ANTI ULCER ACTIVITY OF ETHANOLIC EXTRACT OF 
ACHYRANTHES ASPERA LEAVES IN ALBINO RATS

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ABSTRACT
Gastric Hyper activity and gastro duodenal ulcer is a common global problem. The gastric lesions develops when the delicate Balance between some aggressive and defence factors. The study was to investigate Anti ulcer effect of Achyranthes aspera extract on Pyloric ligation and Aspirin induced ulcer model. EEAA showed concomitant attenuation of gastric secretory volume, acidity, pepsin secretion in ulcerated rats, the EEAA(200mgkg) and reference drug omeperaole(20mgkg). The severity of the reaction of ulcerogenic and reduction of ulcer sie by EEAA.

Keywords: EEAA, gastro duodenal ulcer, Achyranthes aspera.

INTRODUCTION
Herbs are prime medicinal agents in traditional and holistic therapies. Particularly in India an extensive science has been developed. Peptic ulcer disease refers to pathological lesions and ulcers of any portion of gastrointestinal tract exposed to acid activated pepsin. Peptic ulcer disease (PUD) differs from gastritis and erosions in that ulcers typically extend deeper into the muscular is mucosa. There are three common forms of peptic ulcers: Helicobacter pylori(H. pylori)-associated, Non steroidal anti-inflammatory drug (NSAID)-induced, and stress ulcers. The cause of ulceration in patients is mainly due to hyper-secretion of gastric juice and pepsin. The other forms of peptic ulcers are Zollinger-Elliison syndrome, drug associated ulcers and stress ulcers. Treatment options available are use of mucoprotective
agents, antacids, alginates, motility stimulants and acid suppressants. Anti reflux surgery is done in severe cases.

**PLANT PROFILE**

**Synonyms:** Achyranthes aspera (L.)

**Family**: Amarantheaceae

**Leaves:**
Leaves are 3-5cm in size and trifoliate; Leaflets rhomboid- ovate 0.3.-0.7 cm long coriaceous, stellate- pubescent, base deltoid, margin entire, apex acuminate. Petiole 1-3 cm.

**MATERIALS & METHODS**

**Collection and Authentication of plant**
Leaves of Achyranthes asperawere collected from Dharmapuri, Tamilnadu and authenticated by professor, P.JAYARAMAN, Ph.D. Director, National institutes of herbal science, Chennai, Tamilnadu. The voucher specimen No PARC/ /2011/1010) was deposited in the herbarium of the National institute of herbal science.chennai (T.N.).

**Preparation of extract**
The shade dried coarse powder of the leaves (200gm) was packed well in soxhlet apparatus and was subjected for continuous hot extraction with 99.9% ethanol until the completion of the extraction. The extract was filtered while hot and the resultant extract was in vaccum under reduced pressure in order to remove the solvent completely dried and kept in desiccator till experimentation.

**PHARMACOLOGICAL EVALUATION**

**Animal care and handling as per CPCSEA guideline**
Male Wistar albino rats of weight 150-250 grams were selected, procured from. The animals were acclimatized to the standard laboratory conditions in well cross ventilated animal house at temperature 25±2°C relative humidity 44 –56% and light and dark cycles of 12 and 12 hours respectively for 1 week before and during the experiments. The animals were fed with standard diet and water ad libitum.

The experiments were approved by CPCSEA and the institutional ethics committee. Food was withdrawn 18hours before the start of the activity.
Animal detail

Male albino rats weighing between 150-200gms and swiss albino mice were procured from, Padmavaththi college of Pharmacy and Research Institute, Dharmapuri, Tamilnadu. They were maintained at standard housing conditions at a room temperature of 24±1°C, relative humidity of 45-55% with 12:12 hour light/dark cycle. The feeding was done with commercially available rat feed pellets and water was given *ad libitum* during the experiment. The was approved by Institutional ethical committee.

Acute toxicity study and dose selection

The extract was investigated for its acute toxicity studies according to the OECD guidelines (425). The extract was given at different doses to the group of six animals at 100 mg/kg, 200mg/kg, and 400mg/kg orally. The animals were observed for regular three hours after the dose administration and after 24 hours and 48 hours for the changes in behaviour and changes in body weight and mortality. It was found that the extract doesn’t produce any significant toxicity up to the dose of 200 mg/kg. Thus the extract was highly tolerable up to 200 mg/kg.

Experimental design

Ulcer induced models

**Pylorus ligation induced model**

Male albino rats weighing 150-200g were selected for pyloric ligation ulcer model. Rats were divided into four groups, each group consisting of six animals. Animals were fasted for 48 hours.

- Group-I: Normal
- Group-II: PL Control (saline 2 ml/kg)
- Group-III: Treated with Ranitidine (20 mg/kg)
- Group-IV: Treated with EEAA (200 mg/Kg)

The oral treatment was carried out 1 hour before pyloric ligature, respectively. After 48 hours of fasting, ulcer induction was undertaken according to Shay et al.

The rats were and mildly anaesthetized with chloroform and the abdomen was cut open through a midline incision. The pylorus was secured and ligated with silk sutures, after which the wound was closed and the animals were allowed to recover from anaesthesia. After ligation of the pylorus, drinking water was withheld and the gastric examinations were undertaken 18 hours after pylorus ligation. The animals were sacrificed with an overdose of...
chloroform and the stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a magnifier lens (10x) to assess the formation of ulcer as shown in figure I.

Using transpire surgical tapes assessed ulcer area, which was fixed on a light and transparent sheet and the ulcer area was measured for each stomach. The number of erosions formed on glandular portion of stomach was counted and each was given a severity rating on a 1-3 scale. The gastric contents were collected and centrifuged at 1000 rpm for 10 min. One ml of the supernatant liquid was pipette out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer’s reagent as indicator, to the endpoint when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity. Acidity was expressed as:

**Acidity = Volume of NaOH x Normality x 100 mEq/l.**

The mucous was then washed and extent of ulceration was scored as per the method. **Scoring of ulcer:**

- 0 = Normal coloured stomach
- 0.5 = Red colouration
- 1 = Spot ulcer
- 1.5 = Haemorrhagic streaks
- 2 = Deep Ulcers
- 3 = superficial ulcer

**Calculation of ulcer Index**

\[
U1 = UN + US + UP 
\times 10^{-1}
\]

U1 = Ulcer Index

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animal with ulcer

**Aspirin induced ulcer model**

The animals were put in three groups, each group containing 5 rats.

The groups were as follows:

Group-I: Control (Aspirin 200mg/Kg)

Group-II: Ranitidine (20 mg/kg)
Group-III: Ethanolic extract *Achyranthes aspera* (200 mg/kg)

Control received 200 mg/kg Aspirin orally. Standard drug selected was Ranitidine 20mg/kg, orally. The remaining group received calculated effective dose of *Achyranthes aspera* plant extracts orally according to body weight of animals. After 8 days of dosing, animals were fasted for 24 hours and later aqueous suspension of aspirin at a dose of 200mg/kg was given orally. The animals were then sacrificed by euthanasia four hours later of Aspirin administration. The stomachs were dissected and examined for ulcers. Gastric tissues were used for histopathological studies.

**Calculation of ulcer score – ulcer index**

The stomach was opened along the greater curvature and washed slowly under running tap water. It was put on a glass slide and observed under 10X magnification for ulcers. The ulcers were scored as shown in Table II. Mean ulcer score in each group was calculated and was designated as ulcer index and percentage was calculated.

\[
\% \text{ Protection} = \left( \frac{C - T}{C} \right) \times 100
\]

Where C= ulcer index in control group

T= ulcer index in treated group

**Estimation of Pepsin**

1. For estimation of pepsin ,placed 4 test tubes, test tube 1 and 2 containing 5ml of 1% bovine albumin in0.01 M Hcl, test tube 3 and 4 containing 10 ml of 0.35 M Trichloro acetic acid.
2. The gastric juice was mixed with an equal volume of 0.01 M Hcl,warmed to 370c. 1 ml of this mixture was added to each tubes of 1and 4.
3. Incubated for 15 min,at the mixed content of tube 1 with 3. Allow to stand for 4 min. 1+3 gives Test and 2+4 gives blank.
4. The mixture was filtered. To 2 ml of the filtrate, 10 ml of NAOH was added. Then 1 ml of phenol reagent was added and mixed by gentle rotation, after 30 min, the absorbance was measured at 680 nm,difference between test and blank gives the measures of peptic activity.
5. Asstandard, mixed 2 ml of freshly prepared phenol solution containing 50mcg/ml with 10 ml NAOH and 1 ml of phenol reagent was added and measured at 680 nm.
Statistical analysis
Ulcer indice were shown as the mean ± standard error of mean and ulcer protection presented as percentage inhibition. The significance of the differences in mean ulcer indices between Extract and Negative control was calculated by using one way-ANOVA followed by students’t test.

RESULTS AND DISCUSSION
Phytochemical screening tests and results

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Glycosides</td>
<td>+</td>
</tr>
</tbody>
</table>

Effect of Ethanolic Extract of *Achyranthes aspera*on Pylorus ligation induced Ulcer in Rats.

<table>
<thead>
<tr>
<th>Treatmet</th>
<th>Dose (mg/kg) p.o</th>
<th>Volume of gastric juice ml</th>
<th>PH</th>
<th>Free Acidity (mEq/L)</th>
<th>Total Acidity (mEq/L)</th>
<th>Ulcer Index</th>
<th>% ulcer protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (saline)</td>
<td>2ml/kg</td>
<td>3.1±0.23</td>
<td>4.1</td>
<td>5.4±0.08</td>
<td>15.6±0.2</td>
<td>1.6</td>
<td>--</td>
</tr>
<tr>
<td>PL Control (saline)</td>
<td>2ml/kg</td>
<td>4.2±0.18</td>
<td>2.8</td>
<td>4.6±0.05</td>
<td>22.3±0.3</td>
<td>9.03</td>
<td>--</td>
</tr>
<tr>
<td>Standard</td>
<td>20mg/kg</td>
<td>1.9±0.07</td>
<td>7.5</td>
<td>3.1±0.02</td>
<td>6.3±0.16</td>
<td>1.76*</td>
<td>80.5</td>
</tr>
<tr>
<td>EEAA</td>
<td>200mg/kg</td>
<td>2.4±0.11</td>
<td>6.9</td>
<td>3.4±0.03</td>
<td>7.2±0.12</td>
<td>3.63**</td>
<td>59.8</td>
</tr>
</tbody>
</table>

Results are mean ± S.E.M. (n = 6). Statistical comparison was performed by using ANOVA coupled with student’s t’ test., * p<0.05, ** P<0.01 were consider statistically significant when compared to control group.
Pylorus ligation induced ulcer stomach image

Fig.1: Normal Group  Fig.2: Control Group

Fig.1: The figure shows the normal group clear mucosal characterized

Fig.2: The figure shows the pyloric ligation induced control group produced mucosal Damage characterized by multiple haemorrhage red bands

Fig.3: Standard Group  Fig.4: EEAA Group

Fig.3 shows the Ranitidine treated group reduce mucosal damage and stomach Lesions.

Fig.4 The figure shows the EEAA treated group produce moderate mucosal damage with less number of red band.

Effect of EEAA on pepsin content in pylorus ligated rats

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Treatment(mg/kg)</th>
<th>Pepsin content(mcg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>0.89±0.01</td>
</tr>
<tr>
<td>2</td>
<td>Ulcerated control</td>
<td>5.56±0.69</td>
</tr>
<tr>
<td>3</td>
<td>Ranitidine(20mg/kg)</td>
<td>2.39±0.63</td>
</tr>
<tr>
<td>4</td>
<td>EEAA(200mg/kg)</td>
<td>3.33±0.24</td>
</tr>
</tbody>
</table>
Histopathology of pylorus ligation model

Fig. 5 Normal

Normal: Stomach revealing normal surface epithelium and glandular structures

Fig. 6 Control

Control: Necrosis and sloughing of surface epithelium and infiltration of inflammatory cells.

Fig. 7 Standard (Ranitidine)

Drug: Regeneration of surface epithelium and congested blood vessel
Fig.8(EEAA)

Test: Almost normal appearance of surface epithelium and congested blood

Aspirin induced ulcer model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg) p.o</th>
<th>Ulcer Index</th>
<th>% ulcer protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (saline)</td>
<td>2ml/kg</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Control (aspirin)</td>
<td>2ml/kg</td>
<td>8.83±1.1</td>
<td>--</td>
</tr>
<tr>
<td>Standard</td>
<td>20mg/kg</td>
<td>2.56±0.8</td>
<td>71.25</td>
</tr>
<tr>
<td>EEAA</td>
<td>200mg/kg</td>
<td>3.63±1.0</td>
<td>59.02</td>
</tr>
</tbody>
</table>

Effect of ethanolic extract of *Achyranthes apera* on Aspirin induced ulcer in *rats*

Values are mean + SEM of five animals in each group Pa<0.001 Vs Control, b<0.01Vs Control, c<0.05 Vs. Control, using students ‘t’ test.

Aspirin induced ulcer stomach image

Fig.9: Normal Group

Fig.10: Control Group

Fig.9 The figure shows the normal group clear mucosal characterized
**Fig.10:** The figure shows the Aspirin induced control group produced mucosal Damage characterized by multiple haemorrhage red bands

**Fig.11:** Standard Group

**Fig.12:** EEAA Group

**Fig.11** Shows the Ranitidine treated group reduce mucosal damage and stomach Lesions. **Fig.12** The figure shows the EEAA treated group produce moderate mucosal damage with less number of red band.

**HISTOPATHOLOGY OF ASPIRIN INDUCED ULCER MODEL**

**Fig.13** Normal

Normal: Stomach revealing normal surface epithelium and glandular structures
Fig.14 control group

Fig.14. Stomach of control rat showing erosion in the upper part of epithelium with RBCs in eroded portion

Fig.15 standard

Fig.15. Stomach of rat treated with ranitidine showing small erosions with minimum deviation from normal morphology

Fig.16 EEAA

Fig.16 Stomach of rat treated with EEAA showing superficial erosions with minimum deviations from normal morphology
DISCUSSION
The etiology of the peptic ulcer unknown in most of the cases, yet it is generally accepted that it results from imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanism. To rejoin the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis. Pylorus ligation induced ulcer was used to study the effect of extract on gastric acid secretion and mucus secretion the ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcer in the stomach. The original Shay rat model involves fasting of rat for overnight followed by ligation of pyloric end of the stomach. The ulcer index is determined 18 hrs after pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach. In this study ethanolic extracts of Achyranthes aspera showed a significant reduction in PH, Free acidity, Ulcer index, and percent protection when compared to control (p<0.01, p<0.05); this suggested that it is having an antisecretory effect. Pyloric liguation and Aspirin play a major role in the pathogenesis of gastric ulcers, due to increase gastric acid and acetylcholine. The plant extract of Achyranthes aspera produce a significant effect for ulcer protection. The plant (Achyranthes aspera) containing carbohydrates, amino acids, saponinA,B proteins, flavonoids and some organic acids which are responsible for the various types of pharmacological activity of the plants. Sterol, Quercetine, Oleanic acid may be partly responsible for the antiulcerogenic activity of plant. In the present study we used the Pylorus ligation and Aspirin(NSAID) induced ulcer to evaluate the antiulcer effect of the alcoholic (ethanolic) extract of Achyranthes aspera leaves. The model which uses to evaluate percent ulcer protection.

In the Pylorus ligation in rats Ranitidine and 200mg extract of Achyranthes aspera provided ulcer protective effect. Ulcer protection was observed through ulcer score. The ulcer through Aspirininduced wasAchyranthes aspera extract provided ulcer protective effect at 200mg/kg.

CONCLUSION
Here present study was carried out to investigate anti ulcer activity of ethanolic extract of Achyranthes aspera .L leaves in pylorus ligated and aspirin induced ulceration in the rats. pylorus ligation induced ulcer is one of the most widely used method for studying the effect of drug on gastric secretion. pylorus ligation induced ulcers are due to auto digestion of
gastric mucosa and breakdown of the gastric mucosal barrier. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers, and life-threatening perforation and haemorrhage. Prostaglandin E2 and I2 are predominantly synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate. Hydrophobic surfactant-like phospholipids secretion in the gastric epithelial cells is also stimulated by tje prostaglandin. Effect of pylorus ligation has caused the accumulation of gastric secretion. The total acidity, free acidity, ulcer index of gastric secretion were increases. Ranitidine and leaves extract of Achyranthes aspera significantly decreased total acidity, free acidity, ulcer index. In case of aspirin and pylorus ligation induced ulcer the ethanolic extract of Achyranthes aspera leaves possess significant anti-ulcer properties in a manner.

REFERENCE

