ABSTRACT

Background and objectives: Gastric ulcers occur when the mucous layer covering the gastric wall is damaged. Curcuma and licorice are famous ancient herbs that have been widely used as food additives and medicinal herbs. The present study aimed to investigate gastroprotective activity of Curcuma and licorice extract (CLE) against ethanol-induced gastric ulcers in male Wistar rats.

Methods: Fifteen male Wistar rats were randomly divided into five groups and fasted for 24 hours with free access to water. The rats received different doses of CLE (200 and 500 mg/kg) or omeprazole (positive control, 20 mg/kg) by oral gavage. One hour after the induction of gastric ulcer via absolute ethanol administration (5 ml/kg), the rats were sacrificed, and the stomach was opened along the greater curvature. Ulcerative lesions were observed and counted. Total juice acidity was evaluated. Finally, mRNA level of Bax and COX-2 was measured.

Results: The oral administration of CLE (200 and 500 mg/kg) and omeprazole (positive control, 20 mg/kg) to rats remarkably attenuated gastric acidity and the number of ethanol-induced gastric lesions. Further examination of gastric mucosal homogenate revealed significant downregulation of Bax and COX-2 in the CLE-treated groups compared with the lesion control group.

Conclusion: The findings of this study confirm the gastroprotective activity of CLE against ethanol-induced gastric injury.

Keywords: Curcuma, Stomach Ulcer, Omeprazole, Rhizome.
INTRODUCTION

Gastric ulcers are a common disease in which gastric mucosa becomes damaged (1, 2). According to a systematic review and meta-analysis, the prevalence of peptic ulcers among the Iranian population (34%) is higher than the worldwide rate (15%) (3). Previous studies have reported that the balance between proliferation and apoptosis of gastric epithelial cells is vital for maintaining the gastric mucosal integrity. *Helicobacter pylori* infection, alcohol consumption, and smoking are the main causes of peptic ulcers (3). In addition, the most common signs and symptoms are epigastric pain, gastroesophageal reflux, dyspepsia, and hematochezia (3). Proton pump inhibitors, including omeprazole, are active agents used for treatment of gastric ulcers. Herbal medicines have also been used for treatment of gastric ulcers (4, 5). Turmeric root (*Curcuma longa* L.) has been used in Chinese traditional medicine for treatment of peptic ulcer. Some studies demonstrated the gastroprotective activity of *Curcuma* extracts in various ulcer models in vivo (6, 7). It has been reported that curcumin, one of the key components of *C. longa*, prevents ethanol-induced gastric damage by decreasing acid release and increasing expression of superoxide dismutase and cyclooxygenase-2 (COX-2) (6, 8, 9). Licorice (liquorice or sweet wood) is a famous ancient herb derived from the roots and stolons of the genus *Glycyrrhiza* (10). The plant has anti-*H. pylori* effects and protects against acid secretion by covering lesion sites and promoting mucous secretion (10, 11). The present study aimed to analyze protective effects of *Curcuma* and licorice extract (CLE) against ethanol-induced gastric ulcers in male rats.

MATERIALS AND METHODS

In this research, 15 male Wistar rats (200-250 g) were used. The study was approved by the ethics committee of Kharazmi University (approval code: 9806). The animals were kept in cages, at temperature of 25±1°C, and under a 12-hour light/dark cycle. Before experimentation, all rats fasted for 24 hours with free access to water (except for the last 2 hours). In order to prepare the extract, 20 grams of powdered plants in an equal amount (1: 1 ratio) were soaked in 400 ml of water by the Rasmation method. After 48 hours, the mixture was filtered by Whatman paper, concentrated, and finally dried in a rotary evaporator at 37 °C. Following 24 hours of fasting, *Curcuma*, at doses of 200 and 500 mg/kg, was orally administered 1 hour before the induction of gastric damage using absolute ethanol (5 ml/kg). Gastric ulcer was induced by a single oral administration of absolute ethanol in rats. Rats in a control group received the same volume of buffer saline solution. The rats were randomly divided into five groups (n=3) as follows: healthy control group (group 1), gastric ulcer control group (group 2), positive control group (group 3), and two CLE treatment groups (groups 4 and 5). The untreated healthy control group received distilled water orally (1 ml/kg). The gastric ulcer control group received ethanol orally (1 ml/kg). The positive control group received omeprazole orally (20 mg/kg in distilled water). The treated groups received CLE extract orally (200 mg/kg and 500 mg/kg in distilled water).

All rats were sacrificed with an overdose of xylazine and ketamine an hour after the ethanol administration. Their stomach was carefully dissected and opened along the greater curvature. The mucosal surface of the stomach was washed with cold saline isotonic solution, and a photograph was taken from the damaged area of the stomach using a digital microscope (mm²). The ulcerative lesion index was determined as described by Singh et al. (12). The percentage of the protective index was calculated using the following formula in which UC and UT were the gastric ulcer area of the control and treated group, respectively.

\[
\text{Protective index} = \frac{\text{UC} - \text{UT}}{\text{UC}} \times 100
\]

Content of each stomach was collected into a tube and centrifuged at 4,000 rpm for 10 minutes. Subsequently, the supernatant was analyzed for hydrogen ion concentration using a digital pH-meter titration with 0.1 NaOH solution. Part of the stomach was excised and homogenized. The stomach sample was centrifuged at 5,000 rpm for 10 minutes, and the resulting clear supernatant was used for measurement of *Bax* and COX-2 expression. For this purpose, total RNA was extracted using Trizol reagent (Invitrogen, USA).
according to the standard instructions. Reverse transcription reaction was performed for 1 μg of total RNA as template using a cDNA synthesis kit (Takara, Japan). Real-time polymerase chain reaction (PCR) was performed via the Applied Biosystems 7500 Fast Real-Time PCR System using SYBR Green PCR Master Mix (Takara, Japan), cDNA templates, and specific primers. The primer pairs used for detection of Bax, COX-2, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are presented in Table 1. The GAPDH was used as a housekeeping gene. In addition, Bax and COX-2 expression levels were calculated by the 2^(-ΔΔCt) method and normalized relative to the GAPDH mRNA.

Table 1: Sequences of the primers used for the real-time PCR experiment

<table>
<thead>
<tr>
<th>Genes</th>
<th>Sequences</th>
</tr>
</thead>
</table>
| Bax    | Forward: 5'-AAAGACGCTGAGCGAGTGTCT-3'  
Reverse: 5'-CAAGATGGTCACGTCTGCaC-3' |
| COX-2  | Forward: 5'-GATTGACAGCCACAAACTT-3'  
Reverse: 5'-CGGATGAACTCTCCTCTCA-3' |
| GAPDH  | Forward: 5'-CGGAGTCACGGTTTGTCGTAT-3'  
Reverse: 5'-AGCCTTCTCCCATGTTGGAAGAC-3' |

All results were reported as mean ± standard deviation. Differences between the study groups were analyzed using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Effect of different treatments on the pH of gastric content

The acidity of gastric content in rats receiving oral ethanol was significantly increased compared with the healthy control group. After pre-treatment with omeprazole (positive control) and CLE at doses of 200 and 500 mg/kg, the acidity was attenuated in rats with ethanol-induced gastric ulcer (Table 2 and Figure 1).

Macroscopic evaluation of gastric lesions

The administration of ethanol induced noticeable lesions in the gastric walls (Figure 2B) with the ulcer area of 42 mm² (Table 3). As shown in Figure 2, pre-treatment with omeprazole (Figure 2C) and CLE (Figures 2D and E) significantly reduced ulcer areas and the number of gastric lesions compared with the lesion control group. In addition, CLE at dose of 500 mg/kg showed the highest inhibitory effects on the length and number of gastric lesions (Table 3 and Figure 3).

![Figure 1](image-url)

Figure 1- The effects of CLE on gastric juice acidity in male rats. 1: healthy control group; 2: ulcer control group; 3: rats treated with omeprazole; 4: rats treated with 200 mg/kg CLE; 5: rats treated with 500 mg/kg CLE.

Table 2-The effects of CLE on gastric juice acidity in male rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control group</td>
<td>6.4</td>
</tr>
<tr>
<td>Ulcer control group</td>
<td>4.8</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>5.9</td>
</tr>
<tr>
<td>200 mg/kg CLE</td>
<td>5.5</td>
</tr>
<tr>
<td>500 mg/kg CLE</td>
<td>6.1</td>
</tr>
</tbody>
</table>
The protective index indicates the protective effect of CLE against gastric ulcer induction (Table 4). At 500 mg/kg CLE, the protective index was higher than another groups. The lowest protection rate (68%) was recorded for the 200 mg/kg CLE group (Figure 4 and Table 4).

**Effect of different treatments on Bax and COX-2 levels**

To determine the effects of apoptosis and inflammation, we determined the level of Bax and COX-2 expression in gastric tissue homogenate, respectively. After treatment with ethanol, the COX-2 level increased significantly compared with the normal control group ($p<0.05$). The results also showed that pre-treatment with CLE (200 and 500 mg/kg) and omeprazole (20 mg/kg) significantly ($p<0.05$) decreased COX-2 level compared with the gastric ulcer control group (Figure 5A).

![Image](image1.png)

**Figure 2-** The effects of CLE on ethanol-induced gastric ulcer in male rats. 1: healthy control group; 2: ulcer control group; 3: rats treated with omeprazole; 4: rats treated with 200 mg/kg CLE; 5: rats treated with 500 mg/kg CLE.

**Table 3-** The effects of CLE on the number and size of gastric lesions in male rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of lesions</th>
<th>Size of lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control group</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ulcer control group</td>
<td>11</td>
<td>42</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>200 mg/kg CLE</td>
<td>3.5</td>
<td>8</td>
</tr>
<tr>
<td>500 mg/kg CLE</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

![Image](image2.png)

**Figure 3-** The effects of CLE on number of gastric lesions in male rats. 1: healthy control group; 2: ulcer control group; 3: rats treated with omeprazole; 4: rats treated with 200 mg/kg CLE; 5: rats treated with 500 mg/kg CLE.

![Image](image3.png)

**Figure 4-** The effects of CLE on protective index in male rats. 1: healthy control group; 2: ulcer control group; 3: rats treated with omeprazole; 4: rats treated with 200 mg/kg CLE; 5: rats treated with 500 mg/kg CLE.
Ethanol treatment did not change the Bax mRNA level compared with the normal control group. Moreover, CLE (200 and 500 mg/kg) and omeprazole (20 mg/kg) significantly decreased Bax level compared with the gastric ulcer control and healthy control groups \((p<0.05)\) (Figure 5B).

Table 4 - The effects of CLE on the protective index in male rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Protective index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control group</td>
<td>*</td>
</tr>
<tr>
<td>Ulcer control group</td>
<td>0</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>72%</td>
</tr>
<tr>
<td>200 mg/kg CLE</td>
<td>68%</td>
</tr>
<tr>
<td>500 mg/kg CLE</td>
<td>82%</td>
</tr>
</tbody>
</table>

Figure 5 - The effects of CLE on Cox-2 (A) and Bax (B) expression in male rats. 1: healthy control group; 2: ulcer control group; 3: rats treated with omeprazole; 4: rats treated with 200 mg/kg CLE; 5: rats treated with 500 mg/kg CLE.

DISCUSSION

For centuries, herbal medicines have been used for wound healing and treatment of various diseases including cardiovascular disease, urinary tract infection, liver disease, and gastric ulcers \((4, 13)\). According to the World Health Organization, medicinal plants are still consumed by about 70% of the world’s population, and the numbers are still rising \((14)\). In the present study, the protective effect of CLE against gastric ulcer was evaluated in male Wistar rats. Oral administration of ethanol is a contributing factor to development of gastric ulcer \((15-17)\). Ethanol can cause gastric ulcer via numerous mechanisms such as epithelial cell injury, dehydration, gastric micro-vessels disruption, and leukocytes recruitment \((15)\). These effects are probably because of hemorrhagic damage, submucosal edema, lipid peroxidation, formation of free radicals, and induction of apoptosis \((6, 16)\). Therefore, in the present study, ethanol administration was used for induction of gastric ulcer in the rats. Based on the findings, the aqueous extract of CLE, particularly at concentration of 500 mg/kg, had protective effects on ethanol-induced gastric damage. In addition, CLE could effectively inhibit gastric acidity and decrease the size and number of gastric lesions. Previous studies have shown that medicinal plants could suppress gastric acidity and improve gastric wall mucus secretion in patients and animals with gastric ulcers \((7, 18, 19)\). Omeprazole is an effective drug for treating gastroesophageal reflux disease and gastric ulcer in humans and animal models \((20-22)\). Here, the protective effects of omeprazole in rats with gastric damage were similar to those observed in rats treated with CLE extract. It has been reported that administration of ethanol to rats lead to upregulation of Bax \((20, 23)\). Hence, medicinal plants may protect gastric cells against apoptosis by reducing Bax expression and inhibiting cytochrome c translocation from mitochondrial membrane to the cytosol \((8, 24-27)\). Our findings showed that the CLE treatment could downregulate Bax protein compared with the control group. Several studies have reported acute inflammation in the pathogenesis of ethanol-induced gastric ulcers \((28-30)\). The COX-2 enzyme is rapidly upregulated at inflammation sites, leading to prostaglandin production \((31)\). In the present study, ethanol administration increased COX-2 expression, which is consistent with previous studies \((28, 32, 33)\). Our findings also showed that CLE, especially at concentration of 500 mg/kg, induced downregulation of COX-2 mRNA expression.

CONCLUSION

Our findings indicate the beneficial effects of CLE extract in improving ethanol-induced gastric injury. Similar to omeprazole, CLE might target the hydrochloric acid secretory pump. Further studies are required to confirm...
findings of the present study.

ACKNOWLEDGMENTS
The authors would like to thank Dr. Maryam Haj Rezaee and Dr. Mohammad Abbasi for their helpful comments. The Biology Department and Center for Growth and Reproduction of Laboratory Animals of Kharazmi University are greatly acknowledged for their support.

DECLARATIONS
Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

Ethics approvals and consent to participate
The study was approved by the ethics committee of Kharazmi University (approval code: 9806).

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest regarding publication of this article.

REFERENCES
15. Rafiee M, Namazi F, Rajaian H, Nazifi S. Gastroprotective Effects of Various Scrophularia striata Extracts on Ethanol-Induced Gastric Ulcer in Rats. Turkish Journal of Pharmaceutical Sciences. 2016;13(3). [View at Publisher] [DOI: 10.4274/tjps.2016.08] [Google Scholar]


30. Abbasi M, Abazari OO. Probing the Biological evaluations of a new designed Palladium (II) complex using spectroscopic and theoretical approaches: Human Hemoglobin as a Target. Archives of Medical Laboratory Sciences. 2017; 3(3). [View at Publisher] [Google Scholar]


How to Cite:
Panji M, Ghafoori M [Evaluation of Gastroprotective Activity of Licorice and Turmeric Rhizome Aqueous Extract against Ethanol-Induced Gastric Injury in Male Wistar Rats]. mljgoums. 2022; 16(4): 32-38 DOI: 10.29252/mlj.16.4.32

Medical Laboratory Journal, Jul-Aug, 2022; Vol 16: No 4