

Effect of Extract Bitter (Andrographis Paniculata Nees.) of The Uterus Histological Mice (Mus Musculus L.)

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Abstract - Indonesia has a wide variety of germs that are used as a source of traditional medicines that are widely used by the community. One of the commonly used plants is bitter plants. Bitter plants have many specialty but bitter plants also have side effects that can cause undesirable effects if used in large quantities, especially in pregnant women because Bitter has chemical compounds that act as antifertility. Pursuant to this matter done research to know influence of Bitter extract to histologis uterus mouse. This research was an experimental research with complete randomized design (RAL), 4 treatments and 6 replications. The treatment used was control, dose 0,2 g / kgbb, 0,4 g / kg bb, 0,6 g / kg bw done by gavage for 12 days. Parameters observed were uterine weight, long cornua, thick endometrium, myometrium, perimetrium damage and abnormalities in uterine mice. The uterus is made of micro anatomical sedation with hematoxylin-eosin (HE) staining. Data were analyzed by variance (ANOVA) significant level 0,05 and continued with BNT test. The results showed that the Bitter extract had no effect on the weight, length of cornua, thickness, myometrium, perimetrium but effect on endometrial thickness. It can be concluded that Bitter extract can affect fertility in females.

Keywords - Extract; Bitter (*Andrographis paniculata* Nees); Mice (*Mus musculus* L.); Uterus Histology; Antifertility.

I. INTRODUCTION

Indonesia has a diverse range of herbs that are used as a source of traditional medicines and can be cultivated by the community as a live pharmacy. The traditional use of medicines is a legacy of ancestral heredity used by some communities as a traditional herb. That can treat certain diaseas. WHO has recommended the use of traditional medicine in the maintenance of public health, prevention and treatment of diseases, especially for chronic diseases, degenerative diseases and cancer. WHO also supports efforts to improve the safety and efficacy of traditional medicine [19].

The use of traditional medicine in Indonesian society is considered as an alternative because it is considered relatively cheap, affordable to all people, efficient and safer from side effects compared to synthetic drugs [11]. Traditional medicines that are widely used can come from

plants and animals. Traditional medicinal herbs are generally made from natural ingredients such as roots, tubers, rhizomes, wood, tree bark, seeds, leaves, fruit, sap, flowers or from plant extracts. Of the many plants that can be used as medicinal plants one of them is Bitter (*Andrographis paniculata* Ness).

Bitter plants (*Andrographis paniculata* Ness) one of the medicinal plants that have long been used as a traditional ingredient of herbs. Some tests of the efficacy of Bitter against diseases such as fever, gastric infections, respiratory infections, malarial fever, repellent insects, diabetes complications, protect from liver disease, antiviral [15] Overdose of this plant leads to some side effects such as nausea, vomiting and loss of appetite. [7] In addition, the herb Bitter also has side effects as anti fertility, both male and female [21]. According to Dalimartha (1999) the chemical content of Bitter is Laktone which consists of deoksi-andrographolide, andrographolide (bitter substance),

Neoandrographolide, 14-deoxy- 11,12 didehydro-andrographolide and Neoandrohomoandrographolide grafolid, 14-deoxy- 11,12didehydro-andrographolide, and homoandrographolide, also flavonoids, alkanes, aldehyd ketones, minerals (potassium, calcium, and sodium), acid grit and resin. The hallmark of this plant is the bitter taste that is caused because of andrographolida that is not present in other plants [13]. Bitter can be extracted with ethanol or methanol from all parts of the plant, leaves and stems of Bitter contain more than 20 diterpenoids and more than 10 flavonoids [5]. Of the many chemical compounds contained in Bitter compounds that act as anti fertility is andrographolide.

Andrographolid is an active substance in Bitter that serves to prevent cell division (cytokinesis) [4]. Based on Zoha's research, et al. (1989) studied the anti-fertility effects of bitter observed in female mice under different experimental conditions. When the bitter powder is mixed with animal feed at a dose of 2 grams / kgbb per day, then given to the female mice daily for 6 weeks, not one pregnant when mated with male mice. In contrast, most of the control group mice who were not given the drug became pregnant when mated to males of the same kind as in the treatment group, giving birth in normal numbers (average 5 to 6) after the next six marriages.

Based on this background, it is necessary to do research about the effect of giving Bitter extract on histology of mice uterus. This research is done considering Bitter, widely used by the community for traditional medicine such as increasing appetite, but because of the content of Bitter which has side effects as anti fertility then this research needs to be done. Therefore, research has been done to investigate the effect of the extract of Bitter (*Andrographis paniculata* Ness) on the histology of the mice's uterus.

II. REVIEW OF LITERATURE

Bitter is an annual herbaceous plant and is widely cultivated in south Asia, China and some parts of Europe. In some areas in Indonesia Bitter known by various names. The people of Central Java and East Java call it by bidara, sambiroto, sandiloto, takilo, paitan, and Bitter. In West Java called ki oray, takila, or ki purat. In Bali better known as samiroto. The people of Sumatra and most of the Malay people call it with pepaitan or ampadu [20].

Bitter habitats grow wild in the open, such as in gardens, riverside, vacant soil rather damp, or in the yard. Grows in the lowlands to an altitude of 700 m above sea level. Often

grow in groups. This plant grows in hot areas in Asia with tropical and sub tropical climates such as in India, Malaya Peninsula and Indonesia [3].

In traditional medicine the bitter is widely used to get rid of body heat, dissipate toxins from the body, prevent common cold, respiratory infections including sinusitis, fever, antidote to snake venom and insects are also useful in treating cardiovascular diseases, preventing liver toxicity, thus improving heart function and heart. Bitter is also reported to decrease fertility in animals and humans [8]. However Andrographolide has some side effects such as nausea, vomiting, loss of appetite that can only be seen in overdose [2].

Based on the research of Widyawati, (2007) Toxicology test in experimental and human animals showed that andrographolide and other compounds found in Bitter have very low toxicity. In mice given oral extract of oral (10 g / kg BW) once daily for 7 days, none of the mice died. When bitter with a dose of 500 mg / kg body weight is given for 10 days every day in mice, there is no effect on growth, appetite and fecal production. The experimental animals remain energetic and the results of their full blood count are within normal limits.

Mice have the characteristics of a small body shape, white, have a regular estrus cycle of 4-5 days. The condition of the room for the maintenance of the mice should always be clean, dry and away from noise. The maintenance room temperature should also be maintained between 18-19°C and air humidity between 30-70%. Adult female mice with age 35-60 days have weight 18-35 g. Long life 1-2 years, can reach 3 years. Reproductive period of female mice lasts 1.5 years. Female or male mice can be mated at 8 weeks of age. Length of pregnancy 19-20 days. The number of children with an average of 6-15 mice with birth weight between 0.5-1.5 g. Mice are often used in research with the consideration that these animals have several advantages, ie, regular and detectable cycles, relatively short periods of pregnancy, and have many children and there is alignment of growth with the human condition [1].

The uterus or uterus is the right and left oviduct enclosure shaped like a pear and its lower portion is called the cervix [9]. The mice uterus has a different shape than the human uterus. The uterus of mice belongs to the duplex type, where there are two uteus horns with one cervix in each cornua. The type of uterus is an adjustment for the reproduction of children in large quantities [16] The shape of the uterus resembles the letter "Y" with a very short stalk. The largest part of this tissue is a muscle, with the outer wall

being longitudinal and composed of smooth muscle fibers. While the inner wall in the form of circular and also composed by smooth muscle fiber [12].

III. METHODOLOGY

The research will be carried out from January - March 2018 preparatory preparations are carried out in FMIPA UNP Zoological laboratory and histology of uterine mice readings (*Mus musculus* L.) will be done in Anatomical Pathology Laboratory of UNAND Medical Faculty. This research type is experiment with research design in the form of Completely Random Design (RAL).

1. Tools And Materials

The tools used are: scales, blenders, digital analytics scales, Erlenmeyer, 500 ml beaker glass, measuring cups, Petridis, water bath, desiccators, hotplate, animal cage, a set of surgical instruments, syringe, vials, objects, glass cover, brush, microscope, measuring cup, gavage needle, knife, incubator, microtome, stopwatch, refrigerator.

The ingredients used are: dry Bitter, female mice test animal, mice food, aqua bides, 1% CMC (Carboxy Methyl Cellulose), 0.9% NaCl, methanol, eosin solution, haematoxylin solution, Bouin solution, wooden kettle, filter paper, paper label, tissue, paraffin, alcohol 70%, 80%, 90%, 95%, absolute alcohol, Xylol, tap water, aquades, Entellan.

A. procedure

2. Preparation of test animals

Animals used were female mice (*Mus musculus* L) with adult age of 8-11 weeks of mice placed in a rectangular plastic cage with size 30 cm (p) x 20 cm (l) x 10 cm (t) covered with wire and given a wooden cushion that is replaced twice a week. Feed and drinking water are given on ad libitum.

3. Creating Extracts

Bitter (*A. paniculata*) which has been dried selected leaf and branches, then smoothed with a blender, so that the form of dry powder. For the manufacture of bitter extracts made by taking 100 grams of powder Bitter then soaked in methanol as much as 200 ml incubated for 48 hours. After 48 hours methanol added 200 ml. Further filtered by using filter paper so that filtrate (sari) obtained. To produce a concentrated extract, Filtrate was evaporated in a waterbath at 50 ° C. Then dried using incubator to stabilize with temperature 30°C. Bitter extract in the form of a solid plate, pollinated by grinding in a pestle and stored in a desiccator.

4. Preparation of CMC solution 1%

Sedian 1% CMC solution is made by sprinkling 1 gram of CMC into 1000 ml of aquadest, then left for about 15 minutes until it is clear and shaped like a gel. Further stirred to become a homogeneous mass.

5. Phase Determination

Preparing cotton buds, glass objects, eosin and microscope to be used for vaginal pillows. Insert the cotton buds into the vaginal opening to get the mucus, then apply the mucus to the glazed object and given the eosin dye. Afterwards look at the vaginal pillow preparation with a microscope to determine the phase.

6. Mouth Uterus Preparation Mice (*Mus musculus* L.)

Preparation of uterine preparations by paraffin method in the following order:

a. Network retrieval

The tissue taken is the uterus of the mouse

b. Fixation

Network preservation (fixation) is one of the processes selected to preserve the sampling tissue. In addition to preserving the network for a while, also aims to prevent damage to structures and active components of the network. The mice uterus is inserted into a glass vial that has been filled with bouni's solution and leave for 24 hours.

c. Dehydration

Dehydration is a process of withdrawal of water present in the sample tissue using 70% alcohol solution for 3 days, 80% alcohol, 90%, 95% for 1 day (24 hours), and absolute I, absolute II, absolute III for 1 hour . by soaking the sample tissue that has been stored in the tissue box or tissue box

d. Clearing

The material used for xylol cleansing, with immersion for 30 minutes to 1 hour.

e. Infiltration

The process of replacing the purifying medium with a growing medium of paraffin which has been melted in the incubator. Commonly used paraffins are paraffins whose melting point is 480-500C Paraffin soft (Soft) and 580 - 600C Hard paraffin (Hard).

f. Embedding

Planting (Embedding), split from paraffin 3 tissue planted on pagoda cover that had previously been smeared glycerin

and filled paraffin hard. The tissue is grown and filled in paraffin hard and placed at room temperature so that the paraffin hardens. Once it was closed the pagoda was moved into the refrigerator to harden, after hardening the paraffin was cut according to the block used.

g. Sectioning

Paraffin blocks which have been cooled in the refrigerator then taken and attached to the clamp block (block holder) microtome. Then arranged and directed alignment with the cutting surface of the blade microtome. Paraffin block position adjustment by regulating lever clamp blocks that exist in harmony microtome to obtain the cutting position with 4 μ m cutting thickness. Selected pieces were good and then inserted into a water bath which has been filled with distilled water and danganasakan with 38-40oC temperature. After entering the network into the water bath, the network will expand and tissue taken using a glass slide, and labeled in accordance with the original paraffin blocks. Further preparations dried over a hotplate.

7. Staining

Perfomed tissue stained pigment with hematoxylin-eosin, then do Deparafinition using Xylol I, Xylol II for 3 minutes, and Xylol III for 5 minutes. Next Rehydration using Absolute Alcohol I, Absolute Alcohol II, Absolute Alcohol III, for 3 minutes. Next Alcohol 95%, Alcohol 90%, Alcohol 80% For 3 minutes, Alcohol 70% for 5 minutes. Laundry uses tap water. Subsequently sedan was inserted into lartan eosin for 7 minutes and then tap water. The next step is dehydration to dissolve water on the tissue using 70% alcohol, 80% alcohol, 90% alcohol of absolute alcohol I every few seconds, absolute alcohol II for 1 minute, absolute alcohol III, xylol I, xylol II, xylol III During 3 minutes. After doing this step-stage then preparat ready for mounting. Conducted under a microscope and perform documentation. (Sumarmin, 2015).

8. Data Analysis

Data obtained from the research results were analyzed by analysis of variance or analysis of variance (ANOVA) alone. If the F count > F table then followed by LSD (Least Significant Difference) with a 5% significance level. While the histological features in the description presented in the form of photographs.

IV. RESULTS AND DISCUSSION

A. Research Results

The observation of the effects of extracts of bitter against the weight, length cornua, the thickness of the endometrium, myometrium, perimetrium in the mouse uterus is as follows:

1. Data on average weight and length of the uterine cornua

Table 1: Data on the effect of the extract of bitter mouse uterus weight and length for 12 days

Treatment	The average weight of the uterus $\pm$ SD(g)	Mean $\pm$ SD uterine cornua length (cm)
Control	0.292 $\pm$ 0.078	3.629 $\pm$ 0.462
P1 (0.2 g)	0.249 $\pm$ 0.067	3.486 $\pm$ 0.508
P2 (0.4 g)	0.364 $\pm$ 0.096	3.857 $\pm$ 0.657
P3 (0,6 g)	0,267 $\pm$ 0,045	3,829 $\pm$ 0,175

Based on data of control group weight and length of uterine cornua have biggest average 0,292 and 3,629. then a decrease in the treatment to-1 to equal to 0.249 and 3.486. In the 2nd treatment increased the weight of the mice's high uterus by 0.364 and 3.857. Then there was the decline in the 3rd treatment of 0.267 and 3.829. From the data it is the largest average on the weight, and the length of uterine cornua at treatment 2 with the use of dose 0,4g/kb bb.

2. The mean histologic data of the uterus

Tabel 2. Data Average thickness of the endometrial, myometrial and uterine lining of the uterine mice after administration of bitter extract for 12 days

Perlakuan	Rata-Rata ketebalan endometriu m $\pm$ SD (μ m)	Rata-Rata ketebalan miometriu m $\pm$ SD (μ m)	Rata-Rata ketebalan miometriu m $\pm$ SD (μ m)
Kontrol	335,691 $\pm$ 54,007b	117,646 $\pm$ 24,717	111,963 $\pm$ 23,257
P1 (0,2 g)	273,546 $\pm$ 36,143ab	60,3971 $\pm$ 10,472	82,9371 $\pm$ 9,429
P2 (0,4 g)	263,54 $\pm$ 27,632ab	81,7114 $\pm$ 14,005	112,189 $\pm$ 24,400
P3 (0,6 g)	213,503 $\pm$ 33,678a	75,0771 $\pm$ 75,077	99,0429 $\pm$ 24,315

Description: The number followed by the same letter in the same column did not differ significantly according to the BNT test at $\alpha = 5\%$.

Based on Table 2, it shows that the mean thickness of the endometrium, myometrium and perimetrium decreased compared to the average in the control group. Can be seen in figure 1

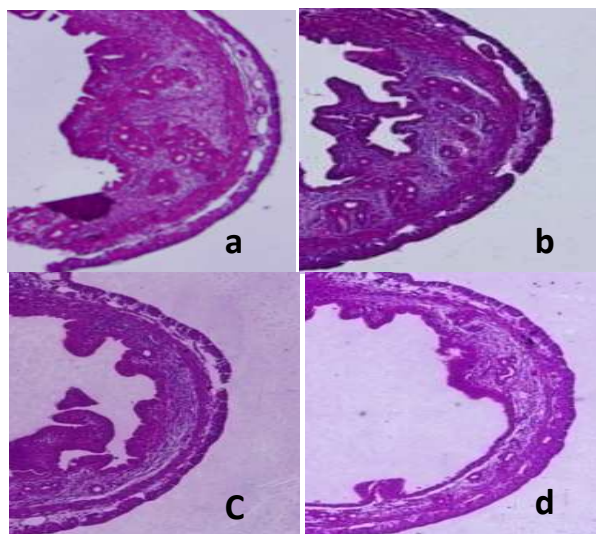


Figure 1. Uterine cross sections showing the thickness of the endometrium, myometrium and perimetrium. Magnification 4 x 10. Description: a. Control (CMC 1%), b. P1 (0.2 g / Kg bb), c. P2 (0.4 g / Kg bb), d. P3 (0.6 g / Kg bb).

B. Discussion

After doing research with giving of Bitter extract to female mice for 12 days with dose 0.2 gr / Kg bb, 0.4 gr / Kg bb and 0.6 gr / Kg bb no effect on uterine weight, uterine cornua length, myometrial thickness, perimetrium but effect on endometrial thickness.

At the thickness of the endometrium can be seen in the table the higher the dose given the more endometrial layer will be depleted, at a dose of 0.6 g / kg bb which more affect the thickness of the endometrium, this is because karsha andrographolide content suspected as an active ingredient antifertilitas in Bitter which can inhibit the occurrence of pregnancy. In addition to andrographolide, the content of flavonoids in Bitter is also suspected to inhibit pregnancy, where the flavonoid group is one of phytoestrogens, it is known that natural estrogen is not only found in animals or humans, but estrogen-like compounds are also found in some plants that are usually called phytoestrogens. flavonoids can affect the production of FSH and LH by pituitary gland. Flavonoids will inhibit the pituitary to remove FSH and LH so that the acid properties in the cervix are maintained.

FSH and LH are hormones that play a role in the reproductive cycle. As described by leny dkk (2012) in her study of interactions by oral contraceptive drugs, the hormones that regulate the menstrual cycle are estrogen and progesterone. Levels of these two hormones are controlled by the Gonadotrophin Releasing Hormone (GnRH) derived from the hypothalamus, to send signals to the pituitary gland, and the pituitary gland is stimulated to excrete Follicle Stimulating Hormone (FSH) and Lutenizing Hormone (LH).

In the Bitter plants also found the existence of alkaloid compounds, saponins, tannins and tripenoids work based on cytotoxic effects that interfere with the development of both ovum cell cells in the ovaries so that the hormone synthesis of progesterone and estrogen will also be disturbed or cells composing the endometrium and myometrial layer. Hormonally, the administration of bitter extract at 0.4g / kg bb triggers the production of FSH (Follicle Stimulating Hormone) by the hypothalamus, thus stimulating the development of follicular granulosa cells that eventually produce estrogen. Increased estrogen levels will trigger endometrial tissue growth by the proliferation of endometrial cells. However, if the dose is given too much or less than 0.4 g / kg bb, phytoestrogens will be antagonistic to estrogenic reactions.

Based on the data presented in the table, it was found that in the control and treatment of 2 doses of 0.4 g / kg bb there was an increase in mean on uterine weight, long cornua, myometrial thickness and perimetrium significantly compared with treatment to 0.2 g / kg bb and treatment to 3 0.6 g / kb bb. At the 1st and 3rd treatments there is a decrease in the average weight of the uterus, the length of cornua, thickness, myometrium, and perimetrium. Improvement in control and treatment of 2 is thought to be caused by the active compound of the bitter extract which can affect the weight of the uterus, the length of cornua, the perimetrium and the endometrium. Presumably, the presence of triterpenoid saponin compounds at low doses of Bitter can increase cell proliferation in endometrial tissue. Besides an increase in control and treatment of P2 dosage of 0.4 g / kbbb, there was a decrease in uterine weight, length of cornua, myometriuml thickness and perimetrium at treatment of 0.2 g / kb bb and treatment of 0.6 g / kb The bb is allegedly derived by compounds derived from the bitter extract which can increase the levels of estrogen in the blood. It is possible that endogenous levels of endogenous hormone in blood are still high because of the mice that normal mice use, so that endogenous estrogen production is still ongoing.

Uterus is one of the female reproductive organs that develop due to the influence of regulation of estrogen hormone in the body. The effect of this estrogen hormone because the uterus has estrogen receptor. The uterus is an organ composed of three layers: the endometrium, the myometrium and the perimetrium. The three most noticeable layers affected by the hormone estrogen is the endometrial layer characterized by the thickening of the coating (Novalinda, 2017).

The endometrial layer is composed of loose connective tissue, endometrial gland and columnar epithelium. Endometrial glands become one of the determinants of endometrial thickness, because the gland undergoes differentiation and proliferation. The endometrium has two developmental phases: proliferation and secretion. The proliferative phase is characterized by an increase in the thickness of the endometrial layer as the number of estradiol hormones increases during the proestrous period until the estrous time. The secretion phase occurs during the matestrous period until the diestrous period marked by secretion from the gland due to the influence of progesterone hormone. The end of the secretion phase is the occurrence of death or necrosis of the endometrium because the spiral artery wall contracts, thus closing the bloodstream and causing ischemia. Novalinda, (2017).

histological damage to the uterus, the endometrium seen in kelenjer, presented in Figure 2

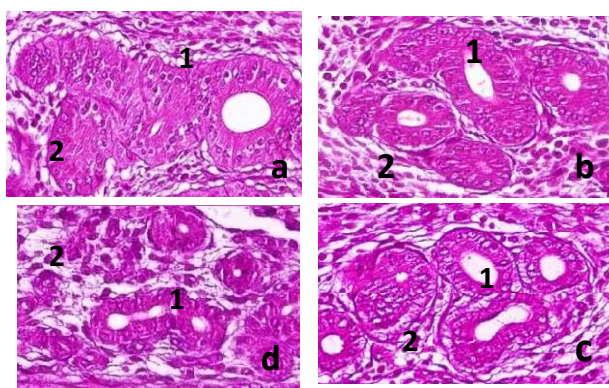


Figure 2. Transverse slices uterus Shows Breakdown in Kelenjer endometrium. 4x10 magnification. a) Control, b) (0.2 g / kg bb), c) (0.4 g / kg bb), d) (0.6 g / kgbb). Description 1. Gland 2.

Based on histological observation of the damage to the endometrium kelenjer, visible changes in the histologic structure kelenjer endometrium after treatment compared

with controls, as shown in Figure 10 kelenjer in control with lobular structure, winding, high columnar epithelium with a central core. In each treatment appears kelenjer simple tubular, kelenjer small size, with low cuboidal epithelium.

Furthermore, based on the histology of the mouse uterus wall can be seen in figure 3

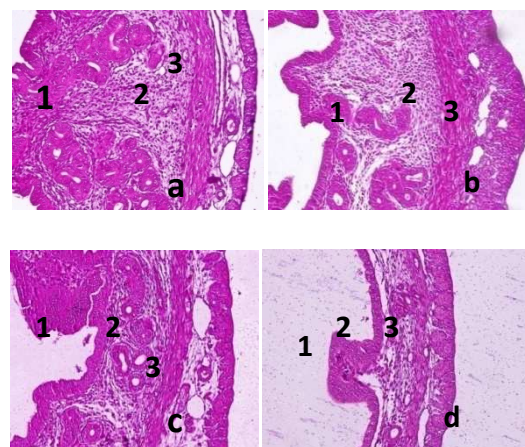


Figure 3. Damage to the endometrial, myometrial and perimetrial layers. a) control b) Dose 0.2 g / kg bb c) Dose 0.4 g / Kg bb d) Dose 0.6 g / Kg bb. Description 1) Endometrium, 2) Myometrium, 3) Perimetrium .

Based on histologic observation of damage to the thickness of the endometrial layer, myometrium, and perimetrium can be seen the occurrence of damage to the lining of the endometrium, myometrium, and perimetrium. There was noticeable decrease in thickness after treatment on endometrial, myometrial, and perimetrial lapsian compared with controls. In each treatment can be seen in the 3rd treatment with a dose of 0.6 g / kg BW looks thinner endometrium thickness.

From the results of this study can be seen that the observation of endometrial thickness showed a significant decrease. Whereas in the thickness of myometrium, perimetrium, uterine weight of mice, the length of uterine cornua did not occur significantly. The cause of the results is not influential on the weight of the uterus, the length of cornua, the thickness of the myometrium and the perimetrium because the dose given to the test animals is not enough to give effect. Furthermore, Bitter extract can also damage the lining of the endometrium wall.

Bitter plants can be developed as a female contraceptive. The antipertility mechanism of the bitter extract is suspected of cytotoxic andrographolide on the bitter leaf. The best dose that can be used as an antifertility drug is at a dose of

0.2 g / kg bb because at a dose of 0.2 has already shown a decrease in uterine weight, long cornua, endometrial thickness, myometrium, and perimetrium. This result is similar to that of Sumarmin et al against male mice (2018), which states that bitter extract (*Andrographis paniculata* Nees.) Decreases the number of ejaculated mice (*Mus musculus* L.) spermatozoa and decreases the number of normal sperm. Based on the results of the test is known that the use of 0.2 g dose most in reducing the number of ejaculatory spermatozoa, and bias used as an anti-fertilizer dose.

Mice used in this study were mice (*Mus musculus* L) in diestrus phase. The diestrus phase is the longest phase in the estrus cycle. In the diaphoric phase of the endometrium thickens, the uterine gland grows, and the uterine muscle shows an increase in progression. This change is shown to supply food substances to the embryo in the event of pregnancy. This condition will continue during pregnancy and the corpus luteum will be maintained until the end of pregnancy [18].

V. CONCLUSION

Based on the research that has been done, it can be concluded that the treatment with the Bitter extract has no effect on the weight of the uterus, the length of cornua, the thickness of the myometrium and the perimetrium but has an effect on the thickness of the endometrium.

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