Role of Melatonin with Lansoprazole in Treating Peptic Ulcer in Rats

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ABSTRACT

Peptic ulcer is common in present days due to stress and anxiety. Peptic ulcer is thought to result from an imbalance between acid-pepsin secretion and mucosal defense factors. Though currently available drugs provide adequate relief from pain, promote healing, the problem of relapse has eluded success. The present study was designed to evaluate the combination effect of melatonin with lansoprazole against pylorus ligation induced ulcer model in rats. Albino Wistar rats of either sex were divided into 4 groups of 6 animals each. The antiulcer effect of the combination of melatonin 0.108 mg/200 g and lansoprazole 0.54 mg/200 g b.w orally was compared with the reference standard lansoprazole 0.54 mg/200 g b.w orally. The ulcer index and other biochemical parameters like volume, pH, free acidity and total acidity of gastric juice were estimated. The ulcer index and other biochemical parameters like volume (***P < 0.001), free acidity (***P < 0.001), total acidity (***P < 0.001) and pH (*P < 0.05), of gastric juice showed reduction when compared to control and standard lansoprazole. The percentage protection of combination was 90% as compared to standard lansoprazole 82.8%. Thus the combination of lansoprazole with melatonin was found to be synergistic in nature when compared to lansoprazole alone.

Keywords: Gastroprotection, Melatonin, Lansoprazole, Pylorus ligation model, Ulcer index.

INTRODUCTION

Peptic ulcer is one of the major ailments affecting about 60% human adults and nearly 80% child population in tropical countries.¹ An acid peptic disease includes hyperacidity, gastroesophageal reflux diseases (GERD’s), stress induced mucosal erosions and peptic ulcers (gastric as well as duodenal). Inflamed break in the lining of the stomach or the duodenum caused due to either increased acid production or damage to the mucus lining of the stomach leads to formation of peptic ulcer, a term that includes both gastric as well as duodenal ulcer. Peptic ulcer arises when the normal mucosal defensive factors (mucus, bicarbonate, prostaglandins etc) are impaired or over powered by the aggressive factors (Gastric acid, pepsin, bile, acetylcholine, histamine, gastrin, H. pylori).²³ A number of drugs including proton pump inhibitors and H₂ receptors antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapse, side effects, and drug interactions. This has been the rational for the development of new antiulcer drugs and the search for novel molecule or drug therapy that offer better protection and decreased relapse.

Melatonin, a close derivative of serotonin,⁴ is a hormone produced by the pineal gland and intestinal enterochromaffin cells which control sleep and gastrointestinal motility.⁵ It is beneficial for treating disorders of the GI tract by exerting antioxidant effect, inhibiting hydrochloric acid and pepsin secretion, and acting as an immunostimulant.⁶⁷ Hence an attempt was made to study the combination effect of melatonin with antiulcer drugs like lansoprazole. Lansoprazole is a proton pump inhibitor that suppresses the gastric acid secretion by inhibiting H⁺K⁺ ATPase pump. The FDA has approved it
for treatment and prevention of recurrence of NSAIDs associated gastric ulcers in patients who continue NSAID use. It is more potent, has longer duration of action, better bioavailability and lesser drug interactions than other drugs. Therefore, the present study was designed to evaluate the combination of melatonin and lansoprazole against pylorus ligation induced ulcer model.

**MATERIALS AND METHODS**

Albino Wistar rats of either sex weighing between 180 to 220 g were procured from central animal house, MR. Medical College, Gulbarga. The animals were acclimatized for seven days and housed under standard conditions of temperature (25 ± 2°C) and relative humidity (30–70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (Hindustan Lever Ltd, Mumbai) and water ad libitum. Approval of the Institutional Animal Ethics Committee (IAEC) of H.K.E.S College of pharmacy, Gulbarga was taken for conducting antiulcer activity. The protocol number was HKE-COP/IAEC/17/2009–10 and the animal studies were performed in accordance to guidelines of CPCSEA. Pure drug samples of lansoprazole and melatonin were procured from Lee Pharmaceuticals (Hyderabad) and Aristo Pharmaceuticals (Mumbai) respectively. The dose calculations were extension of human dose based on body surface area.

In pylorus ligation induced ulcer model, the rats were divided into 3 groups of 6 animals each. The animals of Group I were treated with vehicle and the animals of Group II were treated with standard, i.e., lansoprazole 0.54 mg/200 g b.w orally. Similarly Group III animals were treated with melatonin and lansoprazole i.e., 0.108 mg and 0.54 mg per 200 g b.w orally respectively. The drugs were administered daily for 5 days. On 5th day, the rats were fasted for 24 h before pyloric ligation. Care was taken to avoid coprophagy. At the end of 24 h, the rats were anaesthetized with anesthetic ether. Abdomen was opened by a midline incision. The stomach was lifted out and a ligature was placed at the pyloric sphincter without causing any damage to its blood supply. The stomach was replaced carefully and abdomen wall was sutured in two layers.

After 6 hours, the rats were sacrificed with excess of anesthetic ether, and the stomachs were dissected out. Gastric juice was collected and drained into test tubes and then centrifuged at 1000 rpm for 10 min and the volume of supernatant was noted. The pH of the gastric juice was recorded by pH meter. Then the contents were subjected for the analysis of free and total acidity. The stomachs were opened along the greater curvature then washed under running water to see ulcers in the glandular portion of the stomach. The number of ulcers per stomach was noted and scoring was done microscopically with the help of hand lens (10x). The recording was 0 for normal coloured stomach, 0.5 for red colouration, 1 for spot ulcer, 1.5 for hemorrhagic streaks, 2 for ulcer ≥ 3 ≤ 5 and 3 for ulcer > 5. The mean ulcer score for each animal was expressed as ulcer index and the percentage protection was calculated by using the formula:

\[
\% \text{ Protection} = \frac{[\text{Ulcer score of control} - \text{Ulcer score of treated}]}{\text{Ulcer score of control}} \times 100
\]

**Determination of free acidity and total acidity**

1 ml of gastric juice was pipetted into 100 ml conical flask. It was diluted to 10 ml with distilled water and added 2–3 drops of Topfer’s reagent and titrated with 0.01 N sodium hydroxide until all traces of red color disappear and the color of the solution turns to yellowish orange. The volume of the alkali added was noted. This volume corresponds to free acidity. Then 2–3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears. Again the total volume of alkali added was noted. The volume corresponds to total acidity. Acidity was calculated by using the formula:

\[
\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{100 \, \text{meq/L/100 g}} 
\]

**Histopathological evaluation**

The stomachs were immersed in 10% formalin solution for histopathological examination. These tissues were processed and embedded in paraffin wax. The central part of damaged or ulcerated tissue (if present) was cut off along the long diameter. If the stomach was protected from the damage then the section was taken from basal part using a rotary microtome, sections of thickness of about 5 µm were cut and stained with haematoxylin and eosin. These were examined under the microscope for histopathological changes such as congestion, haemorrhage, necrosis, inflammation, infiltration, erosion and ulcers. The micro photographs were taken for publication.

**Statistical analysis**

The results were expressed as mean ± SEM, (n = 6). Statistical analysis was performed using student 't' test. P value less than 0.05 was considered to be statistically significant.

**RESULTS**

It is evident from Table 1 that the effect of combination group i.e., melatonin and lansoprazole showed reduction
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in ulcer index and all biochemical parameters like volume, free acidity, total acidity and increase in pH of gastric juice when compared to control and standard lansoprazole. The percentage protection of combination group was found to be 90% when compared to control and standard lansoprazole (82.8%) and melatonin (72.0%).

The Histopathological examination using haematoxylene and eosin staining also revealed the protective activity of combination group when compared to control and standard lansoprazole (Figure 5).

DISCUSSION

Peptic ulceration is related primarily to a breakdown of the barrier that normally prevents irritation and autodigestion of mucosa by prolonged and excess gastric acid secretion. A peptic ulcer results from an imbalance between some endogenous aggressive factors that is hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species and defensive factors, which include the function of the mucus-bicarbonate barrier, surface active phospholipids, prostaglandins (PGs), mucosal blood flow, cell renewal and migration, non enzymatic and enzymatic antioxidants and some growth factors.13 Prostaglandins (PG) offer protection to stomach by increasing mucosal resistance and decreasing aggressive factors like acid and pepsin.14 According to Goel and Bhattacharya (1991),15 pylorus ligation ulcers may be due to autodigestion of gastric juice, decreased mucosal blood flow and breakdown of mucosal barrier.

The Shay model9 is simple, reproducible and highly predictable model for the screening and evaluation of anti-ulcer drugs. It utilizes neither the exogenous ulcerogens nor the induced exogenous interfering factors. In case of pyloric ligation, ulcer formation is mainly due to the stasis at the gastric juice and stress16 or there is an excess of acid for a given degree of mucosal defense. Therefore the reduced gastric ulcer in this model may be due to the reduction in acid secretion and increased gastric pH.

In the present study, from Table 1 and Fig. 1 to Fig. 5 the reduction in ulcer index and other biochemical parameters of gastric juice like volume, free acidity, total acidity and increase in pH by combination group suggests that its cytoprotective mechanism may be due to inhibition of gastric secretion and neutralization of reactive oxygen species by one or more mechanism. Lansoprazole, being a potent proton inhibitor,

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Table 1: Effect of Melatonin and Lansoprazole in Pylorus Ligation Induced Gastric Ulcer Model

<table>
<thead>
<tr>
<th>Gr. No.</th>
<th>Treatment</th>
<th>Dose/200 g rat</th>
<th>Vol. of gastric juice (ml)</th>
<th>Free acidity (mEq/L) 100 gm</th>
<th>Total acidity (mEq/L) 100 gm</th>
<th>pH</th>
<th>Ulcer Index</th>
<th>Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Distilled water 0.5 ml</td>
<td>8.133 ± 0.11</td>
<td>112.0 ± 0.73</td>
<td>124.3 ± 0.55</td>
<td>1.800 ± 0.07</td>
<td>5.833 ± 0.10</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Melatonin</td>
<td>0.108 mg</td>
<td>2.000 ± 0.06</td>
<td>80.50 ± 0.38</td>
<td>97.28 ± 0.49</td>
<td>4.448 ± 0.05</td>
<td>1.000 ± 0.00</td>
<td>72.0%</td>
</tr>
<tr>
<td>2</td>
<td>Lansoprazole</td>
<td>0.54 mg</td>
<td>4.567 ± 0.14</td>
<td>56.67 ± 0.55</td>
<td>67.67 ± 0.55</td>
<td>6.767 ± 0.09</td>
<td>1.000 ± 0.18</td>
<td>82.8%</td>
</tr>
<tr>
<td>3</td>
<td>Lansoprazole + Melatonin (0.54 + 0.108) mg</td>
<td>3.800 ± 0.07***</td>
<td>47.67 ± 0.91***</td>
<td>58.00 ± 0.70***</td>
<td>7.133 ± 0.11*</td>
<td>0.6667 ± 0.16</td>
<td>90%</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. of 6 rats/treatment
Significant *P < 0.05 and ***P < 0.001 compared with standard lansoprazole.
Figure 3: Shows stomach of melatonin treated rat in pylorus ligation model.

Figure 4: Shows stomach of standard lansoprazole treated rat in pylorus ligation model.

Figure 5: Shows stomach of melatonin and lansoprazole treated rat in pylorus ligation model.

Figure 6: Histopathological studies of Pylorous ligation induced ulcer model in rat (A) Control negative: Gastric mucosa shows normal histology. (B) Control positive: Gastric mucosa shows redness, congestion, hemorrhagic streaks, edema, ulceration, necrosis and dilation of blood vessels. (C) Melatonin: Gastric mucosa showing ulceration, congestion and mild inflammation. (D) Standard Lansoprazole: Gastric mucosa shows redness, congestion, hemorrhage, mild edema and dilation of blood vessels. (E) Melatonin and Lansoprazole: Gastric mucosa shows mild redness, congestion, mild edema and dilation of blood vessels.
decreases the excess acid secretion, by irreversibly blocking the H+, K+-ATPase of the parietal cells. Melatonin being an antioxidant scavenges OH radical that cause cellular damage. The ulcer healing activity of melatonin probably results from anti-H.pylori and antioxidant action of this indole, resulting in the regression of mucosal inflammation and acceleration of ulcer healing.17

CONCLUSION

From the present study and available results, it can be concluded that the combination group of melatonin and lansoprazole was found to be synergistic in nature and have more cytoprotective and antisecretory effect when compared to the standard drug lansoprazole alone.

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