EVALUATION OF THE PROTECTIVE EFFECT OF AGAR-HP HARD CAPSULE FOR PEPTIC ULCER IN EXPERIMENTAL ANIMALS

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Imbalance between exogenous damaging agents and protective factors in the gastro-duodenum tract can lead to peptic ulcer disease. Herbal medicines could be an effective treatment to human gastric ulcers. This study aimed to investigate the protective effect of AGAR-HP hard capsules on cysteamine-induced gastric-duodenal ulcers in experimental rats. The animals were divided into five treatment groups: Group 1 (control) and group 2 (model) were treated with distilled water, group 3 received esomeprazole at 10 mg/kg, group 4 received AGAR-HP at 0.074 g/kg, and group 5 received AGAR-HP at 0.221 g/kg. All the rats were treated for seven consecutive days. On day 7, peptic-duodenal ulcer were induced by oral cysteamine 400 mg/kg. The number of rats with the ulcer, the number of ulcers per rat, and the ulcer index of each group were recorded. Compared to group 2, AGAR-HP at high dose reduced the mean number of ulcers and the ulcer index under macroscopic and microscopic examinations. These parameters also decreased in the group of AGAR-HP low doses compared to the cysteamine control group. AGAR-HP hard capsules showed some protective effect on cysteamine-induced gastric ulcers in experimental rats.

Keywords: AGAR-HP, peptic ulcer, experimental animals.

I. INTRODUCTION

Peptic ulcer disease (PUD) is a global problem with a lifetime risk of development ranging from 5% to 10%. The lifetime prevalence of PUD in the United States is about 12% in men and 10% in women. Moreover, an estimated 15.000 deaths per year result from complication of PUD.[1] The use of nonsteroidal anti-inflammatory drugs (NSAID) is one of the most well-known causes of PUD. Other factors related to the development of PUD include oxidative stress, alcohol consumption, smoking, and *Helicobacter pylori* infection. Synthetic drugs such as proton pump inhibitors (PPIs) and histamine-2 (H2) receptor antagonists are conventionally used for treating peptic ulcers, but they are costly and associated with adverse effects, relapses, and various drug interactions. Recently, the demand for traditional herbal medicines has increased across the world. Many herbs and plant materials are found to play important roles in protecting against or serving to heal abdomen ulcers.[2] It has often been used in many Asian traditional medicine systems to treat various illnesses, including ulcers.[3] Also, natural compounds from a plant usually have fewer adverse effects compared with synthetic medications.

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Recently, several studies have reported on the effectiveness of herbal medicine in treating and improving clinical symptoms of ulceration.[4] The *Sepiella maindroni,* also known as Cuttlefish bone, is effectively used in treating gastritis and promoting the healing of ulcers; besides that, it is frequently used as a hemostatic agent to astringe and stop bleeding.[5] *Rhizoma Dioscoreae* was demonstrated to be highly effective in improving the gastrointestinal function and restoring the activities of plasma substances and antioxidant enzymes in rats by preventing gastric tissue damage in acute gastric ulcers and.[6] *Aquilariae Lignum* is one of the critical substances in promoting circulation and relieving pain in the clinical practice of Traditional Chinese Medicine.[7] Each of these herbal medicines has pharmacological activities that can be used to treat peptic ulcers.

Several animal models have been used to evaluate anti-ulcer medication.[8–10] Peptic ulcers can be induced by animal species' physiological, pharmacological, or surgical manipulations. Cysteamine has become one of the most widely used animal models for studying ulcer disease.[11]

The safety of a combination of herbal medicine in AGAR-HP has been evaluated but data will be published in another issue of journal. This study aimed to investigate the **gastric-duodenal protective effect** of AGAR-HP hard capsules on cysteamine-induced gastric-duodenal ulcers in experimental rats.

II. MATERIAL AND METHODS

1. Plant materials

The AGAR-HP product is a hard capsule which consisted of the following ingredients: *Agarwood essential oil* 5 mg, 250 g, *Sepiella maindroni* 100mg, *Rhizoma Dioscoreae* 100 mg, and other synthetic ingredients just enough for one hard capsule.

The clinically acceptable dose is three hard capsules per day (equivalent to 615 mg dry extract per day). The AGAR-HP product is manufactured by AGARVINA CO., LTD, and is distributed and marketed by DSV Pharmaceutical Company.

In the study period, AGAR-HP was kept in a cool and dry place. Every morning, these hard capsules were added with a sufficient volume of solvent to make the needed concentration.

2. Animals

Wistar rats (200 ± 20 g) of both sex were purchased from Center provides laboratory animals, Dan Phuong district, Hanoi. All animal studies were acclimated to housing conditions of the laboratory in the Department of Pharmacology, Hanoi Medical University, for seven days before and during the study. The housing condition was 25 ± 2°C and 80% ± 10% humidity under a 12 hrs light/12 hrs dark cycle. The rats were provided with free access to standard diet and tap water ad libitum.

3. Methods

This model was first described by Selye and Szabo.[12] Animals were divided into five groups of ten rats each. Rats fasted for 16-18 hours before the experiment. In addition to the experimental administrations described in table below, cysteamine hydrochloride (400 mg/kg, p.o) was administered orally, two times, at an interval of four hours to produce ulcers.

Groups (n = 10)			Administration	
I	Vehicle control		oral distilled water 1 ml/100 g b.w/day	Oral cysteamine at the dose of 400 mg/kg b.w, twice a day, at an interval of four hours on Day 7th
II	Cysteamine control		oral distilled water 1 ml/100 g b.w/day	
III	Standard group		oral esomeprazole at the dose of 10 mg/kg b.w/day	
IV	AGAR-HP	low dose	oral AGAR-HP at the dose of 0.074 g/kg b.w/day	
V		high dose	oral AGAR-HP at the dose of 0.221 g/kg b.w/day	

After 24 hours of the first dose of cysteamine, rats were sacrificed by cervical dislocation. The abdomen was opened, and the stomach was excised and washed with normal saline to remove gastric contents and blood clots. After, the stomach was examined under 10× magnifier lens to assess the formation of ulcers. The ulcerative index was calculated as follows[13]:

I – Presence of edema, hyperemia, and single submucosal punctiform hemorrhage.

II – Presence of submucosal hemorrhagic lesions with small erosions.

III – Presence of deep ulcer with erosions and invasive lesions.

Ulcer index (UI) = (number of lesion I) x1 + (number of lesion II) x2 + (number of lesion III) x3.

Evaluation criteria:

- The proportion of rats with an ulcer in each group.

- The average number ulcers of each group.

- Ulcer index (UI)

- Macroscopic observation using image of the peptic ulcer.

- Assessment of histopathology lesions by randomly examining the microstructures of 30% of rats in each group. Histopathological tests of peptic ulcers were performed at the Cancer Research and Early Detection Center.

4. Statistical analysis

Data sets were entered, edited, and analyzed using Excel 2010 and SPSS 20. Results were expressed as mean value ± standard deviation (± SD). Appropriate statistical analysis was conducted. A p value less than 0.05 was considered significant.

III. RESULTS

1. The percentage of cysteamine ulcerated rats

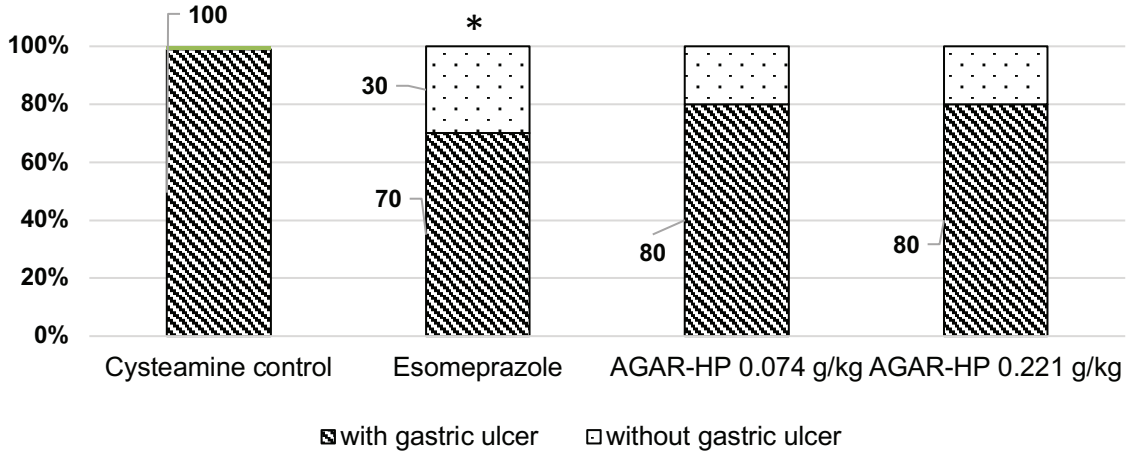

Figure 1. The percentage of rats with gastric ulcer in each group

**p < 0.05 compared with the cysteamine group (Chi-Square test)*

Figure 1 shows the percentage of rats with gastric ulcer in each group. In the cysteamine control group, all rat had gastric ulcers. Oral administration of 10 mg/kg b.w. of esomeprazole resulted in a significant decrease in the percentage of rats with ulcers (p = 0.025). There was no significant difference between the two AGAR-HP groups in the percentages of rats with ulcers.

2. Effect of AGAR-HP on the mean of ulcer and ulcer index

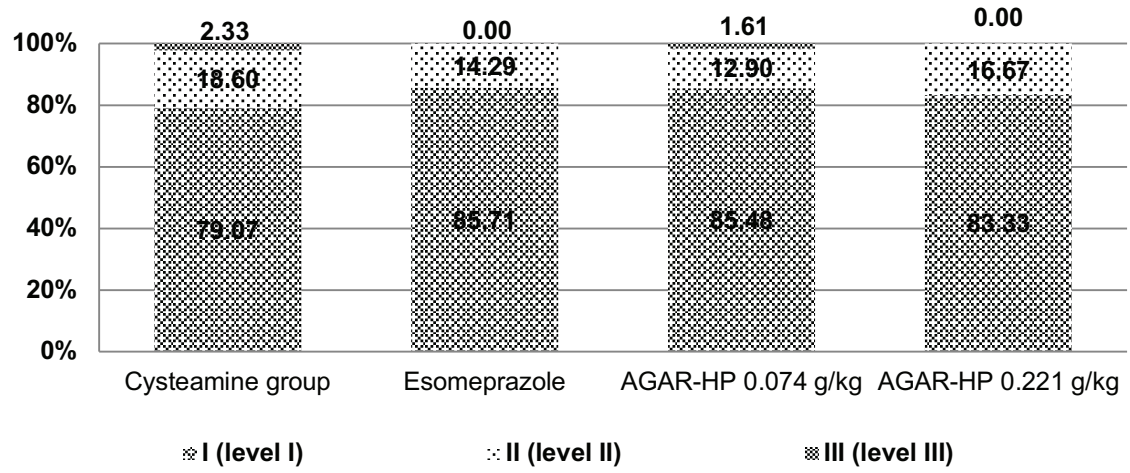

Figure 2. Effect of AGAR-HP on mean ulcerative index

In the cysteamine group, 18.60% of the rats had grade II lesions and 2.33% had grade III lesions. In the group where Esomeprazole 10 mg/kg was used as a positive control, 14.29% of the rats had grade II lesions and there was no grade III lesion. Compared to the cysteamine group, AGAR-HP at both doses improved ulcer severity by reducing the rate of grade II and III lesions and increasing the rate of grade I lesions.

Table 1 showed the pretreatment of esomeprazole and AGAR-HP at a high dose of 0.221 g/kg resulted in a significant reduction ($p < 0.001$) in the mean of ulcer and ulcer index induced by cysteamine. However, these parameters were not statistically considerable compared to the cysteamine control group ($p > 0.05$).

Table 1. Effect of AGAR-HP on the mean number of ulcers and ulcer index

Groups	n	Mean number of ulcers	Ulcer index (UI)
Cysteamine control	10	8.60 ± 3.92	10.60 ± 5.48
Esomeprazole	10	2.80 ± 2.82**	3.20 ± 3.36***
AGAR-HP 0.074 g/kg	10	6.40 ± 4.81	7.90 ± 5.57
AGAR-HP 0.221 g/kg	10	4.20 ± 3.71*	4.90 ± 4.38*

p < 0.05;p < 0.01;***p < 0.001 compared with the cysteamine control group (Mann-Whitney U test)*

3. Evaluation of macroscopic and histopathological changes

Effect of AGAR-HP on macroscopic and histopathological changes of the stomach-duodenal of rats with cysteamine-induced gastritis. (a) Control, (b) cysteamine control, (c) esomeprazole, (d) AGAR-HP 0.074 g/kg, and (e) AGAR-HP 0.221 g/kg. The stomach tissue alterations were confirmed by staining with H&E and then observed at a magnification of 40x.

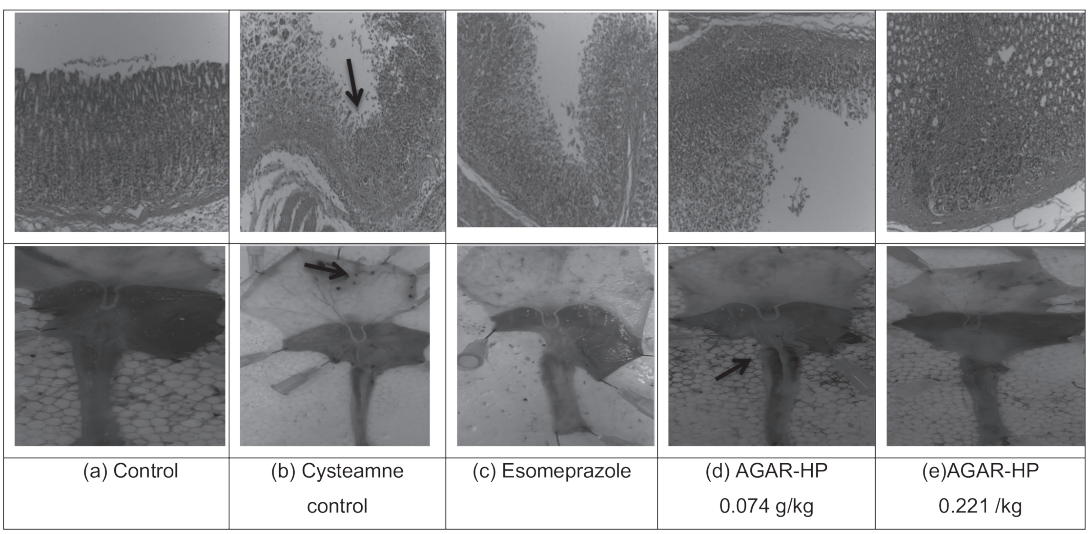

Figure 3. Histological and macroscopical images of stomach-duodenal from rats treated with AGAR-HP (Selected microphotographs HE staining magnification 400X)

The microscopic evaluation of the control group shows typical gastric histoarchitecture with intact epithelium and glands. The cysteamine group displayed several changes in the integrity of the gastric mucosa, necrosis, edema, and dilated gastric glands, along with infiltration of inflammatory cells (neutrophils and eosinophils). Pretreatment with esomeprazole

decreased the gastric lesions compared to the cysteamine control. Treatment with AGAR-HP results in mild edema; the rest of the mucosa showed almost normal gastric glands with limited eosinophilic infiltration compared to ulcer control.

IV. DISCUSSION

Peptic ulcer is a common disease worldwide. In recent years, the ethnomedicinal use of herbal medicines in peptic ulcer management needs to be assessed to improve their effectiveness and possible isolation of lead compounds. This requires the use of appropriate animal models for various ulcers. Various rat ulcer models were found in the literature.[11] Peptic ulcers can be induced by physiological, pharmacological, or surgical manipulations in several animal species; however, most experiments in peptic ulcer studies are carried out in rodents. Several models are used experimentally to evaluate the anti-peptic ulcer activity of drugs/agents, including stress, NSAIDs, ethanol, pylorus ligation, cysteamine, histamine, among others. The model of peptic ulcer induced by cysteamine is used to test natural or synthetic drugs. Selye and Szabo first described a type of peptic ulcer in rats induced by cysteamine HCl, although the mechanism involved in ulcer production has not been fully elucidated.[12] Generally, it is reported that cysteamine given by various routes induced a marked and significant increase in gastric acid secretion in rats by depletion of tissue somatostatin.[11,12] Besides, cysteamine causes the release of gastrin by inhibiting spontaneous Brunner gland secretion, which glands in the proximal duodenum have previously been shown to be an essential factor in the natural defense of the duodenal mucosa. The depression of the Brunner gland secretion may be an important factor in the pathogenesis of cysteamine-induced duodenal ulceration.[14] This study showed administration of cysteamine resulted in the induction of peptic ulcers. Twenty-four hours after the first dose of cysteamine, the mean number of ulcers and UI reached 8.60 ± 3.92 and 10.60 ± 5.48, respectively.

Based on the successful ulcer model, we evaluated the effects of AGAR-HP on the changes in the ulcer parameters. Our results showed animals pre-treated with AGAR-HP given at the dose of 0.074 g/kg had on average lower number of ulcers and UI, but this effect was not statistically significant. Higher doses of AGAR-HP at 0.221 g/kg resulted in a significant reduction in these parameters. In histopathologic assessment, the duodenum of rats treated with AGAR-HP with the dose of 0.221 g/kg was more closely arranged, the connective tissue was thickened, and lesion detectability was improved.

To achieve lower number of ulcers and UI, the traditional medicine components in AGAR-HP play an indispensable role. Especially, *Sepiella maindroni (*also known as Cuttlefish bone) had been shown to be effective in treating stomach ulcers, gastric hyperacidity, and a variety of bleeding.[15] *Sepiella maindroni consists of* 87.3%–91.75% calcium carbonate, chitin, and trace amounts of silicon, aluminum, titanium, manganese, barium, and copper. Previous research established that *Sepiella maindroni* had antioxidant properties, including scavenging hydroxyl radicals and chelating ferrous ions, which were also affiliated with the mechanism of cysteamine-induced acute gastric mucosal lesions. *Sepiella maindroni* is also part of traditional Chinese medicine used to treat gastritis and is frequently used as a hemostatic agent after tooth extraction or rhinoplasty.[5] In addition, according to Lifeng Qiu, *Sepiella maindroni* is a potential therapeutic candidate for preventing and treating indomethacin-induced acute gastric

mucosal lesions by increased secretion of EGF and PGE2, prevention of lipid peroxidation, and activation of radical scavenging enzymes.[16]

Another component of AGAR-HP, *Rhizoma Dioscoreae,* has been used in Asian traditional medicine because of its several biologically beneficial effects like antioxidative capacity (effectively removing free radicals) and in vitro anti-inflammatory activity (down-regulating inflammatory factors). Substances that have been identified in *Dioscoreae Rhizom*a include mucilage, steroidal saponin (dioscin), starch (16%), vitamin C, 3,4-dihydroxy phenylethylamine (dopamine), phytic acid, and polyphenol oxidase glycoproteins. Dioscin isolated from *Dioscorea nipponica* was shown to have a protective effect against ethanol-induced liver injury through the mucosal reduction damage in vivo, presumably through the activation of the antioxidant system and suppression of the inflammatory response.[6,17]

AGAR-HP also has *Aquilariae Lignum* are 2-(2-phenylethyl) chromone and volatile oil, which have been scientifically validated to possess several pharmacological properties of anti-microbial, antioxidant, anti-inflammation.[18] *Aquilariae Lignum* alcohol extracts reduced gastric occurrence and ulcer inhibition rates by up to more than 60% and alleviated the occurrence and development of gastric ulcers via inhibiting oxidation and inflammation.[19] Both medicines were well-cooperated to produce AGAR-HP with preeminent anti-ulcer effects.

V. CONCLUSION

The findings from this study suggest that AGAR-HP at a high dose of 0.221 g/kg (3 times the expected clinical dose equivalent) decreased the average number of ulcers, the ulcer index, and improved macroscopic and microscopic image compared to the model group. AGAR-HP at a dose of 0.074 g/kg/day (the equivalent dose expected for clinical use) resulted in some reduction in the average number of ulcers and the ulcer index, and lower lesions on microscopic and microscopic images compared to the model group.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

The funding body had no role in the study design, collection, analysis, data interpretation, and manuscript writing.

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