INTRODUCTION

Peptic ulcer is one of the most common diseases affecting mankind. It is now well recognized that in peptic ulcer disease, there is shift in balance between mucosal damaging mechanisms to mucosal protecting mechanisms. Pathogenesis of gastric ulcers is mainly attributed to impaired mucosal resistance. Disturbed gastric motility and reduced gastric mucosal blood flow are also important pathogenic elements in gastric ulcer malignancy.

Commiphora caudata (Wight & Arn) Englors, belongs to Burseraceae family, distributed in Srilanka and India [1]. Despite these reports, knowledge of the pharmacological properties of C. caudata is limited and screening studies are necessary to reveal the medicinal properties of the plant. Recently, widespread efforts have been launched to identify novel anti-ulcer drugs from natural resources. A number of models are available in which to test substances for their anti-ulcer effects. Here, we report on the effect of an ethanolic extract from Commiphora caudata barks on gastric lesion induced in different animal models employing necrotizing or stressor agents.

MATERIALS AND METHODS

Collection of plant materials

The bark of the tree was collected in March 2007 from different localities of Namakkal District, Tamilnadu state and was authenticated by Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen has been deposited at Pharmacognosy department under registration number C0024. The barks were cut, washed with distilled water and dried in shade, pulverized by mechanical grinder to get course powder and stored in an airtight container.

Preparation of Plants Extracts

The course powdered bark (200 g) of the plant was submitted to dynamic maceration with ethanol 70% for 4 h. This procedure was repeated three times with the same powder. After filtration, the residue was discarded and the solvent evaporated under reduced pressure and the extract was dried, yielding a dark green powder (37.65 g), designated as the ECC [2,3]. The yield of ethanolic extract was 18.82 %w/w and stored in refrigerator for further use. The concentration used in the experiment was based on the dry weight of the extract. Isolation of some chemical constituents of the bark extract was also undertaken in our laboratory. The extract at doses of 100 and 200 mg/kg was suspended in 0.5% of Carboxy Methyl Cellulose and Cimetidine, the reference antiulcer drug, dissolved in phosphate buffer saline and administered to animal (50 mg/kg body weight).

Experimental animals

Twenty four male Wistar Albino rats, weighed 200–250 g (aged 4 months) were utilized in this study. The animals were obtained from IRT Medical College, Erode district. Tamil Nadu state, India. They were housed in macronol cages under standard laboratory conditions (light period 7.00 A.M. to 7.00 P.M., 21±2 °C, relative humidity 55%). The animals were given standard rat pellets and tap water ad libitum, but they were deprived of food 24 h before the experiments. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. All procedures involving laboratory animal use were in accordance to the Institute Animal
Table 1: effect of ethanol extract of Commiphora caudata on ulcer index and percentage protection in ethanol induced ulcer model.

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-treatment</th>
<th>Ulcer Index</th>
<th>Ulcer area (mm²) (mean±SEM)</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>PBS (diseased control)</td>
<td>13.9531</td>
<td>968.35±42.38</td>
<td>—</td>
</tr>
<tr>
<td>II</td>
<td>C. caudata (100 mg/kg, p.o.)</td>
<td>5.1329</td>
<td>356.23±10.32</td>
<td>63.21</td>
</tr>
<tr>
<td>III</td>
<td>C. caudata (200 mg/kg, p.o.)</td>
<td>3.6311</td>
<td>252.00±10.52*</td>
<td>73.97</td>
</tr>
<tr>
<td>IV</td>
<td>Cimetidine (50 mg/kg, p.o.)</td>
<td>4.0102</td>
<td>278.31±11.56*</td>
<td>71.25</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M., (n=6). *P<0.05 as compared with ethanol.

Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Animal treatment
All rats were fasted for 48 h before the experiment but excess water were allowed and just two hours before starting the experiment water also were removed. Control animals (Group I) each received 5 ml/kg phosphate buffer saline by orogastric intubations; whereas treated animals Group II and Group III each received 100 mg/kg and 200 mg/kg ethanolic extract of C.caudate bark by orogastric intubations, respectively. Group IV animals each received 50 mg/kg cimetidine. Thirty minutes after their pretreatment, all animals were gavaged with absolute ethanol (5 ml/kg B.Wt.). They were sacrificed 15 min later by diethyl ether and their stomach rapidly removed and fixed in 10% buffered formalin.

Gross gastric lesions evaluation
Assessment of gastric lesions was also carried out by gross pathological examination of the stomach mucosa. One hour after ethanolic administration, the animals were euthanized by cervical dislocation. The stomachs were excised, cut along the greater curvature, and gently rinsed under ice cold PBS. Massive gastric hemorrhage was observed in the rats’ stomachs 1 h after oral administration of absolute ethanol. The stomachs were stretched on a frog board with mucosal surface up then to be examined in a standard position for macroscopic examination and the scoring of ulcer was performed with the help of a magnifying hand lens. The mucosa was examined for the number of absolute ethanol-induced mucosal lesions [4]. The length (mm) and the width (mm) of the ulcer on the gastric mucosa were measured by planimeter square (10 X 10 mm) under a dissecting microscope (20X). The ulcer area (UA) was calculated [5]. The total ulcer area (mm²) of each stomach was recorded and the % protection was calculated as follow:

\[
\text{% Protective} = \frac{\text{UA}_{\text{control}} - \text{UA}_{\text{treatment}}}{\text{UA}_{\text{control}}} \times 100
\]

Ulcer index (UI) was calculated as follow:

\[
\text{Ulcer Index} = \frac{10}{X}
\]

Where \(X = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}\)

Histological examination:
Stomach biopsies were processed and assessed for damage by taking a 5µm section, stained with Hematoxylin and Eosin and analyzed under light microscopy.

Statistical Analysis
The results were expressed in terms of mean ± SEM. The significance of difference between mean values for the various treatments were tested using one way analysis of variance test (ANOVA test) followed by Tukey’s multiple range test. P values less than 0.05 were considered significant [6].

RESULTS
Grossly, the results of the current study showed that pretreated rats with ethanolic extract of C.caudata bark extracts or cimetidine significantly reduced the formation of gastric ulcer induced by absolute ethanol compared to animals pretreated with PBS and administered absolute ethanol (Table 1). Histologically, rats pretreated with ethanolic extract of C.caudata bark extracts or cimetidine also significantly inhibited the gastric lesion formation and submucosal edema, induced by absolute ethanol.

DISCUSSION
Administration of absolute ethanol into the gastric lumen induced gross lesions in the glandular part of the stomach [7]. It was also apparent that ethanol caused gastric damage, which was confirmed by significant increase in the number of hemorrhage and gastric erosions [8]. In our study, the ulcerogenesis, pronounced destructive changes associated with hemorrhage in the stomach were observed in the ethanol-induced group. Pretreatment of rats with a single oral dose of C.caudata could partly reduce ulcer area and promote healing of gastric lesions induced by acute intake of ethanol.

The present results demonstrate that the ethanolic extract of C.caudata bark protect the rat gastric mucosa against hemorrhagic lesions produced by absolute ethanol. The cytoprotective effect was confirmed by histological examination showing prevention of mucosal lesions and submucosal edema. Absolute ethanol method of inducing gastric lesions is rapid and convenient way of screening plant extracts for anti-ulcer potency and cytoprotection in macroscopically and microscopically visible lesions. Disturbances in gastric secretion, damage to gastric mucosa, alterations in permeability, gastric mucus depletion and free-radical production are reported to be the pathogenic effects of ethanol [9]. Ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow,
which contributes to the development of the hemorrhagic and necrotic aspect of tissue injury [10]. It is of interest to note that administration of antioxidants inhibit ethanol-induced gastric injury in the rats [11]. It could be conceived that ethanolic extract of *C. caudata* bark exert their anti-ulcer activity through the flavonoids, since flavonoids are reported to protect the mucosa by preventing the formation of lesions by various necrotic agents [12]. We can suggest that it may be possible to use plant bark extract as remedy to prevent ulcers. However, further investigations are required to elucidate their exact mechanism(s) of anti-ulcer activity.

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REFERENCES


[11]. Source of support: Nil, Conflict of interest: None Declared