Research Article

Betalain-Enriched Beetroots Exhibit Antiulcer and Anti-inflammatory Potentials

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Introduction. Recently, plant natural food colorants have received a growing interest due to their therapeutic and preventive activities against various diseases. This study is aimed at evaluating the phytochemical composition by high-performance liquid chromatography with diode array detection (HPLC-DAD) and at evaluating the antiulcer effect of red beetroot betalains (BRB).

Materials and Methods. Ethanol was used for gastric mucosa damage, ulcer, and inflammation induction in rat.

Results. HPLC-DAD data revealed the existence of numerous compounds, including betanidin 5-glucoside (43.22%), isobetanidin 5-glucoside (18.47%), 2,17-bidecarboxy-neobetanin (7.07%), 2-O-glucosyl-betanin (4.03%), 17-decarboxy-betanidin (3.61%), neobetanin (3.24%), and eight other compounds whose yields were too low. In vitro as in gastric tissues in rats, BRB potentially inhibited key enzymes—relation to gastric ulceration and damage as pepsin, lipoxygenase, and hyaluronidase activities. In ethanol-induced gastric ulcer and inflammation, BRB administration at doses 200, 400, and 800 mg/kg significantly \((p \leq 0.05)\) decreases the ulcer areas (UA) and index (UI); increases the curative index (CI) by 78.1, 78.4, and 78%, respectively; and ameliorates the pathological damage induced by ethanol. In addition, BRB administration to rat gastric ulcer rats prevented significantly \((p \leq 0.05)\) the decrease of gastric mucus (GM) content (by 116%) and reduced the stress oxidant, evidenced by a significant \((p \leq 0.05)\) decrease of gastric mucosa thiobarbituric acid reactive species (TBARS) (by 28%) and mucus juice pepsin by 56%. Conclusion. Taken together, BRB exerted potential therapeutic efficacy for gastric ulceration.

1. Introduction

Currently, more than 3000 synthetic components have been used in the food industry as colors, flavors, antioxidants, and preservative agents [1]. In 2014, the global use of pigments is nearly 9.7 million tons [2]. This disease occurs as a result of many factors, such as a reduction in the mucus secretion associated with prolonged ingestion of anti-inflammatory drugs and infection with Helicobacter pylori. In addition, previous studies have reported that synthetic food coloring has caused various and serious health problems such as mutations, disruption of hormones, cancers, hypofertility, and allergic reactions [3–8]. Natural plant colors play an important role in the food industry in the absence of toxicity and also have several beneficial effects for consumers [9–14]. Among these plants, red beetroot pigments have also been used as a natural colorant, aroma, and antioxidant in the food industry [15–18]. In fact, red beetroot is a natural food coloring, because of its richness in red pigments such as betalains [19].

Globally, the prevalence cases of gastrointestinal diseases and ulcer diseases increased to 9584000 in 2019 [20]. Indeed, gastric ulcers and injuries are one of the most common chronic diseases affecting approximately 4.6 million people worldwide. [21]. Several factors have induced ulcers and gastric damage such as alcohol consumption, anti-inflammatory drugs, stress, alcohol consumption, and Helicobacter pylori infection [22]. The use of the antisecretory
therapy or proton pump inhibitors is one strategy to treat ulcers, and they have many side effects [23]. Therefore, the use of medicinal plants is considered a source of various bioactive drugs for treatment and prevention and improvement of ulcer and gastrointestinal diseases without acute or minimal toxicity.

In this context, this study is aimed at identifying the composition of BRB by HPLC-DAD and at assessing the effect of these pigments’ consumption on ethanol-induced gastric ulceration, inflammation, and stress oxidant in the stomach of rats.

2. Materials and Methods

2.1. Extraction of Betalain Pigments from Red Beet and Chromatographic Analysis. Mature fleshy fruit beetroots were collected during the month of May 2022 from Teboulba, Monastir, Tunisia, without damage, and were laved and skinned manually. A voucher specimen (BV-Mo/16) was deposited in the LR11ES39 Laboratory [24]. Optimum extraction of betalains was determined by a mixture of water/methanol/formic acid (84.95/15/0.05) according of the procedure described by Sawicki et al. [25]. 75 mL of the previously described mixture and 25 grams of freeze-dried red beetroot were homogenized and sonicated for 30 seconds before being vortexed. Then, the mixture was sonicated for 30 s, another time vortexed and sonicated, and centrifuged during 10 min (13000 × g at 4°C) during 20 min.

BRB compositions were identified using HPLC-DAD system (Agilent, USA). The main tools for betalain compound tentative identification were the interpretation of the observed MS/MS spectra in comparison with those found in the literature and several online databases (Phenol-Explorer, ChemSpider, MassBank, and Spectral Database for Organic Compounds). To obtain quantitative information, solution of betanin standards (Sigma, 901266) in acetonitrile:water acidified with 0.1% formic acid (1:1, v/v) was employed.

2.2. In Vitro Anti-inflammatory Assay. Lipoxygenase (Sigma-437996-500U) activity was determined by monitoring the formation of conjugated dienes from linoleic acid at 25°C and 234 nm. The mixture of 50 mM sodium phosphate buffer (2.8 mL, pH 7), 10 mM sodium linoleic acid solution (0.1 mL), and crude enzyme solution (0.1 mL) was used. One unit of LOX activity was defined as a change of 0.01 in absorbance per minute at 25°C [26]. The hyaluronidase activity was measured by Sigma protocol. 100 μL of hyaluronidase (4 U·mL⁻¹), 100 μL of sodium phosphate buffer (200 mM, pH 7, 37°C), and 0.01% BSA were mixed and incubated at 37°C for 10 min. The reaction is triggered by the addition of substrate of hyaluronic acid (Sigma, ref 924474). The undigested hyaluronic acid was precipitated
with 1 mL acid albumin solution made up of 0.1% bovine serum albumin in 24 mM sodium acetate and 79 mM acetic acid, pH 3.75 [27].

2.3. Evaluation of BRB Toxicity. The oral toxicity evaluation was conducted as per the 86/609/EEC guidelines. Twelve Wistar male rats were divided into two groups as follows: Group 1 included normal rats that consumed standard diet and allowable free access to water and received 2 mL distilled water by gavage daily. Group 2 included rats that received BRB orally administrated in a daily dose of 2 g/kg bw. Both groups were controlled (toxicity and mortality) during the first 15 days after BRB reception. The general symptoms of in each rat were checked within 48 hours. Fifteen days later, all animals were anaesthetized and sacrificed; the blood samples were initially collected, centrifuged at 3000 rpm for 15 min, and then stored at -80°C for further analysis.

2.4. Protocol of Ethanol-Induced Gastric Mucosa Damage and Ulceration in Rats. The antiulcer action was assessed using the methodology outlined by Izhar et al. [28]. Six groups of male Wistar rats weighing 165 ± 13 g and aged 7 weeks housed under controlled environment and permitted water and food pellets ad libitum were used. Group 1 served as the healthy control group, receiving only water; group 2 served as the gastric ulcer group, receiving ethanol by gastric gavage at a dose of 5 mL/kg body weight; and group 3 served as the ulcerated group, receiving omeprazole at a dose of 20 mg/kg. Gastric ulcer-affected rats in groups 4, 5, and 6 received 200, 400, and 800 mg/kg of BRB as supplements. Absolute ethanol (5 mL/kg) was administered to each rat by gastric gavage one hour later [29]. Absolute ethanol was administered by the gastric gavage route to rats, 1 hour after the BRB or omeprazole ingestion. 90 min later, the rats were sacrificed, and the stomach was detached from rats and was opened along the greater curvature (Figure 1). The stomachs were gently rinsed with iced cold phosphate buffer for cleaning. The present rat experimentation was approved by the National Ethical Committee on Medical and Animal Research of the National Veterinary Medicine School, E.N.M.V of Tunisia (Approval Number: CEEA-ENMV 23/20) and by the Animal Care and Use Committee of Monastir University, Tunisia (Approval No. CER-SVS 0013/202202020-0205)). The study was performed in accordance with the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH publication No. 85–23, revised 1996). All efforts were made to minimize animal suffering and reduce the number of animals used. The rats were sacrificed one hour after receiving an absolute ethanol overdose, and their stomachs were promptly removed, opened, and cleaned with distilled water. The total ulcer area was calculated by inverted microscope with digital camera using the ImageJ software with digital calculable distance (millimeter) by means of an e-ruler. The gastric mucosa content was calculated according to Ofusori et al. [30]. The ulcer index (UI) was determined using the following formula: ulcer index = (ulcer area/total

Table 1: Betalains identified in BRB by LC-MS/MS.

<table>
<thead>
<tr>
<th>Peak compound</th>
<th>RT (mn)</th>
<th>Compounds</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.91</td>
<td>2-O-Glucosyl-betanin</td>
<td>4.03</td>
</tr>
<tr>
<td>2</td>
<td>7.81</td>
<td>Betanin (betanidin 5-glucoside)</td>
<td>43.22</td>
</tr>
<tr>
<td>3</td>
<td>7.99</td>
<td>2-O-Glucosyl-isobetan</td>
<td>0.55</td>
</tr>
<tr>
<td>4</td>
<td>8.83</td>
<td>Isobetanin (isobetanidin 5-glucoside)</td>
<td>18.47</td>
</tr>
<tr>
<td>5</td>
<td>9.47</td>
<td>Betanidin</td>
<td>7.07</td>
</tr>
<tr>
<td>6</td>
<td>9.69</td>
<td>Ferulic acid hexoside (4-glucoside)</td>
<td>1.05</td>
</tr>
<tr>
<td>7</td>
<td>10.95</td>
<td>17-Decarboxy-neobetan</td>
<td>0.47</td>
</tr>
<tr>
<td>8</td>
<td>11.65</td>
<td>Isobetanidin (17-decarboxy-betanidin)</td>
<td>3.61</td>
</tr>
<tr>
<td>9</td>
<td>12.15</td>
<td>Neobetanin</td>
<td>3.24</td>
</tr>
<tr>
<td>10</td>
<td>13.73</td>
<td>17- Decarboxy-betan</td>
<td>0.35</td>
</tr>
<tr>
<td>11</td>
<td>14.19</td>
<td>2,17-Bidecarboxy-betan (isobetanid)</td>
<td>1.57</td>
</tr>
<tr>
<td>12</td>
<td>14.77</td>
<td>2,17-Bidecarboxy-neobetan</td>
<td>7.07</td>
</tr>
<tr>
<td>13</td>
<td>14.87</td>
<td>Isovitexin (apigenin-6-C-glucoside)</td>
<td>2.54</td>
</tr>
</tbody>
</table>

Table 2: Biochemical parameters of control and treated rats, measured during the acute toxicity study. Values are mean ± SEM (n = 5). No significance compared to control.

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control</th>
<th>BRB 2000 mg/kg bw</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver profile</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>145 ± 6.3</td>
<td>136 ± 4.1</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>77 ± 5.1</td>
<td>69 ± 7.3</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.81 ± 0.05</td>
<td>0.65 ± 0.04</td>
</tr>
<tr>
<td><strong>Renal profile</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mg/L)</td>
<td>0.37 ± 0.02</td>
<td>31 ± 0.02</td>
</tr>
<tr>
<td>Creatinine (mg/L)</td>
<td>3.9 ± 0.05</td>
<td>3.2 ± 0.02</td>
</tr>
<tr>
<td><strong>Lipid profile</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.73 ± 0.03</td>
<td>0.69 ± 0.04</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.79 ± 0.11</td>
<td>0.83 ± 0.12</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>0.53 ± 0.04</td>
<td>0.59 ± 0.03</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.21 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>0.98 ± 0.01</td>
<td>0.91 ± 0.05</td>
</tr>
</tbody>
</table>
mucosal TBARS rates were determined according to the protocol described by Buege and Aust [34]. The quantification of the protein was determined using biuret colorimetric method (kits, Biolabo, France, ref K2016). Gastric juice hyaluronidase activity was determined spectrophotometrically by determining the level of n-acetylgalactosamine formed from sodium hyaluronate [35]. The lipoygenase activity was determined according to Wu [36]. Absorbance was measured at 585 nm. The activity of pepsin was calculated as a number of milligram of tyrosine produced by 1 mL of original enzyme solution, and the absorbance of each sample was calculated at 660 nm [37]. TC and HDL-C, TG, total bilirubin, urea, and creatinine levels were measured (kits, Biolabo, France, ref 80106, 80019, and 90206; kits, Biomaghreb, Tunisia, ref 19011, 15013, and 25012). LDL-C rate was determined by the determination of the difference between TC and HDL-C levels. Serum aspartate and alate transaminases were quantified using kits (Biomaghreb, Tunisia, ref 10018 and 11015).

2.6. Statistical Analysis. Experimentation was established using five rats per group. Data obtained was analysed using Fisher’s test (StatView) and one-way analysis of variance (ANOVA). Differences were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Betalain Identification and Quantification by LC-MS/MS. Twelve betalains were newly identified in the red beetroot betalain-rich extract. This study revealed that BRB are composed mainly by betanidin 5-glucoside (43.22%), isobetanin 5-glucoside (18.47%), 17-bicarboxy-neobetanin (7.07%), 2-O-glucosyl-betanin (4.03%), 17-decarboxy-betanin (3.61%), neobetanin (3.24%), apigenin-6-C-

![Table 3: Effect of BRB on lipoygenase and hyaluronidase activities in vitro.](image)

<table>
<thead>
<tr>
<th></th>
<th>Lipoygenase activity</th>
<th>Hyaluronidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (mg/mL)</td>
<td>% inhibition</td>
</tr>
<tr>
<td>BRB</td>
<td>1</td>
<td>23.9 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>43.1 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>81.1 ± 4</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>0.5</td>
<td>39.3 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>52.1 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>75 ± 2.7</td>
</tr>
</tbody>
</table>

![Table 4: Ulcer area and UI and CI levels in BRB-ulcered rats.](image)

<table>
<thead>
<tr>
<th></th>
<th>U</th>
<th>U+400 mg BRB</th>
<th>U+800 mg BRB</th>
<th>U+25 mg/kg Ompz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer area (mm$^2$)</td>
<td>821 ± 37</td>
<td>177 ± 32$^e$</td>
<td>137 ± 41$^e$</td>
<td>232 ± 27$^e$</td>
</tr>
<tr>
<td>Ulceration index</td>
<td>13 ± 0.7</td>
<td>2.8 ± 0.1$^e$</td>
<td>2.16 ± 0.11$^e$</td>
<td>3.67 ± 0.13$^e$</td>
</tr>
<tr>
<td>CI (%)</td>
<td>78 ± 4</td>
<td>83 ± 7</td>
<td>71 ± 6</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Effect of BRB on gastric mucus content and pepsin activity in ethanol-induced gastric ulcer. Each value represents the mean ± SEM for each group ($n = 5$). The statistical analysis is presented as follows: $^p < 0.05$ vs. control; $^{*}p < 0.05$ vs. ethanol-induced gastric ulcer.

<table>
<thead>
<tr>
<th></th>
<th>Stomach pepsin activity (mmol tyrosine liberated/4h)</th>
<th>Mucus content (gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td>5.3 ± 0.4$^e$</td>
<td>4.73 ± 0.3$^e$</td>
</tr>
<tr>
<td>Ulcer rats</td>
<td>28 ± 1.7$^e$</td>
<td>1.71 ± 0.2$^e$</td>
</tr>
<tr>
<td>BRB</td>
<td>400</td>
<td>17 ± 0.9$^e$</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>13.7 ± 1.3$^e$</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>12.3 ± 1.1$^e$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13.8 ± 0.87$^e$</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>10.74 ± 0.6$^e$</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>4</td>
<td>9.73 ± 1.1$^e$</td>
</tr>
</tbody>
</table>

2.5. Biochemical Analysis. The gastric juice pH was calculated using a pH meter. The gastric pepsin activity was determined using the protocol described by [33]. The gastric

mucosa surface area) x 100. The curative index was determined using the following formula: curative ratio = (US control – US treated)/(US control) [31]. The gastric volume was done by the methods described by [31]. Gastric juice was collected by centrifugation of gastric mucus at 3500 x g/5 min/4°C to remove insoluble mucosal gastric. The gastric juice was calculated using graduate tubes. For histological study, fragments of gastric tissues were fixed in formaldehyde 10% and embedding into paraffin wax, were sectioned into 5 µm thick histological sections, and were stained with hematoxylin and eosin solution (H & E) and photographed by an Olympus CX41 light microscope [32].
glucoside (2.54%), 2,17-bidecarboxy-betanin (1.57%), ferulic acid hexoside (1.05%), and four other compounds with lower yields (Table 1).

3.2. BRB Safety. The results of this study showed that rats that received BRB at a dose of 2 g/kg did not experience any physiological toxicity or mortality. Additionally, there was no discernible difference in the levels of TB, urea, creatinine, or AST and ALT activities, as well as in the liver and kidneys. Furthermore, there was no variation in the levels of TG, glucose, LDL cholesterol, HDL cholesterol, or TC during this treatment (Table 2). This finding showed that ingesting BRB is not toxic up to a dose of 2 g/kg body weight.

3.3. Effect of BRB on Lipooxygenase and Hyaluronidase Activities In Vitro. Table 3 shows that the lipooxygenase and hyaluronidase activities were inhibited by BRB. This reduction was estimated with IC$_{50}$ = 1.33 and 1.87 mg/mL, respectively.

3.4. Effect of BRB on Gastric Macroscopic Injury, Mucus Content, and Ulcer Index in Ethanol-Induced Gastric Damage in Rats. The findings of this study showed that the administration of absolute ethanol to rats at a dose of 5 mL/kg body weight resulted in gastric damage and ulceration, as shown by the development of gastric hemorrhagic damage in the rats, a 63% decrease in gastric mucus content, and a significant increase in UI in comparison to normal rats. However, supplementation with BRB at a dose of 800 mg/kg administered via gastric gavage protects against stomach damage, as seen by the significant visual decline in hemorrhagic ulcers and the 78% drop in CI when compared to rats with ulcer not receiving treatment. Furthermore, when BRB was given to rats with gastric ulcers, the amount of gastric mucus increased by 116% compared to the rats that were not given any treatment (Tables 4 and 5 and Figure 2).

3.5. BRB, Ethanol, and Histological Examination of Gastric Tissues. This study showed that the administration of ethanol by gavage caused gastric mucosa ulceration, necrosis, and neutrophil infiltration (Figure 3). However, the BRB ingestion by ethanol-treated rats protects from mucosa gastric damage and inflammation and neutrophil infiltration.

3.6. Effect of BRB on Gastric Pepsin Activity. The results of this study showed that gastric ulceration and damage caused an increase in mucosa pepsin activity by 428% as compared to the normal stomach without injury and ulcer; U: gastric damage ulcer. In ulcered rats treated with BRB at dose 200, 400, and 800 mg/kg bw (U$_{200}$, U$_{400}$, and U$_{800}$), a potential protective effect was observed from mucosa damage and ulcer.

Figure 2: Effect of BRB on ethanol-induced gastric. C: normal stomach without injury and ulcer; U: gastric damage ulcer. In ulcered rats treated with BRB at dose 200, 400, and 800 mg/kg bw (U$_{200}$, U$_{400}$, and U$_{800}$), a potential protective effect was observed from mucosa damage and ulcer.
to normal rats. The consumption of BRB at dose of 800 mg/kg bw attenuates gastric injury, as observed by the decrease in pepsin activity by 56% as compared to rats with ulcers who were not treated (Table 5).

3.7. Effect of BRB on Gastric Lipoxygenase and Hyaluronidase Activities and TBARS Levels. The mucosa lipoxygenase and hyaluronidase activities in gastric ulcer-affected rats significantly increased, as shown in Table 1, by 97, 111, and 91% as compared to the normal rats. Furthermore, ulcer also significantly increased the mucosa TBARS levels by 87%. However, it was found that giving BRB to gastric ulcer-affected rats reversed the activities of lipoxygenase and hyaluronidase in the stomach mucosa by 31 and 35%, respectively. BRB also significantly reduced the TBARS level in the stomach mucosa by 35% (Figure 4).

4. Discussion

The mortality rate of mucosal gastrointestinal diseases and ulcers has been reported to range from 10% to 40% worldwide and to affect a huge population [38–42]. Additionally, the synthetic therapeutic drugs for gastrointestinal diseases and ulcers induced a number of harmful side effects, including arrhythmia, gynecomastia, hypersensitivity, impotence, and hematopoietic changes [43, 44]. Therefore, it is crucial to create natural pharmaceuticals with gastroprotective and antiulcer properties from plants, animals, algae, and microbes. In this context, the safety of gastric mucosa and the reduction of gastric acid secretion by natural’s compounds are the major strategies to treat and prevent gastrointestinal diseases, ulcers, and hyperacidity. Natural pigments from plants, algae, and bacteria are among these natural drugs that are gaining popularity due to their lack of toxicity, extensive use in the food and pharmaceutical industries, sensory acceptability, and curative and preventive effects against a variety of pathologies, as well as the importance of a balanced diet. In our study, the chemical profiling of BRB showed the presence of two major compounds. These are betanidin 5-glucoside (43.22%), isobetanidin (18.47%), 2,17-bidecarboxy-neobetanin (7.07%), 2-O-glucosyl-betanin (4.03%), 17-decarboxy-betanidin (3.61%), neobetanin (3.24%), apigenin-6-C-glucoside (2.54%), 2,17-bidecarboxy-betanin (1.57%), ferulic acid hexoside (1.05%), and four other compounds whose yields were too low. Betanidin 5-glucoside was the most prevalent of the twelve betalain compounds that were found in red beetroot extract, according to the previous studies [45, 46]. Slatnar et al. [46] identified 15 compounds including 2′-O-glucosyl-betanin, betanin, 2′-O-glucosyl-isobetanin, isobetanin, isoprebetanin, betanidin, 2,17-bidecarboxy-neobetanin, 17-decarboxy-neobetanin, 2-decarboxy-neobetanin, neobetanin, and 2,17-bidecarboxy-2,3-dehydro-neobetanin. More recently, Mandal et al. [47] reported eighteen betalains, dominating betanin and isobetanin as previously described [48, 49]. The root peel had the highest betalain concentration. More of 80% of the total pigments of red beetroot are constituted by betacyanins, particularly betanin, and its C15 isomer, isobetanin [48, 49]. According to Sawicki et al. [50], the betacyanins identified were betanin, betanidin, isobetanin, 17-decarboxy-neobetanin, and neobetanin.

In vitro study showed that betalain red beetroot inhibited lipoxygenase and hyaluronidase activities, two important enzymes involved in gastric tissues inflammation with IC₅₀ = 1.33 and 1.87 mg/mL, respectively. In fact, lipoxygenase and hyaluronidase are enzymes that are involved in the conversion of arachidonic acid to leukotrienes and prostaglandins, which are inflammatory mediators. Previous studies have reported that the major compound of our extract as betanidin 5-glucoside (43.22%) protects endothelial cells...
from a cytokine-induced redox imbalance and lowers the expression of the proinflammatory intercellular adhesion molecule 1 (ICAM-1) [51, 52].

In ethanol-induced rat gastric ulcer and damage, this study showed that the administration of BRB suppressed stomach mucosa lipoxygenase and hyaluronidase activities by 31 and 35%, respectively, as compared to untreated rats. Moreover, stomach mucosa TBARS rates were significantly decreased by 35% after ingestion of BRB. Previous research has shown that consuming red beetroot betalains protect from gentamicin-induced increases in NF-κB, TNF-α, and IL-6 levels [53]. Similar studies showed that treatment with red beetroot juice can prevent rats’ liver inflammation induced by N-nitrosodiethylamine [53–58]. Similar to this, betalains reduced the production of cytokines that are precursors of inflammation [59]. Additionally, it has been shown that betalains have the capacity to inhibit the enzymes lipoxygenase and cyclooxygenase-2 [51]. This finding was in accordance with the previous reports that shown betalains can also inhibit the hyaluronidase enzyme [60–63]. Another investigation showed that two of the compounds of our dye as betanidin (7%) and betanin (43%) were able to inhibit key enzymes related to gastric inflammation as COX by interaction with Tyr-385 and Ser-530 residues close to the active site of COX [64, 65]. The anti-inflammatory activity of betalains may also be due to their action against cellular mediators of inflammation, such as intercellular cell adhesion molecule-1 (ICAM-1) significantly repressed by

Figure 4: Effect of the BRB administration on ethanol-induced inflammation and stress oxidant in gastric mucosa by induction of lipoxygenase and hyaluronidase activities and TBARS levels. The supplementation of Opuntia stricta peel and purple polysaccharides inhibits lipoxygenase and hyaluronidase activities and TBARS rates.
Betanin [64, 65]. Furthermore, BRB consumption scavenge ROS and protects from stress oxidant, as evidenced by a much lower rate of TBARS in rats with gastric juice ulcers [66]. In fact, our work demonstrated that giving ethanol orally to rats by gastric gavage led to damage and injury to their stomach tissues, including microscopic hemorrhagic damage to the gastrointestinal mucosa, a 63% drop in gastric mucus content, and a significant rise in UI. This alteration in gastric mucus caused an important increase in the mucosa pepsin activity. Additionally, a significant rise in the activity of the lipoygenase and hyaluronidase enzymes as well as the degree of lipid peroxidation in the mucosa of the stomach is a further proof that drinking alcohol causes inflammation in the tissues of the stomach. Several properties have been attributed to some of these genera: antioxidant; positive effects on metabolic, cardiovascular, and gastrointestinal health in humans; antimalarial; and inhibitors of lipoygenase and cyclooxygenase pathways [64, 67, 68]. Laboratorial investigation has demonstrated diverse anti-inflammatory mechanisms of the major compound of our extract betanin 5-glucoside (43%) as neutralizer of free oxygen species and regulator of nuclear factor (erythroid-derived 2)-like 2-(Nrf2-) dependent signaling pathways [69]. Another study investigates that isobetanin (about 19%) ingestion suppressed lipopolysaccharide-stimulated transcript levels of proinflammatory genes as TNF-alpha and cyclooxygenase-2 [70]. A previous study assessed that betanin (about 7%) ingestion lowers the inflammatory enzymes cyclooxygenase 2 (COX-2) and cyclooxygenase 1 (COX-1) by 97% and 33.5% [65]. Moreover, there has been evidence that betanin has a powerful anti-inflammatory effects by inhibiting the proinflammatory signaling cascades: nuclear factor-kappa B (NF-kB) cascade and also cyclooxygenase-2 and lipoygenase [71].

Previous studies have reported that ethanol caused gastric disturbances in gastric secretion, gastric mucus damage, gastric permeability alterations, and gastric mucus depletion [72–76]. In ulcerated gastric rats treated with BRB, a potential gastroprotective effect was observed against ethanol-induced gastric ulcer, damage, and injury, which is widely approved and used for gastric ulcer treatment. This therapeutic effect of red beetroot betalains was clearly confirmed by macroscopical findings, which showed an important decrease in the hemorrhagic damage in gastric mucosa and gastric mucus content as compared to untreated gastric ulcerated rats. In fact, a significant decrease in the number of lesions by BRB might be related to the stimulation of gastric antioxidant capacity, the suppression of inflammation, and the reduction of gastric acid secretion and pepsin activity in gastric tissues. Similarly, it was observed that betalain at dose 800 mg/kg has a more powerful protective effect against gastric mucosa ulceration and damage, which was comparable with omeprazole. BRB has increased the basicity of gastric contents as compared with gastric ulceration among untreated rats. Our findings is in accordance with the previous study [74]. The protective effect of BRB against inflammation and damage of gastric mucus was confirmed by an important decrease in the mucus pepsin activity. This result is in accordance with Gómez-López et al. [61], Montiel-Sánchez et al. [62], Gómez-López et al. [63], and Reddy et al. [65], who reported that red beetroot pigments protect from ethanol-induced macroscopic gastric hemorrhagic damage, injury, and toxicities. Cai et al. [66] reported that consumption of betalains from fruits as Opuntia ficus-indica protects from gastric damage and injury caused by ethanol, reduces lipid peroxidation level, and stimulates antioxidant capacity in stomach alteration induced by ethanol. Moreover, ulcerated rats pretreated with black chokeberry fruit pigments reduced the UI [77]. Additionally, the present study showed that pretreatment of gastric ulcer rats by BRB caused a considerable decrease in the UI and an important increase in the CI. Biochemical analysis of gastric mucus juice showed that supplementation of red beetroot betalains prevented the increase in the key enzymes related to inflammation in the mucus by the suppression of the mucosa lipoxigenase and hyaluronidase activities and lipid peroxidation level. According to Zielińska-Przyjemska et al. [78] and Jimenez-Gonzalez et al. [79], the administration of red beetroot betalains has a key part in the mechanism of gastroprotection by reducing inflammation in the gastrointestinal system. Furthermore, the results of this study showed that gastric ulcer caused a considerable increase in the mucus pepsin activity as compared to normal rats. This is in agreement with Ahmed et al. [80] and Mandal et al. [47] who found a correlation between stomach ulcers and increased pepsin activity. However, pretreatment with BRB prevented ethanol-induced gastric pepsin activity, suggesting that BRB prevented ulceration and injury to the stomach mucosa.

5. Conclusions

The study’s findings showed that red beetroot betalains reduced gastric mucus and ulcer index (UI) while protecting against the macroscopic hemorrhagic damage that ethanol induced in the gastric mucosa. Additionally, the supplementation that prevented gastric mucus alteration showed an important increase in the mucosa pepsin, lipoygenase and hyaluronidase activities, and lipid peroxidation level.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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