Research on the anti-duodenal ulcer effect of Vien Khoi Tim capsules on Wistar rats with duodenal ulcers caused by cysteamine

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SUMMARY

***Objective**: To evaluate the anti-duodenal ulcer effect of Vien Khoi Tim capsules on Wistar rats.*

***Subjects and methods**: The duodenal ulcer model in rats receiving cysteamine (CYS) orally at a dose regimen of 400 mg/kg/time × 2 times were applied. Rats were divided into 5 groups comprising 12 rats each. In which, the biological proof group only received distilled water while the others were given distilled water, test samples (237.6 or 712.8 mg/kg/day) or famotidine continuously for 10 days before being taken CYS. After 24 hours of treatments, all rats were anesthetized with chloral hydrate before being dissected and observing their duodenums to evaluate the results.*

***Results**: Vien Khoi Tim remarkably reduced rats' ulcer indices compared to those of the model group ($p < 0.05$) with ulcer inhibition rates of 58.5% and 53.7%, respectively. No ulcer lesions or apoptosis were detected in the duodenal microscopic samples of both groups being taken Vien Khoi Tim, while the model group had both ulcer lesions and apoptosis at the rate of 33.33%.*

***Conclusion**: Vien Khoi Tim hard capsules were highly effective against duodenal ulcers in rats at oral dose regimens of 237.6 and 712.8 mg/kg/day × 10 consecutive days.*

***Key words**: Vien Khoi Tim, capsule, anti-duodenal ulcer effect, rat, cysteamine.*

INTRODUCTION

Gastric and duodenal ulcers are of great concern to public health due to their high incidence in many countries around the world. The rate of gastric and duodenal ulcers accounts for 26% of digestive diseases in Vietnam. This disease is often chronic, prone to recurrence, has high treatment costs and can cause some dangerous complications [1]. Therefore, the treatment of gastric and duodenal ulcers often combines both medication and surgical intervention in patients with complications such as gastric bleeding, perforation, or stomach cancer... Nevertheless, long-term use of pharmaceutical medicines can cause several side effects in patients. This makes the health sector of many countries very interested in research and development of new drugs, especially products derived from medicinal herbs.

Currently, many medicinal herbs have good effects in treating gastric and duodenal ulcers, which have been proven experimentally and clinically, such as green tea, Da Cam, Khoi leaves, and turmeric. However, these studies mainly evaluated the effects of individual medicinal ingredients [2],[3]. To date, there have been no studies evaluating the effects of capsule preparations when combining these medicinal herbs.

The remedy consists of medicinal herbs Folia Ardisiae sylvestris, Folia Pseuderanthemi palatiferi, Herba Lactucae indicae, Rhizoma Curcumae longae,

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and Radix et Rhizoma Glycyrrhizae uralensis, which have been widely used in folk medicine to treat duodenal ulcers and have shown good results in improving clinical symptoms. However, there has not been a comprehensive and systematic study to confirm its effectiveness and capsule form. With the goal of developing this medicine into a capsule dosage form for clinical use, we evaluated the anti-duodenal ulcer effect of Vien Khoi Tim hard capsules on Wistar rats with duodenal ulcers induced by cysteamine.

MATERIALS AND METHODS

Sample

Vien Khoi Tim hard capsules were produced by Bavieco Joint Stock Company meeting the basic standard No. 02.2022/TCCS/CVI-BAVIECO. Each hard capsule contains 495 mg of total dry extract, equivalent to the following amount of raw medicinal herbs.

Ingredients of Vien Khoi Tim hard capsules

Ingredients	Contents (mg)
Folia Ardisiae sylvestris	1.400
Folia Pseuderanthemi palatiferi	560
Herba Lactucae indicae	300
Rhizoma Curcumae longae	280
Radix et Rhizoma Glycyrrhizae uralensis	140
Gelatin capsule shell, filler (lactose, microcrystalline cellulose, calcium carbonate), anti-caking agent (magnesium stearate, aerosil, talc), binder (polyvinylpyrrolidone K30 - PVP K30).	Just enough for a capsule

The expected human dose is 2 tablets/time x 2 times/day. Therefore, the equivalent human dose in rats (calculated by a factor of 6) was 237.6 mg/kg/day.

Experimental animals

A total of 60 Wistar rats were selected for the study, meeting the criteria of both breeds, being mature, healthy, and weighing 180 - 220 g. All mice were kept under experimental conditions for 7 days before the study.

Appliances

Blunt-tipped curved needles, a set of surgical instruments, a camera with 24-megapixel resolution, some magnifying glasses, and an Olympus microscope.

Chemicals

Double distilled water; cysteamine (Energy Chemical, China); Famotidine tablets 40 mg (Vidiphar Central Pharmaceutical Joint Stock Company), physiological saline (Braun), chloral hydrate (Shanghai Zhanyun Chemical Co. Ltd, China), formaldehyde and other tissue-dissecting chemicals.

Time and location

This study was conducted between October and December 2023 at Hanoi Medical University.

Method

The experiment was conducted according to the duodenal ulcer model in white rats receiving cysteamine (CYS) orally at a dose regimen of 400 mg/kg/time × 2 times [4],[5]. All rats were coded with numbers, so researchers did not know which group the rats were in to limit errors. Rats were randomly divided into 5 groups, with the same male/female ratio in each one.

- Group 1 (Biological proof, n = 12): Rats were given distilled water with a volume of 10 mL/kg.
- Group 2 (Model, n = 12): Rats were taken 10 mL/kg distilled water and CYS.
- Group 3 (Famotidine, n = 12): Rats were given famotidine 50 mg/kg and CYS.
- Group 4 (Test sample, n = 12): Rats were drunk Vien Khoi Tim Capsules at a dose of 712.8 mg/kg and CYS.
- Group 5 (Test sample, n = 12): Rats were drunk Vien Khoi Tim Capsules at a dose of 237.6 mg/kg and CYS.

Rats were taken test samples or distilled water continuously for 10 days. On day 10, after an hour of drinking test samples, rats in groups 2 to 5 were given CYS at a dose regimen of 400 mg/kg × 2 times with 4 hours apart. Moreover, rats were fasted for 18 hours before receiving CYS. After 24 hours taking CYS, all rats were anesthetized with chloral hydrate before being dissected and observing the duodenum to evaluate the results.

After exposing the rat's stomach and duodenum, the digestive tube from the esophagus (close to the cardia) to the small intestine (3 cm from the pylorus) was cut separately. Then, the duodenum and stomach were opened along the greater curvature. The

observed ulcers were washed with physiological saline, then the surface of the ulcer was infiltrated with 5% formaldehyde. Next, the rat's stomach and duodenum were fixed on the foam sheets with pins and observed with a 10-time magnifying glass to evaluate the extent of the damages and calculate the ulcer indices.

The severity of duodenal ulcers was assessed according to the scale of Szelenyi and Thiemer as follows [6],[7].

- Level-I injury: Edema, congestion and submucosal petechiae.

- Level-II injury: Submucosal hemorrhage and surface lesions.

- Level-III injury: Deep ulcers and invasive lesions.

Evaluation criteria:

- Rats' death rate after taking CYS.

- Percentage of rats with ulcers in each group.

- Ulcer Index (UI):

UI = (number of level-I injuries) × 1 + (number of level-II injuries) × 2 + (number of level-III injuries) × 3

+ Percentage of ulcer inhibition:

% ulcer inhibition = ((UImodel - UItest sample) × 100)/UImodel

+ Microscopic images: In each group, 30% of the rats were assessed for microscopic ulcer damages according to the scale of Simões S et al. [8]. Microscopic damage scores were calculated by the total scores of the evaluation parameters with a maximum score of 15.

Criteria	Score 0	Score 1	Score 2	Score 3
Depth of erosive lesion	Normal cells, no erosive lesion	Up to 1/3 of mucosal thickness	Up to 2/3 of mucosal thickness	Entire mucosa
Depth of ulcerative lesions	Normal cells, no ulcerative lesions	Lesions limited to the muscularis mucosa	Lesions beyond the muscularis mucosa, limited to the submucosa	Deep ulcerative lesions to the muscle layer
Hemorrhage	Normal cells, no bleeding	Local	Mild	Severe
Inflammation	Normal cells, no inflammation	Observable	Mild	Severe
Apoptosis	Normal cells, no apoptosis	Observable	Mild	Severe

Data processing

Data was processed by Excel program (Microsoft XP) according to the method of medical statistics, using Student's t-test and Fisher's exact test to compare the data before and after the test. Also, those data were compared among the control and treated groups. The difference was statistically significant when $p < 0.05$.

Research ethics

The study complied with ethical regulations in biomedical research. Animals were handled properly after the end of the experiment.

RESULTS

Research results showed that the death rates of rats after taking CYS in groups 2 to 5 were not statistically different ($p > 0.05$). In addition, the proportion of rats being drunk famotidine with duodenal ulcers decreased remarkably compared to that of the model group ($p < 0.05$) while the proportions of rats being taken Vien Khoi Tim with ulcers tended to decrease compared to that of the model group; however, the differences were not significantly ($p > 0.05$), table 1.

Table 1. Percentage of dead rats, rats with duodenal ulcers and rats' ulcer indices

Groups	Percentage of dead rats after being given CYS (%)	Percentage of rats with duodenal ulcers (%)	Ulcer indices (UI)	Percentage of ulcer inhibition (%)
Group 1 (Biological proof)	0	0	-	-
Group 2: Model	16.7	70	4.1 ± 2.6	-
Group 3: Famotidine	8.3	18*	2.0 ± 1.4*	51.2
Group 4: Vien Khoi Tim, 712.8 mg/kg/day × 10 days	8.3	45	1.9 ± 1.4*	53.7
Group 5: Vien Khoi Tim, 237.6 mg/kg/day × 10 days	8.3	64	1.7 ± 1.6*	58.5

*(*p<0.05 compared to the model group)*

Rats in groups 3, 4 and 5 had statistically significant decreases in duodenal ulcer indices compared to those of the model group (p<0.05) with ulcer inhibition rates of 51.2%, 53.7% and 58.5%, respectively.

Table 2. Microscopic injury assessment scores

Groups	Total score for microscopic assessment			Average total scores
	Sample 1	Sample 2	Sample 3	
Group 1 (Biological proof)	0	0	0	0.00
Group 2: Model	6	0	2	2.70 ± 3.10
Group 3: Famotidine	6	1	6	4.30 ± 2.90
Group 4: Vien Khoi Tim, 712.8 mg/kg/day × 10 days	3	3	2	2.67 ± 0.58
Group 5: Vien Khoi Tim, 237.6 mg/kg/day × 10 days	3	2	1	2.00 ± 1.00

*(*p<0.05 compared to the model group (Mann-Whitney U test))*

The results of microscopic damage assessment showed that average microscopic damage scores of rats receiving Vien Khoi Tim at a dose of 237.6 mg/kg/day × 10 days tended to decrease compared to that of the model group, but the differences were not notable (p>0.05).

Table 3. Histopathological characteristics

Groups	Histological microscopic images
Group 1 (Biological proof)	All cut samples (100%) had their normal structures and no lesions In which, the mucosa was covered by a single layer of columnar epithelium and had sparse connective tissues underneath it.
Group 2: Model	Two-third of the cut samples (2/3, 66.67%) showed inflammation: + One-third of the cut samples (1/3, 33.33%) showed signs of ulceration with a few scattered spots of necrotic ulcerative lesions that were deep to the muscularis mucosae. Also, the stromal tissue had scattered neutrophils and lymphocytes. + The remaining one (1/3, 33.33%) had erosive inflammation with some areas of peeling mucosa scattered in the 1/3-upper of the epithelial layer. The stroma had scattered neutrophils.
Group 3: Famotidine	+ Two-third of the cut samples (2/3, 66.67%) showed inflammation with a few scattered spots of necrotic ulcerative lesions that were deep to the muscularis mucosae. Also, stromal tissues had scattered neutrophils and lymphocytes. + One excision sample (1/3, 33.33%) showed mild inflammation, and its stromal tissue had scattered neutrophils.

Groups	Histological microscopic images
Group 4: Vien Khoi Tim, 712.8 mg/kg/ day × 10 days	+ Two-thirds of the cut samples (2/3, 66.67%) showed erosive inflammation with scattered areas of mucosa that were peeled off up to 2/3 of the thickness of the epithelial layers. The stromal tissues were infiltrated with scattered neutrophils and lymphocytes. + One cut sample (1/3, 33.33%) showed severe inflammation with the mucosa covered by a layer of benign mucous columnar epithelium. Moreover, its stromal tissue was infiltrated with many neutrophils and lymphocytes concentrated in clusters throughout the entire thickness of the epithelial layer.
Group 5: Vien Khoi Tim, 237.6 mg/kg/ day × 10 days	+ Two-thirds of the cut samples (2/3, 66.67%) showed mild inflammation with some areas of peeled mucosa that scattered in the 1/3-upper of the epithelial layer. In addition, their stromal tissues were infiltrated with scattered neutrophils. + The remaining sample (1/3, 33.33%) had erosive inflammation with some areas of peeled mucosa scattered in the 1/3-upper of its epithelial layer. Besides, its stromal tissue was infiltrated with many lymphocytes and neutrophils.

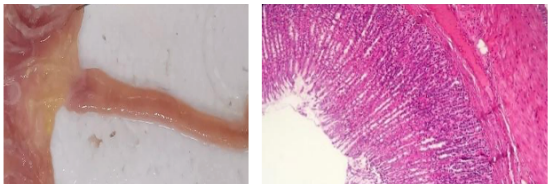

Figure 1. Rat's macroscopic and microscopic images of group 1 (code C01), HE ×100

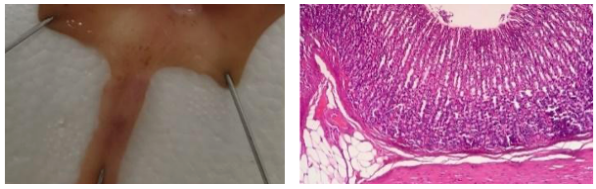

Figure 2. Rat's macroscopic and microscopic images of group 2 (code C16), HE ×100

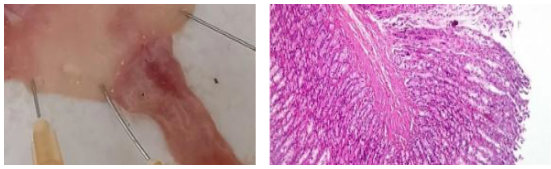

Figure 3. Rat's macroscopic and microscopic images of group 3 (code C22), HE ×100

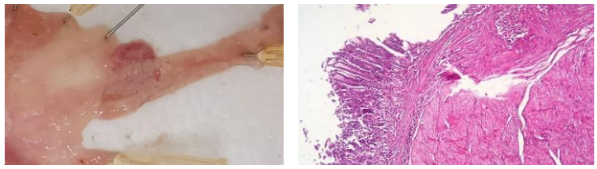

Figure 4. Rat's macroscopic and microscopic images of group 4 (code C96), HE ×100

Figure 5. Rat's macroscopic and microscopic images of group 5 (code C110), HE ×100

DISCUSSION

Gastric - duodenal ulcer is a condition of mucosal damage that penetrates through the muscularis mucosae down to the muscle layer due to an imbalance between attack and protective factors [1]. In this study, we used cysteamine to cause rats' duodenal ulcers with its mechanism of causing vasoconstriction, anemia, and hypoxia before causing ulcers and chose famotidine as a positive control drug.

The results of rats' death rates, ulcer indices and ulcer inhibition rates reveal that we have successfully caused duodenal ulcers in rats with cysteamine at a dose regimen of 400 mg/kg/time × 2 times. Apart from that, with oral doses of 237.6 and 712.8 /kg/day × 10 consecutive days, Vien Khoi Tim capsules tended to reduce the rates of deaths and rats with duodenal ulcers compared to those of the group without sample use (8.3% and 8.3% vs. 16.7%, 64% and 45% vs. 70%, respectively). In addition, at both tested doses, Vien Khoi Tim capsules notably reduced the rats' duodenal ulcer indices compared to that of the model group ($p < 0.05$) with ulcer inhibition rates of 53.7% and 58.5%, respectively. This effect was like

that of the famotidine group at a dose of 50 mg/kg with an ulcer inhibition rate of 51.2% (table 1).

Macroscopic and microscopic images of rats' duodena in all groups ulcerated with cysteamine showed no hemorrhagic lesions, but mainly acute inflammatory lesions. These inflammatory lesions altered the mucosal and submucosal structures of duodenums, causing phenomena such as some areas of mucosa being peeled off, some areas being covered by a layer of benign columnar epithelium and stromal tissues with scattered neutrophils and lymphocytes. These lesions are very specific and obvious. In the group of rats receiving famotidine, most microscopic samples showed ulceration, with scattered necrotic ulcerative lesions that were deep to the muscularis mucosae. In both groups of rats taking Vien Khoi Tim capsules, all rats (100%) did not have ulcerative lesions or apoptosis, but inflammatory lesions. Meanwhile, in the model group, there were ulcerative lesions and apoptosis (in 33.33% of specimens).

The above results are also consistent with a number of other studies evaluating the gastroprotective effects of individual single-ingredient medicinal herbs or preparations containing the medicinal ingredient under study. For instance, Aleandra Orona- Ortiz et al. (2021) studied the gastroprotective effect of extract of turmeric (TEA) (Curcuma longa L.) in acetone solvent and compared it with its own curcuminoids. The results revealed that the proportion of curcuminoids in TAE made an important contribution to protecting the stomach from harm caused by ethanol [2]. Besides, Pham Ba Tuyen et al. (2014) found that Hpmax (including Folium Ampelopsis, Herba Hedyotidis capitellatae, and Folium Ardisiae sylvestris) had anti-duodenal ulcer effects on an ulcer model using cysteamine at a dose of 470 mg/kg [9].

CONCLUSION

The anti-duodenal ulcer effect of Vien Khoi Tim hard capsules was evaluated on rats with duodenal ulcers induced by cysteamine. The results revealed that at oral doses of 237.6 and 712.8 /kg/day × 10 consecutive days, Vien Khoi Tim significantly reduced rats' ulcer indices compared to those of the model group ($p < 0.05$) with ulcer inhibition rates of 58.5% and 53.7%, respectively. No ulcer lesions or apoptosis were detected in the duodenal microscopic samples of both groups being taken Vien Khoi Tim, while the model group had both ulcer lesions and apoptosis at the rate of 33.33%. Furthermore, the microscopic damage assessment index of rats being given Vien Khoi Tim at a dose of 237.6 mg/kg/day tended to decrease compared to that of the model group, but the difference was not remarkable. In short, Vien Khoi Tim capsules were effective against duodenal ulcers in rats at tested doses.

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