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Citation: [AIP Conference Proceedings](#) **1844**, 020003 (2017); doi: 10.1063/1.4983414

View online: <http://dx.doi.org/10.1063/1.4983414>

View Table of Contents: <http://aip.scitation.org/toc/apc/1844/1>

Published by the [American Institute of Physics](#)

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# Effect of Water Decoction of Langsat Bark (*Lansium domesticum* Corr.) on Estrous Cycle and Uterus Weight in Mice (*Mus musculus* L.)

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**Abstract.** The study aims to determine the effect of water decoction of Langsat's bark stew on estrous cycle and uterus weight in mice. Twenty adults, healthy, female mice were divided into four groups, each given 1 mL of Langsat's bark stew with water concentrations of 20%, 40%, and 60%, as well as a control group that was given water ad libitum for twelve days. Estrous phases were determined every day; on the thirteenth day, all mice were euthanized and the uteruses were weighed. The observation of estrous cycles showed that there was no significant difference in the frequency of the phases of the estrous cycle between the control group and the treatment group. Uterine weight treatment groups were higher than in the control group in line with increasing doses, but this was not statistically significantly different. We conclude that water decoction of Langsat's bark stew does not affect the estrous cycle and weight of the mice uterus, and its use as an anti-fertility treatment has not been proven.

## INTRODUCTION

High population growth is still a problem in Indonesia. The Central Bureau of Statistics in Indonesia announced that the Indonesian population in 2010 was more than 237 million people with a growth rate of 1.49 percent/year.<sup>1</sup> To control the growth of the population, the Indonesian government has developed Family Planning Programs. Various kinds of contraception are offered to the public, but none of are fully effective and safe to use. This is a challenge for researchers to find anti-fertility drugs for high population growth which are safe and effective.

Indonesia is rich in biodiversity that can be used as herbal medicines. One of the herbal medicines is Langsat (*Lansium domesticum* Corr.). Langsat (*Lansium domesticum* Corr.) of the family Meliaceae is commonly grow in the lowlands. Various parts of this plant can be used as a drug. Lansiolides triterpenoids were isolated from the bark of Langsat, which can be used as an anti-malarial drug<sup>2</sup> and as an antifeedant ladybird beetle (*Epilachna vigintioctopunctata*) larvae.<sup>3</sup> Langsat fruit peel showed antimicrobial<sup>4,5</sup> and anti-mosquito activity<sup>6</sup>. Water decoction of the Langsat's bark mixed with Angsana's bark (*Pterocarpus indica* Willd.) was used to treat dysentery. Powdered bark was used to treat scorpion stings.<sup>3</sup>

Empirically, water decoction of Langsat's bark stew has been used by rural communities of Borneo as an anti-fertility drug. However, until now there have been no scientific reports to prove it. The study aims to determine the effect of water decoction of Langsat's bark stew on the estrous cycle and uterus weight in mice.

## **MATERIALS AND METHODS**

### **Preparation of Water Decoction of Langsat Bark**

Bark of Langsat obtained from Kota Bangun village, Kutai Kartanegara district of East Kalimantan. One hundred grams of the inner bark of Langsat was boiled with 1 L of water until 250 mL of water remained. The resulting water decoction was filtered and was considered a 100% concentrate. To obtain a water decoction with a concentration of 60%, 40% and 20%, 100% concentrate was diluted with water.

### **Animals**

Female mice were obtained from the Anatomy Laboratory of Faculty of Mathematics and Natural Sciences of Mulawarman University. Their age was 3 months, and the average weight was 28 g. Before the treatment was given, mice were maintained for 1 week in the laboratory. The mice were divided into 4 groups with 5 replicates and maintained under uniform husbandry conditions with natural photoperiod, humidity, temperature (28±2) °C and free access to food and water. The treatment I was used as a control by giving 1 ml distilled water/mouse. Animals in treatments II, III, and IV were given Langsat's water decoction at concentrations of 20 %, 40%, 60%, respectively. The treatment was given at the same time each day for 12 days.

### **Determination of the estrous cycle and weighing uterus**

Estrous cycle was observed by making vaginal smears every day for 12 days during treatment. Vaginal smears are made by rinsing the vagina with a sterile saline using a glass pipette. That recovering solution containing the cells are placed on a preparation slide, fixed with methanol, stained with methylene blue, drained and and observed by using light microscopy. Cells descriptions were used to classify mice based on the stage of the estrous cycle (proestrus, estrus, and diestrus metestrus). Thirteen days after the treatment, mice from each treatment were euthanized and the uteruses were weighed.

### **Data Analysis**

Estrous cycle and uterus weight data were expressed as the mean ±Standard Deviation. One-way analysis of variance (ANOVA) were used to determine difference among groups. Comparisons with P<0.05 were considered to be statistically significant. If there was a significant difference, Fishers LSD was used.

## **RESULT**

### **Estrous Cycle**

The length of the estrous cycle in mice was 4-5 days. In this study, observation of estrous cycles was performed over 12 days, meaning that there will be 2-3estrous cycles. Figure 1 shows the difference in frequency stages of the estrous cycle all treatment groups. Diestrus stage frequency in all treatment groups appears to be lower than the control group, but the statistic analysis showed that there was no significant difference between the control group and all treatment groups. The same was seen in the frequency of proestrus stage. Estrus stage frequency in T II group is highest and the lowest is T III group. Metestrus stage frequency in control group appears lower than the all treatment groups. Statistic analysis results for the estrus stage and metestrus stage also showed no significant difference between the control group and all treatment groups

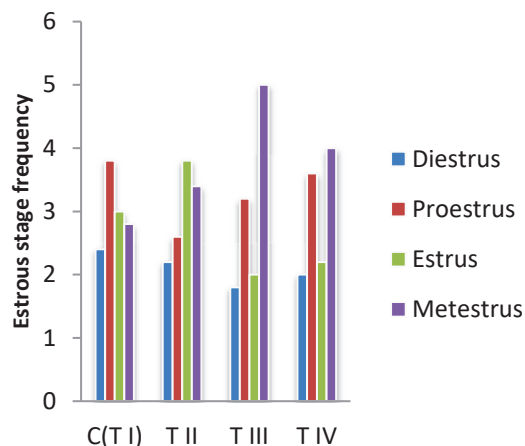


Figure 1. Frequency of appearance of estrous stage for 12 days  
 C(T I) = Control group; T II= concentrate 20% ; T III = concentrate 40%; T III = concentrate 60%

### Uterus Weight

The weight of the mice uterus after treatment on day 13 is illustrated in Table 1. Weight of the uterus of mice from all treatment groups appear higher than the control group. Uterus group treated concentrates 40% and 60% have the same weight. But overall there was no statistical difference.

Table 1. Mean of mice uterus weight after treatment on 13<sup>th</sup> day

Treatment (1 mL/day)	Uterus Weight (grams)
Control(distilled water)	0.05 ±0.025
20% concentrate	0.06 ±0.009
40% concentrate	0.07 ±0.009
60% concentrate	0.07 ±0.009

The values are expressed as mean ± the standard deviation, P<0.05 significantly different compared to control (distilled water)

### DISCUSSION

The reproductive cycle in female mice is known as the estrous cycle. The length of the cycle ranges from 4-5 days. The estrous cycle consists of proestrus, estrus, metestrus and diestrus. Ovulation occurs between the proestrus and estrus phase.<sup>7</sup> This cycle can easily be observed by vaginal smear method. There were changes in the cells making up the epithelial lining of the vagina during every phase of the estrous cycle. Proestrus stage characterized by a predominance of nucleated epithelial cells. These cells may appear in clusters or individually. Occasionally, some cornified cells may appear in the sample. This stage corresponds to the pre-ovulatory day when E2 increases and consequently, during the night, LH and FSH surge and ovulation occurs. Estrus stage is distinctively characterized by cornified squamous epithelial cells, which occur in clusters. There is no visible nucleus; the cytoplasm is granular; and the shape is irregular. Metestrus phase characterized by cornification of epithelial cells and leukocytes. In diestrus phase found nucleated epithelial cells, leucocytes and mucus.<sup>8,9</sup>

This study was conducted to confirm the use of empirical society as anti-fertility drugs. A phytochemical cause antifertility in females by acting as (a) Estrous Cycle Disruptors (b) Anti-estrogenic agents (c) Anti-Implantation agents or (d) Abortifacient agent.<sup>10</sup> Pregnancy takes a function of normal estrous cycle, any interruption in the estrus cycle will show the effect of a compound antifertilitas. Thus, the estrous cycle serves as a surrogate marker and has been frequently used to evaluate the impact of any antifertility agents.<sup>11</sup> Estrous cycle can be disrupted if a compound affects the hormonal system that controls the functions of the ovary and the estrous cycle.<sup>12</sup> Estrous cycle is a process that is controlled by various hormones, either hormones of the hypothalamus-pituitary and ovarian. These hormones

are released hypothalamic GnRH, LH and FSH are released anterior pituitary and estrogen produced by the cells of ovarian follicles.<sup>13</sup>

One of the chemical constituents of Langsat's Bark is Lansiolides triterpenoids.<sup>2</sup> The triterpenoid compounds have effects similar to estrogen.<sup>14</sup> Estrogen induces the proliferation and cornification of epithelial cells. High estrogen levels in the estrus phase cause thickening and cornification of the vaginal epithelium.<sup>15</sup> In this study, there was no significant difference in the appearance of the estrous cycle during treatment with water decoction of Langsat's bark (Fig. 1). Probably, boiling Langsat's bark for too long is suspected to cause a loss of the active substances contained in it, so that treatment did not have an effect on the estrous cycle.

The condition of the uterus is strongly influenced by the stage of the estrous cycle. The estrous phase has high estrogen levels, thus stimulating uterine cells to proliferate and increase the weight of the uterus.<sup>16</sup> If an anti-fertility substance can suppress estrous cycle, it indirectly can also reduce the weight of the uterus. Treatment with water decoction of Langsat's bark had no effect on inducing proliferation, so it did not give a significant effect on uterine weight in mice (Table 1). Some of the compounds found in plants are useful as anti-fertility include steroids, flavonoids and alkaloids.<sup>17</sup> There are no reports that the Langsat's bark containing these components, so use as a drug anti-fertility not appropriate.

## SUMMARY

Water decoction of Langsat bark (*Lansium domesticum* Corr.) was used as an anti-fertility by rural communities in East Kalimantan. This study aims to test the truth of the empirical data. The results of this study showed no significant effect of water decoction of Langsat's bark on the estrous cycle and uterine weight in test animals. Thus, the water decoction of Langsat bark as an anti-fertility is not proven, so it is not recommended for use.

## ACKNOWLEDGMENT

The author would like to thank Prof. Dr. Ir. Estri Laras Arumingtyas, M.Sc.St. for her advice on manuscripts in English class. Thanks also to Mulawarman University for providing research facilities.

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