EVALUATE THE ANTIULCEROGENIC PROPERTIES OF *GUETTARDA SPECIOSA* (L.) IN EXPERIMENTAL ANIMALS

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**ABSTRACT**

*Guettarda speciosa* L., a member of the Rubiaceae family, is used in folk medicine because of its treatment in ulcer, wounds, sores, diarrhoea, febrifugal and anticholinergic applications. The purpose of the present study is to investigate the phytoconstituents, and anti-ulcer activity of the ethanol Extract of *Guettarda speciosa* (L.) (EEGS) leaf extract in albino rats. EEGS at the doses of 200 and 400 mg/kg body weight orally was administered to evaluate anti-ulcer activity by using Ethanol and pyloric ligation (PL) induced gastric ulcer models in Albino rats. Standard drug Omeprazole (20 mg/kg /p.o) were administered 30min prior to induction of gastric ulcer. Ethanol extract of *Guettarda speciosa* dose dependent inhibition in ethanol induced gastric lesions, showed 67.37 % protection at 400 mg/kg, and 54.7% protection at 200 mg/kg. EEGS dose dependent inhibition in pylorus ligation induced gastric ulcer (PL) showed 72.02% protection at 400 mg/kg and 47.86% protection at 200 mg/kg. All the results are found to be statistically significant (p≤0.05). Administration of the extracts led to increases the gastric volume and pH. Hence we suggest that ethanol extract of *Guettarda speciosa* L. possesses anti-ulcerogenic properties. The mechanism of anti-ulcer activity can be attributed to decrease in gastric acid secretory activity along with strengthening of mucosal defensive mechanisms.

**Keywords:** *Guettarda speciosa* L., Traditional Medicine, Antiulcer Activity, Pylorus Ligation, Ethanol Induced Ulcer.

**INTRODUCTION**

Peptic ulcer disease affect a large portion of the world population and are induced by several factors, including stress, smoking, nutritional deficiencies, and ingestion of non-steroidal anti-inflammatory drugs (Nash et al., 1994). In spite of the vast amount of research on ulcer, the cause of chronic peptic ulceration is still not clear. Although in most of the cases the aetiology of the ulcers is unknown, it is generally accepted the pathophysiology of these ulcers involves an imbalance between offensive (acid, pepsin, and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors).

Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection (Hoogerwerf and Pasricha, 2001; Valle, 2005). Modern approach to this includes proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analog (Manonmani et al., 1995). Development of tolerance and incidence of relapses and side effects on clinical evaluation make their efficacy arguable. This has been the basis for the development of new antiulcer drugs, which includes herbal drugs.

*Guettarda speciosa* Linn. (Family: Rubiaceae) is widely distributed from East Africa to India and throughout to Malaysia into the South Pacific. This A decoction of the leaves is used to treat coughs, colds and sore throats. The native practitioners in and around Tirunelveli District, India, have claimed that the inner bark of this plant are being traditionally used in ulcer,

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wounds, sores, diarrhoea, febrifugal and anticholinergic applications. (Weiner, 1984; Weiner, 1971) Upon literature review it was found that the plant contains loganic acid and secologanin. (Inouye et al., 1988; Cambie and Ash, 1994) Anti epileptic and antidiarrhoeal activity of Guettarda speciosa was reported. Previous studies have demonstrated that extracts of these plants was subjected to acute toxicity and then screened for antiepileptic activity on Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats. (Saravanakumar et al., 2009; Gandhimathi et al., 2009) Therefore, the present study was performed to verify the antiulcer activity of Guettarda speciosa in experimental animals.

MATERIALS AND METHODS

Plant collection

The Plant material of Guettarda Speciosa used for investigation was collected from Tirunelveli District, in the Month of August 2007. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany, C.C.R.A.S., Govt. of India. The voucher specimen (CHESA-GS-01) of the plant was deposited at the college for further reference.

Preparation of extracts

Inner bark of the whole plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (60g) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of ethanolic extract of G. Speciosa was found to be 17.5 % w/w.

Preliminary phytochemical screening

The extract was screened for the various phytochemical constituents like steroids, alkaloids, carbohydrates, proteins, flavonoids, tannins and glycosides using the standard methods. (Harborne JB, 1973)

Animals used

Albino wistar rats (150-230g) of either sex were obtained from the animal house in C.L. Baid Metha College of Pharmacy, Chennai. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given ad libitum. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No. IAEC/XIII/ 03 / CLBMCP / 2008-2009).

Antulcer activity

Ethanol induced gastric ulcer

Animals were randomly divided into four groups each of 6 rats. Group I treated with 4% v/v aqueous tween 80 (10 ml/kg p.o), Group II & III treated with ethanol extract of Guettarda speciosa (200and 400mg/kg p.o) respectively for 14 days and Group IV treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the 14th day, Gastric ulcers were induced with ethanol at a dose of 8ml/kg (Mizui et al., 1987) administered to all groups by orally. The animals were anaesthetized 6 h with ether and stomachs were incised along the greater curvature and the ulcer index for each rat was taken as the mean ulcer score.

Pyloric ligation induced gastric ulcer

Animals were divided into four groups each of six rats. Group I treated with of 4% v/v aqueous tween 80 (10 ml/kg p.o), Group II & III treated with ethanol extract of Guettarda Speciosa (200and 400mg/kg p.o) respectively for 14 days and Group IV treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the 14th day, all groups rats were fasted 24 h prior to induction of gastric ulcer. Pyloric ligation was done by ligating the pyloric end of the stomach of rats 1 h after drug administration (Shay et al., 1945). Animals were allowed to recover and stabilized in individual cage and were deprived of water during post-operative period. After 4 h of surgery, rats were sacrificed by cervical dislocation and ulcer index were examined on the dissected stomachs as described below.

Measurement of ulcer index

The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition (%I) was calculated by following formula:

\[
%I = \frac{(USc - USt)}{USc} \times 100
\]

Where USc = ulcer surface area in control and USt = ulcer surface area in treated animals.

Histopathological studies

The freshly excised stomachs were washed with saline and preserved in 10% formaldehyde solution for histopathological studies. The sections of stomachs stained with hematoxylin and eosin, were assessed for histopathological changes such as congestion, edema, hemorrhage and necrosis (Shah and Khan, 1997). The microscopic slides were photographed.
Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett’s test p values less than 0.05 were considered as significance.

RESULTS

Phytochemical screening

The results of preliminary phytochemical screening of the ethanolic extract of inner bark of *G. speciosa* Linn revealed that presence of alkaloids, flavonoids, carbohydrates, tannins, phenols, gums and mucilage and absence of saponins and steroids.

Effect of EEGS on gastric ulcer induced by Ethanol

The EEGS showed significant anti-ulcer effect against ulcers induced by Ethanol in a dose dependent manner. In ethanol induced ulcer model, EEGS at a dose of 200 and 400 mg/kg body weight showed protective effect of 50.49 and 71.61%, respectively, where as Omeprazole showed protection index of 79.09% at a dose of 20 mg/kg body weight (Table -1 & Figure 1).

Effect of EEGS on gastric ulcer induced by pylorus ligation (PL)

The EEGS showed significant anti-ulcer effect against ulcers induced by pylorus ligation in a dose dependent manner. In PL induced ulcer model, EEGS at a dose of 200 and 400 mg/kg body weight showed protective effect of 48.26 and 75.46%, respectively, where as Omeprazole showed protection index of 81.38% at a dose of 20 mg/kg body weight (Table -2 & Figure 2).

Table 1: Effect of ethanol extract of *Guettarda Speciosa* L. (EEGS) in ethanol (8 ml/kg) induced gastric ulcer in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Design of Treatment</th>
<th>Ulcer Index</th>
<th>Percentage Inhibition (% I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (4% v/v aqueous tween 80, 10 ml/kg b.w ) p.o</td>
<td>19.13 ± 1.09</td>
<td>---</td>
</tr>
<tr>
<td>II</td>
<td>EEGS (200mg/kg b.w) p.o</td>
<td>9.47 ± 0.49*</td>
<td>50.49</td>
</tr>
<tr>
<td>III</td>
<td>EEGS (400mg/kg b.w) p.o</td>
<td>5.43 ± 0.31**</td>
<td>71.61</td>
</tr>
<tr>
<td>IV</td>
<td>Omeprazole (20mg/kg b.w) p.o</td>
<td>4 ±0.47**</td>
<td>79.09</td>
</tr>
</tbody>
</table>

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. *P < 0.01 and **P < 0.001 as compared to control (n = 6 in each group). EEGS= Ethanol Extract of *Guettarda speciosa* L. B.W= Body weight.

Table 2: Effect of Ethanol Extract of *Guettarda Speciosa* L. (EEGS) in pylorus ligation Induced ulcer model.

<table>
<thead>
<tr>
<th>Group</th>
<th>Design of Treatment</th>
<th>Ulcer Index</th>
<th>Percentage Inhibition (% I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (4% v/v aqueous tween 80, 10 ml/kg b.w ) p.o</td>
<td>17.24 ± 0.42</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>EEGS (200mg/kg b.w) p.o</td>
<td>8.92 ± 1.13*</td>
<td>48.26</td>
</tr>
<tr>
<td>III</td>
<td>EEGS (400mg/kg b.w) p.o</td>
<td>4.23 ± 0.21**</td>
<td>75.46</td>
</tr>
<tr>
<td>IV</td>
<td>Omeprazole (20mg/kg b.w) p.o</td>
<td>3.21 ± 0.32**</td>
<td>81.38</td>
</tr>
</tbody>
</table>

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett’s multiple comparison test. *P < 0.01 and **P < 0.001 as compared to control (n = 6 in each group). EEGS= Ethanol Extract of *Guettarda speciosa* L. B.W= Body weight.
Figure 1: Effect of Ethanol Extract of *Guettarda Speciosa* L. (EEGS) in ethanol (8 ml/kg) induced gastric ulcer in rats.

Figure 2: Effect of Ethanol Extract of *Guettarda speciosa* L. (EEGS) in pylorus ligation induced ulcer model.

Figure 3. An effect of *Guettarda Speciosa* L. extracts pretreatment on ethanol-induced gastric ulcer in rats. Stomach tissue was stained with hematoxylin and eosin (100 x). (A) Stomach after ethanol treatment. (B) Stomach treated with EEGS-200 mg/kg plus ethanol. (C) stomach treated EEGS-400 mg/kg plus ethanol, and (D) stomach treated with omeprazole-20 mg/kg plus ethanol.
DISCUSSION AND CONCLUSION

The ethanol extracts of *Guettarda speciosa* showed protective effects against ethanol and pylorus ligation induced gastric mucosal damage. The anti-ulcer effect of EEGS was tested against gastric lesions induced by ethanol, the experimental model related to lesion pathogenesis with production of reactive oxygen species. Reactive oxygen species are involved in the pathogenesis of ethanol-induced gastric mucosal injury in vivo (Plummer DI 1985). *Guettarda speciosa* prevented the mucosal lesions induced by ethanol. The gastric mucosal protection against ethanol can be mediated through a number of mechanisms that include enhancement of the gastric mucosal defense through increase in mucus and/or bicarbonate production, reducing the volume of gastric acid secretion or by simply neutralizing the gastric acidity (Antonio et al., 2004). EEGS may either reduce the gastric acid secretion or enhance the barrier defence of the mucosal wall. EEGS dose dependent inhibition in ethanol induced gastric lesions (Table 1 & Figure 1). Histopathological studies suggest that the ethanol damage to the gastrointestinal mucosa starts with microvascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting (Szabo et al., 1985). Hence histopathological studies conformed; EEGS showed dose dependent inhibition in ethanol induced gastric lesions (Figure 3).

In order to probe the effectiveness of EEGS in preventing gastric ulcer and also assess their antisecretory activity, they were tested against pylorus ligation induced ulcer. Pylorus ligation- (Sairam et al., 2003) induced ulcers are results of auto digestion of the gastric mucosal barrier probably due to excess production and accumulation of HCl in the stomach. Gastric acid is an important factor for the genesis of ulceration in pylorus-ligated rats (Shay H et al., 1945). The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligation is believed to increase gastric acid secretion (Baggio CH et al., 2003). The current data clearly demonstrated that, EEGS in a dose-dependent manner decreased hydrogenic concentration suggesting that the pharmacological mechanism has a relationship to antisecretory activity. (Table - & Figure 2). Phytochemical studies of the EEGS revealed the presence of alkaloids, flavonoids, and tannins which may be responsible for the anti-ulcer properties. Many compounds from these chemical classes such as nimbidine, ursolic acid, oleanolic acid, quatein, diosmin, wogonin and sophoradine (Sasajima et al., 1978; Pillai and Santhakumari, 1984; Danielson et al., 2003; Kahrman et al., 2003; Park et al., 2004) have been shown to possess anti-ulcer properties.

The ethanol extracts of *Guettarda speciosa* at a dose of 400mg/kg showed similar activity to that of omeprazole (a proton pump inhibitor). The gastroprotective effect of omeprazole is mediated through block of acid secretion by inactivation of H+/K+-ATPase (Fellenius et al., 1981). This study reveals that the EEGS are potent inhibitors of gastric mucosal lesions caused by ethanol and pylorus ligation in rats.

Further, our results fortify the ethanopharmacological importance of EEGS as an anti-ulcer agent. Etiology of ulcers produced in different ulcer models is diverse. The study was concluded EEGS and its active constituents may emerge as more effective therapeutic agent to counter gastric ulcer incidence.

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REFERENCES


