EVALUATION OF ANTIULCEROGENIC ACTIVITY OF VARIOUS EXTRACTS OF SARACA INDICA BARK ON ASPIRIN INDUCED GASTRIC ULCERS IN ALBINO RATS

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ABSTRACT
Objective: The effect of various extracts of Saraca indica bark was investigated by carrying out acute oral toxic study in albino rats to evaluate the antiulcerogenic activity by using aspirin induced gastric ulcer model. Materials and methods: The extracts of Saraca indica bark were prepared by soxhlet extraction and maceration methods. Four extracts that is ethyl acetate, Hydroalcoholic (50:50), methanolic and aqueous extract were evaluated for their anti-ulcerogenic activity. The animals were divided into ten groups each containing six animals. The control group received normal saline and one group received the standard drug omeprazole(8mg/kg) the other eight groups received the four extracts of each extract in two doses that is 250mg and 500mg. The parameters like macroscopic ulcer index and microscopic ulcer index were considered to assess the antiulcerogenic activity. Results and discussion: The results indicate that the aqueous bark extract of Saraca indica showed significant decrease of ulcers when compared to the other extracts of the bark with respect to control in a dose dependent manner thus scientifically validating the use of Saraca indica bark aqueous extract as an antiulcer agent in folklore medicine.

Keywords: Saraca indica, Omeprazole, Aspirin, anti ulcer, albino rats, gastric ulcer.

INTRODUCTION
Ulcers are one of the commonly prevailing disorders in the present times. Various medications are available for the treatment of ulcers. Many natural products were used in the treatment of ulcers. Of the many plants Saraca indica (family Caesalpinaceae) is the most ancient medicinal tree of India. Almost every part (bark, flower, seeds, fruits, shoot, and leaves) of Saraca indica has various pharmacological activities. Extensive literature survey showed that several pharmacological properties were already reported like: anti-depressant activity (M.Krishnamoorthy et.al)7, anti cancer (Varghese, C.D. et al)7 anti-oxidant and cytotoxic effect (Amar.D.Shinde et.al, Rao BS et.al)8,9 and anti-microbial activity (Sainath et.al)5, (Pal SC et.al)6. It was shown that no previous work has been reported about the anti ulcer activity of Saraca indica. The present research project is an acute study of the anti-ulcerogenic effect of various extracts of Saraca indica.

MATERIALS AND METHODS
Plant material
The bark of Saraca indica was collected from a local ayurvedic vendor (voucher number432) and authenticated by a local Ayurvedic doctor Dr.S.Srinivasa rao.the voucher specimen was preserved for future identification.
Extraction procedure
The collected bark was dried in shade for about 48 hours and was finely powdered. The powdered drug was divided in two parts i.e. one fourth of the drug powder was subjected to maceration with water for seven days. After seven days it was filtered and the collected macerate was distilled off to remove water. The aqueous extract was collected and air dried. The other three fourth part of the powdered drug was subjected to hot percolation process by soxhale apparatus. The solvents used for percolation were ethyl acetate, hydro-alcohol (50:50), methanol. The percolates were collected and were distilled to remove excess solvent. The extracts were collected and dried in a dessicator.

Preliminary phytochemical screening
The extracts of Saraca indica were subjected to preliminary phytochemical screening for the detection of various plant constituents as per the standards. 17,18,21

Animals
Male albino Wister strain rats weighing about 180-220gms were used. The animals were maintained under standard laboratory conditions of 25° ± 1°C temperatures with relative humidity of 45%±10% and 12 hours light cycle and 12 hours dark cycle. All the animals were acclimatized to laboratory conditions for a week before use. They were fed with standard animal feed and water ad libitum. The experimental protocol was approved by institutional animal ethics committee and the animal regulatory body of the government. Case number is 1354/a/10/CPCSEA.

Drugs and chemicals
Omeprazole, Aspirin were procured from lee Parma, Hyderabad. The solvents methanol, ethyl acetate, ethanol, carboxy methyl cellulose were of analytical grade, Merck, procured from National chemicals at Vijayawada, India (voucher number 50). Doubled distilled water was used.

Test compound and standard drug formulation
The extracts of bark of Saraca indica and the standard drug Omeprazole were prepared in 1% sodium CMC solution in distilled water prior to oral administration to animals. The vehicle alone is served as control.

Induction of ulcers
Ulcers were induced by aspirin given orally at a dose of 200mg/kg body weight (George D et al) 2

Surgical procedure
Rats were anesthetized by using inhalational anaesthetic i.e. Chloroform and decapitated. The stomach was cut opened along the greater curvature and the residual matter cleaned off with saline and the inner surface was examined for ulceration by counting the ulcer spots.

Quantification of ulcers
Long lesions were counted and measured along their greater length. Petechial lesions were counted. Each five petechial lesions were taken as 1mm of ulcer (Alkofahi A) 6 The sum of the total length of long ulcers and petechial lesions in each group of rats was divided by its number to calculate the ulcer index (mm).

The macroscopic curative ratio was determined by the formula. Curative ratio is {((Control ulcer index) – (Test ulcer index))/(Control ulcer index)) x 100.Microscopic ulcer index was obtained using published methods (Pandit S) 5 by two pathologists i.e Microscopic ulcer index = (number of lesion 1) + (number of lesion 2) x2 + (number of lesion3) x3 separately and a mean index was calculated. Normal tissue = 0; Local damage to gastric pits cells = 1; Local damage to gastric glands = 2; Deep damage to gastric glands = 3.

Experimental design protocol
The normal rats were divided in to 10 groups of 6 animals each. Ulcers were induced in all the rats by following the above mentioned method. Group1: control; group 2: treated with standard Omeprazole given at a dose of 8mg/kg body weight; group 3: treated with Saraca indica ethyl acetate extract at a dose of 250mg/kg body weight; group 4: treated with Saraca indica ethyl acetate extract at a dose of 500mg/kg body weight; group 5: treated with Saraca indica hydro alcohol extract at a dose of 250mg/kg body weight; group 6: treated with Saraca indica hydro alcohol extract at a dose of 500mg/kg body weight; group 7: ulcer induced rats treated with Saraca indica methanol extract at a dose of 250mg; group 8: treated with Saraca indica methanol extract at a dose of 500mg/kg body weight; group 9: treated with Saraca indica aqueous extract at a dose of 250mg/kg body weight; group 10: treated with Saraca indica...
aqueous extract at a dose of 500mg/kg body weight. All these are administered orally using plastic syringe fitted with a metallic feeding gastric tube one hour before the induction of ulcers.

RESULTS AND DISCUSSION
Acute oral toxicity study
The acute oral toxicity study was carried out by up and down regulation method. The safe limit dose was 2500mg/kg and 5000mg/kg with no mortality observed. One tenth of these doses i.e. 250mg/kg and 500mg/kg were used in subsequent study.

Preliminary phytochemical screening
The phytoconstituents present in the various extracts of Saraca indica include contain carbohydrates, proteins, aminoacids, saponin glycosides, tannins, steroids.

Aspirin induced gastric ulceration
Aspirin at a dose of 200mg/kg body weight showed Petechial lesions in control animals. However, animals treated with hydroalcoholic and aqueous extract of Saraca indica at doses of 250mg/kg and 500mg/kg doses (p<0.001) showed significant decreases in the macroscopic and microscopic ulcer index.

Statistical analysis
The results were expressed as mean ± SD. Differences in ulcer index determined by factorial one way analysis of variance. Individual groups were compared by using Dunnet’s test (multiple comparison tests). Differences with p<0.001 were considered statistically significant.

DISCUSSION
The etiology of peptic ulcer is unknown yet, it is generally accepted that the ulcers arise due to an imbalance between aggressive factors and mucosal integrity by certain endogenous factors. To regain the balance various therapeutic agents such as plant extracts were used. Among those Saraca indica is one such plant that is used in the present study to primarily evaluate the antiulcerogenic activity in aspirin induced ulcers in rats. Ulcers are caused due to increase in gastric acid secretion. The volume of gastric acid is also an important factor in the formation of ulcers due to the exposure of unprotected lumen of the stomach to accumulated acid. Peptic ulcers are induced due to autodigestion of mucosa and mucosal barrier. These ulcers are caused by NSAIDs such as aspirin, phenyl butazone and some anti-inflammatory agents. These drugs induce ulcers by inhibiting the secretion of prostaglandins E2 and I2 that are predominantly released by the gastric mucosa which are responsible for inhibition of acid secretion and release of bicarbonate and mucus.

In the present study of all the extracts aqueous extract showed a significant action in gastric ulcers induced by NSAIDs (11) which are potent inhibitors of mucus secretion in prophylactic studies. The hydroalcoholic and aqueous extracts of bark of Saraca indica showed a significant decrease in the macroscopic and microscopic ulcer index. The ethyl acetate and methanol extracts were not significant. Of the four extracts used the aqueous extract comparatively showed a significant decrease in the ulcer index. Phytochemical investigation revealed the presence of saponin glycosides, tannins, steroids and flavanoids in the extracts. The significant increase in the antiulcer activity may be attributed to saponin and phenolic glycosides, flavanoids and alkaloids present. The cytoprotective effect may be due to the presence of flavanoids and glycosides that enhance mucus, bicarbonate and prostaglandin secretion and counteract the deteriorating effects of reactive oxidants present in the gastrointestinal lumen. So the antiulcer activity of the Saraca indica may be primarily due to the flavanoids and glycosides content. The results of the present study suggest that aqueous extract may be beneficial in the treatment of peptic ulcers showing that the anti-secretory mechanism and cytoprotective effects of the extract must be responsible for its antiulcerogenic activity. Further studies to elucidate the mechanism of action and identify the mechanism of action are recommended.

CONCLUSION
Earlier studies conducted on antiulcer activity of various plant extracts have shown that the pharmacological activity of the extracts was primarily due to the presence of water soluble phenolic glycosides. Phytochemical analysis of extracts showed the presence of water soluble phenolic glycosides in aqueous and methanolic extracts. This is the key aspect contributing to the antiulcerogenic and cytoprotective activity. The present investigation is an acute oral toxic study of antiulcerogenic activity of the aqueous extract of Saraca indica the investigation may be continued by taking up a chronic study to investigate the antiulcer activity for the extracts of Saraca indica.
Table 1:

<table>
<thead>
<tr>
<th>Group no</th>
<th>Drug used</th>
<th>Macroscopic Mean ulcer index (Mean ± SEM)</th>
<th>% ulcer inhibition.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>control</td>
<td>1.300±0.029</td>
<td>-</td>
</tr>
<tr>
<td>Group 2</td>
<td>Omeprazole 8mg/kg body weight</td>
<td>0.4900±0.0140***</td>
<td>62.31%</td>
</tr>
<tr>
<td>Group 3</td>
<td>Saraca indica Ethyl extract-250mg</td>
<td>0.45±0.045***</td>
<td>65.38%</td>
</tr>
<tr>
<td>Group 4</td>
<td>Saraca indica Ethyl extract-500mg</td>
<td>0.3600±0.012***</td>
<td>72.30%</td>
</tr>
<tr>
<td>Group 5</td>
<td>Saraca indica Methanol extract -250mg</td>
<td>0.2800±0.021***</td>
<td>78.46%</td>
</tr>
<tr>
<td>Group 6</td>
<td>Saraca indica Methanol extract -500mg</td>
<td>0.29±0.016***</td>
<td>77.69%</td>
</tr>
<tr>
<td>Group 7</td>
<td>Saraca indica Hydro alcoholic -250mg</td>
<td>0.2600±0.041***</td>
<td>80%</td>
</tr>
<tr>
<td>Group 8</td>
<td>Saraca indica Hydro alcoholic -500mg</td>
<td>0.24±0.031***</td>
<td>81.53%</td>
</tr>
<tr>
<td>Group 9</td>
<td>Saraca indica aqueous extract-250mg</td>
<td>0.2300±0.025***</td>
<td>82.30%</td>
</tr>
<tr>
<td>Group 10</td>
<td>Saraca indica aqueous extract-500mg</td>
<td>0.16±0.033***</td>
<td>87.20%</td>
</tr>
</tbody>
</table>

Table 2:

<table>
<thead>
<tr>
<th>Group no</th>
<th>Drug used</th>
<th>Microscopic Mean ulcer index (Mean ± SEM)</th>
<th>% ulcer inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>control</td>
<td>31.5000±0.7640</td>
<td>-</td>
</tr>
<tr>
<td>Group 2</td>
<td>Omeprazole 8mg/kg body weight</td>
<td>18.67±0.0882***</td>
<td>40.73%</td>
</tr>
<tr>
<td>Group 3</td>
<td>Saraca indica Ethyl extract-250mg</td>
<td>16.830±0.477***</td>
<td>46.57%</td>
</tr>
<tr>
<td>Group 4</td>
<td>Saraca indica Ethyl extract-500mg</td>
<td>15.670±0.615***</td>
<td>50.25%</td>
</tr>
<tr>
<td>Group 5</td>
<td>Saraca indica Methanol extract-250mg</td>
<td>14.170±0.792***</td>
<td>55.01%</td>
</tr>
<tr>
<td>Group 6</td>
<td>Saraca indica Methanol extract-500mg</td>
<td>12.980±0.669***</td>
<td>58.79%</td>
</tr>
<tr>
<td>Group 7</td>
<td>Saraca indica Hydro alcoholic extract-250mg</td>
<td>10.670±0.494***</td>
<td>66.12%</td>
</tr>
<tr>
<td>Group 8</td>
<td>Saraca indica Hydro alcoholic extract-500mg</td>
<td>9.980±0.557***</td>
<td>68.31%</td>
</tr>
<tr>
<td>Group 9</td>
<td>Saraca indica aqueous extract-250mg</td>
<td>8.870±0.630***</td>
<td>71.84%</td>
</tr>
<tr>
<td>Group 10</td>
<td>Saraca indica aqueous extract-500mg</td>
<td>6.180±0.490***</td>
<td>80.38%</td>
</tr>
</tbody>
</table>
Group-1: control, group-2: omeprazole 8mg/kg, group-3: Saraca indica ethyl acetate extract 250mg/kg body weight, group-4: Saraca indica ethyl acetate extract 500mg/kg body weight, group-5: Saraca indica methanolic extract 500mg/kg body weight, group-6: Saraca indica hydroalcohol (50:50) extract 250mg/kg body weight, group-8: Saraca indica hydroalcohol (50:50) extract 250mg/kg body weight, group-9: Saraca indica aqueous extract 250mg/kg body weight, group-10: Saraca indica aqueous extract 500mg/kg body weight,

**Fig 1 and 2:**

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