Original Article

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Gastric ulcer protective effect of the *Curcuma comosa* traditional formula in rats

W. Suvitayavat[1], P. Yaithummasarn[2], A. Sribusarakum[2], A. Pratuangdejkul[2], S. Prathanturarug[3], S. Thirawarapan[1], N. Soonthornchareonnon[4], P. Saralamp[3]*

[1] Department of Physiology, Faculty of Pharmacy, Mahidol University, 447 Sri-ayudthaya road, 10400, Thailand;

[2] Medicinal Plant Information Center, Faculty of Pharmacy, Mahidol University, 447 Sri-ayudthaya road, 10400, Thailand;

[3] Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, 447 Sri-ayudthaya road, 10400, Thailand;

[4] Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri-ayudthaya road, 10400, Thailand;

Abstract

Several Thai traditional herbal medications are used for the relief of menopause symptoms, including the *Curcuma comosa* traditional formula (CCZ). CCZ contains *Curcuma comosa*, *Curcuma aromatica* and *Zingiber montanum* at a ratio of 1:1:1. One of the most prevalent diseases in perimenopausal women, is peptic ulcer. Since gastric ulcer is a common disease in adults of both genders, we hypothesized that this could be used for wider benefit. We investigated the potential gastric ulcer prevention activity of CCZ on gastric ulcers in adult male rats, as compared with the standard-of-care, ranitidine, using three different models. Rats were treated either acutely or chronically with CCZ or ranitidine (positive control), prior to inducing ulcers with either aspirin or hydrochloric acid or water immersion restraint stress (WIR). Ranitidine (50 mg/kg) or CCZ (0.25, 0.5, 1.0 and 2.0 g/kg) were orally administered to the rats 30 minutes before gastric ulcer induction for acute effect, or given once a day for 14 days and the ulcer induced on day 15, for chronic dosing investigations. Our results revealed that in the aspirin-induced model, CCZ single pretreatment showed a tendency to decrease gastric ulcers, although this was not statistically different from vehicle control, while ranitidine significantly reduced ulcer formation. In the HCl–induced gastric ulcers, both the acute and chronic CCZ pretreatments significantly decreased gastric lesions. The therapeutic effect on ulcer formation was dose-dependent for the single dose, and the 2.0 g/kg dose was more efficacious than ranitidine. This dose-dependence was not observed in the long term pretreatment group. In WIR–induced gastric ulcer, only single CCZ pretreatment significantly protected against gastric lesions, while ranitidine significantly decreased gastric lesions after both single and long term pretreatments. Our data demonstrates that CCZ has acute gastric ulcer protection for hypersecretion of acid and stress-induced ulcers and relatively lower protection against the damage induced by nonsteroidal anti-inflammatory drugs such as aspirin. The mucosal protective activity was observed only for acid-induced gastric ulcer after long term pretreatment. Our data further clarify the efficacy of CCZ in protecting the gastric mucosal membrane and reducing ulcer formation in male rats, indicating the potential for this being a treatment option for patients of both genders.

Keyword: *Curcuma comosa* traditional formula, *Curcuma comosa*, *Zingiber montanum*, antiulcer, gastric ulcer protection

1. INTRODUCTION

Peptic ulcers (either gastric or duodenal) are produced by an imbalance between the gastroduodenal mucosal defense mechanisms and damaging factors such as NSIADs, bacteria and stress. Gastric acid and pepsin can damage

***Corresponding author:** E-mail: wisuda.suv@mahidol.ac.th

the mucosa, resulting in ulcers, when mucosal defenses fail. In perimenopausal and menopausal women, an increase in the incidence and aggravation of peptic ulcers has been reported [1-4]. This has been related to a decrease of estrogen [3, 5] as estrogen can alleviate gastric and duodenal ulcers in women[6] and ovariectomized rats[7- 9]. However, estrogen does not appear to affect (worsens or protect) aspirin-induced gastric ulcers in female rats[10].

Several Thai traditional formulas have been used to relieve menopause symptoms. The *Curcuma comosa* traditional formula (CCZ) is one of those traditional formulas. This contains *Curcuma comosa* Roxb. (Wan chak modluk, *Curcuma aromatica* Salisb. (Wan nang kum) and *Zingiber montanum* (Koenig) Link ex Dietr (Plai) in equal concentrations. Wan chak modluk contains phytoestrogen, specifically diarylheptanoids, that have been isolated and evaluated for estrogenic activity[11-13]. Its hexane[14-16] and ethanol[15] extracts and diarylhep tanoids[15] showed anti-inflammation by reducing the expression of iNOS (inducible nitric oxide synthase), MCP-1 (monocyte chemoattractant protein-1), IL-6 (interleukin-6)[15] and COX-2 (cyclooxygenase-2)[16] in LPS-induced microglia and decreased pro-inflammatory cytokines, tumor necrosis factor α (TNF-α) and interleukin-1β in phorbol-12-myristate-13-acetate in stimulated human mononuclear cells[15]. Wan chak modluk also showed anti-gastric ulcer activity in rats[17]. *Zingiber montanum* or Plai has demonstrated both antiulcer[18, 19] and anti-inflammatory activities[20, 21, 22]. In contrast to Wan chak modluk and Plai, Wan nang kum has revealed no anti-inflammatory activity[23] but its curcuminoids can improve the allergic symptoms in mouse[24]. Since CCZ is used in menopausal women, a subpopulation which presents a higher incidence of peptic ulcers, it was interesting to investigate the possibility of gastric ulcer prevention activity of CCZ. Since gastric ulcers occurs in both genders and from adult to elderly. In fact, the male to female prevalence ratio has been reported as 2.2:1, and duodenal to gastric ulcer prevalence ratio as 3.8:1[25]. This clearly indicates that an investigation in males is warranted. Towards this purpose, we used adult male rats for our evaluations, with the goal of further understanding how CCZ counteracts the effects of three different ulcer-causing factors. Moreover these studies are also relevant for the general population, including menopausal women, as the effects of sex hormonal variation during estrous cycle did not confound the data. Using male adult rats as a model, we have determined the antiulcer activity of this formulation on acid-, aspirin- and water immersion stress-induced gastric ulcers.

2. MATERIALS AND METHODS

2.1. Plant Material: Rhizomes of *Curcuma comosa* and *Zingiber montanum* were obtained from Nakhon Pathom Province and *Curcuma aromatica* from Sakon Nakhon Province. Voucher specimens were deposited at the Herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University.

2.1.1. Physicochemical Examination: The rhizomes were sliced and dried at 60° C for 48 hrs and the dried plant material was ground and passed through the sieve (No. 40). The powdered rhizomes were examined for physicochemical properties including loss on drying, total ash, acid insoluble ash, hexane extractive value, dichloromethane extractive value, ethanol extractive value, water extractive value, and volatile oil content using the methods described in Thai Herbal Pharmacopoeia I (26)

2.1.2. TLC examination: TLC chromatograms of the ethanolic extracts were performed using silica gel 60 GF_{254} as stationary phase and a solvent system of dichloromethane and methanol (95:5), detected with UV at 254 nm and 366 nm, anisaldehyde/sulfuric acid and phosphomolybdic acid.

2.1.3. CCZ Formula Preparation: The dried rhizomes from all there plants were ground individually and passed through a sieve (No. 80), prior to mixing at the ratio of 1:1:1. The extracts powder was kelp in refrigerator 4°C.

2.1.4. Preparation of the CCZ formula suspension: The CCZ formula powder was

freshly suspended in 1% sodium carboxy-methyl cellulose (CMC) solution at concentrations of 0.25, 0.5, 1.0 or 2.0 g per 5 ml, on the treatment day.

2.2. Animals

Male Wistar rats weighing between 200 and 220 g were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhon Pathom Province. Rats were housed in an animal room with controlled temperature (23 ± 2°C), under 12 hour light/dark cycles. The animals were fed with standard pellet (C.P. Mice feed; SWT. Co. Ltd., Thailand) and tap water *ad libitum*. Each experimental group consisted of 6 animals. This experimental protocol was approved by the Institutional Animal Care and Use Committee of Faculty of Pharmacy, Mahidol University in accordance with Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes recommended by The National Research Council of Thailand.

2.3. Experimental protocol

The 12 hours fasted rats were orally (PO) administrated 5ml/kg of 1% sodium carboxy-methyl cellulose (CMC, control) or CCZ suspension at doses of 0.25, 0.5, 1.0 or 2.0 g/kg body weight, 30 minutes before gastric ulcer induction for the single pretreatment protocol. In long term pretreatment experiment, one percent CMC (5ml/kg) or CCZ suspension were given to the rats at doses of 0.25, 0.5, 1.0 or 2.0 g/kg body weight, once a day for 14 days. The rats were fasted for 12 hours before gastric ulcer induction on the day 15.

2.3.1. Gastric ulcer induction by aspirin (ASP)

Rats were orally administrated 200 mg/5 ml/kg of aspirin suspended in 1% CMC solution. Five hours later, the rats were sacrificed for gastric ulcer determination[27].

2.3.2. Gastric ulcer induction by hydrochloric acid (HCl)

Rats were orally administrated 6 ml/kg of 0.6 N HCl. Four hours later, the rats were sacrificed for gastric ulcer determination[28].

2.3.3 Gastric ulcer induction by water immersion restraint stress (WIR)

Rats were restrained in stainless steel cages and immersed up to their xiphoid in water maintained at 16 ± 2°C. Five hours later, the rats were sacrificed for gastric ulcer determination[29].

To determine gastric ulcers, the rats were euthanized by carbon dioxide. The abdomen was immediately opened, pylorus and cardia were ligated and the stomach was instilled with 7 ml of 0.5% formalin. The stomach was excised and fixed in 0.5% formalin for 10 minutes and cut along the greater curvature. Gastric ulcers were determined by summary of hemorrhagic erosion length in each stomach for HCl and WIR-induced gastric ulcer. In ASP-induced stomach, the gastric ulcer was determined by the severity score (30): 0 = no pathology; 1 = mucosal edema and petechiae; 2 = 1-5 small ulcer (1-2 mm); 3 = more than 5 small ulcer or 1 medium ulcer (3-4 mm); 4 = two or more than 2 medium ulcers or 1 large ulcer (>4 mm); 5 = perforated ulcer.

The gastric ulcer index in HCl- and WIR-induced model were calculated as average of lesion length per animal[28, 29], whereas aspirin-induced gastric ulcer index was determined as average of severity score per animal[30].

Statistical analysis: The data were expressed as mean ± SEM (standard error of the mean). One way analysis of variance (ANOVA) and Tukey's honesty significant difference (HSD) test were used to compare the difference in the values among various experimental groups. A *p*-value of less than 0.05 ($p<0.05$) was considered statistically significant.

3. RESULTS

3.1. Single pretreatment

The gastric ulcer index of control aspirin-induced rats was 3.33 ± 0.3. The CCZ formula at doses of 0.25 to 2.0 g/kg (ulcer index 2.33 ± 0.51 to 1.5 ± 0.2) slightly decreased aspirin-induced gastric ulcers, whereas ranitidine at a dose of 50 mg/kg significantly prevented gastric ulcer induction, as shown in figure 1. In HCl-

induced gastric ulcer model, CCZ (0.25-2.0 g/kg) significantly inhibited gastric ulcer in a dose dependent manner. CMC treated rats had an ulcer index of 119.3± 9 and CCZ (2.0 g/kg) maximally decreased the ulcer index to 0.8±0.8. Ranitidine also significantly decreased the gastric ulcer index to 45.7±6.2 (Figure 2). The inhibition of ranitidine was significantly lower than CCZ at doses of 1.0 (ulcer index 9.2 ± 1.7) and 2.0 g/kg. On the other hand, CCZ at doses of 0.25 to 2.0 g/kg showed the similar inhibition in WIR-induced gastric ulcer (34±6.5 to 28±3.2) as shown in figure 3. The control group treated with CMC had the gastric ulcer index of 59±6.8 and ranitidine decreased the gastric ulcer index to 21±1.8. The gastric ulcer inhibition of CCZ was not significantly different from ranitidine.

3.2. Long term pretreatment

Since single pretreatment of CCZ did not show significant inhibition in aspirin-induced gastric ulcer rats, the long term pretreatment of CCZ was investigated only in HCl- and WIR-induced gastric ulcer rats. In HCl-induced gastric rats, the CCZ decreased gastric ulcer index from control 135.5±7.3 to 85.3±7.4, 71.5 ± 5.3, 75.8 ± 6.6 and 61.8±5.2 at doses of 0.25, 0.5, 1.0 and 2.0 g/kg/day, respectively. Long term pretreatment of ranitidine decreased gastric ulcer index to 38.2±5.8. The inhibitory effect of ranitidine was significant higher than CCZ at doses of 0.25, 0.5 and 1.0 g/kg/day but not significantly difference from CCZ 2.0 g/kg/day (Figure 4). In contrast to single pretreatment in WIR-induced rats, long term pretreatment of CCZ did not show any inhibitory effect on gastric ulcer. The control rats had ulcer index 51.3 ± 6.4 whereas the CCZ treated rats had ulcer index 47.7± 4.7, 57.0 ± 4.1, 70.0 ± 6.2 and 57.8 ± 6.1 at doses of 0.25, 0.5, 1.0 and 2.0 g/kg/day, respectively. Long term pretreatment of ranitidine inhibited WIR-induced gastric ulcer (from 51.3±6.4 to 10.7±1.8) as shown in figure 5.

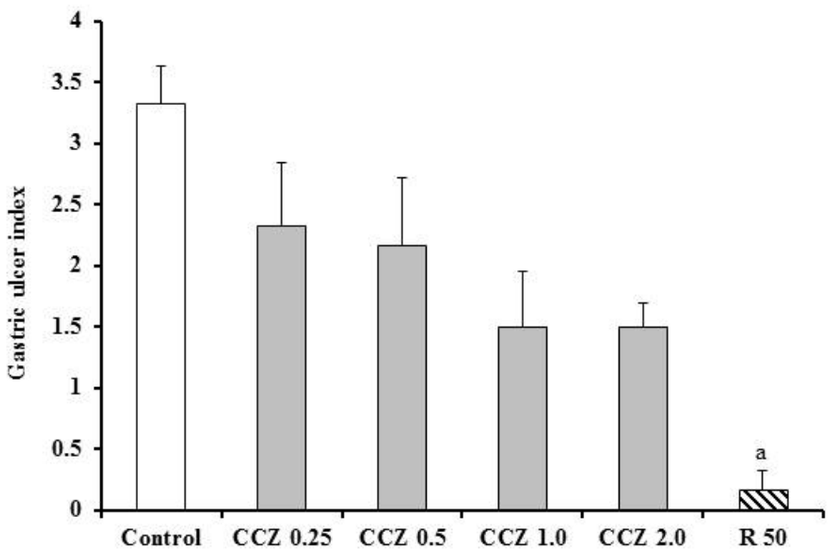

Figure 1. Effect of single dose pretreatment of CCZ (0.25, 0.5, 1 and 2 g/kg) and ranitidine (50 mg/kg) on aspirin-induced gastric ulcer rat (n = 6).
[a] $p < 0.001$: significantly different from the control group

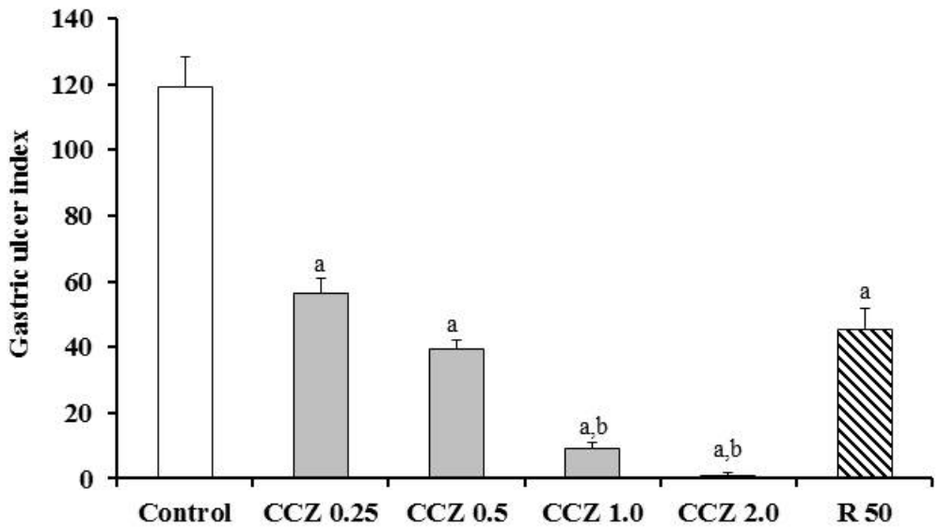

Figure 2. Effect of single dose pretreatment of CCZ (0.25, 0.5, 1 and 2 g/kg) and ranitidine (50 mg/kg) on HCI-induced gastric ulcer rat (n = 6).
[a] $p < 0.001$: significantly different from the control group
[b] $p < 0.001$: significantly different ranitidine treated group

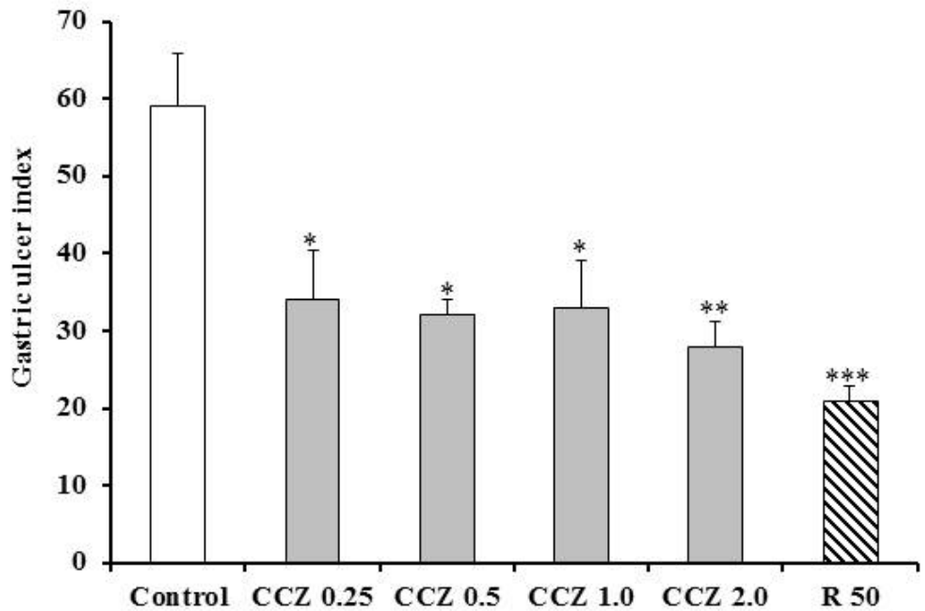

Figure 3. Effect of single dose pretreatment of CCZ (0.25, 0.5, 1 and 2 g/kg) and ranitidine (50 mg/kg) on WIR-induced gastric ulcer rat (n = 6).
[*] $p < 0.005$: significantly different from the control group
[**] $p < 0.01$: significantly different from the control group
[***] $p < 0.001$: significantly different from the control group

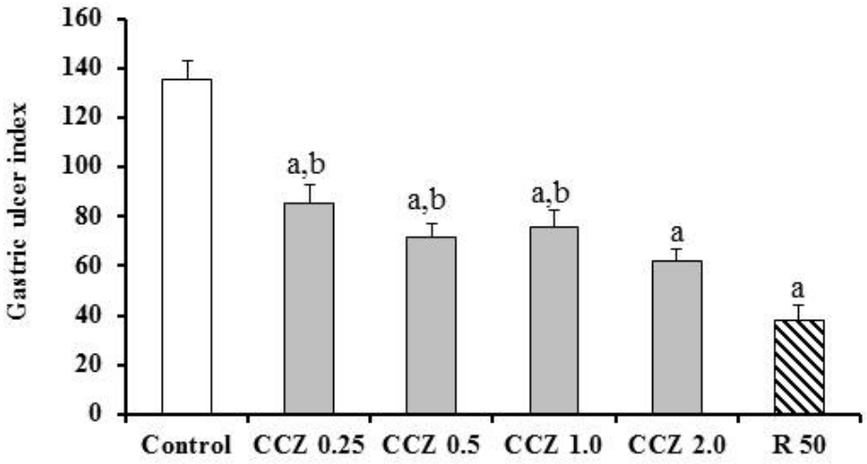

Figure 4. Effect of long term pretreatment of CCZ (0.25, 0.5, 1 and 2 g/kg) and ranitidine (50 mg/kg) on HCI-induced gastric ulcer rat (n = 6).
[a] $p < 0.001$: significantly different from the control group
[b] $p < 0.05$: significantly different from ranitidine treated group

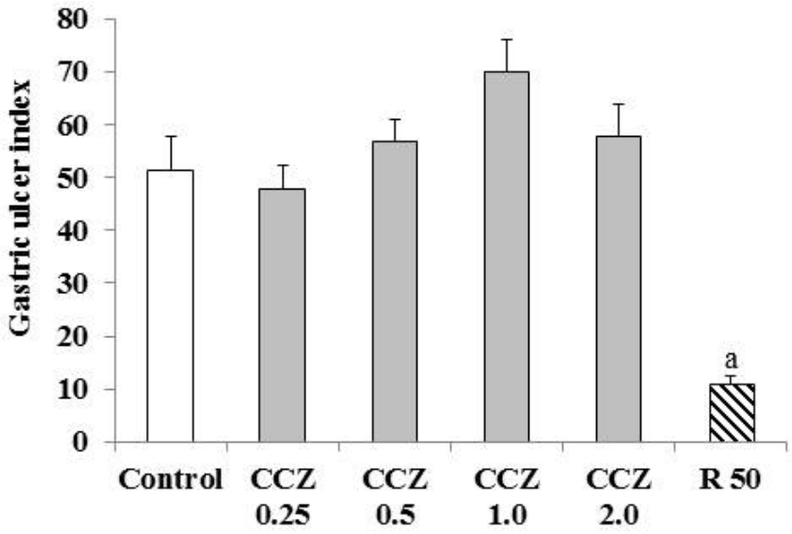

Figure 5. Effect of long term pretreatment of CCZ (0.25, 0.5, 1 and 2 g/kg) and ranitidine (50 mg/kg) on WIR-induced gastric ulcer rat (n = 6).
[a] $p < 0.001$: significantly different from the control group

4. DISCUSSION

Each gastric ulcer induction model we have used has different mechanisms for damaging the gastric mucosa. Acid is a corrosive factor that directly damages gastric mucosa, whereas the ulcer in the water immersion restraint stress model is caused by hydroxyl radical[31] and hypothermia[32]. The mechanism of NSAID-induced gastric ulcer can be prostaglandin-dependent (through cyclooxygenase inhibition) and prostaglandin-independent, such as inhibition of nitric oxide, hydrogen sulphide and polyamines[33].

The CCZ formula showed the most effective gastric ulcer protection against hyperacidity. The gastric ulcer inhibition was almost complete (99.3%) at the dose of 2 g/kg and more effective than the ranitidine at the dose of 50 mg/kg (61.7%). On the other hand, CCZ (2g/kg) treatment inhibited ulcer development by of 42.4%, which was less than the preventative effect of ranitidine (64.4%) in stress ulcer induction. The CCZ did not significantly protect against aspirin-induced ulcers. This demonstrated that CCZ can protect gastric mucosal damage from offensive factors but has relative lower efficacy when challenged with defensive factors.

Curcuma comosa, a component in CCZ formula, has been shown to be effective to reduce gastric ulcer formation in HCl- and ethanol-induced rats but not in indomethacin-induced ulcer[17], similar to CCZ. Wan chak modluk contained diarylheptanoids, identified as phytoestrogen, and has estrogenic activity[11-13]. Its hexane[14-16] and ethanol[15] extracts and diarylheptanoids[15] also demonstrated anti-inflammation properties. Gastric ulcer has been shown to be related to decrease of estrogen[3, 5] and estrogen decreased gastric and duodenal ulcers in both women[6] and ovariectomized rats[7-9]. However, estrogen did not protect against aspirin-induced gastric ulcer in female rats[10]. In addition, *Zingiber montanum* or Plai, the second component of CCZ showed both antiulcer[18, 19] and anti-inflammatory activities[20- 22]. This implies that CCZ formula protects gastric mucosa from damaging factors through phytoestrogen in Wan chak modluk and anti-inflammatory substances from Wan chak modluk and Plai. Although, Plai has demonstrated anti-gastric ulcer activity in both HCl- and indomethacin-induced gastric ulcer in rats[18] and mice[19], CCZ formula showed limited inhibitory effect in aspirin-induced rat. This may be due to the ratio of Plai being only one-third of the CCZ formula, resulting in consequently lower concentrations in the dose. It is also possible that interactions between the multiple components of this formulations, affect some of the activity. To clarify CCZ's antiulcer protective mechanisms, the effects of CCZ on gastric secretion and prostaglandins production should be further investigated.

The CCZ formula showed less gastric protective effect in HCl-induced ulcer (54.4% inhibition at 2.0g/kg) and no significant effect in WIR-induced ulcer after by long term treatment, whereas ranitidine was more effective in both models (71.8% and 79.1% inhibition in HCl-induced and WIR-induced ulcer, respectively). This may be due to the lower concentrations of the active components in the herbal formulation vs. the concentrated chemical. It is also possible that short duration of action and fast metabolism of active substances from the medicinal plant, so it does not show any cumulative effect.

Ranitidine, which is the most widely prescribed drug for ulcers, was the most efficacious against gastric ulcers in aspirin-induced rats and was protective in HCl- and WIR-induced gastric ulcer rats. This result was in contrast to the CCZ formula, implying different mechanisms of ulcer protection. It is possible that ranitidine, in combination with CCZ, can provide additional benefit in gastric ulcer protection.

5. CONCLUSION

The CCZ formula demonstrates acute gastric ulcer protection for hypersecretion of acid and stress induced ulcer and is relatively less protective against nonsteroidal anti-inflammatory drug such as aspirin. Our data clearly demonstrate that CCZ has the potential to prevent gastric ulcers not only in women during perimenopausal and menopausal periods but also across the population.

REFERENCES

1. Pilot ML, Muggia A, Spiro HM. Duodenal ulcer in women. Psychosom Med. 1967; XXIX(6):586-97.
2. Clark DH. Peptic ulcer in women. Bri Med J. 1953; 6:1254-57.
3. Sanweiss D, Harry MD, Saltzstein C. The relation of sex hormones to peptic ulcer. Am J Digest Dis. 1939; 6(1):6-12.
4. Redchitis IV, Petrov EE. The age-related characteristics of the clinical picture of

duodenal ulcer in women. Lik Sprava. 1995; 5-6:149-52.
5. Orlovski VE, Medvedev VN. Gonadotropins and sex steroid hormones in women with exacerbated duodenal peptic ulcer. Vrach Delo. 1959; 8:64-6.
6. Smith A, Contreras C, Ko KH, Chow J, Dong X, Tuo B, et al. Gender-specific protection of estrogen against gastric acid-induced duodenal injury: Stimulation of duodenal mucosal bicarbonate secretion. *Endocrinology.* 2008; 149(9):4554-6.
7. Aguwa CN. Effects of exogenous administration of female sex hormone on gastric secretion and ulcer formation in the rat. Eur J Pharmacol. 1984; 104:491-4.
8. Kurt D, Saruhan BG, Kanay Z, Yokus B, Kanay BE, Unver O, et al. Effect of ovariectomy and female sex hormones administration upon gastric ulceration induced by cold and immobility restraint stress. Saudi Med J. 2007; 28(7):1021-7.
9. Shimozawa N, Okajima K, Harada N. Estrogen and isoflavone attenuate strees-induced gastric mucosal injury by inhibiting decreases in gastric tissue levels of CGRP in ovariectomized rats. Am J Physiol Gastro-intest Liver Physiol. 2007; 292:G615-9.
10. Sangma TK, Jain S, Mediratta PK. Effect of ovarian sex hormones on non-steroidal anti-inflammatory drug-induced gastric lesions in female rats. Indian J Pharmacol. 2014; 46(1):113-6.
11. Suksamrarn A, Ponglikitmongkol M, Wongkrajang K, Chindaduang A, Kittidanairak S, Jankam A, et al. Diarylheptanoids, a new phytoestrogens from the rhizomes of *Curcuma comosa*: isolation, chemical modification and estrogenic activity evaluation. Bioorg Med Chem. 2008; 16:6891-02.
12. Winuthayanon W, Piyachaturawat P, Suksamrarn A, Ponglikitmongkol M, Arao Y, Hewitt SC, et al. Diarylheptanoid phytoestrogens isolated from the medicinal plant *Curcuma comosa*: biologic actions *in vitro* and *in vivo* indicate estrogen receptor-dependent mechanisms. Environ Health Perspect. 2009; 117(7):1155-61.
13. Winuthayanon W, Suksen K, Boonchird C, Chuncharunee A, Ponglikitmongkol M, Suksamrarn A, et al. Estrogenic activity of diarylheptanoids from *Curcuma comosa* Roxb. Required metabolic activation. J Agric Food Chem. 2009; 57:840-45.
14. Jantaratnotai N, Utaisincharoen P, Piyachaturawat P, Chongthammakun S, Sanvarinda Y. Inhibitory effect of plant *Curcuma comosa* on NO production and cytokine expression in LPS-activated microglia. Life Sci. 2007; 78:571-77.
15. Sodsai A, Piyachaturawat P, Sophasan S, Suksamrarn A, Vongsakul M. Suppression by plant *Curcuma comosa* Roxb. Of pro-inflammatory cytokine secretion in phorbol-12-myristate-13-acetate stimulated human mononuclear cells. Int Immunopharmacol. 2007; 7:524-31.
16. Al-Amin M, Sultana GNN, Hossain CF. Antiulcer principle from *Zingiber montanum*. J Ethnopharmacol. 2012; 57-60.
17. Pongpiriyadacha Y, Nuansrithong P, Chantip D, Sirinthraved N. Protective effects of the extract from *Curcuma comosa* on gastric mucosal lesions in rats. 31st Congress on Science and Technology of Thailand at Suranaree Univeristy of Technology, 18-20 October, 2005.
18. Pongpiriyadacha Y, Nuansrithong P, Chantip D. Protective effects of the extract from *Zingiber cassumunar* on gastric mucosal lesions in rats. 32nd Congress on Science and Technology of Thailand at Suranaree Univeristy of Technology, 10-12 October, 2006.
19. Al-Amin M, Sultana GNN, Hossain CF. Antiulcer principle from *Zingiber montanum*. J Ethnopharmacol. 2010; 141:57-60.
20. Jeenapongsa R, Yoovathaworn K, Sriwatanakul KM, Pongprayoon U, Sriwata-nakul K. Anti-inflammatory activity of (*E*)-1-(3,4-dimethoxyphenyl) butadiene from *Zingiber cassumunar* Roxb. J Ethnopharmacol. 2003; 87:143-8.
21. Nakamura S, Iwami J, Matsuda H, Wakayama H, Pongpiriyadacha Y, Yoshikawa M. Structures of new phenylbutanoids and nitric oxide production inhibitors from the rhizomes of *Zingiber cassumunar*. Chem Pharm Bull. 2009; 57(11):1267-72.

22. Kaewchoothong A, Tewtrakul S, Panichayupakarannant P. Inhibitory effect of phenylbutanoid-rich *Zingiber cassumunar* extracts on nitric oxide production by murine macrophage-like RAW264.7 cells. Phytother Res. 2012; 26:1789-92.
23. Tohda C, Nakayama N, Hatanaka F, Komatsu K. Comparison of anti-inflammatory activities of six *Curcuma* rhizomes: A possible curcuminoid-independent pathway mediated by *Curcuma phaeocaulis* extract. eCAM. 2006; 3(2):255-60.
24. Trinh HT, Bae EA, Lee JJ, Kim DH. Inhibitory effects of curcuminoids on passive cutaneous anaphylaxis reaction and scratching behavior in mice. Arch Pharm Res. 2009; 32(12): 1783-7.
25. Rosenstock SJ, Jorgensen T. Prevalence and incidence of peptic ulcer disease in aDanisk County – a propective cohort study. Gut. 1995;36:819-824.
26. Thai Ministry of Public Health. Thai Herbal Pharmacopoeia. Bangkok: Prachachon; 1995.
27. Goel RK, Gupta S, Shankar R, Sanyal AK. Anti-ulcerogenic effect of banana powder (*Musa Sapientum* var paradisiaca) and its effect on mucosal resistance. J Ethnopharmacol. 1986; 18:33-44.
28. Robert A, Nezamis JE, Lancaster C, Davis JP, Field SO, Hanchar AJ. Mild irritants prevent gastric necrosis through adaptive cytoprotection mediated by prostaglandins. Am J Physiol. 1983; 245: G113-
29. Takagi K, Kansuya Y, Watanabe K. Studies on the drugs for peptic ulcer: a reliable method for production stress ulcer in rats. Chem Pharm Bull. 1964;12(4)465-73.
30. Minano FJ, Serrano JS, Pascual F, Bhattacharya SK. Effect of GABA on gastric acid secretion and ulcer formulation in rats. Life Sci. 1987; 41(13):1654-58.
31. Das D, Bandyopadhyay D, Bhattacharjee M, Banerjee RK. Hydroxyl radical is the major causative factor in stress-induced gastric ulcer. Free Radic Biol Med. 1997; 23(1):8-18.
32. Fernandez JL. Analysis of the cold-water restraint procedure in gastric ulceration and body temperature. Physiol Behav. 2004; 81:827-33.
33 Musamba C, Pritchard DM and Pirmohamed M. Review article: cellular and molecular mechanism of NSID-induced peptic ulcers. Aliment Pharmacol Ther. 2009; 30:517-31.