CHAPTER VII

ANTI-ULCER EFFECT OF FLAVONOID GLYCOSIDES AGAINST PYLORIC LIGATION INDUCED GASTRIC ULCER IN RATS
INTRODUCTION

Gastric acid hypersecretion is one of the major pathogenic factors for the induction of gastric ulcer disease. The back diffusion of acid into the mucosa could directly lead to vascular leakage and aggressive damaging effect in the basement membrane of both epithelial and mucosal cells in the gastric wall, which could inhibit the restitution processes in the injured mucosa and induce a progression of apoptosis to deeper layers of the mucosa. Peptic ulcer (encompassing gastric ulcer and duodenal ulcer) is a major health hazard both in terms of morbidity and mortality. Peptic ulcer disease affect a large portion of the world population and are induced by several factors, including stress, smoking, nutritional deficiencies, and ingestion of non-steroidal anti-inflammatory drugs. The pathophysiology of these ulcers involves an imbalance between offensive (acid, pepsin, and \textit{Helicobacter pylori}) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection.

Peptic ulcer disease and gastric dyspepsia-associated with chronic use of therapeuticals such as non-steroidal anti-inflammatory drugs (NSAIDs) and anti-cancer agents are the two major causes that adversely affect the life quality. Presently used antisecretory agents like proton pump inhibitors may represent a key option in peptic ulcer therapy but their prolonged use seems to be associated with high incidence of hip fractures. NSAIDs induced gastropathy remains a major clinical
problem which has not been solved through the introduction of selective inhibitors of cyclooxygenase-2 (COX-2) due to cardiac side effects. Similar to NSAIDs, many cancer chemotherapeutics such as cisplatin, and bisphosphonates like alendronate can induce gastric dyspepsia.

In recent years, there is an active search to discover novel and alternative agents useful to combat gastric dyspepsia, and peptic ulcer disease. Worldwide interest in natural products as preventive and therapeutic agents has led to a greater appreciation of the rich heritage of traditional systems of medicine. Dietary and lifestyle modifications are the basis of Ayurvedic medicine, with herbal formulas rounding out therapeutic programs. Ayurvedic formulas contain many balancing herbs offering a high degree of safety and efficacy. Therefore, the protection of gastric tissue damage has become a rational approach in preventing pylorus ligation induced ulcer. Pyloric ligation induced ulcer represents a unique ulcer model in examining the cause, course, consequence and treatment of peptic ulcer. Pylorus ligation induced ulcer is results of auto digestion of the gastric mucosal barrier probably due to excess production and accumulation of HCl in the stomach. The present study was carried out to evaluate the gastroprotective effect of G1, G2, G3, G4, G5, and G6 against pyloric ligation induced gastric ulcer in rats.
MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately weighing 160–180g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2ºC and 12-hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided and libitum. They were acclimatized to the environment for one week prior to experimental use.

Chemicals

Diethyl ether, Sodium hydroxide and omeperazole were purchased from Sigma chemical company, Mumbai. All other chemicals and reagents used in this study were of analytical grade with high purity and were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai, India.

Pylorus ligation (PL)-induced ulcers

Animals were randomly divided into nine groups each of 6 rats. Each animal was marked for identification and regularly monitoring. Group I served as control group received saline orally. Groups II to IX animals served as ulcerogenic group as Pyloric ligated animals. Group II animals served as ulcerogenic. Groups III to VIII animals received G1, G2, G3, G4, G5, and G6 at a dose of 2ml/kg orally. Group IX was orally
administered 20mg/kg (ip) Omeperazole as a standard drug. The duration of the treatment was 5 days. After 5 days of drug treatment, the rats were anaesthetized using pentobarbitone (35mg/kg, ip). Pyloric ligation was done by ligating the pyloric end of the stomach of rats 1 h after last drug administration without causing any damage to its blood supply. Animals were allowed to recover and stabilized in individual cage and were deprived of water during post-operative period.

After pyloric ligation for 4 hours, all the animals were killed under ether anesthesia. Their stomachs were removed rapidly, and the gastric contents were collected and centrifuged. The supernatant measured and further used for analysis. Gastric contents were analysed for total acidity by titrating against 0.01N NaOH using phenolphthalein as indicator. The pH of gastric juice was measured by using digital pH meter and the pH was recorded for different groups of animals.

**Measurement of ulcer index**

The stomachs were excised and were examined for hemorrhagic lesions in glandular mucosa. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminant, if any. Gastric lesions were evaluated by examining the inner gastric surface with a magnifying glass and the mucosal lesions were counted and scored. The ulcer index for each rat was taken as the mean ulcer score. The percentage of inhibition (%I) was calculated as described by Nguelelack et al.\textsuperscript{461} using the following formula:
\[
\%I = \left(\frac{\text{USc}-\text{USt}}{\text{USc}}\right) \times 100
\]

Where USc is the ulcer surface area in control and USt is the ulcer surface area in treated animals.

**BIOCHEMICAL ESTIMATIONS**

**Determination of gastric juice volume and pH**

The volume and pH of centrifuged gastric juice were measured by pipette and digital pH meter. The volume was expressed as ml.\(^{462}\)

**Determination of total and free acidity**

The total and free acidity were determined by titrating with 0.01N NaOH using phenolphthalein and Topfer’s reagent or methyl orange.

**Reagents**

1. 0.01N NaOH
2. Phenolphthalein
3. Topfer’s reagent or methyl orange

**Procedure**

Pipette 1ml of filtered gastric contents into a small beaker, add 2 to 3 drops of Topfer’s reagent or methyl orange and titrate with 0.01 N NaOH until all trace of the red colour disappears and the colour is yellowish orange. Note the volume of alkali added that indicate free acidity. Then add 2 or 3 drops of phenolphthalein and continue titrating until a definite red tinge reappears. Note the total volume of alkali added that indicate total acidity.

The results are expressed as Meq/l
**Statistical Analysis**

The values were expressed as mean ± SD for six rats in each group and statistically significant differences between the mean values were determined by one-way analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparisons. The results were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 3 was used and P<0.01 was considered to be significant.
RESULTS

The effect of orally administered G1, G2, G3, G4, G5, and G6 on gastric damage induced by pyloric ligation is shown in Table 7.1. It was observed that significant reduction in ulcer lesion in treatment with G1, G2, G3, G4, G5, and G6. It is significant to note that the flavonoid glycosides increased the volume, total acidity and free acidity and decreased pH of gastric juice were observed in ulcer control rats as compared to normal rats. Administration of G1, G2, G3, G4, G5, and G6 decreased the volume, total acidity and free acidity and increased pH of gastric juice were observed as compared to control rats. Animal groups treated with the G1, G2, G3, G4, G5, and G6 (2ml/kg,) exhibited a reduction of gastric damage against pyloric ligation induced gastric ulceration. The percentage of ulcer protection was 77.78% for G1, 88.90% for G2, 88.87% for G3, 76.79% for G4, 66.67 % for G5, 66.68% for G6 and 77.80% for standard. Among the flavonoid glycosides, the significant inhibitions were 88.90% for G2, 88.87% for G3 observed (Table 7.1). Omeperazole, the positive control included for the study also offered significant protection (77.80%) against pyloric ligation induced gastric ulcer (Table 7.1). The G1 and G2 percentage of inhibition was higher than that of standard. Fig. 7.1 shows the photographic representation of control and experimentally induced ulcer model. Fig. 7.1a shows the normal architecture of stomach (Normal). Fig. 7.1b shows the control animals that pyloric ligation induced gastric damage presented clearly produced characteristic haemorrhage, congestion and oedema formation and epithelial lifting (ulcer-induced rats) in stomach sections. Fig. 7.1c to 7.1h and Fig. 7.1i shows the flavonoid glycosides and standard drug treated rats close to normal architecture of stomach.
### TABLE – 7.1
Effect of the flavonoid glycosides G1, G2, G3, G4, G5, and G6 on pH, volume, acidity, ulcer lesion in control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>pH</th>
<th>Volume</th>
<th>Free acidity</th>
<th>Total acidity</th>
<th>Gastric ulcer lesion (No.)</th>
<th>% of Ulcer protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>2.5 ± 0.16</td>
<td>1.4 ± 0.09</td>
<td>222 ± 15.4</td>
<td>260 ± 18.2</td>
<td>1 ± 0.07</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>1.4 ± 0.11</td>
<td>3.4 ± 0.21</td>
<td>322 ± 22.4</td>
<td>360 ± 25.2a</td>
<td>9 ± 0.63a</td>
<td>-</td>
</tr>
<tr>
<td>Group III (G1)</td>
<td>2.3 ± 0.15</td>
<td>1.7 ± 0.04</td>
<td>220 ± 12.8b</td>
<td>240 ± 16.8b</td>
<td>2 ± 0.14b 77.78</td>
<td></td>
</tr>
<tr>
<td>Group IV (G2)</td>
<td>2.6 ± 0.16</td>
<td>1.6 ± 0.04b</td>
<td>214 ± 8.4b</td>
<td>280 ± 12.6b</td>
<td>1 ± 0.08b 88.90</td>
<td></td>
</tr>
<tr>
<td>Group V (G3)</td>
<td>2.5 ± 0.15</td>
<td>1.5 ± 0.05b</td>
<td>216 ± 7.7b</td>
<td>270 ± 11.9b</td>
<td>1 ± 0.07b 88.87</td>
<td></td>
</tr>
<tr>
<td>Group VI (G4)</td>
<td>2.4 ± 0.40</td>
<td>1.7 ± 0.04b</td>
<td>233 ± 9.1b</td>
<td>290 ± 13.3b</td>
<td>2 ± 0.14b 76.79</td>
<td></td>
</tr>
<tr>
<td>Group VII (G5)</td>
<td>2.6 ± 0.18</td>
<td>1.7 ± 0.04b</td>
<td>212 ± 7b</td>
<td>260 ± 11.2b</td>
<td>3 ± 0.21b 66.67</td>
<td></td>
</tr>
<tr>
<td>Group VIII (G6)</td>
<td>2.5 ± 0.17</td>
<td>1.8 ± 0.05b</td>
<td>220 ± 8.4b</td>
<td>270 ± 11.9b</td>
<td>3 ± 0.22b 66.68</td>
<td></td>
</tr>
<tr>
<td>Group IX (Standard)</td>
<td>2.3 ± 0.14</td>
<td>1.9 ± 0.06b</td>
<td>224 ± 8.4b</td>
<td>260 ± 11.2b</td>
<td>2 ± 0.28b 77.80</td>
<td></td>
</tr>
</tbody>
</table>

a - Compared with group I (P<0.01).
b - Compared with Group II (P<0.01).

**G1**---7-methoxy quercetin-3-O-glucuronide  
**G2**---6-acetoxy-4’hydroxy isoflavone-7-O-rhamnopyranoside  
**G3**---Kaempferol 3-O-β-D (4” coumaroyl) rhamnoside  
**G4**---Eriodictyol 7-O-β-D (6”malonyl) neohesperidoside  
**G5**---quercetin-3-O-(6”acetyl)-galactosyl glucoside  
**G6**---4’-methoxy isoflavone 7-O-rhamnopyranoside
Fig. 7.1. The % of ulcer protection of flavonoid glycosides G1, G2, G3, G4, G5, and G6 in experimental rats.
Fig. 7.2. Photographic representation of control and experimentally induced ulcer model.

Fig. 7.2a. Normal

Fig. 7.2b. Control (ulcer induced)

Fig. 7.2c. G1

Fig. 7.2d. G2

Fig. 7.2e. G3

Fig. 7.2f. G4
Fig. 7.2g. G5

Fig. 7.2h G6

Fig. 7.2i. G7 Standard
DISCUSSION

Peptic ulcer is defined as disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation. Despite the constant attack on the gastroduodenal mucosa by a host of noxious agents (acid, pepsin, bile acids, pancreatic enzymes, drugs, and bacteria), integrity is maintained by an intricate system that provides mucosal defense and repair. This intricate biologic system consists of mucus bicarbonate layer, surface epithelial cells and a rich submucosal micro-circulatory bed which provides bicarbonate ions to neutralize the acid generated by parietal cell secretion of hydrochloric acid. Moreover, this microcirculatory bed provides an adequate supply of micronutrients and oxygen while removing toxic metabolic by products.  

Several experimental models were used to assess the anti-ulcerogeinic activity of test drugs in rats. They include Cold restraint stress, Pyloric ligation, Aspirin, Indomethacin, Cysteamine, ethanol etc. Mucus, which continuously coats over the gastric mucosa, is well known as a “mucous barrier” to prevent the injury of luminal acid, bacteria and noxious agents injuries. Mucus might implicate in scavenging oxygen-derived free radicals. Mucus glycoproteins and lipids bound to mucin might involve in the antiradical process.  

The ulcer formation in each of these models occurs by different mechanisms. Pylorus ligation-induced ulcers are caused by enhanced acid pepsin secretion leading to auto-digestion of the gastric mucosa and break down of the gastric mucosal barrier, and the digestive effect of
accumulated gastric juice and interference of gastric blood circulation are responsible for the induction of ulceration.\textsuperscript{466}

The gastroprotective activity of 2\text{ml/kg} dose of G1, G2, G3, G4, G5, and G6 was comparable to that of reference drugs (Omeperazole) used in each experimental model. In the present investigation, it has been demonstrated that G1, G2, G3, G4, G5, and G6 can significantly enhance gastric mucus secretion while reducing the acidity of the gastric juice in rats. Gastric mucus is an important protective factor for the gastric mucosa and it is capable of acting as an anti-oxidant agent and reducing mucosal damage mediated by oxygen free radicals.\textsuperscript{467} However, the protective properties of the mucus barrier depend not only on the gel structure but also on the amount or thickness of the layer covering the mucosal surface.\textsuperscript{468}

G1, G2, G3, G4, G5, and G6 possess marked gastroprotective properties as evidenced by its significant inhibition of the formation of gastric lesions induced by pyloric ligation. Our results are concordant with the earlier report.\textsuperscript{469} Acid is considered as an important factor in the development of acute and chronic gastric mucosal lesions. Suppression of gastric acid by surgical and a variety of pharmacological means\textsuperscript{470} provides effective and rapid healing of ulcer.\textsuperscript{471} Acid reducing property is discussed with several anti-ulcer drugs. The increase in volume in the ulcer control rats is undoubtedly due to increase production of hydrochloric acid as evident from the total acidity and decrease pH value of gastric juice. In the present study, the decrease in volume of the gastric juice and concomitant decrease in the acidity and increase in pH proving
the anti-ulcer activity of G1, G2, G3, G4, G5, and G6 and this result complements the earlier findings reported by Gurbuz et al.\textsuperscript{472} Further evidenced by the reduced edema formation and epithelial lifting were observed in morphometric study (Figs. 1a–1i).

Administration of G1, G2, G3, G4, G5, and G6 significantly increased the amount of mucus produced by the rat gastro mucosa compared to their respective controls. Therefore, the enhanced mucus secretion after administration of G1, G2, G3, G4, G5, and G6 may help to protect against the pyloric ligation induced damage by preventing the action of acid on the stomach mucous epithelium.\textsuperscript{459} Similar mode of action has been reported with several other plants also.\textsuperscript{458} It is also well known that prostaglandins synthesized in large quantities by the gastrointestinal mucosa can prevent experimentally induced ulcers by ulcerogens. Thus, when the ulcer lesions are induced by pyloric ligation, the cytoprotective effect of the anti-ulcer agent can be mediated through endogeneous prostaglandins.\textsuperscript{473} Therefore, it can be thought that G1, G2, G3, G4, G5, and G6 may stimulate the secretion of prostaglandin or possess prostaglandin like substances.

**Conclusion**

Gastroprotective role for G1, G2, G3, G4, G5, and G6 against gastric mucosal damage induced by pyloric ligation were investigated in the present study. Pyloric ligation induced gastric ulcer rats show increased gastric volume, acidity and depleted pH. The observed gastroprotection is possibly mediated to a major extent by a gastric mucosal secretion mechanism as the G1, G2, G3, G4, G5, and G6 were
able to restore the increased volume, acidity and depleted pH by pyloric ligation almost towards normal levels seen in control. This is further evidenced by morphometric study. Other complementary mechanisms may include the activation of capsaicin-sensitive gastric afferents, stimulation of endogenous prostaglandins and nitric oxide, and opening of $K^+$ ATP channels. These combined effects are likely to be accompanied by an increase in gastric microcirculation. The percentage of ulcer protection was 77.78% for G1, 88.90% for G2, 88.87% for G3, 76.79% for G4, 66.67% for G5, 66.68% for G6 and 77.80% for standard. Among the flavonoid glycosides, the significant inhibitions were 88.90% for G2, 88.87% for G3 observed. Omeperazole, the positive control included for the study also offered significant protection (77.80%) against pyloric ligation induced gastric ulcer. The G1 and G2 percentage of inhibition was higher than that of standard.