HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2016.07.012

Effect of *Alpinia officinarum* Hance alcohol extracts on primary dysmenorrheal

Ling Huang, Jun-Qing Zhang, You-Bin Li, Mi Liu, Hui-Ming Deng, Yu-Chao Luo, Yin-Feng Tan, Jie Hou, Gui-Wei Yao, Wei-Wei Guan*

School of Pharmaceutical Sciences, Hainan Medical University, Haikou 571101, Hainan, China

ARTICLE INFO

Article history:
Received 17 May 2016
Received in revised form 16 Jun 2016
Accepted 1 Jul 2016
Available online 26 Jul 2016

Keywords:
Alpinia officinarum Hance alcohol extracts
Relieve
Primary dysmenorrhea

ABSTRACT

Objective: To study the effect of *Alpinia officinarum* Hance (*A. officinarum*) 80% alcohol extract on the primary dysmenorrhea.

Methods: *A. officinarum* 80% alcohol extract were enriched by macroporous adsorption resins. Female mice of primary dysmenorrhea model were established by oxytocin induction; the effects of *A. officinarum* 80% alcohol extract on primary dysmenorrhea were observed by body twist method; and the homogenate level of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin E_2 (PGE_2) and Ca^{2+} in the uterus were observed in oxytocin-induced female mice.

Results: The writhing frequency of primary dysmenorrhea mice was significantly decreased after treatment of *A. officinarum* 80% alcohol extract and the level of $PGF_{2\alpha}$, PGE_2 and Ca^{2+} in mice uterus was significantly decreased ($P < 0.05$, $P < 0.01$) in groups of mice treated with middle and high dosage of *A. officinarum* 80% alcohol extract compared with that of model group.

Conclusions: These findings suggest that *A. Officinarum* 80% alcohol extract can significantly relieve primary dysmenorrhea.

1. Introduction

Primary dysmenorrheal, which is a common gynecological problem, is defined as cramping pain in the lower abdomen occurring just before or during menstruation, and it occurs in up to 50% of menstruating females and causes significant disruption in quality of life [1–4]. Principal pharmacological therapies for primary dysmenorrheal include nonsteroidal anti-inflammatory drugs (NSAIDs) [4–7] or oral contraceptive pills (OCPs) [8,9]. NSAIDs reduce myometrial activity by inhibiting prostaglandin (PG) synthesis and reducing vasopressin secretion. However, the failure rate of NSAIDs is often 20%–25%. Besides, NSAIDs have long-term adverse effects involving disorders of the liver, kidney, and digestive systems [10]. OCPs suppress ovulation and thin the endometrial lining which reduces menstrual fluid volume along with the amount of PG produced, thus reducing the pain associated with uterine contractions [11]. However, OCPs can cause side effects including nausea and water retention and may not be suitable for all women, especially those pursuing pregnancy [12,13].

Because of these limitations of conventional treatments, herbal medicines are considered as feasible alternatives for the treatment of pain or primary dysmenorrhea [14,15]. Herbal medicines used for treating pain or primary dysmenorrhea have been used for long time in China, and currently these therapies are increasingly being used worldwide [16,17]. Herbal medicines are relatively well tolerated by patients because of fewer adverse effects and lower recurrence rates associated with them. During the last few decades, an increasing number of preclinical studies investigating the efficacy of herbal medicines in cell and animal models for primary dysmenorrhea have been published.

Alpinia officinarum Hance (*A. officinarum*) is a perennial medicinal plant that is mainly distributed in the tropical and subtropical regions of Southeast Asia. It is commonly used as a food additive, and a kind of Chinese medicinal material, and has been listed as Affinal Drug and Diet by National Health and Family Planning Commission of the People's Republic of China due to its high safety. The most medicinally active part of the plant is the rhizome, which has important medical value. The rhizomes of *A. officinarum* are widely used in China for

*Corresponding author. Wei-Wei Guan, School of Pharmaceutical Sciences, Hainan Medical University, Haikou 571101, Hainan, China.
E-mail: puer6@163.com

Peer review under responsibility of Hainan Medical College.

Foundation project: This study was supported by the key project supported of Hainan Province (Grant No. ZDZX2013008) and National Natural Science Foundation of China (Grant No. 2014 81403006).

relieving stomach aches, treating colds, invigorating the circulatory system, reducing swelling and invigorating blood circulation and antiemetic [18–21].

Nevertheless, there are little studies that have addressed the pharmacological effects of *A. officinarum* on impairment in primary dysmenorrhea. Taking the above into consideration, this study aims to clarify the pharmacology effects of *A. officinarum* effective parts on impairment in pain perception and dysmenorrhea, and provide theoretical basis for the industrialization development of *A. officinarum* rhizome.

2. Materials and methods

2.1. Experimental animals

Female BALB/c mice (25 ± 5 g, 8–10 week old) were purchased from Medical Experimental Animal Center of Hunan Province and kept in a room at 22–24 °C with alight/dark cycle of 12/12 h and 55%–60% relative humidity in Experimental Animal Center of Hainan Medical University. They had free access to standard rodent chow and clean tap water. All procedures of animal experiments in this study were in accordance with the Regulations of Experimental Animal Administration issued by the Ministry of Science and Technology of the People's Republic of China. The design of animal experiment was approved by Experimental Animal ethics review committee of Hainan Medical University.

2.2. Main instrument

AB-8 macroporous absorptive resin was purchased from Bohong Resin Technology Co. Ltd (Shanghai, China); Waters alliance 2695 high-performance liquid chromatography was sourced from Waters Science and Technology Co. Ltd (Milford, USA); YLS-6B hot plate gauge was purchased from Jinan Yiyan Technology Development Co. Ltd (Shandong, China).

2.3. Chemicals and reagents

The dried root of *A. officinarum* was purchased from Xuwen *A. officinarum* planting base (Xuwen, China); aspirin was purchased from Bayer AG (Leverkusen, Germany); diethylstilbestrol and oxytocin was purchased from Dalian Meilun Technology Co. Ltd (Shenyang, China).

2.4. A. officinarum 80% alcohol extracts preparation

A total of 10 kg dried root of *A. officinarum* was identified by associate professor Jianping Tian from School of Pharmacy, Hainan Medical University (Haikou, China). After crushing, *A. officinarum* powder was refluxing extracted by 12 times volume of water for 2 h, which was repeated twice. The 2 times extraction were filtrated and combined, then *A. officinarum* aqueous extract were obtained, the aqueous extract was subjected to macroporous resin AB-8 column chromatography, then were eluted with 80% alcohol successively, the 80% eluent were collected and concentrated by vacuum drying to obtain the 80% alcohol extracts. A flavonoids and diarylheptanoids enriched extract (280 g) were collected after the 80% alcohol eluent were concentrated by vacuum drying, the total content of flavonoids and diarylheptanoids were more than 65% measured by HPLC.

2.5. Establishment of mice dysmenorrhea model

A total of 50 Balb/c mice were intragastric administrated with diethylstilbestrol (0.8 mg/mL, 0.1 mL/10 g wt) for seven days to establish primary dysmenorrhea models, six hours after medication of diethylstilbestrol from the seventh day, 50 Balb/c mice were randomly divided into 5 groups with 10 mice in each group and administrated with aspirin 0.5 g/kg (positive control group); normal saline (model group); *A. officinarum* 80% alcohol extracts low dose group (crude drugs 0.75 g/kg), medium dose group (crude drugs 3.00 g/kg), high dose group (crude drugs 12.00 g/kg) for another seven days. Other 10 Balb/c mice which were intragastric administrated with normal saline for 13 d (0.1 mL/10 g wt) were considered as normal control group, each group of final volume of administration was 0.1 mL/10 g wt. On the 13th day, each group of mice were treated with intraperitoneal injection of oxytocin 33 U/kg to induce the writhing reaction after administration with *A. officinarum* extracts [22,23].

2.6. Writhing test of A. officinarum 80% alcohol extracts on primary dysmenorrhea

Writhing test were used to observe the effects of *A. officinarum* extracts on primary dysmenorrhea mice. On the 13th day, each group of mice were treated with intraperitoneal injection of oxytocin 33 U/kg to induce writhing reaction 30 min after administration with *A. officinarum* 80% alcohol extract, then the number of writhing was observed. Mice were placed in a box, and the number of writhing was counted for the next 30 min. The writhing was characterized by abdominal contraction, stretching or bending of the body, trunk and/or pelvis ending with limbs extension [24].

Analgesia percentage = (writhing number of model group − writhing number of medicated groups/writhing number of model group) × 100%.

2.7. Preparation of uterine smooth muscle tissue homogenate

Injection of oxytocin in mice after 1 h, intraperitoneal injection of chloral hydrate anesthesia, open the abdominal cavity, and the uterine tissues were then dissected for further investigation. The uterine tissues were homogenized in 9 volumes of normal saline. Following centrifugation at 3 000 rpm for 15 min at 4 °C, the supernatant was used to measure the levels of $PGF_{2\alpha}$, PGE_2 and Ca^{2+}.

2.8. $PGF_{2\alpha}$, PGE_2 and Ca^{2+} enzyme linked immunosorbent assay

The supernatant obtained from 2.7 was used to measure the levels of $PGF_{2\alpha}$, PGE_2 and Ca^{2+} in uterine smooth muscle tissue of mice by enzyme linked immunosorbent assay (ELISA) in accordance with manufactures instructions [14,25,26].

2.9. Statistical analysis

The results were expressed as mean ± SD Statistical comparisons were made using Student's *t*-test and the chosen level of significance was $P < 0.05$.

3. Results

3.1. Writhing test of A. officinarum extracts on primary dysmenorrhea

Mice models of primary dysmenorrhea which were induced by oxytocin were established to investigate the effects of *A. Officinarum* 80% alcohol extract for relieving dysmenorrhea symptoms. As shown in Table 1, the model group mice all showed body writhing reaction compared with normal control group indicating that the primary dysmenorrhea model was successfully established. Compared with model group, the writhing number of positive control group was significantly reduced, and the analgesia percentage was 84.00% ($P < 0.01$). The mice writhing numbers were significantly decreased by *A. officinarum* 80% alcohol extract low, medium and high dose group, and their analgesia percentage were 59.22%, 61.87% and 84.35% respectively ($P < 0.05$, $P < 0.01$).

3.2. $PGF_{2\alpha}$ levels of oxytocin-induced writhing mice

The synthesis and release of PGs increase is one of the important reasons of cause primary dysmenorrhea, and this study tested the effect of *A. officinarum* 80% alcohol extract on the PG level of the dysmenorrhea model mice uterus. As shown in Table 1, compared with the normal control group, the level of uterus tissue $PGF_{2\alpha}$ was significantly increased in model group; compared with model group, the $PGF_{2\alpha}$ level of uterus tissue was significantly decreased in positive control group ($P < 0.05$); $PGF_{2\alpha}$ level of uterine tissue was decreased in some degree after administrated with *A. officinarum* 80% alcohol extract all dose group ($P < 0.05$).

3.3. PGE_2 levels of oxytocin-induced writhing mice

As shown in Table 1, compared with the normal control group, the level of uterus tissue PGE_2 in model group mice was significantly increased; compared with model group, the PGE_2 level was significantly decreased in positive control group uterus tissue ($P < 0.05$), the uterine tissues PGE_2 level was significantly reduced by *A. officinarum* 80% alcohol extract high dose group ($P < 0.05$).

3.4. Ca^{2+} levels of oxytocin-induced writhing mice

As shown in Table 1, compared with the normal control group, the level of Ca^{2+} in uterus tissue was significantly increased in model group ($P < 0.01$); compared with model group, the Ca^{2+} level was significantly decreased in positive control group uterus tissue ($P < 0.01$), and the uterine tissues Ca^{2+} level were significantly reduced by *A. officinarum* 80% alcohol extract high dose group ($P < 0.01$).

4. Discussion

Primary dysmenorrhea is defined as a cramp-like pain in the lower abdomen before or during menstruation without any identifiable pelvic pathology, it may be accompanied by lower back pain, nausea, vomiting, and diarrhea and it is frequently found in young nullipara [27]. Modern medical science holds that the emergence of primary dysmenorrhea is related with many factors. Among them, temporary ischemia of myometrium and endometrium of the uterus plays an important role. The ischemia may result from pressured intermuscular blood vessels induced by forceful contraction of uterine arteries and paroxysmal contraction of uterine smooth muscles [28]. Traditional Chinese Medicine thinks that primary dysmenorrhea is often caused by blood stasis blocking the uterus. In some previous studies, *A. officinarum* showed the effects of regulating the contractive amplitude, frequency, and mobility of gastrointestinal smooth muscle and improving microcirculation. Therefore, we hypothesized that *A. officinarum* 80% alcohol extracts perhaps could alleviate dysmenorrhea. We established the dysmenorrhea mice models by administration of diethylstilbestrol and oxytocin to further study the effects of *A. officinarum* on dysmenorrhea.

After administrated with diethylstilbestrol and oxytocin, the abdominal contraction, stretching or bending of the body, trunk and pelvis ending with limbs extension were observed in all model mice suggesting that mouse dysmenorrhea model was established successfully. The dysmenorrhea latency was remarkably increased by *A. officinarum* 80% alcohol extracts, while after administration of *A. officinarum* in each group, the body writhing reaction caused by oxytocin were decreased. The analgesia effect of *A. officinarum* 80% alcohol extract high dose group was even better than aspirin.

COX enzymes are involved in inflammatory pathways and responsible for formation of pro-inflammatory PGs. PGs which can cause constriction in uterine smooth muscle and sensitize spinal neurons to pain are regarded as the most pain factor to primary dysmenorrhea [29]. $PGF_{2\alpha}$ and PGE_2, two naturally occurring PG, are used to induce labor in medicine. $PGF_{2\alpha}$ and PGE_2 can combined with their receptor on the spiral arterioles increased uterine contractility, resulting in ischemia pain [30]. In the present study, it was found that the $PGF_{2\alpha}$ and PGE_2 levels in uterus were all significantly decreased after treatment with *A. officinarum* 80% alcohol extract medium and high groups.

Table 1

Effects of *A. officinarum* 80% alcohol extract on writhing reaction, analgesia of primary dysmenorrheal model mice, and levels of $PGF_{2\alpha}$, PGE_2 and Ca^{2+} in their uterine tissue ($n = 10$).

Group	Writhing number (30 min)	Analgesia (%)	$PGF_{2\alpha}$ (μg/gprot)	PGE_2 (μg/gprot)	Ca^{2+} (mmol/gprot)
Normal control	0.00 ± 0.00	100.00	198.01 ± 16.20	158.01 ± 14.23	0.14 ± 0.02
Model	61.37 ± 10.47**	0.00	277.81 ± 18.29*	234.12 ± 15.12**	0.29 ± 0.04**
Positive control	9.82 ± 3.81$^{\#\#}$	84.00	208.81 ± 9.76$^{\#}$	178.16 ± 6.45$^{\#}$	0.18 ± 0.06$^{\#\#}$
Alcohol extracts 80% (Low)	25.09 ± 7.73$^{\#}$	59.22	218.3 ± 9.23$^{\#}$	208.45 ± 10.11	0.25 ± 0.04*
Alcohol extracts 80% (Medium)	23.40 ± 6.48$^{\#}$	61.87	199.64 ± 10.24$^{\#}$	205.64 ± 11.16	0.19 ± 0.05$^{\#}$
Alcohol extracts 80% (High)	9.60 ± 3.66$^{\#\#}$	84.35	209.92 ± 12.23$^{\#}$	178.34 ± 9.87$^{\#}$	0.16 ± 0.02$^{\#\#}$

$^{*}P < 0.05$, $^{**}P < 0.01$, compared to normal control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared to model group.

The levels of Ca^{2+} in the dysmenorrheal model mice uterus were also detected. Overload of intracellular Ca^{2+} can cause uterine smooth muscle contraction and reduce the endometrial blood supply, leading to the occurrence of dysmenorrhea. Therefore, calcium channel blocking agents decrease myometrial contractility and beneficial in cases of dysmenorrhea [31,32]. The treatment of *A. officinarum* 80% alcohol extract, the medium and high doses groups, all resulted in the significant decrease of Ca^{2+} levels. This indicated that one mechanism of *A. officinarum* 80% alcohol extracts treating dysmenorrhea may act on Ca^{2+} channel to decrease intracellular Ca^{2+} concentration. The above results provide theoretical evidence that medicines extracted from *A. officinarum* 80% alcohol extract could be produced to treat dysmenorrhea in clinic.

In conclusion, the results of the current study provide evidence that *A. officinarum* might be effective for treating primary dysmenorrhea. Considering the advantages of better tolerability, shorter contraindication list and lower incidence of side effects, *A. officinarum* 80% alcohol extract might be considered as an alternative agent of NSAIDs and analgesic in treatment of primary dysmenorrhea.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This study was supported by the key project supported of Hainan Province (Grant no. ZDZX2013008) and National Natural Science Foundation of China (Grant No. 2014 81403006).

References

[1] Chen WH, Zhao Y, Zeng CC, Zhang DN, Wang YP, Tang L, et al. Pain-induced pulsograph changes in patients with primary dysmenorrhea: a pilot study. *Evid Based Complement Alternat Med* 2015; **2015**: 385136; http://dx.doi.org/10.1155/2015/385136.

[2] Faramarzi M, Salmalian H. Association of psychologic and non-psychologic factors with primary dysmenorrhea. *Iran Red Crescent Med J* 2014; **16**(8): e16307.

[3] Modaress Nejad V, Asadipour M. Comparison the effectiveness of Fennel and Mefenamic acid on pain intensity in dysmenorrhea. *East Mediterr Health J* 2006; **12**(3–4): 423-427.

[4] Smith CA, Zhu X, He L, Song J. Acupuncture for dysmenorrhoea. *Cochrane Database Syst Rev* 2011; **18**(4): CD007854; http://dx.doi.org/10.1002/14651858.CD007854.pub3.

[5] Marjoribanks J, Ayeleke RO, Farquhar C, Proctor M. Nonsteroidal anti-inflammatory drugs for dysmenorrhoea. *Cochrane Database Syst Rev* 2015; **30**(7): CD001751; http://dx.doi.org/10.1002/14651858.CD001751.pub3.

[6] Sugumar R, Krishnaiah V, Channaveera GS, Mruthyunjaya S. Comparison of the pattern, efficacy, and tolerability of self-medicated drugs in primary dysmenorrhea: a questionnaire based survey. *Indian J Pharmacol* 2013; **45**(2): 180-183.

[7] Witt J, Strickland J, Cheng AL, Curtis C, Calkins J. A randomized trial comparing the VIPON tampon and ibuprofen for dysmenorrhea pain relief. *J Womens Health (Larchmt)* 2013; **22**(8): 702-705.

[8] Wong CL, Farquhar C, Roberts H, Proctor M. Oral contraceptive pill for primary dysmenorrhoea. *Cochrane Database Syst Rev* 2009; **7**(4): CD002120; http://dx.doi.org/10.1002/14651858.CD002120.pub3.

[9] Dmitrovic R, Kunselman AR, Legro RS. Continuous compared with cyclic oral contraceptives for the treatment of primary dysmenorrhea: a randomized controlled trial. *Obstet Gynecol* 2012; **119**(6): 1143-1150.

[10] Daniels SE, Torri S, Desjardins PJ. Valdecoxib for treatment of primary dysmenorrhea: a randomized, double-blind comparison with placebo and naproxen. *J Gen Intern Med* 2005; **20**(1): 62-67.

[11] Harel Z. Dysmenorrhea in adolescents and young adults: etiology and management. *J Pediatr Adolesc Gynecol* 2006; **19**(6): 363-371.

[12] Hugon-Rodin J, Gompel A, Plu-Bureau G. Epidemiology of hormonal contraceptives-related venous thromboembolism. *Eur J Endocrinol* 2014; **171**(6): R221-R230.

[13] Gronier H, Robin G. Cardiovascular risks of combined oral contraceptives – beyond the French controversy. *Gynecol Obstet Fertil* 2014; **42**(3): 174-181.

[14] Chen J, Jiang QD, Wu YM, Liu P, Yao JH, Lu Q, et al. Potential of essential oils as penetration enhancers for transdermal administration of ibuprofen to treat dysmenorrhoea. *Molecules* 2015; **20**(10): 18219-18236.

[15] Mirabi P, Alamolhoda SH, Esmaeilzadeh S, Mojab F. Effect of medicinal herbs on primary dysmenorrhoea – a systematic review. *Iran J Pharm Res* 2014; **13**(3): 757-767.

[16] Proctor M, Farquhar C, Stones W, He L, Zhu X, Brown J. Transcutaneous electrical nerve stimulation for primary dysmenorrhoea. *Cochrane Database Syst Rev* 2002; **1**: CD002123; http://dx.doi.org/10.1002/14651858.CD002123.

[17] Kusari P, Kusari S, Spiteller M, Kayser O. Endophytic fungi harbored in *Cannabis sativa* L.: diversity and potential as biocontrol agents against host plant – specific phytopathogens. *Fungal Divers* 2013; **60**(1): 137-151.

[18] Porras-Alfaro A, Bayman P. Hidden fungi, emergent properties: endophytes and microbiomes. *Annu Rev Phytopathol* 2011; **49**: 291-315; http://dx.doi.org/10.1146/annurev-phyto-080508-081831.

[19] Hubbard M, Germida JJ, Vujanovic V. Fungal endophytes enhance wheat heat and drought tolerance in terms of grain yield and second-generation seed viability. *J Appl Microbiol* 2014; **116**(1): 109-122.

[20] Li SB, Huang J, Zhou RC, Xu SR, Jin YX. Fungal endophytes of *Alpinia officinarum* rhizomes: insights on diversity and variation across growth years, growth sites, and the inner active chemical concentration. *PLoS One* 2014; **9**(12): e115289; http://dx.doi.org/10.1371/journal.pone.0115289.

[21] Daitetsu S, Kaoru K, Kiyotaka K, Kakahashi K. Aritiemet principles of *Alpinia officinarum*. *J Nat Prod* 2002; **65**(9): 1315-1318.

[22] Liu F, Xiong J, Huang GY, Wang W. Study on the underlying mechanism of acupuncture in regulating neuroendocrine activity in dysmenorrhea rats. *Zhen Ci Yan Jiu* 2009; **34**(1): 3-8.

[23] Yang YQ, Huang GY. Effect of acupuncture on Cx43 knock-out mice dysmenorrhea response. *Zhen Ci Yan Jiu* 2008; **33**(6): 366-371.

[24] Liu P, Duan JA, Hua YQ, Tang YP, Yao X, Su SL. Effects of xiang-fu-si-wu decoction and its main components for dysmenorrheal on uterus contraction. *J Ethnopharmacol* 2011; **133**(2): 591-597.

[25] Yang L, Cao Z, Yu B, Chai C. An *in vivo* mouse model of primary dysmenorrhea. *Exp Anim* 2015; **64**(3): 295-303.

[26] Altunyurt S, Göl MD, Altunyurt S, Sezer O, Demir N. Primary dysmenorrhea and uterine blood flow: a color Doppler study. *J Reprod Med* 2005; **50**: 251-255.

[27] Xie X, Gou WL. *Obstetrics and gynecology*. 8th ed. Beijing: People's Medical Publishing House; 2013.

[28] Chung D, Caruso RL. Potential role for oxidative stress in 2,2′-dichlorobiphenyl-induced inhibition of uterine contractions but not myometrial gap junctions. *Toxicol Sci* 2006; **93**(1): 172-179.

[29] Siemieniuch MJ, Woclawek-Potocka I, Deptula K, Okuda K, Skarzynski DJ. Effects of tumor necrosis factor-alpha and nitric oxide on prostaglandins secretion by the bovine oviduct differ in the isthmus and ampulla and depend on the phase of the estrous cycle. *Exp Biol Med (Maywood)* 2009; **234**(9): 1056-1066.

[30] Dawood MY, Khan-Dawood FS. Clinical efficacy and differential inhibition of menstrual fluid prostaglandin F2alpha in a randomized, double-blind, crossover treatment with placebo,

acetaminophen, and ibuprofen in primary dysmenorrhea. *Am J Obstet Gynecol* 2007; **196**(1). 35.e1–e5.

[31] Fenakel K, Lurie S. The use of calcium channel blockers in obstetrics and gynecology: a review. *Eur J Obstet Gynecol Reprod Biol* 1990; **37**(3): 199-203.

[32] Heidarifar R, Mehran N, Heidari A, Tehran HA, Koohbor M, Mansourabad MK. Effect of Dill (*Anethum graveolens*) on the severity of primary dysmenorrhea in compared with mefenamic acid: a randomized, double-blind trial. *J Res Med Sci* 2014; **19**(4): 326-330.