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Effect of Giving Young Papaya (Carica Papaya L.) Fruit Extract on Endometrial Histology of Female Rats (Rattus Norvegicus)

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ABSTRACT

Young papaya contains several phytochemicals such as saponins, alkaloids and flavonoids. The active compounds in young papaya are thought to be able to cause disruption of the hypothalamic-pituitary pathway which causes disruption of GnRH secretion and affects the ovarian cycle which affects the normal endometrial cycle. The aim of this study was to determine the effect of giving young papaya fruit extract (Carica papaya L.) on endometrial histology of female rats (Rattus norvegicus). This study uses the Post Test Only Control Group method. Total samples were 24 rats (Rattus norvegicus), 2-3 months old and 200-300 grams in weight. Samples were taken randomly, divided into 4 groups. Group (K) without being given treatment. Group (P1) was given 200 mg / 200gr BW of young papaya extract. Group (P2) was given young papaya fruit extract 400 mg / 200gr BW. Group (P3) was given young papaya fruit extract 600 mg / 200gr BW. After 20 days of treatment, the endometrial thickness and density of the endometrium were measured. The results showed that the administration of young papaya fruit extract (Carica papaya L.) caused changes in the thickness of the endometrium and inhibited endometrial gland density. The results of statistical analysis using the one way ANOVA test showed the effect of giving papaya extract on endometrial thickness (p = 0.009) and the effect of giving papaya extract on gland density. It can be concluded that there is an effect of giving young papaya (Carica papaya L.) fruit extract on endometrial thickness and endometrial gland density.

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1. Introduction

The rate of population growth that continues to increase is the main problem faced by Indonesia as a developing country. Based on the population census, the total population of Indonesia in 2018 was 265 million, with 133.17 million men and 131.88 million women. This number is likely to increase every year, so that the government makes several efforts to reduce the population growth rate (Bappenas, 2018). Young papaya fruit contains several phytochemical substances such as saponins, alkaloids, tannins, flavonoids, triterpenoids, and quinones (Iwuagwu et al, 2013; Nadiyah et al, 2016). The active compound in papaya is thought to be able to work as an anti-fertility compound. Joshi et al, (2011) stated that the active compounds in plants naturally have mild estrogenic properties and strong anti-estrogenic properties so that they may be used as fertility regulating agents. Exposure to anti-fertility compounds can cause zygote destruction, prevention of ovulation, fertilization or implantation. Anti-fertility compounds are thought to work on the action of the hypothalamus-pituitary-gonads so that they affect the secretion of gonadotropin hormones, the secretion of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) so that reproductive hormone synthesis in the ovaries is disrupted (Udoh, 2005). The inhibition of FSH secretion causes the de graaf follicle to experience developmental and maturity barriers, causing a decrease in embryo implantation (Sabrina, 2013). The endometrium is the innermost lining of the uterus which is the site of implantation and development of the embryo. The thickness of the mucous membrane and the vascularization of the endometrium varies according to changes in the ovarian hormones estrogen, progesterone, and pregnancy (Akbar, 2010). Punitha et al. (2015) proved that female rats given a combination of papaya pulp and papaya seed extract showed endometrial epithelial disorders, impaired endometrial folds, shrinking and a reduction in the number of uterine glands. The purpose of this study was to see the effect of giving young papaya fruit extract (Carica papaya L.) on the histological ligands of the endometrium of female rats (Rattus norvegicus). This research is an experimental study with a Post Test Only Control Group design. The thickness of the mucous membrane and vascularity in the endometrium varies according to changes in the ovarian hormones estrogen, progesterone, and pregnancy (Akbar, 2010). Punitha et al. (2015) proved that female rats given a combination of papaya pulp and papaya seed extract showed endometrial epithelial disorders, impaired endometrial folds, shrinking and a reduction in the number of uterine glands. The purpose of this study was to see the effect of giving young papaya fruit extract (Carica papaya L.) on the histological ligands of the endometrium of female rats (Rattus norvegicus). This research is an experimental study with a Post Test Only Control Group design. The thickness of the mucous membrane and the vascularization of the endometrium varies according to

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The study used a sample of 24 female rats (Rattus norvegicus) aged 2-3 months with a body weight of 200-300 grams, which were divided into 4 groups, the control group without extracting, the treatment group 1 dose 200 mg / 200 gr BW, the treatment group. 2 doses of 400 mg / 200 g BW, treatment group 3 doses of 600 mg / 200 g BW. The sample was taken by surgery. The dead female mouse was placed on the fixation board. Perform a laparotomy, identify and cut the uterus, then fix it in 10% BNF solution for 24 - 48 hours. After fixation, it was continued with the paraffin method and observations were made with the field of view to determine the thickness of the endometrium and the density of the endometrial glands.

2. Method

This research is an experimental study with a post test only control group design carried out at the Natural Material Chemistry Laboratory, Faculty of Pharmacy, Andalas University, Biomedical Laboratory and Anatomical Pathology Laboratory, Faculty of Medicine, Andalas University from April 2019 to February 2020. Data measuring endometrial thickness and endometrial gland density were analyzed first. first with the data normality test using the Shapiro Wilk test. The results of the normality test showed that the data were normally distributed (p> 0.05), then statistical analysis was carried out using the one way ANOVA test. The results of statistical tests using the one way ANOVA test showed that the effect of giving young papaya fruit extract (Carica papaya L.) on endometrial thickness with a value (p = 0.009),

3. Result and Discussion

3.1 Effect of Giving Young Papaya Fruit Extract (Carica papaya L.) on Endometrial Thickness in Female Rats (Rattus norvegicus)

In this study, a normality test of endometrial thickness was carried out using the Shapiro Wilk test, and the data were normally distributed (p> 0.05), then statistical analysis was performed using the one way ANOVA test.

Table 1

One way ANOVA test results for Endometrial Thickness of Female Rats (Rattus norvegicus) in Control and Treatment Groups

Subject Group	n	Endometrim thickness Mean ± SD	p
Control	6	438.13 ± 89.40	
Treatment 1	6	371.80 ± 44.76	0.009
Treatment 2	6	349.63 ± 103.3	
Treatment 3	6	277.20 ± 14.30	

Description: Control, without treatment

Treatment 1 (Dose 200mg / 200grBB)

Treatment 2 (Dose 400mg / 200grBB)

Treatment 3 (Dose 600mg / 200grBB)

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Table 1 above shows the results of the one way ANOVA test which shows the average endometrial thickness in the control group, namely 438.13 ± 89.40, treatment group 1 is 371.80 ± 44.76, treatment group 2 is 349.63 ± 103.3, and treatment 3 namely 277.20 ± 14.30. There was a significant difference between the control group and the treatment group 1, 2 and 3 with a value of p = 0.009 (p <0.05). To see the differences in each of these groups, a Bonferoni type Multiple Comparison test (post hoc test) was carried out as seen in Table 3.1.2.

Table 2

Multiple Comparison test (post hoc test) of Bonferoni type to Endometrial Thickness of Female Rats (Rattus norvegicus) in Control and Treatment Groups

Group	Level of Significance Endometrial Thickness			
	Control	**Treatment 1**	**Treatment 2**	**Treatment 3**
Control	-	0.765	0.279	0.006 *
Treatment 1	0.765	-	1,000	0.207
Treatment 2	0.279	1,000	-	0.587
Treatment 3	0.006 *	0.207	0.587	-

Table 2. It can be seen that the results of the Multiple Comparison test (post hoc test) of Bonferoni type showed a significant difference between the control group and the treatment group 3 (p = 0.006).

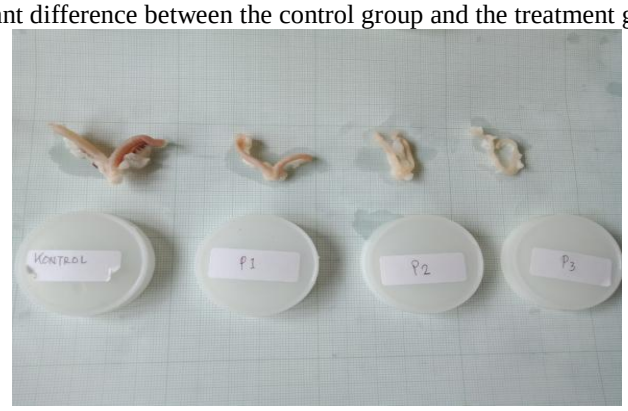

Fig 1 Rat uterus after 20 days of treatment. (K) without treatment, P1 dose 200 mg / 200gr BW, P2 dose 400 mg / 200gr BW, P3 dose 600 mg / 200gr BW

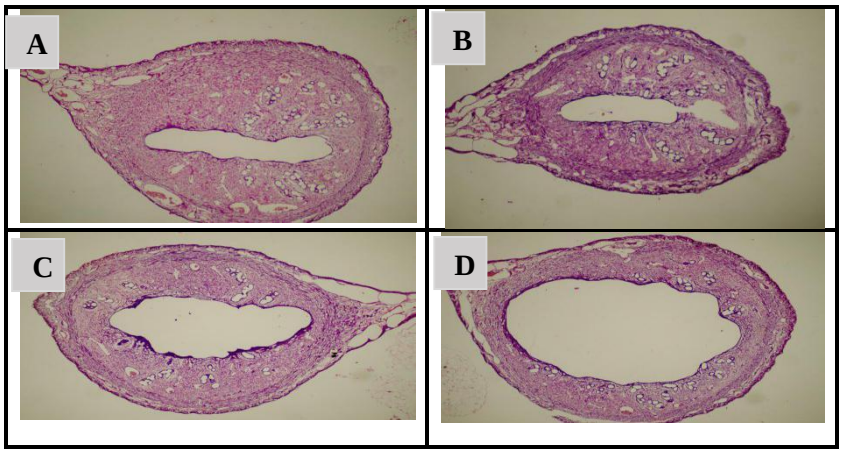

Fig 2 Microscopic Histology of Endometrial Thickness

Information :

a. Control group, without treatment
b. Treatment group 1 (dose 200mg / 200grBB)
c. Treatment group 2 (dose 400mg / 200grBB)
d. Treatment group 3 (dose 600mg / 200grBB)

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3.2 Effect of Giving Young Papaya Fruit Extract (Carica papaya L.) on Endometrial Gland Density in Female Rats (Rattus norvegicus).

The results of the normality test on the density of the endometrium using the Shapiro Wilk test obtained normally distributed data (p> 0.05), then statistical analysis was carried out using the one way ANOVA test.

Table 3

One way ANOVA test results Density of Female Rat Endometrium (Rattus norvegicus) in Control and Treatment Group

Subject Group	n	Endometrial gland density Mean ± SD	p
Control	6	25.30 ± 3.20	
Treatment 1	6	24.87 ± 3.67	<0.001
Treatment 2	6	18.17 ± 3.00	
Treatment 3	6	12.67 ± 2.36	

Table 5 above shows the results of the one way ANOVA test which shows the average endometrial gland density in the control group, namely 25.30 ± 3.20, treatment group 1 is 24.87 ± 3.67, treatment group 2 is 18, 17 ± 3.00, and treatment group 3 namely 12.67 ± 2.36. There was a significant difference between the control group and the treatment group 1,2 and 3 with a value of p = <0.001 (p <0.05). To see the differences in each of these groups, a Bonferoni type Multiple Comparison test (post hoc test) was performed as seen in table 3.2.2.

Table 4

Multiple Comparison test (post hoc test) of Bonferron type to Density of Female Rat Endometrium (Rattus norvegicus) in Control and Treatment Groups

Group	Significance Level of Endometrial Gland Density			
	Control	Treatment 1	Treatment 2	Treatment 3
Control	-	1,000	0.004 *	0,000 *
Treatment 1	1,000	-	0.008	0,000 *
Treatment 2	0.004 *	0.008	-	0.35
Treatment 3	0,000 *	0,000 *	0.35	-

Table 4 It can be seen that the results of the Multiple Comparison test (post hoc test) for the Bonferoni type showed a significant difference between the control group and treatment group 2 (p = 0.004) and treatment group 3 (p = 0.000), there was also a significant difference between treatment group 1 with treatment 3 (p = 0.000).

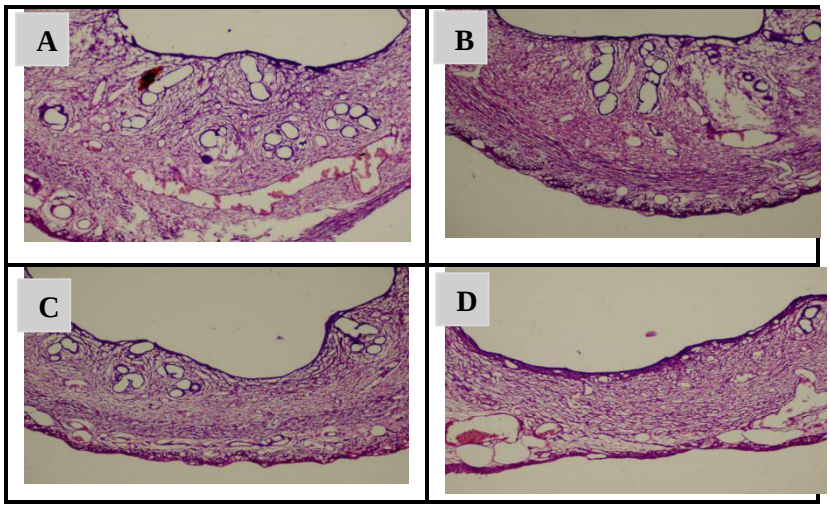

Fig 3 Microscopic Histology of Endometrial Gland Density

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Information :

a. Control group, without treatment
b. Treatment group 1 (dose 200mg / 200grBB)
c. Treatment group 2 (dose 400mg / 200grBB)
d. Treatment group 3 (dose 600mg / 200grBB)

3.3 Effect of Giving Young Papaya Fruit Extract (Carica papaya L.) on Endometrial Thickness in Female Rats (Rattus norvegicus)

The endometrium is a highly regenerative tissue that undergoes multiple cycles of cell proliferation, growth, and apoptosis as a function of the estrous cycle, pregnancy and uterine involution (Szotek et al, 2007; Gargett et al, 2008). During the estrous cycle in most animals, the endometrium shows periods of cell growth, apoptosis, and endometrial loss, without showing bleeding. The endometrium has great cell renewal capabilities (Gargett et al, 2007)

The effect of papaya extract on endometrial thickness is thought to be due to slowing growth of endometrial cells, which causes a decrease in the thickness of the endometrium. This is thought to be due to the presence of anti-proliferative compounds in the extract of young papaya (Carica papaya L.) fruit. The most significant decrease in rat endometrial thickness was found in treatment group 3 at a dose of 600 mg / g BW. So the researchers suspect that the higher the dose given, the more it affects the thickness of the endometrium.

Young papaya contains several phytochemical compounds such as saponins, alkaloids, tannins, flavonoids, triterpenoids, and quinones(Iwuagwu et al, 2013; Nadiyah et al, 2016). The phytochemical compounds in young papaya fruit extract are thought to be able to work as anti-fertility compounds. According to Hediningrat (2002), anti-fertility compounds basically work through two mechanisms, namely cytotoxic effects and through disturbing effects on the balance of the hormonal system.

The decrease in endometrial thickness is probably due to the presence of alkaloid compounds, where in addition to being estrogenic, alkaloids have toxic and antiproliferative properties against cancer cells. The antiproliferative properties of these alkaloids are thought to inhibit the proliferation process in the endometrium. Research conducted by Kataria et al (2011), proves that saponin compounds have an effect in tumor cells, which can be used as agents to control cell proliferation.

Research conducted by Odirichukwu, et al (2016), proves that pProviding methanol extract from young papaya fruit can disrupt the estrous cycle and produce an irregular cycle pattern in the treated rats. The mice showed a prolonged metestrous pattern and diestrous pattern in each cycle, thereby reducing the frequency of the estrous phase. As a result, the frequency of ovulation decreases with the result of decreased fertility. This is thought to be due to the effect of the steroid saponin compounds present in young papaya.

In line with In the research of Shivalingapa et al (2002), saponin extracts showed a significant extension of the entire estrous cycle and most importantly in the proestrous and diestrous phases. These observations confirm an imbalance in the secretion of the hormones FSH and LH, the hormones estrogen and progesterone. This imbalance can lead to infertility in mice.Disruption of the synthesis of the hormone estrogen will inhibit the proliferation of cells that make up the walls of the uterus or endometrium. In addition, the function of the hormone progesterone to support the work of estrogen, namely by thickening the endometrium and growing blood vessels in the endometrium will also be disturbed.

3.4 Effect of Giving Young Papaya Fruit Extract (Carica papaya L.) on Endometrial Gland Density in Female Rats (Rattus norvegicus)

This study shows that there are significant differences between the control group and the treatment group. It can be concluded that giving young papaya extract affects the density of the endometrial glands of female rats*(Rattus norvegicus).* So this is thought to occur because of the active compound from the extract of the young papaya fruit, Carica papaya L) which is anti-proliferative. .

The uterine glands in the endometrium are simple tubular glands that undergo changes during the estrous cycle. The action of the hormone estradiol during the follicular phase causes the proliferation of the lining of the endometrium, including the endometrial glands.Punitha et al (2015), proving that female rats given a combination of papaya pulp and papaya seed extract showed endometrial epithelial disorders, impaired endometrial folds, shrinking and a reduction in the number of uterine glands.

Muchtaromah et al (2019), in their research also showed that the presence of active triterpenoid saponin compounds from the combination of C. asiatica and P. indica extracts affected the thickness of the endometrium, myometrium, oviduct mucosal layer and oviduct smooth muscle layer and the number of glands. This is evidenced by a decrease in the thickness of the rats' endometrial gland and layer at a

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dose of 125-200 mg / kg bw.

In line with previous research conducted by Muchtaromah, it was reported that the administration of high doses of C. asiatica extract (125 mg / kg, 200 mg / kg or 275 mg / kg bw) in rats had an antifertility effect. The results did not find every follicle that reached the graafia follicle stage. As a result, theca cells in the ovarian follicles cannot produce estrogen optimally. Furthermore, low levels of estrogen inhibit the proliferation of endometrial, glandular and myometrial cells (Muchtaromah, 2011).

Whirledge and Cidlowski reported that an adult animal exposed to external estrogen precursors had high levels of estrogen in the blood. In addition, continued endogenous estrogen production will cause negative feedback to the hypothalamus to reduce FSH secretion. As a result, estrogen secretion is also reduced and affects several reproductive organs (Whirledge and Cidlowski, 2010).

4. Conclusion

Based on the results of the research the effect of giving young papaya fruit extract (Carica papaya L.) on the histology of female rats endometrium (Rattus norvegicus), it was found that the effect of giving young papaya fruit extract (Carica papaya L.) on the thickness of the endometrium of female rats (Rattus norvegicus) was found. the effect of giving young papaya (Carica papaya L.) fruit extract on the density of the endometrial glands of female rats (Rattus norvegicus)

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