Evaluation of Anti-Ulcer Activity of Methanolic Leaf Extract of Nyctanthes Arbortristis.

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Antiulcer activity of *N. arbortristis* methanolic leaf extract (250 and 500 mg/kg body weight) was studied on Ethanol induced gastric ulcer and Water immersion stress induced gastric ulcer animal models. Ranitidine was used as standard drug. The antiulcer activity of *N.arbortristis* was evaluated with the help of gastric parameters such as Ulcer Index, Acid Volume, pH, Total Acidity and Free Acidity. Preliminary phyto-chemical screening was also carried out. Results showed that the methanolic leaf extract treatment of *N.arbortristis* prevented ulcer area and gastric secretion in a dose-dependent manner. Preliminary phyto-chemical analysis identified the presence of flavonoids in the methanolic leaf extract of *N.arbortristis*. The extract is non-toxic even at relatively high concentrations. The anti-ulcer activity is probably due to presence of flavonoids.

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INTRODUCTION:
Peptic ulcer or peptic ulcer disease (PUD) is defined as a break in the mucosal lining of the gastrointestinal tract\(^1\). It occurs in that part of the gastrointestinal tract, which is exposed to gastric acid and pepsin, i.e. the stomach and duodenum. The normal gastric mucosa maintains a balance between defensive and aggressive factors\(^2\). Some of the main aggressive factors are gastric acid, abnormal motility, pepsin, bile salts, free radicals, use of alcohol and non-steroidal anti-inflammatory drugs (NSAID), as well as infection with microorganisms (Helicobacter pylori and others). On the other hand, defensive factors, such as mucus secretion, bicarbonate production, gastroprotective prostaglandin synthesis, endogenous nitric oxide and normal tissue microcirculation protect against ulcer formation. Etiology of Peptic ulcer is unknown yet, it is generally accepted that peptic ulcers develop when aggressive factors (endogenous, exogenous and/or infectious agents) over-come mucosal defense mechanisms\(^3\). The incidence of PUD varies with age, gender, geographical location and is associated with severe complications, including haemorrhages, perforations, gastrointestinal obstruction, and malignancy. Thus, this clinical condition represents a worldwide health problem because of its high morbidity, mortality and economic loss\(^4\).

Treatment approaches to control peptic ulceration include potentiation of the mucosal defense along with reduction of acid secretion and its neutralization, enhancement of antioxidant levels in the stomach, stimulation of gastric mucin synthesis and inhibition of H. pylori growth\(^5,6\). The currently used drugs include antibiotics to kill H. pylori, acid blockers, which reduce acid secretion for a prolonged duration (ranitidine, famotidine), proton pump inhibitors (omeprazole), and tissue lining protecting agents (sucralfate, bismuth)\(^7\). These drugs have decreased the morbidity rates, but produce many adverse effects, including relapse of the disease, and are often expensive for the poor population. Use of natural drugs (Ginger root, Onion, Garlic, Licorice, Cabbage juice, a diet rich in fiber) in gastric ulcers is well documented. Most of these drugs augment the mucosal defensive factors, which are thought to be important for protection of gastric mucosa\(^8\).

Nyctanthes arboritris (Oleaceae) is one of the most useful traditional medicinal plant widely distributed in sub-Himalayan regions and southwards to Godavari in India. It is commonly known as Harsingar, Parijat and Night Jasmine\(^9,10,11\). Almost each part of the plant has some medicinal value thus commercially exploitable. It has also been used in Ayurveda, Siddha and Unani system of medicines. The bioactive constituents such as beta-sitosterol, astragaline, nicotiflorin, arbortristoside-A&B, 7-O-trans-cinnamoyl-6β-hydroxyloganin, Nyctanthic acid have been reported from various parts of plant. N.arboritris to possess leishmanicidal, antiplasmodial, antispermatogenic, antiallergic, anti-inflammatory, analgesic activity\(^12,13,14\).

It is evident from the literature and previous investigations that N.arboritris also possesses significant anti-ulcer activity. Present study is done to establish the anti-ulcerogenic and ulcer-healing properties of methanolic leaf extract of N.arboritris in experimentally induced gastric ulcer models including ethanol induced and water immersion stress induced gastric ulcer model.

MATERIALS AND METHODS:

Plant material
Fresh leaves were collected from a N.arboritris plant growing at botanical garden of R.K. Pharmacy College, Azamgarh. The plant was identified taxonomically and authenticated by Prof. N. K. Dubey Assistant Professor “(Dept. of Botany Banaras Hindu University).” A voucher specimen no. Olea. 2021/4 has been reserved in herbarium laboratory. Leaves were washed with water and dried in shade and make coarsely powdered and stored in an airtight container. For extraction 15 gm. of dried leaf powder was loosely packed in thimble of soxhlet apparatus and extracted with methanol for 6 hrs. at 50\(^\circ\)C. The extract was dried using rotary evaporator and weighed and preserive in a petridish covered with aluminium foil and stored at 4\(^\circ\)C for further experiment. For oral administration, the extract was dissolved in distill water at different concentrations\(^14\).
Phytochemical screening
The methanolic leaf extract of *N. arbortristis* was evaluated for the presence of flavonoids, tannins, alkaloids, saponins, glycosides and sterols using methods of Brain *et al*[^14].

Drugs and Chemicals
Ranitidine (Standard drug) was a gift sample from Vellinton Healthcare, Baddi. Ethanol and Methanol purchased from CDH, Delhi. All other chemicals were obtained from local sources and were of analytical grade.

Experimental animals
Wistar albino rats weighed upto 150-200 gm. of either sex were used. They were housed in clean polypropylene cages under standard conditions of humidity (50±5%), temperature (25±2°C), and 12 hour light/12 hour dark cycle and fed with a standard diet (Shree Ganpati Feeds and Foods, Varanasi, India) and water *ad libitum*[^15]. All animals are handled with humane care. Experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee and followed the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India (IAEC Reference No.: 1384/PO/Re/S/10/CPCSEA).

Acute toxicity study
Acute oral toxicity study of methanolic leaf extract of *Nyctanthes arbortristis* was evaluated for acute toxicity at doses of 5, 50, 300, 2000 mg/kg, as per OECD423 guidelines and dose of 2000 mg/kg showed the toxic symptoms. So, it is considered as LD$_{50}$ cut off value. Doses selected for pharmacological studies by fixed dose methods were 125mg/kg, 250mg/kg and 500mg/kg, body weight[^16].

Preparation of test and reference drug solutions
The methanolic leaf extract of *N. arbortristis* was dissolved in distilled water and the aqueous solution was used.

Reference drug Ranitidine was prepared and used as an aqueous solution by dissolved in distill water.

Antiulcer activity
The effects of methanolic leaf extracts of *N. arbortristis* were evaluated in ethanol-, and water immersion stress-, induced gastric ulcer model in wistar albino rats. Ranitidine was used as a standard drug for ethanol and water immersion stress-induced gastric ulcer model for comparing the antiulcer potential of *N. arbortristis*.

Ethanol induced gastric ulceration[^17,18]
Albino wistar rats weighing 150-200gm after acclimatization (6-7 day’s) in the animal quarter’s were randomly divided into five groups of 6 animal (Table 1) each and treated in following way:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment/Dose</th>
<th>Animals Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I : Normal</td>
<td>Saline, Water</td>
<td>6</td>
</tr>
<tr>
<td>Group-II: Toxic Control</td>
<td>Absolute ethanol (1ml/200gm, p.o.) for 7 days</td>
<td>6</td>
</tr>
<tr>
<td>Group-III: Treated with Standard drug</td>
<td>Ranitidine 50 mg /kg +1ml absolute ethanol for 7 days, orally</td>
<td>6</td>
</tr>
<tr>
<td>Group-IV: Treated with Test drug NAMLE.</td>
<td>NAMLE 250 mg /kg + 1 ml absolute ethanol for 7 days, orally</td>
<td>6</td>
</tr>
<tr>
<td>Group-V: Treated with Test drug NAMLE</td>
<td>NAMLE 500 mg /kg + 1 ml. absolute ethanol for 7 days orally</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 1.
All rats were fasted for 24 hrs. but allowed free access to water. The standard drug and test drug were administered orally to the respective groups. After their pretreatment, 30 minutes later all animals gavaged with absolute ethanol and sacrificed humanely 1 hour later with high dose of diethyl ether (anaesthetic), the stomach were excised and opened along the greater curvature. Ulcer formed in the glandular portion of the stomach were measured for the ulcer index.

**Water immersion stress induced gastric ulcer model**

Water immersion stress induced gastric ulcer by forcing the wistar albino rats of either sex to swim in the water to the height of xiphoid level maintained at 22°C for 1 hr. Animals were fasted for 24 hr. prior to the experiment and devided into 5 group, each group have 6 animals (Table 2).

### Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment/Dose</th>
<th>Animal Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I: Normal</td>
<td>Saline, Water</td>
<td>6</td>
</tr>
<tr>
<td>Group-II: Toxic Control</td>
<td>Immersed in water at 22°C for 1 hour</td>
<td>6</td>
</tr>
<tr>
<td>Group-III: Treated with Standard drug</td>
<td>Ranitidine 50mg/kg + immersed in water at 22°C for 1 hour</td>
<td>6</td>
</tr>
<tr>
<td>Group-IV: Treated with Test drug NAMLE.</td>
<td>NAMLE 250mg/kg + immersed in water at 22°C for 1 hour</td>
<td>6</td>
</tr>
<tr>
<td>Group-V: Treated with Test drug NAMLE</td>
<td>NAMLE 500mg/kg + immersed in water at 22°C for 1 hour</td>
<td>6</td>
</tr>
</tbody>
</table>

After this the animals were sacrificed with high dose of anaesthetic diethyl ether, each stomach was opened along the greater curvature and examined for gastric erosions under the microscope (10x) and measure the ulcer index.

**Ulcer Index**

\[
\text{Ulcer Index} = \frac{10}{z}, \quad \text{where } z = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}
\]

**Statistical analysis**

The result of antiulcer activity are expressed as mean±SEM. Results were statistically analysed using one way ANOVA followed by Dunnett’s test. P<0.05 were considered to be significant.

**RESULTS**

### Ethanol induced gastric ulcer in rats

Administration of absolute ethanol in rats damages the gastric mucosa in disease control group of rats. However there was marked reduction in damage in the Ranitidine and *N. arbortristis* leaf extract treatment groups. When compared with disease control group as seen in the photographs (Figure.1a-e). The photograph of stomach of disease control rat showed prominent ulcers while the treatment groups like Ranitidine and methanolic leaf extract of *N. arbortristis* (500 mg/kg, p.o.) showed only redness and no ulcer. The methanolic leaf extract of *N. arbortristis* (250 mg/kg, p.o.) showed a small amount of ulceration. The effects of methanolic leaf extract of *N. arbortristis* 250 and 500 mg/kg treatments on ulcer index, acid volume, pH, total acidity, free acidity is summarized in Table 3.
**Figure 1.** Observations of ulcers in stomach of ethanol and drug treatments in rats: a) Normal control (untreated) group, b) absolute ethanol (1ml/200gm) treatment group, c) Ranitidine (50mg/kg) treatment group, d) NAMLE (250mg/kg) treatment group, e) NAMLE (500mg/kg) treatment group.

**Table 3. Effect of NAMLE on stomach ulcer index, acid volume, pH, total acidity, free acidity in absolute ethanol ulceration in rats.**

<table>
<thead>
<tr>
<th>Gastric Parameters</th>
<th>Normal Control</th>
<th>Disease Control (Ranitidine 50 mg/kg, p.o.)</th>
<th>NAMLE (250mg/kg, p.o.)</th>
<th>NAMLE (500 mg/kg, p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer Index</td>
<td>-</td>
<td>1.84±0.08*</td>
<td>0.88±0.03**</td>
<td>1.11±0.003</td>
</tr>
<tr>
<td>Acid Volume(ml)</td>
<td>1.76±0.07</td>
<td>4.05±0.06*</td>
<td>2.05±0.04**</td>
<td>2.80±0.11</td>
</tr>
<tr>
<td>pH</td>
<td>3.72±0.09</td>
<td>2.97±0.07*</td>
<td>3.62±0.11**</td>
<td>3.24±0.03</td>
</tr>
<tr>
<td>Total acidity(mEq/L)</td>
<td>43.07±1.18</td>
<td>92.18±0.96*</td>
<td>38.77±0.85**</td>
<td>59.48±1.08</td>
</tr>
<tr>
<td>Free acidity(mEq/L)</td>
<td>36.63±1.1</td>
<td>85.44±0.95*</td>
<td>34.12±0.97**</td>
<td>50.73±0.57</td>
</tr>
</tbody>
</table>

Values are expressed in Mean±SEM, n=6 in each group; one-way ANOVA followed by Dunnett’s test for statistical analysis. (*p<0.05 and **p<0.05 when standard and test drug group were compared to disease control group)

**Water immersion stress induced gastric ulcer model**

Water immersion stress was one of the best model of stress in rats to induce ulcer. This model provided emotional as well as physiological stress to the rats. The results of the present study showed that the methanolic leaf extract of *N. arbortristis* possessed antiulcer activity as evidenced by its significant inhibition in the formation of ulcers induced by stress induced ulcer models (Table 4). Microscopical examination of the stomachs removed from the disease control group of animals (not treated with either ranitidine or NAMLE) showed complete ulceration (Figure 2). However a protective effect against ulceration was noticed in rats treated with ranitidine, 250mg/kg and 500mg/kg methanolic leaf extract of *N. arbortristis*.

**Figure 2.** Observations of ulcers in stomach of water immersion stress and drug treatments in rats: a) Normal control (untreated) group, b) disease control group, c) Ranitidine (50mg/kg) treatment group, d) NAMLE (250mg/kg) treatment group, e) NAMLE (500mg/kg) treatment group.
Table 4. Effect of NAMLE on stomach ulcer index, acid volume, pH, total acidity, free acidity in water immersion stress induced ulceration in rats.

<table>
<thead>
<tr>
<th>Gastric Parameters</th>
<th>Normal Control</th>
<th>Disease Control</th>
<th>Standard (Ranitidine 50 mg/kg, p.o.)</th>
<th>NAMLE (250mg/kg, p.o.)</th>
<th>NAMLE (500 mg/kg, p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer Index</td>
<td>-</td>
<td>1.56±0.06*</td>
<td>0.78±0.03**</td>
<td>1.03±0.05</td>
<td>0.86±0.02**</td>
</tr>
<tr>
<td>Acid Volume(ml)</td>
<td>1.70±0.03</td>
<td>3.81±0.10*</td>
<td>1.98±0.05**</td>
<td>2.93±0.11</td>
<td>2.55±0.10**</td>
</tr>
<tr>
<td>pH</td>
<td>3.55±0.08</td>
<td>2.58±0.05*</td>
<td>3.73±0.06**</td>
<td>2.97±0.05</td>
<td>3.46±0.10**</td>
</tr>
<tr>
<td>Total acidity(mEq/L)</td>
<td>34.75±1.44</td>
<td>70.90±1.04*</td>
<td>36.75±0.90**</td>
<td>54.22±1.16</td>
<td>40.76±1.15*</td>
</tr>
<tr>
<td>Free acidity(mEq/L)</td>
<td>28.36±0.97</td>
<td>65.12±0.75*</td>
<td>30.38±1.10**</td>
<td>49.51±1.22</td>
<td>35.41±1.18*</td>
</tr>
</tbody>
</table>

Values are expressed in Mean±SEM, n=6 in each group; one-way ANOVA followed by Dunnett’s test for statistical analysis. (*p<0.05 and **p<0.05 when standard and test drug group were compared to disease control group)

DISCUSSION
Ethanol is responsible for disturbances in gastric secretion, damage to mucosa, alterations in permeability and free radical production. The generation of free radical was produced by continuous release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol. Ethanol induced gastric ulceration may be occurred due to stasis in gastric blood flow which contributes to the development of haemorrhage and necrotic tissue injuries. Alcohol has ability to penetrate the gastric mucosa and causing the cellular damage that leads to cell death and exfoliation of surface epithelium. The results of the study found that NAMLE established a cytoprotective action via antioxidant effect against ethanol induced cellular damage in the gastric mucosa of rats.

In Water immersion model the cause of gastric ulcer is due to stress induced increase in gastric acid secretion and these acid secretion promote ulceration of gastric mucosa.

CONCLUSION
From this study it is clear that N. arbortristis leaf extract have significant anti-ulcer activity in both animal models. The anti-ulcer activity probably due to the presence of flavonoids. Phytochemical screening confirms the presence of proteins, tannins, terpenoids and flavonoids in N. arbortristis leaf extract. Flavonoid exhibit antioxidant property which may contributes as anti-ulcer. Therefore concluded that N. arbortristis possess anti-ulcer activity may be due to flavonoids, tannins and terpenoids.

ACKNOWLEDGEMENT
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