



The Effect of *Anastatica hierochuntica* (Fatimah Grass) on Oxytocin Levels and Uterine Contractions: an Experimental Study

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ABSTRACT

Background: One of the global targets of the Sustainable Development Goals (SDGs) in 2030 is to reduce maternal mortality rate (MMR) to below 70/100.000 live births and infant mortality rate (IMR) to 2/1000 live births. One of the factors that complicates childbirth is inadequate contractions, which is influenced by oxytocin. Oxytocin is a hormone secreted by neurohypophysis that causes uterine contractions during delivery. One of the traditional herbs that can be used to increase contractions is Fatimah grass (*Anastatica hierochuntica*). *A. hierochuntica* was used in Arabs, Indonesia, and Malaysia (63,9%) to shorten labor time, breastfeeding, and reduce postpartum hemorrhage by traditional.

Purpose: This purpose was to identify the potency of *A. hierochuntica* as an oxytocin stimulator by invitro and invivo study.

Methods: The method used a true experiment with a double-blind randomized controlled trial (RCT) and posttest design with a control group design of four groups (G1-G4). The research subject was 28 pregnant mice.

Results: The results of this research were *A. hierochuntica* has flavonoids, terpenoids, steroids, alkaloids, phenolic, tannin, and saponin secondary metabolites. *A. hireochuntica* have activity to increase the oxytocin hormone levels in pregnant mice (G1 averaged 49.79; G2 averaged 83.38; G3 averaged 91.05; and G4 averaged 101.39 pg/ml), increase the contraction of onset time (G2 averaged 17; G2 averaged 14.71; and G4 averaged 13.57 minutes) and shorten labor time.

Conclusion: The use of *A. hierochuntica* decoction significantly increases levels of the oxytocin and uterine contractions. The use of *A. hireochuntica* in delivery women as traditional herbs requires monitoring from health workers because of its strong ability to increase contractions, and must be done carefully with health worker recommendation.

Keywords: Fatimah grass; *Anastatica hierochuntica*; Oxytocin; Stimulator; Contraction

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BACKGROUND

Maternal mortality rate (MMR) and infant mortality rate (IMR) in the world are still relatively high, according to the World Health Organization (WHO), 810 moms worldwide pass away per day due to problems related to pregnancy and childbirth, accounting for 295.000 maternal deaths during pregnancy and childbirth, 94% of all maternal deaths occurring in low- and middle-income countries (Organization., 2019). Pre-eclampsia, eclampsia, infection, hemorrhage, and unsafe abortion are the primary problems that account for around 80% of maternal mortality (Say et al., 2014; WHO, 2014). MMR in Indonesia, according to SUPAS in 2015, decreased from 359 to 305/100.000 live births, but it has not succeeded in achieving the MDG target of 102 per 100.000 live births in 2015, which means that MMR in Indonesia is still three times higher than the MDGs target MMR in Indonesia (2019) was 4.221 maternal deaths, and pregnancy-related hypertension (1.066 instances), infection (207 cases), circulatory system diseases (200 cases), metabolic disorders (157 cases), bleeding (1.280 cases), and other causes (1.311 cases) were the leading causes of maternal death (Kassebaum et al., 2016; Kodan, Verschueren, van Roosmalen, et al., 2017).

One of the global targets of Sustainable Development Goals (SDGs) in 2030 is to reduce MMR to below 70 per 100.000 live births and IMR to 2 per 1000 live births (Kodan, Verschueren, Roosmalen, et al., 2017; Kodan, Verschueren, van Roosmalen, et al., 2017). WHO recommends good practices for delivering and delivering babies without complications in healthcare facilities by improving delivery quality (Baldisserotto et al., 2016; Surwade, 2020). Pregnant mothers will experience labor as a physiological and natural process, and it is a life-changing event, but can be disrupted by complex factors, which sometimes require clinical intervention (Irvani et al., 2015; NICE., 2017). The physiological process of pregnancy can become pathological if the care provided is not carried out properly and according to procedures. Maternal and fetal safety must be prioritized and anticipated in the event of complications in delivery, suddenly (Saleh & Machado, 2012). WHO states that the goal of providing childbirth care is so that mothers and children are born healthy, with as few interventions as possible (Kyu et al., 2018). Most of the deaths of mothers and newborns in the world can be avoided by providing quality health care, by providing delivery care (Kassebaum et al., 2016). Deliveries assisted by experts are the most crucial element in lowering maternal death, while one of the factors that complicates childbirth is inadequate contractions, which is influenced by oxytocin (Abate & Amanuel, 2012).

Oxytocin is a hormone secreted by neurohypophysis that specifically causes uterine contractions in delivery (Acevedo-Rodriguez et al., 2015). Hormones will cause inhibition if the membrane contains inhibitory receptors for the hormone rather than stimulating receptors, and oxytocin is very strong in stimulating the pregnant uterus,

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especially at the end of pregnancy, therefore this hormone is thought to be involved in baby delivery by several obstetricians (Guyton & Hall, 2006). The sensitivity and effectiveness of myometrial contractions to oxytocin are affected by estrogen (Lecomte et al., 2017). Deficiency of the hormones estrogen and oxytocin can lead to prolonged labor because oxytocin can increase inadequate contractions (Çalik et al., 2018; Thornton et al., 2020; Vakilian et al., 2018). One of the traditional herbs that can be used to increase contractions is Fatimah grass (*Anastatica hierochuntica*).

A. hierochuntica, the rose of Jericho is an herbal plant that is found in the Sahara desert, Saudi Arabia, and traditionally used in difficult labor (Algamdi et al., 2011a; Benyagoub et al., 2017; El Sayed et al., 2020; Norulaini et al., 2009). *A. hierochuntica* is used in Arab, Indonesia, and Malaysia (63,9%) to shorten labor time, breastfeeding, and reduce postpartum hemorrhage (Gechev et al., 2014; Tariq et al., 2018). With the presence of natural products, the bioavailability and absorption of active compounds can be increased due to an increase in the surface area of particles and solubility absorbed by the intestinal mucosa (Demetrovici, 2020; Situmorang et al., 2019). Based on the background, further research from *A. hierochuntica* as an alternative treatment of oxytocin receptor stimulator for labor contraction is needed. This research focused on identifying the potency of *A. hierochuntica* as an oxytocin stimulator by invitro and invivo study.

OBJECTIVE

This purpose was to identify the potency of *A. hierochuntica* as an oxytocin stimulator by invitro and invivo study.

METHODS

The material used is *A. hierochuntica* purchased from a local market in Mecca (Fig 1). The invivo study was given to the white Rat, *Rattus norvegicus*, because it is a commonly utilized experimental animal in medical and health research, reproduces more quickly, is easier to raise, calmer in nature, bigger, and more disease-resistant (Suckow et al., 2016). This research was conducted in the Molecular Genetics Clinical Laboratory, Padjadjaran University Hospital. The sample size was a total of 28 mice, with the Federer formula, which was divided into 4 groups (1 controlled group and 3 intervention groups with each doses).

$$(t-1)(r-1) \geq 15$$

$$(4-1)(r-1) \geq 15$$

$$3(r-1) \geq 15$$

$$r \geq 15+1$$

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3

$r \geq 6$

Calculation of sample size by estimating Drop Out at 10%:

$F = \frac{1}{(1-f)^r}$

$F = \frac{1}{(1-0,1)^6}$

$F = 6,67 = 7$

Note: r= number of groups

F= total sample

f=estimating drop out

So the number of samples per group consists of 7 mice, so that the total number of test animals is 7 in 4 groups, with total 28 mice.

This research method used a true experiment with a double-blind randomized controlled trial (RCT) and a post-test design with a control group design. The inclusion criteria in this research were healthy Wistar strain mice, 19 days pregnant, body weight 250-350 grams, not in serious illness. There were 4 groups, namely control group 1 (G1: controlled using aquadest); treatment group 1 (G2: intervention with 10 grams); group 2 (G3: intervention with 30 grams) and group 3 (G4: intervention with 50 grams). This aims to determine the best dose given by increasing the dose every 20 grams. Variable independents in this research were oxytocin hormones (V1), contraction onset time (V2), and baby mice health (V3). Oxytocin hormone was identified by Rat Oxytocin Elisa Kit 96 strip wells (ELK Biotechnology CO., Ltd; Denver, USA; NO. MBS263908, molecular weight 13.427 Da; species reactivity rat; samples rat serum, plasma, or cell culture supernatant; assay type sandwich; sensitivity 5pg/ml; ISO 9001:2015).

The analyzed data used an ANOVA one-way test with Bonferroni type multiple comparisons test (post hoc test) for V1 and V2, while the chi-square test for V3. This research was carried out after completing a code of ethics test by the Health Research Ethics Committee of the Pontianak Ministry of Health Polytechnic.

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RESULTS

Phytochemicals of *A. hierochuntica*

Based on phytochemical analysis, Fatimah grass (*A. hierochuntica*) has secondary metabolites as follows (Table 1):

Based on Table 1, *A. hierochuntica* has flavonoids, triterpenoids or steroids, alkaloids, phenolic, tannin, and saponin as its secondary metabolites.



Figure 1. Fatimah Grass (*A. hierochuntica*) Morphology (private documentation)

Table 1. Secondary Metabolites from *A. hierochuntica*

Secondary Metabolites	Chemical Reagents	Results
Flavonoids	Wilstater (concentrated HCl + Mg powder)	+
	H ₂ SO ₄ 2N	+
	NaOH 10%	+
Triterpenoids/steroids	Lieberman (concentrated H ₂ SO ₄ + acetic acid)	+/+
Alkaloids	Dragendorf	+
	Wagner	+
	Mayer	+
Phenolic	FeCl ₃ 5%	+
Tannin	FeCl ₃ 1%	+
Saponin	Bring to a boil stir and leave for 15 minutes	+

Oxytocin hormones

Fatimah grass (*A. hierochuntica*) has an activity of oxytocin hormone stimulator as follows (Table 2 and Figure 2):

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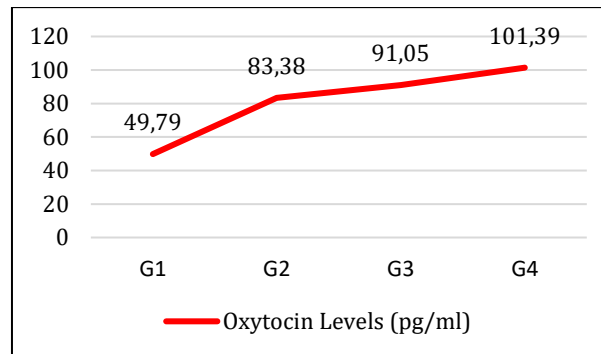


Figure 2. The Oxytocin Levels of G1-G4

Table 2. The Oxytocin Hormone Levels in Pregnant White Rats after Giving *A. hierochuntica* Soaking Water

Variables	Mean \pm SD (pg/ml)	Min-Max (pg/ml)
G1	49.79 \pm 23.79	27,78-71,79
G2	83.38 \pm 28.90	56,64-110,12
G3	91.05 \pm 9.95	81,84-100,25
G4	101.39 \pm 18.69	84,10-118,69

Note: G1 (controlled group 1); G2 (intervention group 1); G3 (intervention group 2); G4 (intervention group 3)

Based on Table 2 and Fig 2, the results showed that oxytocin levels in G1 (controlled using aquadest) averaged 49.79 (minimum 27.78 to maximum value 71.79 pg/ml), while G2 (intervention with 10 grams *A. hierochuntica*) averaged 83.38 (minimum 56.64 to maximum value 110.12 pg/ml), G3 (intervention with 30 grams *A. hierochuntica*) averaged 91.05 (minimum 81.84 to maximum value 100.25 pg/ml), and G4 (intervention with 50 grams *A. hierochuntica*) averaged 101.39 (minimum 84.10 to maximum value 118.69 pg/ml). This data is normally distributed, and bivariate analysis was used one-way ANOVA.

Based on Table 3, the *P* value between those 4 groups was $0.001 < 0.05$, so there was a difference between *Fatimah* grass-soaked water on the levels of the hormone oxytocin in pregnant mice in the treatment group.

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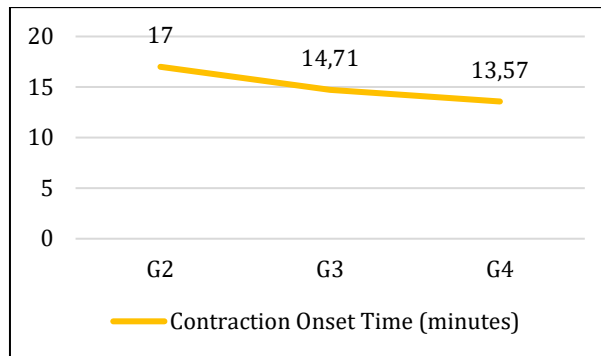


Figure 3. The Contractions Onset Time of G1-G4

Table 3. ANOVA Test and Multiple Comparisons Oxytocin Hormone Levels with Post Host Test (Bonferroni)

Treatment Type	Treatment Type	Mean Diff	Lower Bound	Upper Bound	Sig
G1	G2	-33,59	-66,65	-0,53	0,001
	G3	-41,26	-74,31	-8,20	
	G4	-51,60	-84,66	-18,55	
G2	G1	33,59	0,53	66,65	
	G3	-7,66	-40,72	25,38	
	G4	-18,01	-51,07	15,04	
G3	G1	41,26	8,20	74,31	
	G2	7,66	-25,38	40,72	
	G4	-10,34	-43,40	22,71	
G4	G1	51,60	18,55	84,66	
	G2	18,01	-15,04	51,07	
	G3	10,34	-22,71	43,40	

Contraction onset time

Fatimah grass (*A. hierochuntica*) has an activity of contraction onset time stimulator as follows (Table 4 and Figure 3):

Table 4. Onset Time of Contractions in Pregnant Rats after Giving *A. hierochuntica* Soaked Water

Variables	Mean ± SD (pg/ml)	Min-Max (pg/ml)
G1	NM	NM

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G2	17,00 ± 1,82	15,31-18,68
G3	14,71 ± 3,45	11,52-17,90
G4	13,57 ± 2,63	11,13-16,01

Note: G1 (controlled group 1); G2 (intervention group 1); G3 (intervention group 3); G4 (intervention group 3); NM (cannot be measured)

Based on Table 4 and Fig 3, the results show that the contraction onset time in the control group not measured, treatment group 1 averaged 17 (minimum 15.31 to maximum 18.68 minutes), treatment group 2 averaged 14.71 (minimum 11.52 to maximum 17.90 minutes), and treatment group 3 averaged 13.57 minutes (minimum 11.13 to maximum 16.01 minutes). This data is not normally distributed using Shapiro-Wilk, and bivariate analysis was used one-way ANOVA with Games-Howell.

Based on Table 5, it was shown that the P value was $0.000 < 0.05$, so there was an effect of giving Fatimah grass soaking on the onset time of contractions.

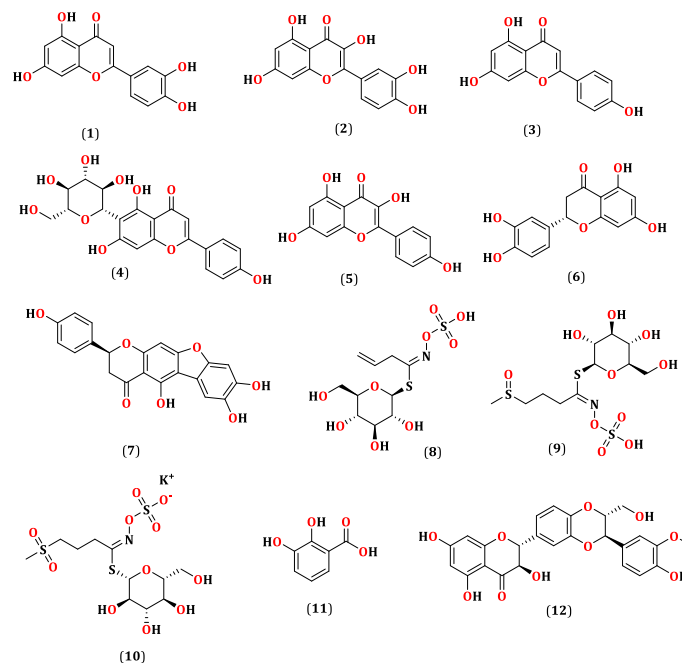


Figure 4. The Chemical Compounds of Fatimah Grass (*A. hierochuntica*)

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Table 5. ANOVA Test and Multiple Comparisons Contraction Onset Time with Post Host Test (Games-Howell)

Treatment Type	Treatment Type	Mean Diff	Lower Bound	Upper Bound	Sig
G1	G2	-17,00	-19,38	-14,61	0,000
	G3	-14,71	-19,22	-10,19	
	G4	-13,57	-17,02	-10,12	
G2	G1	17,00	14,61	19,38	
	G3	2,28	-2,30	6,87	
	G4	3,42	-0,23	7,09	
G3	G1	14,71	10,19	19,22	
	G2	-2,28	-6,87	2,30	
	G4	1,14	-3,78	6,06	
G4	G1	13,57	10,12	17,02	
	G2	-3,42	-7,09	0,23	
	G3	-1,14	-6,06	3,78	

Baby mice health

The condition of baby mice was identified after their birth, as follows (Table 6):

Table 6. The Health Condition of Baby Mice

Variable	Live Birth	Stillbirth	Chi-square
G1	7	0	a
G2	7	0	
G3	7	0	
G4	7	0	
Total	28	0	

Based on Table 6, the results obtained show that the chi-square test in the condition of baby mice cannot be analyzed because all baby mice were born safely and make the results constant.

DISCUSSION

Most of the natural substances utilized in both conventional and modern medicine are derived from plants (Calixto, 2000). Approximately 80% of people on the planet use

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herbal medicines for both normal medical care and therapy, particularly in underdeveloped nations (Kamboj, 2000; Mohankumar et al., 2017). Within the *Brassicaceae* family, *A. hierochuntica* is a monotypic species (Rameshbabu et al., 2020) and is highly valued for its many benefits in the management of various diseases (Al-Khafaji, 2018), such as high blood pressure, gastrointestinal disorders, depression, headaches, fever, infertility, heart disease, epilepsy, and malaria, inflammation, arthritis, pain, diabetes, and considered for fewer side effects (Alatshan et al., 2018; Hegazy & Kabiell, 2007; Jigna et al., 2005).

Fatimah grass (*A. hierochuntica*) is a small gray plant with white pads and flowers, and very hard seeds (Shaban et al., 2011). When dried, *A. hierochuntica* will become like a wooden sphere that will grow and stand back up when immersed in water (Shaban et al., 2011; Siti Rosmani.Md Zin et al., 2018; Siti Rosmani MD Zin et al., 2017). If estrogen increases, either in mice or humans, the connective signal in uterine muscle cells will form electrically slit linkages, which function to hold the uterine smooth muscle cells together, so that contractions become coordinated and uterine responsiveness will trigger labor and proliferative to facilitate labor (Astutik et al., 2020; Lecomte et al., 2017). *A. hierochuntica* also contains lots of Mg, Ca, Mn, phenolic compounds, flavonoids, alkaloids, turbinones, calcosides, alfalfa, about 15% soap and essential oils, <0.5% essential oils, terpenoids, phenolic, tannins, saponins, and has potential antioxidant and free radical scavenger activities (Al-sultany et al., 2019; Faten et al., 2016) Aluminum, potassium, Zn, Fe, carbon, oxygen, silica, calcium, and magnesium (Siti Rosmani MD Zin et al., 2017), Mn, Co, Cu (Kim Sooi & Lean Keng, 2013). *A. hierochuntica* also contains luteolin (1), quercetin (2), apigenin (3), isovitexin (4), kaempferol (5), eriodictyol (6), anastatin (7), glucosinolate (8), glucoiberin (9), glucocheirolin (10), terpenes, caffeoyl acid, dicaffeoylquinic acid, dihydroxybenzoic acid (11), silybin (12), isosilybin, and hierochins (Figure 4) (Abdulfattah, 2013; Algamdi et al., 2011b; Yoshikawa et al., 2003).

Based on Table 1, one of the secondary metabolites was flavonoids, which are naturally occurring substances with an estradiol-like structure and estrogenic activity, are present in *A. hierochuntica*. Elevated estrogen stimulates the uterine smooth muscle cells' connecton signaling. The resulting connections are injected into the myometrial plasma membrane to create gap junctions, which electrically hold the uterine smooth muscle cells together and enable coordinated contractions. Labor is eventually sparked by the uterus's

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increased receptivity to oxytocin as a result of these myometrial alterations. Additionally, High amounts of estrogen stimulate prostaglandin synthesis, which activates the enzymes involved in cervical ripening that break down collagen fibers locally in the cervical tissue (Campbell et al., 2010). Because it includes minerals and phytoestrogens, Fatimah grass is believed to expedite labor. Estrogen stimulates the body's proliferative processes, which can lead to an increase in the number of oxytocin receptors and myometrial cells. Thus, it might increase the myometrium's oxytocin sensitivity and the force of myometrial contractions (Ali et al., 2013).

There are three layers in the uterus, the outermost layer is called serosal, the thick muscular is called myometrium, and the endometrium has a well-differentiated layer (Aguilar & Mitchell, 2010). High levels of estrogen induce connection signals in the uterine smooth muscle cells, when the uterine contractions are formed due to a coordinated connection of the myometrial plasma membrane and uterine smooth muscle cells, labor will occur if there is an increase in myometrial responsiveness to oxytocin, an increase in levels of the hormones estrogen and prostaglandins, so that the cervix becomes thinner and flattens (Campbell et al., 2010). Oxytocin is a peptide hormone and neuropeptide (Standring, 2016; Yang et al., 2013) from nine amino acids that are produced in the supraoptic and paraventricular hypothalamus by magnocellular neurons, and its purpose is to trigger contractions (Chatterjee et al., 2016; Ross et al., 2009); disulfide ring of 6 amino acids and tail with 3 members (Sarli, 2017). The mechanism of action of oxytocin to prevent uterine bleeding is that oxytocin is a nine-amino acid peptide that is synthesized in the hypothalamic nerve and flows to the axons of the posterior pituitary to be secreted into the blood. Oxytocin is also secreted into the brain and from several tissues, and functions to stimulate uterine muscle contractions (Sarli, 2017; Stanton et al., 2013). Oxytocin stimulates uterine contractions through Ca^{2+} dependent and Ca^{2+} independent mechanisms. The Ca^{2+} independent pathway is via the Rho kinase pathway (Sawada et al., 2002). Based on that, intervention with a soaked water of Fatimah grass (*A. hierochuntica*) has the result of the body's oxytocin hormone levels rising, so that by rising oxytocin levels in the body, it will increase the quantity of contractions in pregnant mice, which will give birth on days 19-22.

Rats are mammals, which when they are in labor, are similar to human labor. This physiological process can turn pathological if it is not handled properly. Complications

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in childbirth often appear suddenly and this must be anticipated to ensure the safety of the mother and fetus. The first stage of labor is said to be prolonged if it lasts more than 20 hours in primigravida and 14 hours in multigravida. The cause of the prolonged first stage is the condition of the contraction, the condition of the birth canal, the condition of the fetus, which is often found in the long first stage, namely contraction abnormalities. Inefficient or adequate contraction will result in placental vasoconstriction, so that the supply of O₂ to the fetus will be reduced and the development and growth of the fetus in the uterus will experience abnormalities, and the fetus will experience distress so that its well-being will be disturbed. So the use of *A. hierochuntica* decoction, which can increase oxytocin levels in the body, will specifically cause uterine contractions so that you can give birth to a baby. Fatimah grass contains flavonoid phytochemicals which are natural ingredients with a structure similar to estradiol and show estrogenic activity. The function of the hormone oxytocin is very strong in stimulating the pregnant uterus, especially at the end of pregnancy. Therefore, the use of Fatimah grass is limited to prolonged labour. The use of *A. hierochuntica* during prolonged labor will cause changes in the myometrium, thereby increasing the responsiveness of the uterus to oxytocin and the effectiveness of myometrial contractions, which ultimately trigger labor that was previously inadequate.

CONCLUSION

Fatimah grass (*A. hierochuntica*) has been utilized for a long time in traditional medicine, it has the activity of stimulating the hormone oxytocin in pregnant mice, thus increasing the onset time of contractions which results in faster labor due to the effects of its secondary metabolites. Because of its excellent function in increasing oxytocin levels and uterine contraction in the body, the use of *A. hierochuntica* is better used in pathological labor such as long labor. The use of *A. hierochuntica* in delivery women requires monitoring from health workers because of its strong ability to increase contractions, and must be done carefully with the health worker's recommendation

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