EVALUATION OF ANTI-ULCER ACTIVITY OF HYDROALCOHOLIC EXTRACT OF THE TERMINALIA ARJUNA BARK [ROXB]

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ABSTRACT
The anti-ulcer activity of hydro alcoholic extract of Terminalia arjuna bark Roxb. Family (Combretaceae), HAETA was investigated in Ethanol induced and Stress induced ulcer models in wistar albino rats. In both models the most common parameter determined was ulcer index. HAETA at doses 100, 400 mg/kg produced significant inhibition of gastric lesions induced by ethanol induced gastric ulcer & stress induced gastric ulcers. The extracts 100, 400 mg/kg showed significant P<0.01 reduction in ulcer index, pH, free acidity and total acidity as compared to control, omeprazole 20 mg/kg were employed as reference drug in anti-ulcer studies respectively. The present study suggests that HAETA was found to possess anti-ulcerogenic as well as ulcer healing properties, which might be due to anti-secretory activity.

Keywords: Terminalia arjuna Roxb bark, Ethanol & Stress induced ulcer, Ulcer index Omeprazole 20 mg/kg.

INTRODUCTION
Peptic ulcer is the erosion in lining of stomach or duodenum. The word ‘peptic’ refers to pepsin, a stomach enzyme that breaks down proteins. Peptic ulcer located in stomach is known as gastric ulcer, it is believed that the ulcers are mainly due to imbalance between protective factors (mucous and bicarbonates) and aggressive factors (acid and pepsin) in stomach. [1] Such factors could range from natural causes (gastric cancer) infections (H. pylori), life style (usage of drugs like non steroidal anti-inflammatory agents, alcohol, stress & cigarette smoking.) [2] These agents have been implicated in the process of 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. [3] Current treatment of ulcers in developing countries has been largely based on suppression of pain, with little (or) no strategy aimed to cure the disease. Ulcers are mainly treated with combinations of different kind of anti-biotics, acid reducers and H2-receptor blockers, proton pump inhibitors etc which are expensive to a common man and have prolong side effects also. Herbal medicine is used as fast emerging alternative treatment of ulcers, [4, 5] possible due to low cost, availability, fewer adverse effects and perceived effectiveness. Hence effort is to find a suitable treatment from natural product as a source i.e. hydro alcoholic extract of Terminalia arjuna bark.
(Indicator), Phosphate buffer (buffer), H₂O₂ solution (vehicle) were used, the dose of the hydroalcoholic extract of *Terminalia arjuna* Roob (HAETA) was selected randomly as 100, 400 mg/kg body wt, of the animal. A suspension of HAETA in 5% (w/v) Carboxy Methyl Cellulose was prepared for oral administration.

Animals
A healthy, adult wistar rat of either sex (180-220g) was obtained from the Central animal house facility from Vels University, Chennai. The animals were kept in a well ventilated room and the animals was exposed to 12 hrs day and night cycle with a temperature between 20±3°C and fed with standard animal pellet feed (Hindustan lever limited) and water ad-libitum. The protocol was approved by animal ethics committee constituted for purpose of animal experimentation as per CPCSEA guidelines. (XII/VELS/PCOL/47/2000/CPCSEA/IAEC/11.03.11)

Preparation of animals
The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Stress induced ulcer model
Allbino wistar rats of either sex are divided into four groups of each containing six animals in each group.First Group represented the control group, which received distilled water orally. Second &Third Groups received Hydro alcoholic extract of *Terminalia arjuna* bark at the dose of 100 and 400 mg/kg, Standard drug Omeprazal was administrated at the dose of 20 mg/kg orally for Fourth group. The gastric ulcers were induced in rats by, Cold resistance stress (CRS) induced ulcer to 18 hours fasted rats, Cold resistance stress is produced by strapping the rats on a wooden plank and keeping them for 2 hours at 4° - 6°C. Ulcer scoring will be done after sacrificing the animal by cervical dislocation.[10]

Ethanol induced ulcer model
The ulcer was induced by administering ethanol (1ml/200g). All the animals were fasted for 36 hours before administration of ethanol. The animals were divided into five groups, each consisting of six rats. One Group represented the control group, which received distilled water orally. Second group receive ethanol, Third & Fourth Groups received hydroalcoholic extract of *Terminalia arjuna* bark in the dose of 100 and 400 mg/kg and, Omeprazole, in the dose of 20 mg/kg were administered orally for Fifth group as reference standard drug. The gastric ulcers were induced in rats by administrating absolute ethanol (95%) (1ml/200g) Orally, after 45 min of HAETA and Omeprazole treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1h latter with anaesthetic ether and stomach was incised along the greater curvature and ulceration was scored, similar to pyloric ligation induced ulcer model.[11]

In vivo anti-oxidant estimation from tissue homogenate:
Super oxide dismutase (SOD) activity: Inhibition of reduction of nitro blue tetrazolium (NBT) to blue colored Formosan in presence of phenazine methasulphate (PMS) and NADH is measured at 560 nm using n-butanol as blank. One unit of enzyme activity is defined as the amount of enzyme that inhibits rate of reaction by 50% in one min under the define assay condition and the results have been expressed as unit (U) of SOD activity/mg protein.[13]

**Ulcer index**[13]
The stomach was opened along the greater curvature and fixed on a cork plate. The number and severity of ulcers was registered using the following scores.

- Severity Score:
  - 0 = Normal colored stomach
  - 0.5 = Red coloration
  - 1 = Spot ulcer
  - 1.5 = Hemorrhagic streaks
  - 2 = Ulcers ≥ 3 but ≤ 5
  - 3 = Ulcers > 5.

Ulcer index was calculated as: UI = UN+US+ UP X 10-1
Where,
- UI = ulcer index,
- UN= average of number of ulcers per animal,
- US= average of severity score
- UP=percentage of animals with ulcer.

Statistical analysis
The values expressed as mean ± S.E.M (standard error of the mean). Dunnett- test was used for the evolution of data and p<0.01 accepted as significant.

RESULTS
Plant studies
*Terminalia arjuna* bark was extracted by using 70% methanol and 30% water by cold maceration method and the yield was found to be 2.1%. A qualitative phytochemical study of the extract was performed by using suitable chemicals and reagents to confirm the presence of alkaloids, flavonoids, steroids and tannins, etc.

Stress induced ulcer
Induction of stress by cold resistance method to control group clearly produced the characteristic mucosal lesions, and which may be due to free radical generation. Pre-treatment with HAETA, orally at doses of 100 and 400 mg/kg, showing protection index 51% and 61% and significant reduction in ulcer index, free acidity, total acidity, Pn and inhibition of free radicals generation, the positive control (Omeprazole 20 mg/kg), showed 72% reduction of ulcers. (Results are tabulated in Table-1 &3)

Ethanol induced ulcer
The oral administration of Ethanol solution to the control group clearly produced the characteristic mucosal lesions. Ethanol solution induced long ulcer lesions after a relatively short time. Pre-treatment with HAETA, orally at doses of 100 and 400 mg/kg, showing protection index 66% and 76% significant reduction in ulcer index, free acidity , total acidity, Pn , the positive control (Omeprazole 20 mg/kg), showed 79% reduction of ulcers. (Results are tabulated in Table-2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Ulcer index</th>
<th>P% of gastric content</th>
<th>Free acidity (meq/lit)</th>
<th>Total acidity (meq/lit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Stress</td>
<td>65±2±1.01</td>
<td>2.5±0.12</td>
<td>22.12±0.01</td>
<td>65.0±2.01</td>
</tr>
<tr>
<td>II</td>
<td>HAETA 100mg/kg</td>
<td>32±0.34</td>
<td>51%</td>
<td>3.5±0.16&quot;</td>
<td>16.2±0.56&quot;</td>
</tr>
<tr>
<td>III</td>
<td>HAETA 400mg/kg</td>
<td>25±1.02&quot;</td>
<td>61%</td>
<td>4.4±0.14&quot;</td>
<td>13.1±1.13&quot;</td>
</tr>
<tr>
<td>IV</td>
<td>Stress.Omeprazole</td>
<td>18.80±3.26&quot;</td>
<td>72%</td>
<td>4.71±0.22&quot;</td>
<td>10.1±0.34&quot;</td>
</tr>
</tbody>
</table>

*P<0.01 Vs Control group; *P<0.05 Vs Control group. All values are represented as mean ± S.E.M and statistical significance using one way ANOVA, followed by Dunnett's test where P<0.05 was considered as moderately significant and P<0.01 was considered as a statistically significant.
DISCUSSION

The anti-ulcer activity of the hydroalcoholic extract of *Terminalia arjuna* bark against Stress & Ethanol Induced ulcers was established in this study. Stress can arise from prolonged anxiety, tension, and emotion, severe physical discomfort, hemorrhage, surgical shock, burns and trauma, thereby resulting in severe gastric ulceration. Recently research has shown that resistant cold stress can cause severe hemorrhage ulcer through disarrangement of the mucosal antioxidant enzyme such as superoxide dismutase and peroxides. Stress generate highly reactive OH− radicals that causes oxidative damage of the gastric mucosa and that the radicals is formed by metal catalyzed reactive OH* radicals that causes oxidative damage of the gastric epithelium.

Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion leads to formation of ulcer.

Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion leads to formation of ulcer. The extract shows protection against characteristic lesions of *Terminalia arjuna* bark in the treatment of ulcer.

**REFERENCES**