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The Effect of *Anastatica hierochuntica* L. Extract on the Histology of Myometrial Cells and Prostaglandin Levels (PGE2, PGF2α) in Pregnant Mice

Heny Astutik[1,*], Budi Santoso[2], Mangestuti Agil[3]

[1]*Department of Midwifery, Poltekkes Kemenkes Malang, Malang, Indonesia*
[2]*Department of Medicine Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia*
[3]*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia*
[*]*Corresponding author. Email: henyast21@gmail.com*

ABSTRACT

Anastatica hierochuntica L. is well known as a traditional medicine which brings benefit to the final trimester of pregnancy by increase uterine contractions. This study aimed to analyze the effect of *A. hierochuntica* L. extract on the histology of myometrial cells and prostaglandin levels (PGE2 and PGF2α) of pregnant mice. The mice were divided into three groups: (1) control group, treated by 0.5% CMC solution 10 ml/kg bw/day; (2) two treatment groups that treated by *A. hierochuntica* L. extract with 100 and 150 mg/kg bw/day doses, respectively. Uterine tissue was collected for histological analysis and blood serum was collected to examine the PGE2 and PGF2α levels. It obtained that myometrial hypertrophy increased significantly in the treatment groups ($p = 0.001$) than in the control group. Moreover, there were significant differences in the PGE2 levels ($p = 0.001$) and PGF2α levels ($p = 0.000$) between the control group and the treatment groups. The 100 mg/kg bw/day dose had a greater effect on myometrial hypertrophy than 150 mg/kg bw/day, which resulted in a higher increase of PGE2 and PGF2α levels. Hence, it was concluded that the 100 mg/kg bw/day dose of *A. hierochuntica* L. extract was the appropriate dose for increasing prostaglandin levels.

Keywords: Anastatica hierochuntica L., *myometrial cells, prostaglandins, pregnant mice*

1. INTRODUCTION

Nowadays, advanced technology and medical science still utilizing plant-based medicines [1]. Herbal medicine is considered safe since they are derived from natural products and has fewer side effects. In brief, herbal medicines are mostly utilized in developing countries for routine health care [2] and treatment of gynecological health issues, including Indonesia [3]. *A.hierochuntica* L. is well known as a traditional medicine and in Indonesia, it has been extensively consumed by expectant mothers during the final trimester of their pregnancy to increase uterine contractions to initiate labor. Indonesian pregnant women used to drink the soaking water of *A. hierochuntica* L [4]. Nevertheless, there is no much research about the biological activity of this plant.

A. hierochuntica L., also known as Rose of Jericho, Fatimah grip, [5] Kaff Maryam—or in Indonesia—as Rumput Fatimah, is found in the Sahara-Arab desert where it is widely consumed as an herbal tea drink [6]. It was claimed to be very useful in the antepartum treatment [6] and has been used as a traditional medicine during the end of pregnancy, since it is popularly believed to facilitate the delivery process, [5] reduces uterine bleeding, [7] and treats various health problems [2][6][8][9]. Based on pharmacology studies, *A. hierochuntica* L. contains phenolic substances (51.97 mg/g d.w), flavonoids (42.53 to 46.28 mg/g d.w)10, sterol11, and several minerals including Mg, Ca, Cr, Mn, Fe, Co, Cu and Zn.9 *A. hierochuntica* L. is considered a phytoestrogen plant, with estrogenic activity, due to the presence of flavonoids and another compound.9,11-14 Phytoestrogen—a member of the polyphenols family—is derived from herbal and it functions similar to estrogen in the human reproductive system [15].

A study by Nani [4] resulted that drinking the soaking water containing a dose of 40 grams of *A. hierochuntica* L. increased the frequency of uterine contractions [4]. However, until now, the mechanism that regulates myometrial contraction activity during pregnancy, labor, and birth has not been fully observed [16]. Several studies have obtained an overview of the estrogenic effect of phytoestrogens [17]. The uterine tissue undergoes changes in histological structure that are dynamic based on estrogen levels [18]. Estrogen also increases a series of myometrial changes, including elevated production of the prostaglandins, PGE2 and PGF2α, with a high expression of prostaglandin receptors [19].

Prostaglandins, especially those produced in intrauterine tissue, play a central role in the initiation and development of labor, including myometrial contraction stimulation, metabolism management of the extracellular matrix related to cervical maturation, and upregulation of the fetal HPA-

axis [20]. Prostaglandin (PGs), a uterotonic agent [21] and a stimulator of uterine contractions [3] is synthesized from arachidonic acid by cyclooxygenases and prostanoid synthases [22]. Prostaglandins act by binding to certain G protein-coupled receptors [23] thereby activating intra-cellular signals and gene transcription [24].

In addition to having estrogenic activity, *A. hierochuntica* L. also has gastroprotective activity, [13] hepatoprotective activity, [24] antioxidants activity [9]. Several studies have been conducted to identify the various activities of this plant, but only a few studies are available regarding the histological effects of *A. hierochuntica* L. and its activity during labor. Ethnobotany research aims to investigate and report the herbs for gynecological health problems and their uterotonic activities in several countries, including Indonesia [3]. However, studies related to the biological activity of *A. hierochuntica* L. during labor have not been reported. Therefore, this study aimed to evaluate the effect that *A. hierochuntica* L. has on histological changes in myometrial cells and prostaglandin levels (PGE2, PGF2a) in pregnant mice. Understanding the histological effects of this herb is essential since, in many cases, there is no scientific evidence on the use of *A. hierochuntica* L. plants as a facilitator of labor during childbirth.

2. METHOD

The material used in this study is the whole *A. hierochuntica* L. was obtained from the local market of Saudi Arabia. This plant determined by plant taxonomists from the Indonesian Institute of Science (LIPI), Plant Conservation Center of Bogor and it has also been identified using the liquid chromatography-mass spectrometry (LCMS) method.

The plant was extracted with 96% ethanol using maceration method. The sample was sonicated in an ultrasonic bath for 10 minutes, then filtered, using a Burger funnel which was connected to a vacuum pump to obtain residue and solution. The solution obtained from each filtration step was evaporated using a rotary evaporator at 50 ºC. Furthermore, the extract was weighed and stored in a closed bottle at 4 ºC.

The research protocol for this study was approved by the local ethics committee. Healthy adult female mice (*Mus musculus* strain Balb C), 12–14 weeks old and 25–35 grams in weight, were used for this research. The mice were acclimated for a week then their estrus period was synchronized using 5 IU 0.1 mL of Prostaglandin and 5 IU 0.1 mL of HCG. Afterward, the female mice were placed with fertile male mice, 1:1 (monogamy), for breeding.

All pregnant mice were divided into three groups—the control group (P0) and two treatment groups (P1 and P2). Each group consisted of seven random mice. Treatment was treated on day 14 to 18 of pregnancy. Mice within the control group were treated by 0.5% CMC Na solution 10 mL/kg bw/day for 5 days. Mice belonged to P1 and P2 treatment groups were treated by *A. hierochuntica* L. extract, 100 and a 150 mg/kg bw/day dose of, respectively, for 5 days. On the 19th day, mice were dislocated for the preparation of uterine organ tissue and blood serum collection.

The fetus was removed from the uterus, then one side of the uterine cornu was excised, fixed in a 10% formalin buffer solution for 24 hours, and planted in paraffin. The tissue was stained with Hematoxylin-Eosin (HE staining) and the staining results were observed using a Nikon H600L microscope with a DS Fi2 300 megapixel of a digital camera. The Examination of prostaglandin levels, PGE2 and PGF2α, were measured in intracardiac blood serum of pregnant mice using a sandwich ELISA kit.

All the myometrial cell histology for each sample was assessed semiquantitatively according to the modified Billingham method [25]. The myometrial cell histology was measured within a 0–5.5 score based on histological changes, which was a percentage of hypertrophic myocytes of all observed myocytes. The hypertrophic cells were determined based on the size or diameter of myocyte cells, which more than 6 μm.

The data were analyzed using the Kruskal Wallis test to identify the differences in myometrial histology. Moreover, a one-way ANOVA was conducted to analyze the prostaglandin level ($p < 0.05$). Then, the post-hoc test with LSD was used to determine the differences between the treatments of each group.

3. RESULTS AND DISCUSSION

Myometrium is one of the essential reproductive organs that regulate contractions. This regulation is controlled by estrogen and progesterone.[26] Estrogen has a direct or indirect ability to change the myometrium, as it increases the capacity of contraction power in myometrial cell hypertrophy[27], the contractile potential of myometrium cells, uterotonic receptors, and cell to cell communication [21][28].

Table 1 shows the results of the microscopic examination of myometrium obtained from the uterine tissue of pregnant mice. This identification aimed to determine the hypertrophic degree of myometrial myocytes associated with an estrogenic activity of *A.hierochuntica* L. extract in pregnant mice. It was also expected to have an effect on prostaglandin production (PGF2a and PGE2). The alteration of myometrial cells is related to the administration of *A. hierochuntica* L. extract, as presented in Figure 1.

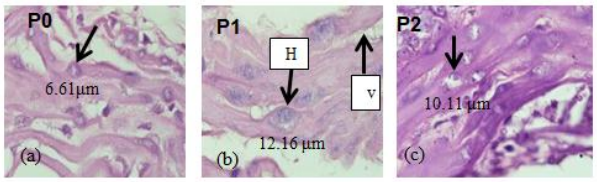


Figure 1. The size difference in the nucleus of hypertrophic myocyte cells (arrows) between treatment groups (μm): (a) Myometrial tissue of the control group (P0), (b) Myometrial tissue of the treated groups (P1) (H= hypertrophy; V= vacuolization), and (c) P2. (M=400x; Nikon microscope 600l; camera ds fi2 300 megapixels).

The histological analysis of myometrium cells of pregnant mice treated with 100 mg/kg bw/day of *A.hierochuntica* L. extract showed that there was an increasing number of hypertrophy cells (H) with larger myocyte cell diameter compared to the control group and the treatment group treated 150 mg/kg/day of the extract (P2) (Figure 1).

Table 1. Microscopic Changes within Myometrial Myocytes Cells in Pregnant Mice Treated Different Doses of *Anastatica hierochuntica* L. (Ah) Extract

Group	Extract dose/kgbw	A hypertrophic score of myocyte cells	Number of hypertrophic myocyte cells
Group 1 (P0)	0 mg	0.71	The average number of hypertrophic myocyte cells was < 5% of observed myocyte cells
Group 2 (P1)	100 mg	3.43	The average number of hypertrophic myocyte cells was 36–50% of observed myocyte cells, whilst some of them had the vacuolization
Group 3 (P2)	150 mg	2.79	The average number of hypertrophic myocyte cells was 36–50% of observed myocyte cells

* Group 1 = control, pregnant mice were treated 0.5% CMC solution, Group 2 = Pregnant mice treated with AH extract dose of 100mg/kg bw/day, Group 3 = Pregnant mice treated with AH extract dose of 150mg/kg bw/day.

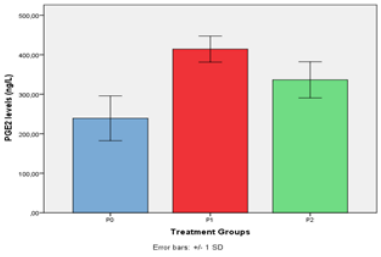
Table 1 shows that the average number of hypertrophic myocyte cells in the control group was < 5% of all observed myocytes cells, while in the pregnant mice group treated with *A. hierochuntica* L. (AH) extract, the number of hypertrophic myocyte cells increased up 36–50% both in the P1 and P2 groups—and some of the cells underwent vacuolization. The difference in the hypertrophic scores of the myometrial cells between the three groups of pregnant mice is shown in Table 2.

Table 2. The Results of the Hypertrophic Score of Myometrial Myocytes Cells in the Treatment Groups Treated Different Doses of *Anastatica hierochuntica* L. Extract.

Group	n	The hypertrophic score of myometrial myocytes cells				*p*-value
		x rank	SD	Min	Max	
P0	7	4.57[a]	1.45324	0.00	2.50	
P1	7	16.07[b]		3.00	4.50	0.001*
P2	7	12.36[b]		2.00	4.50	

Note: *significance α = 0.05 (Kruskal Wallis)
[a,b] It showed differences among the group (Mann-Whitney test)

There was a significant difference in the hypertrophy of myometrial myocyte cells (p < 0.05) (Table 2) between the control group compared to the treatment groups treated a 100 mg/kg bw/day and 150 mg/kgbw/day dose of *Anastatica hierochuntica* L. extract. However, there was no significant difference between the treatment groups in the hypertrophy of myocyte cells.
The average PGE2 level within the control group treated 0.5% CMC and the treatment group treated with *A. hierochuntica* L. extract presented in Figure 2.



P0[a] = Control group CMC 0.5%
P1[c] = Treatment group treated an AH dose of 100 mg/kg body weight/day
P2[b] = Treatment group treated an AH dose of 150 mg/kg body weight/day
[a,b,c] superscript showed difference among groups (*multiple comparisons* LSD)

Figure 2 The PGE2 level among groups.

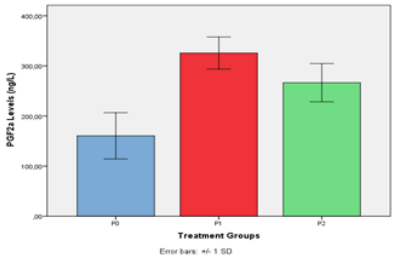
Figure 2 shows that the PGE2 levels in group P1 (100 mg/kg bw/day) increased 1.7× (X1 = 414.36) and in group P2 (150 mg/kg bw/day) 1.4× (X2 = 336.50) higher than the control group (X0 = 239.14). Group P1 also had PGE2 levels that were 1.2× higher than group P2. Furthermore, Table 3 presents the results of the one-way ANOVA test that was performed, with α = 0.05.

Table 3. ANOVA Test of PGE2 Level among the Groups.

Groups	n	PGE2 level				*p*-value
		x	SD	Min	Max	
P0	7	239.14[a]	56.76	177.00	343.50	
P1	7	414.36[c]	32.99	388.00	479.00	0.000*
P2	7	336.50[b]	45.83	266.00	402.50	

Note: *significance α=0.05 (*one-way ANOVA*)
[a,b,c] superscript showed difference among group (*multiple comparisons* LSD)

The results displayed in Table 3 presented that *Anastatica hierochuntica* L. treatment affects the PGE2 levels within pregnant mice. The significance level of PGE2 levels among the control group, group P1 (100 mg/kg bw/day), and group P2 (150 mg/kg bw/day) (P2), through one-way ANOVA statistical analysis, showed a significance level with p = 0.000 (p < 0.05). This result means there were significant differences in the levels of PGE2 between the control group and the treatment group. The results of the post-hoc tests, multiple comparisons of LSD, showed that there was a significant difference in the levels of PGE2 between the control group and group P1, the control group and group P2, and between the P1 and P2 groups.
The average level of PGF2α between the control group, group P1, and group P2 is presented in Figure 3.



P0[a] = Control group CMC 0.5%
P1[c] = Treatment groups treated an AH dose of 100 mg/kg body weight/day
P2[b] = Treatment groups treated an AH dose of 150 mg/kg body weight/day
[a,b,c] superscript showed difference among groups (*multiple comparisons* LSD)

Figure 3. Level of PGF2α among groups.

Figure 3 shows that the average level of PGF2α within-group P1 (100 mg/kgb w/day) and group P2 (150 mg/kg bw/day) increased 2× (X1 = 325.76) and 1.6× (X2 = 266.57), respectively, and both groups had higher levels than the control group (X0 = 160.54). Whilst, the levels in group P1 was 1.2× higher than group P2. Furthermore, Table 4 shows the results of the one-way ANOVA (α = 0.05).

Table 4. ANOVA Test of PGF2α Levels among Groups.

Group	n	PGF2α level				*p*-value
		$\bar{x}$	SD	Min	Max	
P0	7	160.54[a]	46.22	99.70	228.00	
P1	7	325.76[c]	31.95	298.70	391.00	0.000*
P2	7	266.57[b]	38.17	206.00	301.70	

Note: *significance α=0.05 (one-way ANOVA)
[a,b,c] superscript showed difference among groups (multiple comparisons LSD)

The results in Table 4 demonstrated *A. hierochuntica* L. did have an effect on the PGF2α within pregnant mice. The level of significance of the difference in PGF2α levels between the control group and the *A.hierochuntica* L. extract treatment group was p = 0.000 (p < 0.05), demonstrating that there were significant differences of the levels of PGF2α between the control group and the treatment group. The results of the post-hoc tests, multiple comparisons of LSD, demonstrated that there were significant differences in PGF2α levels between the control group and the treatment group, and between treatment groups (P1 and P2).

Hypertrophy of myometrial myocyte cells occurs physiologically within pregnancy. It is associated with increased myometrial growth during late pregnancy. Smooth muscle cell hypertrophy is caused by the mechanical stretch of uterine tissue due to fetal growth [27]. However, the estrogen hormone also has an essential role within the growth of reproductive tissue cells, including the myometrium.

As explained in the previous studies, estrogen stimulation can cause hypertrophy at the end of pregnancy. Several potential mechanisms that regulate myometrial hypertrophy have been proposed within the literature. It has been reported that ovarian hormones, estrogen, and progesterone, could induce myometrial hypertrophy [27]. Phytochemical test results, using the LCMS method, presented that *A. hierochuntica* L. contained flavonoid compounds. Therefore, it is considered to be a phytoestrogen, which is structurally and functionally similar to estradiol, [15][29] with estrogenic function [30].

The administration of *A. hierochuntica* L. affects the increased number of hypertrophic myometrial myocytes cells. The results showed that *A. hierochuntica* L. extract increased hypertrophic myometrial myocyte cells in pregnant mice, which was characterized by a bigger cell size (7 μm–12 μm) compare to the control group (4 μm–6 μm). Moreover, it also increased the number of hypertrophic myocyte cells 36–50% of all observed myocytes. Based on these results, it obtained that the *A. hierochuntica* L. plant has the potential to induce myometrial cell hypertrophy at the end period of pregnancy. This change in process is normal since, physiologically, uterine tissue undergoes dynamic changes in the histological structure based on estrogen hormone levels [18]. Based on the study of Gaete *et al.*, the administration of Genistein (0.5 mg/kg body weight, subcutaneously) causes myometrial cell hypertrophy and uterine luminal epithelium as estradiol [31]. During hypertrophy, the contractile element enlarges and the extracellular matrix expands for growth [32].

The administration of *Anastatica hierochuntica* L. extract also increased the mean PGE2 and PGF2α levels within the blood serum of pregnant mice. The administration of 100 mg/kgbw/day of *Anastatica hierochuntica* L. extract increased the levels of PGE2 and PGF2α by 1.7 and 2×, respectively, compared to the control group. The results following the previous theory that estrogen and progesterone involve in regulating uterine contractions. The membrane is depolarized, increasing the prostaglandin production of PGE2, PGF2α, and oxytocin receptors.[33] Previous research stated that prostaglandin F2α increased in the proestrus phase of the rat cycle when estrogen levels were maximum.

This result proved that estradiol-17β caused an increase in prostaglandin F2α levels in uterine ovariectomized rats. Estrogen acts to control prostaglandin synthesis by regulating the prostaglandin synthetase complex, as it produces a pattern change in the ratio of prostaglandin F to prostaglandin E [34].

The *Anastatica hierochuntica* L. treatment presented a significant difference in PGE2 levels (Table 3) and PGF2α levels (Table 4). These results indicated that the 100 mg/kgbw/day dose was more effective to induce the PGE2 and PGF2α levels within pregnant mice. Increased levels of prostaglandin are expected to be related to cell hypertrophy and the stretching process (Mechanical Stretch) that has an impact on NFkB activation [35]. The NFkB activation process will trigger prostaglandin production via the Cox-2 pathway [36]. The results in this study showed that the administration of *Anastatica hierochuntica* L. extracts at doses of 100 mg/kg bw/day and 150 mg/kgbw/day increased prostaglandin levels compared to the control group—according to the increase of myometrial cell hypertrophy in the group. The association of an increase in prostaglandins, with myometrial cell hypertrophy, needs further analysis.

In the process of labor, prostaglandin has three actions, myometrial contraction stimulation, cervical softening, and gap junction induction [33][37]. Prostaglandin acts by binding to certain G-protein-coupled receptors, thereby activating intra-cellular signals and gene transcription [24]. Prostaglandins (PGs) invove in the initiation and maintenance of labor by acting through specific relaxation or contractile receptors in the myometrium. This study obtained that *A. hierochuntica* L. is an herbal plant that has a role in the reproductive system due to its potential to increase the levels of prostaglandins that are important in pregnancy and childbirth.

4. CONCLUSION

A. hierochuntica L. plant extract had a greater effect on the histology of myometrial cells in pregnant mice and prostaglandin levels in both PGE2 and PGF2α on the administration of a 100 mg/kg bw/day oral dose compared to the 150 mg/kg/day dose. These results provide new scientific evidence for the activity of *Anastatica hierochuntica* L. as a facilitator of labor during childbirth and further research on this plant is needed to evaluate its activity on contractile proteins.

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