Characterization and gastroprotective effects of *Rosa brunonii* Lindl. fruit on gastric mucosal injury in experimental rats – A preliminary study

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Gastric ulcer is the most prevalent disorder affecting a large population. *Rosa brunonii* Lindl. fruit (RBF) has traditionally been used to treat stomach pains. Therefore, the current work aimed to isolate, characterize, and investigate the gastro-protective effect of *Rosa brunonii* Lindl. fruit chloroform extract (RBFCe) against ethanol-induced gastric ulcers in rats. Quercetin 3-O-glucoside (QUE-G) was isolated and characterized by modern spectroscopic techniques. RBFCe was orally administered at 250 mg/kg, 500 mg/kg, and 750 mg/kg doses for ten days. Gastric ulcer was induced by a single dose of absolute ethanol (5 ml/kg) on the last day of the study. Histological changes were calculated, along with ulcer inhibition and the ulcer index (UI). Gastric juice volume, pH, acidity, mucus content, and protein content were evaluated to understand the mechanism underlying its gastroprotective effect. Omeprazole (OMP) was used as the positive control. RBFCe at a dose of 750 mg/kg significantly (<0.01) reduced the UI (3.54) and increased the protection rate (67.63%) compared to the negative (ulcer) control group. Treatment with RBFCe in a dose-dependent manner increased the gastric pH, mucus content, and total protein while decreasing gastric juice volume and total acidity. Histopathological studies showed severe gastric mucosal injury and edema in ulcer control animals compared to extract-treated groups. This study demonstrated that oral administration of RBFCe possesses a significant gastroprotective effect due to its anti-secretory and cytoprotective mechanisms. Our findings support the traditional use of RBF to treat the gastric ulcer.

**Keywords:** gastroprotective, *Rosa brunonii* Lindl. fruit, ulcer, quercetin 3-O-glucoside

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**Abbreviations:** NSAIDs, non-steroidal anti-inflammatory drugs; OMP, Omeprazole; RBFCe, *Rosa brunonii* Lindl. fruit chloroform extract; QUE-G, Quercetin 3-O-glucoside; UI, ulcer index

**INTRODUCTION**

Gastric ulcer is one of the common digestive disorders affecting 10% of world population. It is a complex and multifactorial disease, characterized by pathological lesion in digestive tract, gastrointestinal bleeding, perforations and erosions of gastric mucosa (Saranya et al., 2011). It occurs as a result of imbalance between offensive and defensive factors. Stress, smoking, alcohol consumption, nutritional deficiencies, infection by *Helicobacter pylori* (Yu et al., 2022) and use of non-steroidal anti-inflammatory drugs (NSAIDs) cause imbalance in the gastric acid, pepsin, mucous secretion, prostaglandins and sulfhydryl compounds (Zakaria et al., 2016b). The imbalanced secretions cause mucosal damage, which can lead to perforations and bleeding if not treated properly (Yuan et al., 2006; Balan et al., 2014; Kangwan et al., 2014; Khoder et al., 2016; Kim et al., 2019). Conventional pharmacotherapeutic treatments for gastric ulcers include acid suppressants, that may have certain side effects like impotence, osteoporotic bone fracture, gynecomastia and iron deficiencies (Baubon et al., 2016; Zakaria et al., 2016b; Yu et al., 2017). Furthermore, the symptoms of gastric ulcers may usually reoccur on discontinuation of antulcer therapy (Kangvan et al., 2014). Consequently, antulcer remedies with minimal side effects are required for possibility of their long use (Kang et al., 2009). Mucosal protective agents can be a good alternative as they are considered to have relatively low side effects (Shim et al., 2017). Beside use of synthetic drugs to cure gastric ulcers, people also rely on phytomedicines as an alternative therapeutic source which being natural are considered without side effects (Rozza et al., 2012; Chatjerje et al., 2014).

*Rosa brunonii* Lindl., also called “Himalayan musk rose,” is a member of the Rosaceae family and found in the western Himalayas. Locals use the plant’s roots, known as “Rajatarini,” to cure inflammation in the eyes. Plant flowers are used to produce gulkand, a sweet preserve used as a laxative and a remedy for stomach aches. Literature reported substantial in-vitro antioxidant activity of crude extract from *Rosa brunonii* Lindl. flowers (Ahmad et al., 2020). Some of the compounds such as quercetin-3-O-rhamnoside, astragalin and tiloside were isolated from RBF such as quercetin-3-O-rhamnoside, astragalin with strong antioxidant properties (Ishaque et al., 2017) which increased the medicinal value of RBF. RBFCe possess significant hepatoprotective potential against Rifampicin/Isoniazid induced toxicity in rats. It possess various classes of phytochemicals including cardiac glycosides, flavonoids, steroids, phenolic compounds, terpenoids, anthraquinones and proteins (Ahmad et al., 2020).

*Rosa brunonii* Lindl. is of particular interest due to its widespread use in folklore medicine and the presence of...
highly antioxidant bioactive secondary metabolites. Furthermore, no reports showing gastroprotective effects of RBFCE were found in the literature, which encouraged us to appraise the possible anti-ulcer potential of the plant (Aziz et al., 2023; Saleem et al., 2023; Sana et al., 2022). Given the preceding, the current study sought to isolate and characterize bioactive metabolites from RBFCE and investigate its potential gastroprotective effects in ethanol-induced gastric ulcers in rats. The medium polar chloroform fraction was selected for the study due to the ease of separating bioactive secondary metabolites for further trials.

MATERIALS AND METHODS

Collection of plant material
Fruits and leaves of Rosa brunonii Lindl. were collected from Murree, Punjab Pakistan, identified at Department of Botany, GC University, Lahore (Pakistan) under voucher specimen number, GC.Herb.Bot.3314 for future reference.

Chemicals and Reagents
Omeprazole (Dr. Reddy Pharma, India) and carboxymethylcellulose (CMC) (India) were kindly provided by Next Pharmaceuticals (Pvt) Ltd. Pakistan. Silica gel (Merck), Bovine serum albumin (BSA) and Alcian blue dye 8GX were obtained from BioShop (Canada) and Uni-chem (China). Ethanol, methanol, hexane, dimethyl sulphoxide, chloroform (CHCl₃) and ethyl acetate were obtained from Sigma Aldrich Chemicals (Germany) and of analytical grade.

Preparation of extract
The fruits of Rosa brunonii Lindl. were washed with distilled water, shade-dried, and crushed using a mechanical grinder. Extraction was carried out by reported maceration method with slight modifications (Sambandan et al., 2016). Finely ground powdered material (500 g) was dipped into 5 L of CHCl₃. The extract was filtered, and the solvent was evaporated using a rotary evaporator. The resulting semisolid residue was weighed and kept at –4°C in an airtight container for later use. The semisolid residue was fractionated using a solvent extraction technique. Sample of RBFCE weighing about 187 g was loaded onto the column packed with silica gel. Separation of analytical grade.

Isolation and characterization of compound
Isolation was carried out by using conventionally reported isolation method with slight modifications (Ahmad et al., 2007). Silica gel (60 mesh) was added in hexane to form slurry and loaded into the column. The column was packed carefully by minimizing the bubble interruption. Sample of RBFCE weighing about 187 g was loaded onto the column packed with silica gel. Separation was started with non-polar solvent, hexane as an eluent. Polarity of the eluent was raised by adding 10% CHCl₃ at each step. In order to further raise the polarity of the eluent, 10% ethyl acetate was added at each step. Thin layer chromatography (TLC) was continuously carried out after every 10% rise in polarity of eluent. Fractions showing same Rf values were combined and at the end (1-6), (11-16) and (19-25) fractions were obtained. On the basis of TLC, fractions (19-25) were loaded onto the Sephadex LH-20 column and eluted with ethyl acetate and polarity was increased by adding 25% CH₂OH at each step. At 100% CH₂OH as an eluent, polarity was further raised by adding 1.0% water in the eluent. At polarity of 97.0% CH₂OH and 3.0% water, the pure compound was eluted and showed single spot on TLC plate under UV light at mobile phase ratio of ethyl acetate, methanol and water (10:5:3) and (7:5:3). The isolated compound was weighed using calibrated Sartorous TE214S weighing balance. Melting point of compound was determined using Melting point apparatus (SMP-10). FTIR of the compound was performed on IR Prestige-21, NMR spectra was obtained on NMR, Bruker (500 MHz) and mass was determined by LC-MS/MS (Agilent).

Experimental animals
The experiment was carried out on healthy adult Wistar albino rats of either sex (172–204 g) obtained from a local animal house facility and acclimatized for 1 week under standard environmental conditions with free access to food and water ad libitum. The study was carried out according to the protocols approved by the Animal Ethical Committee of the University of Punjab, College of Pharmacy. The animals were divided into different groups, detailed in the proceeding text.

Acute toxicity study
The acute toxicity of RBFCE was studied to determine the safe dose of the extract. Rats were divided into three groups (n=6 in each) and given a vehicle (CMC, 5 ml/kg), a low dose (1000 mg/kg), a medium dose (2500 mg/kg), and a high dose (5000 mg/kg) of RBFCE. Before dosing, rats were deprived of food for 24 h with free access to water. Food was also withheld for another 4 hours after dosing. Animals were monitored for morbidity and mortality for 4 hours and then daily for 14 days.

Determination of gastroprotective effect
The gastroprotective effect of RBFCE was studied in an ethanol-induced gastric ulcer rat model, which was deprived of water just 2 h before starting the experimental procedure. The animals were randomly divided into six groups (n=6) and treated for 14 days with extract and drug as follows:

Normal control group: Animals received drinking water to show the normal gastric parameters.

Negative control group: Animals were given vehicle solution 5 ml/kg 0.5% CMC. (Hariprasath et al., 2012; Rahim et al., 2014). This group was only included to check the effect of CMC on gastric parameters.

Positive control group: Animals were given OMP (20 mg/kg p.o, OD) for 14 consecutive days (Al-Wajeeb et al., 2016).

Low dose RBFCE group: Animals were given RBFCE (250 mg/kg/p.o, OD) for 14 consecutive days.

Medium dose RBFCE group: Animals were given RBFCE (500 mg/kg/p.o, OD) for 14 consecutive days.

High dose RBFCE group: Animals were given RBFCE (750 mg/kg/p.o, OD) for 14 consecutive days.

All therapies were given through intragastric gavage. After 30 min of above mentioned treatments, 10% ethanol solution was given orally to all animals on the first day of the trial, with the exception of the normal control group. From second day of trial, animals in all other...
groups received 30% (v/v) ethanol orally for 14 days, except normal control group.

Sample collection and processing

On the 14th day, all animals were anesthetized with an overdose of xylazine and ketamine and sacrificed after 1 h of ethanol administration (Ketuly et al., 2013). After cutting stomach tissues along the larger curvature, gastric contents were collected in glass tubes. Gastric mucosa was inspected after stomach tissues were cleaned with ice-cold normal saline (Das et al., 2012). The number of lesions, their details, and their scores was all recorded. Stomach tissues were then fixed in 10% formalin solution to evaluate histopathological parameters (Qaiser et al., 2018).

Macroscopic and microscopic evaluation

The excised stomach tissues were observed under magnifying glass and dissecting microscope to count lesions present on inner side of the stomachs (Dashputre et al., 2011; Qaiser et al., 2018).

Ulcer scoring

Ulcer scoring was carried out as follows on the basis of their intensity (Raju et al., 2009).

<table>
<thead>
<tr>
<th>Ulcer index</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No ulcer</td>
</tr>
<tr>
<td>0.5</td>
<td>reddish mucosa</td>
</tr>
<tr>
<td>1</td>
<td>red spots</td>
</tr>
<tr>
<td>1.5</td>
<td>hemorrhagic streaks</td>
</tr>
<tr>
<td>2</td>
<td>profound ulcers</td>
</tr>
<tr>
<td>3</td>
<td>punctures/perforations</td>
</tr>
</tbody>
</table>

Ulcer index

Ulcer index was calculated using the formula (Ahmad et al., 2015; Gul et al., 2015).

\[
\text{Ulcer Index} = \frac{\text{UN} + \text{US} + \text{UP}}{\text{UP} \times 10^4}
\]

Where UN=average number of ulcers per animal, US=average of severity score, and UP=percentage of animals with ulcer.

Percentage protection

The percentage protection by RBFCE was calculated by the following formula and compared with negative control group (Raju et al., 2009).

\[
\%\text{ Protection} = \frac{\text{Ulcer index of untreated group}}{\text{Ulcer index of treated group}} \times 100
\]

Estimation of gastric content volume, pH and total acidity

Following the stomach opening, the entire gastric contents were put into the test tubes and centrifuged for 10 minutes at 1000 rpm. The volume and pH of the supernatant were determined using a 5 mL burette and a pH metre (Thermo Orion thermoscientific 3-star). The supernatant (1 ml) was titrated against freshly prepared 0.1N NaOH using phenolphthalein as an indicator (Shukla et al., 2017). Correction factor of 0.1 N NaOH was also calculated.

Total activity = \[\frac{\text{[Volume of NaOH used] \times [Normality of NaOH used]}}{100}\]

Results were expressed in terms of the clinical units (mEq/L).

Gastric mucous content determination

Estimation of gastric mucous content was carried out by using standard curve of alcian blue according to the reported protocol (Hajrezaie et al., 2015).

Protein content determination

Protein content was assessed by using standard curve of bovine albumin solution (BSA standard curve) (Markwell et al., 1981).

Histopathological investigations of gastric ulcer

The separated stomachs were sliced along the larger curvature and rinsed in ice-cold normal saline. The stomach tissues were partially preserved in a 10% formalin solution and further processed by embedding them in paraffin wax. For histological investigation, 3–5 mm thick slices were cut and stained with hematoxylin and eosin (Hajrezaie et al., 2015). The sections were photographed after being evaluated under a light microscope for histological changes such as ulceration, decongestion, necrosis, congestion, and erosions on an arbitrary scale (Bancroft et al., 2013).

Statistical analysis

Data was articulated as the mean ± S.E.M., where applicable. Data for gastric content volume, pH, total acidity, percentage protection and protein content were analyzed using one-way analysis of variance (ANOVA). Tukey’s post hoc multiple comparison test was applied for determination of statistical difference among all groups. p<0.05 considered significant. Graph Pad Prism® (Version 8.0.1 (244) for Windows) was used for statistical calculations and plotting graphs.

RESULTS

Acute oral toxicity

During the observation period, no behavioral changes or signs of toxicity were observed in any of the treated rats. During the 14-day observation period following oral administration of all three doses of RBFCE, none of the rats died.

Characterization of isolated compound

Characterization of the isolated compound was carried out by spectral studies such as IR, NMR, and mass spectroscopy and melting range. The observed data was examined and compared with the published data for pos-
sible flavonoid glycoside and found matched with QUE-G. The observations regarding compound under study are as follows in Table 1.

The proton NMR showed peaks in aromatic region and the presence of a sugar moiety. The splitting of aromatic proton exhibited two distinct coupling patterns, one was meta coupling (J = 2.0 Hz) and the other 1,4,5 coupling pattern. As hydroxyl was normally appeared at carbon number 5 in flavonoid that normally hydrogen bonded with carbonyl, therefore meta coupling could be suggested in A ring and 3,4-disubstituted pattern in B ring of flavonoid that was also matched with the published data (Table 2). Anomeric proton appeared at δ 5.2 ppm as doublet and six other protons from 3.60 to 3.25 δ ppm due to possible sugar moiety. The experimental data was matched with the NMR data of QUE-G according to literature.

IR spectrum confirms the characteristic of flavonol system. Peaks at wave numbers 3214 cm⁻¹ indicates OH-stretching and at 1671 cm⁻¹ conforms presence of –C=O group. Similarly, the molecular formula of isolated compound was determined as C₂₁H₂₀O₁₂ by NMR and mass spectrum. The molecular weight (464.30 g/mol) of the isolated compound also confirms the isolated compound as QUE-G (Fig. 1). Melting range of the isolated compound (233–237°C) confirms that compound under study is QUE-G as it has melting point 236°C.

**Macroscopic evaluation of stomach tissue treated with RBFCE**

Macroscopic evaluation of control animals showed the normal mucosa without any signs of erosion/ulceration. Gross appearance of ulcer control tissue exhibited various notable lesions and signs of ulcerations. No remarkable signs of ulcerations were observed in the positive control group. On the other hand, macroscopic evaluation of low dose RBFCE treated group showed marked degree of erosion as compared to medium and high

---

**Table 1. C¹³ and H¹ NMR data of isolated compound**

<table>
<thead>
<tr>
<th>C.No</th>
<th>Multiplicity</th>
<th>Experimental C¹³-NMR (δ)</th>
<th>Reported (Kuruüzüm-Uz et al., 2013) C¹³-NMR (δ)</th>
<th>Experimental H¹-NMR (δ)</th>
<th>Reported (Kuruüzüm-Uz et al., 2013) H¹-NMR (δ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>C</td>
<td>157.61</td>
<td>157.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-2</td>
<td>C</td>
<td>134.22</td>
<td>134.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-3</td>
<td>C</td>
<td>178.08</td>
<td>178.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-4</td>
<td>C</td>
<td>161.63</td>
<td>161.90</td>
<td>6.21, d (J= 2.0 Hz)</td>
<td>6.18 d (J= 2.0 Hz)</td>
</tr>
<tr>
<td>C-5</td>
<td>CH</td>
<td>98.49</td>
<td>98.90</td>
<td>6.40, d (J= 2.0 Hz)</td>
<td>6.37 d (J= 2.0 Hz)</td>
</tr>
<tr>
<td>C-7</td>
<td>CH</td>
<td>93.31</td>
<td>93.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-8</td>
<td>C</td>
<td>157.06</td>
<td>157.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-9</td>
<td>C</td>
<td>104.28</td>
<td>104.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-10</td>
<td>C</td>
<td>121.79</td>
<td>121.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-2'</td>
<td>CH</td>
<td>114.60</td>
<td>114.80</td>
<td>7.73, d (J= 2.5 Hz)</td>
<td>7.70, d (J= 2.0 Hz)</td>
</tr>
<tr>
<td>C-3'</td>
<td>C</td>
<td>144.50</td>
<td>144.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-4'</td>
<td>C</td>
<td>148.44</td>
<td>148.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-5'</td>
<td>CH</td>
<td>116.16</td>
<td>116.40</td>
<td>6.89, d (J=8.6 Hz)</td>
<td>6.86, d (J=8.4 Hz)</td>
</tr>
<tr>
<td>C-6'</td>
<td>CH</td>
<td>121.67</td>
<td>122.00</td>
<td>7.61, dd (J=8.5, 2.5Hz)</td>
<td></td>
</tr>
<tr>
<td>C-1''</td>
<td>CH</td>
<td>102.93</td>
<td>103.20</td>
<td>5.26, d (J=7.5 Hz)</td>
<td>5.22, d (J=7.6 Hz)</td>
</tr>
<tr>
<td>C-2''</td>
<td>CH</td>
<td>74.33</td>
<td>74.60</td>
<td>3.37, t (J=9.5 Hz)</td>
<td></td>
</tr>
<tr>
<td>C-3''</td>
<td>CH</td>
<td>76.98</td>
<td>77.00</td>
<td>3.73, dd (J=2.5, 12.0 Hz)</td>
<td></td>
</tr>
<tr>
<td>C-4''</td>
<td>CH</td>
<td>69.81</td>
<td>70.10</td>
<td>3.59, dd (J=5.5, 12 Hz)</td>
<td></td>
</tr>
<tr>
<td>C-5''</td>
<td>CH</td>
<td>76.71</td>
<td>77.20</td>
<td>3.25, m</td>
<td></td>
</tr>
<tr>
<td>C-6''</td>
<td>CH₂</td>
<td>61.15</td>
<td>61.40</td>
<td>3.51, m</td>
<td></td>
</tr>
</tbody>
</table>

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![Figure 1. Structure of quercetin-3-O-glucoside.](image-url)
Effects of Rosa brunonii Lindl. fruit on gastric mucosal injury

dose treated groups. Gross appearance of gastric mucosa showed milder injuries in low dose extract treated group compared to medium and high dose and negative control groups as shown in Fig. 2.

Effect of RBFCE on ulcer score, ulcer index and percentage protection

Ethanol administration significantly \( (p<0.001) \) increased the ulcer score (Mean=4.91±1.11) and ulcer index (Mean=10.94) in negative control group compared to the normal animals (0 ± 0.00). Low dose of RBFCE decreased \( (p>0.05) \) the ulcer score to –26.80% (Mean=3.60±1.00), ulcer index to –18.72% (Mean=8.90) and increased the percentage protection by 18.72% compared to the negative control group. Likewise, oral administration of medium dose of RBFCE reduced \( (p<0.05) \) the ulcer score to –45.80% (Mean=2.70±0.90), ulcer index to –32.70% (Mean=7.40) and increased the percentage protection by 32.67% compared to the negative control group. Moreover, high dose of RBFCE reduced \( (p<0.01) \) the ulcer score to –61.00% (Mean=1.92±0.70), ulcer index to –45.10% (Mean=6.00) and increased the percentage

Table 3. Title!

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± S.E.M.</th>
<th>Ulcer no</th>
<th>Ulcer Score</th>
<th>Ulcer Incidence (%)</th>
<th>Ulcer index</th>
<th>Ulcer Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative control</td>
<td>4.5±0.67 ***</td>
<td>4.92±0.46***</td>
<td>100</td>
<td>10.94</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Positive control</td>
<td>1.17±0.74</td>
<td>33.33</td>
<td>3.52</td>
<td>68.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low dose RBFCE</td>
<td>3.33±0.30</td>
<td>3.60±1.00</td>
<td>82.00</td>
<td>8.90</td>
<td>18.72</td>
<td></td>
</tr>
<tr>
<td>Medium dose RBFCE</td>
<td>*3.00±0.40</td>
<td>*2.70±0.90</td>
<td>68.00</td>
<td>7.40</td>
<td>32.67</td>
<td></td>
</tr>
<tr>
<td>High dose RBFCE</td>
<td>**2.20±0.60</td>
<td>**1.92±0.70</td>
<td>56.00</td>
<td>6.00</td>
<td>45.10</td>
<td></td>
</tr>
</tbody>
</table>

The results are expressed in the form of mean ± S.E.M. Significant at \( p<0.05 \), 0.01**, and 0.001***, ns=not significant compared to negative control.

Table 4. Effect of RBFCE on gastric juice parameters

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Group Name</th>
<th>Gastric volume (mL)</th>
<th>Gastric pH</th>
<th>Total acidity (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>1.33±0.25</td>
<td>4.03±0.18</td>
<td>27.5±3.45</td>
</tr>
<tr>
<td>2</td>
<td>Negative control</td>
<td>3.97±0.49***</td>
<td>2.9±0.26</td>
<td>89.83±3.0***</td>
</tr>
<tr>
<td>3</td>
<td>Positive control</td>
<td>1.4±0.26</td>
<td>6.48±0.18</td>
<td>37.5±3.80</td>
</tr>
<tr>
<td>4</td>
<td>Low dose RBFCE</td>
<td><strong>2.53±0.30</strong></td>
<td>*3.22±0.34</td>
<td>***67.66±7.80</td>
</tr>
<tr>
<td>5</td>
<td>Medium dose RBFCE</td>
<td>*<strong>2.30±0.29</strong></td>
<td>***3.60±0.40</td>
<td>***58.60±5.10</td>
</tr>
<tr>
<td>6</td>
<td>High dose RBFCE</td>
<td>*<strong>1.95±0.50</strong></td>
<td>***4.40±0.30</td>
<td>***52.00±6.00</td>
</tr>
</tbody>
</table>

Figure 2. Macroscopic examination of:
(a) Normal rats
(b) Rats pre-treated with CMC (negative control group) showing severe mucosal injuries
(c) OMP 20 mg/kg (positive control) showing normal looking mucosa
(d) RBFCE 250 mg/kg treated group indicates surface erosions
(e) RBFCE 500 mg/kg treated group indicates relatively protected mucosal surface with only focal erosion
(f) RBFCE 750 mg/kg treated group indicates no injuries to gastric mucosa.
Effect of RBFCE on Gastric content volume, acidity, pH

Ethanol administration significantly \((p<0.001)\) increased the gastric content volume and acidity \((p<0.001)\) by 197.5% \((\text{Mean}=3.96\pm1.19)\) and 226.70% \((\text{Mean}=89.83\pm7.35)\) while decreased the pH -28.10% \((\text{Mean}=2.90\pm0.64)\), respectively compared to the normal group. Low dose of RBFCE reduced \((p<0.05)\) the gastric content volume to -36.13% \((\text{Mean}=2.53\pm0.30)\), acidity \((p<0.05)\) to -24.70% \((\text{Mean}=67.66\pm7.80)\) and increased the pH \((p<0.05)\) by 10.92% \((\text{Mean}=3.22\pm0.34)\) compared to the negative control group. Likewise, medium dose of RBFCE reduced \((p<0.01)\) the gastric content volume to -42.90% \((\text{Mean}=2.30\pm0.29)\), acidity \((p<0.05)\) to -34.70% \((\text{Mean}=58.60\pm5.10)\) and increased the pH by \((p<0.01)\) 23.0% \((\text{Mean}=3.60\pm0.40)\), compared to the negative control animals. High dose of RBFCE reduced \((p<0.01)\) the gastric content volume to -50.84% \((\text{Mean}=1.95\pm0.50)\), acidity \((p<0.01)\) to -42.10% \((\text{Mean}=52.00\pm6.00)\) and increased the pH \((p<0.01)\) 50.60% \((\text{Mean}=4.40\pm0.30)\) in comparison with negative control group (Table 4).

Effect of RBFCE on mucous and protein content

Ethanol administration to negative control group significantly \((p<0.05)\) decreased the mucous and protein content \((p<0.05)\) by -18.32% \((\text{Mean}=323.33\pm45.46)\) and protein by -47.94% \((\text{Mean}=36.24\pm5.57)\) \((p<0.01)\), respectively, compared to the normal group as shown in Fig. 1 and 2, respectively. Oral treatment of low dose of RBFCE increased \((p<0.05)\) the mucous content by 9.28% \((\text{Mean}=368.17\pm12.27)\), and protein content 10.82% \((\text{Mean}=45.50\pm2.03)\) compared to the negative control group. Likewise, oral administration of medium dose of RBFCE increased the mucous content \((p<0.05)\) 18.60% \((\text{Mean}=380.16\pm10.12)\) and protein content by 29.70% \((\text{Mean}=53.17\pm2.04)\) \((p<0.01)\), compared to the negative control group. Oral treatment of high dose of RBFCE increased the mucous content \((p<0.05)\) by 21.40% \((\text{Mean}=392.66\pm9.01)\) and protein content by 56.92% \((\text{Mean}=56.87\pm2.41)\) \((p<0.001)\) compared to the negative control group as shown in Figs. 4 and 5.
reflected mucosa with focal erosion and inflammation. The histopathological investigations of medium dose RBFCE treated group (500 mg/kg) showed normal surface epithelium having only focal erosion without inflammatory changes. The high dose RBFCE group (750 mg/kg) showed normal looking mucosal epithelium without surface erosion/ulceration and inflammation (Fig. 6).

DISCUSSION

The present study investigated the acute oral toxicity and gastroprotective effect of RBFCE on ethanol-induced gastric ulcer model and its possible mechanism. Characterization of isolated compound, QUE-G confirms the presence of flavonoid class of compound. Flavonoids are safe and effective therapeutic agents for the treatment of gastrointestinal diseases. Previously, QUE-G has been isolated from Azadirachta indica (Tatke et al., 2014), Prunus frutetosa (Razavi et al., 2009), Manihot glaziovii (Hakim et al., 2020), and Byrsocarpus Coccineus etc. (Ahmadu et al., 2007). However, this compound was the first report from RBF. QUE, a naturally occurring bioflavonoid is commonly found as QUE-G in herbs, fruits, and vegetables. Presence of glucose moiety makes QUE more stable compared to aglycone form. Only, a very small quantity of naturally occurring QUE lacks a glycoside chain. Bioavailability of QUE-G is much better then aglycone form (without glycoside chain) (Kâşkci et al., 2016), which is commonly available form of QUE as a supplement. It is a globally recognized safe complementary or alternative medicine used for different a various comorbidities including heart (Patel et al., 2018), liver (Li et al., 2018), and stomach (Ekström et al., 2011). It also possess anticancer (Vafadar et al., 2020) and neuroprotective potential (Khan et al., 2018; Salehi et al., 2020). QUE inhibits several cytochrome P450 isozymes, i.e., CYP3A4, CYP2C8 CYP2C9 and CYP1A2 (Umathe et al., 2008; Samala et al., 2016) and considered to be involved in herb-drug interactions (HDIs). Therapeutic profile of glucoside form is identical to that of aglycone form. QUE also possess significant antiulcer and gastroprotective activity due to its antioxidiant, anti-secretory, antihistaminic and proton pump inhibiting properties (de Lira Mota et al., 2009). QUE-G possesses cardiovascular benefits (Terao, 2023).

The toxicity study demonstrated that rats treated with RBFCE did not show any sign of toxicity or mortality and LD50 value was found above 5000 gm/kg. All the three orally administered once daily doses of RBFCE (250, 500, 750 mg/kg) to rats for 10 days for gastroprotective activity, were safe. OMP, a widely used proton pump inhibitor for the treatment of gastric ulcers, was used as positive control, in line with the literature (Nordin et al., 2014). After one hour on last (14th) day of treatment, single dose (5 ml/kg) of absolute ethanol was orally administered to animals. Ethanol is most widely used in experimental models to assess the gastroprotective activity in rats (Sidahmed et al., 2015). It rapidly penetrates into the gastric mucosa, increases mucosal absorptivity (Sidahmed et al., 2013) and releases vasoactive mediators (histamine, leukotrienes C4 and endothelin-1). The vasoactive mediators cause blood flow stasis in circulation of mucous membrane and increasing lesions in mucosa. In addition, ethanol also reduced the mucus production, gastric mucusal blood flow, bicarbonate secretion, prostaglandin production, tissue levels of DNA, RNA and proteins, which leads to tissue injury. Formation of superoxide and reactive oxygen species generates oxidative stress which, in turn results rupturing of the blood vessels that contributes to the hemorrhage, tissue necrosis and disrupting the protective mucosal layer (Fahmy et al., 2015). In the present study, oral administration of ethanol to rats produced hemorrhagic red streaks of various sizes on the gastric mucosa of the control group. RBFCE pre-treated groups (250, 500, 750 mg/kg) showed protected gastric mucosa and significantly reduced the rate of ethanol-induced damage to the gastric mucosa, compared to the negative control group. RBFCE showed gastroprotective effect in dose dependent manner, comparable to the OMP group (Table 1). Pretreatment with RBFCE significantly reduced UI, maximum being seen at 750 mg/kg, similar to that of the OMP.

Any agent that reduces the gastric acid secretion or increases the mucous secretion is an effective gastroprotective agent (Oliveira et al., 2014). Gastric secretions in rats were studied because they may contribute to RBFCE’s gastroprotective action. The ulcer control group had a lower pH, a higher volume of gastric juice, and total acidity. Pre-treatment with either OMP or different doses of RBFCE significantly decreased gastric juice volume and total acidity, coupled with a significant increase in gastric pH when compared to the ulcer control group. Oral administration of RBFCE for 10 days strengthened the gastrointestinal system in such a way that gastric volume and the total acidity was significantly reduced in the treated rats with a corresponding increase in pH compared to the negative control group. A decrease in gastric juice volume could be due to less acid production, as evidenced by the pH and total acidity of the gastric juice. These findings indicated presence of therapeutically active compounds in RBFCE: that reduced the acidity of gastric acid secretions, which was increased by ethanol administration. Therefore, anti-secretory effect RBFCE could be a possible mechanism of gastroprotection.

The gastric epithelium, which is surrounded by a continuous mucus layer, acts as the first line of mucosal defense against luminal acid by acting as a barrier against luminal pepsin to protect the underlying mucosa from proteolytic digestion. Mucus comprises mucin-type glycoproteins detectable by amount of alcian blue binding (Zakaria et al., 2016a). Microscopic evaluation revealed the comprehensive damage to gastric mucosa in ulcer control group (Fig. 5). Animals treated with RBFCE showed protection to gastric mucosa with significant reduction in inflammation in dose dependent manner. The daily RBFCE treatment for 10 days found to amplify the amount of gastric mucous as compared to the vehicle. Increased mucous content may be liable to gastric cytoprotection. Therefore, it may be another possible mechanism of gastroprotective effect of RBFCE.

Stomach from normal control animals in histopathological examination showed normal-looking mucosal surface which indicates absence of any signs of ulceration in normal control group. The presence of focal mucosal inflammation and metaplastic changes in stomach of negative control animals indicates development of ulceration. Gastric metaplastic cells may transform into precancerous cells which increase the risk of gastric cancer if untreated. While the positive control group revealed mucosa and part of submucosa with only focal erosion, but no mucosal or submucosal inflammation was observable. Stomach section from low dose (250 mg/kg) RBFCE treated animals showed mucosa with focal erosion and mild chronic inflammation while the medium dose RBFCE treated group (500 mg/kg) exhibited normal surface epithelium with mild degree of erosion. Rest of the
mucosa was normal looking without evidence of inflammation. On the other hand, high dose RBFCE treated animals (750 mg/kg) revealed normal looking mucosal epithelium without surface erosion/ulceration, metaplastic and inflammatory changes which indicates protective potential of RBFCE at medium and high doses. A high dose of RBFCE could significantly reduce gastric lesions and histological damage in a dose-dependent manner comparable to OMP. The present findings supported the ethnopharmacological use of RBF and highlighted its potential to be used as herbal gastroprotective medicine. Some of the limitations of the research work include (1) study was performed in rats but not in humans (2) only preliminary data was collected (3) study was performed on extract, not on isolated compound.

CONCLUSIONS

RBFCE showed a significant gastroprotective effect against ethanol-induced gastric ulcer, as reflected by a decreased gastric juice volume and acidity, parallel to an increased gastric mucus secretion. The above activity could be attributed to QUE-G, major bioactive flavonoid isolated from RBFCE in this study. These findings deliver considerable evidence in favor of the folk use of RBF in the treatment of gastric disorders.

Declarations


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Data Availability statement: We have presented all our data in the form of figs. and tables. The datasets supporting the conclusions of this article are presented in the paper. The Rosa brunonii Lindl. fruits were collected from Murree, Punjab, Pakistan and identified by an expert taxonomist at Department of Botany, GC University, Lahore, Pakistan. A voucher specimen (GC Herb.Bot.3315) was deposited in the Herbarium of GC University, Lahore, Pakistan for future reference.

Compliance with Ethical Standards: All the animal studies were carried out according to internationally accepted protocols, which were approved by the institutional animal ethical committee College of Pharmacy, University of Punjab (AEC/PUCP/1077) dated 03–05–2018.

Conflicts of Interest: The authors declare no conflict of interest.

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