International Journal of Research and Review
[Indexed, Peer Reviewed Journal]
Volume 12; Issue: 1; January 2025
Website: www.ijrrjournal.com
Research Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Estrogenic Activity of Purple Sweet Potato (*Ipomoea batatas* L.) Ethanol Extract on the Estrous Cycle of Wistar Rats in a Menopause Model

I Putu Bintang Pradita Kirana[1], Ida Ayu Dewi Wiryanthini[2], I Wayan Gede Sutadarma[3], Ni Wayan Tianing[2]

[1]Undergraduate of Medicine Study Program, Faculty of Medicine, Udayana University, Bali, Indonesia,
[2]Department of Biochemistry, Faculty of Medicine, Udayana University, Bali, Indonesia,
[3]Department of Clinical Nutrition, Faculty of Medicine, Udayana University, Bali, Indonesia.

Corresponding Author: I Putu Bintang Pradita Kirana

DOI: https://doi.org/10.52403/ijrr.20250139

ABSTRACT

Menopause is a condition in which menstruation ceases due to ovarian follicles no longer producing estrogen. This condition can reduce the quality of life by causing physical and psychological disturbances. While hormone therapy is commonly used, its side effects necessitate alternative approaches, such as phytoestrogens derived from purple sweet potato anthocyanins. This study evaluates the estrogenic activity of ethanol extract from purple sweet potato (*Ipomoea batatas* L.) on the estrous cycle of menopausal Wistar rats. A post-test-only control group design was employed using preserved biological materials. The study sample consisted of 5 rats per group, with a total of 3 groups: a negative control group (aquadest), an intervention group with ethanol extract of purple sweet potato at a dose of 400 mg/kgBW/day, and another intervention group at a dose of 600 mg/kgBW/day. Observations were conducted using a microscope to count the average number of cells in 10 fields of view. Subsequently, the estrous phases were identified to determine the duration of the estrous cycle. The data were analyzed using one-way ANOVA and Kruskal-Wallis tests. The results showed p-values for the duration of the estrous cycle, proestrus phase, estrus phase, metestrus phase, and diestrus phase as 0.289 ($p>0.05$), 0.368 ($p>0.05$), 0.310 ($p>0.05$), 0.174 ($p>0.05$), and 0.929 ($p>0.05$), respectively. Despite the lack of statistical significance, descriptive variations in cycle duration were observed among groups. It can be concluded that there is estrogenic activity from the ethanol extract of purple sweet potato on the estrous cycle of Wistar rats in a menopause model.

Keywords: Estrous cycle, Menopause, Phytoestrogen, Purple sweet potato

INTRODUCTION

Women naturally experience the cessation of their menstrual cycle, known as menopause. The end of the menstrual cycle leads to a decline in estrogen levels in the body during menopause. This decrease in estrogen causes vaginal dryness, mood changes, and vaginal atrophy.[1] Additionally, menopause can reduce women's quality of life due to physical and psychological disorders.[2]

Unlike humans, who experience menstrual cycles, most other female mammals undergo an estrous cycle. The estrous cycle is a periodic physiological process influenced by reproductive hormones.[3,4] Signs of estrous in

animals include swelling and redness of the vulva, decreased appetite, and clear vaginal mucus.[3] Along with aging, female rats experience a phase called estropause, marked by irregular estrous cycles and prolonged cycle duration.[5] Rats can also exhibit estropause patterns similar to human menopause when undergoing ovariectomy, which reduces estrogen and progesterone levels, mimicking the hormonal changes observed in humans.[6] Therefore, using rats as a research model facilitates studying the physiological mechanisms of menopause relevant to human conditions.

One of the management strategies for decreased estrogen during menopause is hormone therapy. This therapy is the most effective method for reducing menopausal symptoms.[7] However, hormone therapy carries the risk of uterine hyperplasia and cancer due to unopposed estrogen or excessive estrogen stimulation.[8,9] A safer alternative therapy for managing estrogen decline during menopause is the use of phytoestrogens.[8]

Phytoestrogens are compounds with chemical structures similar to estrogen, exhibiting estrogen-like activity. They have antioxidant, antimutagenic, and antiangiogenic properties. Phytoestrogens are a safe alternative to hormone therapy because they do not elevate the risk of blood clots in postmenopausal women.[10] One natural food source of phytoestrogens is purple sweet potato, which contains anthocyanins.[11]

Purple sweet potato (*Ipomoea batatas* L.) plays an essential role in global food and energy security. This crop is widely cultivated on 60 million hectares worldwide, yielding over 860 million tons in 2019.[12] In Indonesia, sweet potato is a common and affordable crop found in many markets, ranking fourth as a carbohydrate source after rice, corn, and cassava. It is rich in carbohydrates and calories and contains anthocyanin compounds.[13] The accessibility of purple sweet potato in Indonesia makes it an economical and sustainable alternative.

Anthocyanins are a subgroup of flavonoids proven to have antioxidant and phytoestrogenic activity. Sugiritama et al. investigated the impact of ethanol extract from purple sweet potato on the mRNA expression of estrogen receptor alpha (ERα) and superoxide dismutase (SOD) in a menopausal animal model, finding that purple sweet potato possesses phytoestrogen and antioxidant activity.[11] Another study found that phytoestrogens in purple sweet potato ethanol extract exhibit estrogenic activity and have the potential to replace estrogen hormones.[14] In addition to being used for menopause therapy, phytoestrogens from purple sweet potato can enhance animal productivity through estrous cycle management. Extending the estrous cycle via phytoestrogens can increase reproductive opportunities in animals, positively impacting the livestock industry. Prolonged estrous cycles and phases can be utilized for animal breeding by extending the mating phase duration and increasing the probability of mating success.[15]

The decline in estrogen levels during menopause can cause physical and psychological disorders that affect women's quality of life. Hormone therapy carries side-effect risks, necessitating safer alternatives such as phytoestrogens. Purple sweet potato, which is rich in anthocyanins, has the potential to serve as a natural, safe, and accessible phytoestrogen source. These compounds can also extend the estrous cycle in female animals and improve reproductive success. Therefore, this study will discuss the estrogenic activity of ethanol extract from purple sweet potato on the estrous cycle of Wistar rats in a menopause model.

MATERIALS & METHODS

A post-test-only control group design was employed using Stored Biological Material (SBM) from vaginal smears of bilaterally ovariectomized Wistar rats. The rats were divided into three groups: K0 (negative control with distilled water), K1 (intervention with purple sweet potato ethanol extract at 400 mg/kgBW/day), and K2 (intervention with purple sweet potato ethanol extract at 600 mg/kgBW/day). The research was conducted at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University, between June and November 2024.

The observed SBM from vaginal smears included 5 samples per group, totaling 15 samples. Observations were conducted using a light microscope to identify the number of cornified epithelial cells, nucleated epithelial cells, and leukocyte cells. The estrous cycle phase, estrous cycle phase durations, and the total estrous cycle duration were determined through cell count calculations. The data were analyzed using descriptive analysis, the Shapiro-Wilk test for normality, the Levene test for homogeneity, and hypothesis testing with one-way ANOVA or the Kruskal-Wallis test.

RESULT

The study was conducted from June 3, 2024, to November 11, 2024, at the Integrated Biomedical Laboratory. The samples consisted of 5 samples per group (15 in total). Each research sample comprised 4 vaginal smear SBM preparations, labeled I, II, III, and IV. These labels indicated the time of vaginal smear collection in 3-hour intervals, following Roman numeral designations. Observations were carried out using a light microscope on 10 fields of view per preparation. Cell counts were performed on each preparation to determine the estrous cycle duration.

The research results were presented through descriptive analysis, normality testing, homogeneity testing, and hypothesis testing. Descriptive analysis included the duration of estrous cycle phases and the overall cycle duration. These data were obtained through microscopic observations of the vaginal smear SBM preparations. Cell counting was performed to identify differences in the average number of leukocytes, cornified epithelial cells, and nucleated epithelial cells to determine the estrous cycle phases and their respective durations. Meanwhile, normality testing, homogeneity testing, and hypothesis testing were conducted to analyze the effects of purple sweet potato ethanol extract on the estrous cycle duration in menopausal Wistar rats.

Table 1. Duration estimation of the estrous cycle in each group

Groups	Sample	Duration Estimation (Hour(s))				
		Estrus Phase				Estrus Cycle
		Proestrus	Estrus	Metestrus	Diestrus	
K_0	1	0	0	3	9	6
	2	0	0	3	6	6
	3	0	3	3	3	6
	4	0	3	0	6	6
	5	0	6	3	3	6
K_1	1	0	3	0	9	6
	2	0	3	0	6	6
	3	0	3	3	6	9
	4	3	3	0	3	9
	5	0	6	3	3	9
K_2	1	0	3	3	6	6
	2	0	3	0	9	3
	3	0	3	0	6	6
	4	0	12	0	0	12
	5	0	6	0	3	9

According to Table 1, samples in K_0 exhibited an estimated estrous cycle duration ranging from 6 to 9 hours. In samples 1, 2, and 3, the metestrus phase was observed at the 3rd hour, characterized by a higher average of leukocytes and cornified epithelial cells compared to nucleated epithelial cells, followed by the diestrus phase, which showed increased leukocyte dominance. Sample 4 displayed an initial diestrus phase, followed by estrus, and concluding again with diestrus. In sample 5, the estrus phase was dominant, leading to a longer estrous cycle duration compared to the other samples.

The cell morphology of the estrous cycle in Group K_0 can be observed in Figure 1. The identified phases included estrus (E), metestrus (M), and diestrus (D). The proestrus phase was not detected. Cornified epithelial cells (green arrows), leukocytes (yellow arrows), and nucleated epithelial cells (blue arrows) were observed in each phase.

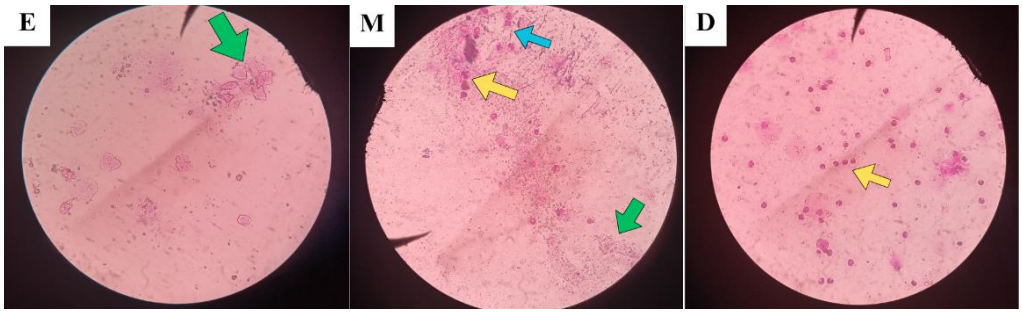

Figure 1. Estrous Cycle Cells in K_0

In Group K_1 (Table 1), where rats received 400 mg/kgBW/day of purple sweet potato ethanol extract, the estrous cycle phases varied among samples, with an estimated duration of 6 to 9 hours per cycle. In samples 1 and 2, the predominant phases were estrus and diestrus, indicated by dominance of cornified epithelial cells and leukocytes. Sample 3 exhibited a metestrus phase, while sample 4 showed a proestrus phase alongside estrus. Sample 5 remained in the estrus phase longer, as indicated by a high average of cornified epithelial cells.

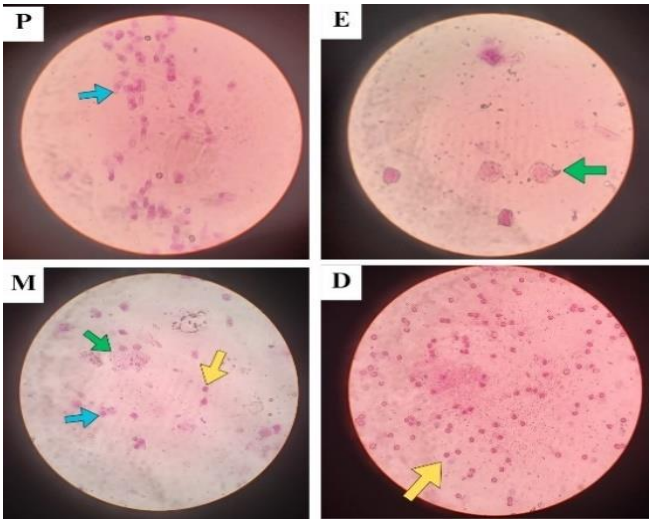

Figure 2. Estrous Cycle Cells in Group K_1

Figure 2 displays the cell types observed in Group K_1's estrous cycle. All estrous cycle phases, including proestrus (P), were detected. Upon observation, cornified epithelial cells (green arrows), leukocytes (yellow arrows), and nucleated epithelial cells (blue arrows) were identified within the sample.

In Group K2 (Table 1), where rats received 600 mg/kgBW/day of purple sweet potato ethanol extract, the estimated cycle duration ranged from 3 to 12 hours. In samples 1, 2, and 3, the diestrus phase dominated the cycle, marked by a high average of leukocytes. The estrus phase was observed intermittently. In sample 4, the cycle was entirely in the estrus phase, with a high average of cornified epithelial cells. In sample 5, the diestrus and estrus phases alternated.

Figure 3 shows the estrous cycle phases observed in K_2. The proestrus phase was not detected, but other phases were present. Cornified epithelial cells, leukocytes, and nucleated epithelial cells were observed and are indicated by green, yellow, and blue arrows, respectively.

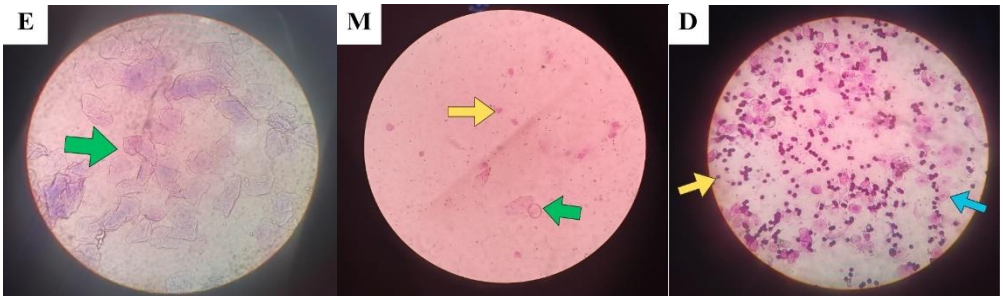

Figure 3. Estrous Cycle Cells in Group K_2

Normality Test

Normality testing using the Shapiro-Wilk test was performed to assess data distribution. Significant values were obtained for each group across the dependent variables. Normally distributed variables included estrous cycle duration in K_2, estrus phase duration in K_0, and diestrus phase duration across all groups. Other variables showed a non-normal distribution.

Homogeneity Test

Homogeneity testing using the Levene test revealed homogeneity in the estrous cycle duration, metestrus phase duration, and diestrus phase duration. However, the proestrus phase duration and overall estrous cycle duration exhibited non-homogeneous data.

Hypothesis Test

Hypothesis testing was selected based on the results of the normality and homogeneity tests. The Kruskal-Wallis test was used for estrous cycle duration, proestrus phase duration, estrus phase duration, and diestrus phase duration, as the data were not normally distributed. Because the metestrus phase duration followed a normal and homogeneous distribution, the one-way ANOVA test was applied.

Hypothesis testing using one-way ANOVA was conducted on the variable of diestrus phase duration, as shown in Table 2. The obtained p-value was 0.929, indicating no significant difference in the duration of the diestrus phase between the groups because the p-value is above 0.05.

Table 2. Hypothesis Testing with One-Way ANOVA Test

Variable	P-value
Duration of Diestrus Phase	0.929*
* = The variable data does not have a statistically significant difference.	

Table 3 shows the Kruskal-Wallis test results regarding estrous cycle duration, proestrus phase duration, estrus phase duration, and metestrus phase duration. The significance values obtained were 0.289, 0.368, 0.310, and 0.174, respectively. All values exceeded 0.05 ($p > 0.05$), suggesting that there were no significant differences among the groups for these variables.

Table 3. Hypothesis Testing with Kruskal-Wallis Test

Variable	P-value
Duration of Estrus Cycle	0.289*
Duration of Proestrus Phase	0.368*
Duration of Estrus Phase	0.310*
Duration of Metestrus Phase	0.174*
* = The variable data does not have a statistically significant difference.	

DISCUSSION

Estrogenic Activity of Ethanol Extract of Purple Sweet Potato on Estrous Cycle Duration

The duration of the estrous cycle in rats serves as an indicator of estrogenic activity. According to the study, no significant variations in estrous cycle duration were observed across the different groups. This finding aligns with the study by Izzati et al. which examined the fertility effects of Hibiscus sabdariffa L. extract on Sprague Dawley rats and found no significant difference in the length of the estrous cycle between groups.[16]

In contrast, the study by Wiratmini et al. found a significant relationship between the administration of *Leucaena leucophala* (Lam.) de Wit leaf extract and the duration of the estrous cycle. In that study, the group receiving a higher dose exhibited a longer estrous cycle duration compared to those receiving a lower dose and the control group.[17] Research by Safrida et al. also demonstrated an increase in estrous cycle duration in ovariectomized rats given tempeh flour. This was attributed to the isoflavones in tempeh flour, which increase estradiol and endogenous estrogen levels, thereby prolonging the estrous cycle.[15]

This study also found that the durations of the proestrus, estrus, metestrus, and diestrus phases showed no significant differences among groups. This suggests that the treatment administered did not have a strong enough impact to influence the durations of these phases in the estrous cycle. However, research conducted by Wiratmini et al. showed different results, indicating significant differences among groups in the lengths of each estrous cycle phase.[17]

One sample from the intervention group receiving 600 mg/kg body weight/day of purple sweet potato ethanol extract experienced a continuous estrus phase for 12 hours. This aligns with the findings of Hidayati and Nofianti, who noted that the estrous cycle phase in the group given the fruit extract of takokak was descriptively different from the negative control group.[18]

The differing results of this study compared to previous research may be influenced by the observation time of the samples. This study was conducted with a total observation time of 12 hours, which is significantly shorter than the normal estrous cycle duration in rats, typically lasting 4–5 days or approximately 96–120 hours. This short duration may have led to the unidentification of some phases of the estrous cycle.[3] The study by Wiratmini et al. reported a total estrous cycle duration in ovariectomized rats in the negative control group of 109.3 hours, while the intervention group with phytoestrogens had a cycle duration ranging from 110 to 122.66 hours.[17] Another study found that the estrous cycle duration in ovariectomized rats in the control group was 72 hours, while the phytoestrogen intervention group had a cycle duration of up to 108 hours.[15]

The dosage of phytoestrogens in the purple sweet potato extract may also influence the estrogenic response, resulting in increased durations of the estrous cycle phases and the total cycle duration. In this context, the anthocyanins contained in purple sweet potatoes are known to bind to estrogen receptors, both ERα and ERβ.[11] Research by Prasetyo indicated that low doses of phytoestrogens result in minimal binding to ER, leading to lower estrogenic activity.[19] Low estrogenic activity can result in the absence of the estrus phase because this phase requires high estrogen levels. The obstruction of the estrus phase also hinders the progression of other phases in the estrous cycle.[15]

Excessively high doses of phytoestrogens may also disrupt the interaction between phytoestrogens and their estrogen receptors. Research by Lorenzana-Martínez et al. found that administering 100 mg/kgBW of Hibiscus sabdariffa extract resulted in lower levels of ERα mRNA compared to a dose of 50 mg/kgBW. This was due to excess phytoestrogens potentially causing a decrease in ERα transcription regulation to prevent excessive receptor production and inappropriate signaling. Nevertheless, the group with the higher dose exhibited greater ERα protein levels in comparison to the lower dose group. The differences in mRNA and ERα protein levels indicate desensitization of ERα when

overstimulated, leading to recycling or degradation.[20]

Estrus Phase in the Negative Control Group

The results of the study indicate the presence of the estrus phase in the negative control group, characterized by a relatively higher number of keratinized epithelial cells compared to other cell types. This contrasts with the findings of Safrida et al., who reported that the control group did not experience the estrus phase. The absence of the estrus phase in ovariectomized rats given pellets may be due to low endogenous estrogen levels, which are necessary for the estrus phase to occur.[15]

The emergence of the estrus phase in the control group may be influenced by the timing of vaginal smear sample collection post-ovariectomy. A study using rabbits found that vaginal smear samples taken pre-operatively, on day 7, and day 14 post-ovariectomy did not show significant differences in estradiol levels. This would also affect the number of nucleated epithelial cells and keratinized epithelial cells, which indicate the estrous cycle. Significant differences were observed on days 30, 60, and 90 post-ovariectomy compared to earlier groups, resulting in the absence of keratinized epithelial cells in that study.[21] This indicates that observation timing affects the identification of the estrus phase in negative control rats.

Limitations of the Study

The limitations of this study include the observation duration of only 12 hours, which risks missing important phases in the estrous cycle, especially in ovariectomized rats. Additionally, the influence of the purple sweet potato extract dosage needs to be tested further to determine the appropriate dosage for eliciting estrogenic effects.

CONCLUSION

The ethanol extract of purple sweet potato (*Ipomoea batatas* L.) shows estrogenic activity on the estrous cycle of Wistar rats in a menopause model. While descriptive differences in the duration of the estrous cycle among groups were observed, no statistically significant differences were found in the duration of the estrous cycle or its phases (proestrus, estrus, metestrus, and diestrus) among treatment groups. This may be due to the short observation time (12 hours) and the dosage of the purple sweet potato ethanol extract being insufficient to significantly impact estrous cycle regulation.

Based on the study results, it is recommended that future research explore a wider range of dosages for purple sweet potato ethanol extract to determine the optimal dosage and extend the observation duration to at least match the normal estrous cycle duration (4–5 days) to observe long-term effects on the estrous cycle and its phases.

Declaration by Authors

Ethical Approval: Ethical approval, with an ethical clearance letter numbered 0872UN14.2.2.VII.14/LT/2024, obtained from the Research Ethics Committee of the Faculty of Medicine, Udayana University.

Acknowledgement: None

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Doshi S, Agarwal A. The Role of Oxidative Stress in Menopause. J Midlife Health 2013;4(3):140.
2. El Hajj A, Wardy N, Haidar S, Bourgi D, El Haddad M, El Chammas D, et al. Menopausal Symptoms, Physical Activity Level and Quality of Life of Women Living in The Mediterranean Region. PLoS One 2020;15(3):1–16.
3. Ajayi AF, Akhigbe RE. Staging of The Estrous Cycle and Induction of Estrus in Experimental Rodents: An Update. Fertil Res Pract 2020;6(1):1–15.
4. Caligioni CS. Assessing Reproductive Status/Stages in Mice. Curr Protoc Neurosci 2009;Appendix 4:Appendix 4I.
5. Koebele S V, Bimonte-Nelson HA. Modeling Menopause: The Utility of Rodents in Translational Behavioral Endocrinology Research. Maturitas 2016;87:5–17.
6. Acosta JI, Mayer L, Talboom JS, Tsang CWS, Smith CJ, Enders CK, et al. Transitional Versus Surgical Menopause in

a Rodent Model: Etiology of Ovarian Hormone Loss Impacts Memory and the Acetylcholine System. Endocrinology 2009;150(9):4248–59.
7. Sugiritama IW, Adiputra IN. Potensi Antosianin Dalam Manajemen Menopause. J Kesehat Andalas 2019;8(1):158.
8. Peacock K, Ketvertis KM. Menopause [Internet]. Treasure Isl. StatPearls Publ.2022 [cited 2023 Oct 11];Available from: https://www.ncbi.nlm.nih.gov/books/NBK507826/#article-24984.s6
9. Oliva MM, Gambioli R, Forte G, Porcaro G, Aragona C, Unfer V. Unopposed Estrogens: Current and Future Perspectives. Eur Rev Med Pharmacol Sci 2022;26(8):2975–89.
10. Desmawati D, Sulastri D. Phytoestrogens and Their Health Effect. Open access Maced J Med Sci 2019;7(3):495–9.
11. Sugiritama IW, Wahyuniari IAI, Ratnayanti IGAD, Linawati NM, Wiryawan IGNS, Komalasari NLGY, et al. The Effect of Purple Sweet Potato (Ipomea batatas L) Ethanol Extract on Estrogen Receptor Alpha (ERα) and SOD mRNA Expression in The Menopause-Liver Animal model. Bali Med J 2020;9(3 SE-ORIGINAL ARTICLE): 538–41.
12. Nguyen NTN, Asben A, Syukri D. Effects of purple sweet potato (Ipomoea Batatas L.) starch addition on characteristics of peel-off gel preparation. IOP Conf Ser Earth Environ Sci 2023;1182(1).
13. Agatha VB, Noerhartati E, Bon AT Bin. Analysis of Design Product Entrepreneursip Syrup Based Purple Sweet Potato (Ipomoea batatas). Proc Int Conf Ind Eng Oper Manag 2019;0(July):2092–9.
14. Putra BA, Santosa KB, Niryana IW, Golden N, Duarsa GWK, Suryawisesa IBM. The Effects of Purple Sweet Potato (Ipomoea Batatas L.) Ethanol Extract on Bladder Urothelial Layer and Smooth Muscle Thicknesses in Menopausal Female Wistar Rats. Folia Medica Indones 2023;59(2): 180–6.
15. Safrida, Solihin DD, Sabri M. Correlation Between Estrogen Hormone Concentration and Estrous Cycle of Rat Fed Soybean Flour and Tempeh Flour. J Kedokt Hewan - Indones J Vet Sci 2019;13(1):24–30.
16. Izzati I, Luqman M, Nizam WA. Fertility and Teratogenic Study of the Aqueous Extract of Hibiscus Sabdariffa L. in Female Sprague Dawley Rats. Food Res 2024;8(5):289–302.
17. Wiratmini NI, Sukmaningsih A, Setyawati I. Estrous Cycle and Uterine Weight of Ovariectomized Menopause Female Rat Models After Treatment With Leucaena leucocephala (Lam.) de Wit Leaf Extract. Folia Medica Indones 2024;60(2):119–26.
18. Hidayati NLD, Nofianti T. Penelusuran Potensi Antifertilitas Buah Takokak (Solanum torvum Swartz) melalui Skrining Fitokimia dan Pengaruhnya terhadap Siklus Estrus Tikus Putih (Rattus norvegicus). J Kesehat Bakti Tunas Husada 2014;11(1):94–103.
19. Prasetyo D. Gambaran Siklus Estrus Tikus Putih (Rattus norvegicus) Ovariektomi Setelah Pemberian Infusa Buah Adas (Foeniculum vulgare Mill). Inst. Pertan. Bogor2016;
20. Lorenzana-Martínez G, San Juan-García CA, Santerre A, Andrade-González I, Bañuelos-Pineda J. The Phytoestrogenic Effect of Hibiscus sabdariffa Involves Estrogen Receptor α in Ovariectomized Wistar Rats. Plant Foods Hum Nutr 2023;78(2):426–31.
21. Dewi TIT, Noviana D, Priosoeryanto BP, Gunanti. Sitologi Vagina dan Kadar Estradiol pada Hewan Model Hipoestrogenik Kelinci (Oryctolagus cuniculus) (Vaginal Cytology and Estradiol Levels on Hypoestrogenic Animal Models of Rabbit (Oryctolagus cuniculus)). Acta Vet Indones 2023;12(1):40–6.

How to cite this article: I Putu Bintang Pradita Kirana, Ida Ayu Dewi Wiryanthini, I Wayan Gede Sutadarma, Ni Wayan Tianing. Estrogenic activity of purple sweet potato (*Ipomoea batatas* L.) ethanol extract on the estrous cycle of wistar rats in a menopause model. *International Journal of Research and Review*. 2025; 12(1): 304-311. DOI: *10.52403/ijrr.20250139*

******