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Antisecretory Effect of the Combination of Pegagan (*Centella Asiatica*) and Sambiloto (*Andrographis paniculata*) Leaves Ethanol Extracts on Pyloric Ligation-Induced Gastric Ulcer in Rats

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ABSTRACT

Pegagan (*Centella asiatica*) and sambiloto (*Andrographis paniculata*) contain flavonoid compounds that can provide anti-ulcer effect. This study aims to investigate the antisecretory effect of the combination of *C. asiatica* and *A. paniculata* leaves ethanol extract on pyloric ligation-induced gastric ulcer in rats. The animals were treated with a combination of 100:75, 100:150, 200:75 and 200:150 mg/kg BW extract for 7 days after fasting for 48 hours prior to pyloric ligation. Furthermore, the animals were sacrificed 19 hours after pyloric ligation while the stomach was isolated for macroscopic, microscopic and gastric secretion studies. The combination of *C. asiatica* and *A. paniculata* leaves ethanol extract with 100:75, 100:150, 200:75, and 200:150 mg/kg BW doses showed antisecretory effect against ulcers induced by pyloric ligation. There was a significant difference ($p < 0.05$) between the combination of the extract and the control group in terms of ulcer score, ulcer and mucus indexes, volume, pH and acidity of gastric secretion, which showed a fair healing effect of 78.08%. Based on the results, the combination of *C. asiatica* and *A. paniculata* leaves ethanol extract have antisecretory effect with the most effective dose being 200:150 mg/kg BW.

Keywords: Antiseretory effect, Combination, *Andrographis paniculata*, *Centella asiatica*, Gastric ulcers, Pyloric ligation

Introduction

Ulcers occur due to an imbalance between aggressive stomach acid and mucosal defense factors.[1] The digestive tract is lined with a mucous membrane which protects the main tissues against corrosion due to high gastric acid secretion. However, when the amount of acid persists, pH is significantly reduced, or mucous membrane layer becomes thin, the acid damages the tissue and ulcer occurs. The possible causes of gastric ulcers include *Helicobacter pylori* infection, non-steroid anti-inflammatory drug (NSAIDs) and stress-related mucosal damage.[2] In addition, factors such as stress, smoking, spicy foods and nutritional deficiencies also contribute to ulcer development.[1,2] Some commonly used antiulcer drugs include sucralfate and omeprazole. Sucralfate causes side effects such as constipation and hypophosphatemia while omeprazole is nephrotoxic and causes acute hypersensitivity reactions.[3,4]
Centella asiatica (*C. asiatica*) has been used as a traditional medicine for healing wound[5] and also as neuroprotectant.[6] The major compounds of the plant are triterpenes, asiatic acid[7] together with madecassic acid and derivatives such as ester glycosides, asiaticoside and madecassoside.[8] Previous studies stated that *C. asiatica* has anti-inflammatory,[9] antioxidant[10] and anti-gastric ulcers effects.[11–14] Meanwhile, *Andrographis paniculata* (*A. paniculata*) contains andrographolide diterpenoid glycosides,[15] flavonoids,[16] quercetin, formononetin and biochin.[17]
A. paniculata has been reported to have anti-lipid peroxidation and anti-inflammatory as well as antioxidant effects.[18–20]
The gastroprotective potential of the plant extract is associated with high content of flavonoids secondary metabolites and other polyphenols. The secondary metabolite function to protect the cells from damage, as antioxidant or cytoprotective. The protective effect of these cells is relevant to ulcer condition and is called gastroprotective. Meanwhile, the gastroprotective effects of *C. asiatica* and *A. paniculata* leaves ethanol extracts have not been reported. Therefore, this study aims to investigate the gastroprotective effects of the extracts combination on pyloric ligation-induced ulcer model.

Materials and Methods

Collection of plant material
A. paniculata was collected from Pancur Batu, Deli Serdang, Sumatera Utara Province, Indonesia while *C. asiatica* was collected from Berastagi, Sumatra Utara Province, Indonesia. The samples were collected in February 2019 and were identified in Herbarium Medanense (MEDA), Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatra Utara (identification number 4601/MEDA/2019 and 4602/MEDA/2019). The fresh leaves used in the study were then dried in a drying cabinet.

Preparation of ethanol extract
The *C. asiatica* and *A. paniculata* leaves extraction was carried out using percolation method with 96% ethanol. One kilogram of powdered sample was soaked with 10 L of 96% ethanol in a closed container for a minimum of 3 hours and the mixture was transferred gradually into the percolator. The solvent was poured sufficiently into the percolator until the liquid began to drip and maintained a layer of solvent over the sample for 24 hours. The liquid was allowed to drip with a speed of 20 to 60 drops/minute. Afterwards, percolation was

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carried out until the ethanol extraction of *C. asiatica* and *A. paniculata* was completely obtained and then concentrated using a rotary evaporator. The extract was further evaporated in a water bath until a concentrated extract was obtained.[21]

Experimental animals
A total of 32 male Wistar rats aged 8 to 15 weeks and weighing 150-200 g were obtained from the Pharmacology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. The animals were acclimatized for a week and were fed standard pellets and provided water *ad libitum*. All the experimental protocols were approved by the Animal Research Ethics Committee (AREC), Universitas Sumatera Utara (Approval number 0631/KEPH-MIPA/2019).

Pyloric ligation induced gastric ulcer in rats
The rats were divided into 8 groups each consisting of 4 animals and were pretreated orally with standard drugs, extracts or vehicles for 7 days.
Group 1, which served as the normal control group was treated with normal diet; Group 2 as the ulcer control was treated with a normal diet; Group 3, as vehicle control group was treated orally with carboxymethyl cellulose sodium suspension; Group 4, as a positive control was treated with ranitidine 27 mg/kg BW; Groups 5, 6, 7 and 8 were treated with a combination of *C. asiatica* leaves (CAEE) and *A. paniculata* leaves ethanol extract (APEE) at doses of 100:75, 100:150, 200:75 and 200:150 mg/kg BW, respectively. Meanwhile, pyloric ligation was done for all the groups except group 1.
On the 7th day the rats were fasted for 48 hours prior to pyloric ligation and 1 hour after the last dose. The animals were anaesthetized with ketamine while pyloric ligation was carried out without causing a disruption in blood supply to the pylorus. Furthermore, the abdomen was sutured, and the animals were restrained for 19 hours. Animals were sacrificed under chloroform anesthesia. The stomach was isolated while gastric juices were collected and centrifuged.

Gastric macroscopic examination
Macroscopic observations consisted of a number of gastric ulcers, ulcer score and index as well as ulcer inhibition percentage. The gastric ulcer index data were used to calculate the percentage of ulcer inhibition.

Gastric microscopic studies
The cleaned gastric tissue was fixed in 10% formalin solution for slide preparation. Gastric tissue was embedded in paraffin blocks and slides were stained with hematoxylin-eosin and were observed under a light microscope.[21]

Gastric secretion studies
Gastric secretion parameters included gastric secretion volume and pH, mucus production and gastric HCl concentration. The volume and pH were measured from the gastric fluid collected and centrifuged at 3000 rpm for 10 minutes.[22,23] Thereafter, gastric mucus was gently scrapped from the stomach surface with a glass and weighed. The mucus index was calculated from the stomach weight percentage[24] and the acidity of gastric fluid was measured by titrating 1 mL of centrifuged gastric fluid with 0.01 N NaOH solution using a phenolphthalein indicator.[23] Meanwhile, the acidity of gastric fluid was calculated from the equation:

$$Acidity\ (mEqL^{-1}) = \frac{(Volume\ of\ NaOH\ x\ Normality\ x\ 100)}{0.1}$$

Statistical analysis
The data were presented as mean ± standard error of mean. The data were analyzed using one-way analysis of variance with the SPSS 22.0 program, followed by Tukey post-test with a confidence level of 95%.

Results and Discussion

Gastric macroscopic studies
The result showed that the administration of *C. asiatica* (CAEE) and *A. paniculata* ethanol extract (APEE) significantly reduced the number of ulcers, ulcer scores and indexes (Figure 1). There were no ulcers in normal control group, while the ulcer control group which was induced by pyloric ligation showed the highest mean number of ulcers compared to other groups.
A decrease in the mean number indicated a positive ulcer healing effect. The positive control group which was treated with ranitidine 27 mg/kg BW had the lowest mean number of stomach ulcers. Compared to the ulcer control group, the mean number of ulcers in the combination of CAEE and APEE decreased as the dose increased. Meanwhile, the best effect was observed at the dose of CAEE 200: APEE 150 mg/kg BW with a significant reduction of all ulcer parameters (Table 1).

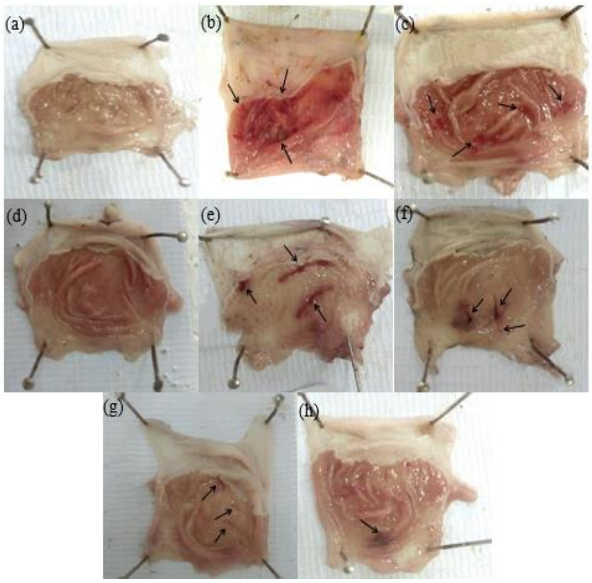

Figure 1: Gastric macroscopic studies:
(a) normal control group; (b) ulcer control group; (c) vehicle control group; (d) positive control group; (e) CAEE 100: APEE 75 mg/kg BW; (f) CAEE 100 : APEE 150 mg/kg BW; (g) CAEE 200 : APEE 75 mg/kg BW; (h) CAEE 200 : APEE 150 mg/kg BW. Black arrow indicates a lesion.

Table 1: Macroscopic studies of gastric after treatments (Mean ± SEM)

Group of treatments	Number of ulcers	Ulcer score	Ulcer index
Normal control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Ulcer control	6.75 ± 0.85^{d}	4.53 ± 0.21^{d}	6.12 ± 0.53^{d}
Vehicle control	4.75 ± 0.48^{cd}	4.00 ± 0.25^{cd}	3.47 ± 0.41^{c}
Ranitidine 27 mg/kg BW	2.00 ± 0.41^{ab}	1.54 ± 0.36^{b}	0.23 ± 0.05^{a}
CAEE 100 : APEE 75 mg/kg BW	4.25 ± 0.48^{b}	3.48 ± 0.21^{cd}	3.60 ± 0.32^{c}
CAEE 100 : APEE 150 mg/kg BW	3.75 ± 0.48^{bc}	3.24 ± 0.16^{cd}	2.57 ± 0.35^{bc}
CAEE 200 : APEE 75 mg/kg BW	3.75 ± 0.48^{bc}	3.53 ± 0.54^{cd}	2.18 ± 0.21^{bc}
CAEE 200 : APEE 150 mg/kg BW	3.00 ± 0.41^{bc}	2.92 ± 0.42^{bc}	1.34 ± 0.13^{ab}

BW = body weight; different superscript notation = significantly different ($P < 0.05$)

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Table 2: Ulcer inhibition (Mean ± SEM)

Group of treatments	% Ulcer Inhibition
Ranitidine 27 mg/kg BW	96.33 ± 1.07^{d}
CAEE 100 : APEE 75 mg/kg BW	41.22 ± 5.27^{a}
CAEE 100 : APEE 150 mg/kg BW	58.01 ± 5.71^{ab}
CAEE 200 : APEE 75 mg/kg BW	64.11 ± 3.55^{bc}
CAEE 200 : APEE 150 mg/kg BW	78.08 ± 2.17^{c}

BW = body weight; different superscript notation = significantly different (P<0.05)

The test groups showed a decrease in mean ulcer score which indicated a reduction in the area of ulcer compared to the negative control. Moreover, the combination, of ethanol extract dose of CAEE 200: APEE 150 mg/kg BW had the lowest mean ulcer score. The effectiveness of gastric ulcer healing process depends on APEE from the decrease of ulcer index. Hence, decrease in ulcer index results in better gastric ulcer healing effect. The data showed that the administration of the CAEE and APEE combination had an ulcer index with a significant difference from the ulcer control group (Table 1).

Percentage of ulcer inhibition showed the ability of the combination of CAEE and APEE dose to cure and inhibit ulcer formation in the stomach. In addition, the doses CAEE 200: APEE 150 mg/kg BW showed the highest ulcer inhibition percentage, however with a better effect at the dose of CAEE 200: APEE 75 mg/kg BW.

Gastric secretion studies

The gastric acid secretion plays an important role in the formation of gastric ulcers. In contrast, it is assumed that substances that suppress gastric acid secretion, such as proton pump inhibitors accelerate the healing process of gastric lesions or inhibit the mucosal injury formation.[25] Moreover, gastric acid secretion inhibition is the main strategy for most therapeutic agents used for gastric ulcer treatment. Currently, the CAEE and APEE combination prevent gastric ulcers by reducing the number of ulcers, ulcer score and index, gastric secretion volume and acidity compared to the ulcer control group.

Pretreatment with the combination of extract before gastric lesions induction showed an increase in pH of gastric secretion and mucus index. The combination of extract also showed an increase in the ulcer inhibition percentage which indicated its ability in ulcer healing.

The test group pH showed that CAEE and APEE combination increases the pH and mucus index. Besides, the ulcer control group had the lowest mean mucus index (Table 3).

Table 3: Gastric secretion after the treatments (Mean ± SEM)

Group of treatments	Volume of gastric secretion (ml)	pH of gastric secretion	Acidity (mEq L^{-1})	Mucus index
Normal control	2.48 ± 0.18^{a}	5.40 ± 0.39^{e}	40.13 ± 3.60^{a}	0.05 ± 0.02^{a}
Ulcer control	6.15 ± 0.38^{d}	2.03 ± 0.18^{a}	63.25 ± 4.48^{b}	0.03 ± 0.01^{a}
Vehicle control	5.68 ± 0.53^{cd}	2.18 ± 0.26^{ab}	51.20 ± 2.81^{ab}	0.05 ± 0.01^{a}
Ranitidine 27 mg/kg BW	3.18 ± 0.51^{ab}	4.55 ± 0.54^{de}	42.03 ± 2.81^{a}	0.12 ± 0.03^{b}
CAEE 100 : APEE 75 mg/kg BW	5.20 ± 0.33^{cd}	2.88 ± 0.17^{abc}	47.63 ± 2.25^{a}	0.05 ± 0.02^{a}
CAEE 100 : APEE 150 mg/kg BW	5.10 ± 0.31^{cd}	3.25 ± 0.30^{abcd}	50.18 ± 2.35^{ab}	0.05 ± 0.02^{ab}
CAEE 200 : APEE 75 mg/kg BW	4.50 ± 0.26^{bcd}	3.43 ± 0.17^{bcd}	44.58 ± 1.25^{a}	0.08 ± 0.01^{ab}
CAEE 200 : APEE 150 mg/kg BW	4.05 ± 0.26^{abc}	3.78 ± 0.11^{cd}	43.20 ± 2.96^{a}	0.09 ± 0.00^{ab}

BW = body weight; different superscript notation = significantly different (P<0.05)

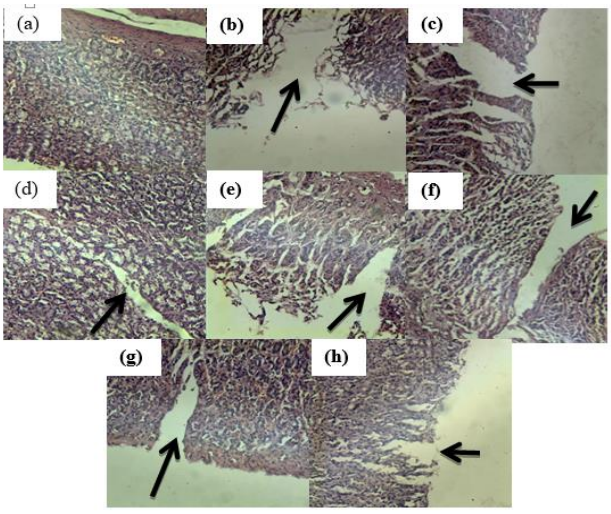

Figure 2: Hematoxylin and eosin-stained sections of gastric mucosal tissues for gastric ulcer and mucosal erosion observation. (a) normal control group; (b) ulcer control group; (c) vehicle control group; (d) positive control group; (e) CAEE 100 : APEE 75 mg/kg BW; (f) CAEE 100 : APEE 150 mg/kg BW; (g) CAEE 200 : APEE 75 mg/kg BW; (h) CAEE 200 : APEE 150 mg/kg BW. Black arrow indicates a lesion magnification of 100x and mucosal damage is shown by the arrows.

The combination of extract protects the stomach from gastric ulcers due to increased mucosal defensive factors which were expressed in a high mucus index.

This is in line with the previous study which stated that the extract increased gastric mucus level.[26] Mucus forms a gel that covers the mucosal membrane and physically protects the mucosa from abrasion.[27] Meanwhile, the administration of the extract combination significantly increased the amount of mucus adhering to the gastric mucosa. This gastroprotective effect results from the strengthening of the mucosal defensive factors.[28]

Histopathological examination

The histopathological examination in the normal group showed good gastric surface cells without erosion which indicated that there were no ulcers in the gastric mucosal tissue. Furthermore, the ulcer induction and vehicle control groups showed that the gastric mucosa epithelial cells had been damaged (Figure 2. a-c). The positive control group showed that the gastric mucosal epithelial cells were intact and no erosion, which indicated that the gastric surface cells had been protected by administering ranitidine 27 mg/kg BW to test animals for 7 days. The results of histopathological examination in the group treated with the extracts combination showed an inhibitory effect on the occurrence of gastric epithelial damage. Meanwhile, the administration of the extract dose of CAEE and APEE for 7 days prior to ulcer induction by pyloric ligation showed that the gastric cells were not protected and the density between cells was decreased. Furthermore, microscopic results of gastric epithelial cells in extracts of CAEE 200: APEE 150 mg/kg BW doses showed that the cohesion between epithelial cells of gastric mucosa was very good, and no

erosion occurred. The results showed that the combination of extract plays a role in protecting gastric mucosal cells (Figure 2. d-h).

Conclusion

Based on the results, the combination of *C. asiatica* and *A. paniculata* leaves ethanol extracts has gastroprotective effect.

Conflicts of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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