Anticancer activity of *Utleria salicifolia* rhizome extract

Ch.V. Rao 1,a, S.K. Ojha 1, K. Radhakrishnan 1, R. Govindarajan 1, S. Rastogi 1, S. Mehrotra 1, P. Pushpangadan 1

1 Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Council of Scientific and Industrial Research, Rana Pratap Marg, P.O. Box 438, Lucknow 226 001, Uttar Pradesh, India

Received 28 April 2003; received in revised form 3 December 2003; accepted 23 December 2003

Abstract

The effect of 50% ethanolic extract of *Utleria salicifolia* (USE) was assessed in different acute and chronic gastric ulcer models in rats. USE, 50–200 mg/kg administered orally, twice daily for 5 days showed dose-dependent ulcer protective effect in pylorus ligation (14.48–51.03% protection, *P* < 0.01), cold-restraint stress (21.22–77.14% protection, *P* < 0.01), and ethanol-induced ulcer (15.22–60.74% protection, *P* < 0.01), and acetic acid (20.1–84.37% protection, *P* < 0.01) induced acute and chronic ulcers. USE also significantly (*P* < 0.01) reduced the ulcer incidence (50 and 10%) and severity (67.83 and 91.34% protection) of duodenal ulcer, induced by cysteamine. USE offered protection (53.52 and 60.58%) against ethanol-induced depletion of gastric wall mucus. However, USE reduced the ulcer index with significant change in plasma corticosterone (25.53 and 39.52% protection, *P* < 0.01) and increased in catalase (28.42 and 71.0% protection, *P* < 0.01), and superoxide dismutase (15.80 and 26.61% protection, *P* < 0.01) and increased in catalase (28.42 and 71.0% protection, *P* < 0.01) activity, respectively. Preliminary phytochemical screening of the USE gave the positive test for steroids, alkaloids, terpenoids, saponins and tannins. The HPTLC studies in the toluene: ethyl acetate: formic acid and the densitometric scanning at 254 nm gave three major spots with area corresponding to 28.16, 17.17, and 13.79% at 0.69, 0.78, and 0.88 Rf values, respectively. The results indicate that USE possesses anticancer activity. © 2004 Elsevier Ireland Ltd. All rights reserved.

Keywords: *Utleria salicifolia*; Ulcer; Mucin; Antioxidant

1. Introduction

*Utleria salicifolia* Bedd. Ex. Hook. F. (Periplocaceae) is a branched shrub endemic to South Western Ghats of peninsular India. The species is known from three localities in South Western Ghats viz. Anamalais (Kerala and Tamil Nadu), Nelliampathy and Marayoor forests (Kerala) (Radhakrishnan et al., 1998). The ethnomedical use of *Utleria salicifolia* was recorded from the Malasar, Kadar and Mudhuvar tribes inhabiting these areas. These tribes call *Utleria salicifolia* as ‘Mahali kizhangu’. ‘Mahali’ refers to the Hindu Goddess of wealth ‘Mahalekshmy’ and ‘Kizhangu’ root tubers. The Malasar and Kadar tribes use the tuber chips boiled in water for the preparation of pickles which are said to be good for intestinal ailments like colic and bleeding due to ulcer (Radhakrishnan et al., 1998).

Gastric hyperacidity and ulcer are very common causing human suffering today. It is an imbalance between damaging factors within the lumen and protective mechanisms within the gastric duodenal mucosa. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still very poorly understood (Rao et al., 2000). Oxygen derived free radicals have been implicated in the pathogenesis of a wide variety of clinical disorders and gastric damage is caused by physical, chemical and physiological factors that leads to gastric ulceration in human and experimental animals (Rao et al., 1999). Most of the available drugs are thought to act on the offensive factors which neutralize acid secretion like antacids, H2 receptor blockers like ranitidine, famotidine, anticholinergics like pirenzepin, telipazine, proton pump blockers like omeprazole, lanaprazole, etc. which interfere with acid secretion. Recently the involvement of neural mechanism in the regulation of stress responsiveness and complex neurotransmitter interactions were reported causing gastric ulceration (Sairam et al., 2001). To the best of our knowledge there were no scientific...
They were kept in the departmental animal house at 26 animal house of Central Drug Research Institute, Lucknow.

2.4. Test animals

These plates were observed at UV 254 and were scanned on toluene:ethyl acetate:formic acid (5:5:1) as the mobile phase. silica gel plate (Merck 60 F 254) as the stationary phase and (USE) were carried out on pre-coated silicic acid (HPTLC) studies of the 50% ethanolic extract of steroidal saponins, and tannins. The high performance thin layer chromatogra-

phy (Anshu- thier et al., 2003) of the rhizomes of Ulteria salicifolia gave the positive test for steroids, alkaloids, terpenoids, saponins, and tannins. The high performance thin layer chromatography (HPTLC) studies of the 50% ethanolic extract of Ulteria salicifolia (USE) were carried out on pre-coated silica gel plate (Merck 60 F 254) as the stationary phase and toluene:ethyl acetate:formic acid (5:5:1) as the mobile phase. The extract was spotted using a Camag Linomat IV spotter. These plates were observed at UV 254 and were scanned on TLC scanner III using CAT software.

2.3. Phytochemical screening

Preliminary qualitative phytochemical screening (Anshu-rathi et al., 2003) of the rhizomes of Ulteria salicifolia gave the positive test for steroids, alkaloids, terpenoids, saponins, and tannins. The high performance thin layer chromatography (HPTLC) studies of the 50% ethanolic extract of Ulteria salicifolia gave the positive test for steroids, alkaloids, terpenoids, saponins, and tannins. The high performance thin layer chromatography (HPTLC) studies of the 50% ethanolic extract of Ulteria salicifolia (USE) were carried out on pre-coated silica gel plate (Merck 60 F 254) as the stationary phase and toluene:ethyl acetate:formic acid (5:5:1) as the mobile phase. The extract was spotted using a Camag Linomat IV spotter. These plates were observed at UV 254 and were scanned on TLC scanner III using CAT software.

2.4. Test animals

Sprague-Dawley rats (140–180 g) were procured from the animal house of Central Drug Research Institute, Lucknow. They were kept in the departmental animal house at 26±2 °C and relative humidity 44–56%, light and dark cycles of 10 and 14h, respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18–24 h before the experiment though water was allowed ad libitum. All experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmerman, 1985).

2.5. Experimental procedure

USE in doses of 50, 100, and 200 mg/kg and H2 receptor blocker, ranitidine, in the dose of 50 mg/kg were adminis-
tered orally twice daily at 10:00 and 16:00 h, respectively, for 5 days for acute and up to 5 or 10 days for chronic ulcer protective studies. Reduced glutathione (RG) 150 mg/kg was injected intraperitoneally twice: once before 20 h and another 1 h prior to subjecting the animals to cold-restraint stress and reported to exerts its antioxidant defense mech-
amism (Das and Banerjee, 1993). Control group of animals received suspension of 1% carboxymethyl cellulose in distilled water (10 ml/kg).

2.6. Aspirin (ASP)-induced ulcers

ASP in dose of 200 mg/kg (20 mg/ml) was administered to the animals on the day of the experiment and ulcers were scored after 4 h (Goel et al., 1985). The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by a person unaware of the ex-
perimental protocol in the glandular portion of the stomach. Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer after histological con-
firmation as follows (Sanyal et al., 1982): 0, no ulcer; +, pin point ulcer and histological changes limited to super-
ficial layers of mucosa and no congestion; ++, ulcer size less than 1 mm and half of the mucosal thickness showed necrotic changes; ++++, ulcer size 1–2 mm with more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscularis remaining unaffected; +++++, ulcer either more than 2 mm in size or perforated with complete destruction of the mucosa with necrosis and hemorrhage, muscularis still remaining unaffected. The pooled group ulcer score was then calculated according to the method of Sanyal et al. (1982).

2.7. Cold-restraint stress (CRS)-induced ulcers

Rats were deprived of food, but not water, for about 18 h before the experiment. On day six, the experimental rats were immobilized by strapping the fore and hind limbs on a wooden plank and kept for 2 h, at temperature of 4–6 °C (Gupta et al., 1985). Two hours later, the animals were sac-
rificed by cervical dislocation and ulcers were examined on the dissected stomachs as described above.

2.8. Pylorus ligated (PL)-induced ulcers

Drugs were administered for a period of 5 days as de-
scribed above and the rats were kept for 18 h fasting and reports available in support of its traditional claims. There-
fore, present study was designed to demonstrate the effect of Ulteria salicifolia extract (USE) on physical and chemical factors induced gastric ulceration in rats.
care was taken to avoid coprophagy. Animals were anaesthetized using pentobarbitone (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post-operative period (Shay et al., 1945). After 4 h, stomachs were dissected out and cut open along the greater curvature and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach as mentioned in aspirin induced ulcers.

2.9. Ethanol (EtOH)-induced ulcers

The gastric ulcers were induced in rats by administrating 100% EtOH (1 ml/200 g, i.p.) (Hollander et al., 1985) and the animals were sacrificed for intensity, using a scale of 0–3, where 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, and 3 = perforated or penetrated ulcer (into the pancreas or liver).

2.12. Determination of gastric wall mucus

Gastric wall mucus was determined according to the method of Corne et al. (1974). The glandular segments from stomachs were removed, weighed and incubated in tubes containing 1% Alcian blue solution (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8) for 2 h. The alcian blue binding extract was centrifuged at 3000 rpm for 10 min and the absorbency of supernatant was measured at 498 nm. The quantity of alcian blue extracted (gram per gram of glandular tissue) was then calculated.

2.13. Estimation of lipid peroxidation (LPO)

The fundic part of the cold-restraint stress (CRS)-induced ulcer stomach was homogenized (5%) in ice-cold 0.9% NaCl with a Potter–Elvehjem glass homogenizer for 30 s. The homogenate was centrifuged at 800 × g for 10 min and the supernatant was again centrifuged at 12,000 × g for 15 min and the obtained mitochondrial fraction was used for the following estimations (Das and Banerjee, 1993). A volume of the homogenate (0.20 ml) was transferred to a vial and was mixed with 0.2 ml of a 8.1% (w/v) sodium dodecyl sulfate solution, the abdomen was closed in two layers and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (square millimeters per rat).


2.11. Cysteamine-induced duodenal ulcers

The method described by Szabo (1978) was followed. Duodenal ulcers were induced by administrations of two doses of cysteamine hydrochloride, 400 mg/kg, p.o. in 10% aqueous solution at an interval of 4 h. USE at dose levels of 100 and 200 mg/kg, ranitidine (50 mg/kg, p.o.) were administered 30 min before each dose of cysteamine hydrochloride. All the animals were sacrificed 24 h after the first dose of cysteamine and duodena were excised carefully and opened along the antimesentric side. The duodenal ulcers were scored for intensity, using a scale of 0–3, where 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, and 3 = perforated or penetrated ulcer (into the pancreas or liver).
2.15. Estimation of plasma corticosterone (PC)

The animals were lightly anesthetized with ether and blood was collected from the supraorbital plexus using the microcapillary technique in CRS-induced ulcer model. Three hundred microlitres of isooctane was added to 100 µl of plasma. After mixing and centrifugation, the isooctane was discarded. Six hundred microliters of chloroform was added to each tube and after extraction 400 µl of chloroform was transferred to another stoppered tube. To this 800 µl of acid–alcohol (50%) solution (2:1) was added. After 1 h, acid layer fluorescence was measured at 462 nm (excitation) and (emission) using a spectrofluorimeter and expressed as micrograms per deciliter (Glick et al., 1964).

2.16. Statistical analysis

All the data were presented as mean ± S.E.M. and analyzed by Wilcoxon Sum Rank Test (Padmanabha pillai et al., 1982) and unpaired Student’s t test for the possible significant interrelation between the various groups. A value of *P* < 0.05 was considered statistically significant.

3. Results

The preliminary HPTLC studies revealed that the solvent system toluene:ethyl acetate:formic acid (5:5:1) was ideal and gave the well-resolved peaks of the sample (Fig. 1). The spots of the chromatogram were visualized at 254 nm with a 400 k filter at 0.10, 0.20, 0.59, 0.78, and 0.88 *Rf*. The densitometric scanning at 254 nm gave major three spots with area of 28.16, 17.17, 13.79% at 0.69, 0.78, and 0.88 *Rf* values, respectively.

Effects of USE at doses of 50–200 mg/kg, twice a day for 5 days prevented the acute gastric ulcers in a dose related manner. The range of percent protection were PL 14.48–51.03% (*P* < 0.01), ASP 28.80–56.52% (*P* < 0.05), EtOH 13.22–60.74% (*P* < 0.05 to *P* < 0.001) and CRS 21.22–77.14% (*P* < 0.05 to *P* < 0.001), respectively. The

![Fig. 1. HPTLC fingerprint profile of Utleria salicifolia extract.](image)
The gastric wall mucus was significantly a cytoprotective property (Table 2). In chronic ulcers induced by 50% acetic acid, USE reduced ulcer index significantly percent protection of ranitidine ranged from 57.44–80.0% (P < 0.05 to P < 0.001), respectively, in various gastric ulcer models (Table 1). Secretion of mucus and bicarbonate by surface epithelial constitute a mucus–bicarbonate barrier, which is regarded as first line of defense against potential ulcerogens. The gastric wall mucus was significantly < 0.05 compared to respective CRS group. †† P < 0.001 compared to respective EtOH group. † P < 0.05 compared to respective control group.

### Table 2
Effect of *Utleria salicifolia* extract (USE, twice daily for 5 days) on 50% acetic acid-induced chronic ulcers in rats

<table>
<thead>
<tr>
<th>Treatment and dose (mg/kg)</th>
<th>Ulcer index (square centimeters per rat)</th>
<th>Percent protection</th>
<th>Gastric wall mucus (gram per gram wet glandular tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0 ± 0.0</td>
<td>–</td>
<td>268.7 ± 15.1</td>
</tr>
<tr>
<td>EtOH</td>
<td>24.1 ± 5.1</td>
<td>–</td>
<td>171.2 ± 12.9</td>
</tr>
<tr>
<td>USE 100</td>
<td>11.2 ± 4.2</td>
<td>53.52</td>
<td>207.4 ± 13.8</td>
</tr>
<tr>
<td>USE 200</td>
<td>9.5 ± 2.7**</td>
<td>60.38</td>
<td>277.3 ± 14.6**</td>
</tr>
<tr>
<td>Ranitidine 50</td>
<td>10.1 ± 2.7*</td>
<td>58.09</td>
<td>213.8 ± 10.1**</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. for six rats.

† † † P < 0.001 compared to respective CRS group.
* P < 0.05 compared to respective control group.

### Table 3
Effect of *Utleria salicifolia* extract (USE, twice daily for 5 and 10 days) on 50% acetic acid-induced chronic ulcers in rats

<table>
<thead>
<tr>
<th>Treatment and dose (mg/kg)</th>
<th>Acetic acid-induced chronic ulcers</th>
<th>Percent incidence of perforations</th>
<th>Ulcer index</th>
<th>Percent incidence of perforations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Five days treated</td>
<td></td>
<td>Ten days treated</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>22.5 ± 2.2</td>
<td>70.5</td>
<td>12.8 ± 1.5</td>
<td>35.3</td>
</tr>
<tr>
<td>USE 100</td>
<td>18.0 ± 1.5</td>
<td>48.9</td>
<td>7.5 ± 1.4†</td>
<td>5.1</td>
</tr>
<tr>
<td>USE 200</td>
<td>12.1 ± 1.3**</td>
<td>26.7</td>
<td>2.0 ± 0.4**</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. for six rats.

† † P < 0.05 compared to respective control group.
* P < 0.01 compared to respective control group.

### Table 4
Effect of *Utleria salicifolia* extract (USE) on cysteamine-induced duodenal ulcers in rats

<table>
<thead>
<tr>
<th>Treatment and dose (mg/kg)</th>
<th>Ulcer incidence</th>
<th>Ulcer score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Percent</td>
</tr>
<tr>
<td>Control</td>
<td>8/10</td>
<td>80</td>
</tr>
<tr>
<td>USE 100</td>
<td>5/10</td>
<td>50</td>
</tr>
<tr>
<td>USE 200</td>
<td>1/10</td>
<td>10</td>
</tr>
<tr>
<td>Ranitidine 50</td>
<td>2/10</td>
<td>20</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. for 10 rats.

† † P < 0.001 compared to respective control group.
* P < 0.01 compared to respective control group.

### Table 5
Effect of *Utleria salicifolia* extract (USE, twice daily for 5 days) on plasma corticosterone (PC), lipoperoxidation (LPO), catalase (CAT), and superoxide dismutase (SOD) activities in cold-restraint stress (CRS)-induced ulcers

<table>
<thead>
<tr>
<th>Treatment and dose (mg/kg)</th>
<th>Ulcer index</th>
<th>PC</th>
<th>LPO</th>
<th>CAT</th>
<th>SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0 ± 0.0</td>
<td>21.7 ± 3.3</td>
<td>0.35 ± 0.01</td>
<td>33.1 ± 2.0</td>
<td>98.9 ± 10.0</td>
</tr>
<tr>
<td>CRS</td>
<td>24.0 ± 3.5†</td>
<td>37.2 ± 4.1†</td>
<td>0.48 ± 0.02†</td>
<td>18.3 ± 1.5†</td>
<td>215.3 ± 11.3†</td>
</tr>
<tr>
<td>USE 100</td>
<td>14.5 ± 2.9*</td>
<td>27.5 ± 2.6</td>
<td>0.39 ± 0.01**</td>
<td>25.5 ± 1.3*</td>
<td>181.3 ± 5.7*</td>
</tr>
<tr>
<td>USE 200</td>
<td>5.6 ± 1.3***</td>
<td>22.5 ± 2.3*</td>
<td>0.25 ± 0.01***</td>
<td>31.3 ± 1.9***</td>
<td>158.4 ± 4.9***</td>
</tr>
<tr>
<td>Reduced glutathione 150</td>
<td>4.5 ± 2.0***</td>
<td>21.7 ± 1.3**</td>
<td>0.28 ± 0.02***</td>
<td>31.1 ± 1.8***</td>
<td>149.1 ± 5.1***</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. for six rats.

† † P < 0.001 compared to respective control group.
* P < 0.05 compared to respective CRS group.
* * P < 0.01 compared to respective CRS group.
* * * P < 0.001 compared to respective CRS group.
Cysteamine produced duodenal ulcers in 80% of the control rats. Usually two ulcers were produced close to the pylorus, the larger on the anterior and the smaller on the posterior wall of the duodenum. They were elongated extending longitudinally down to the duodenum. Treatment with USE (100 and 200 mg/kg) produced a significant (P < 0.001) and dose-dependent reduction in the severity and incidence of cysteamine induced duodenal ulcers. However, the H2 receptor blocker ranitidine (50 mg/kg) also produces a significant protective effect (Table 4).

A summary table (Table 5) indicates to indicate the severity of ulcer index as well as enzyme activities. While studying the role played by the reactive oxygen species on CRS-induced gastric damage, lipid peroxidation, and SOD were increased significantly of the ulcerated stomachs (P < 0.001). Pretreatment with USE and a general antioxidant, reduced glutathione, significantly reduced the ulcer index, LPO, SOD levels and increased in CAT activity in comparison to the CRS ulcers (P < 0.05 to P < 0.01). Henke (1979) stated that the central nervous system played an important role in stress ulceration and regulation of plasma corticosterone. USE almost completely protected gastric ulceration by scavenging the free radicals that involved in the endocrinological plasma corticosterone.

4. Discussion and conclusion

The present study showed that the ethanolic extract of *Utleria salicifolia* possess gastroprotective activity as evidenced by its significant inhibition in the formation of ulcers induced by various physical and chemical agents. Pylorus ligation-induced ulcers are due to autodigestion of the gastric mucosa and break down of the gastric mucosal barrier (Saram et al., 2002). Synthetic NSAIDs like aspirin cause mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and break diffusion of H+ ions (Rao et al., 2000). The incidence of ethanol-induced ulcers is predominant in the glandular part of stomach was reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products (Oates and Hakkinen, 1999). Ethanol-induced depletion of gastric wall mucus has been prevented by USE. It implies that a concomitant in-crease in prostaglandins (Pihan et al., 1986) or sulfhydryl ions (Rao et al., 1999); are involved in genesis of stress induced ulcers. Complex neurochemical mechanisms are involved in the organism’s biological response to noxious stimuli like stress. The pathologic alterations occur with the changes in the synthesis, actions and degradation of hormones, neurotransmitters and neuromodulators. The central nervous system plays an important role in stress ulceration and regulation of plasma corticosterone (Henke, 1979).

As etiopathogenesis of these ulcer models are different, mechanism of USE should then include number of predisposing factors. On the other hand, the mucosal protection induced by non-prostanoid compounds was perhaps mediated through the mobilization of endogenous prostaglandins (Konturek et al., 1987).

Gastric ulcer is often a chronic disease and it may persist for 10–20 years characterized by repeated episodes of healing and re-exacerbations. Acetic acid-induced ulcer better resembles clinical ulcers in location, chronicity and severity and servers as the most reliable model to study healing process (Okabe and Pfeiffer, 1972). USE significantly healed the penetrating ulcers induced by acetic acid after 5 and 10 days treatment.

Free radicals affect lipids by initiating peroxidation. Superoxide (O2−), hydrogen peroxide (H2O2) and hydroxyl radical (OH•) are important ROS causing tissue damage (Fridovich, 1986); and lipid peroxide level is an indicator for the generation of ROS in the tissue. The experimental data stated that the cold-restraint stress aggravated the ulcer severity, lipid peroxidation, and plasma corticosterone as compared to unstressed rats. The higher lipid peroxidation and SOD levels indicated increased production of O2− within the tissue as elevated O2− level was thought to increase the concentration of cellular radical level. These radicals functioned in concert to induce cell degeneration via peroxidation of membrane lipids, breaking of DNA strands and denaturing cellular proteins (Hallowell and Gutteridge, 1985). This effect was significantly reversed by prior administration of USE providing a close relationship between free radical scavenging activity and the involvement of endocrinological (plasma corticosterone) responses. The more work is required for the clear understanding of the mechanism of action with chemically identified active principles. However, in the present study the plant shows a potent antulcer activity, which justifies the ethnomedical claims.

References


Glick, D., Redlich, D.V., Levine, S., 1964. Fluorimetric determination of corticosterone and cortisol in 0.02–0.05 ml of plasma or submilligram samples of adrenal tissue. Endocrinology 74, 653–655.


