Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.5

ANTI-INFLAMMATORY AND ANTI-ULCEROGENIC ACTIVITIES OF CHANTALEELA RECIPE

Seewaboon Sireeratawong,[1*] Parirat Khonsung,[2] Pritsana Piyabhan,[3] Urarat Nanna,[1] Noppamas Soonthornchareonnon,[4] Kanjana Jaijoy,[5]

[1]Division of Pharmacology, Department of Preclinical Science, Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand., [2]Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.
[3]Division of Physiology, Department of Preclinical Science, Faculty of Medicine, Thammasat University, Pathumthani 12120, Thailand., [4]Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand.
[5]Research Unit of Pharmacology and Toxicology of Natural Products, Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand.
***E-mail:** seewaboon@gmail.com

Abstract

Chantaleela recipe is indicated for relieving fever in Thai traditional folk medicine. In the present study, Chantaleela recipe was investigated for anti-inflammatory, analgesic, antipyretic and anti-ulcerogenic activities. In preliminary investigation Chantaleela recipe was found to exert an inhibitory activity on the acute phase of inflammation as seen in ethyl phenylpropiolate-induced ear edema as well as in carrageenan-induced hind paw edema in rats. The results suggest that the anti-inflammatory activity of Chantaleela recipe may be due to an inhibition via cyclooxygenase pathway. In the analgesic test, Chantaleela recipe showed a significant analgesic activity in both the early and late phases of formalin test, but exerted the most pronounced effect in the late phase. The analgesic activity of Chantaleela recipe may act via mechanism at peripheral and partly central nervous system. In antipyretic test, Chantaleela recipe significantly decreased rectal temperature of brewer's yeast-induced hyperthermia rats, probably by inhibiting synthesis and/or release of prostaglandin E_2 in the hypothalamus. Therefore, the key mechanism of anti-inflammatory, analgesic, and antipyretic activity of the Chantaleela recipe likely involves the inhibition of the synthesis and/or release of inflammatory or pain mediators, especially prostaglandins. The oral administration of the Chantaleela recipe reduced ulcer formation in acute gastric ulcer models (EtOH/HCl-, indomethacin-, and stress-induced gastric lesions). In contrast, this recipe did not reduce the secretory rate, total acidity, and increase pH in rat stomach. These results indicated that Chantaleela seem to possess anti-ulcerogenic effect. This activity may be due to the increase of gastric mucosal resistance or potentiation of defensive factors and/or the decrease of aggressive factors but did not associate the anti-secretory activity. Moreover, the high oral doses treated did not cause acute toxicity in rats and the long term oral administration did not produce gastric and ileum lesions.

Key words: Chantaleela recipe, Anti-inflammatory, Analgesic, Antipyretic, Anti-ulcerogenic.

Introduction

Chantaleela recipe is composed of eight kinds of herbal plants including Koad-Kamao (*Atractylodes lancea* (Thunb) DC.), Koad-So (*Angelica dahurica* (Fisch.ex Hoffm.) Benth. et Hook. f.), Koad-Chulalumpa (*Artemisia vulgaris* Linn.), Chan-Daeng (*Dracaena loureiri* Thoms. & Gagnep.), Pra-Lai-Peark (*Eurycoma longifolia* Jack.), Ka-Dom (*Gymnopetalum cochinense* Kurz H.C.), Nutmeg (*Myristica fragrans* Houtt.), and Bor-ra-pet (*Tinospora crispa* Miers ex. Hook. f.). This recipe has long been used in Thai traditional folk medicine for relieving fever, which was prepared in powder or tablet form (National Drug Committee, Ministry of Public Health, 2006). The ethnobotanical data and traditional use or biological activities of plants are shown in Table 1.

The normal dose of Chantaleela for alleviation of fever is 750 mg orally every 8 hours. *In vivo* study, Chantaleela at the dose of 400 mg/kg showed the antipyretic action in white rabbits, and its efficacy and duration of action were equivalent to paracetamol at the dose of 200 mg/kg (Thongpraditchote et al., 2001). Clinical study, normal dose of Chantaleela for alleviation of fever does not have an effect on either platelet aggregation or platelet numbers (Itthipanichpong et al., 2010). Normally, inflammatory reaction produces the synthesis and release of the inflammatory mediators, such as histamine, bradykinin, prostaglandins (PGs), interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α), which their effects are related to pain and fever (Ivanov and Romanovsky, 2004; Engblom et al., 2002). However, less scientific data are available to support the anti-inflammatory, analgesic and antipyretic activities of Chantaleela recipe. Therefore, anti-inflammatory, analgesic and antipyretic activities of Chantaleela recipe were pursued.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of inflammatory diseases. The mechanism of NSAIDs involves the inhibition of the synthesis and/or release of inflammatory or pain mediators, especially prostaglandins (PGs). The side effects are nausea, vomiting, diarrhea, constipation, decreased appetite, rash, dizziness, headache, and drowsiness. Moreover, NSAIDs may also cause fluid retention, leading to edema. The most serious side

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.5

effects are kidney failure, liver failure, ulcers and prolonged bleeding after an injury or surgery (Waller et al., 2005). If the activities of Chantaleela recipe seem to be similar to those of NSAIDs, the gastric ulcer may be one of the potential side effects of Chantaleela recipe.

Table 1: The ethnobotanical data and traditional use or biological activities of plants in Chantaleela recipe

Species	Family	Part of used	Biological activities or Traditional use
A. lancea	Compositae	rhizome	Anti-oxidant (Cai et al., 2004), Anti-inflammatory (Ul'chenko et al., 2005)
A. dahurica	Umbelliferae	root	Anti-inflammatory (Lin et al., 2002; Kang et al., 2007)
A. vulgaris	Compositae	whole plant	Anti-inflammatory and Anti-oxidant (http://www.stuartxchange.com/Damong.html)
D. loureiri	Agavaceae	stem	Anti-oxidant (Kongcharoensuntorn et al., 2005), Anti-inflammatory (Sawasdee, 2001), Antinociceptive and Antipyretic (Reanmongkol et al., 2003)
E. longifolia	Simarubaceae	root	Antipyretic (Bhat and Karim, 2010)
G. cochinense	Cucurbitaceae	fruit	Antipyretic (Kumklang, 1999)
M. fragrans	Myristicaceae	seed	Anti-oxidant (Khatun et al., 2006; Jin et al., 2005)
T. crispa	Menispermaceae	stem	Anti-oxidant (Cavin et al., 1998), Anti-inflammatory (Higashino et al., 1992), Antipyretic (Saralamp et al., 1996)

The gastrointestinal effects of four kinds in this recipe have been reported. *A. lancea* have been described as appetizer, carminative and digestive agent. *G. cochinchinense* is a digestive agent. *M. fragrans* is a carminative. *T. crispa* have been described as appetizer. However, the effect of this recipe on gastrointestinal tract has not yet been studied. The purposes of the present study were to investigate the effect of Chantaleela recipe on GI tract and anti-ulcerogenic activities of as well as its possible mechanism(s) of action.

Materials and Methods

Plant material and Preparation of Chantaleela Recipe

The eight plant materials were kindly provided by Thai traditional medicine doctor. The preparation of the crude extract of Chantaleela recipe was developed in our laboratory by a consecutive extraction with three solvents including hexane, 95% ethanol and water, and then spray dried. The quality control of the raw materials and crude extracts was followed by Thai Herbal Pharmacopoeia (organoleptic examination, % loss on drying, extractive values, total ash and acid insoluble ash). The percent amount of volatile oil, type of chemical constituents in oils (detected by GC/MS), chemical constituents (flavonoids, lactone, terpnoids and tannins) in raw materials and extracts, and TLC finger prints were also to studied.

Experimental animals

Male Sprague-Dawley rats, weighing 40-60, 120-150, 200-250, 250-300 g as well as male ICR mice weighing 30–40 g were obtained from the National Laboratory Animal Center (NLAC-MU), Nakorn Pathom, Thailand. They were housed under standard environmental conditions of temperature at 24 ± 1°C under a 12 h dark-light cycle, and allowed free access to drinking water and standard pellet diet. All animals were deprived of food except water 16-18 hour prior the experiments. The Animal Ethics Committee of Faculty of Medicine, Thammasat University, Pathum Thani, Thailand approved all experimental protocols (No.0002/2006 and No. 0003/2008).

Test drugs and drug administration

For anti-inflammatory, analgesic, antipyretic and anti-ulcerogenic experiments all test substances were diluted in distilled water. They were orally administered in an equivalent volume of 0.1 ml/100 g body weight of the rats and in a volume of 0.1 ml/10 g body weight of the mice, except in the ear edema model where a local application of the test drug to outer and inner surfaces of the ear was performed. Control groups received vehicle only in the same volume and same route of administration.

Ethyl phenylpropiolate (EPP)-induced ear edema in rats (Brattsand et al., 1982)

Male rats (40–60 g) were used. Test substances dissolved in dimethylsulfoxide (DMSO) and acetone (1:1) were administered topically (20 µl/ear) just before EPP (1 mg/20 µl/ear) to the inner and outer surfaces of both ears. Control group received vehicle (20 µl/ear), and the reference group received 1 mg/20 µl/ear of phenylbutazone in acetone. The test group received Chantaleela recipe at the doses of 1, 2 and 4 mg/20 µl/ear. The thickness of each ear was measured with vernier calipers before and at 15, 30, 60 and 120 min after edema induction.

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.5

Carrageenan-induced hind paw edema in rats (Winter et al., 1962)

Male rats (120–150 g) were used. The test groups were received Chantaleela recipe (300, 600, 1,200 mg/kg), aspirin (300 mg/kg) and distilled water (control). Test substances were given 1 h prior to carrageenan injection (1% in normal saline solution). Paw edema was induced by an intradermal injection of carrageenan into the plantar surface of the right hind paw of the rats at a volume of 0.05 ml. The edema volume was determined using a plethysmometer (model 7140, Ugo Basile, Italy) prior to and 1, 3 and 5 h after carrageenan injection.

Formalin test (Hunskaar and Hole, 1987)

Male mice (40-60 g) were used. Control group received distilled water. Aspirin (300 mg/kg) and morphine (10 mg/kg) were used as reference groups. Chantaleela recipe was administered at the doses of 300, 600 and 1,200 mg/kg. In the early phase assessment, 20 µl of 1% formalin in saline solution was injected subcutaneously into the right dorsal hind paw of the mouse 60 min after test drug administration. Then, between 0 and 5 min after formalin injection, the time in seconds the mouse spent for intensive licking the right dorsal hind paw was determined. In the late phase assessment, another set of mice was used. The formalin was injected 40 min after test drug administration and the licking time was determined between 20 and 30 min after formalin injection.

Antipyretic activity (Teotino et al., 1963)

Male rats (200-250 g) were used. Rats were injected subcutaneously of 1 ml/100 g body weight of 25% brewer's yeast. Rectal temperatures were recorded using a twelve channel electric thermometer (LETICA, model TMP 812 RS, Panlab S.L., Spain) at the initial and 18th h after yeast injection. Those animals which show a rise in rectal temperature of more than 1°C were used. Control group received distilled water. Aspirin (300 mg/kg) was used as a reference group. Chantaleela recipe was administered at the doses of 300, 600 and 1,200 mg/kg. Test substances were administered orally and the rectal temperatures of animals were recorded at 30 min interval for 2 h following drug treatment.

Anti-ulcerogenic activity
Preparation of rats for anti-ulcerogenic activity study

Male rats were fasted 48 h, but had free access to water. The water was withdrawn 1 h before starting the experiment. Control group received distilled water. Cimetidine (100 mg/kg) was used as a reference drug. Chantaleela recipe was administered at the doses of 150, 300 and 600 mg/kg. Test substances were given orally to the rats 1 h before induction of gastric lesions.

Methods used to induce gastric lesions
Ethanol/hydrochloric acid (EtOH/HCl)-induced gastric lesions (Mizui and Doteuchi, 1983)

Each rat was administered 1 ml of EtOH/HCl (absolute ethanol 60 ml + HCl 1.7 ml + distilled water 38.3 ml) orally. One hour later, the rats were sacrificed for determination of gastric lesions.

Restraint water immersion stress-induced gastric lesions (Takagi et al., 1963)

Rats were restrained in stainless steel cages and immersed up to their xiphoid in a water bath maintained at 20 ± 2 °C. Five hours after restraint in cool water, the rats were sacrificed for determination of gastric lesions.

Indomethacin-induced gastric lesions (Hayden et al., 1978; Djahanguiri, 1969)

Suspension of indomethacin in 5% Tween80 was administered intraperitoneally with a single dose of 30 mg/kg to the rats. Five hours later, the rats were sacrificed for determination of gastric lesions.

Evaluation of gastric lesions

After each rat was sacrificed, the stomach was removed and opened along the greater curvature, rinsed with isotonic saline and pinned out on a wax plate. The glandular portion of the stomach was then examined for lesions. The length (mm) of each lesion was measured under a dissecting microscope (10x). The sum of the total length of lesions in each group divided by the number of rats in that group was expressed as the gastric lesions. The percent inhibition of gastric lesion formation was calculated.

Pylorus ligation (Shay et al., 1945)

One hour after drug administration, rats were lightly anesthetized by ether. The abdomen was opened and the pylorus was ligated. The abdomen was closed by suturing. The animals were sacrificed 5 h later by an overdose of ether. The stomach was removed and its content was subjected to measurement of volume, pH and total acid output. The gastric juice was centrifuged and the total acidity of the supernatant was be determined by titration with 0.1 N NaOH to an end point of

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.5

pH 7.4 using phenolphthalein as an indicator. Total acidity of gastric juice was expressed as ml and µEq per 100 g body weight of rat per hour, respectively

Acute oral toxicity (OECD, 2001; WHO, 2000)

Ten rats were randomly divided into two groups of 5 animals per sex. Chantaleela extract at a single dose of 5,000 mg/kg body weight was given orally to the treated group, while the control group received water vehicle. Body weight, signs of toxicity and mortality were observed after the administration at the first, second, fourth and sixth hour and once daily for 14 days. On the 15th day, all rats were sacrificed for necropsy examination.

Long term effect on GI tract

Chantaleela recipe at the dose of 2,400 mg/kg was given orally to the rats daily for 90 days. At the end of experiment, the stomach and ileum were removed and observed for gross lesions. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination.

Statistical analysis

Data were reported as mean ± standard error of mean (S.E.M.) and were compared using one-way analysis of variance (ANOVA), followed by post hoc least-significant difference (LSD) test using SPSS software, and *p* values less than 0.05 were considered to be significant.

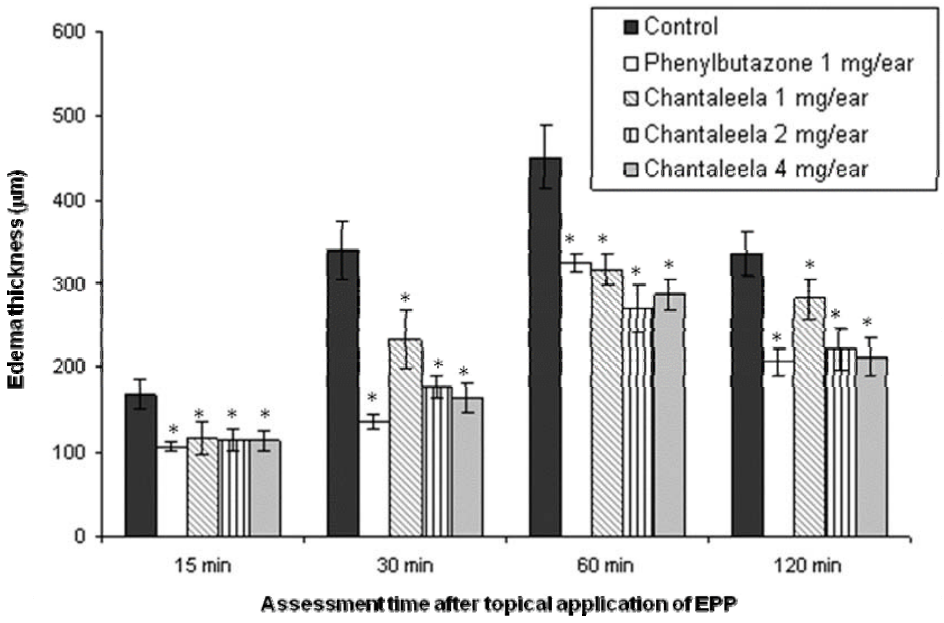
Results

Standardized of Chantaleela recipe

The raw materials in Chantaleera recipe contained 8 varieties of plants (*G. chinens*, *A. lancea*, *A. dahurica*, *A. annua*, *S. album*, *D. loureiri*, *T. crispa* and *E. longifolia*), there were 2 plants which contained volatile oil (*A. Lancea* and *S. album*). The chemical constituents in the raw materials were mainly flavonoids, terpenes and fluorescence compounds. The extract had no residue of hexane and ethanol. Quality control of the extract was maintained using several parameter such as % Loss on drying (2.7), % Total ash (7.35), % Acid insoluble ash (0.22). The absence of microbial, aflatoxin, and heavy metal was substantiated.

EPP-induced ear edema in rat

Chantaleela recipe possessed significant inhibitory effect on EPP-induced ear edema at all assessment times, as shown in Figure 1. The percent inhibition on the edema formation increased as the dose increased. The result showed that a marked effect of Chantaleela recipe was obtained with the highest dose (4 mg/ear) used in this experiment.



Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.5

Figure 1 Effect of Chantaleela recipe on EPP-induced ear edema formation in rats. *Significantly different from the control group, $p<0.05$.

Carrageenan-induced hind paw edema in rat

The inhibitory activity on carrageenan-induced rat hind paw edema, caused by the oral administration of Chantaleela recipe at various assessment times after carrageenan injection is shown in Figure 2. Chantaleela recipe at the doses of 300, 600 and 1,200 mg/kg body weight also possessed significant inhibitory effect on carrageenan-induced paw edema at all recorded times. Aspirin, a cyclooxygenase inhibitor, at the dose of 300 mg/kg body weight exhibited significant edema inhibition.

Formalin test in mice

Inhibition of licking response of the test drugs in the early phase and late phase of the formalin test is shown in Table 2. In the early phase, Chantaleela recipe at the doses of 300, 600 and 1,200 mg/kg body weight significantly inhibited the licking response. Aspirin at a dose of 300 mg/kg body weight also significantly reduced the licking time. In late phase, Chantaleela recipe at doses of 300, 600 and 1,200 mg/kg body weight markedly decreased the licking time, aspirin at a dose 300 mg/kg body weight could intensively inhibit the licking response.

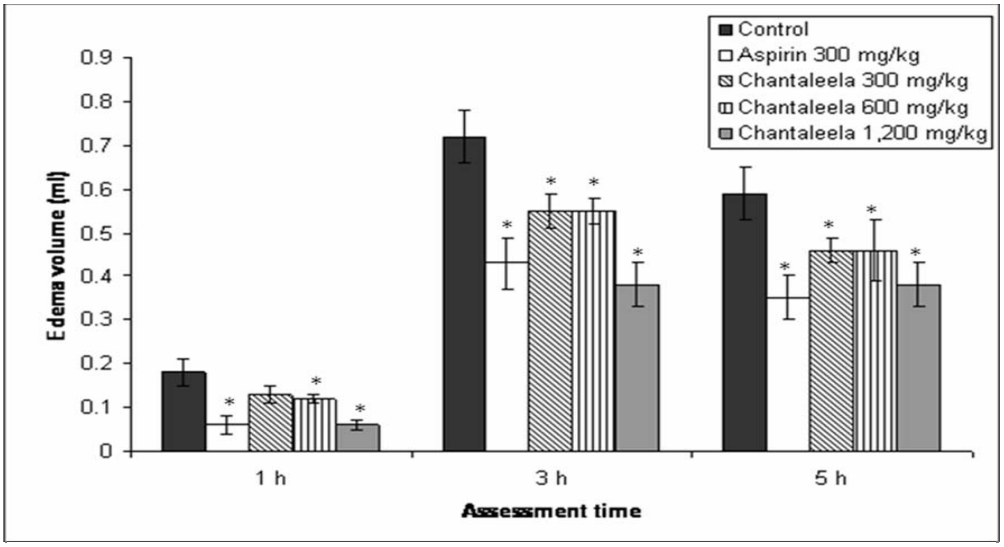


Figure 2 Effect of Chantaleela recipe on carrageenan-induced paw edema in rats. *Significantly different from the control group, $p<0.05$.

Table 2: Effect of Chantaleela recipe on early phase and late phase of the formalin test in mice

Group	Early phase		Late phase	
	Licking time (sec)	% Inhibition of licking response	Licking time (sec)	% Inhibition of licking response
Control	100.33 ± 11.09	-	91.00 ± 10.79	-
Aspirin 300 mg/kg	77.67 ± 8.24*	23	0.00 ± 0.00*	100
Morphine 10 mg/kg	00.00 ± 0.00*	100	0.00 ± 0.00*	100
Chantaleela 300 mg/kg	66.00 ± 11.58*	34	6.83 ± 3.19*	92
600 mg/kg	57.33 ± 8.21*	43	0.00 ± 0.00*	100
1,200 mg/kg	32.17 ± 7.09*	68	0.00 ± 0.00*	100

Values expressed as mean ± S.E.M. ($n = 6$)
* Significantly different from the control group, $p<0.05$.

Yeast-induced hyperthermia in rats

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.5

The antipyretic effect of aspirin and Chantaleela recipe is shown in Figure 3. Aspirin at the dose 300 mg/kg could reduce the rectal temperature of the rats when measurement was made 30, 60, 90 and 120 min after drug administration. Chantaleela recipe at the dose of 1,200 mg/kg caused a reduction of the rectal temperature of hyperthermic rats.

Anti-ulcerogenic activity

As showed in Table 3, Chantaleela at the doses of 150, 300 and 600 mg/kg, and cimetidine (a histamine-2 receptor antagonist) at the dose of 100 mg/kg significantly reduced the formation of gastric lesions induced by EtOH/HCl-, restraint water immersion stress- and indomethcin-induced gastric lesions in rats in comparision with that of the control group ($p<0.05$). All doses of Chantallela recipe did not reduce the secretory rate, total acidity, and increase pH in rat stomach. The secretory rate and total acidity of cimetidine (100 mg/kg) group were significantly reduced when compare with those of the control group ($p<0.05$), as showed in Table 4.

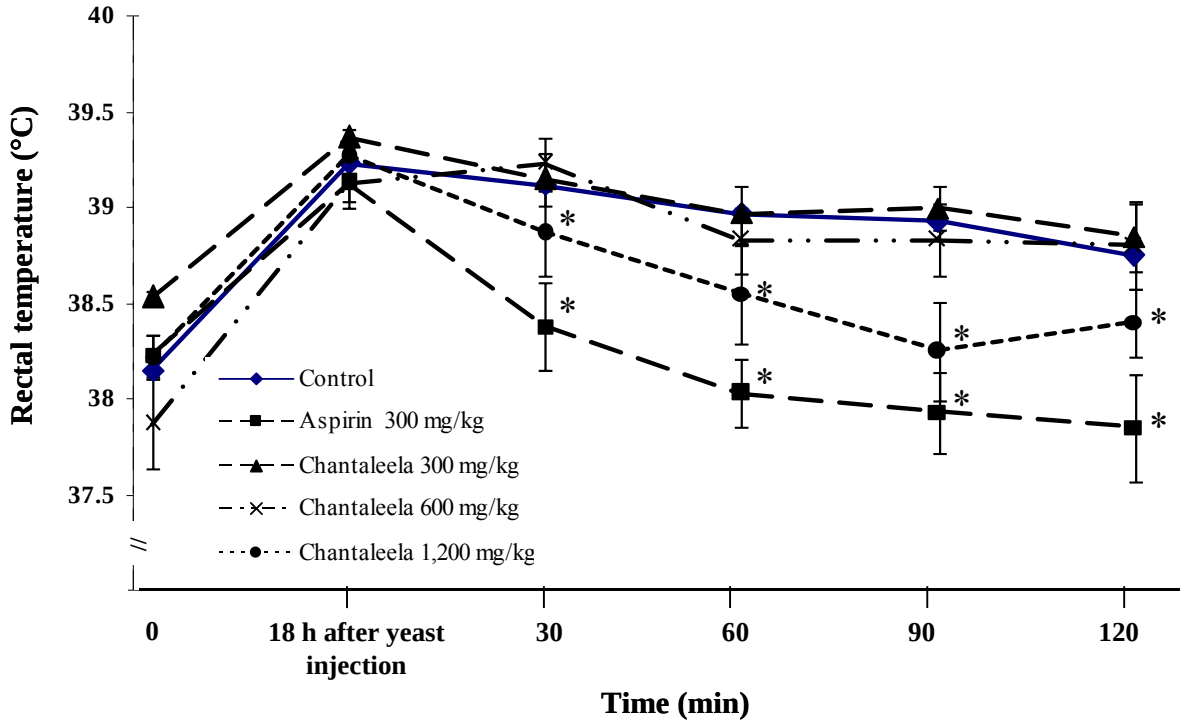


Figure 3 Effect of Chantaleela recipe on yeast-induced hyperthermia in rats. * Significantly different from the control, $p<0.05$

Table 3: Effects of Chantaleela recipe and cimetidine on EtOH/HCl-, restraint water immersion stress- and indomethcin-induced gastric lesions in rats

Group	Dose (mg/kg)	Gastric lesions (mm)	Inhibition (%)
Control + EtOH/HCl	-	81.52 ± 7.12	-
Cimetidine + EtOH/HCl	100	20.02 ± 3.48*	75
Chantaleela + EtOH/HCl	150	42.17 ± 8.30*	48
	300	28.68 ± 5.32*	65
	600	22.20 ± 4.42*	73
Control + Stress	-	17.42 ± 2.36	-
Cimetidine + Stress	100	0.80 ± 0.28*	95
Chantaleela + Stress	150	5.40 ± 0.42*	69
	300	4.02 ± 0.83*	77
	600	2.85 ± 0.48*	84
Control + Indomethacin	-	11.30 ± 0.59	-
Cimetidine + Indomethacin	100	0.40 ± 0.27*	96
Chantaleela + Indomethacin	150	10.43 ± 1.64	8
	300	4.30 ± 1.21*	62
	600	2.08 ± 0.49*	82

Values expressed as mean ± S.E.M. ($n = 6$)

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.5
* Significantly different from the control group, $P<0.05$.

Acute oral toxicity

Chantaleela recipe at a single dose of 5,000 mg/kg was orally given to the rat. The results showed no toxicity in terms of general behavior change, mortality, or change in gross appearance of internal organs (data not shown).

Long term effect on GI tract

The long term oral administration of Chantaleela recipe did not produce gastric and lieum lesions of both gross and histopathological examination (data not shown)

Table 4: Effects of Chantaleela recipe and cimetidine on pyrolus ligation in rats

Group	Gastric volume (ml)	Secretory rate (ml/100 g/5 h)	pH	Total acidity (mEq/100 g/5 h)
Control	3.25 ± 0.35	1.54 ± 0.19	1.95 ± 0.32	29.20 ± 2.65
Cimetidine 100 mg/kg	2.37 ± 0.16	0.87 ± 0.05*	5.06 ± 0.79*	13.30 ± 2.35*
Chantaleela				
150 mg/kg	4.47 ± 0.74	1.47 ± 0.11	1.58 ± 0.18	28.61 ± 3.26
300 mg/kg	3.97 ± 0.63	1.45 ± 0.22	2.48 ± 0.56	34.47 ± 4.52
600 mg/kg	2.83 ± 0.12	1.09 ± 0.09	1.85 ± 0.11	30.94 ± 2.79

Values expressed as mean ± S.E.M. ($n = 6$)
* Significantly different from the control group, $p<0.05$.

).

Discussion

The anti-inflammatory study of Chantaleela recipe was evaluated by using EPP-induced ear edema as well as carrageenan-induced paw edema. EPP causes release of many inflammatory mediators such as kinin, serotonin and PGs, which leads to fluid accumulation and edema characteristic of the acute inflammatory response (Brattsand et al., 1982). Chantaleela recipe exhibited an inhibitory effect on the ear edema formation induced by EPP. It is suggested that Chantaleela recipe probably possessed anti-inflammatory action by inhibition of the inflammatory mediators of the acute phase of inflammation. Moreover, carrageenan-induced inflammation is useful to detect orally active anti-inflammatory agents and has frequently been used to assess the anti-edematous effect of natural products. The edema is produced by a sequential release of pharmacological mediators, histamine, serotonin, kinins and PGs (Sedgwick and Willoughby, 1989). Chantaleela recipe showed a significant inhibition of carrageenan-induced hind paw edema. The results in this test model support the possible mechanism of action of Chantaleela recipe on the cyclooxygenase pathway and on other inflammatory mediators, which are involved in paw edema caused by carrageenan.

The formalin test is a valid and reliable model of nociception. The response of early phase (0-5 min after injection) is believed to represent a direct chemical stimulation of nociceptors (Tjolsen et al., 1992; Hunskaar et al., 1985; Dubuisson and Dennis, 1977), due to the irritant effect of formalin on sensory C fibers (Tjolsen et al., 1992; Heapy et al., 1987). The late phase response (20-30 min after injection) is most likely secondary to the development of an inflammatory response and the release of algesic mediators (Hunskaar and Hole, 1987). Moreover, the experimental results have indicated that substance P and bradykinin participate in the early phase, while histamine, serotonin, PGs, and bradykinin are involved in the late phase (Shibata et al., 1989). Chantaleela recipe showed analgesic activity on both phases of the formalin test, which suggests that the analgesic on both direct effect on the nociceptor and an inhibition of inflammatory pain, which its effect on the synthesis and/or release of PGs and/or other pain mediators.

In antipyretic test, the injection of exogenous pyrogens have been shown to induce the production of pro-inflammatory cytokines, such IL-1β, IL-6, interferon-α (IFN-α) and TNF-α, which enter the hypothalamic circulation and stimulate release of local PGs, resetting the hypothalamic thermal setpoint (Dalal and Zhukovsky, 2006). Aspirin and some nonsteroidal anti-inflammatory drugs (NSAIDs) exhibit the inhibitory effect on the biosynthesis and/or the releases of PGE_2 into the preoptic area of the anterior hypothalamus caused by endogenous pyrogens (Dalal and Zhukovsky, 2006; Li et al., 2001). Thus, the antipyretic effect of Chantaleela recipe may be due to the inhibition of the synthesis and/or release of local PGE_2 into hypothalamus.

EtOH/HCl-induced gastric lesions model is commonly employed for determining whether the anti-ulcerogenic activity involves the effects on gastric mucosal protective factors. Substance with cytoprotective activity will be able to prevent gastric lesions in this model. These lesions are due to a direct necrotizing effect to gastric mucosa (Miller and Henagan, 1984). EtOH/HCl may impair the defensive factors such as mucus secretion (Kuwata et al., 1985), and bicarbonate secretion (Flemstrom, 1987) and of mucosal circulation (Trier et al., 1987). Gastric ulcers can be induced in experimental animals and humans by physical or psychological stress (Takagi and Okabe, 1968; Grossman, 1981). The main factors in the gastric lesion formation include: increase of acid secretion (Kitagawa et al., 1979; Menguy, 1969; Brodie et al., 1962) and

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.5

increase of gastric motility (Garrick et al., 1986; Yano et al., 1978; Watanabe, 1966), decrease of gastric mucosal blood flow (Hase and Moss, 1973; Guth, 1972; Menguy, 1969; Guth PH, Kozbur, 1968) and decrease of alkaline secretion (Takeuchi et al., 1990).

The injurious gastrointestinal effects of NSAIDs such as indomethacin are largely caused by the inhibition of cyclooxygenase-1 (COX-1). NSAIDs disrupt the normal gastric mucosal barrier of bicarbonate and hydrophobic mucus via disturbance of PGs synthesis by inhibition of constitutive COX isoenzyme, COX-1 (Hawkins and Hanks, 2000; Hayllar and Bjarnason, 1995; Selling et al., 1987; Vane, 1971).

In the present study, Chantaleela recipe reduced gastric lesion induced by EtOH/HCl, restraint water immersion stress and indomethacin. These results indicated that Chantaleela seem to possess anti-ulcerogenic activity. This activity may be due to the increase of gastric mucosal resistance or potentiation of defensive factors and/or the decrease of aggressive factors. The pyrorus ligation is the model for determination of the anti-secretory activity. The ligation caused an accumulation of intraluminal HCl, leading to gastric mucosal damage (Shay et al., 1945). In addition, the gastric acid output as well as volume of gastric content can also be determined. In the present study, Chantallela did not reduce the secretory rate, total acidity, and increase pH in rat stomach. In contrast, cimetidine showed the significantly increased of the pH in the rat stomach. Thus, these results indicated that anti-ulcerogenic activity of Chantaleela recipe did not relate to its anti-secretory activity

In acute toxicity study, rats fed with Chantaleela recipe at the dose of 5,000 mg/kg did not show any sign of toxicity over 14-day period of observation. Moreover, necropsy and gross examinations of the internal organs revealed no pathological abnormality. These results suggest that Chantaleela recipe is not toxic to the rats after an acute exposure at the dose of 5,000 mg/kg. Moreover, the long term oral administration of Chantaleela recipe did not produce gastric and lieum lesions of both gross and histopathological examination.

In conclusion, the anti-inflammatory, analgesic and antipyretic activities of Chantaleela recipe seem to be similar to those of NSAIDs. Inhibitory effect on the synthesis and/or release of inflammatory or pain mediators (especially the products derived via COX pathway) may be the main mechanisms of action of Chantaleela recipe. This study provides evidence that the anti-ulcerogenic activity of Chantaleela recipe may be due to the increase of gastric mucosal resistance or potentiation of defensive factors and/or the decrease of aggressive factors but not associated with the anti-secretory activity. Moreover, this recipe at the high oral doses treated did not cause acute toxicity in rats and the long term oral administration of Chantaleela recipe did not produce gastric and lieum lesions.

Acknowledgements

We are gratefully acknowledged and also financially supported by the National Research Council of Thailand.

References

1. Bhat, R. and Karim, A.A. (2010). Tongkat Ali (*Eurycoma longifolia* Jack): a review on its ethnobotany and pharmacological importance. Fitoterapia. 81(7): 669 – 679.
2. Brattsand, R., Thalen, A., Roempke, K., Kallstrom, L. and Gruvstad, E. (1982). Influence of 16 alpha, 17 alpha-acetal substitution and steriod nucleus fluorination on the topical to systemic activity ratio of glucocorticoids. J. Steroid. Biochem. 16(6): 779 – 786.
3. Brodie, D.A., Marshall, R.W. and Moreno, O.M. (1962). Effect of restraint on gastric acidity in the rat. Am. J. Physiol. 202: 812 – 814.
4. Cai, Y., Luo, Q., Sun, M. and Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life. Sci. 74: 2157 – 2184.
5. Cavin, A., Hostettmann, K., Dyatmyko, W. and Potterat, O. (1998). Antioxidant and lipophilic constituents of *Tinospora crispa*. Planta Med. 64(5): 393 – 396.
6. Dalal, S. and Zhukovsky, D.S. (2006). Pathophysiology and management of fever. J. Support. Oncol. 4(1): 9 – 16.
7. Djahanguiri, B. (1969). The production of acute gastric ulceration by indomethacin in the rat. Scand. J. Gastroenterol. 4: 265 – 267.
8. Dubuisson, D and Dennis, S.G. (1977). The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain. 4(2): 161 – 174.
9. Engblom, D., Ek, M., Saha, S., Ericsson-Dahlstrand, A. and Jakobsson, P.J. (2002). Prostaglandins as inflammatory messengers across the blood-brain barrier. J. Mol. Med. (Berl) 80(1): 5 – 15.
10. Flemstrom, G. (1987). Gastric and duodenal mucosal bicarbonate secretion. In: Johnson, L.R. (ed). Physiology of the Gastrointestinal Tract, 2nd ed. Raven Press, New York, pp 1011 – 1029.
11. Garrick, T., Buack, S. and Bass, P. (1986). Gastric motility is a major factor in cold restraint-induced lesion formation in rats. Am. J. Physiol. 250: G191 - G199.
12. Grossman, M.I. (1981). Peptic ulcer: a guide for the practicing physician. Year Book Medical Publishers, Chicago.
13. Guth, P.H. (1972). Gastric blood flow in restraint stress. Am. J. Dig. Dis. 17: 807 – 813.
14. Guth, P.H. and Kozbur, X. (1968). Pathogenesis of gastric microcirculatory and mast cell changes in restraint stress. Am. J. Dig. Dis. 13: 530 – 535.
15. Hase, T. and Moss, B.J. (1973). Microvascular changes of gastric mucosa in the development of stress ulcer in rats. Gastroenterology. 65: 224 – 234.

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.5

16. Hawkins, C. and Hanks, G.W. (2000). The gastroduodenal toxicity of nonsteroidal anti-inflammatory drugs: a review of the literature. J. Pain. Symptom. Manage. 20: 140 – 151
17. Hayden, L.J., Thomas, G. and West, G.B. (1978). Inhibitors of gastric lesions in the rat. J. Pharmacol. 30: 244 – 246.
18. Hayllar, J. and Bjarnason, I. (1995). NSAIDs, COX-2 inhibitors, and the gut. Lancet. 346: 521 – 522.
19. Heapy, C.G., Jamieson, A. and Russell, N.J.W. (1987). Afferent C-fibre and A-delta activity in models of inflammation. Br. J. Pharmacol. 90: 164p.
20. Higashino, H., Suzuki, A., Tanaka, Y. and Pootakham, K. (1992). Inhibitory effects of Siamese *Tinospora crispa* extracts on the carrageenin-induced foot pad edema in rats (the 1st report). Nihon. Yakurigaku. Zasshi. 100(4): 339 – 344.
21. http://www.stuartxchange.com/Damong.html. Philippine Medicinal Plants. Damong maria [September 6, 2011].
22. Hunskaar, S., Fasmer, O.B. and Hole, K. (1985). Formalin test in mice, a useful technique for evaluating mild analgesic. J. Neurosci. Methods. 14(1): 69 – 76.
23. Hunskaar, S. and Hole, K. (1987). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain. 30(1): 103 – 114.
24. Itthipanichpong, R., Lupreechaset, A., Chotewuttakorn, S., Akarasereenont, P., Onkoksoong, T., Palo, T., Kongpatanakul, S., Chatsiricharoenkul, S., Thitilertdecha, P., Punpeng, P. and Laohapand, T. (2010). Effect of Ayurved Siriraj Herbal Recipe Chantaleela on Platelet Aggregation. J. Med. Assoc. Thai. 93(1): 115 – 122.
25. Ivanov, A.I. and Romanovsky, A.A. (2004). Prostaglandin E_2 as a mediator of fever: synthesis and catabolism. Front. Biosci. 9: 1977 – 1993.
26. Jin, D.Q., Lim, C.S., Hwang, J.K., Ha, I. and Han, J.S. (2005). Antioxidant and anti-inflammatory activities of macelignan in murine hippocampal cell line and primary culture of rat microglial cells. Biochem. Biophys. Res. Commun. 331: 1264 – 1269.
27. Kang, O.H., Lee, G.H., Choi, H.J., Park, P.S., Chae, H.S., Jeong, S.I., Kim, Y.C., Sohn, D.H., Park, H., Lee, J.H. and Kwon, D.Y. (2007). Ethyl acetate extract from *Angelica dahuricae* Radix inhibits lipopolysaccharide-induced production of nitric oxide, prostaglandin E2 and tumor necrosis factor-alphavia mitogen-activated protein kinases and nuclear factor-kappa B in macrophages. Pharmacol. Res. 55(4): 263 – 270.
28. Khatun, M., Eguchi, S., Yamaguchi, T., Takamura, H. and Matoba, T. (2006). Effect of thermal treatment on radical scavenging activity of some spices. Food Sci. Tech. Res. 12: 178 – 185.
29. Kitagawa, H., Fujwara, M. and Osumi, V. (1979). Effect of water immersion stress on gastric secretion and mucosal blood flow in rats. Gastroenterology. 77: 298 – 302.
30. Kongcharoensuntorn, W., Chomosot, N., Jindamol, J., Arrayasillapathon, J., Charadram, P., Ounarom, S. and Pittayaphasertgul, A. (2005). Screening of some Thai medicinal plants for antioxidant activity and antimicrobial activity of microorganisms. J. Med. Tech. Assoc. Thailand. 33(1): 947 – 958.
31. Kumklang, A. (1999). Local vegetables and herbs. Rajamangala University of Technology, Ministry of Education, Thailand.
32. Kuwata, H., Ishihara, K., Kakei, M., Ohara, S., Okabe, H. and Hotta, K. (1985). Correlation of quantitative cal changes of gastric mucosal glycoproteins with ethanol-induced gastric damage in rats. Nippon. Shokakibyo. Gakkai. Zasshi. 82: 28 – 33.
33. Li, S., Ballou, L.R., Morham, S.G. and Blatteis, C.M. (2001). Cyclooxygenase-2 mediates the febrile response of mice to interleukin-1 beta. Brain. Res. 910(1-2): 163 – 173.
34. Lin, C.H., Chang, C.W., Wang, C.C., Chang, M.S. and Yang, L.L. (2002). Byakangelicol, isolated from *Angelica dahurica*, inhibits both the activity and induction of cyclooxygenase-2 in human pulmonary epithelial cells. J. Pharm. Pharmacol. 54(9): 1271 – 1278.
35. Menguy, R. (1969). Gastric mucus and the gastric mucous barrier. A review. Am. J. Surg. 117: 806 – 812.
36. Miller, T.A. and Henagan, J.M. (1984). Indomethacin decreases resistance of gastric barrier to disruption by alcohol. Dig. Dis. Sci. 29: 141 – 149.
37. Mizui, T. and Doteuchi, M. (1983). Effect of polyamines on acidified ethanol-induced gastric lesions in rats. Jpn. J. Pharmacol. 33: 939 – 945.
38. National Drug Committee, Ministry of Public Health. (2006). National List of Essential Medicines of Thailand 2006.
39. Organization of Economic Co-operation and Development. (2001). OECD guideline for testing of chemical: 420 Acute Oral Toxicity. France.
40. Reanmongkol, W., Subhadhirasakul, S. and Bouking, P. (2003). Antinociceptive and antipyretic activities of extracts and fractions from *Dracaena loureiri* in experimental animals. Songklanakarin. J. Sci. Technol. 25: 467 - 476.
41. Saralamp, P., Chuakul, W., Temsiririrkkul, R. and Clayton, T. (1996). Medicinal plants in Thailand Volume I. Amarin Priting and Publishing Public Co., Ltd, Bangkok.
42. Sawasdee, K. (2001). Inhibitors of cyclooxygenase-2 from *Dracaena loureiri* stem. Thesis, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok.
43. Sedgwick, A.D. and Willoughby, D.A. (1989). Animal models for testing drugs on inflammatory and hypersensitivity reaction. In Dale MM, Foreman JC, eds. Textbook of immunology, 2nd ed. Blackwell Scientific, Oxford, pp 253 – 261.
44. Selling, J.A., Hogan, D.L., Aly, A., Koss, M.A. and Isenberg, J.I. (1987). Indomethacin inhibits duodenal mucosal bicarbonate secretion and endogenous prostaglandin E2 output in human subjects. Ann. Intern. Med. 106: 368 – 371.
45. Shay, H., Komarow, S.A., Fels, S.S., Meranze, D., Gruenstein, H. and Siplet, H. (1945). A simple method for the uniform production of gastric ulceration in rat. Gastroenterology. 5: 43 – 61.

Publisher: African Networks on Ethnomedicines
Web page: /http://journals.sfu.ca/africanem/index.php/ajtcam/index
http://dx.doi.org/10.4314/ajtcam.v9i4.5

46. Shibata, M., Ohkubo, T., Takahashi, H. and Inoki, R. (1989) Modified formalin test: characteristic biphasic pain response. Pain. 38(3): 347 – 352.
47. Takagi, K., Kasuya, Y. and Watanabe, K. (1963). Studies on the drugs for peptic ulcer. A reliable method for producing stress ulcer in rats. Chem. Pharma. Bull. 12: 465 – 472.
48. Takagi, K. and Okabe, S. (1968). The effect of drugs on the production and recovery process of stress ulcer. Jpn. J. Pharmacol. 18(1): 9 – 18.
49. Takeuchi, K., Furukawa, O., Okada, M., Niida, H. and Okabe, S. (1990). Influences of stress on gastric alkaline secretion in rats. J. Pharmacol. Exp. Ther. 252: 1228 – 1233.
50. Teotino, U.M., Friz, L.P., Gandini, A. and Bella, D.D. (1963). Thio derivatives of 2, 3- dihydro-4H-1, 3-benzoazin-4-one synthesis and pharmacological properties. J. Med. Chem. 6: 248 – 250.
51. Thongpraditchote, S., Wongkrajang, Y., Temsiririkkul, R., Nagsa-nga, R. and Kongsaktrakoon, B. (2001). Antipyretic activity of Chantaleela formula. Thai J. Phytopharm. 8: 20 – 26.
52. Tjolsen, A., Berge, O.G., Hunskaar, S., Rosland, J.H. and Hole, K. (1992). The formalin test: an evaluation of the method. Pain. 51(1): 5 – 17.
53. Trier, J.S., Szabo, S. and Allan, C.H. (1987). Ethanol-induced damage to mucosal capillaries of rat stomach. Ultrastructural features and effects of prostaglandin F2 beta and cysteamine. Gastroenterology. 92: 13 – 22.
54. Ul'chenko, N.T., Khushbaktova, Z.A., Bekker, N.P., Kidisyuk, E.N., Syrov, V.N. and Glushenkova, A.I. (2005). Lipid from flowers and leaves of *Artemisia annua* and their biological activity. Chem. Nat. Comp. 41: 280 – 284.
55. Vane, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat. New. Biol. 231: 232 – 235.
56. Waller, D.G., Renwick, A.G. and Hillier, K. (2005). Non-steroidal anti-inflammation drugs. In: Medical pharmacology and therapeutics, 2nd ed. Harcourt Publishers Limited, Spain, pp 305 – 314.
57. Watanabe, K. (1966). Some pharmacological factors involved in formation and prevention of stress ulcer in rats. Chem. Pharm. Bull. (Tokyo). 14: 101 – 107.
58. Winter, C.A., Risley, E.A. and Nuss, G.W. (1962). Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drug. Proc. Soc. Exp. Biol. Med. 111: 544 – 547.
59. World Health Organization. (2000). General guidelines for methodologies on research and evaluation of traditional medicine. Switzerland.
60. Yano, S., Akahane, M. and Harada, M. (1978). Role of gastric motility in development of stress-induced gastric lesions of rats. Jpn. J. Pharmacol. 28: 607 – 615.