

# The Influence of Ethanol Extract of Leaves on Dragon Scales (*Pyrrosia piloselloides*) to the Histology of The Uterus Female Rats *in vivo*

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**Abstract**— The diversity of plants found in Indonesia is vast, and many are used for traditional medicine. One such plant is dragon scale (*Pyrrosia piloselloides*), which contains phytoestrogen compounds, such as flavonoids. These phytoestrogens can bind to estrogen receptors, producing estrogenic effects similar to endogenous estrogen. Therefore, a study was conducted to investigate the effect of ethanol extract of dragon scale leaves (*Pyrrosia piloselloides*) on the histology of the uterus of female white rats (*Rattus norvegicus*) *in vivo*. The research was experimental, using a completely randomized design (CRD). The treatments were grouped into six groups, each consisting of four female white rats. The treatments included a control group (no treatment) and rats given ethanol extract of dragon scale leaves (*Pyrrosia piloselloides*) with five different doses: 0 mg/200g body weight (P1), 25 mg/200g body weight (P2), 50 mg/200g body weight (P3), 75 mg/200g body weight (P4), 100 mg/200g body weight (P5), and 125 mg/200g body weight (P6). After administration, the rats' uterine organs were collected and prepared for histological observation with hematoxylin and eosin (HE) staining. The observed parameters were the number of endometrial glands and the thickness of the uterine layers. The results showed that the ethanol extract of dragon scale leaves (*Pyrrosia piloselloides*) affected the increase in the number of endometrial glands at doses of 25 mg/200g body weight up to 50 mg/200g body weight, but a decrease in the number of endometrial glands was observed at doses of 75 mg/200g body weight up to 100 mg/200g body weight. Meanwhile, the ethanol extract of dragon scale leaves (*Pyrrosia piloselloides*) did not affect the thickness of the endometrium, myometrium, and perimetrium in the histology of the female white rat uterus *in vivo*.

**Keywords**— Ethanol extract of dragon scale leaves (*Pyrrosia piloselloides*), uterus, glands, endometrium, myometrium, perimetrium, *in vivo*.

## I. INTRODUCTION

The biodiversity in the region of Indonesia is incredibly diverse and most of them are utilized for traditional medicine by the ancestors of the Indonesian. Traditional medicine not only follows the development of the times, but has become a lifestyle.

Indonesia is one of the mega biodiversity countries known as a warehouse of medicinal plants. It is estimated from around 30,000 types of flora in the tropical forests of Indonesia, there are around 9,600 species that are known to have medicinal properties. From these number, 283 species are recorded as important medicinal plants for the

traditional medicine industries. Raw materials for traditional medicine can be obtained from various types of plants that contain several compounds that are used by humans as a means of health. One of these plants is dragon scales (*Pyrrosia piloselloides*).

Dragon scale leaves are applied by the community for traditional medicine, including as a bleeding stopper, laxative, anti-inflammatory, cough medicine, antiseptic and antibacterial [1], and used as a breast cancer drug [2]. It is known that the application of traditional medicine derived from dragon scale leaves has several active compounds that can be utilized for various human needs. Hariana [2] and Dalimartha [3] stated that dragon scales leaves contain essential oils, flavonoids, alkaloids, tannins, sugars, saponins, triterpenoid-steroids, and polyphenols. One of these active compounds has the potential as a fertility regulator. The active constituents in plants naturally have mild estrogenic properties and strong anti-estrogenic properties so that they have the potential to be aimed as fertility regulators. Various bioactive substances in plants, especially the steroid, alkaloid, isoflavonoid, triterpenoid and xanthone compounds, have properties as fertility regulators.

The potential of dragon scale leaf extract on the reproductive system of female animals can be observed from the regularity of the estrus cycle of test animals. This is because the estrus cycle describes the fluctuation of reproductive hormones in the animal's body and occurs repeatedly, marked by physiological and behavioral changes. These physiological changes will affect various reproductive organs, one of which is the uterus.

The uterus consists of 3 layers, namely the endometrium (tunica mucosa-submucosa), myometrium (tunica muscularis) and perimetrium (tunica serosa). The endometrium is the layer that is most responsive to the varying circulating levels of the hormones. Its cyclic structural changes in this layer can be used as an indicator of hormonal fluctuations and various other changes [4]. The changes that occur can be influenced by exposure to active compounds from plants that have mild estrogenic properties and strong anti-estrogenic properties so that they have the potential as the fertility regulators. Isoflavones from the flavonoid, saponin and alkaloid groups can stimulate the formation of estrogen in mammals, and from their structure there are similarities to the hormone estrogen. According to Gruber et al. [5], the estrogenic

effect is initiated by the bond between phytoestrogens and estrogen receptors, resulting in activation of estrogen receptors.

An *in vivo* study of the administration of dragon scale extract carried out on female mice needs to be investigated. Thus, the effect of administering dragon scale leaf extract containing active constituents as a fertility regulator on the reproductive status of female mice can be evaluated by the uterine histology. This refers to several researches that have the same compounds in the form of flavonoids in dragon scale using other plants as fertility regulators. This study is expected to find the right dose that has the potential as antifertility or a fertility support in female mice which can later be converted into a dose for humans.

## II. MATERIALS AND METHODS

This study is experimental research using a Completely Randomized Design (CRD). The treatments were grouped into 6 groups, with each group consisting of 4 female white rats. The treatment groups were as follows:

- P1 (Na-CMC 0.5%)
- P2 (Na-CMC 0.5% + Dragon Scale Extract dose 25mg/200g BB)
- P3 (Na-CMC 0.5% + Dragon Scale Extract dose 50mg/200g BB)
- P4 (Na-CMC 0.5% + Dragon Scale Extract dose 75mg/200g BB)
- P5 (Na-CMC 0.5% + Dragon Scale Extract dose 100mg/200g BB)
- P6 (Na-CMC 0.5% + Dragon Scale Extract dose 125mg/200g BB)

The tools and materials used included: female white rats, dragon scale leaves, prostaglandin hormone (Capriglandin brand), Na-CMC 0.5% (Carboxy Methyl Cellulose), distilled water, 10% formalin, ethanol, paraffin, running tap water, xylene, Canada balsam, Mayer's albumin, 1% hematoxylin and 1% eosin, Giemsa stain, animal housing cages, stomach tube, glassware set, microscope slides, cover slips, analytical scale, stopwatch, Buchner funnel, rotary evaporator, 100 ml volumetric flask, 10 ml measuring cylinder, 50 ml beaker glass, 500 ml beaker glass, 500 ml Erlenmeyer flask, glass stirrer, hot plate, glass funnel, dropper pipette, surgical instruments, Nikon E binocular microscope, and computer microscope.

**Test Animals and Estrus Cycle Synchronization** The test animals used were 24 female white rats. Before being treated, they underwent estrus cycle synchronization using 10 IU PMSG hormone. This hormone was administered via intraperitoneal injection at a dose of 0.2 ml (10 IU). After hormone administration, the estrus cycle was checked 17 hours later.

### A. Estrus Cycle Observation

The estrus cycle was determined by examining vaginal smears stained with Giemsa. The estrus phase was determined by observing the ratio of nucleated epithelial cells, cornified epithelial cells, leukocytes, and mucus in the vaginal smear.

### B. Treatment Administration

The results of estrus phase observation were used as an indicator for oral administration of dragon scale extract according to the treatment group.

### C. Sample Collection

After the rats were given the dragon scale extract according to the specified doses, the estrus phase was checked on day 16. The rats were then euthanized, and their uterus organs were collected. Histological preparations were made with a thickness of 5  $\mu$ m and stained with HE stain.

### D. Measurement of Endometrium, Myometrium, and Perimetrium Thickness

The thickness of the endometrium, myometrium, and perimetrium was measured by determining the layer that directly borders the uterine lumen up to the boundary between the endometrium and myometrium layers using the Image Raster application. Measurements were taken starting from the lumen edge to obtain the layer thickness value. The thickness of the endometrial layer was obtained from the average of four measurements taken from the upper, lower, right, and left parts of the endometrium. The observations were made under a microscope with 4 x 10 magnification.

### E. Counting Endometrial Glands

The number of endometrial glands was determined by counting the glands in each uterine section. The observations and counting were done by sorting based on the position of the glands in each uterine slice. Glands that had already been counted in previous sections were not counted again in subsequent sections. New cross-sections of glands in the next slice were counted as new glands. This process was carried out from the beginning to the end of the organ sections.

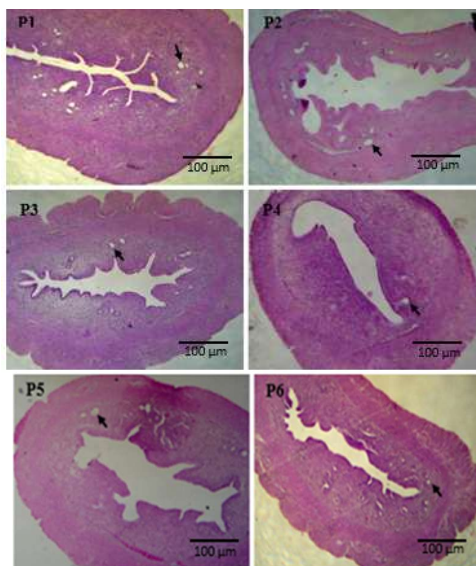
### F. Data Analysis

The obtained results were tested for normality and homogeneity, and then analyzed using One-Way ANOVA at a 5% significance level. If significant differences were found, further testing was done using the BNT 5% test.

## III. RESULT AND DISCUSSION

### A. The Effect of Dragon Scale Leaf Extract (*Pyrrosia piloselloides*) on the Number of Endometrial Glands in Female White Rats (*Rattus norvegicus*) *In vivo*

The visualization of uterine tissue shows the number of endometrial glands in the different treatment groups, which can be seen in Figure 1.



**Figure 1.** Cross-Section of the Uterus Showing the Number of Endometrial Glands. Magnification 4 x 10. Note: P1 (NaCMC 5%), P2 (25 mg/200g BW), P3 (50 mg/200g BW), P4 (75 mg/200g BW), P5 (100 mg/200g BW), P6 (125 mg/200g BW). The arrow (→) indicates the endometrial glands.

Observations of this parameter were conducted to determine the condition of the uterus in rats for each treatment group. Each treatment group displayed uterine glands located in the endometrial layer, tubular in shape, opening at the surface, and representing invaginations of the epithelium [6]. Based on the images of cross-sections of the female white rat's uterus, differences in the number of glands across the treatments were observed. The glands observed had characteristics as described by Akbar [6], being tubular with a hollow lumen. The control treatment (P1) and the 50 mg/200g BW dose (P3) showed a higher number of glands compared to the other treatments. In contrast, the 100 mg/200g BW dose (P5) and 75 mg/200g BW dose (P4) showed fewer glands than the other treatments.

Endometrial glands are a key factor in determining the thickness of the endometrial layer because these glands undergo differentiation and proliferation. The number of endometrial glands influences the thickness of the endometrial layer, as these glands change throughout the estrus cycle. The observed effect is attributed to the presence of phytoestrogens in the dragon scale leaf extract, which was administered in different doses across the treatment groups. The first treatment (P1) as the control is categorized as normal because it did not receive the dragon scale leaf extract. The 50 mg/200g BW dose (P3) treatment showed a significant increase in the number of endometrial glands.

**TABLE 1.** SUMMARY TABLE OF BNT 5% REGARDING THE EFFECT OF DRAGON SCALE LEAF EXTRACT (PYRROSIA PILOSELLOIDES) ON THE NUMBER OF ENDOMETRIAL GLANDS IN FEMALE WHITE RATS (RATTUS NORVEGICUS) IN VIVO

| Treatment            | Average $\pm$ SD   | Notation |
|----------------------|--------------------|----------|
| Dose 100 mg/200gr BW | 15 $\pm$ 6         | A        |
| Dose 75 mg/200gr BW  | 16,25 $\pm$ 8,5    | A        |
| Dose 25 mg/200gr BW  | 17,25 $\pm$ 7,37   | A        |
| Dose 0 mg/200gr BW   | 20,50 $\pm$ 5,07   | A        |
| Dose 125 mg/200gr BW | 27,33 $\pm$ 6,66   | Ab       |
| Dose 50 mg/200gr BW  | 39,25 $\pm$ 15, 54 | B        |
| BNT 5% = 13,73       |                    |          |

**Note:** Different notations indicate significant differences.

The results show the differences in the effects of dragon scale leaf extract on the number of endometrial glands in the uteruses of female white rats. The best treatment in this observation was the 50 mg/200g BW dose, as indicated by the highest average number of endometrial glands, marked with notation ( $\alpha=0.05$ ) as b. This treatment shows a significant difference in the number of glands between the 50 mg/200g BW dose and the 0 mg/200g BW, 25 mg/200g BW, 75 mg/200g BW, and 100 mg/200g BW doses. However, there was no significant effect on the 125 mg/200g BW dose. Furthermore, Table 4.2 also shows that at doses between 25 mg/200g BW and 50 mg/200g BW, the dragon scale leaf extract increased the number of endometrial glands. On the other hand, the extract at doses of 75 mg/200g BW to 100 mg/200g BW decreased the number of endometrial glands, suggesting its potential use as an antifertility agent.

#### B. The Effect of Dragon Scale Leaf Extract (*Pyrrosia piloselloides*) on the Thickness of Endometrium, Myometrium, and Perimetrium in the Histology of the Uterus of Female White Rats (*Rattus norvegicus*) In vivo

The results of uterine tissue visualization showing the thickness of the endometrium, myometrium, and perimetrium across the different treatment groups after 15 days of dragon scale leaf extract administration can be seen in the figure 2.

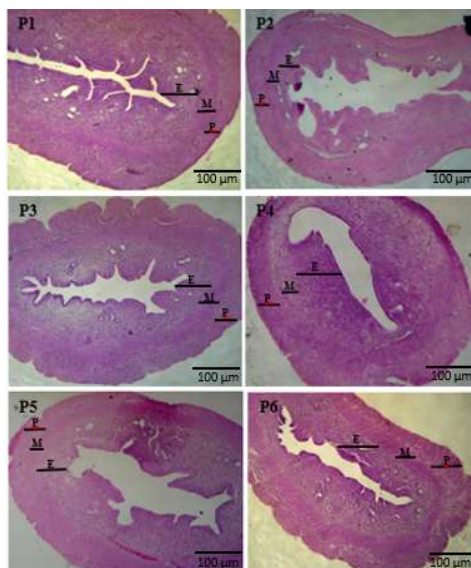


Figure 2. Cross-Section of the Uterus Showing the Thickness of the Endometrium, Myometrium, and Perimetrium. Magnification 4 x 10. Note: P1 (NaCMC 5%), P2 (25 mg/200g BW), P3 (50 mg/200g BW), P4 (75 mg/200g BW), P5 (100 mg/200g BW), P6 (125 mg/kg BW). (E: Endometrium; M: Myometrium; P: Perimetrium)

Based on the observations of cross-sections of the uterine organ in Figure 2, the structure of the uterus from all treatment groups is visible. The uterine structure in all groups contains the endometrium, myometrium, and perimetrium, with a clean lumen. The endometrium in P1, P4, and P6 appeared thicker and bordered by a relatively narrow lumen compared to the other treatments (P2, P3, and P5). The myometrium layer in P1, P3, and P6 appeared thicker than in P2, P4, and P5. The outermost uterine layer, the perimetrium, in P6 was the thickest compared to the other treatment groups. The changes observed in the three uterine layers were caused by the administration of dragon scale leaf extract, which led to structural changes in the cells of each layer. These structural changes could affect the ability of embryo implantation, especially in the endometrium. The next observation was to measure the thickness of each layer in the treatments.

The observations showed that the thickness of the uterine layers, in descending order, was: endometrium, myometrium, and perimetrium. However, this was not observed in the 75 mg/200g BW (P4) and 125 mg/200g BW (P6) treatments, where the perimetrium was thicker than the myometrium.

The results of the One-Way ANOVA test on each parameter showed that the calculated F value was less than the F table value, meaning that the administration of the combination of dragon scale leaf extract did not significantly affect the thickness of the endometrium, myometrium, or perimetrium. This might be due to the low doses of the treatment given to the test animals, which were insufficient to induce significant changes in the thickness of the uterine layers.

Phytoestrogens have a lower affinity for estrogen receptors compared to estradiol. The phytoestrogen content

in dragon scale leaf extract at certain doses could cause both antagonistic and agonistic effects, such as inhibiting the growth and development of follicles, thus reducing the production of endogenous estrogen. This results in many estrogen receptors in the target cells, particularly in the uterus, not binding with estrogen. The absence of estrogen receptors due to a lack of endogenous estrogen is filled by phytoestrogens that bind to estrogen-beta receptors, which are then translocated to the cell nucleus, causing protein synthesis and cellular responses [7].

The response caused by the binding of estrogen receptors and phytoestrogens is weaker than the binding of estrogen receptors with endogenous estrogen. The competition between phytoestrogens and endogenous estrogen in the body causes cellular responses such as thickening of the uterine layers, which do not occur optimally [8]. The treatment with dragon scale leaf extract (*Pyrrosia piloselloides*) in this study did not significantly affect the thickness of the uterine layers, including the endometrium, myometrium, and perimetrium. This may be due to the insufficient phytoestrogen content in the dragon scale leaf extract to produce a significant response in increasing the thickness of the uterine wall.

#### IV. CONCLUSION AND SUGGESTION

Based on the results of the research conducted, it can be concluded that ethanol extract of dragon scale leaf (*Pyrrosia piloselloides*) has an effect on increasing the number of endometrial glands at doses of 25 mg/200g BW to 50 mg/200g BW, but there was a decrease in the number of endometrial glands at doses of 75 mg/200g BW to 100 mg/200g BW. However, ethanol extract of dragon scale leaf (*Pyrrosia piloselloides*) did not significantly affect the thickness of the endometrium, myometrium, and perimetrium in the histology of the uterus of female white rats in vivo.

Several suggestions for improving future research include the need for identification and measurement of the active compounds in the ethanol extract of dragon scale leaf using spectrophotometry. In addition, dose changes should be considered, such as increasing the dose beyond 125 mg/200g BW, with a wider interval between doses exceeding 25 mg/200g BW.

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