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The Effect of Cucumber (*Cucumis Sativus*) Feeding on the Estrus Cycle in Female Mice (*Mus musculus*)

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Abstract

Cucumbers (*Cucumis sativus*) are plants that contain bioactive compounds such as flavonoids and phytoestrogens that resemble the hormone estrogen, so they have the potential to affect the reproductive system. One of the indicators of the balance of reproductive hormones in female animals is the etrus cycle. An imbalance of the hormones estrogen and progesterone can cause changes in the duration of the etruscan cycle. This study aims to determine the effect of cucumber extract administration on the estrus cycle of female rats (*Mus musculus*). This study is an experimental study with Complete Random Design (CRD). A total of 9 female rats were divided into three groups, namely the control group, the treatment group with a dose of 200 mg/kg body weight, and the treatment group with a dose of 300 mg/kg body weight. Cucumber extract is administered orally for 15 days. Observation of the estrus cycle is carried out daily by the vaginal swab method. The data were analyzed using a one-way analysis of variance test. The results showed that the average duration of the estrus cycle of the control group was 4.8 days, the 200 mg/kg BB dose treatment group was 6.1 days, and the 300 mg/kg BB dose treatment group was 4.6 days. The results of statistical analysis showed that the administration of cucumber extract did not have a significant effect on the estrus cycle of female mice ($p > 0.05$). It can be concluded that administration of cucumber extract at doses of 200 mg/kg BB and 300 mg/kg BB did not have a significant effect on the estrus cycle of female rats, although there is a tendency to alter the length of the estrus phase in some individuals.

Keywords: *Cucumis sativus*; Estrus Cycle; *Mus Musculus*.

INTRODUCTION

Indonesia is known as a country with very high biodiversity and has great potential as a source of natural herbal medicinal ingredients. LIPI 2021 states that Indonesia has around 15,000 species of plants that have the potential to be used as medicines, but only about 7,000 species are used as raw materials for medicine. One of the plants that is easy to find and often consumed by the community is cucumber (*Cucumis sativus*), which in addition to being used as a food ingredient is also known to have various health benefits. Cucumbers contain secondary metabolite compounds in the form of alkaloids, phenolics, flavonoids, steroids, terpenoids, and saponins that act as natural antioxidants (He et al., 2023). The content of vitamin C and flavonoids in cucumbers is able to capture free radicals so that they have the potential to maintain the body's physiological balance. In addition, cucumbers are also hypotensive and have a high water content, so they play a role in maintaining hydration and electrolyte balance in the body (Christine et al., 2021). The high water content and bioactive compounds are indirectly suspected to affect the reproductive system through hormonal regulation mechanisms in the body.

The reproductive system in female mice is characterized by the presence of the estrus cycle which is the main physiological indicator in mammalian reproductive activity. The estrus cycle is controlled by the hormones estrogen and progesterone which work in a balanced manner (Lee et al., 2021). In one estrus cycle, there are four main phases, namely proestrus, estrus, metestrus, and diestrus, which take place sequentially and repeatedly (Purday, 2025). The proestrus phase is characterized by an increase in estradiol hormone due to follicle development, the estrus phase is the peak period of fertility when ovulation occurs, the metestus phase is the post-ovulation phase affected by the hormone progesterone, while the diestrus phase is the longest phase characterized by the dominance of the progesterone hormone before the cycle returns to the proestrus phase (Lee et al., 2021). The change in these phases can be observed through the vaginal smear method based on the characteristics of the epithelial cells seen in each phase.

Cucumbers contain phytoestrogen compounds derived from flavonoids and Phytoestrogens, although in relatively low amounts compared to other plants. Phytoestrogens have a similar structure to the hormone estrogen so that they are able to bind to estrogen receptors in the body and affect the balance of reproductive hormones (Helppi et al., 2020). The content of flavonoids, terpenoids, and saponins in cucumbers is also suspected to have biological activity that can affect the regulation of the hormones estrogen and progesterone (Fernandez-Valdivia et al., 2010). Thus, cucumber consumption has the potential to have an effect on the regulation of the estrus cycle in female mice, both in maintaining the stability of the cycle and causing the elongation or shortening of certain phases. However, until now, research on the effect of cucumbers on the estrus cycle is still very limited, compared to studies on other plants with higher phytoestrogen content.

Based on these conditions, the problem in this study arises from the suspicion that the content of bioactive compounds in cucumbers, especially flavonoids and phytoestrogens, can affect the balance of reproductive hormones that play a role in regulating the etruscan cycle. According to previous research, Wang et al., (2025) the content of flavonoids and phytoestrogens in cucumbers has an effect that can prolong the proestrus phase by slowing down the maturation of follicles and slowing down the metestrus phase. This study was conducted experimentally using the vaginal smear method to observe the change in the phase of the estrus cycle after the administration of cucumber extract with two dose variants. This study aims to determine the effect of cucumber administration on the estrus cycle of female mice.

MATERIALS AND METHODS

Study Design

This research included experimental research carried out from May to June 2025 by making a preparation for vaginal smear at the UPT Integrated Laboratory of Sebelas Maret University of Surakarta. This research protocol had been approved by the Health Research Ethics Committee of the Dr. Moewardi Regional General Hospital Surakarta with letter number 1.322/VI/HREC/2025.

Materials

The tools used in testing the effect of cucumbers on the estrus cycle of mice were lumpang alau or blender, gauze, measuring cups, bottles (for cucumber extract containers), oral probes, glass objects, cover glass, microscopes, cotton buds, mice cage. The materials used in testing the effect of cucumbers on the estrus cycle of mice are fresh cucumbers, 70% alcohol, Methylene blue, aquades, husks (wood powder/rice), mouse feed, this study used 9 female mice weighing 25 grams (0.025 kg) aged three months.

Procedures

Making Cucumber Extract

Cucumbers used were approximately 1125 mg or 112.5 ml. Cucumbers were crushed by grinding them using a pestle or blender, making extracts do not need to add water because cucumbers already contain water, after that strain the pulp with gauze and copy it into a bottle. Treatment 1 mice were given at a dose of 200 mg/kg BW while Treatment 2 mice were given at a dose of 300 mg/kg BW.

Preparation of Animals

The animals used were female mice aged three to four months with a body weight of 27-33 grams, in a physically healthy condition, not deformed, and in a condition of not pregnant. Before starting the study, the mice were acclimatized for 15 days, aiming for the mice to be able to adjust to their environment. For 15 days, mice were given enough food and drink every day and husk (wood powder/rice) which was changed regularly for three days.

Treatment of Animals

Treatment of animals was carried out after the completion of the acclimatization period for 15 days. Mice are divided into three groups, namely

- Control (C): No cucumber extract was given, but only enough food was given.
- Treatment 1 (T1) : Cucumber extract is given at a dose of 200 mg/kg BW
- Treatment 2 (T2): Cucumber extract is given at a dose of 300 mg/kg BW

To determine the dosage based on body weight, the test animals were weighed daily, but the mice's weight data was taken once every two days. Feeding and feeding time is routinely carried out every day, namely in the afternoon for 15 days, and husk replacement was also routinely carried out so that rats do not get stressed and die. Cucumber administration was carried out orally using an oral probe by attaching it to the upper palate of the rat, then slowly inserting it into the esophagus.

Estrus Cycle Observation

Observation of the estrus cycle in mice was carried out using the vaginal smear method. Vaginal smear was obtained by applying cutton buds that have been moistened with aquades on the vagina of female mice that had been treated repeatedly. Leave the glass object and cover glass with 70% alokohol, then this vaginal smear was placed on the glass object by applying the results of the vaginal smear in one direction. Drip methylene blue with a dropper pipette on a glass object, leave it for a while, then water it with aquades and dry it.

After that, observe the estrus cycle under a microscope with the appropriate magnification, then document, then identify the estrus cycle that occurs. Observation of the estrus cycle in mice was carried out by randomly selecting mice in each treatment group. Observation 16 was carried out daily after giving treatment to mice for 15 days.

Data Analysis

After the preparation of the vaginal smeal, the next phase in the estrus cycle was identified by classifying the forms of nucleated epithelial cells, horned epithelial cells, and leukocytes. In the proestrus phase it was characterized by rounded intermediate epithelial cells and there is a nucleus that is located in the middle of the cell and does not have leukocytes (SEi), in the estrus phase it was characterized by epithelial cornelization cells where the cells are large but nucleated very small or without nuclei and many epithelial cells that are horned, the metestrus phase was characterized by the presence of cornecized epithelial cells and many leukocytes. The metestrus phase is characterized by the presence of horned epithelial cells (HEc), nucleated epithelial cells (NEc) and leukocytes (L), and the diestrus phase was characterized by the presence of nucleated epithelial cells (NEc) and leukocytes (Fatmala et al., 2022). After identifying the phases in the etruss cycle, the data obtained in this study was tested quantitatively, then analyzed the data obtained using the one-way analysis of variance test. The Research Design used is CRD (Complete Random Design) using experimental tests. The test calculation uses SPSS. The data obtained from this study would be analyzed using the one-way analysis of variance test to determine the significant differences between treatment groups on the observed variables, with the significance level used being $p < 0.05$. If the test results show a significance value of less than 0.05, then it can be concluded that there is a real effect of cucumber extract administration on the parameters studied. Conversely, if the p value ≥ 0.05, then there is no significant difference between the treatment groups. This test is suitable for use because 20 there is only one treatment factor, namely the administration of cucumber extract with two different doses.

RESULTS AND DISCUSSION

Length of Estrus Cycle

The analysis of the length of the estrus cycle was carried out by calculating the average length of the estrus cycle of each individual mouse in Three groups. The length of the estrus cycle is calculated starting from the estrus phase to before the next estrus phase. The higher the dose of treatment given, the longer the etruscan cycle. The following is a table of calculations of the average length of the etruss cycle.

Table 1. The average length of a mice's estrus cycle is 15 days.

Length of Estrous Cycle ($\bar{x}$±SD)	Number of Repetitions			Total	Average
	1	2	3		
C (Control)	5,5	4,6	4,3	14,4	4,8 ± 0,62
T1 (200 mg/kg BW mice)	6	4,3	8	18,3	6,1 ± 1,85
T2 (300 mg/kg BW mice)	4,3	4	5,5	13,8	4,6 ± 0,79

Note: Data presented as mean ($\bar{x}$) ± standard deviation (SD)
BW = Body Weight

The data showed that the T1 group had the highest average estrus cycle length (6.1 days), followed by the K group (4.8 days) and the T2 group as the lowest (4.6 days). This indicates that the treatment in the P1 group had the greatest influence on the estrus cycle prolongation compared to the other groups. Then the data was carried out with a one-way analysis of variance test because this study only used one treatment factor, namely the administration of cucumber extract with two different doses. Before the one-way analysis of variance test, it is mandatory to carry out a normality test, then when the normal normality test (>0.05) is followed by a homogeneous test when the homogeneous test states homogeneous data (>0.05), it can be continued with the one way analysis of variance test test. The following is a table of one-way analysis of variance test results.

Table 2. One-way analysis of variance test estrus cycle.

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	6.069	2	1.362	1.362	.325
Within Groups	13.367	6			
Total	19.436	8			

The results the analysis of variance test showed an F value of 1.362 with a significance value (Sig.) of 0.325, which is greater than 0.05. This value shows that different treatments in each group do not have a

noticeably different effect on the observed variable. In other words, the treatment of each group did not cause a significant change in the outcome of the Number variable.

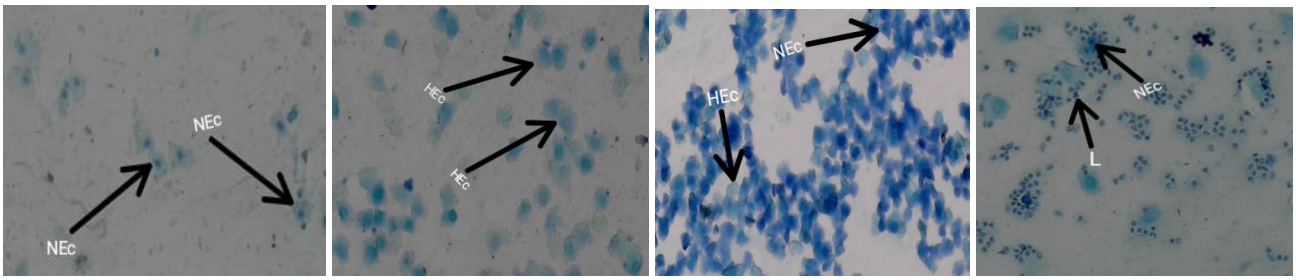

Figure 1. Phases of the Estrus Cycle.
Note: (A) Proestrus phase; (B) Estrus phase; (C) Metestres phase; (D) Diestrus Phase; (Nec) Nucleated Epithelial Cells; (Hec) Horned Epithelial Cells; (L) Leukocyte.

Body Weight of Mice

Weighing the weight of mice is carried out once every two days. Another focus of this observation of the weight of the mice is to find out the extent to which the variation in cucumber extract treatment affects the difference in the average weight of the mice observed in each group. The data collection of the weight of these mice was taken before and after treatment with a span of two days for 15 days. The following is a table of the average weight of mice.

Table 3. Analysis Body Weight of Mice.

Treatment	Initial treatment (x̄+SD)	Final treatment (x̄ +SD)	Body weight changes
C (Control)	29,6 ± 0,58	32 ± 1,0	+ 2,4
T1 (Cucumber extract dosage 200 mg/kg BW)	30,6 ± 1,15	31,3 ± 0,57	+ 0,7
T2 (Cucumber extract dosage 300 mg/kg BW)	29,6 ± 1,52	28,3 ±4,7	-1,3

Note: Data presented as mean (x̄) ± standard deviation (SD)
BW = Body Weight

The weight data of mice showed clear differences between treatment groups. The Control Group (C) experienced the highest weight gain (+2.4 g), reflecting normal growth. The T1 treatment (cucumber extract dose of 200 mg/kg BW) still caused weight gain (+0.7 g), but it was much lower than control. In contrast, the T2 treatment (cucumber extract dosage of 300 mg/kg BW) was the only group that showed average weight loss (-1.3 g). This indicates that higher doses of cucumber extract (T2) have the most significant effect in suppressing or reducing the weight of mice. The research on the weight of mice was also carried out a one-way analysis of variance test. The following are the results of the one-way analysis of variance test of mouse weight.

Table 4. One-way analysis of variance test weight mice.

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	16202.667	2	8101.333	.458	.653
Within Groups	106235.333	6	17705.889		
Total	122438.000	8			

The results of the analysis of variance test of the weight of mice showed an F value of 0.458 with a significance value (Sig.) of 0.653, which is greater than 0.05. This value shows that different treatments in each group do not have a noticeably different effect on the observed variable. In other words, the treatment of each group did not cause a significant change in the outcome of the Number variable.

Discussion

Physiologically, the normal estrus cycle in female mice lasts for 4–5 days with a regular sequence of proestrus, estrus, metestrus, and diestrus phases (Pestana & Graham, 2024). Freeman (2006), also asserts that the stability of the sequence and duration of the estrus cycle reflect healthy endocrine conditions in untreated animals. This is reinforced by Ajayi & Akhigbe (2020), who stated that mice with normal physiological status will

maintain a stable pattern of estrus, and that cyclical changes generally only occur when there is exposure to substances that affect hormonal balance. Based on this theory, the stability of the estrus cycle in the control group in this study showed that the mice were in normal physiological conditions without the presence of hormonal disorders. The changes in the estrus cycle that occur in the treatment group can be explained by the content of bioactive compounds in cucumbers. According to Okafor et al (2023), the flavonoids and phytosterols in cucumbers have activities that resemble estrogen and progesterone. Physiologically, progesterone acts as a hormone that inhibits reproductive readiness by suppressing the release of estrogen and inhibiting the work of FSH and LH hormones from the pituitary gland, resulting in slower follicle maturation (Fatmala et al., 2022). This mechanism causes the proestrus phase to become longer and the transition to the next phase is delayed, which further impacts the elongation of the metestrus phase. This condition explains the prolongation of the proestrus and metestrus phases in treated mice. In addition, according to (Narulita et al., 2017), the diestrus phase is characterized by high levels of progesterone which plays a role in maintaining the condition of the uterus after ovulation. If progesterone levels remain high, then the transition to the next phase of proestrus will be delayed, so ovulation is also inhibited and the diestrus phase becomes longer. Therefore, the administration of cucumbers has the potential to have an antiovulatory effect on rats through increasing or maintaining progesterone levels in the body. The phytoestrogenic activity of cucumbers also plays a role in the change in the etruss phase. Persistent estrus can occur when estrogen levels persist for a longer period of time than normal so that the estrus phase lasts longer and its frequency increases (Goldman et al., 2007). The imbalance between estrogen and progesterone, specifically estrogen dominance, will hinder the normal transition to the metestrus and diestrus phases (Nelson, 2018). On the other hand, very high estrogen levels can also speed up the proestrus phase so that it lasts very short and is difficult to detect through vaginal smear (Conneely & Lydon, 2000). This explains why in some estrus cycle patterns the proestrus phase is not always clearly visible.

Physiologically, the proestrus phase occurs due to an increase in the hormone estrogen which plays a role in follicle maturation and ovulation preparation. The elongation of the proestrus phase indicates that estrogen levels persist longer than normal conditions so that the transition to the estrus phase is delayed (Nalbandov, 1990). This condition is strongly suspected to be strongly influenced by phytoestrogen compounds from cucumbers that have estrogen-like activity, thereby prolonging the dominance of estrogen in the body of mice (Taylor, 1994).

The changes that arise at different doses of treatment are also in line with the concept of the optimal dose effect, that is, at a given dose the body of the test animal provides an optimal hormonal response in regulating the estrus cycle (Minasian et al., 2014). However, if the dose is too high, the active compounds in cucumbers can actually cause hormonal imbalances that play a role in the reproductive cycle (Korn et al., 2023). This suggests that administration of cucumbers at certain doses can still maintain hormonal balance, but at higher doses it has the potential to cause disruption of the estrus cycle pattern.

The weight stability of control mice occurred due to the absence of treatment so that physiological balance and homeostasis mechanisms were maintained (Ariens et al., 1986). In the treatment group, low amounts of additives are generally neutralized by the metabolic system so that they do not trigger significant changes in weight (Deny, 2008). Mild weight loss in mice is thought to be related to the high content of water and cucumber fiber which can reduce appetite and energy intake (Lu, 1995). while at high doses it can accelerate

The use of energy reserves due to increased metabolism or mild electrolyte disorders, with variations in responses between individuals affected by the initial condition of the body, the efficiency of nutrient absorption, and genetic factors (Triwahyuni et al., 2019).

CONCLUSIONS

The use of cucumber extract in this study did not have a significant effect on the difference in the length of the estrus cycle of mice between groups. Although, there were changes in the estrus cycle in the treatment group 1 which was longer compared to the control group. However, the changes in the estrus cycle in group 2 tended to be faster than in the Control group. This happened because Treatment 2 received a dose of cucumber extract that exceeded the optimal dose triggering a more significant hormonal imbalance than Treatment 1. At these higher doses, cucumber phytoestrogen compounds have the potential to cause strong estrogen dominance that would drastically disrupt hormonal feedback mechanisms. As a result, instead of prolonging the dose cycle, it results in an acceleration of the cycle average or triggers a different endocrine response. Meanwhile, in the weight parameters, the treatment with the highest dose caused a weight loss of 1.3 grams. However, the weight loss cannot be said to be a statistically significant difference.

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