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PEGAGAN (*Centella asiatica*) EXTRACT INCREASES VAGINAL WALL THICKNESS IN MENOPAUSAL RATS

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ABSTRACT

Painful intercourse (dyspareunia) is one of the complaints that are often found in menopausal women due to decreased kolagen.dan atrophy vaginal wall. This can lead to domestic disharmony because it can lead to divorce, affairs and domestic violence. Menopausal women growing more and more year, because of the higher life expectancy. To prevent these complaints need estrogen therapy or phytoestrogens. Centella asiatica is a phytoestrogen that can increase proliferation and collagen synthesis, is expected to occur with the administration of Centella asiatica collagen thickening and increase in vaginal wall so that the vagina becomes more elastic and repair cells that atrophy so painful intercourse can be avoided. Mechanism of thickening and increased collagen vaginal wall has not been clear. This study aims to explain the mechanism of thickening due to proliferation and maturation of epithelial and collagen synthesis increased vaginal wall Rattus norvegicus strain Wistar rats that experienced a given ovariectomy Indian pennywort extract. The research method is experimental design with posttest only control group design, by 35 female rats of Wistar strain Rattus norvegicus age of 4 months, weight 290-300 grams, conducted randomized into 4 groups, each group of seven rats. All groups performed ovariectomy. Preliminary research to determine the atrophy of the vaginal wall (on day 21). Group 1, not given the extract of Centella asiatica. Group 2, 3, 4 were given extracts of Centella asiatica on day 22 post-ovariectomy with a dose of 30mg, 60mg, 120 mg/Kbw/day for 40 days orally. Immunohistochemical examination to see the expression of estrogen receptor beta-producing cells (ER-ß) and collagen-producing cells. Histological examination to see thickening of the vaginal wall with Hematoxylin eosin staining. Results were analyzed using univariate analysis showed an increase in epithelial proliferation, maturation of the vaginal wall was significant (p <0.05), increased estrogen receptor beta and collagen was significantly (p, 0.05). Conclusion: It can be concluded that administration of Centella asiatica extract can increase epithelial proliferation of the vaginal wall through the mechanism of increased estrogen receptor beta, and collagen

Keywords: *Centella asiatica, vaginal epithelial proliferation, collagen synthesis, phytoestrogen*

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INTRODUCTION

Painful intercourse (dyspareunia) is one of the complaints that are often encountered in menopausal women because the hormone estrogen decreases. Ovarian hormones estrogen decreases because it is not functioning optimally. These conditions resulted in resulted in the vaginal wall thinning, dry and less elastic (vaginal dryness) because there is a process of epithelial atrophy and a decrease in collagen (Cuozi et al 1995, Baziad 2003, Al-Baghdadi and Ewies 2009). Complaints menopause can be reduced by a lot and regularly eat vegetables and fruits that contain phytoestrogens (estrogen from herbs) such as peanuts, soybeans, tofu, tempeh, yam and fruit wines. Food as above only can reduce hot flashes (hot flushes), coronary heart disease and osteoporosis, but does not affect the urogenital complaints such as painful intercourse. Research on Japanese women are menopausal and often consume fruit and vegetables, such as peanut, soy, and tofu, proven to reduce complaints of hot flushes (Davis 2001), but has not proven to affect the elasticity of the vaginal repair (Noerpramana 2005). Similarly, the meta-analysis study with the use of other phytoestrogens such as Black cohosh, Red Clover only help in the problem of hot flushes, night sweats, heart health and slow the loss of bone mass, but there is no evidence that an effect on the improvement of dyspareunia complaints (Noerpramana 2005) .

From year to year life expectancy of women in Indonesia is increasing (Samil 1986) because of better health facilities. It is estimated that in 2020, the number of menopausal women will increase by 30 million people or 11.5% of the total population of Indonesia.

Based on these data the incidence of risk will also increase vaginal atrophy (Baziad 2003, Fadhilah 2005, Al-Baghdadi and Ewies 2009). According to research on the incidence of vaginal dryness during perimenopause as much as 3%, whereas the complaint in the post menopausal vaginal dryness and painful intercourse increased to 48% (Dennerstein 2000, Anne 2007). In Indonesia there are no accurate reports about the data the incidence of vaginal dryness and painful intercourse (Baziad 2003). If conditions are not handled properly, then sexual activity will decline, thus causing disharmony households, which can cause domestic violence (domestic violence), adultery, polygamy and even divorce, so the child as a successor generation will be displaced, such conditions will can cause drug abuse or juvenile delinquency. From the survey in the Office of Religious Affairs Surakarta that divorce most young married couples, and the second at 45-60 year-old couple who for reasons no longer fit and less hormonis in the household, because of infidelity, polygamy and domestic violence (KUA 2008).

In Sumatra, particularly in the Aceh area have the habit of consuming leaves of *Centella asiatica* (peugaga) as a typical meal there, but it is also widely consumed after giving birth in an effort to accelerate the delivery of wound healing. According to research, *Centella asiatica* or *Centella asiatica*, commonly found in Indonesia, which has the efficacy to increase collagen synthesis and proliferation of epithelial-maturation (Winarto and Surbakti 2005, Lee 2006). It has also been evidenced by Coldren et al (2003) and Lee (2006) in experimental animals that made the wound be quickly healed with administration of *Centella asiatica*. Effect of *Centella asiatica* that can increase collagen synthesis and proliferation of epithelium, the provision of *Centella asiatica* on menopausal women is possible to reduce vaginal atrophy, so that with the increase in collagen synthesis and proliferation of epithelial expected vaginal wall becomes thicker and elastic. Until now the mechanism of thickening and elasticity of the vaginal wall in menopausal women with the extract of *Centella asiatica* remains unclear.

Centella asiatica plants that contain phytoestrogens include beta-sitosterol and efficacy have triggered the synthesis of collagen type-1 in wound healing process (Coldren et al 2003, Lee 2006), but has not been proven to affect the vagina. Based on this, the researchers tried to use gotu kola as a basis to trigger collagen synthesis and epithelialization of the vagina in women who experience thinning of the vaginal epithelium and decreased collagen in postmenopausal conditions. Menopausal women must undergo a process of degeneration, especially thinning of the epithelium occurs and decrease of elasticity of the vaginal wall due to the decrease of estrogen so that it can affect psychological and physical factors of the women concerned, so it needs to get some attention. One of the efforts is to improve the quality of constituent cells of the vaginal wall. To improve the quality of those currently carried out by the hormone estrogen, but the use of estrogen negative effect of increasing incidence of breast cancer and cervical cancer in long-term use, also the price is expensive, so difficult to reach by the low socioeconomic communities. As a successor is sought from herbal estrogen namely phytoestrogens. Because phytoestrogens did not influence the increased incidence of breast and cervical cancer. Giving phytoestrogens gotu kola is expected to affect the proliferation and maturation of epithelial cells (squamous epithelial), and triggers the synthesis of collagen that eventually the vaginal wall is expected to become more thick and elastic.

Cells generally have estrogen receptors, therefore, when the cells were induced with phytoestrogens, particularly those derived from *Centella asiatica*, the phytoestrogens will bind to receptors on cell surfaces, resulting in electrical activation occurs intra-cellular signals that lead to increased calcium ion. It will then form a complex with calcineurin, these materials can inhibit the inhibitor kappa beta (IKB) so that the nuclear factor kappa beta (NF-kB) to be active and translocated into the nucleus of cells (Alexande et al 2002, Shakir and Derek 2004). Furthermore, the process of transcription in efforts to establish a variety of proteins that plays a role in cell division cycle. Menopausal women also experience mitochondrial dysfunction that generated a lot of free radicals, resulting in cells experiencing oxidative stress. To protect damaged proteins from the effects of free radicals, the cells which express the stress protein heat shock protein-70 (HSP-70) to protect cells from damage. *Centella asiatica* is a powerful antioxidant (Shukla 1999), which can reduce the effects of free radicals and is also a material that can induce fibroblasts to synthesize collagen by TGF-ß receptors (Maquart et al 1990, Lee 2006). Therefore, when in menopausal women given gotu kola is expected to suppress free radicals and increase collagen synthesis and epithelial proliferation in the entire body including the vagina, so the vagina becomes better quality due to the thickening and increased collagen which is expected to increase elastistasnya. To prove the effect of *Centella asiatica* on the increase in collagen and thickening of the vaginal wall in menopausal women is necessary to do some research. Therefore, this research material sample of the vaginal wall is not taken from human tissue due to obstructed ethical factors, the researchers used animal models of rats *Rattus norvegicus* strain Wistar. Objectives Research shows increased wall thickening, increased collagen vaginal estrogen receptor

and post-ovariectomy Wistar rats given *Centella asiatica* extract solution.

MATERIALS AND METHODS

This type of research is experimental. The design of this experiment was control group post test only design (Zainuddin 2000). Therefore, this study is not possible in humans. Therefore, the author used experimental animal models of white rat (*Rattus norvegicus*) Wistar strain. Samples are female rats (*Rattus norvegicus*) Wistar strain aged 4 months, weighing 290-300 grams, healthy and who have been ovariectomized as an animal model of menopause. Atrophy in experimental animals occurred on day 21 after ovariectomy, with the observation of the vaginal wall live layer of parabasal epithelial cells. New research conducted after the rats had atrophy on day 21. The sample size using the formula of the sample from Higgins, Kleinbaum (1985). The calculation of the minimum sample obtained at 7.0, rats per group. There are four groups in this study. Group 1 was not given the extract of the handle for 40 days. Group 2 was given gotu kola extract dose of 30 mg/Kbw per day, for 40 days. Group 3 received the extract of *Centella asiatica* 60 mg/Kbw per day, for 40 days. Group 4 were given doses of gotu kola extract 120 mg/Kbw per day, for 40 days

Inclusion criteria were female rats (*Rattus norvegicus*), Wistar strain, aged 4 months, weighing 290-300 grams, healthy (bright eyes, nimble) and were ovariectomized until there is atrophy such as menopausal conditions. Exclusion criteria were animal death/pain during treatment. To make the animal try to menopause, the treatment performed by ovariectomy. To see the effect of low estrogen on animal examination of the vaginal wall every week until there is a change towards atrophy, mean hipoestrogen effects have occurred, according to preliminary research results obtained by the incidence of atrophy on day 21 after ovariectomy. Preparation of animal models of menopause do ovariectomy bilateral. Postoperative therapy given gentamicin injection at a dose of 60-80 mg/kg/day for 3 days. Natural Materials Science Research Laboratory Faculty of Pharmacy, Airlangga University School of Medicine, Pathology Laboratory In Vitro Faculty of Veterinary Medicine, Airlangga University, for the manufacture of gotu kola leaf extract. To analyze the thickness of the vaginal wall, increased estrogen receptor beta and type-1 kologen using anova analysis. Statistical calculation was performed with computer software SPSS 15.0 series.

RESULTS

The results of the 35 white rats (*Rattus norvegicus*), Wistar strain, female, 4 months old, weighing approximately 290-300 grams, which is divided randomly into 5 groups. One group for the preliminary study and 4 groups for treatment of post ovariectomy. Performed univariate tests (ANOVA) to see the difference between dosing on proliferation of epithelial-maturation (Thickening of the vaginal wall) showed significant differences due to increased doses of extract of *Centella asiatica* ($p < 0.05$). In Table 1 shows the proliferation and maturation of epithelial tend to rise (in micrometers) due to *Centella asiatica* extract appropriate dose increase, compared with no given the extract of *Centella asiatica* showed a significant difference ($p < 0.05$).

In Table 2 shows the increase in estrogen receptor beta and collagen type-1, at all doses the results significant with $p < 0.05$ This suggests that administration of *Centella asiatica* extract affects the increase in estrogen receptor beta and collagen type 1. In Figure1 shows the increase in proliferation-maturation (thickening) of the vaginal wall epithelium in all treated groups, after increasing dose of extract of *Centella asiatica* as compared with the control group 1, without the extract of *Centella asiatica*. In Figure 3 (A). Looks vaginal epithelium have shown atrophy, thinning after ovariectomy 21 days compared with vaginal tissue in the preliminary study (figure 3 A). Figure 3 (B) after 40 days were given extract of *Centella asiatica* seems there are changes in the vaginal wall thickening, appearing intermediate and superficial layers.

Table 1. Mean and Standard Deviation proliferation-maturation of research data

Variables	Group 1		Group 2		Group 3		Group 4		p
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Proliferation of epithelial maturation (μ)	10.286	.5872	18.229	1.1856	25.943	5.6824	30.029	5.5790	.000

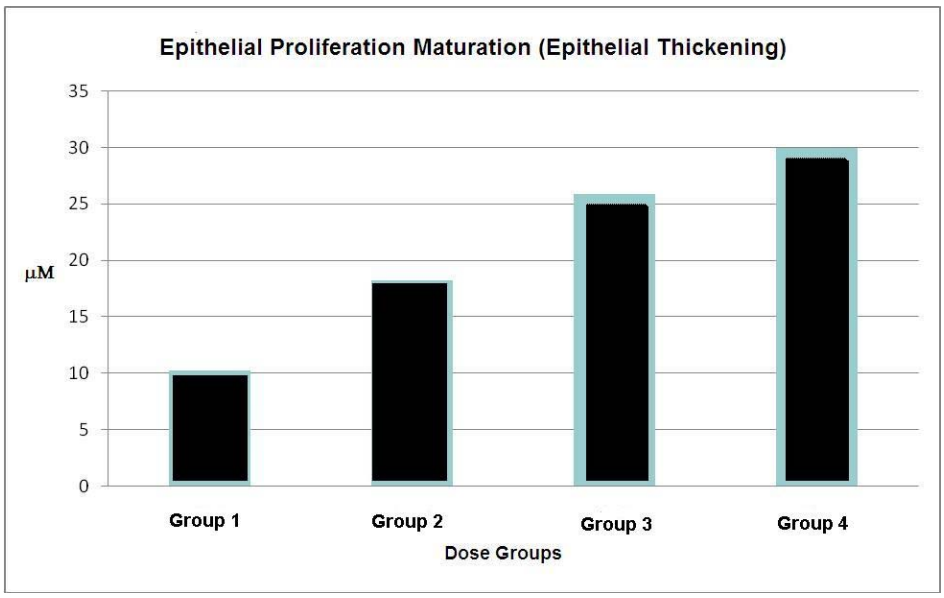
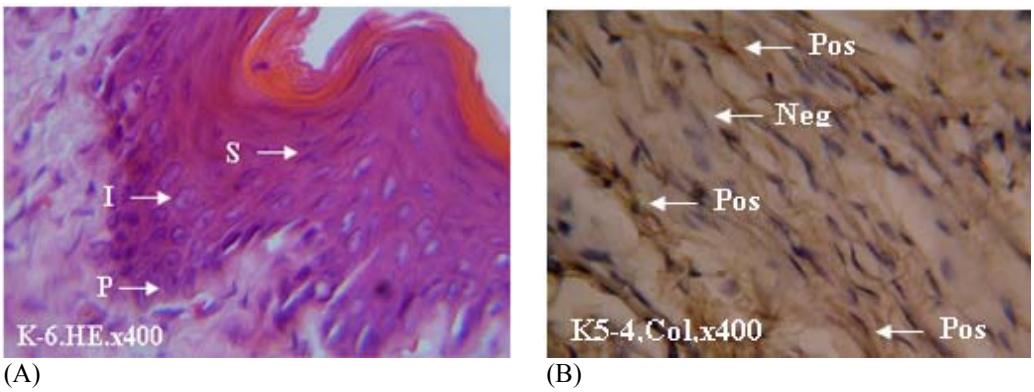


Figure 1 Mean and Standard Deviation proliferation-maturation of research data.

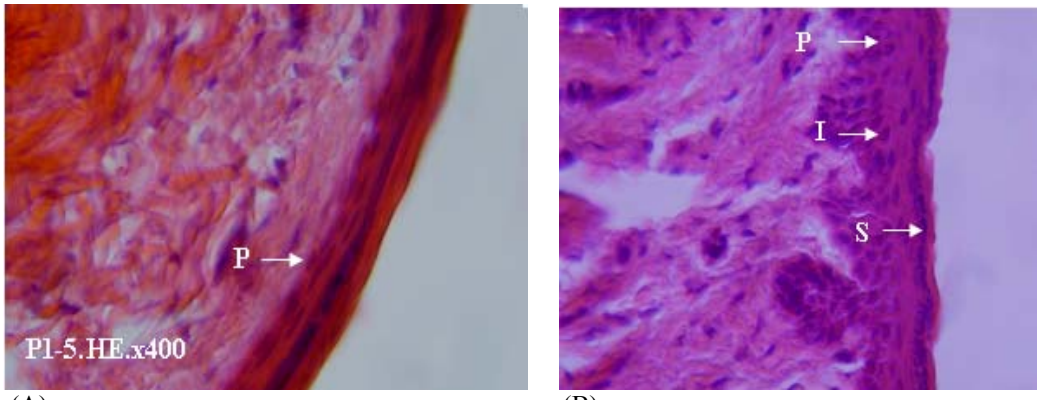
Table 2. The mean and standard deviation of estrogen receptor beta and collagen type-1

Variables	Group 1		Group 2		Group 3		Group 4		p
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
REβ	0.7429	.700	1.2000	1.194	1.4286	.243	2.8571	.276	0.0000
KT.1	1.1429	.2992	1.1143	.4298	1.7714	.1799	2.8286	.3729	0.0000



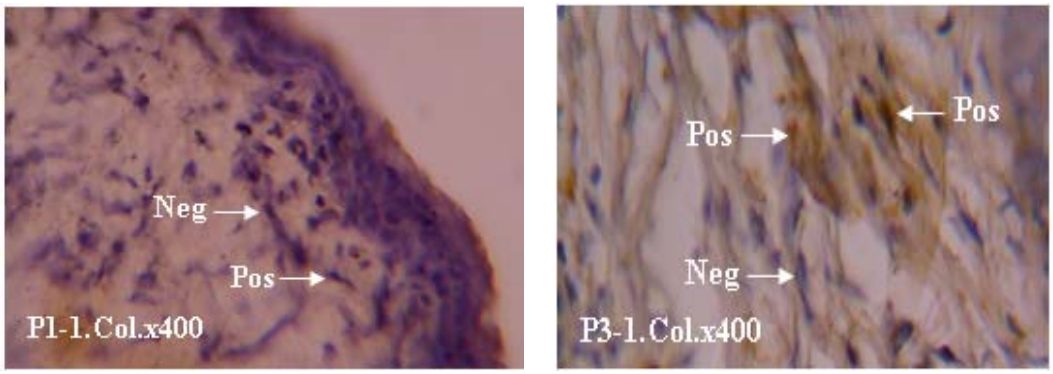
(A) (B)

Figure 2. Preview epithelial lining of the vaginal wall and the collagen fiber in normal mice. Figure 2 (A): Shows the composition of the vaginal epithelium enthroned in normal experimental animals P: parabasal cells, I: intermediate, S: superficial (HE, x 400) while (B): arrows indicate collagen fiber which gives a positive reaction (to provide color brown) against collagen type-1 ABM



(A) (B)

Figure 3. Picture of vaginal epithelial layer experimental animals that experienced atrophy and who have obtained extracts of *Centella asiatica*. Figure 3 (A). shows the epithelial layer thinning (atrophy), which was taken from vaginal tissue of Wistar 21 days after ovariectomy (HE, x 400), whereas (B) shows a picture of the epithelium of ovariectomized Wistar gotu kola extract and then given a dose of 120 mg/kg, for 40 days , P: parabasal cells, I: epithelial intermedia, S: superficial epithelium (HE, x 400)



(A) (B)

Figure 4 (A). The arrows indicate the collagen fiber, which gives a reaction to the ABM collagen type-1, taken from Wistar vaginal tissue 21 days after ovariectomy (CPI, x 400), whereas (B) shows the picture of the collagen fiber which gives a reaction to the ABM-1 collagen type taken from the vagina of ovariectomized Wistar gotu kola extract and then given a dose of 120 mg/kg, for 40 days (CPI, x 400). In Figure 4 shows that collagen type-1 tended to increase in animals who have been trying to get the extract of *Centella asiatica* (B) compared with not getting the extract of *Centella asiatica* (A)

DISCUSSION

This was an experimental study to explain the effects of *Centella asiatica* extract on vaginal wall thickening and elasticity. In this study, experimental animals rat *Rattus norvegicus* strain Wistar female who is 4 months old and weighed between 290-300 grams of 35 ovariectomized tail. Based on a sample size calculation, obtained n = 7 per group. In this study required 4 groups of experimental animals were allocated to each group randomly (allocation random sampling). Preliminary study was done to determine the observed atrophy of the vaginal wall. This time is found on day 21. Distribution of 4 groups to see the effect ovariectomy and dose effects of *Centella asiatica* extract on the thickening of the vaginal wall. The variables of this study include cell expression of estrogen receptor ß (ER-ß), and Collagen Type-1 (KT1) and histological examination to determine the thickening of the vaginal wall. Therefore this study can not be done in humans, then there are animal models that made the menopause. As a way of creating experimental animals performed ovariectomy menopause in both ovaries.

Until now, the effective dose of phytoestrogens there is no consensus among scholars or researchers. In general, starting from a low dose of 20 mg to 100 mg/kbw/day. Doses used in this study based on previous research on the effects of phytoestrogens on rat experimental animals is dose 30mg and 60 mg/kbw/day (Ananingati 2007) and the researchers add a dose of 120 mg/kbw/day with the intention of looking for effects of phytoestrogens when the dose was increased. Group 1, is ovariectomy rats not given the extract of *Centella asiatica*. Group 2, 3, and 4 were ovariectomy rats fed extracts of *Centella asiatica* with each dose of 30 mg, 60 mg, 120 mg/kg/day. Until the day-to-61 group of post ovariectomy mice not given the extract of *Centella asiatica Centella asiatica* extract and fed sacrificed. Samples were taken of the vaginal wall and immunohistochemical examination to see producing cells (expression) RE-ß, Collagen type-1 and histological examination to see the thickening of vaginal epithelium.

In the ANOVA analysis variable RE-ß, and KT-1 between groups 1, 2, 3 and 4 showed significant ($p = <0.05$, Table 2). In the control group 1, namely the group not given the extract of *Centella asiatica*. shows the number of producing cells (expression) Collagen type-1 ($X = 1.142; \pm 0.299$) and (ER-ß = 0.742 ± 0.700 and (Table 2). The condition is similar to menopause (Baziad 2005). In group 2, the group given a dose of gotu kola extract 30mg/Kgbb/day, indicate the number of RE-ß-producing cells ($X = 1.200 \pm 1194$), then the number of cells producing the KT-1 ($X = 1114 \pm 0.429$)

The number of RE-ß-producing cells in the group 2 higher than the group 1. The presence of RE-ß high in the vaginal epithelium due to exposure to phytoestrogens extracted from *Centella asiatica*. Phytoestrogens bound by the RE-ß on the surface of epithelial cells. The existence of ties between phytoestrogens *Centella asiatica* with its receptor (ER-ß), causing the electrical signal that begins with the activation of intracellular enzymes (phospholipase-C). This enzyme will change fosfatidil inositol in the phosphate (P1-2p) to fosfatidil inositol triphosphate (PI-3p). PI-3p receptors are located on the surface endoplasmik reticulum (ER). The existence of ties between the PI-3p with these receptors lead to calcium gate open, so that an increase in intra-cellular calcium ions. Calcium ions would bind with calsineurin in the cytoplasm. The existence of this complex calsineurin inhibit the activity of inhibitor kappa beta (Ik-ß), so that the nuclear factor kappa beta (NFk-ß) is active, then having translocation into the cell nucleus, and trigger the transcription process. The process of transcription produces mRNA that is transferred to the cytoplasm (ribosomes). In the ribosome translation process occurs to produce several proteins such as CDK, cyclin that acts on cell division cycle and proteins to increase the number of RE-ß-producing cells. (Alexande 2002, Lu et al 2004, Shakir & Derek 2004). At this dose (30 mg) the amount of collagen-producing cells (KT-1) do not look real, new possibilities initiation process, so it does not seem change the number of KT-1-producing cells.

In group 3 that extracts of gotu kola group was fed a dose of 60 mg/kbw/day. Indicates the number of KT-1-producing cells ($X = 1.771 \pm 0.179$) the highest, then the number of RE-ß-producing cells ($X = 1.428 \pm 0.243$). According to the conceptual framework, mechanisms and elasticity of the vaginal wall thickening by *Centella asiatica* through antioxidants. On increasing the dose to 60mg/kbw/day, there is increased collagen type-1 is more striking than in 30mg/kbw/day dose group. It is possible occurred when extracts of gotu kola is captured by the estrogen receptor acts as an antioxidant that will reduce ROS that damage collagen and beta estrogen receptors can be inhibited. This condition causes the protein that is estrogen receptor beta and collagen type-1 increased (Table 2). In group 4 that extracts of gotu kola group was fed a dose of 120 mg/kbw/day, showed a picture of RE-ß-producing cells ($X = 2.857 \pm 0.276$) and followed the highest number of cells producing collagen type-1 ($X = 2.828 \pm 0.372$). At doses 120 mg/kbw/day amount of estrogen receptor beta-producing cells and collagen-producing cells at doses much higher than the dose 120mg/kbw/day 60mg/kbw/day. This is possible when the extract of *Centella asiatica* function more as anti-oxidants that prevent damage both of these proteins. Changes in the vaginal wall from the control, doses of 30, 60 to 120 mg/kbw/day showed more and thicker, on the basis of these changes, the dose of gotu kola is a dose of 120 best among the three doses by seeing an increase in the highest of RE-ß and collagen type-1, which means the synthesis of collagen is very striking compared to the lower dose (Table 2).

On microscopic observation of the vaginal wall incision with Hematoxylin eosin staining show an increase in vaginal wall thickening in experimental animals after a given *Centella asiatica* extract, especially at doses 120mg/kbw/day compared with the vaginal wall in experimental animals are not given the extract of *Centella asiatica*. On immunohistochemical examination showed an increase in RE-ß, Collagen type-1 (KT-1) after the experimental animals given gotu kola extract compared with experimental animals are not given the extract of *Centella asiatica*, this proves a good response occurred in the extract of *Centella asiatica* in improving thickening and elasticity vaginal wall in menopausal conditions.

CONCLUSION

Giving oral gotu kola extract in rats of Wistar strain *Rattus norvegicus* menopause with a dose of 30 mg, 60 mg and 120 mg/kgbw/day, can increase estrogen receptor ß, proliferation of the vaginal wall maturation, and increased collagen, causing thickening and increased collagen vaginal wall.

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