**การประชุมเสนอผลงานวิจัยระดับบัณฑิตศึกษา มหาวิทยาลัยสุโขทัยธรรมาธิราช ครั้งที่ 2**

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# การศึกษาเบื้องต้นของฤทธิ์ต้านแผลในกระเพาะอาหารและการศึกษาความเป็นพิษเฉียบพลันของสารสกัดเอทานอลจากเหง้าขิงแครง

# Preliminary Study of Antiulcer Activity and Acute Toxicity Study of *Zingiber Simaoense* Y. Y. Qian Rhizome Ethanol Extract

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**Abstract**

*Zingiber simaoense* Y. Y. Qian is one of plants in genus Zingiber. In Thailand, it is called "Khing Krang" and is used for treating flatulence, bloating and relieving abdominal pain in Thai traditional medicine. The purposes of the present study were to verify the antiulcer activity and the safety, using acute toxicity study, of *Z. simaoense* Y. Y. Qian rhizome ethanol extract in rats.

The method for gastric ulcer prevention model, rats were pretreated with vehicle (5% tween80), standard drug (misoprostol and cimetidine) or one of various doses of *Z. simaoense* extract (ZSE) by oral administration. Then the gastric ulcer formation was induced by ethanol/hydrochloric acid. Finally the stomach was removed and opened for determination of gastric ulcer. The method for acute toxicity, rats received vehicle or ZSE at a dose of 2,000 mg/kg. Visual observations of signs and symptoms were made and recorded at 1, 2, 4, 6 h and once daily for 14 days after the administration. Survival rats were sacrificed on the 15th day to examine any gross pathological changes of the internal organs.

The results showed that the extract at the doses of 60, 125, 250 and 500 mg/kg significantly inhibited gastric ulcer formation induced by ethanol/hydrochloric acid (EtOH/HCl). In addition, no signs of toxicity or mortality in any of rats were found. These findings indicate that the ethanol extract of rhizome of *Z. simaoense* exerts antiulcer activity without acute toxic effect. Further studies to find out dose-related activity and its mechanisms of action should be performed.

**Keywords:** Antiulcer activity, Acute toxicity, *Zingiber simaoense*, Ethanol extract

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**Introduction**

Peptic ulcer disease is one of the most common diseases affecting the gastrointestinal tract, in which lesions in the stomach and duodenum are gastric ulcer and duodenal ulcer, respectively. It causes inflammatory injuries in mucosa, with extension related the submucosa into the muscularis mucosa. The etiologies of gastric ulcer are multifactorial and developed when aggressive factors overcome protective mechanisms, but the two major etiologies for peptic ulcers are *Helicobacter pylori* (*H. pylori*) infection and nonsteroidal anti-inflammatory drugs (NSAIDs) intake. Even if gastric ulcer is a common disease, a diagnosis is difficult because it has a wide spectrum of clinical presentations (Barros et al., 2008 ; Brunton, Lazo, Parker, Gilman, & Goodman, 2006; Chan & Leung, 2002 ; Konturek, Konturek, Brzozowski, Konturek, & Pawlik, 2005; Wolfe & Sachs, 2000).

Peptic ulcer is a chronic disease that results from an imbalance between endogenous protective factors of gastric mucosa (mucus and bicarbonate secretion, adequate blood flow, prostaglandin E2, nitric oxide, sulfhydryl compounds and antioxidants enzymes, and others) and aggressive factors (acid and pepsin secretions)(Brunton et al., 2006). Behavioural and environmental factors such as smoking, poor diet, alcohol and non-steroidal anti-inflammatory drugs ingestion, and *Helicobacter pylori* infection, among others have also been implicated in the etiology of gastric ulcer (Konturek et al., 2005)

The goals of therapy for gastric ulcer are the relief of pain, promotion of healing and prevention of recurrence. Drugs used in the prevention and treatment of gastric ulcer are aimed to balance aggressive factors against defensive factors. They include neutralizing gastric acid agents such as antacids [e.g., $Al(OH)_3$, $Mg(OH)_2$ and $Ca(CO_3)$], gastric anti-secretory agents such as $H_2$-receptor antagonists, proton pump inhibitors, muscarinic antagonists, octreotide and mucosal protective agents such as sucrafate, prostaglandin analogs, colloidal bismuth compounds, carbenoxolone, and agents that eradicate *H. pyroli* (Kenneth, 2007). At the same time, each of these drugs confers mild to severe side effects such as enterochromaffin-like (ECL) cell hyperplasia (occurs with omeprazole) and gynaecomastia (occurs with cimetidine) (Rang, Dale, & Ritter, 1995). Antiulcer effect provided by medicinal plants may be a better and safer alternative prevention and treatment of peptic ulcers.

The genus Zingiber, a member of the family Zingiberaceae, comprises about 100-150 species. This genus is distributed in tropical and subtropical Asia, and it has been under cultivation in India, China and Southeast Asian countries for a long time (Theilade, 1999; Wu & Larsen, 2000). It is one of the important spices and medicinal plants of Asian countries. The rhizomes of many species of Zingiber contain essential oils, curcuminoids and diarylheptanoids (Akiyama et al., 2006; Ma, Jin, Yang, & Liu, 2004; Masuda, Jitoe, & Mabry, 1995) which have been shown to have medicinal properties such as anti-inflammatory (Jeenapongsa, Yoovathaworn, Sriwatanakul, Pongprayoon, & Sriwatanakul, 2003; Jiang et al., 2006), anti-allergic (Tewtrakul & Subhadhirasakul, 2007) and anti-ulcerogenic activities (Al-Amin, Sultana, & Hossain, 2012; Al-Yahya, S. Rafatullah, Ageel, Parmar, & Tariq, 1989; Minaiyan, Ghannadi, & Karimzadeh, 2006; Yamahara, Hatakeyama, Taniguchi, Kawamura, & Yoshikawa, 1992; Yamahara, Mochizuki, Rong, Matsuda, & Fujimura, 1988 ).

Recent researches have pointed out that plants in the family Zingiberaceae have gastroprotective (Jamal, Javed, Aslama, & Jafri, 2006) and antiulcer effects (Al-Amin et al., 2012; Minaiyan et al., 2006).

*Zingiber simaoense* Y. Y. Qian is one of plants in genus Zingiber, under its common Chinese name as "Si Mao Jiang". In Thailand, it is called "Khing Krang". Its height is about 0.7-1.2 m. Its rhizomes are gray-brown, fleshy and roots ending in tubercles. Rhizomes have a mild aroma and have been used as a spice. In Thai traditional medicine, *Z. simaoense* is used for treating flatulence, bloating and relieving abdominal pain. However, the scientific evidences for its pharmacological activities including its effects on gastric ulcer prevention and its safety have not been reported yet. This study aimed to investigate the antiulcer activity and acute toxicity study of *Z. simaoense* in rats.

**Purpose of the study**

The purposes of the present study were to verify the antiulcer activity and the safety, using acute toxicity study, of *Z. simaoense* Y. Y. Qian rhizome ethanol extract in rats.

**Materials and methods**

**Preparation of the ethanol extract of *Z. simaoense***

The rhizomes of *Z. simaoense* were collected from Chiang Rai Province, Thailand. The ethanol extract of *Z. simaoense* rhizomes were prepared, identified, and authenticated at School of Health Science, Mae Fah Luang University, Chiang Rai, Thailand.The dried powdered rhizomes were macerated in 95% ethanol, and then filtered, and the filtrate was evaporated under reduced pressure using a vacuum rotary evaporator. The Z. simaoense extract (ZSE) was then lyophilized and its yield was used in all experiments.

**Laboratory Animals**

Male Sprague-Dawley rats weighing 250-300 g and female Sprague-Dawley rats weighing 180-200 g were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakorn Pathom, Thailand. All animals were kept in an animal room maintained under environmentally controlled conditions of 24±1 ˚C and 12 h light-12 h dark cycle. All animals had free access to drinking water and standard pelleted diets (082 C.P. MICE FEED, S.W.T. Co., Ltd., Samut Prakan, Thailand). They were acclimatized at least one week before starting the experiments. All experiments were approved by The Animal Ethics Committee of Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

**Experimental model**

**Gastric ulcer prevention study**

**Ethanol/Hydrochloric acid (EtOH/HCl)-induced gastric ulcer (Mizui & Doteuchi, 1983)**

The 48 h fasted rats were divided into 7 groups of 5 rats. The control group received 5% tween80 at the volume of 5 mL/kg, reference groups received either misoprostol at the dose of 100 µg/kg or cimetidine at

the dose of 100 mg/kg (both suspended in 5% tween80), and test groups received ZSE (suspended in 5% tween80) at the dose of 60, 125, 250 or 500 mg/kg. One hour later, induction of gastric ulcer was done in all rats by oral administration of 1.0 mL EtOH/HCl (60 mL ethanol : 1.7 mL hydrochloric acid : 38.3 mL distilled water). After one hour, all rats were sacrificed for determination of gastric ulcer.

***Evaluation of gastric ulcer***

The abdomen was excised and opened along the greater curvature. After washing with normal saline, the gastric lesion was quantified using a binocular magnifier (10X). The lesion index and percent inhibition of gastric ulcer were assessed after that.

***Length of lesion***

The length in millimeter of lesion was determined by measuring each lesion along its greater diameter (Chattopadhyay, Bandyopadhyay, Biswas, Maity, & Banerjee, 2006). The sum of the total length in each group divided by the number of rats in that group was expressed as an ulcer index.

$$\text{The ulcer index} = \frac{\sum \text{Total length of lesions in each group}}{\text{Sum of the number of rats in that group}}$$

***The percent inhibition of gastric ulcer***

The percent inhibition of gastric ulcer was measured as described by Takagi et al. (Takagi, Okabe, & Saziki, 1969 ) using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Ulcer index}_{\text{control}} - \text{Ulcer index}_{\text{treated}}) \times 100}{\text{Ulcer index}_{\text{control}}}$$

**Acute toxicity study**

The procedure was conducted according to the Oganization of Economic Cooperation and Development (OECD) guideline (Test No. 420) for testing of chemicals ("OECD guidelines for the testing of chemicals. Acute oral toxicity-Fixed dose procedure 420," 2001) with slight modification. Adult (7 weeks) female Sprague Dawley rats weighing 150-170 g were randomly divided into 2 groups of 5 rats each. Rats were deprived of food but not water for 16-18 h before administration of test substances. 5% tween80 at the volume of 5 mL/kg was given to the rats in vehicle control group. In test group, ZSE was administered by gavage at a dose of 2,000 mg/kg. Visual observations of signs and symptoms such as changes in the skin, fur, eyes, and mucous membrane were made and recorded at 1, 2, 4, 6 h, and then once daily for 14 days after the administration of test substances. Survival rats were sacrificed on the 15th day to examine any gross pathological changes of the internal organs. Any changes of the intestinal organs compared with those of the control group were recorded.

**Data analysis and Statistical methods**

The data from the experiment were expressed as mean ± standard error of mean (S.E.M.). Statistical comparison between groups were analyzed by using ANOVA and post hoc least-significant difference (LSD) test and *p* values less than 0.05 were considered significant.

**Drugs and chemicals**

**Drugs:** indomethacin (Sigma Chemical Company, St. Louis, U.S.A.)**, c**imetidine (Sigma Chemical Company, St. Louis, U.S.A.)

**Chemicals**: absolute ethanol (MERCK, Darmstadt, F.R. Germany), hydrochloric acid (BDA Laboratory Supplies, Poole, England)

**Results**

**EtOH/HCl-induced gastric ulcer in rats**

An oral administration of EtOH/HCl to 48 h fasted rats resulted in severe gastric mucosa damage. In the control group, hemorrhagic elongated bands in the glandular segment of stomach were clearly observed. Rats received pretreatment with misoprostol (prostaglandin analog), cimetidine ($H_2$-receptor antagonist) or ZSE had less gastric lesions than those of the control group. ZSE at all doses (60, 125, 250 and 500 mg/kg), misoprostol at the dose of 100 µg/kg and cimetidine at the dose of 100 mg/kg significantly inhibited gastric ulcer formation (Table 1).

**Table 1** Effect of the ethanol extract of *Z. simaoense* on EtOH/HCl-induced gastric ulcer in rats

| Group | Dose | Ulcer index (mm) | Ulcer inhibition (%) |
|---|---|---|---|
| **Control** | - | 101.70 ± 8.00 | - |
| **Misoprostol** | 100 µg/kg | 0.38 ± 0.09* | 99.63 |
| **Cimetidine** | 100 mg/kg | 38.34 ± 3.78* | 62.30 |
| **ZSE** | 500 mg/kg | 0.38 ± 0.29* | 99.63 |
| | 250 mg/kg | 2.12 ± 1.38* | 97.92 |
| | 125 mg/kg | 4.36 ± 2.03* | 95.71 |
| | 60 mg/kg | 11.96 ± 8.50* | 88.24 |

Data are expressed as mean ± S.E.M. (n=5)

Significantly different from control group: (*$p < 0.05$)

**Acute toxicity**

The extract at a dose of 2,000 mg/kg produced no treatment-related signs of toxicity or mortality in any of the animals during 14 days of the study. In addition, no weight loss was detected (Table 2) and all internal organs examined at necropsy were free from any gross pathological changes.

**Table 2** Body weight, weight changes and % weight changes on day 14 of rats in the acute toxicity

| Group | Body weight (g) | | | Weight change on day 14 (g) | % Weight change on day 14 |
|---|---|---|---|---|---|
| | Day 0 | Day 7 | Day 14 | | |
| **Female** | | | | | |
| **Control** | 160 ± 4 | 174 ± 4 | 192 ± 5 | 32 ± 4 | 20 |
| **ZSE 2 g/kg** | 163 ± 4 | 177 ± 5 | 190 ± 5 | 27 ± 4 | 17 |

Data are expressed as mean ± S.E.M. (n=5)

**Discussion**

*Z. simaoense* is used widely in Thai traditional medicine for treating many disorders. In the present study, the ethanol extract of *Z. simaoense* showed antiulcer activity in EtOH/HCl-induced gastric ulcer. ZSE at all doses were as effective as misoprostol but with greater gastric ulcer formation inhibition than that of cimetidine. Since ZSE at all doses showed nearly the same percentage of inhibition (88.24 to 99.63) that seemed to be its ceiling effect, further study with decreased doses of ZSE to appropriate doses that could show its dose-response relationship should be performed. In addition, the studies to find out its mechanisms of action should be performed as well.

In the acute toxicity study, ZSE at a dose as high as 2,000 mg/kg, caused neither treatment-related signs of toxicity nor mortality during 14 days of the study. Therefore, it is safe to state that its oral LD50 is greater than 2,000 mg/kg. This resulted in classifying the extract as “Category 5” or “Not Classified” in the acute toxicity hazard categories according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) ("OECD guidelines for the testing of chemicals. Acute oral toxicity-Fixed dose procedure 420," 2001).

**Conclusion**

The present study indicates that the ethanol extract of rhizome of *Z. simaoense* exerts antiulcer activity without acute toxic effect. Further studies to find out dose-related activity and its mechanisms of action should be performed.

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