Evaluation of Antiulcer Activity of Different Extracts of *Clitoria ternatea* Leaves on Experimental Animals

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**Abstract**

We planned to evaluate the antiulcer effects of four different extract of *Clitoria ternatea* leaves. Gastric lesions were induced in rats by oral administration of Indomethacin (10 ml/kg). The antiulcer activity of Petroleum ether, Chloroform, Methanol and aqueous extract of *Clitoria ternatea* leaves (200 mg/kg and 400 mg/kg body wt) was compared with standard drugs. The parameters studied were ulcer index, gastric juice volume and pH of gastric juice. *Clitoria ternatea* leaves showed a dose dependent curative ratio compared to ulcer control groups. Pretreatment with extract displayed significant antiulcer activity which is almost equal to that of the standard drug. The volume of acid secretion, and ulcer index were decreased and pH of the gastric juice was increased compared to ulcer control group. The petroleum extract showed maximum antiulcer activity compared to other extract.

**Keywords:** Ulcer induction, *Clitoria ternatea*, Indomethacin, Antiulcer activity

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1 Introduction

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility.

Many experimental investigations have been undertaken to elucidate the etiology of development of ulcers induced by various means, however ulcer healing consists of reconstruction of mucosal architecture and is a dynamic, active process of filling the mucosal defects with epithelial and connective tissue cells. It encompasses cell proliferation, division and migration. Prostaglandins (PGs) and growth factors play an important role in healing of ulcers. Synthesis of PGs is governed by the expression of inducible cyclooxygenase-2 (COX-2) isoenzyme in gastric mucosa during healing process. Further, COX-2 expression is enhanced in gastric epithelial cells after treatment with growth factors *in vitro* and *in vivo* during acetic acid induced gastric damage. Among clinically established drugs, H₂ receptor blockers (ranitidine etc) and proton pump inhibitors (omeprazole etc) are most widely used as anti-ulcer drugs in addition to the cytoprotective agents like sucralfate and misoprostol. Efficacy of sucralfate is due to its protective effect by forming a coat over ulcer base, which prevents the direct effect of acid on the ulcer. It neither affects acid secretion and ulcer index were decreased and pH of the gastric juice was increased compared to ulcer control group. The petroleum extract showed maximum antiulcer activity compared to other extract.

*Clitoria ternatea* commonly known as Butterfly pea belonging to the family Fabaceae, is a perennial leguminous twiner. It comprises 60 species distributed mostly within the tropical belt with a few species found in temperate areas. The mostly frequently reported species is *Clitoria ternatea*. The plant is mainly used as a forage as it is highly palatable for live-stock and it is well adapted to various climates. The juice of flowers is reported to be used in insect bites and skin diseases. The roots and leaves are useful in asthma, burning sensation, ascites, inflammation, leucoderma, leprosy, hemicrania, amenia, pulmonary tuberculosis, ophthalmology and reported as bitter,
refrigerant, ophthalmic, laxative, diuretic, cathartic, aphrodisiac, tonic. Consequently they are used in the treatment of a number of ailments including body-aches, infections, urinogenital disorders and as antihelmintic and antidote to animal stings. Seeds are cathartic and useful in visceralgia. They are considered safe for colic, dropsy and enlargement of abdominal viscera. The root, stem and flower are recommended for the treatment of snakebite and scorpion sting in India.

However there are no reports on the antiulcer activity of the plant hence the present study was designed to verify the claims of the native practitioners. Hence we planned to assessed the antiulcer activity of Clitoria ternatea leaves on rats.

2 Materials and Method

2.1 Plant material

The fresh leaves of Clitoria ternatea was collected during the month of September 2011, from the Pratap Nursury, Karamchari Nagar, Bareilly, UP, India. The leaves of the plant was taxonomically recognized and legitimate by Dr. Umesh Chand Pandey, HOD and in charge Botany Department, Bareilly college, Bareilly (BCB/BOT /376/24-01-2012).

2.2 Extract preparation

The leaves of Clitoria ternatea were shad dried until cracking sound was observed during breakage, and then leaves were grinded to make into roughly powdered. The powder form of the leaves (500 gram) was macerated with each different solvents petroleum ether, chloroform, methanol and distilled water (1500 ml) at room temperature for 72 hours with occasionally stirring.

The extracts were separated from the residues by filtering through several layers of muslin cloth for coarse filtration and then through what man No. 1 filter paper. The residue was further extracted using the same procedure. The filtrates obtained were combined and then evaporated to dryness at temperature not exceeding 40 °C on water bath. The extracts were kept at cool place till use. At the time of dose administration the crude extract mixed with distilled water during experiment.

2.3 Animals

Male albino rats weighed by using pan balance, 150-200 gm body weight rats used for experiment. All the rats were kept in fresh metabolic cages, and provided with free access to water and standard pellet diet (GMB, Lipton India Ltd.). The protocol of the study was permitted by Institutional animal ethics committee (1452/Po/a/11/CPCSEA).

2.4 Experimental Procedure

2.4.1 Indomethacin-induced ulcer

Male albino rats weighing 150-200 g were divided into ten groups, of six animals each, as given below. They were fasted for 24 hours prior to the experiment in perforated steel cages to avoid coprophagy.

The animals received the following drugs and control orally:

- Group A (control group) received 10 mg/kg Indomethacin
- Group B received 200 mg/kg methanol extract
- Group C received 400 mg/kg methanol extract
- Group D received 200 mg/kg aqueous extract
- Group E received 400 mg/kg aqueous extract
- Group F received 200 mg/kg chloroform extract
- Group G received 400 mg/kg chloroform extract
- Group H received 200 mg/kg petroleum ether extract
- Group I received 400 mg/kg petroleum ether extract
- Group J (standard group) received 20 mg/kg omeprazole

Thirty minutes following the respective treatment, ulcer was induced with indomethacin 10 mg/kg orally as determined in the preliminary test. Six hours after this, the animals were sacrificed and their stomachs were examined for ulcers. The gastric juice volume and pH of gastric juice were determined.

2.5 Statistical analysis

The values are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of One way ANOVA, followed by Dunnett’s test where P<0.05 was considered statistically significant.

3 Results

Effect of petroleum ether, chloroform, methanol and aqueous extract of Clitoria ternatea leaves (200 mg/kg and 400 mg/kg body wt) on Indomethacin induced ulceration is shown in table 1. The Indomethacin leads the accumulation of gastric secretions of 10.3±0.25 ml with pH 1.5±0.57 in a control group. The ulcer index of the control group was found to be 12.8±1.24. Pretreatment with the petroleum ether, chloroform, methanol and aqueous extract of Clitoria ternatea leaves, significantly (P<0.05) reduced the volume of gastric secretions compared to control group. pH of the gastric fluid was significantly (P<0.05) elevated only at higher dose of the extract compared to control group, but methanol and aqueous extract failed to significantly increase pH of gastric fluid. The lower dose (200 mg/kg body weight) of petroleum ether, chloroform, methanol and aqueous extract exhibited an inhibition percentage of 59.88, 38.18, 55.03 and 54.88, respectively. The higher dose (400 mg/kg body weight) of petroleum ether, chloroform, methanol and aqueous extract exhibited an inhibition percentage of 61.59, 49.21, 58.37 and 58.59, respectively. The standard drug, Omeprazole, exhibited an inhibition percentage of 68.75; significantly decreased the gastric juice and significantly increased the pH of gastric juice compared to control group. The gastroprotection...
offered by the test extract was comparable to that of the standard drug.

Table 1: Effect of Clitorea ternatea leaves extract on various parameters Indomethacin induced gastric

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses (mg/kg body weight)</th>
<th>Ulcer index</th>
<th>Protection (%)</th>
<th>Gastric juice vol. (ml)</th>
<th>pH of gastric juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>10</td>
<td>12.8±1.24</td>
<td>--</td>
<td>10.3±0.25</td>
<td>1.5±0.57</td>
</tr>
<tr>
<td>Group B</td>
<td>200</td>
<td>5.5±0.31*</td>
<td>55.03</td>
<td>6.6±0.30</td>
<td>3.0±0.38</td>
</tr>
<tr>
<td>Group C</td>
<td>400</td>
<td>5.2±0.33*</td>
<td>58.37</td>
<td>5.9±0.92</td>
<td>3.4±0.36</td>
</tr>
<tr>
<td>Group D</td>
<td>200</td>
<td>6.0±0.15*</td>
<td>54.88</td>
<td>5.0±0.48</td>
<td>2.8±0.47</td>
</tr>
<tr>
<td>Group E</td>
<td>400</td>
<td>5.3±2.43*</td>
<td>58.59</td>
<td>4.8±0.08*</td>
<td>3.6±0.68</td>
</tr>
<tr>
<td>Group F</td>
<td>200</td>
<td>7.4±0.12*</td>
<td>38.18</td>
<td>7.3±0.65</td>
<td>2.9±0.37</td>
</tr>
<tr>
<td>Group G</td>
<td>400</td>
<td>6.7±0.78*</td>
<td>49.21</td>
<td>6.8±0.15*</td>
<td>3.2±0.89*</td>
</tr>
<tr>
<td>Group H</td>
<td>200</td>
<td>5.2±0.15*</td>
<td>59.88</td>
<td>4.9±0.32*</td>
<td>3.0±0.47</td>
</tr>
<tr>
<td>Group I</td>
<td>400</td>
<td>4.9±0.24*</td>
<td>61.59</td>
<td>3.2±0.57*</td>
<td>4.1±0.68*</td>
</tr>
<tr>
<td>Group J</td>
<td>20</td>
<td>4±1.45*</td>
<td>68.75</td>
<td>3±0.34*</td>
<td>4.8±0.74*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with control group

Group A: Control group received 10 mg/kg Indomethacin; Group B received 200 mg/kg methanol extract, Group C received 400 mg/kg methanol extract, Group D received 200 mg/kg aqueous extract, Group E received 400 mg/kg aqueous extract, Group F received 200 mg/kg chloroform extract, Group G received 400 mg/kg chloroform extract, Group H received 200 mg/kg petroleum ether extract, Group I received 400 mg/kg petroleum ether extract, Group J (standard group) received 20 mg/kg omeprazole

4 Discussions

This study revealed antiulcer effect of extract of Clitoria ternatea leaves in experimental models of gastric lesion induced by a non-steroidal, antiinflammatory drug, Indomethacin.

Anti-inflammatory drugs like Indomethacin administered in toxic doses (10 mg/kg), produce visible gastric ulcers in animals. Indomethacin is a potent inhibitor of prostaglandin biosynthesis. Prostaglandins are known to play an important role in maintaining mucosal integrity\textsuperscript{12,13}. An Increase in certain endogenous prostaglandins can enhance gastric mucosal resistance to ulcerogenic agents. The mechanisms involved in prostaglandin action are multiple, including stimulation of mucus and bicarbonate output, gastric mucosal blood flow, decreasing gastric motility, increasing the release of endogenous mediators of gastric in jury vasoactive amines and leucotrienes and stimulation of cellular growth and repair\textsuperscript{14-18}. In the present study, the effect of the extract on prostaglandin biosynthesis was not evaluated, but an increase in resistance to the necrotizing effect of Indomethacin was noted.

The extract did show a significant, cytoprotective effect against the gastric lesions induced by necrotizing agents, which suggests a direct, protective effect on the gastric mucosa. The mechanisms underlying the protective action of the extract against ethanol and indomethacin induced gastric lesions are unclear. Further studies using more specific methods are required to explore the compounds responsible for the protective effect, and the mechanism of this activity.

5 Conclusions

The present study showed that pretreatment with petroleum ether, chloroform, methanol and aqueous extract of Clitoria ternatea leaves caused a beneficial effect on Indomethacin induced induced gastric lesion in rats, as evidenced by the reduction in the ulcer scores. The petroleum ether extract exhibited relatively better antulcer activity compared to other extracts. The difference in the antulcer activity could be due to the number or quantity of phytoconstituents present in these extracts.

6 Conflicts of Interests

We have not declared any conflict of interest.

7 Author’s contributions

AS, AM and VS designed the experimental work and performed; PM carried out literature review of this study. Authors read and approved the final manuscript.

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