GASTROPROTECTIVE EFFECT OF VIEN KHOI TIM CAPSULES ON INDOMETHACIN-INDUCED GASTRIC ULCERS IN RATS

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Available anti-ulcer drugs unveil partial effectiveness and numerous adverse reactions. Plants offer an alternative strategy in the search for new drugs in the therapy and prevention of peptic ulceration. The present study investigated the possible protective effect of the herbal formulation Vien Khoi Tim (VKT) on indomethacin-induced gastric mucosal damage in rats. VKT was tested at two doses (1.44 & 0.48 capsules/kg/d po) ten days before the indomethacin single-dose challenge (40 mg/kg po). Animals were sacrificed six hours after indomethacin administration, and gastric tissues were collected for gross observation and histopathological analyses. The results revealed that the administration of indomethacin caused evident gastric mucosal damage with morphological and histological manifestations. VKT pretreatment tended to avert the rise in lesion numbers, reduce the ulcer index, and improve the severity of bleeding streaks and epithelial sloughing in gastric mucosa on the macroscopic examination compared to the model group. It is worth noting that no ulcerative lesions were observed in the gastric tissues of rats receiving VKT upon microscopic examination. Our results indicated that Vien Khoi Tim capsules might possess a protective role against indomethacin-induced gastric ulcers. Additional research is needed to better understand the mechanism by which Vien Khoi Tim capsules exert their gastroprotective effect.

Keywords: Vien Khoi Tim, ulcer, indomethacin, rat.

I. INTRODUCTION

Peptic ulcer disease (PUD) is one of the most common diseases seen worldwide. PUD is defined as damage to the mucosa of the upper gastrointestinal tract due to acid-peptic digestion leading to the formation of an ulcer that extends beyond the muscularis mucosa into the submucosa.[1] *Helicobacter pylori* infection and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) are the most important causes of PUD.[1] Thanks to their effectiveness in reducing pain and inflammation, NSAIDs are among the most commonly used drugs, confirming their place on the WHO Model List of Essential Medicines.[2] NSAIDs are considered to not only cause stomach damage, but through varied mechanisms, they slow down the healing process of ulcers.[3] NSAID use is responsible for about half of all ulcer perforations, occurring most commonly in older patients taking aspirin or other NSAIDs for cardiovascular or joint disease.[4] Therefore, preventing gastric ulcers caused by NSAIDs is extremely important for both medical professionals and researchers. Natural herbs and their phytoconstituents with potent antioxidant, anti-inflammatory, and antiapoptotic effects may offer good gastrointestinal protection.

Previously, indomethacin (IND) was more

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likely to cause stomach damage than common NSAIDs.[5] Hence, in this study, we aimed to mimic this condition using an indomethacin-induced gastric ulcer model to evaluate the possible protective effect of Vien Khoi Tim capsules against NSAID-associated peptic ulcers. Functional food Vien Khoi Tim (abbreviated as VKT) is a product derived from five medicinal herbs, including *Ardisia silvestris* Pitard, *Pseuderanthemum palatiferum* (leaves), *Lactuca indica*, *Curcuma longa*, and *Glycyrrhiza uralensis*, which is distributed by Bavieco Joint Stock Company. *Ardisia silvestris* Pitard, a precious medicinal herb widely used in folk medicine, is the main medicinal herb of VKT. According to traditional medicine, *Ardisia silvestris* Pitard contains the principal ingredients tannins, glucosides, saponins, alkaloids, fats, carotenes, flavonoids, which have anti-inflammatory effects, astringent ulcers, heal scars and reduce the increase in stomach acid.[6,7] Additionally, *Pseuderanthemum palatiferum* and *Curcuma longa* have also demonstrated anti-gastric ulcer activity in some studies.[8,9]

Considering the beneficial properties of the medicinal ingredients in VKT capsules, we tried to test the anti-ulcer effect of these polyherbal capsules on indomethacin-induced gastric ulcers in rats.

II. MATERIALS AND METHODS

Vien Khoi Tim capsules

Vien Khoi Tim is produced by CVI Pharmaceutical Joint Stock Company and distributed by Bavieco Joint Stock Company. Each hard capsule contains Bavieco Khoi Tim extract equivalent to 495 mg of crude herbal mixture extracted from 5 herbal ingredients including *Ardisia silvestris* Pitard (1400 mg), *Pseuderanthemum palatiferum* (leaves) (560 mg), *Lactuca indica* (300 mg), *Curcuma longa* (280 mg), and *Glycyrrhiza uralensis* (140 mg).

The predicted human dose of VKT was 2-3 capsules twice daily. VKT was suspended in distilled water and administered to the rats by oral gavage at dose levels of 1.44 capsules or 712.8 mg herbal extract (high dose) and 0.48 capsules or 237.6 mg herbal extract (low dose) per kg b.w/day (*based on the conversion from an equivalent dose of 4 capsules/day and 12 capsules/day for patients in the clinic, respectively)*. Dose formulations were prepared fresh daily before administration. The intra-gastric gavage procedure was performed without anesthesia by gently holding the animal to immobilize the head and keep the animal in an upright position while inserting the gavage needle along the side of the mouth.

Chemicals and Reagents

Indomethacin (IND) (Indomethacin 25 mg tablets; Kwality Pharmaceutical, India); Misoprostol (MIS) (Misoprostol STELLA 200 mcg tablets, STELLAPHARM, Vietnam) were obtained.

Induction of gastric ulcer

Gastric ulcers were induced in animals according to the procedure described by Guzmán-Gómez O et al.[10] Briefly, ulcers were induced by a single oral dose of IND (40 mg/kg body weight). Animals were food deprived but had free access to water 18 hours before ulceration. Varying degrees of ulceration manifested six hours after indomethacin administration.

Animal grouping and treatment

The experiment used adult *Wistar* rats weighing 180-250 grams. The animals were acquainted with the laboratory for 7 days before the start of the experiment at normal room temperature (22^0C) under appropriate conditions. The study was conducted from July to August 2023 at the Department of

Pharmacology, Hanoi Medical University. All animal procedures were performed under the recommendations for the proper care and use of laboratory animals.

Fifty-five rats were arbitrarily into five groups of eleven rats each. Group 1 (normal control) animals received only distilled water. Rats in Group 2 (ulcerated control) were given only IND. Animals in group 3 were administered IND after pretreatment with MIS (50 μg/kg b.w.). Groups 4 and 5 comprised ulcerated rats pretreated with VKT (1.44 capsules/kg b.w.), and VKT (0.48 capsules/kg b.w.) respectively. Treatments with the reference drug and test capsules lasted for 10 days prior to IND administration. These were orally administered once daily using gastric gavage with ad libitum provision of food and water throughout the experimental period. All rat groups (except for Group 1) were gavaged 40 mg/kg IND one hour after the last application of the test item.

Gastric lesion evaluation

Six hours after giving IND, the animals were sacrificed by cervical dislocation. The abdomens were opened, and the stomachs were excised from the esophagus (close to the cardia) to the small intestine (3 cm from the pylorus). The gastric pouches were opened along the greater curvature, rinsed with cold normal saline, blotted dry between filter papers, and stapled flat on a polystyrene foam board to examine gross lesions. Each gastric cavity was thoroughly examined under a 10× magnifying glass and the degree of ulceration was graded as follows:[10]

- 0, no lesions (normal stomach); 0.5, hyperemia (red coloration);
- 1, hemorrhagic spots;
- 2, 1–5 small ulcers;
- 3, many small ulcers;
- 4, many small and large ulcers;
- 5, stomach full of ulcers with perforations.

The ulcer index (UI) for each animal is the sum of the macroscopic score. The protective index (PI) was calculated as the following: (ulcer index of the ulcerated group − ulcer index of treated group × 100)/ulcer index of the ulcerated group.

For evaluation of histopathological changes, gastric tissues of 4 animals in each group were fixed in formalin 10%, sectioned into 4-6 µm slices, and stained with hematoxylin and eosin (H&E). Histological features included the following parameters: depth of tissue erosion, depth of ulcerative lesions, and presence of hemorrhage, inflammation, and apoptosis. Micro-assessment score evaluated according to the description of Simões S et al.[12] and modified is presented in Table 1. Representative histological images of each group were photographed digitally at 100x.

Table 1. Microscopic score evaluation

	Score 0	Score 1	Score 2	Score 3
Depth of the erosion	No erosion	Up to 1/3 of total mucosa depth	Up to 2/3 of total mucosa depth	Total mucosa
Depth of the ulceration	No ulceration	Limited to the muscularis mucosae	Beyond the muscularis mucosae, limited to the submucosa	Deep into the muscle layer

	Score 0	Score 1	Score 2	Score 3
Hemorrhage	No hemorrhage	Focal	Mild	Severe
Inflammation	No inflammation	Light	Mild	Severe
Apoptosis	No apoptosis	Light	Mild	Severe

Statistical analysis

The data were processed in Microsoft Excel and analyzed using IBM SPSS Statistics software. The results were expressed as the Mean ± Standard Deviation (SD) and presented in tables, graphs, and images. Statistical differences between the groups were determined using the Chi-square test and Mann-Whitney U test. A p-value less than 0.05 was regarded as significant.

III. RESULTS

1. Effects of VKT capsules pretreatment on macroscopic lesions

Table 2. Effects of VKT on the mean number of lesions

Group	n	Ulcer ratio	Lesion numbers (± SD)
Normal	11	0/11	0
Indomethacin	11	11/11	9.00 ± 2.41
Misoprostol + Indomethacin	11	10/11	6.55 ± 2.88
High dose VKT + Indomethacin	10	9/10	8.50 ± 3.21
Low dose VKT + Indomethacin	11	11/11	8.27 ± 2.05

Table 3. Effects of VKT on the ulcer index and protective index

Group	n	Ulcer index (± SD)	Protective index (%)
Indomethacin	11	4.50 ± 0.63	---
Misoprostol + Indomethacin	11	3.18 ± 1.27**	29.29
High dose VKT + Indomethacin	10	4.00 ± 1.43	11.11
Low dose VKT + Indomethacin	11	4.32 ± 0.84	4.04

***p < 0.01 as compared with ulcerated control (Mann-Whitney U test)*

The gastric tissues of control animals had normal gross morphology (Figure 1A). Animals administered IND showed prominent mucosal folds and severe erosions, while marked ulcers admixed with hemorrhages were observed in the gastric mucosa (Figure 1B), with the highest average lesion numbers coupled with the uppermost ulcer index (Table 2, 3). Macroscopic examination of the stomachs of the MIS (50 *μ*g/kg) pretreated group revealed mild edema in the serosa, whereas mild erosions and bleeding in the gastric mucosa were observed. Based on macroscopic findings, the calculated ulcer index was significantly lower in the IND + MIS ($p < 0.01$) compared to the IND group (Table 3). These data verified the protective

effect of misoprostol on gastric ulceration. Macroscopically, the administration of VKT (1.44 and 0.48 capsules/kg) tenderly reduced the cruelty of visible lesions: milder edema in the serosa, slighter erosions in the mucosa, and bloody streaks less severe. (Figure 1D, 1E). Corresponding to gross observations, VKT at both doses tended to reduce the number of lesions, and calculated ulcer index compared to the ulcerated control, however, the difference was not statistically significant (p>0.05) (Table 2, 3).

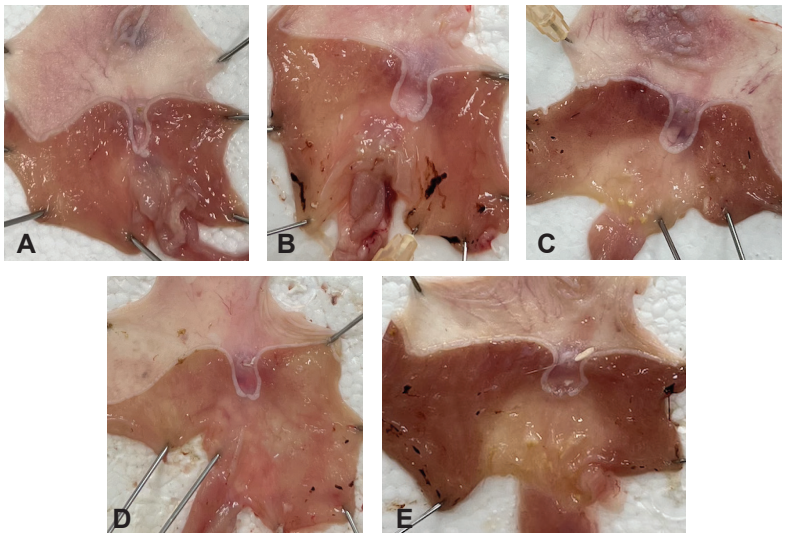

Figure 1. Gastric mucosa appearance in stomachs of the normal control group (A), indomethacin-induced ulcer group (B), misoprostol-treated group (C), high-dose VKT-treated group (D), and low-dose VKT-treated group (E).

2. Effects of VKT capsules pretreatment on microscopic lesions

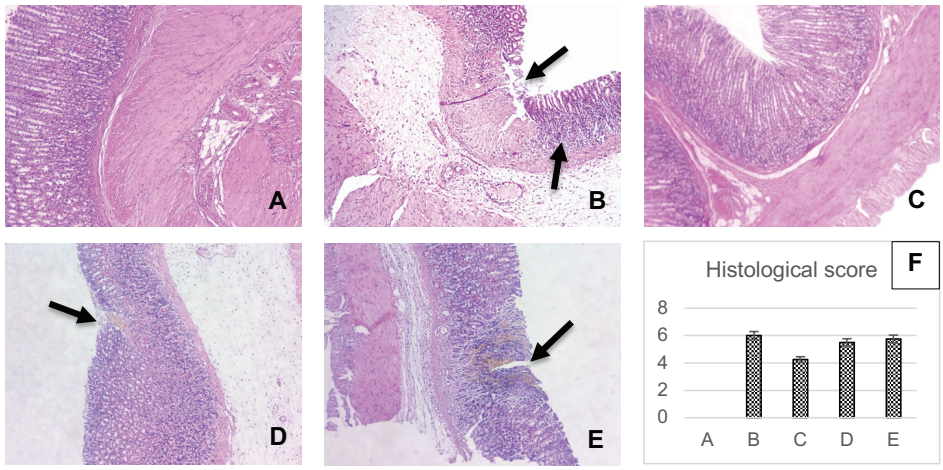

Figure 2. Histopathological changes of gastric tissue stained with hematoxylin and eosin at ×100 magnification. (A) Stomach from the normal control group. (B) Stomach from the IND-treated group. (C) Stomach from IND + MIS (50 μg/kg). (D) Stomach from IND + VKT (1.44 capsules/kg). (E) Stomach from IND + VKT (0.48 capsules/kg). (F) Histological score (n = 4). The data are presented as mean ± SD

Regarding histopathological examination as shown in Fig. 2A, the control group showed normal gastric histological architecture of the mucosa, submucosa, muscularis mucosa, and serosa. However, indomethacin-exposed rats showed reduced mucosal thickness with desquamation of the epithelial lining associated with focal ulceration and necrosis, as well as infiltrated with many inflammatory cells into stromal tissues (arrow), manifested quantitatively by the highest evaluation of histological score (Fig. 2B). Group pretreated with misoprostol (Fig. 2C) showed milder destruction of the lining epithelium of the mucosa with scattered erosions up to 1/3 of the thickness of the epithelium, reduced inflammatory cell infiltration, as quantified by lower histological score. Commendably, both doses of VKT resulted in some positive histological changes (Fig. 2D, E), with no observable ulceration and less severe epithelial sloughing (erosion up to 2/3 of the total mucosal depth), however, many inflammatory cell infiltrates in the mucosa and submucosa layer were still detected (arrow). Respectively, the micro-assessment scores in the rats' stomachs that received the test capsules tended to decrease, although these values presented no significant difference compared to the ulcerated control group.

III. DISCUSSIONS

Preventing or treating gastric ulcers is a major challenge for health authorities nowadays. With inherent side effects and the high cost of synthetic drugs, the exploitation of plant-based products that are considered non-toxic, effective, and affordable will be most suitable for preventing and treating stomach ulcers. This study was carried out to explore the possible gastroprotective effect of VKT hard capsules containing a mixture of five herbal extracts versus indomethacin-induced gastric ulcers in rats.

Many animal models have been used to induce gastric ulcers, in which the NSAID-induced gastric ulcer model is considered one of the most common gastric ulcer models.[12,13] NSAIDs are known to cause ulcers by inhibiting prostaglandin synthetase in the cyclooxygenase pathway. Prostaglandins are found in many tissues including the stomach, where they play a vital protective role through stimulating bicarbonate and mucus secretion, maintaining mucosal blood flow, and regulating regeneration and repair. Thus, inhibition of prostaglandin synthesis by NSAIDs leads to increased susceptibility to mucosal damage and subsequent gastric ulceration.[13] In the present study, indomethacin exposure resulted in severe morphological lesions with extremely hemorrhagic ulcerated mucosal layer, the highest gastric lesion numbers coupled with the uppermost ulcer index, as well as negative histopathological changes in the rat stomach with destructed lining epithelium of mucosal layer and excessive inflammatory cells infiltration in the mucosa and submucosal layers. As a synthetic analog of prostaglandin E1, misoprostol, in the role of a reference drug, has been shown to protect the animal stomachs exposed to indomethacin, as demonstrated by a reduction in the mean number of macroscopic gastric lesions, significantly scaling down the ulcer index with a protection index of 29.29% (Table 2, 3). Correlating with the degree of visible injuries, marked improvements in microscopic inspections were observed in the misoprostol-treated rat stomachs, quantified by lower histopathological scores, compared to the ulcerated group.

VKT pretreatment at doses (0.48 and 1.44 capsules/kg) showed limited injuries relative to the indomethacin-exposed group. Macroscopically, the administration of VKT at tested dosages tenderly reduced the gastric

lesion severity with milder edema in the serosa, slighter erosions in the mucosa, and bloody streaks less severe (Figure 1D, 1E). Microscopically, although the inflammation has not improved, less extreme epithelial sloughing and especially no ulcerative lesions were recognized in the VKT groups. Quantitively, both macro- and microscopic scores of VKT groups were lower than those of IND-treated animals, however, the statistical evaluation did not exhibit a significant in the treated groups in comparison to ulcerated control groups.

The above findings suggested the potential attenuation of gastric affronts of indomethacin by administration of VKT capsules at 0.48 and 1.44 capsules/kg b.w regimens. The exact mechanism of gastric protection of VKT has been not yet elucidated. Generally, the protection afforded by VKT capsules against indomethacin-induced gastric ulcers may be related to the beneficial pharmaceutical properties of the medicinal herbs contained in Bavieco Khoi Tim extract, mainly the role of *Ardisia silvestris* Pitard, *Pseuderanthemum palatiferum* (leaves), and *Curcuma longa*.

The anti-gastric ulcer activity of *Payawanorn* (*Pseuderanthemum palatiferum*) water extract (PPE) was evaluated using three gastric ulcer models:

(1) ethanol/hydrochloric acid (EtOH/HCl),

(2) restraint water immersion stress, and

(3) indomethacin. This study showed that the group treated with PPE reduced gastric volume but had no effect on gastric pH, total acidity, or rate of gastric acid secretion.

This suggests that the anti-gastric secretion effect is unlikely to be an anti-gastric ulcer effect of PPE. Meanwhile, PPE significantly promoted gastric mucus content in the EtOH/HCl model, which suggests that the main gastroprotective activity of PPE is related to gastric mucus protection.[8] The protective effect of the mucus barrier of PPE is consistent with the mechanism of causing ulcers due to reduced mucus secretion related to prostaglandins of indomethacin.

Constituents of *Curcuma longa* have also shown gastroprotective effects through stimulation of gastric wall mucus in a study conducted by Rafathullah S and coworkers. These authors reported that turmeric extract not only increased the gastric wall mucus significantly but also restored the non-protein sulfhydryl (NP-SH) content in the glandular stomachs of the rats.[14] It may thus be beneficial in protecting the gastric mucosa from irritants. Besides the effect of enhancing shielding factors, the findings from the study by Kim DC et al. suggested that *C. longa* extract specifically inhibits gastric acid secretion, an aggressive factor, by competitively blocking histamine H(2) receptors.[9]

Alongside its prostaglandin synthesis inhibition, there are studies suggesting that indomethacin causes gastric injury in rats by inducing the reactive oxygen species (ROS) level.[13] It has been reported that compounds with anti-inflammatory and antioxidant properties can prevent indomethacin-induced gastric mucosal damage in vivo models. *Ardisia silvestris* Pitard, the main medicinal herb of VKT, contains the principal ingredients tannins, glucosides, saponins, alkaloids, fats, carotenes, and flavonoids, which have anti-inflammatory effects, astringent ulcers, heal scars and reduce the increase in stomach acid.[6,7] These benefits may be related to the ability to scavenge free radicals and exert potent antioxidant properties confirmed using ABTS, DPPH, FRAP, and CUPRAC assays in previous studies.[6,15] Based on the IC_{50} values (46 µg/mL and 13 µg/mL) of *A. silvestris* ethanol extract (As-EE) for DPPH and ABTS, Huang L et al. assumed that this plant may have higher

antioxidant activity than other plants such as *Malus baccata*, *Canarium subulatum*, *Licania macrocarpa*, *Atriplex halimus* and *Euphorbia Resinifera*, with IC_{50} values ranging from 50 to 200 µg/mL. The rutin and quercetin contents in As-EE were calculated to be 0.53 and 0.03%, respectively, using the standard area curves of these compounds. These results proposed that the antioxidant properties of As-EE may be beneficial as the main pharmacological activities and that rutin and quercetin may be considered active ingredients in As-EE.[15] Not only *A. silvestris*, but the remaining herbal ingredients of the tested capsule also showed antioxidant activity in many studies.[17,18,19,20]

IV. CONCLUSIONS

From the above discussion, it can be suggested that treatment with Vien Khoi Tim hard capsules at 0.48 and 1.44 capsules/kg b.w regimens can improve the gastric damage caused by indomethacin in Wistar rats. This finding could lead to strategies for treating gastric ulcers using more novel therapies based on herbal-derived preparations. Additional research is needed to better understand the mechanism by which Vien Khoi Tim capsules exert their gastric protective effect.

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