

RESEARCH PAPER

***In vitro* uterine relaxant activity of Fire Ha Kong Recipe in rats**

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Abstract

Fire Ha Kong recipe in a concentration range of 10-40 µl/ml was used to test in an *in vitro* uterine contraction inhibition. Contraction frequencies and amplitudes of the normal uterine of estrogen-primed non-pregnant rats were monitored. A decline in the frequencies of uterine contractions of 43.8 - 93.6% was detected. Addition of Fire Ha Kong at the final concentrations of 20-40 µl/ml inhibited the amplitudes of uterus contraction by 57.1% and 85.7%, respectively. The reductions in area under the curve (AUC) of contractions were 57.1% and 85.7% for the added concentrations. Fire Ha Kong recipe revealed inhibitory effects on the uterine response to oxytocin (10^{-11} M) 31.9% and 29.3% only in frequency but not in amplitude and AUC in comparison to oxytocin alone. These results indicate that the tocolytic potential of Fire Ha Kong recipe may involve oxytocin receptor-dependent pathway or reduce the intracellular availability of calcium ions.

Introduction

The use of herbal medicines during pregnancy and postpartum period is common among the Asian cultures. It has gained a popularity among young highly-educated Chinese women (Chuang *et al*, 2009). Thai traditional postpartum practices

relate to the notion of restoring energy after the child delivery (Kaewsarn *et al*, 2003). Heating is the most simple way of restoring energy, and to do so, there have been various postpartum practices, including lying by fire, taking hot

baths and drinks, taking foods with 'hot taste' spices, etc.

Fire Ha Kong, a Thai name of one of its traditional recipes meaning 5 piles of fire, is composed of an equal amount each of 5 'hot' spices or hot-tasted herbs, i.e. dried fruits of *Acacia concinna* D.C., young seeds of black pepper (*Piper nigrum* Linn.), dried roots of red leadwort (*Plumbago indica* Linn.), rhizomes of ginger (*Zingiber officinale* Roscoe.) and alum. Fire Ha Kong has been on the National List of Essential Medicines of Thailand since 2011 (Thai FDA, 2014) with an indication to be a herbal remedy for gynaecologic treatment. It is believed to strengthen postnatal mothers, potentially to warm up the bodies, clear uterine discharge and regain normal sizes of the uteri. Pepper seeds have been shown to possess spasmolytic activity in uterus of rats (Naseri and Yahyavi, 2007), aqueous extract of ginger rhizome could inhibit uterine smooth muscle contraction in rats (Ghayur and Gilani, 2007) but *Plumbago indica* showed no effect on uterine contraction and no reports about *Acacia concinna* and Alum.

Uterine contraction is one of the substantial indicators for parturition or delivery. After parturition, these contractions help the uterine muscle to continue to constrict the uterine blood vessels, and bring about a decrease in the amount of vaginal bleeding after delivery and cause pains in post-partum periods. Uterine contraction is also used to evaluate the effect of oxytocin (Challis *et al* 2000), and thus could be used to screen the effect of a herbal remedy on uterus which should be an immediate action required for postpartum treatment.

The effect of Fire Ha Kong on the uterine contraction has not been well explained, though its use has been clinically accepted. This explanation should provide substantial information for sustainably improving and promoting its optimal use.

Therefore, the aim of this study was to investigate the effect of Fire Ha Kong recipe on the uterine contraction of non-pregnant rats and oxytocin-induced uterine contraction *in vitro*.

Materials and methods

Animals : Female rats weighted 150-160 g, were obtained from Salaya Animal Center, Mahidol University, Nakornprathom, Thailand. Animals were housed, in a maintained condition at $25 \pm 1^\circ\text{C}$, 60% RH, a 12 h light-dark cycle, in groups of 5 per cage, and acclimatized for with free access to food and water about 1 week in the laboratory animal room before starting the experiments. All experiments were carried out in accordance to the guidelines for care and use of experimental animals by the approval of Institutional Animal Care and Use Committee, Faculty of Pharmacy, Mahidol University (PYR012/2553).

Plant material: The plants were collected as follows: dried fruits of *Acacia concinna* and black pepper from Chanthaburi province, dried roots of red leadwort from Suphanburi province, rhizomes of ginger from Ratchaburi province and alum from herbal drug store in Mahasarakham province. They were identified by comparison with the specimens at Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and

Environments, Bangkok. The voucher specimens (*A. concinna*: MSU.PH-AC1, *P. nigrum*: MSU.PH-PN1, *P. rosea*: MSU.PH-PR1, *Z. officinale*: MSU.PH-ZO1) were deposited at the Department of

Pharmacognosy, Faculty of Pharmacy, Mahasarakham University, Mahasarakham, Thailand.

Preparation and extraction of Fire Ha Kong:

Dried crude samples of *A. concinna*, *P. nigrum*, *P. rosea*, *Z. officinale* and alum, 20 g of each, were pulverized and mixed in a roller mill and the pulverized mix was sieved through a sieve no. 80 to obtain about 100 g of fine powder of Fire Ha Kong. A sample of Fire Ha Kong was extracted in 70% ethanol for 7 days, followed by condensing and freeze-drying to obtain dried brown powder (a yield of about 22%).

Preparation of drugs and Fire Ha Kong for *in vitro* testing:

Oxytocin (Syntocinon ampule 10 I.U/ml= 20 µg/ml, Sandoz Company, Switzerland) was diluted to 10^{-11} M by Krebs's solution. Estradiol benzoate injection (China Chemical & Pharmaceutical, Taiwan) was used as received.

Fire Ha Kong was dissolved in 4% dimethyl sulphoxide (BDH, England) in Krebs's solution to obtain a stock solution of Fire Ha Kong of 2 mg/ml, which was further diluted to 10, 20 and 40 µg/ml by Krebs's solution for use.

***In vitro* uterine contraction:** Each rat was intramuscularly injected by estradiol benzoate at a dose of 1 mg/kg for 24 h to induce the estrous stage, followed by sacrifice by CO₂ inhalation and removal of the uterus (avoiding its stretching). A 3-cm

strip of the uterus was cut, tied both ends by silk threads and bathed in Krebs's solution (37°C, 95%O₂/5% CO₂). The bottom end of the uterus strip was attached to an aeration tube fixed in the organ bath and other end was connected to the gauge coupler of the oscillograph (Gould, USA). The uterine strip was stretched to the basal tension at 1 g. After a 30-min equilibration period, Fire Ha Kong which was prepared in dilutions was added and incubated for 5 min followed by 5 min resting. During the 5-min resting period, Krebs solution was used to rinse the uterus strip 3 times. Then, oxytocin (10^{-11} M) was added and incubated for 5 min. The frequency (number of contraction cycles in 5 min), amplitude (average peak height in cm) and area under the curve (AUC in cm²) were recorded.

Statistical analysis: Statistical analysis of the data was performed using paired Student's t test as well as one way ANOVA followed by Tukey-Kramer multiple comparison test. P<0.05 was chosen as a criterion for significance.

Result and discussion

Figure 1 and Table 1 revealed that addition of Fire Ha Kong to the organ bath at concentrations of 20-40 µg/ml produced significant reductions in the uterine contraction of estrogen-primed non-pregnant rats *in vitro*. The decreases in the normal frequency of contraction were 74.9% and 93.6%, while the reductions were 56.9% and 85.7% for amplitude. The inhibitions in the normal AUC of uterine contraction were amounted to 57.1% and 85.7% for the added concentrations respectively.

Figure 1 Representative tracing record of contractions of rat uterus strips after control and treated with Fire Ha Kong at 3 final concentrations

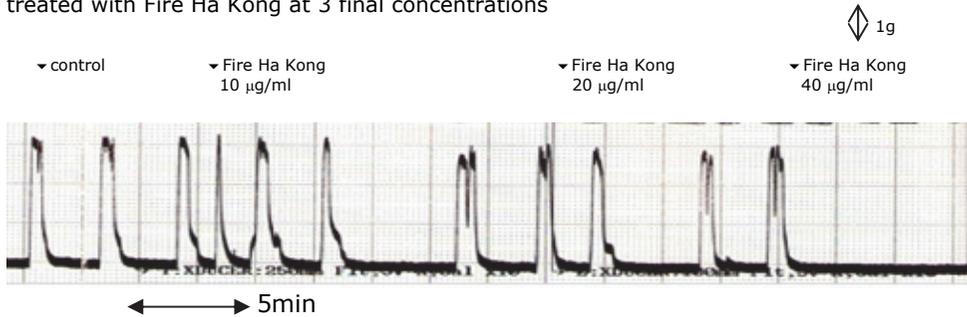


Table 1 Mean (\pm SEM) of the effect of Fire Ha Kong (FHK) on rat oxytocin-induced uterine contractions (n = 5-6)

	Amplitude(cm)	AUC (cm ²)	Frequency (cycles per 5 min)
Control	2.67 \pm 0.51	5.39 \pm 1.54	2.67 \pm 0.52
Vehicle	2.65 \pm 0.45 (-0.7%)	5.26 \pm 1.83 (-2.4%)	2.33 \pm 0.82 (-2.7%)
FHK	2.31 \pm 0.43	4.27 \pm 1.28	1.50 \pm 0.84*
10 µg/ml	(-13.5%)	(-20.8%)	(-43.8%)
20 µg/ml	1.15 \pm 1.28**,+ (-56.9%)	2.31 \pm 2.56* ,+ (-57.1%)	0.67 \pm 0.82***,+ (-74.9%)
40 µg/ml	0.38 \pm 0.94***,++ (-85.7%)	0.77 \pm 1.88** ,++ (-85.7%)	0.17 \pm 0.41***,++ (-93.6%)

* p<0.05, ** p< 0.005, *** p< 0.001 compared to control
+ p<0.05, ++ p<0.005, +++ p< 0.001 compared to 10 µg/ml

Figure 2 and Table 2 showed that oxytocin (10^{-11} M)- induced contraction of rat uterus incubated with Fire Ha Kong recipe in the above added concentrations was markedly declined only in the

frequency of contraction as 37.9% and 29.3% as compared to the values obtained after addition of oxytocin alone but no significant change both in amplitude of contraction and AUC.

Figure 2 Representative tracing showed the effect of Fire Ha Kong on oxytocin-induced uterine contractions

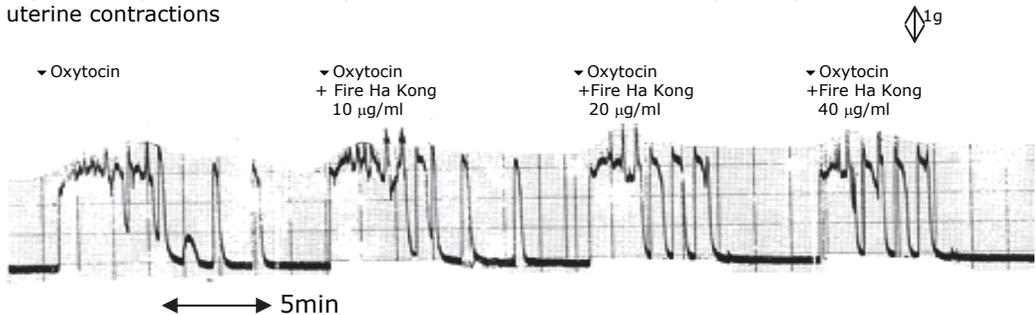


Table 2 Mean (\pm SEM) of the effect of Fire Ha Kong (FHK) on rat oxytocin-induced uterine contractions (n = 5-6)

	Amplitude(cm)	AUC (cm ²)	Frequency (cycles per 5 min)
Oxytocin	3.04 \pm 0.37	7.62 \pm 1.20	19.33 \pm 1.97
oxytocin + FHK (10 μ g/ml)	3.26 \pm 0.41 (7.2%)	7.52 \pm 1.39 (-1.31%)	16.50 \pm 4.18 (-14.6%)
oxytocin + FHK (20 μ g/ml)	3.15 \pm 0.52 (3.6%)	6.85 \pm 1.27 (-10.1%)	13.17 \pm 3.87* (-31.9%)
oxytocin + FHK (40 μ g/ml)	3.01 \pm 0.31 (-0.99%)	6.47 \pm 1.20 (-15.1%)	13.67 \pm 3.01* (-29.3%)

* p < 0.05 compared to oxytocin alone.

The *in vitro* uterine contractions were recorded for 3 min immediately after addition of oxytocin 10^{-11} M incubated with vehicle or Fire Ha Kong in the organ bath followed by three times washing with Krebs solution during 5 min rest period.

It is well-known that estrogen stimulates proliferation in the myometrium, accomplishing the considerable myometrium growth that is necessary for the forceful contractions of labor. Thus, the estrogen-primed uterus is a good model for the research about uterine contractility in the non-pregnancy condition. Data of the present study showed that Fire Ha Kong recipe produced marked inhibition in the spontaneous uterine contraction of estrogen-primed non-pregnant rats in frequency, amplitude and area under the curve which support the medicinal use of the formula. The results are in agreement with those obtained by PSU report 2010 that Fire Ha Kong recipe could reduce frequency of uterine contraction at at doses of 0.1-10 μ g/ml but not amplitude of contraction. In this study the dose range was increased to 40 μ g/ml and showed more effect in amplitude of contraction. Spontaneous uterine contraction is closely dependent on the cytoplasmic concentration of free calcium ions (Bolton, 1979) and drugs that reduce the intracellular

availability of calcium ions depress the contractile activity of isolated human uterine muscle (Poli, 1990). Thus, Fire Ha Kong recipe might reduce the intracellular availability of calcium ions in myometrium.

Oxytocin, a hormone involved in numerous physiologic processes, plays a central role in the mechanisms of parturition and lactation. It acts through its receptor, which belongs to the G-protein-coupled receptor superfamily, while Gq/phospholipase C (PLC)/inositol 1,4,5-triphosphate (IP₃) is the main pathway via which it exerts its action in the myometrium (Vrachnis et al, 2011). It is fundamentally known that oxytocin-induced uterine contraction is mediated through a typical class of G protein-coupled receptor which is primarily coupled via G_q proteins to phospholipase c-beta (Kiss and Mikkelsen, 2005). Moreover oxytocin-induced uterine contraction is attributed to an increase of cytosolic free calcium originating from inositol triphosphate (IP₃)-sensitive intracellular stores and calcium influx via receptor-operated channels (Phillippe and Chien, 1995; Trujillo et al., 2000). Fire Ha Kong recipe also revealed the inhibitory effects on the uterine response to oxytocin (10^{-11} M) only in frequency but not in amplitude and AUC in comparison to oxytocin alone.

In view of what mentioned, the resultant inhibitory effect of Fire Ha Kong recipe on the uterine contractility might be explained due to the inhibition of G protein-coupled receptor and/or the blocking activity of oxytocin receptors and/or reduce the intracellular availability of calcium ions in the rat myometrium. Further pharmacological investigation on the mechanism of action of the extract should be done in future studies.

Conclusion

Fire Ha Kong recipe exhibited potential tocolytic (uterine relax) effects in the rat uterus possibly through antagonizing receptor-dependent mechanism (oxytocin-induced contraction), an effect that could be beneficial in women with uterine contraction in premature labor and also promotes the use of the formula for the tocolytic potential in postpartum mothers.

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