A study on the gastroprotective effect of *Andrographis paniculata* and andrographolide in rats subjected to pylorus ligation

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**ABSTRACT**

The study investigated the anti ulcer effect of *Andrographis paniculata* (AP) (Acanthacea) and andrographolide (AGL), a chief compound present in the leaves of AP in male albino Wistar rats. Rats pretreated with hydroalcoholic extract of *Andrographis paniculata* (HAEAP) (100, 200 and 500 mg/kg b.wt) or AGL (1, 3 and 5 mg/kg b. wt) orally for a period of 30 days were subjected to pylorus ligation (PL). We have found that HAEAP (200 mg/kg b.wt) or AGL (3 mg/kg b.wt) markedly decreased the incidence of ulcer. The test drugs were found to reduce the activities of pepsin, H+K+ATPase and myeloperoxidase when compared to PL rats without drug treatment. HAEAP or AGL was found to maintain the level of GSH, mucin and enzymatic antioxidants. The results indicate that the gastroprotective activity of AP and AGL may probably due to its antisecretory and antioxidant property.

**Key words:** *Andrographis paniculata*, andrographolide, gastric ulcer, H+K+ATPase, mucin, myeloperoxidase.

**INTRODUCTION**

Gastric hyperacidity and ulceration of the stomach mucosa due to various factors are serious health problems of global concern. 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. The pathophysiology of ulcer involve an imbalance between offensive (acid, pepsin and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors).

Although a number of anti-ulcer drugs such as H$_2$ receptor antagonists, proton pump inhibitors and cytoprotectants are currently used for the treatment, their use is limited due to side effects. This demands the search for natural medicines from plant sources. This has been the basis for the development of new anti-ulcer agents, which include herbal substances. In traditional practice of medicine, several plants and herbs have been used to treat gastrointestinal disorders, including gastric ulcers.

*Andrographis paniculata* also known as ‘king of bitters’ is the member of plant family Acanthacea and has been used for centuries in Asia to treat gastrointestinal discomfort, liver disorders and a variety of other chronic and infectious diseases. It is included in twenty six ayurvedic formulations shown in ancient Indian pharmacopoeia. In Traditional Chinese Medicine (TCM), AP is considered as the herb possessing an important “cold property” useful to treat the heat of body in fevers, and to dispel toxins from the body. In Scandinavian countries, it is commonly used to prevent and treat common cold and flu.

AGL, the major constituent of the leaves, is a bicyclic diterpenoid lactone, claimed for the pharmacological effects of AP. Leaf extract of AP and AGL were found to be effective in preventing carbon tetra chloride induced liver damage in rats and mice. The present study was aimed to investigate the anti-ulcer potential of AP and AGL in rats subjected to PL.

**MATERIAL AND METHODS**

**Plant collection and identification:**

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

**Hydroalcoholic extract of Andrographis paniculata (HAEAP)**

The air-dried 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 hrs. The extract was evaporated to dryness and stored at 4º C. The filtrate was lyophilized and the dry material obtained was used for the study.

**Isolation of AGL:**

The shade dried leaves (100 g) of AP were macerated in methanol and kept at room temperature for 3 days. After filtration, the methanol was evaporated to dryness. 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 AGL in the precipitate (0.6 g) was chromatographed on a silica gel column using chloroform/methanol as a solvent to yield pure AGL (0.2 g) and tested by TLC by comparing with the pure compound. The identification of AGL was further confirmed by comparing its spectral data in the literature.

**Animals:**

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).

**Dose response study:**

An initial dose response study was conducted in rats treated with 100, 200 and 500 mg/kg b.wt of HAEAP or 1, 3 and 5 mg/kg b.wt of AGL orally for 30 days, to find out the optimal ulcer protective dose against pylorus ligation induced gastric ulcer in rats. A dose of 200 mg/kg b.wt of HAÆAP or 3 mg/kg b.wt of AGL was then selected on the basis of reducing ulcer score for further studies. Similar dose response study was conducted with 15, 30, 50 mg/kg b.wt of ranitidine and 30 mg/kg b.wt was found to be the optimum dose.

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**Treatment protocol for antiulcer activity**

Rats were divided into five groups of six animals each.

**Group I** - Control rats.

**Group II** - Rats subjected to pylorus ligation.

**Group III** - Rats pretreated with 200mg/kg b. wt of HAEAP or 3mg/kg b. wt of AGL for 30 days followed by pylorus ligation.

**Group IV** - Rats pretreated with 30mg/kg b. wt of standard drug ranitidine for 30 days followed by pylorus ligation.

**Group V** - Rats treated with 200mg/kg b. wt of HAEAP or 3mg/kg b. wt of AGL for a period of 30 days.

**Pylorus ligation (PL)**

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 anesthetized 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. After 4 h, the stomach was dissected out, the contents collected and ulcer score determined.

**Determination of ulcer score:**

The ulcer index of gastric mucosal lesions was evaluated by the score system reported by Nie et al., 2003. 

**Determination of acid secretory parameters:**

The animals were sacrificed by sinus puncture, stomach was dissected out and the gastric juice collected was centrifuged for five min at 2000 rpm and the volume of the supernatant was expressed as ml/120 g b.wt 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.

**Determination of pepsin activity:**

Pepsin was assayed according to the method of Shay et al., 1945 using hemoglobin as substrate. The absorbance of the solution was read at 650 nm. The pepsin content was expressed as µM of tyrosine liberated/ml.

**Determination of gastric mucin content:**

The barrier mucous of gastric tissue was estimated by the method of Corne et al., 1974. 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 solutions were shaken with equal volumes of diethyl ether and optical density of aqueous phase was measured at 605 nm. The barrier mucus 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., 1982. 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. The protein concentration was determined by using bovine serum albumin as standard.

The H+K+-ATPase activity in the gastric tissue was assayed by the method of Reyes- Chilpa et al., 2006. 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 analyses.

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. 

Glutathione (GSH) content of gastric tissue was determined by the method of Moron et al., 1979. Glutathione peroxidase (GPx) was assayed by the method of Flohe and Gunzler, 1984. 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., 1984. The inhibition of reduction of nitroblue tetrazo- lium (NBT) to blue colored formazan in the 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. Decomposition of H2O2 in the presence of CAT was followed at 240 nm. The enzyme activity was expressed as µM of H2O2 consumed/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 ± S.E.M.

**RESULTS AND DISCUSSION**

**Ulcer index**

Ulcer index, a measure of gastric mucosal lesion is used to assess the anti-ulcerogenic efficacy of any new drugs to be investigated. The pylorus ligation induced gastric damage was characterized by marked gross mucosal lesion including hemorrhagic bands, spots and lesions of different sizes along the long axis of glandular stomach. Ulcerated rats pretreated with HAEAP or AGL or ranitidine showed very mild lesions. The rats received the test drugs showed a significant reduction in ulcer index and was comparable to that of the standard drug ranitidine (Table1).

Table 1: Ulcer score in rats subjected to pylorus ligation

<table>
<thead>
<tr>
<th>Rats pretreated with HAEAP or AGL or Ranitidine for 30 days (dose in mg/kg b.wt)</th>
<th>Pylorus ligation</th>
<th>Ulcer score % protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water)</td>
<td>4.8±0.52</td>
<td>-</td>
</tr>
<tr>
<td>HAEAP (100)</td>
<td>3.7±0.44*</td>
<td>23</td>
</tr>
<tr>
<td>HAEAP (200)</td>
<td>1.3±0.19*</td>
<td>73</td>
</tr>
<tr>
<td>HAEAP (500)</td>
<td>1.25±0.15*</td>
<td>74</td>
</tr>
<tr>
<td>AGL (1)</td>
<td>3.5 ± 0.42*</td>
<td>27</td>
</tr>
<tr>
<td>AGL (3)</td>
<td>1.1±1.04*</td>
<td>77</td>
</tr>
<tr>
<td>AGL(5)</td>
<td>1.0± 0.13*</td>
<td>79</td>
</tr>
<tr>
<td>Ranitidine (15)</td>
<td>3.2±0.40*</td>
<td>33</td>
</tr>
<tr>
<td>Ranitidine (30)</td>
<td>1.25±0.17*</td>
<td>74</td>
</tr>
<tr>
<td>Ranitidine (50)</td>
<td>1.2±1.05*</td>
<td>75</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group. Statistically significant difference is expressed as *p<0.001, #p<0.01. Groups are compared as: PL vs HAEAP or AGL or ranitidine.

Pyloric ligation induced ulcers are due to imbalance between offensive and defensive mucosal factors and is the ideal model to infer the mechanism by which a drug works as an anti-ulcerogenic agent. Gastric ulcer in this model occurs because of an increase in acid pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion and breakdown of the gastric mucosal barrier. A copious amount of mucus is secreted during superficial damage and provides favorable microenvironment for repair process. Hence estimation of gastric acidity, pepsin and mucin content is a valuable part of the study to clarify the mechanism of action of the drug under trial.

**Effect of HAEAP and AGL on several parameters in rats with PL induced gastric ulcer**

Table 2 shows the effect of HAEAP and AGL on the acid secretory parameters, mucin, H+K+-ATPase and pepsin activity. Gastric acid is produced by parietal cells (also called oxyntic cells) in the stomach. Parietal cells contain an extensive secretory network (called Canaliculi) from which the gastric...
Acid is secreted into the lumen of the stomach. These cells are part of epithelial fundic glands in the gastric mucosa. The pH of gastric acid is 2 to 3 in the human stomach lumen, the acidity being maintained by the proton pump \( H^+K^-ATPase \). In ulcer control rats, there was a significant increase in the volume of gastric juice when compared to those which received the drug prior to ulcer induction. The pH of gastric fluid collected at the time of sacrifice showed decrease in pH and increase in titrable acidity in ulcerated rats without drug treatment. Ulcer induced rats pretreated with the test drugs showed significant increase in pH and decrease in acidity as shown in Table 2.

Gastric acid activates pepsinogen into pepsin, which helps in digestion of peptide bond linking the aminocids, a process known as proteolysis. Pepsin, the proteolytic enzyme present in gastric lumen that digests the food protein was found to be elevated significantly in ulcer induced animals without drug treatment in such an extent to digest the mucosal protein to cause inflammation. Pretreatment with the test drugs were found to reduce pepsin concentration to near normal level and the effect was comparable to that of ranitidine (Table 2).

Proton pump inhibitors (PPIs) have emerged as the treatment of choice for acid related diseases such as peptic ulcer and gastroesophageal reflux diseases. The responsible ion pump for acid secretion is the \( H^+K^-ATPase \) located in the apical membrane of the parietal cells and transports \( H^+ \) into the parietal cell canaliculus ion exchange for \( K^+ \). \( H^+K^-ATPase \) inhibitors such as omeprazole, rabeprazole and lansoprazole are irreversible inhibitors of the enzyme, which are activated in the acidic environment. The activity of \( H^+K^-ATPase \) in ulcerated rats was found to be elevated and it was significantly decreased in test drugs treated rats (p<0.01) but not lesser than that of control rats (Table 2). We have previously reported that AP inhibited the activity of gastric mucosal \( H^+K^-ATPase \), in vitro and the effect was comparable to that of the standard drug omeprazole. So it is possible that inhibition of the enzyme activity may lead to reduction in gastric acidity by AP. In the present study, increase in pH, decrease in acidity and pepsin concentration were evidenced in ulcerated animals pretreated with the test drugs, which is highly desirable for gastroprotective and anti-ulcer effects.

Gastric wall mucus is thought to play an important role as defensive factors against gastric mucosal damage. The gastric wall mucus that contains mucin is used as an indicator for gastric wall integrity. Mucin acts as an antioxidant and thus can reduce the mucosal injury mediated by oxygen free radicals. When cells containing mucus are damaged by extracellular oxygen radicals, intracellular mucus may be released into the gastric tissue and prevent additional damage by scavenging these radicals. Gastric adherent mucin content of ulcerated rats was significantly reduced when compared to that of control rats and the level was found to be preserved in test drugs received rats (Table 2).

### Table 2: Effect of HAEAP and AGL on gastric acidity and the activity of \( H^+K^-ATPase \) subjected to pylorus ligation

<table>
<thead>
<tr>
<th>Rats pretreated with</th>
<th>pH</th>
<th>Volume of gastric juice (ml/120-g)</th>
<th>Titratable acidity (mEq/ml)</th>
<th>Mucin content (µg Alcian blue of glandular tissue)</th>
<th>Pepisin concentration (µm of tyrosine liberated/min/mg protein)</th>
<th>( H^+K^-ATPase ) (nM of Pi liberated/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.17 ± 0.31</td>
<td>3.40 ± 0.36</td>
<td>92.43 ± 9.96</td>
<td>470.0 ± 49.8</td>
<td>443.45 ± 35.21</td>
<td>1.87 ± 0.20</td>
</tr>
<tr>
<td>PL</td>
<td>1.19 ± 0.12</td>
<td>4.56 ± 0.56</td>
<td>151.33 ± 11.50</td>
<td>242.0 ± 30.49</td>
<td>634.22 ± 71.03</td>
<td>2.44 ± 0.30*</td>
</tr>
<tr>
<td>HAEAP (200) + PL</td>
<td>2.32 ± 0.25*</td>
<td>0.97 ± 0.11*</td>
<td>85.13 ± 7.81*</td>
<td>479.35 ± 41.72</td>
<td>437.17 ± 38.19*</td>
<td>1.75 ± 0.20*</td>
</tr>
<tr>
<td>AGL (3) + PL</td>
<td>3.16 ± 0.23*</td>
<td>2.20 ± 0.13*</td>
<td>84.21 ± 8.14*</td>
<td>467.60 ± 48.49</td>
<td>440.0 ± 44.55</td>
<td>1.72 ± 0.17*</td>
</tr>
<tr>
<td>Ranitidine (30) + PL</td>
<td>3.41 ± 0.36*</td>
<td>3.53 ± 0.35*</td>
<td>93.99 ± 9.41</td>
<td>476.65 ± 47.65</td>
<td>452.12 ± 46.7*</td>
<td>1.77 ± 0.18*</td>
</tr>
<tr>
<td>AGL (3)</td>
<td>3.23 ± 0.33NS</td>
<td>3.42 ± 0.40NS</td>
<td>96.04 ± 11.62NS</td>
<td>482.01 ± 54.94NS</td>
<td>449.21 ± 45.32NS</td>
<td>1.90 ± 0.23NS</td>
</tr>
<tr>
<td>HAEAP (200)</td>
<td>3.37 ± 0.37NS</td>
<td>3.50 ± 0.42NS</td>
<td>97.07 ± 11.45NS</td>
<td>474.11 ± 53.10NS</td>
<td>445.34 ± 44.35NS</td>
<td>1.92 ± 0.20NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals in each group. Statistically significant difference is expressed as *p<0.001, †p<0.05 and NS - non significant. Groups are compared as: Control vs PL, HAEAP or AGL or ranitidine vs PL, control vs HAEAP or AGL.

### Effect of HAEAP and AGL on MPO activity and TBARS and GSH levels in gastric tissue

### Table 3: Effect of HAEAP and AGL on MPO activity and TBARS and GSH levels in gastric tissue

<table>
<thead>
<tr>
<th>Rats pretreated with HAEAP or AGL or Ranitidine for 30 days (dose in mg/kg b.wt)</th>
<th>Myeloperoxidase (U/g tissue)</th>
<th>TBARS (nmol/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7.62 ± 0.78</td>
<td>1.03 ± 0.10</td>
<td>6.16± 0.64</td>
</tr>
<tr>
<td>PL</td>
<td>14.21 ± 1.06*</td>
<td>1.62 ± 0.18*</td>
<td>150.03 ± 18.02*</td>
</tr>
<tr>
<td>HAEAP (200) + PL</td>
<td>7.11 ± 1.01*</td>
<td>1.13 ± 0.12*</td>
<td>232.92 ± 27.95*</td>
</tr>
<tr>
<td>AGL (3) + PL</td>
<td>6.61 ± 0.69*</td>
<td>1.10 ± 0.15*</td>
<td>233.18 ± 29.38*</td>
</tr>
<tr>
<td>Ranitidine (30) + PL</td>
<td>6.19 ± 0.96*</td>
<td>0.99 ± 0.13*</td>
<td>233.18 ± 29.38*</td>
</tr>
<tr>
<td>AGL (3)</td>
<td>7.26 ± 0.74*</td>
<td>1.20 ± 0.13*</td>
<td>231.10 ± 30.04*</td>
</tr>
<tr>
<td>HAEAP (200)</td>
<td>7.43 ± 0.76*</td>
<td>0.98 ± 0.11*</td>
<td>231.12 ± 27.73*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals in each group. Statistically significant difference is expressed as *p<0.001, †p<0.05 and NS - non significant. Groups are compared as: Control vs PL, HAEAP or AGL or ranitidine vs PL, control vs HAEAP or AGL.

### Table 4: Effect of HAEAP and AGL on enzymatic antioxidants in gastric tissue

<table>
<thead>
<tr>
<th>Rats pretreated with HAEAP or AGL or Ranitidine for 30 days (dose in mg/kg b.wt)</th>
<th>GPXs (nmol of GSH oxidized/min/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (µmol of ( H_2O_2 ) decomposed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>212.01 ± 28.62</td>
<td>46.28 ± 5.33</td>
<td>4.01 ± 0.46</td>
</tr>
<tr>
<td>PL</td>
<td>107.11 ± 11.65*</td>
<td>34.91 ± 2.70</td>
<td>2.27 ± 0.23*</td>
</tr>
<tr>
<td>HAEAP (200) + PL</td>
<td>151.01 ± 17.66*</td>
<td>43.16 ± 4.29</td>
<td>3.21 ± 0.35*</td>
</tr>
<tr>
<td>AGL (3) + PL</td>
<td>132.06 ± 16.63*</td>
<td>44.43 ± 4.66</td>
<td>3.45 ± 0.39*</td>
</tr>
<tr>
<td>Ranitidine (30) + PL</td>
<td>194.18 ± 20.0*</td>
<td>43.52 ± 4.73</td>
<td>3.73E ± 0.84*</td>
</tr>
<tr>
<td>AGL (3)</td>
<td>221.11 ± 24.98*</td>
<td>45.09 ± 5.04</td>
<td>4.35 ± 0.45*</td>
</tr>
<tr>
<td>HAEAP (200)</td>
<td>219.01 ± 29.34*</td>
<td>45.02 ± 5.41</td>
<td>3.87 ± 0.30*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals in each group. Statistically significant difference is expressed as *p<0.001, †p<0.05 and NS - non significant. Groups are compared as: Control vs PL, HAEAP or AGL or ranitidine vs PL, control vs HAEAP or AGL.

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damage the biomolecules by peroxidation. Lipid peroxidation can be measured as diene conjugates or TBARS in tissues and body fluids. A reactive free radical generated in the body reacts with non-radicals and results in propagation of new free radicals. The level of TBARS in the test drugs treated rats were found to be significantly lower than that of pylorus ligated rats (Table 3) without drug treatment.

Glutathione is a tripeptide and contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain. Glutathione, as an antioxidant, protect cells from the harmful reaction of reactive oxygen species such as free radicals and peroxides. Thiol groups are kept in a reduced state at a concentration of approximately ~5 mM in animal cells. In effect, glutathione reduces any disulfide bond formed within cytoplasmic proteins to cysteines by acting as an electron donor. In the process, glutathione is converted to its oxidized form glutathione disulfide. In the present study, we have observed that treatment with the test drugs maintain the level of GSH in the rat gastric tissue when compared to that of ulcer control rats.

Effect of HAEAP and AGL on enzymatic antioxidants in gastric tissue Table 4 shows the enzymatic antioxidants measured in the gastric tissue of experimental animals. Severity of ulcer is known to increase with significant decrease in antioxidant status. Various antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase control the accumulation of free radicals.

Preventive antioxidant enzymes such as SOD and CAT provide the first line of defense against ROS. SOD scavenges the super oxide radical $O_2^-$, one of the reactive oxygen species responsible for lipid peroxidation. This reaction leads to increase in generation of peroxyl radical HOO which is also capable of producing more oxidative damage.

CAT and other peroxidases further reduce $H_2O_2$. Metal ions like copper and zinc play important role in the activity of superoxide dismutase and the interaction of hydroxyl radicals was reported to induce loss of activity.

The toxic action of free radicals is largely dependent upon the activity of these enzymes in gastric tissue in which they are formed. In the present study we have observed that the HAEAP and AGL significantly restored the enzyme activities (Table 4). This shows the preventive effect of AP and AGL on free radical formation during ulcerogenesis.

This study concludes that AP and AGL act as ulcer preventive agents probably by decreasing the action of offensive factors and increasing the protective action of mucin, antioxidants and thiols.

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