Effect of Flavonoid Rich Root Extract Of *Glycyrrhiza glabra* on Gastric Emptying and Gastrointestinal Transit in Albino Wistar Rats

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Abstract

**Background:** *Glycyrrhiza glabra* is often used in the management of gastrointestinal disorders. The mechanism for its beneficial actions in gastrointestinal system remains to be elucidated.

**Objective:** To evaluate the gastroprokinetic effect of flavonoid rich root extract of *Glycyrrhiza glabra* (*GutGard*) and glabridin in albino Wistar rats using phenol red meal model.

**Materials and Methods:** Rats were orally treated with GutGard or glabridin for 8 consecutive days. On 8th day, rats were administered phenol red meal 2 h post GutGard or glabridin administration. Percentage gastric emptying and gastrointestinal transit were measured immediately and 20 minutes after phenol red meal administration.

**Results:** Rats treated with GutGard exhibited statistically significant increase in percentage gastric emptying as well as gastrointestinal transit over the normal control group. Gastric emptying of GutGard and glabridin is comparable to domperidone, a potent prokinetic agent.

**Conclusion:** The study findings revealed that GutGard can alleviate gastrointestinal symptoms of functional dyspepsia via prokinetic activity as evident from increase of gastric emptying and gastrointestinal transit. This prokinetic effects of *Glycyrrhiza glabra* might be contributed by galbridin.

**Key words:** Prokinetic activity; Functional dyspepsia; *GutGard*; Phenol red; *Glycyrrhiza glabra*

Introduction

Functional dyspepsia (FD) is a disorder characterized by upper abdominal pain or discomfort in the absence of organic disease but often linked to a motility disorder; occurs very commonly in the general population. Delayed gastric emptying is considered as a major pathophysiological mechanism in FD, and it has been identified in 29% – 59% of patients with FD [1]. Although several agents have been evaluated for FD, drugs approved by FDA are not available for treatment of FD. Agents that improve motility (prokinetic agents) have been recommended as empirical treatment [2].

Prokinetic agents such as antidopaminergics (eg: metoclopramide, domperidone, levosulpiride) and serotonin 5-HT4 receptor agonists (eg: tegaserod) have shown to have modest effects in the treatment of FD. However they have their own disadvantages due to limited clinical effects and unwanted side effects like prolonged QT intervals etc. This has led to focus on phytotherapy that could improve gastric emptying thereby ameliorate FD symptoms [2,3].

GutGard, a flavonoid rich root extract of *Glycyrrhiza glabra* has been developed and proved to be beneficial for the management in ameliorating the symptoms of FD. However the mechanisms behind the effectiveness of GutGard for the management of FD were not explored. This study is to test the hypothesis that, GutGard would have ameliorated FD symptoms via improving gastric emptying and gastrointestinal transit. The present study was conducted to evaluate the gastroprokinetic effect of flavonoid rich root extract of *Glycyrrhiza glabra* (*GutGard*) in rats using phenol red meal model. Further, the effect of glabridin on gastric emptying and gastrointestinal transit were also explored.

Material And Methods

**Drugs and chemicals**

Domperidone (Cipla Ltd., India), phenol red sodium (Loba Chemie Pvt. Ltd., India.) were used and all the other chemicals used in the experiment like carboxy methyl cellulose monosodium salt (CMC), sodium hydroxide and trichloroacetic acid were purchased from Hi-Media Laboratories Pvt. Ltd., India.

**Test Substance**

GutGard is a flavonoid rich, root extract of *Glycyrrhiza glabra* developed by Natural Remedies, Bangalore, India. GutGard has following phytochemical specifications, namely,
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glabridin (≥3.5%w/w), glabrol (≥0.5%w/w), eicosanyl caffeate (≥0.1%w/w), docosylcaffeate (≥0.1%w/w), glycyrrhizin (≥0.5%w/w), and total flavonoids (≥10%w/w) [5].

**Animals**

Male albino Wistar rats (190-200 g) bred at R&D Centre, Natural Remedies Pvt Ltd, was acclimatized for 7 days prior to experimentation. The animals during experimentation were housed under standard husbandry conditions of temperature (22 ± 3°C), relative humidity (30% - 70%) and light: dark cycle of 12:12 h. Rats were allowed free access to pellet feed and UV purified water. All the experimental protocols (Protocol approval number: IAEC/PCL/06/06.15) were approved by the Institutional Animal Ethics Committee (IAEC) of Natural Remedies Private Limited, Bangalore, and conducted according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

**Experimental procedure**

**Preparation of Phenol Red Test Meal**

Carboxy methyl cellulose was dissolved in water at about 80°C and prepared to a final concentration of 1.5%. The solution was stirred until dissolved and phenol red (50 mg/100ml) was added to the stirring solution. The mixture was then brought down to 37°C.

**Study Design**

Male albino Wistar rats were randomly allotted to eight groups each consisting of six animals. Group I was administered with CMC and Group II was administered Domperidone (10 mg/kg) as a single dose on day 8, while Group III, IV, V were administered various dose levels of GutGard (6.25, 12.5 and 25 mg/kg) respectively and Group VI, VII, VIII were administered various dose levels of Glabridin (2.5, 5 and 10 mg/kg) respectively for 8 consecutive days. On day 8, sixteen hours fasted rats were administered phenol red meal (2 ml/animal) 2 h post the test substance or reference drug administration. Immediately and 20 minutes after phenol red meal administration, rats from each group were sacrificed by cervical dislocation under anesthesia. Abdomen was opened, gastroesophageal junction and the pylorus ends were clamped, then stomach was dissected out and placed in 100 ml of 0.1 N NaOH and homogenised. The homogenate is kept at room temperature for an hour and 5 ml of the supernatant were added to the stirring solution. The mixture was then brought down to 560nm wavelength. The percentage of gastric emptying in 20 minutes was calculated by the formula:

\[
\text{Percentage Gastric Emptying} = \left(1 - \frac{X}{Y}\right) \times 100
\]

where:

- X: absorbance of phenol red recovered from stomach of rats sacrificed 20 minutes after test meal
- Y: absorbance of phenol red recovered from stomach of rats sacrificed at 0th minute (immediately after test meal)

**Gastrointestinal Transit (GIT)**

Intestine was dissected out from duodenum to ileo-cecal junction and the distance travelled by phenol red and total length of the intestine (from duodenum to ileo-cecal junction) was recorded. The gastrointestinal transit (GIT) was calculated by the formula:

\[
\text{GIT} = \left(\frac{\text{Distance travelled by phenol red meal in cm}}{\text{Total length of the small intestine in cm}}\right) \times 100
\]

**Statistical Analysis**

The data were processed using statistical software IBM SPSS version 20. Percentage gastric emptying and gastrointestinal transit were expressed as mean ± SEM and analyzed statistically using One Way ANOVA followed by post-hoc Bonferroni test. P ≤ 0.05 was considered to be statistically significant.

**Results**

**Percentage Gastric Emptying (%)**

Twenty minutes after the administration of phenol red meal to the rats, the percentage gastric emptying in control group was found to be 53.33% and group administered with single dose of domperidone (10 mg/kg p.o.) was found to be 87.03%. Groups administered with GutGard and glabridin at all dose levels exhibited statistically significant increase of gastric emptying over control group (Table 1).

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Percentage Gastric Emptying (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (0.5% CMC; 10 ml/kg p.o.)</td>
<td>53.33 ± 3.45</td>
</tr>
<tr>
<td>Domperidone (10 mg/kg p.o.)</td>
<td>87.03 ± 2.00**</td>
</tr>
<tr>
<td>GutGard (6.25 mg/kg p.o.)</td>
<td>83.11 ± 4.10*</td>
</tr>
<tr>
<td>GutGard (12.5 mg/kg p.o.)</td>
<td>86.14 ± 4.86*</td>
</tr>
<tr>
<td>GutGard (25 mg/kg p.o.)</td>
<td>88.13 ± 4.06*</td>
</tr>
<tr>
<td>Glabridin (2.5 mg/kg p.o.)</td>
<td>86.52 ± 4.71*</td>
</tr>
<tr>
<td>Glabridin (5 mg/kg p.o.)</td>
<td>87.80 ± 5.94*</td>
</tr>
<tr>
<td>Glabridin (10 mg/kg p.o.)</td>
<td>89.71 ± 1.91*</td>
</tr>
</tbody>
</table>

Effect of GutGard and glabridin on Percentage gastric emptying in rats administered with phenol red test meal. Data were expressed as mean ± SEM (Group I – V; n=12 (pooled data from two independent experiments) and Group VI-VIII; n=6). *p < 0.05 significantly different from normal control group; **p < 0.01 significantly different from normal control group.
Gastrointestinal transit (%)

Groups administered with domperidone and GutGard at all dose levels demonstrated statistically significant increase in gastrointestinal transit (%) compared to the control group. Glabridin treated groups showed non-significant increase in gastrointestinal transit (%) compared to the control group. Gastrointestinal transit (GT) of control group was found to be 62.62 % and group administered with single dose of domperidone (10 mg/kg p.o.) was found to be 88.24%. GutGard treated groups (6.25, 12.5 and 25 mg/kg p.o.) showed significant increase (77.76%, 79.38% and 83.60%) of gastrointestinal transit compared to the control group. Groups treated with Glabridin (2.5, 5 and 10 mg/kg p.o.) exhibited non-significant increase (71.25%, 72.83% and 73.87%) of gastrointestinal transit compared to control group (Figure 1).

Discussion

Dyspepsia in the absence of a clinically identifiable structural lesion is addressed as functional dyspepsia (FD). FD remains one of the most common and costly medical conditions in primary care as well as in gastroenterology. The impact of FD remains stressful and leads to an enormous medical expenses. In addition, direct and indirect economic stress due to FD was found to be high and also has considerable impact on productivity. The health related quality of life is impacted significantly in patients with FD when compared to general healthy population [6,7]. A disturbance in gastric emptying has been reported to be a major pathophysiological mechanism of FD [1].

Gastric emptying (GE) is the process of transferring the gastric content to the small intestine as the result of motor activity of the stomach, pylorus and duodenum under the control of inhibitory and stimulatory mechanism [8,1]. Drugs that enhance GE are effective in the treatment of FD [2]. With the globally increasing popularity of medicinal plants for treating diseases, many herbal extracts/preparations are evaluated for the management of gastrointestinal disorders. 

Glycyrrhiza glabra also known as licorice have been traditionally used for many centuries. GutGard is a flavonoid rich, root extract of Glycyrrhiza glabra has been reported to possess a variety of pharmacological activities like anti-dyspeptic, anti-ulcer & anti-oxidant activities that can be attributed to the beneficial effects of GutGard on gastrointestinal system [4,9]. Clinically Gutgard was effective in reducing the symptoms of functional dyspepsia in double blind placebo controlled human clinical trial [4]. With this background on the beneficial effects of Glycyrrhiza glabra on gastrointestinal disorders specifically for anti-dyspeptic activity the present study was conducted to determine the effects of GutGard on gastric emptying and gastrointestinal transit in normal rats. In addition, the effects of glabridin a phytoactives of GutGard on gastric emptying and gastrointestinal transit were also investigated.

Phenol red recovery model has been widely used method to measure the gastric emptying and gastrointestinal transit in rodents. The model has been used to measure gastric emptying and gastrointestinal transit for both synthetic as well as herbal products [10-15]. Hence, the current study employed phenol red meal model to investigate the possible prokinetic effects of GutGard.

Gastric emptying was measured after administration of a non-absorbable dye (phenol red) as a marker. Gastric emptying was calculated as the percentage of dye remaining in the stomach relative to the total amount of dye recovered in a group of rat that were sacrificed immediately after gavage [16]. The total length of the small intestine and the distance travelled by phenol red meal was measured for obtaining percentage gastrointestinal transit [14]. The percentage gastric emptying and gastrointestinal transit...
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The study findings indicate that, GutGard a flavonoid rich root extract of Glycyrrhiza glabra accelerates both gastric emptying and gastrointestinal transit in rats and the effects of GutGard might be contributed by glabridin. GutGard could be a potential lead as prokinetic agent in the management of gastrointestinal disorders which need accelerated gut motility.

Conclusion

The study findings indicate that, GutGard a flavonoid rich root extract of Glycyrrhiza glabra accelerates both gastric emptying and gastrointestinal transit in rats and the effects of GutGard might be contributed by glabridin. GutGard could be a potential lead as prokinetic agent in the management of gastrointestinal disorders which need accelerated gut motility.

Declarations

Ethical approval

All the experimental protocols (Protocol approval number: IAEC/PCL/06/06.15) were approved by the Institutional Animal Ethics Committee (IAEC) of Natural Remedies Private Limited, Bangalore, and conducted according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

References