Antiulcer activity of *Andrographis paniculata* (Burm.f.) Wall. against cysteamine-induced duodenal ulcer in rats

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Antiulcer activity of *Andrographis paniculata* was evaluated by cysteamine induced duodenal ulcer model in rats. Male albino Wistar rats were pre-administered with 200 mg/kg body wt. of hydroalcoholic extract of *Andrographis paniculata* (HAEAP) orally, for 30 days prior to i.p. administration of 420 mg/kg body wt. of cysteamine as a single dose. Rats pre-administered with 30 mg/kg body wt. of ranitidine served as standard drug. Ulcer index, thiobarbituric acid reactive substances, mucin, glutathione peroxidase and myeloperoxidase activities, reduced glutathione/oxidized glutathione (GSH/GSSG) ratio, glycoproteins and membrane bound enzyme activities were measured in duodenum of experimental animals. The ulcer score and myeloperoxidase activity were significantly minimized in rats treated with HAEAP. Mucin content was found to be preserved in rats treated with the extract. GSH/GSSG ratio and glutathione peroxidase activities were found to be maintained by the HAEAP. Level of lipid peroxidation products was found to be significantly low in HAEAP treated rats compared to ulcer control rats. The basolateral and brush border membrane bound enzyme activities which were depleted significantly in ulcer control rats were found to be maintained in rats pre-treated with the extract. The ulcer preventing effect was comparable to that of ranitidine treated rats. Level of glycoproteins was also found to be preserved in rats treated with the extract. The normal rats treated with the HAEAP did not show any abnormal alterations in the parameters studied. Histopathological observations also showed the ulcer preventing effect of the HAEAP. It is suggested that the ulcer preventing effect may be due to its mucin preserving and antioxidant nature.

**Keywords**: *Andrographis paniculata*, Basolateral membrane, Brushborder membrane, Cysteamine, Duodenal ulcer, Mucin

Duodenal ulcer is characterized by inflammation, perforation and erosion of the duodenal linings that affects about 10% of the world population with multifactorial etiology including genetic factors. Hypersecretion of gastric acid and its defective neutralization by bicarbonate in the lumen of duodenum and *H. pylori* infection are the major causes of duodenal ulcer in human. Loss of mucosal resistance to acidity has been implicated in the pathogenesis of duodenal ulcer. Duodenal mucosa is frequently exposed to partially digested food mixed with gastric acid. Biliary and pancreatic alkaline secretions act in concert with duodenal mucosal secretion to neutralize the luminal contents. Despite variable acidic condition in the duodenal lumen, the surface epithelium is maintained at neutral $pH^{1,2}$. This is caused by bicarbonate transport by the mucosa into a layer of mucus adherent to the surface of the duodenal epithelium.

Although the role of acid secretion in duodenal ulcer has not been fully elucidated, clinical evidence indicates that the acidity may be minimized by proton pump inhibitors and histamine receptor antagonists which accelerates ulcer healing$^{3,4}$. Ranitidine, omeprazole and sucralfate have been the drugs of choice for duodenal ulcer. Though they are potent acidity reducers long term use has been associated with mild side effect and ulcer relapse in few cases. Hence, identification of new therapeutic agents from medicinal plants that are non-toxic and cost effective is warranted.

*Andrographis paniculata* (Burm.f.) Wall. a medicinal plant, well known as “Kalmegh” and “Green chiratta” and form the principal ingredient of the domestic medicine “Alui” (Bengali). It is referred as a wonder drug in Siddha and Ayurvedic formulations, used for liver and gastrointestinal ailments$^5$. Pharmacological and clinical studies suggest that *Andrographis paniculata* possess anti-inflammatory$^6$, anti-pyretic$^7$, anti-viral$^8$, immunostimulatory, hepato protective and cardio protective activities$^9$. In the present study, gastroprotective effect

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of hydroalcoholic extract of *Andrographis paniculata* (HAEAP) in rats administered with cysteamine induced inflammation and ulceration which mimic human duodenal ulcer has been reported.

**Material and Methods**

*Plant material*—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).

*Hydroalcoholic extract of Andrographis paniculata*—Air-dried whole aerial parts of *Andrographis paniculata* were ground into homogenous powder and freeze-dried. The freeze-dried material was extracted by refluxing 40-60 times with 70% ethanol for 6-8 h. The ethanol extract was evaporated to dryness to one third of the original volume and stored overnight at 4°C. The filtrate was lyophilized and the dry material obtained was used for the study.

*Animals and experimental groups*—Male albino Wistar rats (120-140 g) were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. They were acclimatized to animal house conditions for 10 days and maintained in a 12 h light/dark cycle at 25°±2°C and fed commercial pelleted rat chow (Hindustan Lever Ltd., Bangalore, India) and drinking water *ad libitum*. Animals were maintained according to the guidelines of Institutional Animal Ethics Committee (290/04/V/CPCSEA/IAEC/PHA-24-27). Rats were divided into 5 groups with 6 animals in each group. Group I which was treated with water served as control. Group II rats were administered with cysteamine to induce ulcer. Group III animals were pretreated with HAEAP (200 mg/kg body wt. for a period of 30 days) before ulcer induction. Group IV animals were pretreated with ranitidine (30 mg/kg body wt. for a period of 30 days) before ulcer induction. Group V animals were treated with HAEAP only (200 mg/kg body wt. for a period of 30 days) before ulcer induction. Group IV animals were pretreated with ranitidine (30 mg/kg body wt. for a period of 30 days) before ulcer induction. Group V animals were treated with HAEAP only (200 mg/kg body wt. for a period of 30 days). The dose responsive study was conducted before fixing the dose of 200 mg/kg body wt.

*Cysteamine-induced duodenal ulcer*—Animals were administered orally with 200 mg/kg body wt. of HAEAP once daily for 30 days and cysteamine (420 mg/kg) was administered after the last dose through i.p injection and the rats were fasted for 24 h with water given *ad libitum*. Last dose of HAEAP was given 30 min before the administration of cysteamine HCl. All the animals were killed 24 h after cysteamine treatment and the duodenum was excised carefully and opened along the antimesentric side to determine ulcer score. The dose was selected from the results of dose response study conducted earlier.

**Determination of ulcer index**—The ulcer index of duodenal lesions was evaluated by the score system reported by Makovee et al. The intestine mucosa was opened along the greater curvature and rinsed with 0.1 mol/L ice-cold phosphate buffered saline (pH 7.4). The mucosa was then examined with a 100x magnification to observe erosions and made scores as 1-5: 1. small round hemorrhagic erosion, 2. hemorrhagic erosion <1 mm, 3. hemorrhagic erosion = 1-2 mm, 4. hemorrhagic erosion = 2-3 mm, 5. hemorrhagic erosion > 4 mm. The score was multiplied by 2 when width of the erosion was lesser than 1 mm.

Duodenal mucosa was scrapped out for the isolation of basolateral and brush border membranes. Duodenum was dissected out, washed in ice – cold saline and 10% homogenate was prepared using 0.1M Tris – HCl buffer (pH 7.4) and used to assess the biochemical parameters.

**Biochemical investigations**

**Determination of mucin content**—Mucin content was determined by the method of Corne et al. The duodenum was excised and opened down along the lesser curvature. The reverted duodenum was soaked in 0.1% alcian blue (0.16 M sucrose buffered with 0.05 M sodium acetate). The uncomplexed dye was removed by 2 successive washes of 15 and 45 min in 0.25 M sucrose solution. The dye complexed with mucus was diluted by immersion in 10 ml of 0.5 M magnesium chloride for 2 h. The resulting blue solution was shaken briefly with equal volume of diethyl ether and optical density of the aqueous phase was measured at 605 nm. The mucin content of the sample was determined from the standard curve obtained with different concentration of mucin.

**Determination of myeloperoxidase activity**—Myeloperoxidase (MPO) activity in the duodenal mucosa was measured according to the method of Bradley et al. Pre-weighed tissue was homogenized (1: 10 wt/vol) in 0.5% hexadecyltrimethyl ammonium bromide in 50 mM potassium phosphate buffer (pH 6.0) before sonication in an ice bath for 20 sec. Three freeze/thaw cycles were performed followed by
sonication (20 sec in ice bath). The samples were centrifuged at 17000 g (5 min, 4°C) and myeloperoxidase in the supernatant was assayed by mixing 0.1 ml of supernatant and 2.9 ml of 50 mM potassium phosphate buffer (pH 6.0) containing 0.167 g/L o-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured for 4 min using an UV ELICO spectrophotometer.

Estimation of lipid peroxides (TBARS), reduced and oxidized glutathione (GSH and GSSG) and glutathione peroxidase (GPx)—The excised duodenal 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 homogenates was used for the estimations of TBARS, GSH, GSSG and GPx.

Lipid peroxides in terms of thiobarbituric acid reacting substances (TBARS) was estimated using 1, 1, 3, 3-tetra methoxypropane as the standard and expressed as nM/mg protein. GSH and GSSG were determined by the method of Moron et al. and Hansen et al. respectively. GSH was used as a reference standard and expressed as nM/mg protein. Glutathione peroxidase (GPx) was assayed by the method of Flohe and Gunzler . The activity of GPx was expressed as nM GSH oxidized/min/mg protein.

Determination of glycoprotein components in duodenal mucosa—Glycoproteins in duodenal mucosal tissues were precipitated, hydrolysed and the protein bound hexose, hexosamine, fucose and sialic acid were estimated. The known amount of defatted tissue was hydrolysed with 1.0 ml of 2 N HCl and 1% phosphotungstic acid at 100°C for 4 h to liberate the protein–bound compounds. The hydrolysate was neutralized with 4 N sodium hydroxide and was used for the estimation.

Estimation of hexose was done by the method of Niebes using orcinol reagent. The colour developed was read at 540 nm and values were expressed as mg/g tissue. Hexosamine was estimated by the method of Wagner using acetyl acetone reagent and Ehrlich’s reagent and read at 540 nm. The content of hexosamine was expressed as mg/g tissue. Analysis of sialic acid was carried out by the method of Warren . Periodate solution and thiobarbituric acid was added and the absorbance was read at 540 nm. The level of sialic acid was expressed as mg/g tissue. Fucose was estimated by the method of Winzler using H2SO4 reagent and cysteine- HCl. The absorbance was read at 396 nm. The fucose content was expressed as mg/g tissue.

Preparation of small intestinal brush border membrane—Small intestinal brush border membrane from control and experimental rats was prepared by the method of Schmitz et al. slightly modified by Kessler et al. and the membrane suspension used for the assay of disaccharides and alkaline phosphatases.

Isolation of basolateral membrane—The isolation of basolateral membrane was carried out by the method of Mircheff & Wright. The isolated basolateral membrane was used for the assay of ATPases.

Assay of total adenosine triphosphatase (Total ATP phosphohydrolase, EC 3.6.1.3)—Adenosine triphosphatase catalyses the hydrolysis of ester phosphates in the presence of cations such as Ca2+, Na2+, K+ etc., and release inorganic phosphorus. The amount of inorganic phosphorus liberated was the measure of enzyme activity and quantified by Fiske & Subbarow method.

Assay of sodium, potassium- dependent adenosine triphosphatases: (Na+, K+ ATPases) (Sodium, potassium-ATP phosphohydrolase, EC 3.6.1.3)—Na+,K+ -ATPases catalyse the hydrolysis of phosphate esters only in the presence of Na+ and K+ ions. The amount of inorganic phosphorus liberated in the presence of Na+ and K+ ions was the measure of enzyme activity and quantified as described earlier.

Assay of calcium-dependent adenosine triphosphatase (Ca2+-ATPases) (Calcium, ATP phosphohydrolase, EC 3.6.1.3)—The enzyme catalyzes the hydrolysis of phosphate esters only in the presence of Ca2+ ion. The amount of inorganic phosphorus liberated in the presence of Ca2+ ions was the measure of enzyme activity and quantified as described earlier.

Assay of Sucrase (Sucrose α-glucoside glucohydrolase, E.C. 3.2.1.48)—Sucrase activity was determined by the method of Dahlqvist . The reducing sugar produced by the enzymatic hydrolysis of non-reducing substrate sucrose was determined by the colour reaction with 3,5-dinitrosalicylic acid. The enzyme activity was expressed as n mol of glucose liberated/min/mg protein.

Assay of Maltase (β-D-glucoside glucohydrolase, E.C. 3.2.1.20)—The activity of intestinal brush border membrane bound maltase was determined by the method of Dahlqvist with the use of the coloring reagent as prescribed by Tauber & Klenier and Benedict. The enzyme activity was expressed as n mol of glucose liberated/min/mg protein.
Assay of alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC:3.1.3.1)—The procedure of Bowers & McComb\textsuperscript{14} was adopted for the assay of alkaline phosphatase (ALP). The rate of formation of 4-nitrophenol from 4-nitrophenyl phosphate was the basis of this analytical procedure.

**Histopathological examination**

A portion of duodenum obtained from all experimental groups were washed immediately with saline and then fixed in 10\% formalin. After fixation, the duodenal tissue was processed and embedded in paraffin. After dehydrating and cleaning, the sections were mounted and stained with hematoxylin and eosin and observed under light microscope (100\×). The histo-architectural changes were photographed for documentation.

**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 ± S.E.M. A \( P \) value of <0.05 was considered as statistically significant.

**Results**

*Effect of HAEAP on ulcer index*—Ulcer index in the duodenal mucosa observed in all the experimental animals is presented in Table 1. Rats treated with 200 mg/kg body wt. of HAEAP showed significant reduction in ulcer index when compared to those of rats left without HAEAP treatment.

*Effect of HAEAP on the levels of adherent mucin content, the activities of myeloperoxidase, lipid peroxidation products, GSH/GSSG ratio and GPx*—Table 2 represent the activity levels of myeloperoxidase and GPx, concentration of TBARS, mucin and GSH/GSSG ratio in the duodenum of experimental animals. The malondialdehyde concentration and myeloperoxidase activity were found to be elevated in cysteamine treated rats. A significant reduction was seen in the level of mucin content, GSH/GSSG ratio and GPx in ulcer induced animals. In HAEAP pretreated rats there was no significant elevation when compared to that of normal rats.

*Effect of HAEAP on the level of glycoproteins*—The levels of glycoproteins in the duodenal mucosa were shown in Table 3. The levels of glycoproteins such as hexose, hexosamine, fucose and sialic acid were significantly decreased in cysteamine treated rats, whereas rats pretreated with HAEAP restored to near normal level.

*Effect of HAEAP on the levels of brush border and basolateral membrane*—Table 4 shows the activities of adenosine triphosphatases in the duodenal basolateral membrane and dissacharidases in the duodenal brush border membrane of experimental and control animals. In HAEAP pretreated rats there was no significant reduction was seen in the level of mucin and GSH/GSSG ratio.

### Table 1—Ulcer score in experimental animals treated with cysteamine

<table>
<thead>
<tr>
<th>Treatment protocol</th>
<th>Ulcer index (mm)\textsuperscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats treated with</td>
<td></td>
</tr>
<tr>
<td>Cysteamine</td>
<td>30.65 ± 2.16</td>
</tr>
<tr>
<td>HAEAP 200 mg/kg b. wt for 30 days + Cysteamine</td>
<td>10.15 ± 0.17*</td>
</tr>
<tr>
<td>Ranitidine 30 mg/kg b. wt for 30 days + Cysteamine</td>
<td>9.80 ± 0.10*</td>
</tr>
</tbody>
</table>

Statistically significant difference is expressed as \*\(P<0.001\), \# \(P<0.01\) and ns - non significant. Groups are compared as: control vs cysteamine, cysteamine vs HAEAP (200 mg/kg body wt.) and ranitidine, control vs HAEAP (200 mg/kg body wt.)

### Table 2—Levels of myeloperoxidase, TBARS, mucin, GPx activity and GSH/GSSG ratio in the duodenum of experimental animals

<table>
<thead>
<tr>
<th>Treatment protocol</th>
<th>Myeloperoxidase (U/g tissue)</th>
<th>TBARS (nM/mg protein)</th>
<th>Mucin content (µg alcian blue/g of glandular tissue)</th>
<th>GSH/GSSG ratio</th>
<th>GPx (nM GSH oxidized/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats treated with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (Water)</td>
<td>5.8±0.61</td>
<td>0.97±0.09</td>
<td>600.06±66.01</td>
<td>15.29±1.63</td>
<td>252.01±26.46</td>
</tr>
<tr>
<td>Cysteamine 420 mg/kg body wt.</td>
<td>7.17 ± 0.79*</td>
<td>2.51±0.26*</td>
<td>420.02±44.11*</td>
<td>6.32±0.68*</td>
<td>109.89±12.09*</td>
</tr>
<tr>
<td>HAEAP 200 mg/kg body wt. for 30 days + Cysteamine</td>
<td>5.1 ± 0.56*</td>
<td>0.92±0.09*</td>
<td>540.13±60.49*</td>
<td>12.71±1.48*</td>
<td>232.13±24.37*</td>
</tr>
<tr>
<td>Ranitidine 30 mg/kg body wt. for 30 days + Cysteamine</td>
<td>5.3 ± 0.59*</td>
<td>0.87±0.10*</td>
<td>560.22±61.19*</td>
<td>12.53±1.37*</td>
<td>235.13±25.85*</td>
</tr>
<tr>
<td>HAEAP 200 mg/kg body wt. for 30 days</td>
<td>6.1 ± 0.63\textsuperscript{**}</td>
<td>0.94±1.11\textsuperscript{**}</td>
<td>620.01±68.20\textsuperscript{**}</td>
<td>14.63±1.75\textsuperscript{**}</td>
<td>243.21±26.02\textsuperscript{**}</td>
</tr>
</tbody>
</table>

Statistically significant difference is expressed as \*\(P<0.001\), \# \(P<0.01\) and ns - non significant. Groups are compared as: control vs cysteamine, cysteamine vs HAEAP (200 mg/kg body wt.) and ranitidine, control vs HAEAP (200 mg/kg body wt.)
control groups. The enzyme activities was found to be depleted significantly in ulcerogen treated rats. The HAEAP treated animals significantly reduced these alterations. The animals received only the HAEAP showed the normal activity levels of intestinal basolateral and brush border membrane bound enzymes.

**Histological studies**—The cysteamine induced duodenal ulcer was characterized by marked mucosal lesion, including hemorrhagic spots and inflammatory changes. On gross examinations these spots and lesions were characterized by different sizes. Ulcerated rats pretreated with HAEAP show very mild lesions and rats treated with HAEAP alone showed normal duodenal mucosa. Histological observations of duodenum extracted from control and experimental rats were presented in Fig 1. Rats administered with cysteamine showed mucosal and submucosal edema with erosion and mononuclear cell infiltration in lamina propria. Rats pretreated with the HAEAP and standard drug showed normal intact epithelium. Rats which received only the HAEAP did not show any significant alterations in the duodenal tissue architecture when compared with those of normal control rats.

**Discussion**

Cysteamine induced duodenal inflammation in rats offer a simple and reliable model for the investigation of new drugs against ulcers. Many reports have

<table>
<thead>
<tr>
<th>Treatment protocol</th>
<th>Hexose (mg/g tissue)</th>
<th>Hexoseamine (mg/g tissue)</th>
<th>Sialic acid (mg/g tissue)</th>
<th>Fucose (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (Water)</td>
<td>14.57±1.51</td>
<td>8.72±0.89</td>
<td>1.83±0.18</td>
<td>3.23±0.33</td>
</tr>
<tr>
<td>Cysteamine 420 mg/kg body wt.</td>
<td>8.49±0.87*</td>
<td>4.07±0.41*</td>
<td>0.61±0.06*</td>
<td>1.22±0.12*</td>
</tr>
<tr>
<td>HAEAP 200 mg/kg body wt. for 30 days +Cysteamine</td>
<td>13.92±1.43*</td>
<td>8.5±0.87*</td>
<td>1.67±0.17*</td>
<td>3.17±0.32*</td>
</tr>
<tr>
<td>Ranitidine 30 mg/kg body wt. for 30 days +Cysteamine</td>
<td>13.98±1.45*</td>
<td>8.42±0.85*</td>
<td>1.72±0.18*</td>
<td>3.29±0.34*</td>
</tr>
<tr>
<td>HAEAP 200 mg/kg body wt. for 30 days</td>
<td>14.62±1.54ns</td>
<td>8.78±0.898ns</td>
<td>1.83±0.18ns</td>
<td>3.31±0.36ns</td>
</tr>
</tbody>
</table>

Statistically significant difference is expressed as *P<0.001 and ns - non significant. Groups are compared as: control vs cysteamine, cysteamine vs HAEAP (200 mg/kg body wt.) and ranitidine, control vs HAEAP (200 mg/kg body wt.).

<table>
<thead>
<tr>
<th>Treatment protocol</th>
<th>Na⁺,K⁺ ATPase (n mol of phosphorus liberated/min/mg protein)</th>
<th>Ca⁺⁺ ATPase (n mol of phosphorus liberated/min/mg protein)</th>
<th>Total ATPase (n mol of phosphorus liberated/min/mg protein)</th>
<th>Sucrase (n mol of glucose liberated/min/mg protein)</th>
<th>Maltase (n mol of glucose liberated/min/mg protein)</th>
<th>Alkaline phosphatase (µ mol of phenol liberated/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (Water)</td>
<td>8.79 ± 0.96</td>
<td>2.63 ± 0.29</td>
<td>22.02 ± 2.51</td>
<td>6.01 ± 0.65</td>
<td>2.56 ± 0.26</td>
<td>6.71 ± 0.71</td>
</tr>
<tr>
<td>Cysteamine 420 mg/kg body wt.</td>
<td>7.73 ± 0.82†</td>
<td>1.87 ± 0.20*</td>
<td>14.31 ± 1.52</td>
<td>3.54 ± 0.37</td>
<td>1.91 ± 0.20*</td>
<td>4.27 ± 0.56*</td>
</tr>
<tr>
<td>HAEAP 200 mg/kg body wt. for 30 days +Cysteamine</td>
<td>8.65 ± 0.93†</td>
<td>2.35±0.21*</td>
<td>20.01 ± 2.22*</td>
<td>4.93 ± 0.52*</td>
<td>2.27 ± 0.22*</td>
<td>5.72 ± 0.61*</td>
</tr>
<tr>
<td>Ranitidine 30 mg/kg body wt. for 30 days + Cysteamine</td>
<td>8.77 ± 0.95†</td>
<td>2.31 ± 0.24*</td>
<td>19.89 ± 2.19*</td>
<td>4.98 ± 0.55*</td>
<td>2.39 ± 0.24*</td>
<td>6.19 ± 0.71*</td>
</tr>
<tr>
<td>HAEAP 200 mg/kg body wt. for 30 days</td>
<td>8.75 ± 1.01ns</td>
<td>2.52 ± 0.27ns</td>
<td>21.83 ± 2.29ns</td>
<td>5.73 ± 0.59ns</td>
<td>2.41±0.25ns</td>
<td>7.15 ± 0.81ns</td>
</tr>
</tbody>
</table>

Statistically significant difference is expressed as *P<0.001, †P<0.01, ‡P<0.05 and ns - non significant. Groups are compared as: control vs cysteamine, cysteamine vs HAEAP (200 mg/kg body wt.) and ranitidine, control vs HAEAP (200 mg/kg body wt.).
Fig. 1—Photomicrographs of intestinal mucosa in control and experimental rats (H&E 100×). (a)-control : normal mucosal villi; (b)-cysteamine treated group : duodenum with focal disruption of mucosal epithelium with mucosal and submucosal edema and erosion; (c)-HAEAP+cysteamine treated group : very mild mononuclear cell infiltration in lamina propria; (d)-Ranitidine + cysteamine treated group : normal pattern of tissue architecture with mild inflammation; (e)-HAEAP treated group : normal mucosal layer.
revealed that cysteamine administration depletes the immuno reactive somatostatin in gastric and duodenal mucosa which is essential for the maintenance for the cell integrity and production of duodenal bicarbonate. So the bioavailability of somatostatin is reduced accompanied by increase the gastrin level and gastric acid secretion due to cysteamine treatment. Cysteamine has also shown to reduce bicarbonate secretion that is essential to neutralize the gastric acid released to the duodenum. In the present investigation a significant decrease has been observed in the ulcer index in rats pretreated with HAEAP when compared to that of ulcer control rats which did not receive HAEAP.

Cysteamine induced duodenal ulcer was characterized by marked inflammatory changes and hemorrhagic spots in the duodenum. Ulcer induced rats pretreated with HAEAP showed very mild lesions giving more evidence for the ulcer preventing nature of Andrographis paniculata. The ulcer reducing potential of HAEAP was found to be similar to that of standard drug ranitidine. The mode of action of ranitidine is reported to act as H₂ receptor antagonist to control the acid production in stomach.

A significant elevation in the activity of myeloperoxidase in the duodenum of cysteamine received rats has been observed. Myeloperoxidase is a major neutrophil protein and also present in monocytes. When tissues are subjected to oxidative insult, the neutrophils and monocyte accumulate at the site of inflammation. The heme containing enzyme that uses the superoxide and hydrogen peroxide generated by the neutrophils and produce hypochlorous acid and other reactive oxidants during phagocytosis to kill the pathogens. Klebanoff has shown that the myeloperoxidase system is strongly bactericidal and considered as an important component of neutrophil associated antimicrobial mechanism.

Increased formation of reactive oxygen metabolite has been implicated in the pathogenesis of many inflammatory condition including gastrointestinal tract disorders and peptic ulcer. An important observation is that the pathogenicity of H. pylori in the upper gastrointestinal tract might be related to stimulation of neutrophils that cause reactive oxygen metabolite production. The result of the present investigation showed that the elevation in the level of TBARS in cysteamine received rats was significantly minimized in HAEAP pretreated rats. This might be due to the lipid peroxidation preventing role of the HAEAP during ulcerogenesis. HAEAP has been shown to enhance antioxidant potential in experimental animals. The HAEAP has been reported to contain rich amount of flavonoids such as quercetin, Biochin A and Formononetin and these flavonoids can be claimed for antilipid peroxidative effect observed in this study. Prabha et al. have reported that the antiulcer activity of Pongamia pinnata may be attributed to the presence of flavonoids that act as antioxidants.

Reduced glutathione along with glutathione peroxidase and glutathione S transferase (GST) plays a central role in the defense against free radicals. Thiols such as glutathione are able to counteract free radicals and may influence the physical properties of mucous since its subunits are joined by disulphide bridges. A significant depletion in the ratio of GSH:GSSG and GPx in cysteamine treated rats has been observed. GSH/GSSG ratio is an indicator of thiol redox status in tissues and the ratio was found to be significantly low in cysteamine administered rats showing the influence of reduced thiols on the inflammatory changes induced by cysteamine. Mukherjee et al. have also proved that the antioxidant property of licorice may be assurance for its gastroprotective activity.

Mucin is a viscous glycoprotein with physiochemical properties producing relatively resistant acid barrier. It makes up the major part of the mucus, an important pre-epithelial factor that acts as a first line of defense against ulcerogenesis. In duodenum, gene Muc2 is responsible for production of mucin to offer protection against offensive factors such as gastric acidity and free radicals. The thiol redox status was maintained to provide antioxidant support in HAEAP treated rats.

The high molecular weight of this glycoprotein is responsible for viscous and gel forming characteristics of the mucus. The importance of the mucus secretion as a response to gastric mucosal trauma has long been recognized. Mucus also protects the mucosal and the sub mucosal layer from inflammatory reaction. Mucosal defense agents are a new dimension in the treatment of gastro-duodenal diseases.

The levels of fucose, sialic acid, hexose and hexosamine were significantly low in ulcerogen treated rats and the levels were found to be preserved in HAEAP treated rats. Fucose containing glycans
exist in the terminal region or serve as an attachment point for adding other sugars. In human, fucose is most commonly linked by α-1,6 to the reducing terminal β-N-acetylgalactosamine. Sialic acid is found widely distributed in animal tissues and to a lesser extent in other species ranging from plants and fungi to yeasts and bacteria, mostly in glycoprotein and gangliosides. Sialic acid-rich glycoproteins bind selectins in humans and other organisms. These glycoproteins are essential for the maintenance of membrane integrity in mucosal cells of the duodenum. The membrane glycoprotein preserving nature of the antiulcer drug Pongamia pinnata has been reported.

The brush border membrane is essentially important for digestion and transport of nutrients and other substrates by virtue of its unique biochemical composition. It contains digestive enzymes such as disaccharidases, viz., sucrase, lactase and maltase and alkaline phosphatase, dipeptidase, enterokinases etc. The diminution in the activity of disaccharidases and alkaline phosphatase has also been reported in various gastrointestinal disorders. A significant decrease in the activities of these enzymes may result in the reduced nutrient supply for energy production. In HAEAP received rats these activities were found to be near normal.

Duodenal ion transport processes are supported by ATPase enzymes in basolateral membranes of the enterocytes. Transmembrane ATPases import many of the metabolites for cell metabolism and export toxins, wastes, and solutes that can hinder cellular processes. An important example is the sodium-potassium exchanger (or Na+/K+ATPase), which establishes the ionic balance that maintains the cell potential. Cysteamine treated rats showed depletion in the Na+K+ ATPase, Ca2+ ATPase and total ATPases whereas drug pretreated rats did not show much significant alterations and the activities were comparable with that of standard drug ranitidine.

It may be concluded that the HAEAP treatment resulted in the reduction of ulcer index and related damage in the duodenal mucosa. The HAEAP has been found to reduce the ulcer score and the extent of inflammation associated with reduced level of free radical formation. The antioxidant role and the mucin protective effect of Andrographis paniculata may be claimed for the above mentioned therapeutic action as an ulcer preventing agent.

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