rats subjected to forestomach and pylorus ligation.

Groups	TBARS (Nm of MDA /mg of protein)	Protein carbonyl (nmoles/ml)	GSH*10 ⁴ (mg %)	SOD (unit of SOD/mg of protein)	Catalase (nM of H ₂ O ₂ /min/ mg of protein)
Group-I	0.72±0.01***	62.66±3.02***	2.16±0.11***	7.68±2.83	20.11±2.53
Group-II	6.72±0.07	128.10±22.28	0.97±0.10	3.56±1.22	6.21±0.83
Group-III	2.87±0.01***	85.48±9.11*	1.62±0.03***	3.03±1.23***	15.21±4.03***
Group-IV	1.89±0.04***	81.98±10.23**	2.06±0.09	6.31±2.31	21.22±2.38***
Group-V	1.07±0.01***	74.73±12.83**	2.08±0.24***	6.05±2.77***	22.53±2.12***

Each group contains six animals. Data is represented as Mean±SD. Statistical significance compared to toxic control using one-way ANOVA followed by Bonferroni test.

DISCUSSION

A momentous increase in protein carbonyl content was determined in toxic control as analogous of sham control and combination therapy afforded considerable inhibition of oxidative stress. Similarly the GSH level in toxic group (0.97±0.10 mg %) reduced as compared to control group (2.16±0.11 mg %), after combination therapy is restored (2.06±0.09 mg %). The glutathione (GSH) is a universal tripeptide, which is the most abundant low molecular weight thiol in almost all cells and is tangled in an extensive range of enzymatic reaction. A major role of GSH is to serve as a reductant in oxidation reduction processes, a function causing the formation of glutathione disulfide (GSSG). Free radical destruction leads to consumption of GSH in the first few hours of oxidative stress, leading reduced GSH level, a marker of short term oxidative stress, and treatment with pantoprazole and pregabalin has significantly facilitated to re-establish the same.^{34,35}

In the same way experimental esophagitis diminished the level of SOD 3.56±1.22, in comparison of normal group 7.68±2.83, combination therapy enhanced it further 6.05±2.77 unit of SOD/mg of protein. SOD is a free radical scavenging enzyme it counterpoises

superoxide-free radicals produced during the metabolism of drug, hence its concentration in the tissue reduces with increase in the time. SOD functions as an antioxidant and diminishes oxidative stress in the experimental rats. SOD scavenges the H₂O₂ to form water and molecular oxygen.³⁶ The procedure includes the formation of hydroxyl and molecular oxygen free radical as the intermediate products.³⁷ The SOD in conjugation with catalase organizes the foremost protection against free radicals.

Correspondingly, in toxic group catalase protein condensed 6.21±0.83 from 20.11±2.53 (sham control) and in union therapy is re-established to 22.53±2.12 nM of H₂O₂/min/mg of protein. Catalase is a hemeprotein which catalyses the reduction of hydrogen peroxide and shield the membrane from extremely reactive hydroxyl radical.³⁸ The catalase protein production increased by the treatment of this fusion therapy.

It is remarkable that pregabalin shows better healing effect alone as well as in combination with pantoprazole without any untoward effect, which is not the case for present therapeutic options for GERD. Pregabalin illustrates ameliorative effects on esophageal tissues.

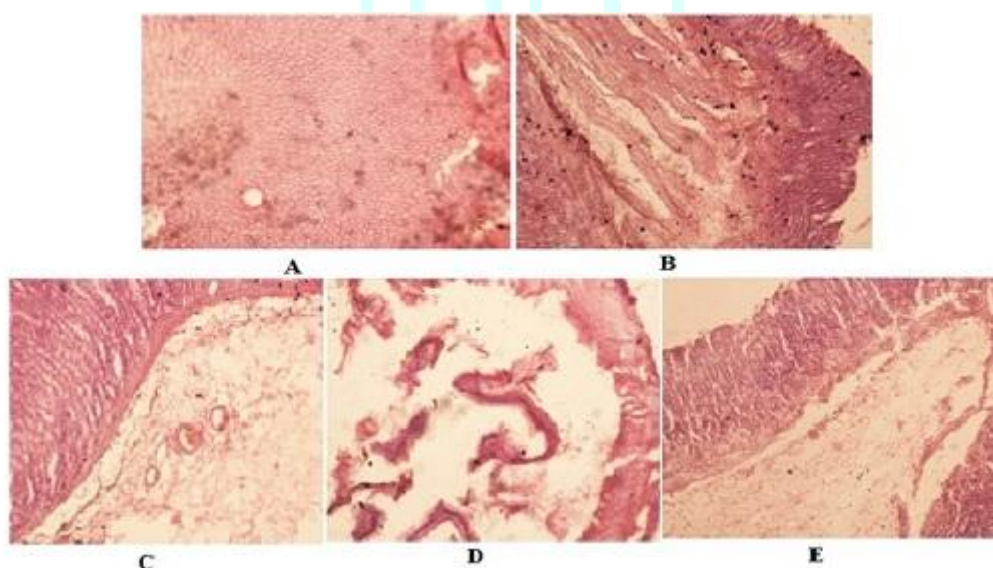


Figure 2: Morphological changes of esophagus in different groups as observed through Microscopy: (A) Sham Control, (B) Toxic Control (3.0 ml/kg normal saline), (C) Pantoprazole(30 mg/kg), (D) Pregabalin (30mg/kg) and (E) Pantoprazole (30mg/kg)+ Pregabalin (30mg/kg).

The ultra-structural changes including dilated intracellular spaces and mucosa degeneration is a well-studied phenomenon and same was observed when studied microscopically. Marked ultra-structural damage with mucus degeneration was observed in toxic group comparison to sham control; treatment with amalgamation therapy demonstrated marked microscopic protection. The figure depicts that the combination therapy of pantoprazole and pregabalin significantly reduced the ulceration as compared to the individual therapy (**Fig. 2**).

This study revealed that the unification therapy of pantoprazole and pregabalin elucidates reasonable protection against the surgical esophagitis. Ligating the forestomach and pylorus developed reflux esophagitis in all the rats blotted by macroscopically visible necrosis and substantial ulceration in the esophagus. Dealing with pantoprazole and pregabalin suggestively inhibited the ulcer formation in esophagus. Pantoprazole is a proton pump inhibitor showing H⁺K⁺ ATPase and carbonic anhydrase inhibitory activity.³⁹ Pantoprazole is an engrained drug used for the treatment of peptic ulcer, which prevents the secretion from the gastric cells and helps in providing relief in GERD.⁴⁰ Pantoprazole covalently binds to cysteine residue of proton pump and obstructs the secretion of gastric acid.⁴¹ It inhibits the secretion from gastric cells and helps in effecting consolation in reflux disease and mucosal curing in gastric ulcer and GERD. The beneficial effects of

pregabalin perceived in the current study could be accredited to its capability to condense TLESR by increasing esophageal sphincter basal pressure and accelerated gastric emptying.⁴² The combination of pantoprazole and pregabalin subdued the esophagitis index, diminished the volume of gastric juices, and condensed the pH to a major level, signifying the thinkable synergistic effect. Thus, the effect against GERD could be congregately attributed to the antisecretory action of pantoprazole and reduction of TLESR by pregabalin.

CONCLUSIONS

This preclinical study concludes that the fusion therapy of pantoprazole and pregabalin is showing synergistic effect in the treatment of GERD. Pregabalin alone as well as in combination with pantoprazole can heal the esophagitis symptoms. We can find more analytical data by doing clinical studies on the same combination.

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CONFLICT OF INTEREST: No conflict of interest.

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