EVALUATION OF ANTI ULCER ACTIVITY OF *PITHECELLOBIUM DULCE* (SEEDS) IN RATS USING PYLORUS LIGATION

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ABSTRACT

The present investigation was designed to evaluate a novel gastroprotective drug from indigenous plant *Pithecellobium dulce* which may be potent and nontoxic or minimal toxic. The anti ulcer effect was investigated in the seeds of *Pithecellobium dulce*. The powder of *Pithecellobium dulce* seeds was successively extracted with Petroleum ether, Ethyl alcohol and water. The preliminary phytochemical tests were done and the LD₅₀ value for both alcohol and aqueous extract was determined. The anti ulcer activity of the alcoholic (100 mg / kg. p.o.) and aqueous extract (100 mg / kg. p.o.) were assessed in pyloric ligation induced gastric ulcer. The results indicate that alcoholic and aqueous extract of *Pithecellobium dulce* (100mg/kg) seeds was effective in protecting ulcers in pyloric ligated rats and significantly decreased the gastric volume, total acidity, free acidity and ulcer index when compared with the standard group. The experimental investigation on *Pithecellobium dulce* seeds extract revealed that it possesses remarkable anti ulcer effect and it may be used for the effective treatment of peptic ulcer disease.

Key Words: *Pithecellobium dulce*, Pyloric ligation, Gastric Volume, Peptic ulcer, Ranitidine.

INTRODUCTION

Medicinal plants are of great value in the field of treatment and cure of diseases. Natural product is a source for bioactive compounds and has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control¹. Peptic ulcer disease refers to pathological lesions and ulcers of any portion of gastrointestinal tract exposed to
acid activated pepsin. There are three common forms of peptic ulcers: *Helicobacter pylori* (H. pylori) associated Nonsteroidal anti-inflammatory drug (NSADI) Induced, and stress ulcers. The *Pithecellobium dulce* a small to medium size, semi evergreen shrub or tree, 5-20 m in height, with a short trunk. Pods are 10-15 x 1.5 Cm. The color becoming reddish brown as they ripen. Each pod contains 5-10 shiny black seeds up to 2 cm long distribution of plant in Argentina, Bolivia, Brazil, United States of America, Mexico, India, Indonesia, Jamaica and Philippines.

The plant has been used traditionally. The seed and pulp are made into a sweet drink similar to lemonade and also in preparation of curries in India. Seeds contain greenish oil, which after refining and bleaching can be used for food or in the making of soap can substitute ground nut seed oil. Root and bark decoctions are taken orally against diarrhea, fruit pulp taken orally to stop blood flow in case of haemoptysis, the seed juice is inhaled into the nostrils against chest congestion and pulverized seeds are ingested for internal ulcers. Leaves, when applied as a plaster, can allay pain of venereal sores and taken with salt to cure indigestion, neuro disorders but can also produce abortion. The root bark may be used to cure dysentery. The bark is used medicinally as a febrifuge.

Study of the hydro alcoholic seeds extract of *Pithecellobium dulce* was found to good antioxidant activity. Hence present study was aimed to investigate the anti ulcer activity of plant extract of *Pithecellobium dulce* on pylorus ligation induced gastric ulcer in wistar rat.

**MATERIALS**

**Drugs and chemical**

Ranitidine was obtained from Glaxo-Smithkline laboratories Limited, the kits for all biochemical estimation were purchased from VMCP, Salem, Tamilnadu, India. The solvent and chemicals were used of analytical grade.

**METHODS**

The seeds of *Pithecellobium dulce* were collected from the Hosur, Tamilnadu. The plant was identified & authenticated by Professor V.RAVI, Head, Post graduate and research department of Botany, Govt. Arts College (Men) Krishnagiri, Tamilnadu. The seeds of *Pithecellobium dulce* were shaded dried at room temperature and then powdered with a mechanical grinder. The powder was passed through sieve no.40 and stored in an air tight container for further use. The solvent used Petroleum ether (60-80°C), Alcohol 90% w/v and
distilled water for decoction. The Petroleum ether extract was obtained using soxhlet apparatus. Alcoholic extract was obtained with ethyl alcohol 90% w/v for 18 hrs using soxhlet apparatus. The aqueous extract was prepared with the remaining mass by maceration process for 7 days. The extract was dried at 55 ºC in a water bath. The Percentage yield of alcoholic and aqueous extract was 9.2% and 9.1% respectively (Table 1).

Table 1: Extractive Values of *Pithecellobium dulce*

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Part used</th>
<th>Method of extraction</th>
<th>Yield in percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pithecellobium dulce</em></td>
<td>Seeds</td>
<td>Continuous hot percolation</td>
<td>Petroleum Ether extract 7 9.2 9.1</td>
</tr>
</tbody>
</table>

Phytochemical Screening
The preliminary phytochemical screening of alcoholic and aqueous extract was carried out as per standard procedure (Table 2).

Table 2 : Preliminary phytochemical screening for the extracts of *Pithecellobium dulce*

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Petroleum ether Extract</th>
<th>Alcohol (95%) extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALKALOIDS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FLAVANOIDS</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CARBOHYDRATE</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>SAPONINS</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>TRITERPENS</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>STEROLS</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>TANNINS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GLYCOSIDES</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ VE = Present     - VE = Absent

Animals
Male & Female Swiss albino mice weighing between 20-25 gm and male wistar rat weighing between 150-220 gm were used for this study. The animal were placed randomly and allocated to treatment groups in propylene cages with paddy husk as bedding. Animals were housed at a temperature of 24± 2°C and relative humidity 45-55% with 12:12 hour’s
light/dark cycle. The animals had free assist to food and water. The animals were habituated to laboratory conditions for 48 hours prior to the experimental protocol to minimize any non-specific stress. All the experimental process and protocols used in this study were reviewed by the institutional animal ethical committee (Pharmacology project no. 1143/ac/07/cpcesa /pcp /iaec /m.pharm/120/13) and were in according with the guideline of the CPCSEA.

**Acute Toxicity Study**

Acute oral toxicity of alcoholic and aqueous extract was determined using nulliparous, non-pregnant female mice. The animals were fasted for 3 hours before experiment and were administered a single dose of extract dissolved in 2% w/v tween 80 and observed for mortality up to 48 hours. Based on short term toxicity the dose of next animal was determined as per OECD guideline by 423 procedures (OECD 2000). All the animals are also observed for long tern toxicity.

**Experimental Protocol**

Test compound the alcoholic & aqueous extract of *Pithecellobium dulce* seeds (100mg/kg body weight) and standard drug Ranitidine (50 mg/kg P.O) were used. The following chemicals were obtained from the indicated commercial.

**Experimental Setup**

Wistar rats (150-200 gm) used in the present studies divided into four groups of 6 animals in each. All the animals were acclimatized for a week before use. The alcoholic & aqueous extract of *Pithecellobium dulce* was dissolved in 2% w/v tween 80.

- **Group I:** Normal Control
  - Control animals were received normal saline 2ml/kg

- **Group II:** Standard Group
  - Ranitidine (50 mg/ kg p.o) for 5days + pyloric ligation

- **Group III:** Test Group- I
  - Alcoholic extract of *Pithecellobium dulce* (100mg/kg/p.o) + Pyloric ligation

- **Group IV:** Test Group- II
  - Aqueous extract of *Pithecellobium dulce* (100mg/kg p.o) + Pyloric ligation
Statistical Analysis
Results are expressed as mean ± S.E.M Statistical analysis was done by one-way analysis of variance (ANOVA), followed by tukey’s multiple comparison test. The $p$-value < 0.05 was considered as statistically significant.

EVALUATION OF ANTI ULCER ACTIVITY
Estimation of Ulcer index and Percentage Protection
Ulcer index will be then calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach $^{11}$.

Scoring of ulcer will be made as follows

<table>
<thead>
<tr>
<th>Score</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal colored stomach</td>
</tr>
<tr>
<td>0.5</td>
<td>Red coloration</td>
</tr>
<tr>
<td>1</td>
<td>Spot ulcers</td>
</tr>
<tr>
<td>1.5</td>
<td>Hemorrhagic streaks</td>
</tr>
<tr>
<td>2</td>
<td>Ulcers &gt; 3 mm but &lt; 5 mm</td>
</tr>
<tr>
<td>3</td>
<td>Ulcers &gt; 5 mm</td>
</tr>
</tbody>
</table>

Mean ulcer score for each animal is expressed as ulcer index. The percentage protection calculated by the using the formula

\[
\text{Percent protection} = 100 - \frac{Ut}{Uc} \times 100
\]

Determination for free acidity and total acidity
The gastric contents centrifuged and take out 1ml into a 100 ml of conical flask added 2-3 drops of Topfer’s reagent and titrated with 0.01 NaOH until red colour turn to yellowish to orange colour. Then few drops of phenolphthalein and till definite red ring appears. Again the total volume of alkali added was noted. The volume corresponds to total acidity.

Determination of $\text{P}^H$
An liquor of 1ml of gastric juice was diluted with 1ml of distilled water and $\text{P}^H$ of the solution was measured using $\text{P}^H$ meter.

Histopathological analysis of Stomach
Histopathology data of scarified animal taken under the supervision of histo pathologist.
RESULTS
Preliminary phytochemical studies revealed the presence of flavonoids, glycosides, saponins, fixed oils and fats according (Table 2). Treatments of rats with pylorus ligation produce an ulcer (Table 3 & 4).

Table 3: Effect of different extract of *Pithecellobium dulce* on Pylorus Ligation induced gastric ulceration

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>-</td>
<td>13.5 ± 0.156</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Standard Group</td>
<td>50</td>
<td>4.5 ± 1.11**</td>
<td>66.6</td>
</tr>
<tr>
<td>III</td>
<td>Test Group - I</td>
<td>100</td>
<td>6.0 ± 0.41***</td>
<td>55.56</td>
</tr>
<tr>
<td>IV</td>
<td>Test Group - II</td>
<td>100</td>
<td>5.25 ± 0.85**</td>
<td>61.11</td>
</tr>
</tbody>
</table>

**P<0.01 ***P<0.001 Vs Control respectively, Values are represented as mean ± S.E.M (n=6). One-way ANOVA followed by Student-Newman-Keuls post test (P< 0.001) is used.

Table 4: Effect of different extract of *Pithecellobium dulce* on Pylorus Ligation induced gastric ulceration (Gastric volume & Acidity)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Gastric Volume (ml)</th>
<th>Free acidity Meq/l/100gm</th>
<th>Total acidity Meq/l/100gm</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>4.2 ± 0.18</td>
<td>4.6 ± 0.05</td>
<td>22.3 ± 0.2</td>
<td>2.8 ± 0.07</td>
</tr>
<tr>
<td>II</td>
<td>Standard Group</td>
<td>2.95 ± 0.07**</td>
<td>3.1 ± 0.02**</td>
<td>6.8 ± 0.16</td>
<td>7.5 ± 0.19</td>
</tr>
<tr>
<td>III</td>
<td>Test Group – I</td>
<td>2.7 ± 0.11**</td>
<td>3.4 ± 0.03*</td>
<td>5.9 ± 0.12</td>
<td>6.9 ± 0.18</td>
</tr>
<tr>
<td>IV</td>
<td>Test Group - II</td>
<td>2.5 ± 0.28**</td>
<td>2.1 ± 0.18*</td>
<td>6.3 ± 0.27*</td>
<td>6.4 ± 0.12</td>
</tr>
</tbody>
</table>

**P<0.01 ***P<0.001 Vs Control respectively, Values are represented as mean ± S.E.M (n=6). One-way ANOVA followed by Student-Newman-Keuls post test (P< 0.001) is used.

Alcoholic and aqueous extract shows significant decrease in ulcer compare to control group. Pretreatment with Ranitidine alcoholic and aqueous extract significantly prevent the no. of ulcer, induced by pylorus ligation. Results indicated 66.6% gastro protection with Ranitidine, 55.56% gastro protection with alcoholic extract of *Pithecellobium dulce* 100mg/kg and 61.11% with aqueous extract of *Pithecellobium dulce* 100mg/kg as compared with ulcerated control. In the histopathological examination, stomach of control rat shows erosion in the upper part of epithelium and RBCs are seen in the eroded portion (Fig 1).

Stomach of rats treated with standard drug (Ranitidine) showed small erosions with a minimal deviation from normal morphology (Fig 2). Stomach of rats treated with alcoholic
extract showed small superficial erosion with minimal deviation from normal morphology (Fig 3) and stomach of rats treated with aqueous extract showed superficial erosions with minimum deviations from normal morphology (Fig 4).

**Histopathological analysis of Stomach**

![Fig: 1 Stomach of Normal Control rat](image1)

![Fig: 2 Stomach of Standard Group rat](image2)
DISCUSSION

The present study provided preliminary data for the seeds of *Pithecellobium dulce* possess significant anti ulcer activity in animal models. It has a gastric anti secretory effect that is comparable to reference drug Ranitidine. The anti ulcer activity is probably due to presence of bioactive compounds like Flavonoids - Quercetin, Rutin, Kaempferol, Naringin, Daidzein\(^1\). Antiulcer activity of seeds of *Pithecellobium dulce* due inhibition of H, K-ATPase activities comparable to Pylorus ligation. Phytochemical screening yielded flavonoids - observation justifies the ethanomdeical uses of the plant as anti ulcer agent and as antacid in addition to its nutritional values. Among the extracts aqueous extract is more effective compared to alcoholic extract\(^1\).
CONCLUSION
This present study concluded that *Pithecellobium dulce* possessing Anti ulcer activity according to the data and further research work can be carried out to different formulations. However there is a shortage of clinical trial regarding its potency and efficacy.

AKNOWLEDGEMENT
Authors are thankful to all management and staff for providing the necessary facilities to conduct this study and are thankful to Ms. Divya for his help pertaining to biochemical study. Authors are also thankful to all persons whose help in this work.

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