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Effectiveness Tapak Dara Leaf Extract (*Catharanthus roseus*) Against Corpus Luteum Formation in Mice (*Mus musculus*) Female

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Abstract: This research aims to determine the effectiveness of Tapak Dara leaf extract (*Catharanthus roseus*) to corpus luteum formation in mouse ovaries (*Mus musculus)* female. This research uses a true experiment and Posttest-Only Control with a quantitative approach. The sample was determined using a random sampling technique with a randomized group design (RAK) research design. The formation of the corpus luteum in the ovaries of mice is known by counting the number of corpus luteum in the mouse ovary incision. The data obtained were analyzed using the one-way ANOVA (Analysis of Variance) test analysis method at a significance level of 5% ($\alpha = 0.05$), then continued with the Least Significant Difference (BNT) further test at a significance level of 5% ($\alpha = 0.05$). Hypothesis test results state F Count(3.094) > F table (3.006). So, this research can conclude that Tapak Dara leaf extract (*Catharanthus roseus*) is significantly effective in the formation of corpus luteum in female mice (*Mus musculus*).

Keywords: Corpus Luteum; Mice (*Mus musculus*); Tapak Dara Leaf Extract.

Introduction

Virgin site plant (*Catharanthus roseus*) contains phytoestrogen compounds, whose structure is similar to the structure of estrogen, so that it can exhibit agonist properties on the Estrogen Receptor (ER). The ability of phytoestrogens to mimic the effects of estrogen is based on the presence of compounds with a molecular weight equivalent to the molecular weight of estrogen (272 g/mol), a phenolic ring as a binding site, and a core with two hydroxyl groups with a distance of 11.0-11.5Å. Phytoestrogens can influence the ovarian cycle through the growth and development of egg follicles and the formation of the corpus luteum [1]. Phytoestrogens are molecules derived from plants that can interact with estrogen receptors (ERs) or modulate the action of the estrogen hormone in vivo [2]. Phytoestrogen group compounds can bind to estrogen receptors, thereby disrupting the endocrine system in the body in various animal species. Phytoestrogens can interact with enzymes and receptors due to their stable structure and low molecular weight, which allows them to pass through cell membranes [3]. Saponin, tannin, flavonoid, terpenoid, alkaloid, sterol, and triterpenoid compounds can suppress fertility levels by disrupting the function of the ovaries, uterus, or vagina [4]. According to [5], the content of flavonoids and saponins increases estrogen and antifertility activity. [6] stated that the antifertility ingredients triterpenoids and saponins work on the hypothalamic, pituitary, and gonadal axes, thereby influencing the secretion of gonadotropin hormones. Saponin directly inhibits the action of genes that play a role in steroidogenesis and suppresses cell development in the ovaries, which is regulated by Follicle follicle-stimulating hormone (FSH) [7]. The results of the phytochemical screening of tapak dara leaves (Catharanthus roseus) show that they contain alkaloids, flavonoids, saponins, tannins, and steroids [8]. In [9], flavonoids have an estrogenic effect; namely, they can work like estrogen by occupying estrogen receptors. In the ovaries, estrogen will occupy the α and β estrogen receptors, while in the uterus, it will occupy the α estrogen receptors so that proliferation occurs in the ovaries and uterus.

The formation of the corpus luteum in the ovaries is influenced by reproductive hormones, especially Luteotropic Hormone (LTH). Reproductive hormone disorders will result in impaired corpus luteum formation. The growth and development of the corpus luteum greatly determines reproductive status in animals because the corpus luteum can produce the hormone progesterone. The function of the progesterone hormone, besides influencing the proliferation of the uterine wall, also regulates the reproductive cycle through negative feedback on the hypothalamus and anterior pituitary. Disruption of the folliculogenesis process will result in the follicles not being able to mature, resulting in no ovulation and formation of the corpus luteum [10]. The corpus luteum is formed from the remains of de Graafian follicles that have undergone ovulation [11].

Follicle-stimulating hormone (FSH) can influence the growth and development of follicles so that if FSH is disturbed, the process of follicle development will also be disturbed [11].

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Phytoestrogens can cause natural estrogen to be unable to bind to its receptors and will increase the amount of free estrogen in the blood [12]. High levels of estrogen in the blood can cause the secretion of FSH (Follicle follicle-stimulating hormone) to be inhibited so that the growth and development of follicles and the formation of the corpus luteum in the ovaries are also hampered. This inhibited follicular development will cause the formation of atretic follicles [13]. According to [14], antifertility ingredients that work on the hypothalamus-pituitary-ovary axis have gonadotropin activity, with a negative feedback mechanism from the hypothalamus, which causes a decrease in GnRH (Gonadotropin gonadotropin-releasing hormone) production. This will affect the secretion of FSH and LH/LTH from the anterior pituitary so that the secretion of FSH and LH/LTH is low, where these two hormones greatly influence the formation, development, and maturation of ovarian follicles, the ovulation process, and the formation of the corpus luteum. Even so, the use of phytoestrogens in the world of health, according to [15] and [16], still has many pros and cons. This is because the dose-effect and the level of affinity of phytoestrogens in the bodies of test animals have not yet been determined. Therefore, this research needs to be carried out to assess the effectiveness of *tapak dara* leaf extract containing phytoestrogens on the formation of corpus luteum in female mice (*Mus musculus*).

Research Method

This research method is a true experiment and Posttest-Only Control with a quantitative approach. Sampling was carried out randomly (random sampling) with a research design, namely a randomized group design (RAK). The formation of the corpus luteum in the ovaries of mice was analyzed using the One-way ANOVA test analysis method. If there were differences, continue with the BNT (Least Significant Difference) test. Both tests have a significance level of 5% ($\alpha = 0.05$). Preparation of tapak dara leaf extract using the maceration method. Tapak dara leaf powder is soaked in 95% alcohol for 3 days with repeated stirring. The maceration results of tapak dara leaves were evaporated using an evaporator at a temperature of 50^{O}C.

The percentage yield of tapak dara leaf extract needs to be considered. The yield is the result of comparing the number of metabolites found after the extraction procedure with the weight of the sample used. The yield of tapak dara leaf extract obtained was 15.5%, which means that the quality of the tapak dara leaf extract was good. The minimum yield must be 7.2% according to the requirements of the Indonesian Herbal Pharmacopoeia [17]. Five groups were tested in this study; one control group (K0) received 0.9% NaCl while the other four groups, namely K1-K4, received *tapak* leaf extract at respective doses (50, 100, 150, 200) mg/kg. Starting in the diestrus phase of the mice, the treatment was given orally, and a probe was used for 14 days. Twenty-five fertile female Balb/C strain mice, aged 8 weeks or less and weighing 20–30 grams, constituted the entire sample in this study. After receiving treatment in the form of giving tapak dara leaf extract for 14 days, which corresponds to the three estrous cycles of mice [18], the animals were sacrificed by neck dislocation. The dead mice were placed on a section board, and the ovaries were surgically removed using a section tool. Clean the ovaries from fat before putting them in a petri dish with 0.9% NaCl solution by making an ovarian incision using paraffin techniques and Hematoxylin-Eosin staining with the aim of counting the number of corpus luteum in the ovaries [19]. The effectiveness of giving tapak dara leaf extract on the formation of corpus luteum was seen by counting the number of corpus luteum in histological incisions of the ovaries. The corpus luteum is a temporary endocrine gland that forms after ovulation as a result of the arrangement of granulosa cells and internal theca follicles of the ovary [20].

Results And Discussion

The results of observing the formation of the corpus luteum are known by counting the number of corpus luteums, summarized in Table 1 and Figure 1.

Table 1. Description of the Effectiveness of Tapak Dara Leaf Extract (*Catharanthus roseus*) Against the Formation of the Ovarian Corpus Luteum of Mice (*Mus musculus*)

Group	Lots of Mice	Mean	Std Dev	Minimal	Maximum
Control Group	5	4.8	1.92353841	2	7
Dosage 50 mg/kgBB	5	4	1.22474487	3	6
Dosage 100 mg/kgBB	5	3.6	1.51657509	1	5
Dosage 150 mg/kgBB	5	2.6	1.14017543	1	4
Dosage 200 mg/kgBB	5	2.2	1.4832397	0	4

Based on Table 1 and Figure 1, it can be seen that the highest average amount of corpus luteum was in the control group, while the lowest average amount of corpus luteum was in treatment group 4 (200 mg/kg BW).

Table 2. Shows the normality test for corpus luteum formation in mouse ovaries (*Mouse muscle*) with hypothesis:
H0: Sample quantity body The mouse ovary *luteum* is normally distributed

Ha: Sample quantity body The mouse ovary *luteum* is not normally distributed
Is known:
a = 0,05
L table = 0.337

Calculation of the Liliefors corpus luteum normality test in all treatment groups shows L count < L

table so that H0 is accepted and Ha is rejected, which means the sample size body The mouse ovary *luteum* is normally distributed.

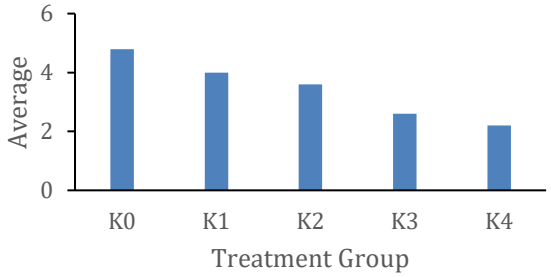

Figure 1. Effectiveness of Tapak Dara Leaf Extract (*Catharanthus roseus*) Against the Formation of the Ovarian Corpus Luteum of Mice (*Mus musculus*)

Table 2. Normality Test for the Effectiveness of Tapak Dara Leaf Extract (*Catharanthus roseus*) Against Corpus Luteum Formation in Mice Ovaries (*Mus musculus*)

Group	L count (Corpus Luteum)
K0	0.23051
K1	0.23051
K2	0.23051
K3	0.263339
K4	0.263339

Table 3. Homogeneity Test for the Effectiveness of Tapak Dara Leaf Extract (*Catharanthus roseus*) Against Corpus Luteum Formation in Mice Ovaries (*Mus musculus)*

Number of Corpus Luteum				
IS	K1	K2	K3	K4
6	6	4	4	3
4	3	5	1	4
7	3	4	3	2
5	4	4	3	0
2	4	1	2	2

Sample	db = (n-1)	Varians (S^2)	db S^2	log S^2	db log S^2
K0	4	3.7	14.8	0.568	2.272
K1	4	1.5	6	0.176	0.704
K2	4	2.3	9.2	0.361	1.446
K3	4	1.3	5.2	0.113	0.455
K4	4	2.2	8.8	0.342	1.369
Amount	20	11	44	1.562	6.249

S^2 gave : db S^2/20 = 2.2
log S^2 gave : log(2.2) = 0.342422681
B : 0.342422681*20 = 6.848453616
Chi count : log(10)*(6.85-6.25) = 0.598906207
Chi table : (see table) = 31.41

Table 3 shows the homogeneity test on corpus luteum formation in mouse ovaries (*Mus musculus*) with hypothesis:
H0: Sample quantity body homogeneous mouse luteum
Ha: Sample quantity body mouse luteum is not homogeneous
Is known:
a = 0.05
X^2 table = 31.41

Calculation of corpus luteum homogeneity test in mouse ovaries in all treatment groups determines X^2 count < X^2 table so that H0 is accepted and Ha is rejected, which means the sample size is homogeneous mouse ovary luteum.

Table 4. One Way ANOVA Test on the Effectiveness of Tapak Dara Leaf Extract (*Catharanthus roseus*) Against Corpus Luteum Formation in Mice Ovaries (*Mus musculus*)

TREATMENT	YELLOW BODY					TOTAL	MEAN
	I	II	III	IV	IN		
K0	6	4	7	5	2	24	4.8
K1	6	3	3	4	4	20	4
K2	4	5	4	4	1	18	3.6
K3	4	1	3	3	2	13	2.6
K4	3	4	2	0	2	11	2.2
AMOUNT	23	17	19	16	11	86	3.44

ANOVA RAK

SK	DB	JK	KT	F count	F table 0.05 (F 5%)	Information
Treatment	4	22.16	5.54	3.094	3.006	*
Block/Group	4	15.36	3.84	2.145	3.006	TN
Error/Remainder	16	28.64	1.79			
Total	24	66.16				

Table 5. BNT Test on the Effectiveness of Tapak Dara Leaf Extract (*Catharanthus roseus*) Against Corpus Luteum Formation in Mice Ovaries (*Mus musculus)*

BNT Test Formula		sd	T 5%	BNT
sd = sqrt (2*KTGalat)/repetition		0.378	1.746	0.660
TREATMENT	MEAN	MEAN + BNT		NOTATION
K0	2.2	2.869673511		a
K1	2.6	3.260673511		a
K2	3.6	4.260673511		b
K3	4	4.660673511		b
K4	4.8	5.460673511		c

Information:
Treatment with the same notation symbol means that it is not significantly different.

Table 5 shows the test*One Way* ANOVA on the number of corpus luteum in mouse ovaries (*Mouse muscle*) with hypothesis:
H0: F count < F table, there is no difference between the five groups
Ha: F count > F table, there are differences between the five groups
Is known:
a = 0.05
F table = 3.006

F count (3.094) > F table (3.006), then H0 is rejected and Ha is accepted, so that the data on the number of corpus luteum in the ovaries of mice are different from each other, which means that there is effectiveness of tapak dara leaf extract on the formation of corpus luteum. Next, further tests were carried out using the BNT test, which can be seen in Table 5. Based on the BNT test, it can be seen that the administration of tapak dara leaf extract to the control group, treatment group 1 (50 mg/kgBB), treatment group 3 (150 mg/kgBB), and treatment group 4 (200 mg/kgBB) was different from treatment group 2 (100 mg/kgBB).

The leaves of Tapak Virgin (*Catharanthus roseus)* contain more than 70 types of chemicals such as alkaloids, ajmalicine, serpentine, and reserpine, acid caffeoylquinic, and glycoside flavonoid as an antioxidant [21]. Alkaloids are very potential active compounds in*Catharanthus rosesus*. More than 400 types of alkaloids contained in this plant are used as medicinal ingredients, especially to control fertility in animals. Based on the results of data analysis using the ANOVA *One Way* test, it shows that F count (3.094) > F table with (3.006). This indicates that the amount of corpus luteum in the ovaries of mice is significantly different in each group, so it can be concluded that there is a significant effectiveness of giving tapak dara leaf extract on the amount of corpus luteum formation in the ovaries of mice. Based on the BNT further test in Table 5, it can be seen that the administration of tapak dara leaf extract in the control group (NaCl 0.9%) was different from treatment group 1 (50 mg/kgBB), treatment group 2 (100 mg/kgBB) and the treatment group 3 (150 mg/kgBW) and treatment group 4 (200 mg/kgBW). This data shows that the highest corpus luteum count was in the control group, while the lowest was at a dose of 200 mg/kgBB. The number of corpus luteum decreased as the dose of tapak dara leaf extract increased. This means that the higher the dose given, the less chance of corpus luteum formation occurring as a result of the active compounds contained in tapak dara leaf extract, namely alkaloids, saponins, tannins, flavonoids, and isoflavonoids which can disrupt the hypothalamus-pituitary-ovary hormonal system by causing bait come back negative [11]. These results are similar to research conducted by [13] explaining that administration of canary leaf extract containing flavonoids can interfere with the mechanism of action of the LH hormone by inhibiting LH binding to its receptor so that the cellular effects of LH do not occur. The absence of cellular effects from LH prevents ovulation from happening so that it does not form the body's *luteum*. In addition, this is also linear with the presence of follicles, the count, which is when follicles count. If you don't ovulate, then nothing body luteum is formed. [22] further stated that giving tapak dara leaf extract, which contains phytoestrogens, can reduce the average number of growth and development of follicles in the ovaries. It is thought that this occurs because the phytoestrogens have a reasonably high affinity for estrogen receptors (17-β estradiol), which can bind to estrogen receptors and have a mechanism of action similar to estrogen. The hormone estrogen works in a negative feedback manner, namely inhibiting FSH secretion, so that the growth and development of follicles are also hampered, accompanied by the formation of a corpus luteum [23]. [24] also reported that high levels of phytoestrogens can damage cell membranes. Damage to the cell membrane causes the estrogen receptors in the cell membrane to be damaged so that phytoestrogens cannot bind to their receptors, which makes estrogen levels in the blood too high. [25] explained that estrogen released into the blood inhibits the hypothalamus and anterior pituitary in a negative feedback manner. Estrogen decreases the sensitivity of cells that produce the hormone gonadotropin, especially FSH-producing cells. [26] and [27] explained that the estrogenic effect produced by phytoestrogens is 102 to 103 times lower than the estrogenic effect of 17β-estradiol which is an endogenous estrogen in the body, thus affecting the growth and development of follicles and the formation of the corpus luteum in the ovaries of mice.

Conclusion

Based on the results of data analysis and discussion, this research can conclude that tapak dara leaf extract (*Catharanthus roseus*) is significantly effective in the formation of corpus luteum in female mouse ovaries (*Mus musculus*).

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