ANTIULCEROGENIC ACTIVITY OF ALCOHOLIC EXTRACT OF THE LEAVES OF TAMARINDUS INDICA (L) ON EXPERIMENTAL ULCER MODELS

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Summary

Tamarindus indica (Leguminosae) is a large, broad-leaved, tropical tree found in Haryana and other parts of India and Asia. Which is used in ayurveda for the management of gastric, inflammation and digestion problem. Therefore the purpose of the study was to investigate the anti-ulcer effect of leaves of the Tamarindus indica (l) (TI) extract on Cold restraint stress (CRU), Indomethacin, (IND), Pyloric Ligation induced ulcer models in rats. The treatment TI (200 mg/kg and 400 mg/kg) at varies doses significantly (P<0.001) inhibited the gastric lesions induced by CRU (68.14%), IND (73.8%) and PL (76.14%) respectively, with equal potency as compared to Std drug. TI showed concomitant attenuation of gastric secretory volume, Acid secretion in ulcerated rats. The results further suggest that TI was found to possess anti-ulcerogenic as well as ulcer healing properties, which might also be due to its anti-secretory and its antioxidant property.

Keywords: Tamarindous indica, Gastric acid, Antisecretory, Gastrin, Pylorus, Indomethacin.

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Introduction

Gastric hyperacidity and gastro-duodenal ulcer is a very common global problem today. Gastric lesions are develop, when the delicate imbalance between some gastro offensive and defensive factor is lost .Major offensive factors are Acid, Pepsin, Helicobacter pylori and bile salts. Defensive factors mainly involve mucus bicarbonate secretion and prostaglandins[1].Hyper secretion of gastric acid is a pathological condition which occurs due to uncontrolled secretion of HCl acid from parietal cells of the gastric mucosa through the proton pumping H⁺K⁺ ATPase[2].Even the normal rate of acid secretion may cause ulceration in the breached mucosa when some gastro protective factors are lost.

Imli or Tamarind (Tamarindus indica), also called Indian Date, is a large, broad-leaved, tropical tree found in Haryana and other parts of India and Asia. The word Tamarind is from Arabic 'tamar-ul-Hind', meaning, "the date palm of India". The tree can grow up to 25 meters with a spread of 12 m, and stays evergreen in regions without a dry season. Tamarind are many and it is used extensively in the Indian system of medicine, Ayurveda Tamarind preparations are universally recognized as refrigerants in fevers and as laxatives and carminatives. Tamarind leaves and flowers, dried or boiled, are used as poultices for swollen joints, sprains and boils. Extracts made from them are used in treating conjunctivitis, as antiseptics, as vermifuges, treatments for dysentery, jaundice, erysipelas and hemorrhoids and various other ailments. The aim of the present study was to evaluate the pharmacological activity of Tamarindus indica in experimental models of gastric ulcer [3].

Materials and methods

Drugs and Chemicals
Indomethacin and Omeprazole(sigma chemicals) , methanol were used in this study. All substances were prepared immediately before use and the reagents were used as analytical grade.

Plant Materials
The leaves of TI used in this study were collected from Srivilliputur(virudhunagar dist,Tamilnadu,India.). The plant was authenticated by Dr.Stephen ,dept of botany American college, madurai.

Extract preparation
TI leaves were shade dried and coarsely powdered. One kilogram of the powdered plant material was soaked in 2L of ethanol for 5 days and then extracted in soxlet apparatus with ethanol for 10hr .The last traces of the solvent were removed and concentrated to dryness under vacuum by using a rotary evaporater. The dried extract was weighed and then kept at -4OC until ready for use. The yield of the extract was 17.8%(w/w)of powdered methanol extract. In each experiment, the extract was diluted with water to desired concentration.
Phytochemical screening

A preliminary phytochemical screening of TI was conducted to determine the presence or absence of alkaloids, tannins, phenols, saponins, volatile oil, ascorbic acid, carbohydrates and glycosides according to the methods described by [4].

Animals

Adult male albino rats of wistar strain weighing about 120-150g were used for the study. They were maintained in clean, sterile, polypropylene cages and fed with commercial pellet rat chow (M/S Hindustan lever limited, Bangalore, India) and water ad libitum. The study was approved by the institutional ethical committee, which follows the guidelines of CPSCEA (Committee for the Purpose of Control and Supervision of Experimental on Animals).

Toxicity studies

For acute oral toxicity studies, rats were divided into four groups of six animals each. Group I served as control rats that received only distilled water while groups II, III, and IV orally fed with TI at a dose level of 1.0, 2.0, and 4.0 g/kg body weight (b.w) for 14 days. On 14th day the animals were sacrificed, blood was collected by sinus puncture and analyzed for red blood cell (RBC), white blood cell count (WBC), Hemoglobin (Hb), Mean corpuscular volume (MCV), and blood urea was assessed by diacetylmonoxime (DAM) method [5].

Antiulcer study

Indomethacin (IND) induced ulcers

On day 3, indomethacin (30 mg/kg b.w) suspended in 0.5% carboxymethyl cellulose was given as a single i.p dose to induce gastric ulcers [6], after 30 min of TI or OMP treatment. After 5 h the animals were killed and lesions in the gastric mucosa were scored. After identification of ulcer areas, the length of the ulcer was measured along the greater diameter. Number of hemorrhagic spots was considered equivalent to 1 mm of ulcer [7]. The mean ulcer size was calculated by dividing the total length (in mm) of ulcers for all the animals divided by total number of animals.

Cold Restraint Induced Ulcer

On day 3, after 30 min of TI or OMP treatment, rats were immobilized in a stress cage and were placed at 4 -6°C in an environmental cage [8]. The animals were sacrificed 2 h later and ulcer index was calculated.

Pyloric Ligation (PL)-induced Ulcers

Pyloric Ligation was applied by legating the pyloric end of the stomach of rats on 3rd day under ketamine Hcl anesthesia at a dose of 10 mg/kg after 30 min of TI and omeprazole treatment [9]. Animals were allowed to stabilize in individual cage and were deprived of water during postoperative method. After 4 h, rats were scarified with over dose of ketamine and gastric juice was collected for performing gastric scoring in stomach.
Statistical analysis
The results are presented as mean ±SD. The data were also analyzed by ANOVA (one-way analysis of variance). The statistical analysis was performed using Dunnett’s T3 multiple comparison test for all parameters. The values were considered significant at the levels of p<0.05, p<0.01 and p<0.001.

Results

Phytochemical analysis
The screening procedures were positive for alkaloids, carbohydrates, steroids, anthocyanin, saponins, tannins, ascorbic acid, β-carotene and volatile oil and negative for xanthones and anthrquinones.

Toxicity Studies
The results of the oral administration of TI in various doses of 1.0, 2.0 and 4.0 g/kg b.w for 14 days on different hematological and biochemical parameters in rats are presented in Table (1). The TI alone treated rats even at the dose of 4.0 g/kg b.w showed non significant effect on RBC, WBC, HB, HCT, MCV, blood sugar and urea as compared to the control group indicating no adverse effect. The values were shown in Table (1).

Table 1: Toxicological evaluation of TI in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>TI(1.0 g/kg b.w)</th>
<th>TI(2.0 g/kg b.w)</th>
<th>TI(4.0 g/kg b.w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC(×10³/ml)</td>
<td>6.24±0.52</td>
<td>6.31±0.25</td>
<td>6.34±0.42</td>
<td>6.35±0.48</td>
</tr>
<tr>
<td>RBC(×10⁶/ml)</td>
<td>8.47±0.28</td>
<td>8.49±0.31</td>
<td>8.51±0.46</td>
<td>8.54±0.56</td>
</tr>
<tr>
<td>Hb%(gmdl)</td>
<td>12.25±0.62</td>
<td>12.28±0.67</td>
<td>12.31±0.72</td>
<td>12.38±0.86</td>
</tr>
<tr>
<td>MCV(FL)</td>
<td>42.42±1.2</td>
<td>42.46±1.8</td>
<td>42.51±2.1</td>
<td>42.98±2.3</td>
</tr>
<tr>
<td>HCT(%)</td>
<td>34.21±0.9</td>
<td>34.26±1.4</td>
<td>34.28±1.4</td>
<td>34.35±1.5</td>
</tr>
<tr>
<td>Sugar(mg/dl)</td>
<td>118.28±2.3</td>
<td>118.42±2.4</td>
<td>118.38±4.8</td>
<td>118.45±3.4</td>
</tr>
<tr>
<td>Urea(mg/dl)</td>
<td>15.25±0.82</td>
<td>15.46±1.3</td>
<td>15.49±1.7</td>
<td>15.49±0.9</td>
</tr>
</tbody>
</table>

Results were expressed as mean ±S.D(n=6). TI treated groups showed non significant changes as compared with control rats.

Effect of TI on gastric Lesion Studies
Effect of TI on the various types of gastric lesion models was shown in Table (2). In ulcerogen treated animals were shown extensive gastric lesions in the stomach of all the experimental models. Stress provoked haemorrhagic form of lesions in the stomach with adequate evidence with intraluminal bleeding where as IND caused mostly petechial ulcers and erosions. TI (200 and 400 mg/kg b.w) given orally for 3 days showed a dose dependent protective effect against gastric ulcers induced by CRU and PL and was comparable with that of OMP.
Effect of TI on acid secretion studies

In 4hr Pylorus – ligated rats, TI (200 and 400 mg/kg b.w) was showed a dose – dependently decreased the gastric Juice volume and gastric acid output, omeprazole also showed significant (P<0.001) reduction in concentration and output of acid secretion in pylorus ligated rats. The values were shown in Table (3).

Table 2. Effect of TI on CRU, IND, PL induced ulcers in rats.

<table>
<thead>
<tr>
<th>Treatment Dose (mg/kg b.w) for 3 days</th>
<th>Ulcer Index</th>
<th>Protection(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRU induced Ulcers Control(CRU)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TI(200)+CRU</td>
<td>16.3±2.4</td>
<td>---</td>
</tr>
<tr>
<td>TI(400)+CRU</td>
<td>10.4±0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.2</td>
</tr>
<tr>
<td>OMP(10)+CRU</td>
<td>5.1±0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.14</td>
</tr>
<tr>
<td>IND induced Ulcers Control(IND)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TI(200)+IND</td>
<td>28.3±1.2</td>
<td>---</td>
</tr>
<tr>
<td>TI(400)+IND</td>
<td>19.3±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.8</td>
</tr>
<tr>
<td>OMP(10)+IND</td>
<td>7.4±0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.8</td>
</tr>
<tr>
<td>PL induced Ulcers Control(PL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TI(200)+PL</td>
<td>21.8±1.5</td>
<td>---</td>
</tr>
<tr>
<td>TI(400)+PL</td>
<td>14.6±0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.02</td>
</tr>
<tr>
<td>OMP (10)+PL</td>
<td>5.2±0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.14</td>
</tr>
<tr>
<td></td>
<td>4.3±0.32</td>
<td>80.21</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.D (n=6): <sup>a</sup>P<0.01 Vs Control; <sup>b</sup>P<0.001 Vs Control
Table 3: Effect Of TI on the volume of gastric juice, acid concentration and acid output of gastric Juice in Pylorus ligation induced ulcer Model:-

<table>
<thead>
<tr>
<th>Treatment (mg/kg b.w.,)</th>
<th>Volume (ml/100g)</th>
<th>Acid Cone (µequiv/ml)</th>
<th>Acid Output(µequiv/4h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(PL)</td>
<td>3.20±0.31</td>
<td>120.8±6.4</td>
<td>249.4±12.8</td>
</tr>
<tr>
<td>TI(200)+PL</td>
<td>2.13±0.16a</td>
<td>86.4±4.5a</td>
<td>195.2±8.7a</td>
</tr>
<tr>
<td>TI(400)+PL</td>
<td>1.82±0.18b</td>
<td>53.6±3.2b</td>
<td>120.2±6.3b</td>
</tr>
<tr>
<td>OMP(10)+PL</td>
<td>1.41±0.14b</td>
<td>41.5±2.3b</td>
<td>99.6±7.5b</td>
</tr>
</tbody>
</table>

Results are presented as mean ± SD, n=6 in each group
  a . P<0.01  Vs  Control(PL)
  b . P<0.001  Vs  Control(PL)

Discussion

The results of the present study state that the ethanolic extract of TI has capacity to significantly (P>0.001) inhibit the basal gastric secretion and ulcerogenicity induced by pylorus ligation, indomethacin and cold restraint stress in rats as compared to control. Pylorus ligation(PL) induced ulcer occurs due to an increases in acid-pepsin accumulation due to pylorus obstruction and subsequence mucosal digestion [10]. Moreover, gastric acid is an important factor for the genesis of ulceration in pylorus-lighted rats [9]. The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hypersecretion model of ligature is believed to increase gastric acid secretion.

Indomethacin is an established ulcerogen especially in an stomach [11], and elevated gastric acid secretion, possibly contributing to its ability to interfere with gastric ulcer healing [12]. The incidence of indomethacin-induced ulceration is mostly on the glandular mucosal part of stomach. Although the underlying etiologic mechanisms of indomethacin-induced gastric mucosal lesions are still unclear, but indomethacin is known to inhibit endogenous prostaglandin formation and increase gastric motor activity. The view is supported by the fact that prostaglandins normally serve as protective function in stomach by maintaining gastric microcirculation [13] and causes gastric secretion of bicarbonate and mucus[14]. The extract inhibited both the indomethacin and necrotizing agents induced ulceration in rats. It has been proposed that mucosal protection induced by extract may be mediated through the generation of endogenous prostaglandins [15]. It is possible the observed antilulcer activity of the extract against indomethacin injury might be related to antisecretory effect [16] or its ability to mobilize prostaglandins in gastric mucosa or through an unknown mechanism. Stress ulcers are due to both physiological and psychological factors. Which is Crucial for gastrointestinal defense and increased accumulation of acid and pepsin leading to auto digestion of the gastric mucosa [17].
In CRU, Incidence of ulcers is mainly due to increased acid secretion and generation of free radicals. The protective efficacy against CRU may be due to its antioxidant activities of TI.

The phytochemical analysis of the TI revealed the presence of saponin, tannins, alkaloids, carbohydrates, steroids, anthocyanin. The anti ulcer effect of the TI extract could be related to their antioxidant properties [18]. Additionally Saponin and tannins are known to affects the integrity of mucus membranes. Tannins with its protein precipitating and vaso constricting effects could be advantageous in preventing ulcer development [19]. Tannins also being an astringent may have precipitated ,Micro proteins on the site of ulcer there by forming an protective film over the lining to prevent absorption of toxic substances and resist the attach of proteolytic enzymes[20].

The results of the present study proved that the crude extract of TI posses a antiulcer activity against experimentally induced acute and chronic gastric ulcer models. Hence , it can be suggested that the antiulcer activity of the extract may be attributed to its ant secretory and antioxidant activities.

References