Evaluation of anti-ulcer activity of aqueous and ethanolic extracts of whole plant of *Byttneria herbaceae* in rats

Bharathi K N, Ankitha M

**Abstract:** The present study was carried out to evaluate the anti-ulcerogenic activity of aqueous and ethanolic extracts of whole plant of *Byttneria herbaceae* against pylorus ligation model and ethanol-induced gastric mucosal damage in rats. Single dose of 200mg and 400 mg/kg b.w.p.o. of extracts were used in the study. The antiulcer activity was assessed by determining volume of gastric content, pH, total acidity, ulcer score, ulcer index and percentage protection in pylorus ligation model. Omeprazole (30 mg/kg b.w.p.o.) was used as standard drug. In the ethanol induced mucosal damage ulcer score, ulcer index and percentage protection was determined. Sucralfate (100 mg/kg b.w.p.o.) was used as standard. Both aqueous and ethanolic extracts of whole plant of *Byttneria herbaceae* exhibited antulcer activity as there was significant ($P<0.001$) reduction in ulcer score induced by pylorus ligation model compared to negative control. Extracts also protected the mucosal damage induced by ethanol as there was significant ($P<0.001$) reduction in ulcer scores, ulcer index compared to negative control. Antiulcer activity of extracts were found to be dose dependent and ethanolic extract was more potent than aqueous extract in ethanol induced ulcer model. Anti-ulcer activity of *Byttneria herbaceae* was due to its cytoprotective activity. Presence of phytochemical constituents such as flavonoid, tannins, saponins, phenolic compounds and triterpenoids are responsible for anti-ulcer activity. As per previous study, *Byttneria herbaceae* was found to have antimicrobial activity against gram negative bacteria, which further substantiate its use in the treatment of gastric ulcers.

**Index Terms** – *Byttneria herbaceae*, Anti-ulcer activity, Pylorus ligation and mucosal damage

**1. Introduction**

Peptic ulcer disease (PUD) is one of the most common gastrointestinal disorder can be seen all over the world with significant mortality and morbidity (Mekonnen, A.N, 2020). It occurs as a wound inside the stomach or duodenum, due to localized destruction of the inner wall (mucosa). Peptic ulcer occurs when an imbalance between offensive (acid, pepsin and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) (Vishali, K, 2011).

Treatment for ulcer includes proton pump inhibitors, antacids, sucralfate, prostaglandins, mucarincin and histaminic antagonists. These treatments may cause side effects like hypersensitivity, arrhythmia, weakness, and hematopoietic disorders. Hence search for natural active and better alternative for the treatment of peptic ulcer with fewer side effects is an emergency need (Jalilzadeh-Amin, G et al, 2015).

In traditional herbal medicine, various parts of *Byttneria herbaceae* is used as an ethno veterinary medicine to cure dysentery, impaction, to treat leprosy, cholera, and asthma (Ansari, J.A, 2013). Presence of tannins, flavonoids , phenolic compounds, triterpenoids and saponins in general have been reported to have anti-ulcer activity in plants like *Tribulus terrestris L* (Ansari, J.A, 2013), *Glycyrrhiza glabra L* (Jalilzadeh-Amin, G, 2015), *Croton macrostachyus Hocsht* (Mekonnen, A.N, 2020) and *Osyris quadripartite Decne* (Abebaw, M, 2017) etc. Since these constituents are present in *Byttneria herbaceae* it was evaluated for the antiulcer activity in this study.
2. Material and methods

2.1. Plant material

Whole plant of *Byttneria herbaceae* was collected from the Savandurga, Ramanagara District, Karnataka, India in the month of August. The samples were authenticated by the University of Trans-Disciplinary Health Science and Technology, Bangalore – 560064.

2.2. Extraction of plant

Plant was washed with water for removal of adhering pulp material and dried under shade at room temperature. Dried sample was pulverized to a coarse powder using a grinder. About 150 g of coarse powder was soaked separately in 1000 ml of distilled water and 1000 ml of 95% ethanol respectively for aqueous and ethanolic extraction, for 24 hrs with occasional stirring. After 24 hrs the preparation was placed in heating mantle connected with reflex condenser. Filtrate was collected and heated in the water bath for the evaporation of the solvent. The residue was then collected, weighed and stored in a sealed container.

2.3. Experimental Animal

Inbred albino rats of Wistar strain of either sex, weighing 150 - 200 g, maintained on a 12 ±1 hrs day and night schedule, fed with standard diet and water ad libitum were used in the present study. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC), Visveswarapura Institute of Pharmaceutical Sciences, Bangalore -560070 (Registration no: 152/Po/ReBi/S/99/CPCSEA). IAEC approval number: VIPS/IAEC/25-08-2021/09-KNB

2.4. Drugs and chemical

Thiopental sodium was procured from NEON laboratories limited, Mumbai. Omeprazole and Sucralfate were procured from Dr Reddy’s Laboratories Ltd, India. Propranolol – Abbott Healthcare Pvt, Solan, Himachala Pradesh, India. Ltd. Loperamide - Prakruti Life Science Pvt, Ltd, India. Furosemide - Zentiva Private limited, Gujarat, India, adrenaline and atropine sulphate Harson Laboratories. Akota, Baroda, India.

2.5. Anti-ulcer Activity

**Dose selection:** *Byttneria herbaceae* aqueous and ethanolic extract of doses 200 and 400 mg/kg, b.w.p.o., was selected based on earlier studies (Sarkar, L et al, 2012).

2.6. Pyloric ligation induced ulcer model

In Pylorus ligation model 36 albino rats of Wistar strain of either sex weighing between 150-200 g were divided into six groups of six animals each. Animals fasted for 24 hrs before the surgery but free access to water. Under thiopental sodium (40mg/kg i.p) anesthesia a midline abdominal incision was made. The pylorus was ligated, care being exercised that neither damage to the blood supply nor traction on the pylorus occurs. The stomach was replaced carefully and the abdomen wall was closed by sutures. The test compounds were administered orally at once. Group I animals (negative control) received vehicle. Group II to V were administered with 200 and 400 mg/kg,b.w., of aqueous and ethanolic extracts respectively. Group VI received standard *omeprazole* 30 mg/kg,b.w., (Mekonnen, A.N, 2020).

After 6 hrs of pyloric ligation Animals were sacrificed by euthanasia. The abdomen was opened, the stomach was removed, and the contents were drained in a centrifuge tube. The volume of gastric juice was measured after centrifugation (Remi Instruments LTD-R 24) at 1000 rpm for 10 minutes (Mekonnen, A.N, 2020). The supernatant was then subjected to analysis to measure pH using pH meter and total acidity (Mekonnen, A.N, 2020).

2.6.1. Macroscopic evaluation of stomach.

Along with the greater curvature the stomach was opened and rinsed with distilled water and pinned on a cork plate and examined for number of ulcers (Vishali, K, 2011), (Jalilzadeh-Amin, G et al, 2015). Severity scoring of ulcer was made as follows:

- Normal colored stomach (0)
- Red coloration (0.5)
- Spot ulcer (1)
- Hemorrhagic streak (1.5)
- Ulcers ≥ 3 and ≤ 5 (2)
- Ulcers ≥ 5 (3)

The ulcer index was determined as follows:

\[
\text{Ulcer index (UI)} = U_N + U_S + U_P \times 10^{-1}
\]

Where,

- \(U_N\) = average number of ulcers per animals,
- \(U_S\) = average of severity score,
- \(U_P\) = percentage of animals with ulcers. (Sahoo, S.K , 2016)
2.6.2. Ulcer inhibition was calculated as below (Mekonnen, A.N, 2020).

\[
\text{Ulcer inhibition (\%) = \left( \frac{UI_{\text{control}} - UI_{\text{treatment}}}{UI_{\text{control}}} \right) \times 100 \text{ acidity (mEq/L)}}
\]

2.6.3. Determination of Total acidity (Mekonnen, A.N, 2020).

An aliquot of 1 ml gastric juice diluted with 1 ml of distilled water was taken in to a 50 ml conical flask, and two drops of phenolphthalein indicator was added to it and titrated against 0.01N NaOH, until a permanent pink color was observed. The volume of alkali consumed was noted. The acidity was expressed as mEq/L by the following formula:

\[
\text{Acidity (mEq/L) = } V_{\text{NaOH}} \times N \times 100 \text{ mEq/L}
\]

Where

\( V = \) Volume

\( N = \) Normality

2.7. Ethanol induced mucosal damage in rats

In this model 36 albino rats of Wistar strain of either sex weighing between 150-200 g were divided in to six groups of six animals each. Animals fasted for 24 hours before the study with water ad libitum. The single dose of test compounds and standard were administered orally. Group I (Negative Control) received vehicle. Group II to V were administered with 200 and 400 mg/kg.b.w., of aqueous and ethanolic extracts respectively. Group VI received standard Sucralfate 100 mg/kg.

After 60 minutes of respective treatment, animals of all the groups were administered 4 ml/kg of absolute ethanol (99.8%) by oral route to induce gastric ulcers. After 1 hour the animals were sacrificed by euthanasia (Vishali, K, 2011) & (Jalilzadeh-Amin, G, 2015). The stomachs were dissected and opened along greater curvature for evaluating the severity of ulcer and percentage protection as in pylorus ligation model (Ansari, J.A, 2013).

2.8. STATISTICAL ANALYSIS

Values were expressed as mean ± S.E.M. Statistical data was analyzed by One-way analysis of variance (ANOVA). \( P<0.05 \) was considered as statistically significant. Post hock analysis was carried out using tukey multiple comparison test.

3. Results

3.1. Percentage yield of aqueous and ethanolic extract of whole plant of *Byttneria herbaceae* was found to be 9.4% w/w and 6.25% w/w respectively. Extracts were investigated for phytochemical constituents by qualitative test and found to contain the following constituents (Table 1)

<table>
<thead>
<tr>
<th>Active constituents</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+: Presence of active constituents,
- : Absence of active constituents.
Table 2: Effect of aqueous and ethanolic extracts of whole plant of *Bytteneria herbaceae* in pylorus ligation ulcer model in rats

<table>
<thead>
<tr>
<th>Group number and treatment</th>
<th>Volume of gastric juice (ml)</th>
<th>pH</th>
<th>Total acidity</th>
<th>Ulcer score</th>
<th>Ulcer index</th>
<th>% Ulcer inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Negative Control (vehicle)</td>
<td>1.58±0.25</td>
<td>3.68±0.21</td>
<td>64±8.74</td>
<td>10.33±1.86</td>
<td>9.7±1.14</td>
<td>_</td>
</tr>
<tr>
<td>II. Aq <em>B. herbaceae</em> 200 mg/kg.b.w.,</td>
<td>2.31±0.75</td>
<td>4.17±0.29</td>
<td>44.8±10.5</td>
<td>3.5±0.54***</td>
<td>8.54±1.12</td>
<td>14.3</td>
</tr>
<tr>
<td>III. Aq <em>B. herbaceae</em> 400 mg/kg.b.w.,</td>
<td>2.54±0.64</td>
<td>4.0±0.37</td>
<td>50.8±9.64</td>
<td>3±1.09**,++</td>
<td>8.7±1.2</td>
<td>15.7</td>
</tr>
<tr>
<td>IV. Et <em>B. herbaceae</em> 200mg/kg.b.w.,</td>
<td>1.49±0.17</td>
<td>3.53±0.09</td>
<td>69.5±9.63</td>
<td>0.60±0.65***</td>
<td>7.61±1.1</td>
<td>20.11</td>
</tr>
<tr>
<td>V. Et <em>B. herbaceae</em> 400mg/kg.b.w.,</td>
<td>1.62±0.24</td>
<td>4.29±0.37</td>
<td>47±14.4</td>
<td>0.58±0.58***,+1,##</td>
<td>6.15±1.54</td>
<td>23.3</td>
</tr>
<tr>
<td>VI. Omeprazole 30mg/ kg.b.w.,</td>
<td>1.48±0.30</td>
<td>7.87±0.16***</td>
<td>00</td>
<td>0.16±0.25***,++++,###</td>
<td>7.58±1.15</td>
<td>29.9</td>
</tr>
</tbody>
</table>

n=6, Values are expressed as mean ± SEM, one way ANOVA, followed by Tuckey’s multiple comparison test, **P<0.01, ***P<0.001 compared to negative control, **P<0.01, ***P<0.001 compared to Aq 200 mg/kg *B. herbaceae*. *P<0.05, ###P<0.001 compared to Aq 400 mg/kg *B. herbaceae*, ***P<0.001 compared to all the groups. Aq = Aqueous extract, Et = Ethanolic extracts.

As shown in table 2, all the groups treated with extracts and standard showed significant and dose dependent decrease in ulcer scores compared to negative control. Ethanolic extracts showed significantly (P<0.01, P<0.05) greater reduction in ulcer scores compared to aqueous extract. Ethanolic extract showed higher percentage inhibition of ulcer compared to aqueous extract. There was decrease in hemorrhagic streak, spot ulcers and red coloration in treatment and standard group compared to negative control as shown in figure 1.

Figure 1: Effect of crude extracts of *Bytteneria herbaceae* on pylorus ligation induced ulcer model in rats. Symbol indicates hemorrhagic streaks and deep ulcer, indicates spot ulcers and indicated red coloration. As the dose increased from lower to higher there was significant decrease in ulcer formation. B: *Bytteneria*, Aq: Aqueous extract and Et: Ethanolic extract.
Table 3: Effect of aqueous and ethanolic extracts of whole plant of Bytteneria herbaceae on ethanol induced mucosal damage in rats.

<table>
<thead>
<tr>
<th>Group number and Treatment</th>
<th>N=6</th>
<th>Ulcer score</th>
<th>Ulcer index</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Negative control</td>
<td>19 ± 1.12</td>
<td>15.25 ± 0.27</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>II. Aq B. herbaceae 200 mg/kg.b.w.p.o.,</td>
<td>6.83 ± 0.74***</td>
<td>11.31 ± 0.16***</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>III. Aq B. herbaceae 400 mg/kg.b.w.p.o.,</td>
<td>2.83 ± 0.47***,+++</td>
<td>10.66 ± 0.11***</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>IV. Et B. herbaceae 200 mg/kg.b.w.p.o.</td>
<td>5.66 ± 0.61***</td>
<td>11.05 ± 0.14***,+++</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td>V. Et B. herbaceae 400 mg/kg.b.w.p.o.</td>
<td>2.5 ± 0.42***,##,++</td>
<td>10.41 ± 0.05***</td>
<td>31.7</td>
<td></td>
</tr>
<tr>
<td>VI. Sucralfate 100 mg/kg</td>
<td>0.83 ± 0.27***,###,+++</td>
<td>10.15 ± 0.04***,###,+++</td>
<td>33.4</td>
<td></td>
</tr>
</tbody>
</table>

n=6, Values are expressed as mean ± SEM, one way ANOVA, followed by Tuckey’s multiple comparison test. ***P<0.001 compared to control, **P<0.01, +++P<0.001 compared to Aq 200 mg/kg B. herbaceae and ##P<0.01, ###P<0.001 compared to Et 200 mg/kg B. herbaceae. Aq = Aqueous extract, Et = Ethanolic extracts, B = Bytneria

As shown in figure 2, the absolute ethanol at doses of 4 ml/kg showed superficial ulcer and hemorrhagic streak formation in the negative control compared to treatment and standard groups. Significant (P<0.001) decrease in the ulcer scores and ulcer index was observed in rats pretreated with extracts and standard compared to negative control. Both extracts exhibited dose dependent activity. Percentage protection of extracts were comparable to standard as shown in the table 3.

![Control](image1)
![Negative control](image2)
![Aq 200 mg/kg B. herbaceae](image3)
![Aq 400 mg/kg B. herbaceae](image4)
![Et 200 mg/kg B. herbaceae](image5)
![Et 400 mg/kg B. herbaceae](image6)
Figure 2: Effect of crude extracts of *Bytteneria herbaceae* on ethanol induced mucosal damage in rats. Symbol indicates hemorrhagic streak. Symbol indicates spot ulcers and indicated red coloration. As the dose increases from lower to higher there is significant decrease in ulcer formation. B : *Bytteneria*, Aq : Aqueous extract and Et : Ethanolic extract.

Sucralfate 100 mg/kg

Control

Negative control

Aq 200 mg/kg *B. herbaceae*

Aq 400 mg/kg *B. herbaceae*

Et 200 mg/kg *B. herbaceae*

Et 400 mg/kg *B. herbaceae*
Discussion

In the present study aqueous and ethanolic extracts of *Bytteneria herbaceae* was evaluated for anti-ulcer activity. Single dose of extracts 200 and 400 mg/kg, b.w.p.o., were selected as lower and higher doses based on the earlier studies (Sarkar, L et al, 2012). Both aqueous and ethanolic extracts of *Bytteneria herbaceae* showed the presence of phytoconstituents such as flavonoid, saponins, terpenoids, tannins, glycosides, steroids and phenolic compounds. Peptic ulcer occurs when there is imbalance between the aggressive factors (HCl, pepsin, and gastrin) and defensive factors (mucus, bicarbonate) with *Helicobacter pylori* infection. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucosal production, stabilizing the surface epithelial cells blocking apoptosis or interfering with the prostaglandin synthesis (Sahoo, S.K et al, 2016). Ulcer can be induced by different mechanisms in rats. The most commonly used method for induction of ulcer was pylorus ligation (SHAY) rat model for screening anti-secretory and anti-ulcer activity of drugs. Pyloric ligation will result in accumulation of gastric acid and activation of pepsin causing mucosal damage in the wall of stomach and duodenum leading to formation of ulcers. Mucosal digestion also decreases the synthesis of prostaglandin E2 and I2 which are important factors for the inhibition of gastric acid secretion (Ali, E., Arshad, N et al, 2020). There is also the involvement of histamine in the formation of pyloric ligated ulcers (Mekonnen, A.N, 2020). In the pyloric ligation model *Bytteneria herbaceae* extracts were evaluated for their effect on volume of gastric juice, pH, total acidity, ulcer scores and ulcer index. Though treatment of extracts showed altered gastric volume, increase in pH, reduction in total acidity and ulcer index compared to negative control but were not significant. Whereas all the treatment groups and standard showed significant (P<0.001) and dose dependent decrease in ulcer scores compared to negative control [Table 2]. Therefore antiulcer activity of extracts was may be due to cytoprotective effect. Ethanolic extract showed significantly (P<0.01 and P<0.05) greater cytoprotective effect compared to aqueous extract, may be due to the higher concentration of phytochemical constituents. Cytoprotective property of extracts was further proved in ethanol induced ulcer model. Administration of ethanol reduces gastric blood flow and decreases secretion of bicarbonate and gastric mucus (Sistani Karampour, N et al, 2014). As a result causes gastric necrotic damage with subsequent inflammatory cell infiltration. Rats were pretreated with the same doses of *Bytteneria herbaceae* extracts as in pylorus ligation model. Both aqueous and ethanolic extracts significantly (P<0.001) decreased the ulcer scores and ulcer index compared to negative control. Both extracts showed dose dependent activity and was comparable with standard sucralfate (100 mg/kg). Table [Table 3]. Histopathological evidence also supported cytoprotective effect of extracts as there was reduction in inflammatory cell infiltration and damage of gastric cells in in treated groups compared to negative control [Figure 3].

Plants such as *Tribulus terrestris* L (Ansari, J.A, 2013), *Glycyrrhiza glabra* L (Jalilzadeh-Amin, G, 2015), *Croton macrostachyus* *Hocsht* (Mekonnen, A.N, 2020) and *Osyris quadripartite* *Decne* (Abeaw, M, 2017) etc. were reported to have anti-ulcer activity due to the presence of phytoconstituents such as tannins, flavonoid, phenolic compounds, terpenoids and saponins. Same constituents present in *Bytteneria herbaceae* has contributed for anti-ulcer activity. Flavonoids will increases the mucus secretion by increasing mucosal prostaglandin content. It also inhibits H+/K+ATPase, decreases histamine secretion from mast cells by inhibition of histidine decarboxylase (Abeaw, M, 2015). Tannins will protect the outermost layer of mucosa by precipitating the microproteins at the site of ulcers thus forming protective layer. This makes the mucosal layer more resistant to damage by acid, pepsin and other aggressive factors. Phenolic compounds will also promote prostaglandin synthesis, antioxidant enzyme synthesis and stress defense. Saponins and terpenoids will increase mucus production (Mekonnen, A.N, 2020) and Steroids will also reduce the development of gastric ulcer (Jalilzadeh-Amin, G et al, 2015).
In previous studies it was proved that *Bytteneria herbaceae* had several properties such as anti-inflammatory (Sarkar, L. 2012), anti-oxidant and wound healing (Bharathi K N, Vidyashree N, 2015) and also exhibit anti-histaminergic action in anti-asthmatic activity (Bharathi, K.N., *et all.*, 2016) etc. These properties supports the use of *Bytteneria herbaceae* in treatment of ulcer as there is inflammation, wound, necrosis and infection (*H pylori*) in gastric ulcers. Further *Bytteneria herbaceae* was also found to have anti-microbial activity against gram negative bacteria such as Escherichia-coli and staphylococcus aureus (Bharathi K N, Vidyashree N, 2015). Since *Helicobacter pylori* is involved in the pathogenesis of peptic ulcer which is a gram negative bacteria, use of *Bytteneria herbaceae* would be justified in the treatment of peptic ulcer. In the present study it was proved that *Bytteneria herbaceae* possesses anti-ulcer activity by exhibiting cytoprotective effect against mucosal damage, attributed to the presence of flavonoids, terpenoids, saponins and tannins.

**Conclusion**

Results of the present study proved that both extracts of *Bytteneria herbaceae* at doses used exhibited significant and dose dependent anti-ulcer activity. Ethanolic extract was more potent than aqueous extract due to higher concentration of phytoconstituents. Phytoconstituents such as flavonoids, saponins, tannins, and triterpenoids present in *Bytteneria herbaceae* have attributed for anti-ulcer activity due to their antisecretory, cytoprotective, anti-inflammation and antimicrobial effects. Results of the present study can be extrapolated to humans and validates the use of *Bytteneria herbaceae* for gastric ulcer. Further studies shall focus on isolation of specific phytoconstituents and elucidating their mechanism of action.

**Reference**