Effects of adlay hull extracts on uterine contraction and Ca\(^{2+}\) mobilization in the rat

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1Cardinal Tien College of Healthcare and Management; 2Graduate Institute of Food Science and Technology, Center for Food and Biomolecules, College of Bioresources and Agriculture, National Taiwan University, Taipei; 3Tsuiki Institute for Traditional Medicine, College of Pharmacy, China Medical University, Taichung; 4Department of Physiology, School of Medicine, National Yang-Ming University, Taipei; and 5Department of Medical Research and Education, Taipei City Hospital, Taipei, Taiwan

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Hsia S-M, Kuo Y-H, Chiang W, Wang PS. Effects of adlay (Coix lachryma-jobi L. var. ma-yuen Stapf.) hull extracts on uterine contraction and Ca\(^{2+}\) mobilization in the rat. Am J Physiol Endocrinol Metab 295: E719–E726, 2008. First published June 24, 2008; doi:10.1152/ajpendo.90367.2008.—Dysmenorrhea is directly related to elevated PGF\(_{2\alpha}\) levels. It is treated with nonsteroid antiinflammatory drugs (NSAIDs) in Western medicine. Since NSAIDs produce many side effects, Chinese medicinal therapy is considered as a feasible alternative medicine. Adlay (Coix lachryma-jobi L. var. ma-yuen Stapf.) has been used as a traditional Chinese medicine for treating dysmenorrhea. However, the relationship between smooth muscle contraction and adlay extracts remains unclear. Therefore, we investigated this relationship in the rat uterus by measuring uterine contraction activity and recording the intrauterine pressure. We studied the in vivo and in vitro effects of the methanolic extracts of adlay hull (AHM) on uterine smooth muscle contraction. The extracts were fractionated using four different solvents: water, 1-butanol, ethyl hexane; the four respective fractions were AHM-Wa, AHM-Bu, AHM-EA, and AHM-Hex. AHM-EA and its subfractions (175 µg/ml) inhibited uterine contractions induced by PGF\(_{2\alpha}\), the Ca\(^{2+}\) channel activator Bay K 8644, and high K\(^+\) in a concentration-dependent manner in vitro. AHM-EA also inhibited PGF\(_{2\alpha}\)-induced uterine contractions in vivo; furthermore, 375 µg/ml of AHM-EA inhibited the Ca\(^{2+}\)-dependent uterine contractions. Thus 375 µg/ml of AHM-EA consistently suppressed the increases in intracellular Ca\(^{2+}\) concentrations induced by PGF\(_{2\alpha}\) and high K\(^+\). We also demonstrated that naringenin and quercetin are the major pure chemical components of AHM-EA that inhibit PGF\(_{2\alpha}\)-induced uterine contractions. Thus AHM-EA probably inhibited uterine contraction by blocking external Ca\(^{2+}\) influx, leading to a decrease in intracellular Ca\(^{2+}\) concentration. Thus adlay hull may be considered as a feasible alternative therapeutic agent for dysmenorrhea.

Dysmenorrhea is characterized by pain, cramping, and backache occurring during menses. It has been reported to lead to increased prostaglandin production, which may result in the contraction of blood vessels and myometrium and insufficient blood flow to the endometrium (32). Dysmenorrhea is a common gynecological complaint. After their first menstrual period, 30–60% of American women experience some level of discomfort (35, 47); this menstrual pain may be accompanied with nausea, vomiting, diarrhea, or headache. However, the cause of dysmenorrhea remains unclear. Many methods are applied to alleviate or treat the symptoms, such as fomentation and the use of nonsteroid antiinflammatory drugs (NSAIDs). Most women worldwide select NSAIDs as the first therapeutic option for their dysmenorrhea (36). Although the action of NSAIDs is rapid and effective, it has many side effects that affect the digestive, hepatic, renal, and cardiac systems (13, 36). Because of these side effects, Chinese medicinal therapy is considered as a feasible alternative medicine for dysmenorrhea. For example, in the previous clinical study, the Chinese herbal formula Four-Agent Decoction (Si Wu Tang) was found to be an effective therapeutic agent for primary dysmenorrhea (48). Coix lachryma-jobi L. var. ma-yuen Stapf. (a Chinese medicinal plant named Yi-yi-lan), commonly known as adlay (Job’s tears), is an annual crop. It has long been consumed as either an herbal medicine or a food supplement. It is widely cultivated in Taiwan, Japan, and China and is considered a healthy food supplement. Since ancient times, adlay has been used in Asian countries for the treatment of rheumatism, warts, neuralgia, and disorders of the female endocrine system (29). Our previous studies (6, 14, 15) have also demonstrated the effects of adlay on the endocrine system. Although adlay has many biological functions, the Traditional Chinese Medicine Classic specifically suggests that it be used with caution during pregnancy (29). Furthermore, different parts of adlay and different extraction fractions of adlay gave various effects (5, 6, 14–16, 23, 24, 27). The adlay hull extracts have more biological functions in different study models (5, 6, 15, 16, 23, 24).

Although adlay has long been used in Asia, its action on uterine contractions and the underlying mechanisms remain unclear. Only one study has demonstrated that the water extract of adlay seed increased the expressions of cyclooxygenase-2 (COX-2), ERK1/2, and PKC-α, all of which enhance uterine contractility during pregnancy in the rat (44). The purpose of the present study was to examine the direct effects of adlay hull extracts on uterine smooth muscle contraction in the rat in vivo...
and in vitro. The mechanisms underlying the effects of adlay hull extracts on agonist-induced uterine contractions and intracellular Ca\(^{2+}\) mobilization in the rat were also investigated.

**METHODS**

**Drugs and solutions.** DMEM-F-12 (#56949C), fatty acid-free BSA (#A7511), trypsin (#T1426), penicillin G (#P3032), sodium bicarbonate (#S5761), oxytocin (#O6379), streptomycin sulfate (#S9137), HEPES (#H3375), PFG\(_{2\alpha}\) (#P5069), glucose (#G7528), caffeic acid (#C0625), ferulic acid (#46278), gallic acid (#G7384), p-coumaric acid (#C9008), vanillic acid (#49770), syringaldehyde (#S7602), 3,4-dihydroxybenzoic acid (#P5630), quercetin (#Q0125), naringenin (#S9893), and DMSO (#D2660) were purchased from Sigma Chemical (St. Louis, MO).

**Plant material and methanol extractions of adlay seeds.** Adlay was purchased from a local farmer who planted the Taichung Shuenyu No. 4 (TSC4) variety of Coix lachryma-jobi L. var. ma-yuen Stapf. in Taichung, Taiwan, in March 2005 and harvested it in July of the same year. The purification method for the adlay seed extracts has been previously described (24). Briefly, the seeds were air dried, and each adlay seed was separated into the following four different parts: adlay hull, adlay testa, adlay bran, and polished adlay. Each part was blended to obtain its powder that was subsequently screened through a 20-mm sieve (0.94-mm aperture). Each sample powder (100 g) was extracted with 1-liter of methanol by stirring on a Thermolyn Nuova stirring/heating plate (Dubuque, IA) at room temperature for 24 h. The contents were filtered through no. 1 filter paper (Whatman, Hillsboro, OR), and the filtrate was concentrated to dryness under vacuum conditions to obtain a dried methanolic extract; the extract was then stored at -20°C. The methanolic extracts obtained from the different parts of the adlay seeds were termed AHM (hull), ATM (testa), ABM (bran), and PAM (polished adlay).

**Fractionation of AHM.** The adlay extracts were fractionated using a previously described method (15, 23). First, to identify and characterize the medicinally relevant chemical compounds present in adlay hulls, we performed liquid extraction by using different solvents with increasing polarity. Since adlay hull contains a myriad of active and nonactive compounds located in different parts of the plant cell, we used solvents of different polarity to dissolve the compounds and then quantified the active compounds present in adlay hulls. Each sample powder (20 kg) was extracted three times with 60 liters of methanol at room temperature over a period of 15 days (5 days for each extraction). To minimize methanol consumption in the large-scale adlay hull methanol extractions, we prolonged the duration of extraction instead of using additional methanol. The plant material was filtered and removed, and the methanolic extracts were combined and concentrated under low pressure by using a rotary vacuum evaporator. The obtained dry extract (AHM) was divided into four parts; one part was suspended in H\(_2\)O, while the remaining three parts were extracted in identical volumes of n-hexane, ethyl acetate, and 1-butanol. This yielded the following four fractions: AHM-Hex (n-hexane fraction), AHM-EA (ethyl acetate fraction), AHM-Bu (1-butanol fraction), and AHM-Wa (water fraction). Next, AHM-EA (147.29 g) was chromatographed on a silica gel column using Hex/EA/MeOH gradient system to obtain the following 11 subfractions: A (10% EA/Hex, 90.92 g), B (20% EA/Hex, 2.33 g), C (30% EA/Hex, 1.73 g), D (40% EA/Hex, 2.25 g), E (50% EA/Hex, 2.31 g), F (60% EA/Hex, 2.88 g), G (70% EA/Hex, 1.83 g), H (80% EA/Hex, 4.10 g), I (90% acetone, 3.28 g), J (100% EA, 3.16 g), and K (100% MeOH, 32.5 g). The total yield was 94.89%. The effects of these AHM-EA subfractions (AHM-EA-A—AHM-EA-K) on uterine smooth muscle contraction/relaxation were then investigated. For the in vitro treatment of uterine smooth muscle cells and uterine segments, AHM was dissolved in DMSO to prepare a stock solution. The final concentration of DMSO was <0.1%.

**HPLC analysis of AHM and AHM-EA subfractions E and F.** The components of the adlay extracts were quantified using a previously described method (15, 21, 26) with slight modifications. The AHM and the most active subfractions E and F were subjected to HPLC analysis by using a mobile phase of KH\(_2\)PO\(_4\)-CH\(_3\)CN. A Cosmosil 5C18-MS column (5 μm, 25 cm × 4.6 mm) was used to quantify the components of AHM, AHM-EA-E, and AHM-EA-F. AHM was found to contain caffeic acid, ferulic acid, gallic acid, p-coumaric acid, vanillic acid, syringaldehyde, 3,4-dihydroxybenzoic acid, naringenin, and quercetin. AHM-EA-E was found to contain caffeic acid, ferulic acid, gallic acid, p-coumaric acid, syringaldehyde, 3,4-dihydroxybenzoic acid, and quercetin, while AHM-EA-F was found to contain p-coumaric acid, vanillic acid, syringaldehyde, 3,4-dihydroxybenzoic acid, and naringenin (Table 1).

**Uterine preparations and measurement of uterine contraction.** Female Sprague-Dawley (SD) rats weighing 200–300 g were housed in a temperature-controlled room (22 ± 1°C) with 14-h day artificial illumination (0600–2000), and food and water were provided ad libitum. The use of the animals was approved by the Institutional Animal Care and Use Committee of the National Yang-Ming University. The animals received humane care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals, published by the National Science Council, Taiwan, ROC.

In the experiment, the rats at estrus stage, which was confirmed by microscopic examination of a vaginal smear, were decapitated; both uterine horns were surgically removed and placed in a petri dish containing Krebs’s solution (113 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl\(_2\), 18 mM NaHCO\(_3\), 1.2 mM KH\(_2\)PO\(_4\), 1.2 mM MgSO\(_4\), 5.5 mM glucose, 30 mM mannitol, and pH adjusted to 7.4). After the adherent fat and mesenteric attachments were removed, each uterine horn was cut into equal-length (≥50 mm) segments; these were used for the measurement of uterine oscillatory contraction. The preparations were placed in isolated organ baths, incubated in a physical solution at 37°C, and bubbled with 95% O\(_2\)-5% CO\(_2\). The preload was 1 g, and the equilibration period was not <60 min. Contractions were recorded by force displacement transducers (PowerLab recorder ML785; Castle Hill, NSW, Australia) by using Chart 5 software (Castle Hill).

**Uterine smooth muscle cell preparation.** The uterine smooth muscle cells were isolated using the protease and collagenase dispersion method as previously described, with some modifications (30). The uterine segments were prepared and incubated under rotation (80 rpm) at 37°C for 15 min in a HEPES buffer solution (140 mM NaCl, 5 mM KCl, 2 mM CaCl\(_2\), 5.5 mM glucose, 10 mM HEPES, and pH adjusted to 7.4) containing 0.2% protease. The solution was then replaced with a HEPES buffer solution containing 0.2% collagenase and 0.2% trypsin inhibitor, and the segments were incubated under rotation (80 rpm) at 37°C for 60 min. The supernatant was strained through a 70-μm nylon mesh, and the cells were purified by centrifugation. The isolated myometrial cells were seeded in a 24-well plate and incubated at 37°C in DMEM-F12 containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. After an ~24-h incubation, the uterine

**Table 1. Contents of pure compounds in AHM and AHM-EA subfractions**

<table>
<thead>
<tr>
<th>Compound</th>
<th>AHM, μg/g</th>
<th>AHM-EA-E, μg/g</th>
<th>AHM-EA-F, μg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid</td>
<td>22.5 ± 1.5</td>
<td>12.57 ± 1.93</td>
<td>32.49 ± 4.09</td>
</tr>
<tr>
<td>p-Coumaric cid</td>
<td>232 ± 9</td>
<td>41.31 ± 0.43</td>
<td>32.49 ± 4.09</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>136.1 ± 0.5</td>
<td>3.16 ± 0.02</td>
<td>10.98 ± 0.31</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>462 ± 12</td>
<td>9.83 ± 0.34</td>
<td>28.95 ± 0.32</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>58 ± 2</td>
<td>0</td>
<td>10.98 ± 0.31</td>
</tr>
<tr>
<td>Syringaldehyde</td>
<td>68.2 ± 3.4</td>
<td>10.94 ± 0.12</td>
<td>28.95 ± 0.32</td>
</tr>
<tr>
<td>3,4-Dihydroxybenzoic acid</td>
<td>62.1 ± 8.9</td>
<td>4.21 ± 0.21</td>
<td>3.24 ± 0.12</td>
</tr>
<tr>
<td>Naringenin</td>
<td>26.3 ± 0.1</td>
<td>0</td>
<td>11.63 ± 0.11</td>
</tr>
<tr>
<td>Quercetin</td>
<td>66.1 ± 1.7</td>
<td>14.25 ± 0.27</td>
<td>0</td>
</tr>
</tbody>
</table>

Reported values are means ± SD (n = 3). AHM, extracts of adlay hull.
smooth muscle cells were harvested with trypsin-EDTA and intracellular calcium mobilization was measured. To verify that our cultured cells were uterine smooth muscle cells, the uterine smooth muscle cell phenotype was identified by photomicroscopy. Also, we used immunostaining to evaluate the ability of uterine smooth muscle cells to express smooth muscle specific protein α-actin with monoclonal anti-α-smooth muscle actin (42; see Supplemental Fig. S1; the online version of this article contains supplemental data).

Measurement of intracellular Ca\(^{2+}\) concentration. The uterine smooth muscle cells were treated with the adlay extracts or PGF\(_{2\alpha}\) for 24 h. They were harvested using trypsin-EDTA and washed twice with culture medium DMEM-F-12 supplemented with 10% FBS. Cell suspension (1 × 10\(^6\) cells/ml) was loaded with 5 mg of Fura 2-AM (Fluka Chemical, Milwaukee, WI) dissolved in 5 ml of DMSO. A fluorescent probe was used for monitoring intracellular calcium concentrations ([Ca\(^{2+}\)]\(_i\)). The cells were incubated in the dark for 30 min at 37°C. After being washed extensively, 1 × 10\(^6\) cells were resuspended in 2.5 ml of loading buffer (152 mM NaCl, 1.2 mM MgCl\(_2\), 2.2 mM CaCl\(_2\), 4.98 mM KCl, and 10 mM HEPES). Fluorescence emission at 505 nm was monitored at 37°C by a dual-wavelength spectrometer system, with excitation at 340 and 380 nm. Free [Ca\(^{2+}\)] was calculated using the method developed by Grynkiewicz et al. (11) from the ratio of fluorescence intensities obtained every 1 s with a K\(_d\) of 135 nM. The dye was considered saturated after lysis with digitonin at the final concentration of 0.16 mM. Minimum fluorescence was determined by adding 0.5 ml EGTA (Sigma Chemical) to the final concentration of 8 mM.

Measurement of uterine contraction in vivo. In the experiment, the rats at estrus stage, which was confirmed by microscopic examination of a vaginal smear, were used. They were anesthetized with pentobarbital (18 mg in 0.3 ml ip). A small mid-portion of a uterine horn and associated mesometrium was obtained through a ventral incision made in the skin and body wall. A 1- to 2-mm incision was made at the distal end of the exposed uterus, and a thin, finger-shaped latex balloon was attached to a polyethylene catheter (Becton-Dickinson, Franklin Lakes, NJ), which was a modification of a method described in a previous study (1, 17, 18, 33). The catheter was connected to a transducer (PowerLab recorder ML785; Castle Hill); contractions were recorded by this transducer by using Chart 5 software (Castle Hill). The spontaneous contraction were recorded after the addition PGF\(_{2\alpha}\) (0.2 mg/kg ip) and AHM-EA (5 or 10 mg/kg ip).

Statistical analysis. Data are presented as the means ± SE of several preparations from different animals. Statistical significance of difference between the groups was analyzed by one-way ANOVA by using SPSS system, version 11.0 (SPSS, Chicago, IL). Comparisons between the mean values of groups were performed using one-way ANOVA and Duncan’s multiple range test. For comparison between two groups, Student’s t-tests were used. The difference between two mean values was considered statistically significant when \(P < 0.05\) and highly significant when \(P < 0.01\).

RESULTS

Effects of adlay extracts on PGF\(_{2\alpha}\)-induced uterine contractions in the rats. PGF\(_{2\alpha}\) is the major substance that induces uterine contractions during dysmenorrhea (8, 9, 32). Several studies reported that adlay hull extracts exhibited various biological effects. Hsia et al. (14, 15) reported that administration of adlay hull extracts at the doses of 10–200 μg/ml decreased the secretion of progesterone and estradiol in rat granulosa cells. The higher concentrations of adlay hull extracts (160–800 μg/ml) decreased the secretion of coticosterone in rat zona fasciculata-reticularis cells (44). Adlay hull extracts display multiple antioxidant effects and induce apoptosis of malignant human cells (U937) at 250 μg/ml (24). Furthermore, adlay hull extracts showed the inhibitory effect on lung cancer cell proliferation with 333 μg/ml (5). Because we did not know the effective concentration of adlay hull extracts on uterine contractions, we referred to previous studies and then tested a broad range of adlay hull extracts concentrations (25–500 μg/ml) determining that 175 μg/ml was optimal. To identify the types of adlay extracts that exert an effect on PGF\(_{2\alpha}\)-induced uterine contractions in the rat, we observed the effects of adlay extracts (175 μg/ml) on uterine contractions induced by PGF\(_{2\alpha}\) (10\(^{-6}\) M) in vitro. The administration of different types of adlay extracts (PAM, ABM, ATM, or AHM; 175 μg/ml) along with PGF\(_{2\alpha}\) (10\(^{-6}\) M) indicated that AHM and ATM at 175 μg/ml exerted an inhibitory effect on uterine contractions in the rats (\(n = 4\); \(P < 0.01\) or \(P < 0.05\); Duncan’s multiple range test; Fig. 1A) compared with the control group. Neither ABM (175 μg/ml) nor PAM (175 μg/ml) exerted any inhibitory effect on uterine smooth muscle contraction (Fig. 1A).

Effects of AHM subfractions on rat uterine smooth muscle contraction. To confirm the inhibitory effect of the major active adlay hull fraction AHM on uterine contractions, AHM was further separated into four fractions: AHM-Wa, AHM-Bu, AHM-EA, and AHM-Hex. The administration of these fractions at 175 μg/ml along with PGF\(_{2\alpha}\) (10\(^{-6}\) M) indicated that 175 μg/ml AHM-EA inhibited uterine contractions in the rats (\(n = 4\); \(P < 0.05\) or \(P < 0.01\); Duncan’s multiple-range test; Fig. 1B) compared with the 0 μg/ml AHM. To study whether AHM has a linear dose-response relationship with the inhibition of PGF\(_{2\alpha}\)-induced uterine contractions, AHM-EA was administered at different doses (0, 25, 75, 175, 375, and 500 μg/ml) along with PGF\(_{2\alpha}\) (10\(^{-6}\) M). AHM-EA at 75–500 μg/ml was found to exhibit a dose-dependent inhibition of the PGF\(_{2\alpha}\)-induced uterine contractions (\(n = 8\); \(P < 0.05\) or \(P < 0.01\); Duncan’s multiple-range test; Fig. 1C). To study whether AHM has a linear dose-response relationship with the inhibition of oxytocin-induced uterine contractions, AHM-EA was administered at different doses (0, 25, 75, 175, 375, and 500 μg/ml) along with oxytocin (10\(^{-6}\) M). AHM-EA at 75–500 μg/ml was found to exhibit a dose-dependent inhibition of the oxytocin-induced uterine contractions (\(n = 8\); \(P < 0.05\) or \(P < 0.01\); Duncan’s multiple-range test; Fig. 1D). The decrease in uterine contraction was not attributed to the cytotoxicity of the adlay extracts. We also assessed cell viability using a recovery study. We determined that by removing the inhibitory substance and washing the tissue with Krebs’s solution for 20 min and then reanalyzing the tissue in the presence of a stimulus (PGF\(_{2\alpha}\) 10\(^{-6}\) M). From our results, we found that PGF\(_{2\alpha}\) could reverse the inhibition after removing the AHM-EA (Supplemental Fig. S2).

Effect of AHM-EA on PGF\(_{2\alpha}\)-induced uterine contractions in vivo. To confirm the inhibitory effect of AHM-EA on uterine contractions in vivo, we measured the uterine pressure. The uterine muscles were treated with AHM-EA (5 and 10 mg/kg ip) along with PGF\(_{2\alpha}\) (0.2 mg/kg ip; Fig. 2A). AHM-EA treatment (5 or 10 mg/kg) significantly reduced the PGF\(_{2\alpha}\) induced uterine contractions in vivo (\(n = 4\); \(P < 0.05\) or \(P < 0.01\); Duncan’s multiple range test; Fig. 2B). Furthermore, the well-being of the animals was not effected by the exposure to 10 mg/kg AHM-EA.

Effect of AHM-EA on Ca\(^{2+}\)-dependent contractions. As reported by previous studies, a Ca\(^{2+}\) channel activator Bay K 8644 and high K\(^+\) have been reported to induce uterine contractions. Administration of AHM-EA (25, 75, 175, 375,
and 500 μg/ml) along with KCl or Bay K 8644 resulted in a dose-dependent inhibition of uterine contraction. \( n = 8; P < 0.05 \) or \( P < 0.01; \) Duncan’s multiple-range test; Fig. 3). To investigate whether the inhibition of contractions by AHM-EA is due to the blockade of external Ca\(^{2+}\) influx, we performed the experiments in external Ca\(^{2+}\)-free medium. In the absence of external Ca\(^{2+}\), the spontaneous contractions were abolished. Further, the application of increasing concentrations of Ca\(^{2+}\) from 0.05 to 5 mM restored the spontaneous contractions (Fig. 4).

**Effect of AHM-EA on \([\text{Ca}^{2+}]_i\).** To study whether AHM-EA inhibits the increases in \([\text{Ca}^{2+}]_i\), similar to its inhibitory effect on muscular contraction, the uterine smooth muscle cells were treated with AHM-EA (25, 50, and 100 μg/ml) along with PGF\(_{2\alpha}\) (200 nM). AHM-EA treatment (50 or 100 μg/ml) significantly reduced the PGF\(_{2\alpha}\)-induced \([\text{Ca}^{2+}]_i\), \( n = 3; P < 0.01; \) Duncan’s multiple-range test; Fig. 5). The decrease in \([\text{Ca}^{2+}]_i\) was not attributed to the cytotoxicity of AHM-EA. The administration of AHM-EA (25~500 μg/ml) caused no release of lactate dehydrogenase from uterine smooth muscle cells (Supplemental Fig. S3).

**Effect of AHM-EA subfractions on rat uterine smooth muscle contraction.** To confirm the inhibitory effect of the major active AHM-EA fraction on uterine contractions, AHM-EA was further fractioned using silica gel column chromatography with gradient elution, and 11 subfractions were produced that were assigned letters A-K. The uterine muscles were treated with the fractions AHM-EA-A to AHM-EA-K (375 μg/ml) along with PGF\(_{2\alpha}\) (10\(^{-6}\) M). The subfractions AHM-EA-B~J significantly reduced the PGF\(_{2\alpha}\)-induced uterine contractions \( n = 3; P < 0.01; \) Duncan’s multiple range test; Fig. 6). Among all subfractions, AHM-EA-D, AHM-EA-E, and AHM-EA-F were more effective in reducing the PGF\(_{2\alpha}\)-induced uterine contractions. However, from our preliminary study, TLC showed the presence of several compounds in fraction AHM-EA-E and AHM-EA-F (data not show). HPLC analysis was performed to further quantify these chemicals. AHM-EA-E was found to contain caffeic acid, ferulic acid, gallic

![Fig. 1. Effects of different parts of adlay extract (A), methanolic extracts of adlay hull (AHM) fractions (B), and AHM-EA (C and D) on PGF\(_{2\alpha}\)- or oxytocin-induced uterine contractions in the rats. \( * P < 0.05, ** P < 0.01 \) vs. control group, as assessed by Duncan’s multiple range test. All columns are means ± SE.](http://ajpendo.physiology.org/)

![Fig. 2. Effects of AHM-EA (5 or 10 mg/kg) on PGF\(_{2\alpha}\)-induced (0.2 mg/kg) uterine contractions in vivo (A and B). Results represent the records of 4 experiments. \( ** P < 0.01 \) vs. PGF\(_{2\alpha}\)-treated group, as assessed by Duncan’s multiple range test. \( # P < 0.05 \) vs. vehicle-treated group, as assessed by Student’s t-test. All columns are means ± SE.](http://ajpendo.physiology.org/)
acid, *p*-coumaric acid, syringaldehyde, 3,4-dihydroxybenzoic acid, and quercetin, while AHM-EA-F was found to contain *p*-coumaric acid, vanillic acid, syringaldehyde, 3,4-dihydroxybenzoic acid, and naringenin (Table 1).

Effects of pure compounds (*caffeic* acid, *ferulic* acid, *gallic* acid, *p*-coumaric acid, *vanillic* acid, syringaldehyde, 3,4-dihydroxybenzoic acid, quercetin, and naringenin) present in AHM-EA-E and AHM-EA-F on rat uterine smooth muscle contraction. Several studies reported that caffeic acid, *ferulic* acid, *gallic* acid, *p*-coumaric acid, vanillic acid, syringaldehyde, 3,4-dihydroxybenzoic acid, quercetin, and naringenin exhibited various biological effects. Ajay et al. (2) reported that administration of flavonoids (quercetin and naringenin) at the doses of $10^{-7}$–$10^{-3}$ M decreased the vascular smooth muscle contractions. Furthermore, the previous investigation was to determine which group(s) of polyphenols (caffeic acid, ferulic acid, *gallic* acid, *p*-coumaric acid, and vanillic acid) is able to cause endothelium dependent vasorelaxation at $10^{-7}$–$10^{-3}$ M (3). Because we did not know the effective concentration of compounds on uterine contractions, we referred to previous studies and then tested a range of compound concentrations ($10^{-6}$–$10^{-4}$). To confirm the inhibitory effects of the different compounds present in AHM-EA on uterine contractions, different doses of the compounds [naringenin, quercetin, vanillin, syringaldehyde, gallic acid, syringic acid, and *p*-coumaric acid ($10^{-6}$–$10^{-4}$ M)] were administered along with PGF$_{2\alpha}$ ($10^{-6}$ M). Naringenin and quercetin exerted

![Fig. 3. Effects of AHM-EA on high K$^+$ (KCl) or Bay K 8644-induced uterine contractions in the rats. **$P < 0.01$ vs. control group, as assessed by Duncan’s multiple range test. All columns are means ± SE.](image)

![Fig. 4. Inhibitory actions of AHM-EA on Ca$^{2+}$-dependent contractile responses. Muscle segments were initially pretreated in a Ca$^{2+}$-free medium containing the vehicle (top) or AHM-EA (375 μg/ml), and calcium (0.05–5 mM) was then cumulatively applied to trigger muscle contraction. Results represent the records of 4 experiments.](image)

**Fig. 3.** Effects of AHM-EA on high K$^+$ (KCl) or Bay K 8644-induced uterine contractions in the rats. **$P < 0.01$ vs. control group, as assessed by Duncan’s multiple range test. All columns are means ± SE.

**Fig. 4.** Inhibitory actions of AHM-EA on Ca$^{2+}$-dependent contractile responses. Muscle segments were initially pretreated in a Ca$^{2+}$-free medium containing the vehicle (top) or AHM-EA (375 μg/ml), and calcium (0.05–5 mM) was then cumulatively applied to trigger muscle contraction. Results represent the records of 4 experiments.
the most potent inhibitory effect on uterine contractions in the rats ($n = 3$; $P < 0.05$ or $P < 0.01$; Duncan’s multiple-range test; Fig. 7).

**DISCUSSION**

In the Orient, adlay is a component of many traditional herbal medicines prescribed for gynecological dysfunction. The present study is the first to demonstrate that adlay hull extracts markedly suppress PGF$_{2\alpha}$-induced uterine contractions. The results demonstrated that 1) AHM-EA treatment inhibited PGF$_{2\alpha}$- and oxytocin-induced uterine contractions in the rats; 2) AHM-EA inhibited uterine contractions stimulated by the Ca$^{2+}$ channel activator and depolarization in response to high K$^+$; 3) AHM-EA inhibited PGF$_{2\alpha}$-induced and high K$^+$-induced increases in [Ca$^{2+}$]; 4) AHM-EA could mimic Ca$^{2+}$ channel blockers, which block Ca$^{2+}$ influx through plasma membrane voltage-operated Ca$^{2+}$ channels; 5) both quercetin and naringenin present in AHM-EA inhibited the PGF$_{2\alpha}$-induced uterine contractions in the rats; and 6) AHM-EA inhibited PGF$_{2\alpha}$-induced uterine contractions in the rats in vivo. Thus our results provide evidence to support the notion that adlay acts as a traditional remedy for gynecological complications. This study demonstrates that adlay hull may be of potential use in the treatment of primary dysmenorrhea. To our knowledge, this is the first study demonstrating the effects of adlay hull extracts on uterine contraction in vivo and in vitro, thus partially explaining the modulatory effects of adlay extracts on female reproductive functions. Conceivably, the different components of adlay seed extracts may possess different chemicals that regulate uterine function.

Dysmenorrhea has been reported to lead to increased prostaglandin (PGF$_2$ and PGE$_2$) production, which may result in the contraction of the blood vessels and myometrium, and insufficient blood flow to the endometrium (8, 9, 31). Some studies (8, 37) found that PGF$_{2\alpha}$ levels are elevated in women with primary dysmenorrhea. Therefore, the role of prostaglandins is implicated in dysmenorrhea. It is well established that PGF$_{2\alpha}$ increases the [Ca$^{2+}$], and then stimulates uterine contraction (4, 7, 45). In the present study, we found that the AHM and AHM-EA fractions inhibited the PGF$_{2\alpha}$-induced uterine contractions both in vivo and in vitro. Inflammation is also involved in the pathogenesis of dysmenorrhea. A previous study (36) showed that NSAIDs could treat dysmenorrhea. Several studies (12, 37) have evaluated the effect of a COX-2 inhibitor in treating primary dysmenorrhea. A previous study (16) has shown that an adlay extract could inhibit COX-2 in a lung cancer cell line; this result indicated the anti-inflammatory activity of the adlay extract and that dysmenorrhea could be treated with the adlay extract.

An increase in the [Ca$^{2+}$], in the uterine smooth muscles induces uterine contractions. Studies (19, 20, 38, 39) have indicated that [Ca$^{2+}$], is regulated by two different Ca$^{2+}$ channels: receptor- and voltage-operated channels (ROCs and VOCs) in the uterine smooth muscles. In the ROCs, uterotonic hormones (PGF$_{2\alpha}$ and oxytocin) that induce uterine contraction increase the [Ca$^{2+}$], via both the influx of extracellular Ca$^{2+}$ through the Ca$^{2+}$ channels and the release of intracellular stored Ca$^{2+}$. In the VOCs, both Ca$^{2+}$ channel activator (Bay K 8644) and membrane depolarization caused by high K$^+$ (high concentration of KCl) can increase Ca$^{2+}$ influx through the VOCs. Therefore, the effect of AHM-EA on uterine contraction could be considered to interfere with ROCs or/and VOCs. Our present study indicates that AHM-EA could inhibit the increases in [Ca$^{2+}$], induced by PGF$_{2\alpha}$, KCl, and Bay K 8644 and block the Ca$^{2+}$ influx through depolarization and/or the VOCs.

AHM is a crude extract containing many specific chemical components that regulate endocrine functions. To confirm which chemical compounds inhibit the PGF$_{2\alpha}$-induced uterine contractions, we separated AHM-EA into 11 fractions. The results indicated that AHM-EA-E and AHM-EA-F have a greater potential to decrease the PGF$_{2\alpha}$-induced uterine contractions. It has been reported that AHM possesses some low-molecular-weight and moderately polar substances that have antioxidative effects (23). Moreover, at least of the following six classes of chemical constituents of AHM have been demonstrated: lignan, phenolic acid, phytosterols, polyphenols, polysaccharides, and flavonoids (15, 22, 34, 43). In our previous study (15), we quantified the components of the AHM-EA fraction; it contained naringenin, quercetin, vanillin, syringaldehyde, gallic acid, syringic acid, and p-coumaric acid. In the present study, we demonstrate that naringenin and quercetin (both of which are flavonoid phytochemicals) inhibit the PGF$_{2\alpha}$-induced uterine contractions. Naringenin has previously been shown to inhibit HMG (3-hydroxy-3-methylglutaryl-CoA reductase) in vitro (25, 26, 28); it could also inhibit progesterone secretion in rat granulosa cells (15) and the aromatase activity in human endometrial stromal cells and ovary granulosa cells (15, 40). In vascular smooth muscle cells, the vasorelaxant effect of the naturally occurring flavonoid (naringenin) on endothelium-denuded vessels was due to the activation of Ca$^{2+}$-activated K$^+$ channels in myocytes (41). Quercetin could inhibit Ca$^{2+}$ influx and release of Ca$^{2+}$ from intracellular stores in isolated rat thoracic aorta (2) as well as both the phasic and tonic components of anaphylactic contraction in a concentration-dependent fashion in guinea pig ileal smooth muscles (10). Taken together, these results suggest that AHM contains several different components that inhibit PGF$_{2\alpha}$-induced uterine smooth muscle contraction in the rat.

![Fig. 7. Effects of the pure compounds (vehicle, naringenin, quercetin, gallic acid, ferulic acid, p-coumaric acid, vanillic acid, caffeic acid, syringaldehyde, and 3,4-dihydroxycinnamic acid) present in AHM-EA-E and AHM-EA-F on PGF$_{2\alpha}$-induced uterine contractions in the rats. *P < 0.05, **P < 0.01 vs. the control group, as assessed by Duncan’s multiple range test. All columns are means ± SE.](http://ajpendo.physiology.org/doi/abs/10.1152/ajpendo.00165.2008)
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Our results provide evidence to explain why adlay could be used for the treatment of dysmenorrhea.

In summary, the present data demonstrate that the obtained fractions of adlay extract could inhibit the PGF$_2$alpha-induced uterine smooth muscle contraction both in vitro and in vivo. The inhibition of uterine smooth muscle contraction is, in part, due to the blockade of the ROCs and VOCs in the rat. Thus adlay hull extract containing naringenin and quercetin seems to be of potential use in the treatment of dysmenorrhea.

GRANTS

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REFERENCES


46. Wang SC. Quantification and Comparison of Physiological Components in Adlay Seeds (M. S. thesis). Taipei, Taiwan, ROC: Graduate Institute of Food Science and Technology National Taiwan University, 2002.
