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Potencial Antifertility of *Centella asiatica* Leaf Extract

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ABSTRACT

The ideal requirements for contraceptive materials are effective, having minimal side effects, reversible, economical, easily to be used and available. However, no much ideal contraceptive are currently available, so it is necessary to find alternative contraceptive materials in order to gain these criteria. One of the materials is *Centella asiatica* (L.) Urban). This study aimed to determine the effect of *C. asiatica* leaf extract on ovarian histology and weight of mice (*Mus musculus*). This research used a completely randomized design with 5 treatments and 5 replications. 25 of fertile female mice were treated with several doses of *C. asiatica* leaf extract, 0 (Control), 125 (T1), 200 (T2), 275 (T3) and 350 mg/kg body weight (bw) (T4). Parameters observed were total of primary, secondary and tertiary follicles, corpus luteum, thickness of theca cell and ovarian weight. ANOVA Test and BNT analysis were used to analyze data. Statistical analysis showed that *C. asiatica* leaf extract affected the ovarian histology and weight of mice. Dose of 275 mg/kg showed the effective treatment that can optimally reduce the number of primary, secondary and tertiary follicles, thickness of theca cells, the number of corpus luteum and mice ovarian weight.

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INTRODUCTION

The increasing of Indonesian population became big issues in relating with the welfare of a decent and healthy life. To control the population, several efforts have been made by government such as contraception. The contraception methods commonly used by women are in the form of hormonal pills, injections, implants; non-hormonal form of Intra Uterine Device (IUD) and tubectomy, while the requirements for ideal contraceptive drug are effective, having minimal side effects, reversible, economical, easily available and practical use (Santoso, B., 1993). However, no much ideal contraceptive methods are recently available, and the contraception methods commonly chosen by Indonesian woman are hormonal contraceptives that can cause side effect. Therefore, it is very important to search other materials derived from nature that are easily obtained, usable and safe, one of these material is gotu kola (*Centella asiatica* (L.) Urban). Fitriyah (2009) reported that *C. asiatica* extract with doses of 25 and 75 mg/kg bw effectively increase the number of primary, secondary and tertiary follicles in the ovaries, but at a dose of 100 and 125 mg/kg bw tend to decrease the number of primary, secondary and tertiary follicles. Based on the reason mentioned above, it is needed to do further investigation on

potential *C. asiatica* extract at doses above 125 mg/kg bw as oral contraceptives in females.

Methodology:

Animals used in this study were female mice (*Mus musculus*) balb/c with \pm 4 months old and 20-30 g in body weight. Before used for experimental study, animals were acclimatized for 2 weeks, fed and watered ad libitum. The simultaneity of estrus was intramuscularly performed by giving 0.1 mg prostaglandin hormone per mice. After acclimation, mice were randomly divided into 5 groups of treatments and 5 replication. Group 1 (control) was given by 0.5% CMC, and the other groups (Group 3-5) were given *C. asiatica* leaves extract as much as 125 mg/kg bw (T1), 200 mg/kg bw (T2), 275 mg/kg bw (T3) and 350 mg/kg bw (T4). The administration of *C. asiatica* leaves extract was performed as much as 0.5 ml for 30 days. The powder of *C. asiatica* leaves was macerated by using 70% ethanol for 24 - 72 hours, filtered and the filtrate obtained was concentrated by using a rotary evaporator at temperature 40 °C. Animal surgery was performed by chloroform at day 31. The ovary was removed and weighed, and then used for histological preparation. Histological section of mice ovary was observed under Microscope Olympus CX31 by using 400 x magnification in five visual fields. Parameters

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observed were total of primary, secondary and tertiary follicles, corpus luteum, thickness of theca cell and ovarian weight. ANOVA Test and BNT analysis were used to analyze data.

RESULTS AND DISCUSSION

C. asiatica Leaves Extract Decrease the Number of Ovarian Follicles, Thickness of Theca Cells and Corpus Luteum of Mice Ovary:

Statistical analysis showed that treatment with dose 125 mg/kg bw (T1) can reduce the number of primary, secondary and tertiary follicles in the mice

ovary. The similar result was also observed in T2, T3 and T4 that were significantly different from controls (Table 1). *C. asiatica* contains triterpenoid saponins one of the derivatives of steroids (Ruszymah, B.H.I., *et al.*, 2012). Steroid and triterpenoid are active materials that work as antifertility factor. These both active ingredients are able to cause disturbances in the hypothalamic pituitary pathways that subsequently lead to impaired secretion of GnRH, and finally contribute to the formation, development and maturation of follicles (Palermo, R., 2007). The higher the dose given, the higher the active ingredients contained.

Table 1: The effect of *C. asiatica* extract on the number of follicles and weight of mice ovary

Pegagan extract	Primary follicle	Secondary follicle	Tertiary follicle	Thickness of theca cell (μ m)	Number of Corpus luteum	Ovarian weight (mg)
T4	3,2 \pm 2,08 a	3,2 \pm 2,70a	1,8 \pm 0,83a	11,76 \pm 2,52a	2,2 \pm 1,41a	11,02 \pm 2,37a
T3	4,0 \pm 3,78 a	3,6 \pm 1,51ab	1,8 \pm 1,04a	14,34 \pm 4,26a	2,6 \pm 2,25a	15,88 \pm 3,90ab
T2	4,4 \pm 1,51 a	4,4 \pm 1,14abc	2,4 \pm 1,63b	17,89 \pm 1,75b	3,0 \pm 1,21a	20,04 \pm 1,07bc
T1	5,4 \pm 0,70 ab	5,6 \pm 1,14bc	4,6 \pm 2,31b	19,07 \pm 5,79b	3,8 \pm 0,81ab	22,98 \pm 1,45c
C	8,0 \pm 0,44 b	6,6 \pm 1,09c	5,2 \pm 2,	19,51 \pm 1,48b	5,6 \pm 1,32b	29,48 \pm 1,30d

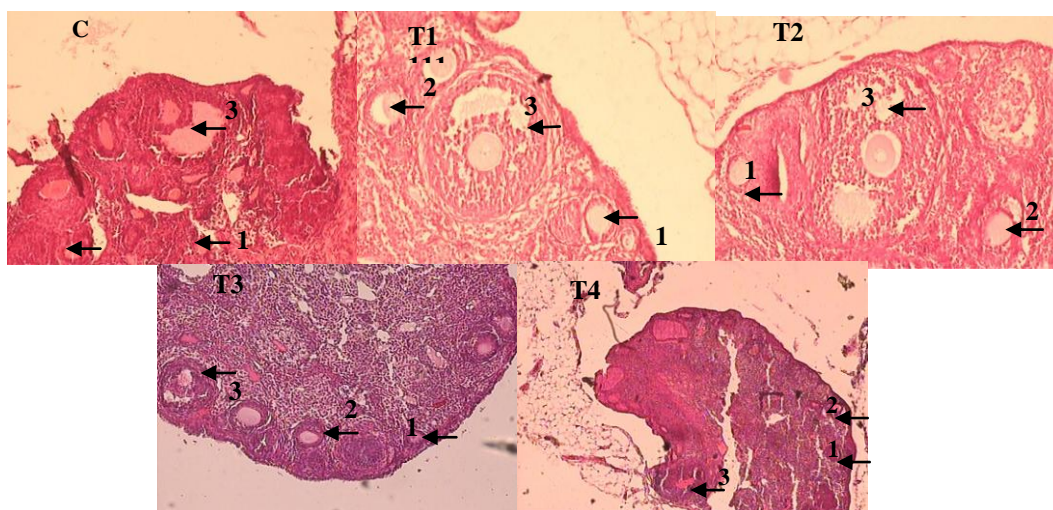


Fig. 1: Histological section of mice ovary (magnification: 100X). 1. Primary follicle 2. Secondary follicle 3. Tertiary follicle.

Figure 1 and 2 showed that the number of primary, secondary and tertiary follicles and corpus luteum decrease in all treatments especially in T4. The decreasing in follicle development is due to an active compound in *C. asiatica* which causes the inhibition of follicular maturation. One of the active ingredients of *C. asiatica* is triterpenoids saponins which can disrupt egg mitotic process and results in the failure of eggs maturation, so that the follicles undergo atresia. In addition, there are several other protein hormones derived from the ovary that also affect the development of ovarian follicles, one of which is inhibidin. Tajima *et al.*, (2007) stated that the main source of inhibidin in ovarian are granulosa cells. Inhibidin suppress pituitary to produce FSH which led to disturb the follicular development. Follicular maturation is strongly influenced by the

gonadotropin hormones FSH and LH. FSH stimulation on adenohipofisa can cause the number of follicles vesicular began to grow. While follicles are developing, the much number of estrogen is produced by the theca interna and will be absorbed into the body's circulation and also into the follicular fluid. If the secretion of these hormones are inhibited, the development of follicles are also disturbed, and finally, the number of follicles (fig. 1) and corpus luteum (fig. 2) as well as the thickness of theca cells (fig. 3) decrease (Guyton, A.C., J.E. Hall, 2006). Ovarian weight decreased at T1, T2 did not differ from C but in contrast to T3 and T4 (Table 1). Ovarian atrophy, ie shrinking the size of the ovary. Atrophy occurs due to reduced ovarian tissue constituent such as the number of follicles, theca cells thickness and other tissues.

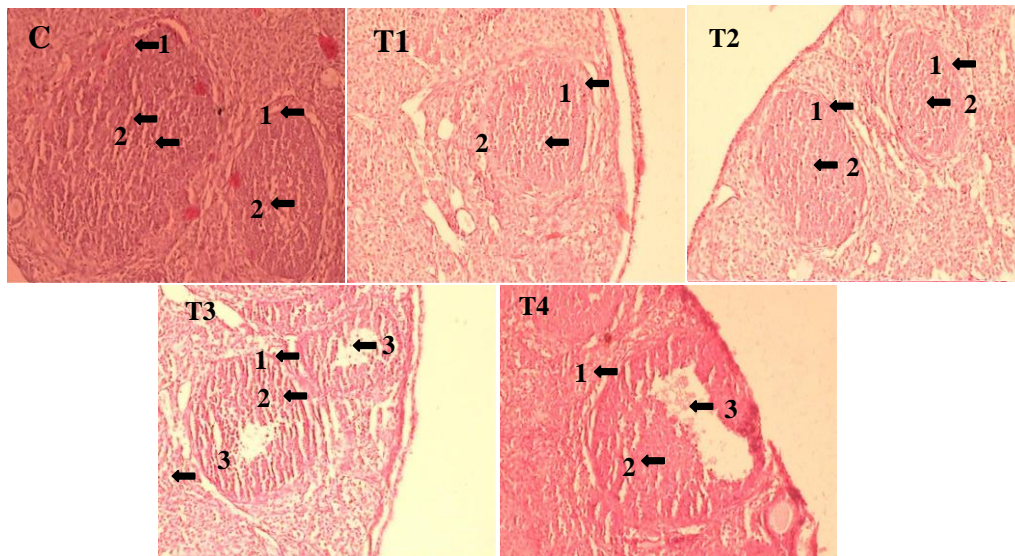


Fig. 2 : Corpus Luteum (magnification: 100X). 1. Theca cells 2. Granulosa cells, 3. Antrum

Figure 2 showed the thickness of theca and granulosa cells, where the thickness of theca cells in all treatments was not significantly difference with control. This might be due to the toxicity of saponins and flavonoids compounds in *C. asiatica* that caused the decreasing of follicles number. While steroidal

alkaloids in *C. Asiatica* interfere the balance of gonadotropin hormone. Tajima *et al.*, (2007) stated that the decreasing of ovarian theca cells thickness is affected by asiatic acid. Asiatic acid is part of triterpenoids which results in excessive apoptosis in cells that begins with mitochondrial damage.

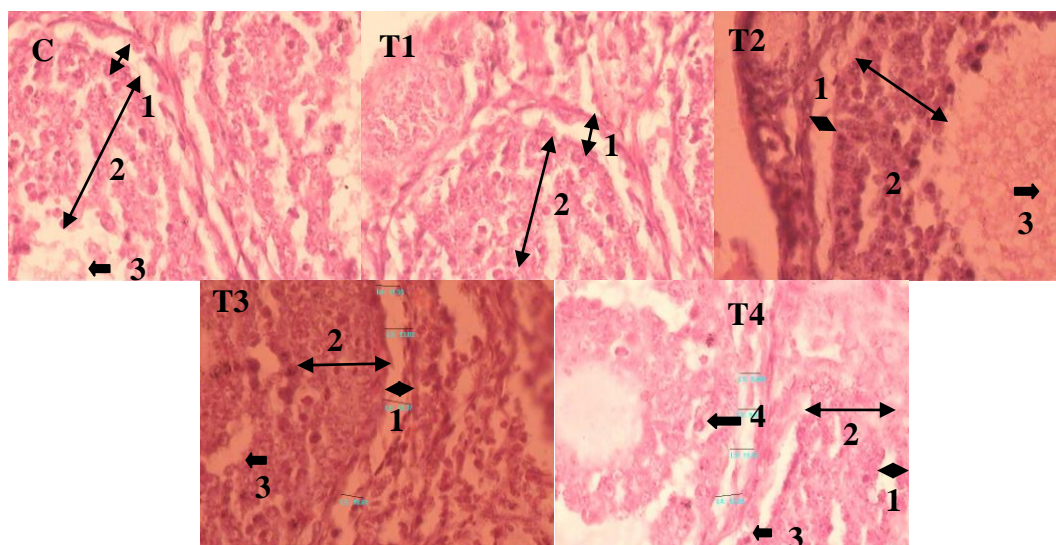


Fig. 3: Tertiary follicle of ovary (magnification: 400X). 1. Thickness of theca cells 2. Thickness of granulosa cells, 3. Antrum 4. Oosite

Theca cells express LH receptors, which stimulate the growth of the corpus luteum. Theca cells also secrete androgens, which are converted into estrogen by the granulosa cells under influenced of FSH. High estrogen will do a positive feedback on the anterior pituitary gland, causing the LH surge then ovulation occurs. Decreasing thickness of theca cells will certainly leave the LH receptor in theca

cells, then decrease LH secretion and lead to failure of ovulation (Palermo, R., 2007; Guyton, A.C., J.E. Hall, 2006).

Corpus luteum secretes large amounts of progesterone and estrogen. While the secretion of LH in granulosa and theca cells causes luteinization. Lutein cells of corpus luteum, which is newly formed, has a strong feedback effect on the anterior

pituitary gland especially in maintaining the secretion speed of FSH and LH. Lutein cells will also secrete small amounts of the inhibidin hormone. This hormone inhibits the secretion of anterior pituitary gland, especially FSH. As a result, the concentration of FSH and LH in the blood become lower than usual. In addition, the loss of this hormone causes the degeneration of corpus luteum, and as a whole is called as corpus luteum involution (Palermo, R., 2007).

Conclusion:

Leaves extract of *C. asiatica* with doses 125, 200, 275 and 300 mg/kg bw could decrease the number of follicles, corpus luteum, the thickness of theca cells and weight of mice ovary, where the most effective dose is 275 mg/kg bw. It is recommended to do further research related with hormonal tests such as estrogen and progesterone.

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