INTRODUCTION

In the present scenario, natural medicines are gaining prominence, because they are economical, easily available and relatively free from side effects. The use of plants extract and pure compounds isolated from natural sources provides foundation to modern pharmaceutical compounds. The well known Indian systems of medicine namely, Ayurveda, Siddha and Unani have used plant based raw material[1].

There are 50 species of genus Artocarpus (Moraceae) of evergreen and deciduous trees. The genus is important source of edible fruit and is widely used in traditional medicines. A. integrifolia (Moraceae) is mentioned in Ayurveda and is reported to possess antibacterial, anti-inflammatory, ant diabet, antioxidant and immunomodulatory properties.

A. integrifolia is reported to contain artoicosides, norartocarpines, betulic acid, cycloheteroxphyllin. triterpenic compounds like cycloartenyl acetate, cycloartenone, betuic acid, artocarpesin, norartocarpesterol. Flavonoids, triterpenes and phenolic compounds including flavanoids, jacline, apiasterin, artepillin, c, cinnamoyl acetate, cinnamoyl cinnamate, cinnamoyl flavone, cinnamoyl flavones A and B, β-sitosterol, phenolic compounds including flavonoids, jacline, (lactone) and arylbenzofuron.[2].

Since 1960, ulcer-related physician visits, hospitalizations, operations, and deaths have declined in India by more than 50%, primarily because of decreased rates of PUD (peptic ulcer disease) among men. Although the overall mortality from PUD has decreased, death rates have increased in patients older than 75 years of age, most likely as a result of increased consumption of NSAIDs and an ageing population.[3]. Ulcer is a conglomerate of heterogeneous disorders, which manifests itself as a break in the lining of the gastrointestinal mucosa bathed by acid and/or pepsin.[4]. WHO has encouraged studies for treatment and prevention of ulcer disease using traditional medical practices. The present study is aimed to evaluate the traditional claim of anti-peptic ulcer activity of plant A. integrifolia.

MATERIALS AND METHODS

This study was conducted in the pharmacology laboratory, department of pharmacology, Rajiv Academy for Pharmacy, Mathura, India.

Collection and extraction

The fresh leaves of A. integrifolia were collected from local area of Mathura, India in the month of August 2012. The material was taxonomically identified, confirmed and authenticated by Dr. Sunita Garg (Chief Scientist and Head of Raw Material Herbarium and Museum), NISCAIR, New Delhi. A Voucher specimen NISCAIR/RHMD/Consult/2013/2217/223 is deposited there. The collected leaves were washed 2-3 times with tap water to remove adhering dust and allowed to dry in shade. The dried material was crushed to coarse powder with mechanical grinder. It was then passed through the 40 No. mesh sieve. The powder was stored in air tight container.

A weighed quantity (150 g) of the powder was subjected to continuous hot extraction in soxhlet apparatus with methanol as a solvent and extracted till the solvent became colourless. Extract was evaporated under reduced pressure using rotary evaporator at a low temperature of 40–60°C until the extract turned syrupy and then this syrupy extract was transferred to an evaporating dish for drying on a water bath.

Animals

Healthy albino wistar rats weighing 200-250 g were used for the study. The animals were housed under standard environmental conditions (22±3°C and 35-60% relative humidity). Food was withdrawn 24 hours before the experiment, but animals were allowed free access of water.

The experimental protocol was approved by the Institutional animal Ethical committee for animal experimentation of Rajiv Academy for pharmacy, Mathura, India, with registration No.-IAEC/RAP/3962.

Preliminary phytochemical screening of extract

Qualitative chemical tests were conducted for methanolic extract of A. integrifolia to identify the various phytoconstituents employing standard screening test [5]. Methanolic extract of A. integrifolia gave positive test for flavonoids, tannins and phenols.

Chemicals and Reagents

All the drugs and chemicals were of analytical grade. Standard Omeprazole and Topfer’s reagent were procured as gift sample from Centron Research labs, New Delhi.

Experimental Design

Albino wistar rats were divided into 3 groups, each group containing 6 rats. Group-I received vehicle control (1% SCMC, sodium carboxy methyl cellulose 1 ml/kg, p.o.).Group-II received standard drug (Omeprazole - 20mg/kg, p.o.). Group-III received methanolic extract of A. integrifolia (MEAI) leaves (250mg/kg, p.o.).
Pylorus ligation model

Extract of *A. integrifolia* leaves was screened for antiulcer activity by pylorus ligation method [6]. In this method albino rats were fasted in individual cages for 24 hours prior to pyloric ligation. *A. integrifolia* methanolic extract of leaves, standard drug and control vehicle was administered 45 minutes prior to pylorus ligation under light ether anaesthesia. The abdomen was cut open by midline incision process, pyloric portion of the stomach was slightly lifted out and ligated avoiding damage to its blood supply[7,8].

The stomach was replaced carefully and the abdominal wall closed by sutures. After 3 hours of pylorus ligation, rats were sacrificed by an overdose of anaesthetic ether. The abdomen was cut open by midline incision process, pyloric portion of the stomach was slightly lifted out and ligated avoiding damage to its blood supply[7,8].

The stomach was then cut open along the greater curvature and washed slowly under running tap water and inner surface was examined for ulceration by giving score number. The severity of the ulcer was scored microscopically by 10X magnification lens. Mean ulcer score for each animal was expressed as ulcer index[9-11].

### Ulcer Index (U.I.): Score

<table>
<thead>
<tr>
<th></th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td>No coloured stomach</td>
<td>0</td>
</tr>
<tr>
<td>Red coloration-</td>
<td>0.5</td>
</tr>
<tr>
<td>Spot ulceration-</td>
<td>1</td>
</tr>
<tr>
<td>Hemorrhagic streak-</td>
<td>1.5</td>
</tr>
<tr>
<td>Ulcer-</td>
<td>2</td>
</tr>
<tr>
<td>Perforation-</td>
<td>3</td>
</tr>
</tbody>
</table>

### Percentage inhibition

Percentage inhibition was calculated using the following formula.

\[
\text{Percentage protection} = \frac{\text{Control U.I} - \text{Test U.I}}{\text{Control U.I}} \times 100
\]

### Statistical analysis

The results were expressed as Mean ± SEM, (n=6). Statistical analyses were performed by using one way ANOVA followed by Dunnett’s test multiple comparison, where P<0.001, P<0.01, P<0.05 was considered statistically significant. Data were analyzed through graph pad software.

### RESULTS

#### Phytochemical screening

The preliminary phytochemical screening of methanolic extract of *A. Integrifolia* (MEAI) leaves showed the presence of carbohydrates, flavonoids, tannins and phenols.

#### Antiulcer activity by pylorus ligation method

MEAI at 250 mg/kg body weight treated rats showed significant (P<0.01) reduction in ulcer index when compared with control. The percentage protection against ulcer by Omeprazole, MEAI were found to be 61% and 50.90% respectively. MEAI produced significant (P<0.01) reduction in the volume of gastric acid secretion and an increase in the pH of the gastric juice. In addition, total acidity and free acidity were also reduced significantly as (P<0.01, P<0.05) shown in Table 1 and 2.

#### Table 1: Effect of Omeprazole and methanolic extraction of *A. integrifolia* leaves on gastric volume, pH, free acidity and total acidity in pylorus ligated model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Gastric volume</th>
<th>Gastric pH</th>
<th>Total acidity</th>
<th>Free acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.3±0.32</td>
<td>2.4±0.33</td>
<td>10±1.8</td>
<td>79.5±1.04</td>
</tr>
<tr>
<td>Std. (Omeprazole 20 mg/kg)</td>
<td>2.1±0.28***</td>
<td>6.3±0.24***</td>
<td>29±0.91***</td>
<td>21.0±0.9 **</td>
</tr>
<tr>
<td>MEAI 250 mg/kg</td>
<td>4.3±0.24**</td>
<td>2.7±0.10**</td>
<td>39±0.85**</td>
<td>33.75±0.85*</td>
</tr>
</tbody>
</table>

Results were expressed as mean ± SEM for 6 rats, Statistical comparison was performed by Graph pad software using ANOVA with Dunnet's test. *** P<0.001, **P<0.01, *P<0.05 were considered statistically significant when compared with the control group.

#### Table 2: Effect of omeprazole and methanolic extraction of *A. integrifolia* leaves on ulcer index and percentage of ulcer protection in pylorus ligated model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer index</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11±0.24</td>
<td>----</td>
</tr>
<tr>
<td>Std. Omeprazole 20mg/kg</td>
<td>4.2±0.13**</td>
<td>61%</td>
</tr>
<tr>
<td>MEAI 250mg/kg</td>
<td>5.4±0.17**</td>
<td>50.90%</td>
</tr>
</tbody>
</table>

Results were expressed as mean ± SEM for 6 rats, Statistical comparison was performed by Graph pad software using ANOVA with Dunnet's test. *** P<0.001, **P<0.01, *P<0.05 were considered statistically significant when compared with the control group.

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Fig. 1: Images of pylorus ligation induced ulcer model (A) Control, (B) Standard (Omeprazole- 20 mg/kg), and (C) MEAI – 250mg/kg.
DISCUSSION

Plants constitute a major remedy in the traditional medical system. The practice continues today due to their biomedical benefits, non toxic nature, their easy access and a place in cultural benefits. In the present scenario, medicinal plants have been used as a source of drugs and as a potential source of remedy for diseases that are currently difficult to treat/cure. Due to some reported side effects of available antiulcer drugs, focus has shifted towards natural products as the new sources of antiulcer agents [12].

Review of the available literature revealed that majority of the antiulcer studies so far conducted have used pylorus ligation model [13]. The different constituents like flavonoids, tannins, phenols, steroids, saponins, alkaloids and glycosides have been reported to be responsible for anti ulcer activity [14].

Peptic ulcer describes a condition in which there is a discontinuity in the entire thickness of the gastric and duodenal mucosa that persists as a result of acid and pepsin in gastric juice. Peptic ulcer is caused by an imbalance between aggressive factors (e.g. acid, bacteria, NSAID’s, dietary factors) and defensive factors (e.g. mucus, bicarbonate, prostaglandins). [15,16].

*A. integrifolia* extract is one such herbal drug used in the present study primarily to evaluate the anti ulcer activity by using pylorus ligation model in rats [17].

The causes of gastric ulcer in pyloric ligation is believed to be due to stress induced increase in gastric hydrochloric acid secretion. The volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid [18]. Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucusal barrier. Pylorus ligation models are usually employed to observe the potential of anti ulcer drugs for their anti acid-secreting activity by checking the gastric volume and its effect on gastric pH, total acidity and free acidity.

The antiulcer property of *A. integrifolia* in pylorus ligation model is evident from its significant reduction in gastric volume, free acidity, total acidity and ulcer index and also, an increase in the pH, it is suggested that *A. integrifolia* has anti-secretory activity.

CONCLUSION

Anti ulcer activity of MEAI at dose 250 mg/kg decreased gastric acid volume and showed significant reduction in free acidity and total acidity in rats treated with it, and also indicate anti-secretory properties. From the results it can be concluded that the leaves of the plant of *A. integrifolia* possesses gastro protective potential which may be due to the presence of tannins, flavonoids, and sterols present in it. The present study indicates a significant decrease in ulcer index and increased percentage inhibition of ulceration.

ACKNOWLEDGEMENTS

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REFERENCES