A Biochemical Study on the Gastroprotective Effect of Andrographolide in Rats Induced with Gastric Ulcer

P. SARANYA, A. GEETHA* AND S. M. K. NARMADHA SELVAMATHY
Department of Biochemistry, Bharathi Women's College (Affiliated to University of Madras), Chennai-600 108, India

Saranya, et al.: Gastroprotective Effect of Andrographolide

The major objective of the study was to evaluate the gastroprotective property of andrographolide, a chief component of the leaves of *Andrographis paniculata* in terms of the ulcer preventive effect in rats. An acute toxicity test was conducted with different concentrations of andrographolide to determine the LD_{50} value. The dose responsive study was conducted in rats pretreated with andrographolide (1, 3 and 5 mg/kg) for a period of 30 days, prior to ulcer induction by administering ethanol, aspirin or by pyloric ligation. The ulcer protective efficacy was tested by determining the ulcer score, pH, pepsin, titrable acidity, gastric mucin, lipid peroxides, reduced glutathione, and enzymatic antioxidants superoxide dismutase, catalase and glutathione peroxidase in gastric tissue. The activities of H^+ -K^+ ATPase and myeloperoxidase were also determined in gastric tissue. The LD_{50} value was found to be 48 mg/kg b. wt and the effective dose was found to be 3 mg/kg. We have observed a significant reduction in the ulcer score in rats pretreated with 3 mg of andrographolide/kg body weight. A favourable increase in the pH and decrease in titrable acidity were observed in the gastric fluid of rats pretreated with the test drug. The gastric tissue H^+ -K^+ ATPase and myeloperoxidase activities were elevated in ulcer-induced animals. The elevation in the enzyme activity was significantly minimized in the andrographolide received animals. The antioxidants and mucin levels were significantly maintained in the gastric tissue of drug-pretreated animals. Andrographolide did not produce any toxic effects in normal rats. This study reveals that the ulcer preventive efficacy of andrographolide may probably due to its antioxidant, cytoprotective and antiacid secretory effects.

Key words: *Andrographis paniculata*, andrographolide, gastric ulcer, H^+ -K^+ ATPase, lipid peroxides, mucin, myeloperoxidase

Gastric ulcer is one of the common disorders of human gastrointestinal system, affecting 10% of the world population and characterized by gastrointestinal bleeding, perforation and erosion of the mucosal wall due to excess acid secretion[1]. It is a disease of multifactorial etiology including *H. pylori* infection, use of antiinflammatory drugs, cigarette smoking, chronic alcohol consumption, stress and altered prostaglandin E metabolism[2,3]. There is a wide variation in the etiology of gastric ulcer in India and different experimental ulcer models have been applied to test the efficacy of new antiulcer drugs in our country.

Currently many allopathic drugs are being used for the treatment of ulcer that includes proton pump inhibitors and hydrogen receptor antagonists, which reduce acid secretion[4]. The therapeutic action of many antiulcer drugs is due to the inhibition of potassium ATP channel opening, free radical scavenging and prostaglandin-elevating actions. The antiacid secretory drugs on frequent usage are associated with adverse effects and ulcer relapse in many cases. From the time of its invention, ulcer has been treated traditionally by using medicinal plants. But the experimental proof is lacking for many herbal medicines used for ulcer treatment. A number of medicinal plants such as *Cistus laurifolius*, *Hypericum Scabrum*, *Plantago major*, *Sambuous ebulus* and *Spartium junceum* are experimentally proved to have antiulcer efficacy. The ever-increasing problem of gastric ulcer due to the advancement in life style and stress demands the identification and screening of many more gastroprotective medicinal plants, which are less toxic and cost effective in nature. The present study is an attempt to evaluate the gastroprotective efficacy of andrographolide isolated from *Andrographis paniculata*, a traditional medicinal plant widely
used in folklore medicine to treat gastrointestinal complications.

*Andrographis paniculata* (Acanthaceae) popularly known as kalmegh of ayurveda and green chirata, is a bitter plant so named as *Bhumi Neem*. *Andrographis paniculata* has been used as a remedy for liver complications, fever and respiratory tract infections. The plant is also well known for its immunostimulant, antimalarial, antithrombogenic, antiinflammatory and antipyretic activities[5]. Andrographolide, the chief constituent of leaves is a water-soluble lactone claiming for many pharmacological effects of the plant. Andrographolide itself has been claimed for antiinflammatory, antimicrobial[6], antiplatelet aggregation[7], hepatoprotective[8] and antiHIV activities.

Andrographolide has aroused the interest of many pharmacologists to unravel its hidden medicinal properties and research work has been undertaken in many parts of the world. New investigations on the pharmacokinetics have been reported after oral administration of andrographolide[9]. Since the leaves of *Andrographis paniculata* have been traditionally consumed for gastric complications, the present investigation is focused on the evaluation of ulcer protective efficacy of andrographolide by testing with various experimental ulcer models in rats.

**MATERIALS AND METHODS**

Ethanol, aspirin, alcian blue, diethyl ether, ATP and NADH were purchased from SRL Chemical Company, India. All other reagents and chemicals used were of analytical grade. Male albino Wistar rats (120-140 g) were obtained from Kings Institute, Chennai, India. They were acclimatized to animal house conditions, fed commercial pelleted rat chow (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. Animals were maintained according to the rules and regulations laid down by the Institutional Ethics Committee (290/04/V/CPCSEA/IAEC/PHA-24-27).

**Plant collection and identification:**
The plant was purchased from the local market in Chennai and authenticated by Dr. Jayaraman, Taxonomist, Plant Anatomy Research Centre, Chennai (Voucher No: of the Specimen: PARC/ 2008/ 185).

**Isolation of andrographolide:**
The shade dried leaves (100 g) of *Andrographis paniculata* were macerated in methanol and kept at room temperature for 3 days[10]. After filtration, the methanol was evaporated under reduced pressure. The residue (6.8 g) was partitioned between ethyl acetate and water (1:1). The water-soluble portion was extracted with n-butanol and filtered to obtain butanol soluble portion (1.5 g). The crude andrographolide in the precipitate (0.6 g) was chromatographed on a silica gel column using chloroform/methanol as a solvent to yield pure andrographolide (0.2 g) and tested by TLC by comparing with the pure compound. The identification of andrographolide was further confirmed by comparing its spectral data in the literature.

**Treatment protocol for acute toxicity study:**
The acute toxicity study was carried out as per guidelines revised by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Acute toxicity studies were performed on male Wistar albino rats. Rats were administered with 10, 20, 50 and 100 mg/kg of andrographolide as a single oral dose and physical and biochemical changes were recorded. The maximum non lethal dose was found to be 48 mg/kg. Hence 1, 3 and 5 mg/kg of andrographolide were taken for dose responsive study.

**Dose responsive study and dosage fixation:**
An initial dose responsive study was conducted in rats treated orally with 1, 3 and 5 mg/kg of andrographolide for 30 days to find out the optimal ulcer protective dose against ethanol, aspirin and pylorus ligation induced gastric ulcer in rats. A dose of 3 mg/kg of andrographolide was then selected on the basis of optimal ulcer protective effect for further studies. Among the three models studied ethanol induced ulcer model was chosen for the evaluation of gastroprotective activity of andrographolide.

**Study protocol:**
Rats were divided into V groups of six animals in each group. Group I rats treated with vehicle DMSO (50 mM-0.5 ml) served as control. Group II rats were administered with EtOH to induce ulcer. Group III animals were pretreated with andrographolide in (3 mg/kg for a period of 30 days) DMSO (50 mM-0.5 ml) prior to ulcer induction. Group IV animals
were pretreated with ranitidine (30 mg/kg for a period of 30 days) prior to ulcer induction. Group V animals were treated with andrographolide (3 mg/kg for a period of 30 days).

**Ethanol induced ulcer:**
Gastric ulcer was produced by oral administration of ethanol (1 ml/200 g) to rats after 12 h fasting. The animals were sacrificed after 1 h of ethanol administration[^11].

**Aspirin induced ulcer:**
Aspirin was administered at the dose of 200 mg/kg and ulcer score was determined after 4 h. The stomach was cut open along the greater curvature and ulcer index was scored by a person unaware of the experiment protocol in the glandular portion of the stomach[^12].

**Pylorus ligation induced ulcer:**
Test or the standard drug was administered to rats for a period of 30 days. On day 30, after the last dose, the rats were kept for 18 h fasting. Rats were anaesthetized using diethyl ether, the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. After replacing the stomach carefully, the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the postoperative period[^13]. After 4 h, stomach was dissected out and the contents collected and ulcer score determined. The ulcer index of gastric mucosal lesions was evaluated by the score system reported by Nie et al[^14].

**Determination of acid secretory parameters:**
The animals were sacrificed by sinus puncture, stomach was dissected out and the gastric juice collected was centrifuged for 5 min at 2000 rpm and the volume of the supernatant was expressed as ml/100 g and pH was measured using pH meter. Total acid output was determined by titrating the contents with 0.01N NaOH, using phenolphthalein as indicator and was expressed as mEq/l/100 g.

**Determination of pepsin activity:**
Pepsin was assayed according to the method of Shay et al[^15], using haemoglobin as substrate. The pepsin content was expressed as µM of tyrosine liberated/ml.

**Determination of gastric mucin content:**
The barrier mucus of gastric tissue was estimated by the method of Corne et al.[^16]. The dissected stomach was soaked for 2 h in 0.1% Alcian blue. Dye complexed with mucus was diluted by immersion in 10 ml aliquots of 0.5 M MgCl₂ for 2 h. The resulting blue solution was shaken with equal volume of diethyl ether and optical density of aqueous phase was measured at 605 nm. The mucus content was expressed in terms of µg of Alcian blue/g of glandular tissue.

**Determination of myeloperoxidase activity:**
Myeloperoxidase (MPO) activity was assayed according to the method of Bradley et al[^17]. The MPO activity was measured by following the oxidation of O-dianisidine dihydrochloride by H₂O₂. Results were expressed as U/g tissue.

**Determination of H⁺-K⁺ ATPase activity:**
Proton potassium ATPase was prepared from gastric parietal cell extract[^18]. The protein concentration was determined by using bovine serum albumin as standard[^19]. The H⁺-K⁺ ATPase activity in the parietal cell extract was assayed by the method of Reyes-Chilpa et al[^20]. The amount of inorganic phosphorous released from ATP was determined spectrophotometrically at 640 nm. The enzyme activity was expressed as nM of Pi liberated/min/mg protein.

**Estimation of lipid peroxides, reduced glutathione and antioxidant enzymes:**
The excised stomach tissue was treated with 5 ml of 0.1M Tris-HCl buffer, pH 7.4, homogenized on ice using Potter-Elvehjem glass homogenizer for 15 min. The homogenate was used for the estimations. Lipid peroxides in terms of thiobarbituric acid reacting substances (TBARS) was estimated using 1,1',3,3'-tetramethoxypropane as the standard and expressed as nM/mg protein[^21]. Glutathione (GSH) content of gastric tissue was determined by the method of Moron et al[^22]. Glutathione peroxidase (GPx) was assayed by the method of Flohe and Gunzler[^23]. The activity of GPx was expressed as nM GSH oxidized/min/mg protein.

Superoxide dismutase (SOD) activity was measured according to the method of Kakkar et al[^24]. The inhibition of reduction of nitroblue tetrazolium (NBT) to blue colored formazan in presence of phenazine methosulphate (PMS) and NADH was measured at 560 nm using n-butanol as blank. The enzyme activity was expressed as units/mg protein. Catalase
(CAT) activity was determined by monitoring the decomposition of $H_2O_2$, measured at 240 nm. The enzyme activity was expressed as $\mu$M of $H_2O_2$ decomposed/min/mg protein.

**Statistical analysis:**
Data were analyzed by using a commercially available statistics software package (SPSS for window V.7.5). Student’s t test was performed and results were presented as mean+SEM and statistical significance was evaluated a $P<0.05$.

**RESULTS AND DISCUSSION**

Ulcers are formed due to over production of gastric acid or decrease in gastric mucosal protective mechanisms. It is already known that the suppression of prostaglandin synthesis by NSAIDs, such as indomethacin results in increased susceptibility to mucosal injury and causes gastric ulceration. Some of the anti-ulcerogenic and ulcer-healing drugs are known to possess the property of inhibiting acid secretion in gastric mucosa.

Table 1 presents the ulcer score measured in the gastric wall of the experimental animals subjected to ulcer induction. The ulcer score was significantly reduced in rats pretreated with andrographolide. Ethanol induced ulcers are due to direct necrotizing effect of ethanol on gastric mucosa. Ethanol toxicity is associated with necrosis of superficial cells of gastric mucosa and erosion. Aspirin induced ulcers are due to mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of $H^+$ ions and thus leading to breaking up of mucosal barriers.

In pylorus ligation, ulcers are developed due to accumulation of gastric acid and pepsin, which leads to auto-digestion of gastric mucosa. Pepsin and HCl play major role in the formation of pylorl ligation induced ulcer. Ulcer score was reduced in all the experimental models of ulcer. The ulcer reducing effect was comparable with that of the standard drug ranitidine. Optimum effect was seen in the rats received 3 mg of andrographolide per kg. Hence 3 mg of andrographolide/kg was used as the effective dosage against ethanol induced ulcer model for further study.

The ulcer score was minimized significantly and the bloody streaks, oozing of blood into the lumen of the stomach observed in ulcerogen treated animals were significantly minimum in andrographolide-pretreated rats. Therefore, we assessed the protective effect by investigating the biochemical parameters such as gastric mucin content, pepsin, myeloperoxidase, $H^+-K^+$ ATPase, TBARS, GSH and antioxidant enzymes CAT, SOD and GPx in the ulcerated stomach of andrographolide/ranitidine pretreated rats.

Ulcer induced animals by ethanol showed a significant decrease in gastric pH when compared to that of normal control rats. Treatment with andrographolide was found to enhance the pH, which is a measure of reduction in acidity. Volume of gastric juice was found to be reduced in drug treated animals.

In this study the titrable acidity was found to be decreased significantly in drug treated animals when compared to that of ulcer induced animals without any treatment. The effect was comparable to that of the standard drug ranitidine (Table 2). The acidity reducing effect may be due to the influence of andrographolide on the acid secreting mechanism or the inhibitory action on the enzyme $H^+-K^+$ ATPase.

<p>| TABLE 1: ULCER SCORE IN ULCER-INDUCED ANIMALS PRETREATED WITH ANDROGRAPHOLIDE |
|---------------------------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Treatment protocol</th>
<th>Ethanol (Ulcer score)</th>
<th>Aspirin (Ulcer score)</th>
<th>Pylorus ligation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats treated with ulcerogens</td>
<td>4.63±0.46</td>
<td>4.01±0.44</td>
<td>4.70±0.52</td>
</tr>
<tr>
<td>Rats pretreated with 1 mg/kg b wt of andrographolide for 30 days +Ulcerogen</td>
<td>3.0±0.33*</td>
<td>2.9±0.36*</td>
<td>3.5±0.42*</td>
</tr>
<tr>
<td>Rats pretreated with 3 mg/kg b wt of andrographolide for 30 days +Ulcerogen</td>
<td>1.0±0.12*</td>
<td>1.0±0.13*</td>
<td>1.11±0.14*</td>
</tr>
<tr>
<td>Rats pretreated with 5 mg/kg b wt of andrographolide for 30 days +Ulcerogen</td>
<td>1.0±0.14*</td>
<td>0.97±0.23*</td>
<td>1.0±0.13*</td>
</tr>
<tr>
<td>30 mg/kg b wt of ranitidine for 30 days +Ulcerogen</td>
<td>1.19±0.12*</td>
<td>1.20±0.15*</td>
<td>1.25±0.17*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group. Statistically significant difference is expressed at *$P<0.05$. Groups are compared as: Control vs 1, 3 and 5 mg/kg of andrographolide + Ulcerogen.
myeloperoxidase and pepsin concentration in rats. The mucin content was found to be depleted significantly in ulcer induced animals without any treatment.

The gastric epithelium is covered by a continuous mucus layer, which adheres to the mucosal surface. This adherent mucus gel, together with bicarbonate secreted by the epithelium, serves as an unstirred buffering barrier against luminal acid. Endogenous PGE\textsubscript{2} plays an important role in maintaining gastric mucus synthesis and secretion. Andrographolide has possible role in enhancing mucosal resistance to acid and this could have offered gastroprotective effect against ethanol induced ulcer. This effect is evidenced from the mucin preserving nature of andrographolide. The animals pretreated with andrographolide showed a high reserve of mucin content in the gastric tissue.

H\textsuperscript{+}-K\textsuperscript{+} ATPase is a prime enzyme that influences secretion of acid in the stomach to aid digestion of food proteins whose activity was found to be elevated significantly in ulcer induced rats without any drug treatment. We have found that the andrographolide suppress H\textsuperscript{+}-K\textsuperscript{+} ATPase activity in ulcerogen treated rats and the effect was comparable with that of standard drug ranitidine which is a histamine H\textsubscript{2} receptor blocker.

Gastric H\textsuperscript{+}-K\textsuperscript{+} ATPase is a key proton pump that exchanges H\textsuperscript{+} and K\textsuperscript{+} ions across the canalicular membrane in acid secreting parietal cells. Proton pump inhibitors (omeprazole, lansoprazole, rabeprazole and pantoprazole) are initially absorbed via the small intestine and distributed to the gastric parietal cells and get activated in the acidic environment. The proton pump is the main component responsible for the acidic environment of the stomach and drugs those inhibit this enzyme represent the principal pharmacological treatment for ulcer. The modern approach to control gastric ulceration comprised of agents which act by scavenging reactive oxygen species, inhibiting H\textsuperscript{+}-K\textsuperscript{+} ATPase pump, preventing excess acid secretion and eradication of \textit{H. pylori} infection. In rats pretreated with the test drug the enzyme activity was not found to be elevated significantly.

Pepsin and myeloperoxidase activities were also found to be elevated in ulcer-induced animals without andrographolide treatment.

### Table 2: Effect of Andrographolide on pH, Volume of Gastric Fluid and Titrable Acidity in Rats Induced with Ulcer by Ethanol

<table>
<thead>
<tr>
<th>Treatment Protocol</th>
<th>pH</th>
<th>Volume of Gastric Juice (ml/100g b. wt.)</th>
<th>Titrable Acidity (mEq/l/100g/b. wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.13±0.32</td>
<td>3.99±0.38</td>
<td>92.41±9.98</td>
</tr>
<tr>
<td>EtOH</td>
<td>1.3±0.15*</td>
<td>3.88±0.45*</td>
<td>139.1±15.71*</td>
</tr>
<tr>
<td>Rats pretreated with 3 mg/kg b wt of andrographolide for 30 days + EtOH</td>
<td>2.42±0.32*</td>
<td>2.63±0.14*</td>
<td>113.0±4.91*</td>
</tr>
<tr>
<td>Rats pretreated with 30 mg/kg b wt of ranitidine for 30 days + EtOH</td>
<td>3.23±0.37*</td>
<td>3.62±0.40*</td>
<td>94.6±9.51*</td>
</tr>
<tr>
<td>3 mg/kg b wt of andrographolide for 30 days</td>
<td>3.23±0.33\textsuperscript{NS}</td>
<td>3.42±0.40\textsuperscript{NS}</td>
<td>94.0±11.62\textsuperscript{NS}</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group. Statistically significant difference is expressed at *P<0.05 and NS non significant. Groups are compared as: Control vs EtOH, EtOH vs andrographolide (3 mg/kg), EtOH vs ranitidine, control vs andrographolide (3 mg/kg).

### Table 3: Effect of Andrographolide on Mucin and Pepsin Content and the Activities of H\textsuperscript{+}-K\textsuperscript{+} ATPase and Myeloperoxidase in Gastric Tissue

<table>
<thead>
<tr>
<th>Treatment Protocol</th>
<th>Mucin Content (µg Alcian blue/g of glandular tissue)</th>
<th>Pepsin (µM of tyrosine liberated/ml)</th>
<th>H\textsuperscript{+}-K\textsuperscript{+} ATPase (nM of Pi liberated/min/mg protein)</th>
<th>Myeloperoxidase (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>470.0±49.8</td>
<td>442.12±4.38</td>
<td>1.74±0.18</td>
<td>3.00±0.34</td>
</tr>
<tr>
<td>EtOH</td>
<td>261.12±29.76*</td>
<td>660.11±81.19*</td>
<td>2.63±0.29*</td>
<td>11.92±1.38*</td>
</tr>
<tr>
<td>Rats pretreated with 3 mg/kg b wt of andrographolide for 30 days + EtOH</td>
<td>420.11±39.69*</td>
<td>460.01±60.72*</td>
<td>1.8±0.11*</td>
<td>6.51±0.69*</td>
</tr>
<tr>
<td>Rats pretreated with 30 mg/kg b wt of ranitidine for 30 days + EtOH</td>
<td>478.12±48.1*</td>
<td>462.35±48.2\textsuperscript{NS}</td>
<td>1.78±0.19*</td>
<td>3.20±0.35*</td>
</tr>
<tr>
<td>3 mg/kg b wt of andrographolide for 30 days</td>
<td>474.11±53.10\textsuperscript{NS}</td>
<td>447.04±60.35\textsuperscript{NS}</td>
<td>1.65±0.18\textsuperscript{NS}</td>
<td>3.21±0.34\textsuperscript{NS}</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group. Statistically significant difference is expressed at *P<0.05 and NS non significant. Groups are compared as: Control vs EtOH, EtOH vs andrographolide (3 mg/kg), EtOH vs ranitidine, control vs andrographolide (3 mg/kg).
pepsin has been reported to protect the gastric mucosa even at a very low pH of 1.3\cite{35}. The degree of gastric ulceration has been associated with increase in the intragastric pressure, acidity and pepsin proteolytic activity. The role of pepsin in the pathogenesis of ulcer has been the subject of intense study and debated for many years. There are two difficulties that inherent in distinguishing between the role of acid alone Vs acid and pepsin, because acid containing gastric juice always contain pepsin and hydrogen ion concentration is a major determinant of the activity of pepsin\cite{36}. The activity of pepsin is significantly decreased in andrographolide pretreated and ulcer induced rats. The effect was comparable with that of the standard drug ranitidine.

Neutrophil infiltration into gastric mucosal tissues is also a critical process in the pathogenesis of variety of gastric ulcers. The neutrophil infiltration into the gastric mucosal tissues was checked by MPO activity. MPO level has been widely used as an index of neutrophil infiltration in various experimental gastric injuries\cite{37}. The release of MPO from gastric cells is another indication of the degree of ulceration. NSAIDs such as indomethacin exhibiting their effects via the inhibition of MPO activity\cite{39}. MPO activity was found to be increased in all the ulcer models studied. Andrographolide pretreated rats have shown a significant decrease in the activity of myeloperoxidase in gastric tissue.

In the present study, decrease in acidity and pepsin concentration and increase in pH were observed in ulcerated animals pretreated with andrographolide. These modulating effects are highly desirable for gastroprotective and anti ulcer effect of any drug.

Reactive oxygen species and other free radicals have been implicated in the pathogenesis of many gastrointestinal disorders including ulcer. Many antioxidant enzymes counteract with reactive oxygen species to prevent oxidative stress. Table 4 show the concentrations of TBARS and antioxidants measured in the gastric tissue of experimental animals. The level of TBARS in andrographolide treated rats was found to be significantly lower than that of rats received only ethanol.

The metabolism of ethanol generates superoxide radicals, which in turn promote lipid peroxidation\cite{39}. The stomach and the upper gastrointestinal tract are the main sites of ethanol metabolism, and recent studies have implicated the role of ethanol-generated free radicals to its toxicity. Ethanol induced lipid peroxidation and depletion of glutathione level has been stated as a mechanism of alcohol induced gastric injury\cite{27}. TBARS concentration was found to be elevated significantly with the compromised levels of antioxidants, glutathione and the enzymes GPx, CAT and SOD in ulcer induced animals without any drug treatment.

GSH is an important component of the intracellular protective mechanism against various noxious stimuli including oxidative stress. In the present study, we have observed a significant decrease in the concentration of glutathione with concomitant increase in lipid peroxide level. Diethyl malate has been reported to deplete gastric glutathione and causes TABLE 4: EFFECT OF ANDROGRAPHOLIDE ON TBARS, GSH AND ENZYMATIC ANTIOXIDANTS IN GASTRIC TISSUE

<table>
<thead>
<tr>
<th>Treatment protocol</th>
<th>TBARS (nM/mg protein)</th>
<th>GSH (nM/mg protein)</th>
<th>GPx (nM GSH oxidized/min/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (µM H₂O₂ decomposed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.0±0.10</td>
<td>6.10±0.64</td>
<td>212.0±28.62</td>
<td>46.01±5.3</td>
<td>3.99±0.46</td>
</tr>
<tr>
<td>EtOH</td>
<td>2.39±0.33*</td>
<td>3.46±0.46*</td>
<td>101.0±11.41*</td>
<td>39.11±4.49*</td>
<td>2.01±0.2*</td>
</tr>
<tr>
<td>Rats pretreated with 3 mg/kg b wt of andrographolide for 30 days + EtOH</td>
<td>1.25±0.15*</td>
<td>6.39±0.71*</td>
<td>196.1±20.08*</td>
<td>42.22±4.7*</td>
<td>3.47±0.38*</td>
</tr>
<tr>
<td>Rats pretreated with 30 mg/kg b wt of ranitidine for 30 days + EtOH</td>
<td>0.95±0.09*</td>
<td>6.08±0.62*</td>
<td>202.1±21.42*</td>
<td>45.01±4.59*</td>
<td>3.83±0.42*</td>
</tr>
<tr>
<td>EtOH</td>
<td>0.98±0.11*</td>
<td>6.20±0.71*</td>
<td>219.0±29.3*</td>
<td>45.0±5.4*</td>
<td>3.87±0.43*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group. Statistically significant difference is expressed at *P<0.05 and NS non significant. Groups are compared as: Control vs EtOH, EtOH vs andrographolide (3 mg/kg), EtOH vs ranitidine, control vs andrographolide (3 mg/kg).
severe gastric ulceration indicating the defensive role played by glutathione in ulcer formation\(^{[40]}\). This alteration was minimized in ulcer-induced rats pretreated with andrographolide.

Superoxide dismutase inactivates superoxides and catalyzes prevents the accumulation of \( \text{H}_2\text{O}_2 \). SOD detoxifies the superoxide anions to produce less toxic \( \text{H}_2\text{O}_2 \) which is scavenged by CAT\(^{[41]}\). Hence decrease in CAT and SOD levels could have lead to accumulation of these reactive products and thereby to free radical mediated tissue damage in ulcerogen treated animals. The results of this study showed that andrographolide restored the activities of antioxidant enzymes. This shows the preventive effect of andrographolide on free radical formation during ulcerogenesis.

In this study we have proved the potent ulcer preventing effect of andrographolide without any toxic side effects. The structure of andrographolide elucidated by Medforth \( \text{et al.}^{[42]} \), revealed that the bicyclic diterpene lactone contains three hydroxyl groups and two methyl groups. Generally the polar hydroxyl groups are good scavengers of ROS. So the effect of andrographolide on free radicals and the prevention of ulcer might be attributed to its chemical nature.

The results of the present investigation indicate that andrographolide isolated from \( \text{Andrographis paniculata} \) has potent antiulcer property by decreasing the gastric acid secretion by \( \text{H}^+\text{-K}^-\text{ATPase} \), decreasing the free radical formation and also by protecting the gastric mucin from acid and free radical induced damage. The test drug has been found to be non toxic when tested with normal rats. The present study revealed that andrographolide can be claimed for the gastroprotective effect of \( \text{Andrographis paniculata} \) the traditional medicinal plant used for gastric complications.

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