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Effects of garlic extract on the contraction of isolated human uterine muscle.

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The effects and mechanism of action of garlic extract on the contraction of isolated human uterine muscle were investigated. Myometrial specimens were obtained from 54 women, 32-52 years of age, during the proliferative phase of their menstrual cycle. The contractile activity in terms of force, rate, and form was recorded with a dynograph. The results revealed that garlic extract exhibited both excitatory and inhibitory effects on the force of contraction of the circular layer, while rate and form were not affected. In the presence of various antagonists, it was found that contractile response to garlic extract was not inhibited by either atropine or phentolamine. The effect was not enhanced by propranolol. Verapamil and nifedipine demonstrated inhibitory effects on the contractile response to garlic extract that were significant ($p < 0.05$). Consequently, the study concluded that garlic extract may not exert its action via muscarinic, alpha-adrenergic, or beta-adrenergic receptors, but through the induction of increased intracellular free calcium.

Key words : *Allium sativum*, garlic, human uterine muscle, proliferative phase, menstrual cycle.

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จุไรพร สมบุญวงศ์, สมศักดิ์ บวรสิน, ราตรี สุดทรง. ผลของสารสกัดกระเทียมต่อการหดตัวของกล้ามเนื้อดลูกของคนที่แยกออกมา. จุฬาลงกรณ์เวชสาร 2536 เมษายน; 37(4) : 227-236

ศึกษาผลและกลไกการออกฤทธิ์ของสารสกัดกระเทียมต่อการหดตัวของกล้ามเนื้อดลูกของคนที่แยกออกมา จากสตรีจำนวน 54 ราย ที่มีอายุระหว่าง 32-52 ปี และอยู่ในระยะ *proliferative phase* ของรอบประจำเดือน ทำการศึกษาในหลอดทดลองโดยใช้เครื่อง *Dynograph* บันทึกผลทั้งความแรง (*force*) อัตรา (*rate*) และรูปลักษณะ (*form*) ของการหดตัว

ผลการทดลองพบว่าสารสกัดกระเทียม มีผลทั้งเพิ่มและลดความแรงของการหดตัวของกล้ามเนื้อดลูกชั้น *circular* ของคน ขึ้นอยู่กับปริมาณของสาร ส่วนอัตราและรูปลักษณะของการหดตัวไม่เปลี่ยนแปลง ผลการศึกษากลไกการออกฤทธิ์ของสารสกัดกระเทียมโดยใช้ *receptor antagonists* พบว่าฤทธิ์ของสารสกัดกระเทียมไม่ถูกยับยั้งโดย *atropine* และ *phentolamine* และไม่ถูกเสริมฤทธิ์โดย *propranolol* แต่สามารถถูกยับยั้งโดย *verapamil* และ *nifedipine* ($p < 0.05$)

จากผลการทดลองนี้สรุปได้ว่าสารสกัดกระเทียมมีผลต่อความแรงของการหดตัวของกล้ามเนื้อดลูกชั้น *circular* ของคนโดยไม่ได้ออกฤทธิ์ผ่าน *muscarinic*, *alpha-adrenergic* หรือ *beta-adrenergic receptor* แต่ออกฤทธิ์โดยอาจเหนี่ยวนำให้มีการเพิ่มของแคลเซียมอิสระภายในเซลล์

Garlic, *Allium sativum* Linn, is widely used as a condiment and food. In ancient times, garlic was used, for inducing what, the ancients perceived to be effect on the reproductive system : namely, as an emmenagogue⁽¹⁾ (for inducing menstruation), abortifacient⁽²⁾ (for inducing abortion), and aphrodisiac⁽³⁾ (for stimulating the libido). *In vivo* and *in vitro* studies in animals have demonstrated the estrogenic effect of garlic.^(1,4-7) In humans, alcoholic extract of garlic has been found increase contraction of the non-pregnant uterus.⁽⁸⁻¹⁰⁾ Nevertheless, it has been reported controversially that water extract of garlic decreased uterine motility in pregnant and non-pregnant albino rat and guinea pig.⁽¹¹⁾ Ninety-five percent alcoholic extract and petroleum ether extract of garlic at doses of 150-200 mg/kg were reported to have no antifertility activity in female albino rats.⁽²⁾

Studies on the effects of garlic on uterine contraction have been undertaken mostly in animals, whereas humans have been only rarely studied for such effect. Also, little information is available on the possible mechanism of its action.

The objectives of this study are as follows. First, it is to determine the effects of garlic extract on the contraction of isolated human uterine muscle during the proliferative phase of the menstrual cycle. Second, it is to investigate the mechanism of action in order to determine whether garlic extract exerts its action through muscarinic, alpha-adrenergic, beta-adrenergic receptors, or calcium channels.

Materials and Methods

1. Myometrial Specimens

The myometrial specimens used in this study were obtained from 54 women, aged 44.13 ± 5.41 (32 to 52) years, who had undergone hysterectomy because of benign diseases under general (n = 12) or spinal anesthesia (n = 42) at the Department of Obstetrics and Gynecology, Chulalongkorn Hospital. The indications for hysterectomy were myoma uteri (n = 38), recurrent dysfunctional uterine bleeding (n = 8), adenomyosis (n = 5), left ovarian cyst (n = 1), and prolapsed uterus (n = 2). All myometrial specimens were obtained and selected from women during the proliferative phase (days 5-14) of the menstrual cycle. Muscle strips were taken from the fundus of the anterior wall of the uterus. The specimens were proved to be normal by gross and microscopic examination; they were also studied to verify the proliferative status of endometrium.

2. Preparation of Myometrial Tissue

The muscle strips were immediately immersed in cold Tyrode's solution (NaCl, 0.8%; KCl, 0.02%; CaCl_2 , 0.01%; NaHCO_3 , 0.1%; MgCl_2 , 0.01%; NaH_2PO_4 , 0.005%; and glucose, 0.1%; pH 7.4). The specimens were taken to the laboratory and then bubbled with a gas mixture of 95% oxygen and 5% carbon dioxide.

The circular layer of myometrium was dissected into pieces about 2.0 x 0.3 x 0.3 cm in size. The long axis of each strip was cut in the direction of the muscle bundles. The dissection was done in a petri dish containing Tyrode's solution bubbled with a gas mixture of 95% oxygen and 5% carbon dioxide.

3. Instruments

Organ bath (double-walled Churchill type), thermostatic bath, dynograph (Beckman type RM), isotonic transducer (Statham UC 3), and blender.

4. Garlic and Chemicals

Garlic cloves, chloroform (Standard Lab), povidone (PVPK), D (+) - glucose - monohydrate (Merck), sodium chloride (NaCl, Merck), sodium dihydrogenphosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, Merck), sodium hydrogen carbonate (NaHCO_3 , BDH), calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, Merck), magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, M & B), potassium chloride (KCl, BDH), atropine sulfate (Sigma), phentolamine (Ciba-Geigy), propranolol hydrochloride (Sigma), verapamil (Knoll), and nifedipine (Bayer).

5. Extraction of Garlic

The procedures of garlic extraction followed were those described by Poolsanong (1984).⁽¹²⁾ Garlic cloves purchased from a local market were used for garlic extraction. The dry outer scales were removed and 100 gm of the cloves washed and dried; after adding 120 ml of chloroform, the cloves were thoroughly blended until a good mixture was obtained. Then the mixture was filtered through a four-layer fine muslin cloth and through Whatman filter-paper No. 1, respectively. Chloroform was separated from the filtrate by rota vaporization at 55°C, leaving behind a yellowish oily liquid residue. The garlic extract was then preserved with 1.2 gm of povidone and stored in a refrigerator. This method of preservation made the effect stable for about a year. For subsequent use, the garlic extract was diluted in normal saline solution to make a final concentration of 80 mg/ml.

6. Experimental Procedures

One end of the muscle strip was sewn by No. 60 thread and attached to a glass hook held in an organ bath containing 20 ml of Tyrode's solution thermostatically controlled at a temperature of 37°C and bubbled

with a gas mixture of 95% oxygen and 5% carbon dioxide. The other end of the muscle strip was tied to a displacement force transducer (Statham UC 3). The contractile activity was recorded isotonicly on a dynograph (Beckman type RM). The initial load was set at 2 gm. In every experiment, the tissue was allowed to equilibrate in the bath for 30 minutes and washed three times with the solution every 15 minutes. The spontaneous contractile activity was recorded after the equilibration.

The experiments were performed as the following :

6.1 Study of the effects of garlic extract on the contraction of isolated human uterine muscle during the proliferative phase of the menstrual cycle.

Six doses of the garlic extract (0.2, 0.3, 0.4, 0.5, 0.6 and 0.8 ml of 80 mg/ml) were tested in the experiments. Contractile responses to various doses of the garlic extract were recorded in terms of force, rate and form.

6.2 Study of the mechanism of action of the garlic extract.

The following blockers were used as pre-treatment: atropine sulfate as a muscarinic antagonist, phentolamine as an alpha-adrenergic antagonist, propranolol as a beta-adrenergic antagonist, verapamil and nifedipine as calcium blocking agents, at concentrations of 10^{-4} , 10^{-4} , 10^{-4} , 10^{-5} and 10^{-5} M, respectively. Only one antagonist was used on each piece of tissue; 0.4 ml of the garlic extract (80 mg/ml) was then added to the bath after each pre-treatment. Contractile responses to the garlic extract in the presence of various blockers were recorded.

7. Statistical Analysis

The results were presented as mean \pm SEM. Student's unpaired t-test was used to evaluate the levels of significant difference between all of the mean values. The probability values that were less than 0.05 were considered to be significant.

Results

Most myometrial preparations exhibited persistent spontaneous contractile activity after equilibration. The tracing of spontaneous contraction of isolated human myometrium during the proliferative phase of the menstrual cycle is shown in figure 1a. After the tissue was washed following each treatment, the contractile activity did not completely recover to that of the equilibration period. All the contractile responses shown in figures 3 to 7 are presented as stress, that is, force/cross-sectional area of the muscle strips.

1. Contractile Responses of Isolated Human Myometrium to Various Doses of the Garlic Extract

The tracings of contractile responses of isolated human myometrium after applications of 0.4, 0.6, and 0.8 ml of the 80-mg/ml garlic extract are shown in figures 1b, c, and d, respectively. There show that the force of contraction was affected while the rate and forms remained unchanged. As shown in figure 2, the garlic extract at doses of 0.2, 0.3, and 0.5 ml of 80 mg/ml slightly increased the force and stress of contraction without significant effect. At the dose of 0.4 ml of 80 mg/ml, the garlic extract significantly ($p < 0.05$) increased the force and stress of contraction. At doses of 0.6 and 0.8 ml of 80-mg/ml garlic extract, significant decreases ($p < 0.05$) in force and stress were observed.

2. Contractile Responses to Garlic Extract in the Presence of Various Antagonists

2.1 Atropine as a muscarinic antagonist.

As shown in figure 3, 0.2 ml of 10^{-4} M atropine significantly ($p < 0.05$) decreased the force and stress of contraction. Atropine elicited no significant effect on the contractile responses to garlic extract.

2.2 Phentolamine as an alpha-adrenergic antagonist.

As shown in figure 4, 0.2 ml of 10^{-4} M phentolamine significantly ($p < 0.05$) decreased the force and stress of contraction. Phentolamine elicited no significant effect on the contractile responses to garlic extract.

2.3 Propranolol as a beta-adrenergic antagonist.

As shown in figure 5, there was no significant effect of 0.2 ml of 10^{-4} M propranolol on the force and stress of contraction. Propranolol produced no significant effect on the contractile responses to garlic extract.

2.4 Verapamil as a calcium channel blocker.

As shown in figure 6, 0.2 ml of 10^{-5} M verapamil significantly ($p < 0.05$) decreased the force and stress of contraction. Verapamil elicited a significant inhibitory effect on the contractile responses to garlic extract ($p < 0.05$).

2.5 Nifedipine as a calcium channel blocker.

As shown in figure 7, 0.2 ml of 10^{-5} M nifedipine significantly ($p < 0.05$) decreased the force and stress of contraction. Nifedipine elicited a significant inhibitory effect on the contractile responses to garlic extract ($P < 0.05$).

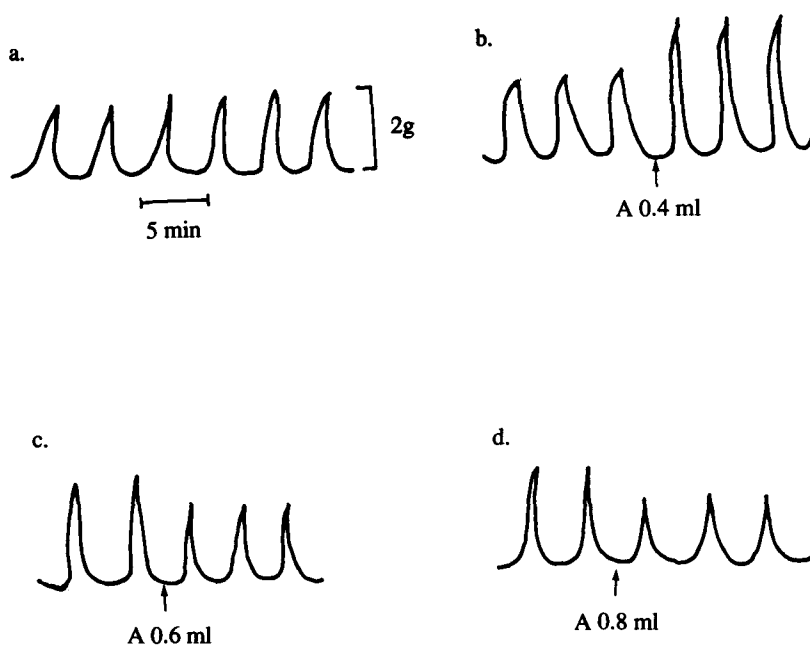


Figure 1. a. Tracing shows spontaneous contraction of a strip of human myometrium during proliferative phase of the menstrual cycle.
b-d. Contractile responses after the applications of 0.4, 0.6, and 0.8 ml of garlic extract (80 mg/ml).

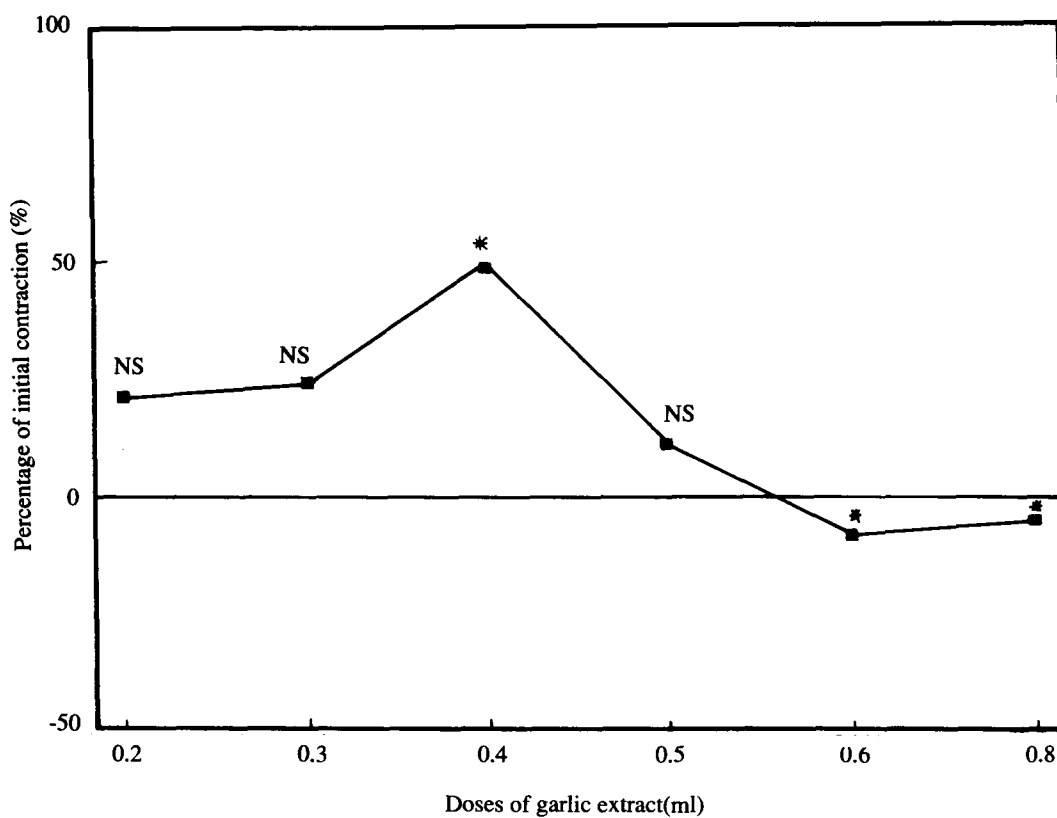


Figure 2. Contractile responses to various doses of garlic extract (0.2-0.8 ml of 80 mg/ml) on strips of human myometrium during proliferative phase of the menstrual cycle (n = 13-36); *p<0.05, NS = nonsignificant difference.

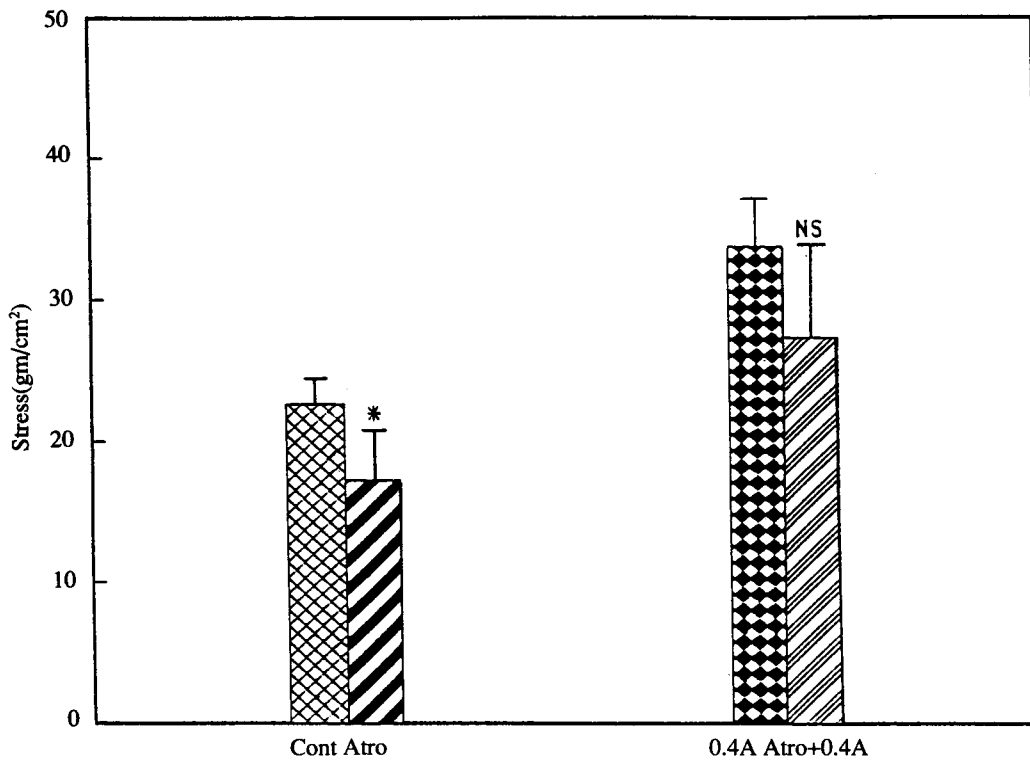


Figure 3. Contractile responses to garlic extract (A) at a dose of 0.4 ml of 80 mg/ml in the presence (n = 6) and absence (n = 15) of 0.2 ml of 10^{-4} M atropine (Atro.) on strips of human myometrium during proliferative phase of the menstrual cycle. Values are expressed as mean \pm SEM; * $p < 0.05$, NS = nonsignificant difference.

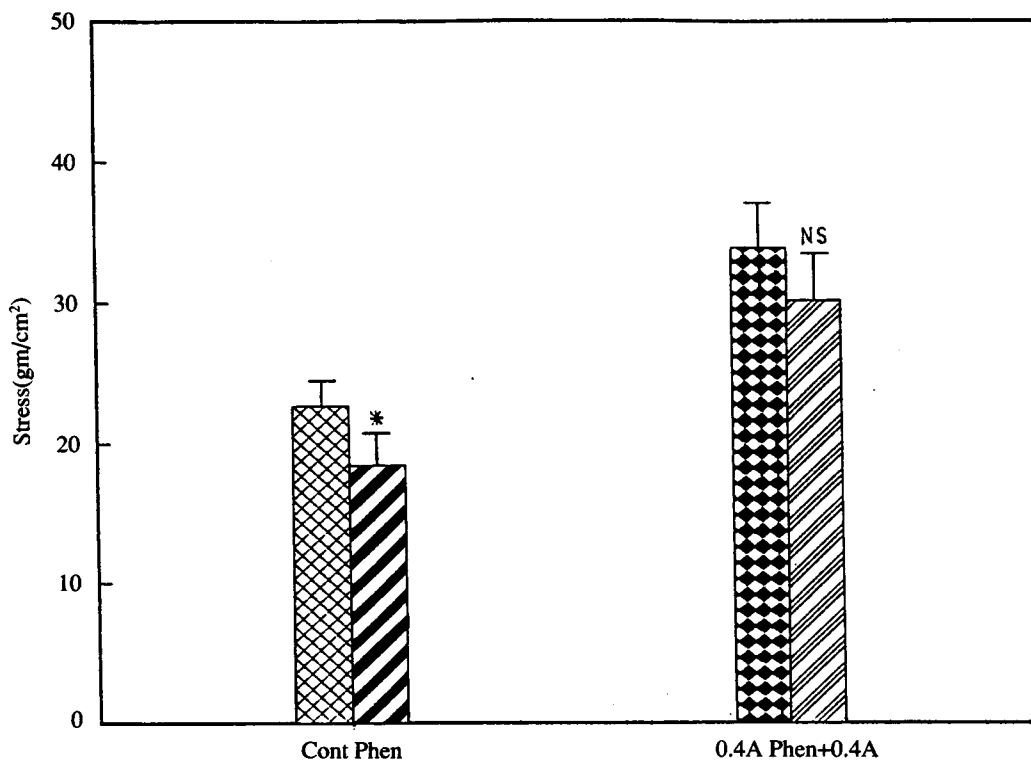


Figure 4. Contractile responses to garlic extract (A) at a dose of 0.4 ml of 80 mg/ml in the presence (n = 6) and absence (n = 15) of 0.2 ml of 10^{-4} M phentolamine (Phen) on strips of human myometrium during proliferative phase of the menstrual cycle. Values are expressed as mean \pm SEM; * $p < 0.05$, NS = nonsignificant difference.

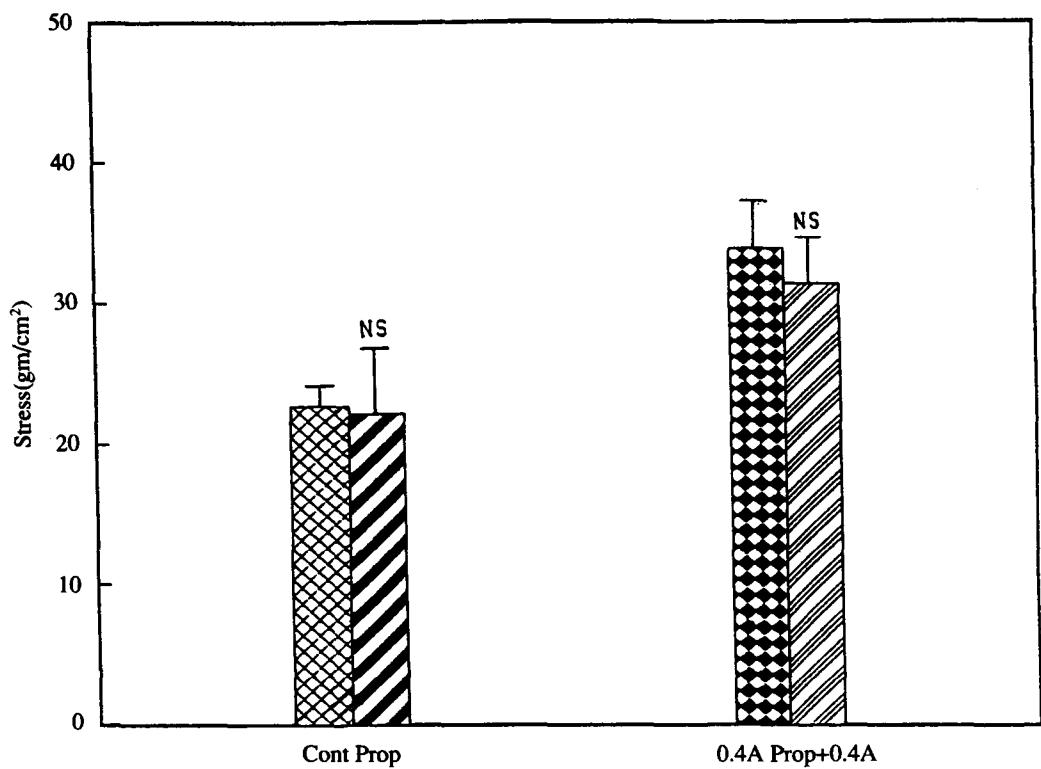


Figure 5. Contractile responses to garlic extract (A) at a dose of 0.4 ml of 80 mg/ml in the presence (n = 6) and absence (n = 15) of 0.2 ml of 10⁻⁴ M propranolol (Prop.) on strips of human myometrium during proliferative phase of the menstrual cycle. Values are expressed as mean ± SEM; NS = nonsignificant difference.

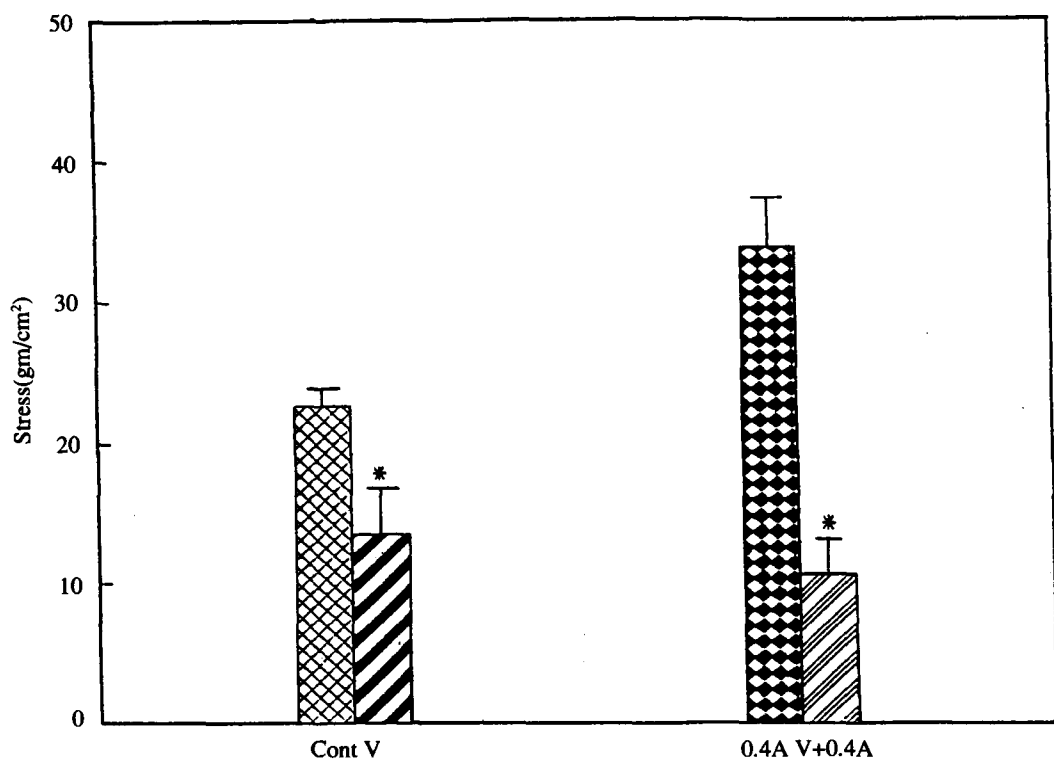


Figure 6. Contractile responses to garlic extract (A) at a dose of 0.4 ml of 80 mg/ml in the presence (n = 6) and absence (n = 15) of 0.2 ml of 10⁻⁵ M verapamil (V) on strips of human myometrium during proliferative phase of the menstrual cycle. Values are expressed as mean ± SEM; *p<0.05.

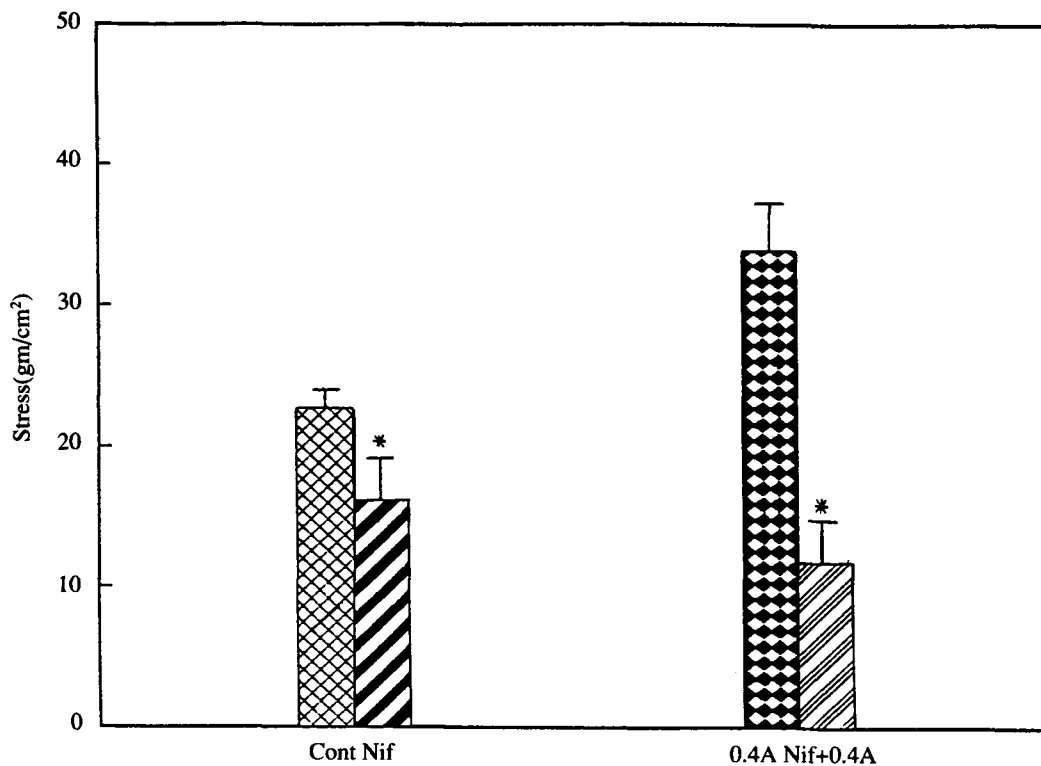


Figure 7. Contractile responses to garlic extract (A) at a dose of 0.4 ml of 80 mg/ml in the presence ($n = 6$) and absence ($n = 15$) of 0.2 ml of 10^{-5} M nifedipine (Nif.) on strips of human myometrium during proliferative phase of the menstrual cycle. Values are expressed as mean \pm SEM; * $p < 0.05$.

Discussion

In the first part of the present study, most of the myometrial preparations exhibited spontaneous contractile activity. The pattern of the contraction of specimens obtained from women during the proliferative phase of their menstrual cycle appeared mostly to be of high frequency and low amplitude, which is consistent with that observed by Lohsiriwat and Anumanrajadhon (1986).⁽¹³⁾ Garlic extract demonstrated both excitatory and inhibitory effects on the contraction of non-pregnant human myometrial preparations from women during the proliferative phase of their menstrual cycle. It was shown that the contraction response was significantly increased ($p < 0.05$) only at the dose of 0.4 ml of 80-mg/ml garlic extract. However, rate and wave-forms were not affected.

In this study, the dose-response manner of myometrial contraction to garlic extract seems to have been selective, resembling that described by Borvonsin, Rerksngarm, and Chumpolbunchorn (1989),⁽¹⁴⁾ even though the doses, phase and species model are different. Such a study was conducted on rat uterine motility during diestrus, which is approximately the same period as that of the menstrual phase in humans. Three doses (2, 4, and 8 mg) of garlic solution were fed to the animals. It was

shown that garlic solution at doses of 2 and 4 mg increased the amplitude and regulated the rhythmicity and form of uterine contraction whereas a dose of 8 mg did not affect uterine contraction. The effect was more potent at a dose of 4 mg of garlic solution.

Contrary to the present study, the experiment in isolated rat uterus obtained from the estrus phase of the estrous cycle of rats, 8 to 10 weeks of age, showed that 3.5 mg/ml of allicin extracted from garlic significantly ($p < 0.05$) increased the amplitude of contraction at doses of 0.4, 0.8, and 1.6 ml in dose-dependent manner while the rate and wave-forms of contraction were not affected (Permpintong, 1991).⁽¹⁵⁾ The contractile responses of myometrial preparations to various doses of garlic extract in the present study do not express the same manner as that mentioned above.⁽¹⁵⁾ This is probably due to differences in species, since the innervations of the human and rat uterus are not similar with regard to the density of nerves.⁽¹⁶⁾

In the second part of the present study, the mechanism of action of garlic extract on the contraction of isolated human myometrium was investigated. Atropine and phentolamine were used as a muscarinic and alpha-adrenergic antagonist, respectively. It was shown

that atropine and phentolamine significantly ($p < 0.05$) decreased the force and stress of myometrial contraction (figures 3, 4). These results are in accord with the finding by electrical field stimulation study⁽¹⁶⁾ that human myometrium is innervated by cholinergic and alpha-adrenergic excitatory motor nerves. 0.2 ml of 10^{-4} M atropine and phentolamine were without significant effects on the response to 0.4 ml of 80-mg/ml garlic extract. As a consequence, it is suggested that garlic extract acts on neither a muscarinic nor an alpha-adrenergic receptor.

Using propranolol as a beta-adrenergic antagonist at a dose of 0.2 ml of 10^{-4} M (as previously described by Morizaki et al., 1989), it was found that propranolol only slightly affected the contraction of human myometrium (figure 5). The contractile response to garlic extract was not enhanced by propranolol (figure 5), suggesting that garlic extract does not exert its action via beta-adrenergic receptor. The findings are in agreement with those described by Permpintong (1991).⁽¹⁵⁾

The data presented here show that 0.2 ml of 10^{-5} M verapamil and nifedipine significantly inhibited the force and stress of myometrial contraction (figures 6, 7). The findings are in agreement with those of Odum and Broughton Pipkin (1988),⁽¹⁷⁾ and Permpintong (1991).⁽¹⁵⁾ Verapamil and nifedipine (0.2 ml of 10^{-5} M) also had significant inhibitory effects on myometrial response to 0.4 ml of 80-mg/ml garlic extract (figures 6, 7). In this study, verapamil completely blocked the contractile responses to garlic extract while partial blockade was observed by Permpintong (1991).⁽¹⁵⁾ Since the effect of garlic extract at a dose of 0.4 ml of 4 mg/ml, which induced a maximal contractile response, was completely blocked by verapamil and nifedipine, it is therefore indicated that garlic extract may exert its action by the induction of increased intracellular calcium (Ca^{2+}). It possibly has direct and/or indirect action through calcium channels. Otherwise, it may act through other receptors such as PGE_2 or $PGF_{2\alpha}$ receptors, resulting in increased intracellular Ca^{2+} , and hence muscle contractile stimulation.

Conclusion and suggestion

The results in the present study indicate that garlic extract exhibited both excitatory and inhibitory effects, depending on the doses, on the contraction of the circular layer of human uterine muscle during the proliferative phase of the menstrual cycle. With regard to the study of the mechanism of action of garlic extract by the application of various receptor antagonists as pre-treatment, it is suggested that garlic extract dose not exert its

action via muscarinic, alpha-adrenergic or beta-adrenergic receptors, but through an induction of increased intracellular free calcium.

Further investigations are suggested on the factors that might be involved in the contractile responses of human myometrium to garlic extract, including age, parity and phases of the menstrual cycle.

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