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Short Communication

Effect of Fenugreek Seed Extract on the Improvement in Folliculogenesis of Female Rats Exposed to Valproic Acid

Arni Kusuma Dewi[1], Bambang Purwanto[2], Widjiati Widjiati[3]*, Viski Fitri Hendrawan[4]

[1]Faculty of Medicine, Airlangga University, (Campus A) Prof. DR. Moestopo No.47 street Pacar Kembang, Surabaya city, East Java, Indonesia 60132; [2]Department of Physiology and Medical Biochemistry, Faculty of Medicine, Airlangga University, (Campus A) Prof. DR. Moestopo No.47 street Pacar Kembang, Surabaya city, East Java, Indonesia 60132; [3]Departement of Veterinary Medicine, Faculty of Veterinary Medicine, Airlangga University, (Campus C) Dharmahusada Permai No.1 street, Mulyorejo, Surabaya city, East Java, Indonesia 60115; [4]Department of Veterinary reproduction, Brawijaya University, Puncak Dieng street, Kunci-kalisongo, Malang, East Java, Indonesia 65151.

Abstract | Chronic administration of valproic acid in psychiatric patient could disturbed menstrual cycle. The menstrual cycle is closely related to the folliculogenesis and , uterine phase. There was study that chronic consumtion of valproid acid increase atretic follicle, but there is no evidence if short term administration. Fenugreek seeds extract have effect to endocrine reproductive system, but there is no research about the effect fenugreek seed to folliculogenesis at rat ovarium exposed valproid acid. The objective to evaluation folliculogenesis on ten days (short administration) of valproid acid and protective effect of fenugreek extract. Twenty-eight female wistar strain rats, were divided into 4 groups. The control group (K), group two was administered with valproic acid only (VPA), group three was administered with valproic acid and 100 mg/kg fenugreek seed extract (VPA+100) and group four was administered with valproic acid and 200 mg/kg fenugreek seed extract (VPA + 200). Assessment of follicle performed during proestrous phase. Administration VPA on 10 days decrease corpus luteum (CL) and increase atretic follicle (FA). Fenugreek seed extract (especially 200 mg/kg) restored CL. Fenugreek seeds extract mitigate VPA-induced folliculogenesis disruption.

Keywords | Fenugreek seed extract, Rat ovary, Folliculogenesis, Estrous cycle, Traditional medicine

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***Correspondence** | Widjiati, Widjiati, Departement of Veterinary Medicine, Faculty of Veterinary Medicine, Airlangga University, (Campus C) Dharmahusada Permai No.1 street, Mulyorejo, Surabaya city, East Java, Indonesia 60115; **Email:** widjiati@fkh.unair.ac.id
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INTRODUCTION

Valproid acid is a drug used in psychiatric cases, especially for bipolar disorder. In the general population, the prevalence of bipolar disorder is around 2% to 3%, especially among women. Clinical symptoms of bipolar can appear in girls, pregnant women, and even women who have given birth. Those who receive valproic acid medication must take it for a long period of time (Masters *et al*., 2022; Carli *et al*., 2023). Long-term use of valproic acid in women with bipolar disorder is a risk factor for Polycystic ovary syndrome (PCOS). which is marked with ovulatory disfunction and excess production of androgens by ovaries (Ibrahim *et al*., 2019).

Ovulatory disfunction and or excess production of androgens have an impact on folliculogenesis and oogenesis. The theory suggests that excess androgens can cause insulin resistance and metabolic changes that form free radicals (Awonuga, Camp and Abu-Soud, 2023).

Chronic consumption of valproic acid, histologically, shows a picture of incomplete folliculogenesis, namely the presence of many atretic follicles and the absence of corpus luteum (Ibrahim *et al*., 2019). The valproate acid study determined that valproate was administered for 90 days, meaning 18 estrous cycles in rats. Research into the effect of valproate on folliculogenesis when administered for just two cycles (10 days) has not yet been conducted. Fenugreek seeds are a part of the fenugreek plant that has been proven to be used as a traditional medicine in treating diabetes and dyslipidemia (Gupta and B, 2001; Khorshidian *et al*., 2016). There are few studies to reveal the benefits of fenugreek seed extract on the reproductive organ system. Those studies that have been conducted are to explore that phytopharmaceutical contents of fenugreek seeds, on endocrine metabolism related to gynecology such as estradiol and prolactin hormones, and insulin (Tamura *et al*., 1997), but still not to explore for the folicullogenesis.

Ovulatory closely related to folliculogenesis. Based on histological structure, the ovum is located within the ovarian follicle. Therefore, oogenesis influences follicle development, a process called folliculogenesis. The stages of folliculogenesis and oogenesis influence the uterine phase. Follicular development begins with the formation of primordial follicles during fetal development. On primordial follicle, ovum begins meiosis I prophase I. These condition of ovum arrested until puberty but in this phase, ovum could be growth. Primordial follicles are then activated into primary follicles at puberty. In rodent species, puberty begins at 35 days postnatal age (Hernandez-Medrano and Belmpa, 2024). If a primordial follicle is activated and becomes a primary follicle, the next stage of development is ovulation or atresia. The primary follicle then becomes secondary follicle (preantral follicle). This changes depends on parakrin hormone that secretion by granulosa cell and Theca cell. When fluid fill cavity in follicle is called antral follicle or growing follicle. On this phase, folliculogenesis start influenced by gonadotrophin hormone. The growing follicle becomes a Graafian follicle. The Graafian follicle becomes a corpus luteum, accompanied by ovulation, which releases the ovum into the uterus. If fertilization occurs during ovulation, the corpus luteum is maintained; however, if fertilization does not occur, the corpus luteum becomes corpus albicans. Atretic follicle is a primary or secondary follicle that does not grow and develop (Hernandez-Medrano and Belmpa, 2024). Folliculogenesis and oogenesis are influenced by endocrine and paracrine signals (Ibrahim *et al*., 2019).

Fenugreek seeds contain phytopharmaceutical ingredients that are synergistic with each other. The major phytopharmaceutical content of fenugreek seed extract is steroid saponin, alkaloid and flavonoid (Lavle *et al*., 2016). Based on Tamura *et al*. (1997) study that extract fenugreek seeds have effect on endocrine and radical oxidation, so we hypothese that extract fenugreek seed might be prevent and improve the effect of valproid acid on folliculogenesis.

Therefore, in this study, we analyze the effect of 10 days (2 estrus cycles) of only valproic acid consumption on folliculogenesis and analysis of the effect of fenugreek seed extract on improving folliculogenesis with valproic acid exposure. We will calculate the mean number of growing follicles, follicle de graaf, corpus luteum and atretic follicles on proestrous phase.

MATERIALS AND METHODS

We used twenty-eight female Wistar rats were obtained from Professor Niddom Fondation, aged 60 days with an average weight of 184.29 (10.19 gr) and in good health. Animals were treated accordingly received ethical approval from the Animal Care and Use Committee (ACUC) with approval number 2. KEH.182.12.2023. After acclimatization, animals were randomly divided into 4 groups (N=7): Valproic acid 400 mg/kg for 10 days (VPA), group two was administered with valproic acid 400 mg/kg and fenugreek seed extract 100 mg/kg for 10 days (VPA+100) and group three was administered with valproic acid 400 mg/kg and fenugreek seed extract 200 mg/kg for 10 days (VPA + 200). Valproic Acid Sodium Salt 10 g manufactured by Sigma No P4543 taken 2800 mg add aqua 28 mL. One hundred gram of dried Fenugreek seeds are soaked in a 70% ethanol solution for 24 hours. Then, they are centrifuged, and the supernatant is used as medicine. To make a 100 mg/kg dose, take 1400 mg and add 70 mL of aqua. To make a 200 mg/kg dose, take 2800 mg and add 70 mL of aqua. Ovarian tissue was taken on the 11th day by mouse termination. The ovarian tissue was then made into histopathology preparations for H.E. staining. Preparat histopathology of ovarium dilihat dengan 200x magnification with a Nikon light microscope with NIS Element F.55.22.00 software. Number of growing follicles, follicle de graaf, corpus luteum and atretic follicles carried out in 5 fields of view were calculated. The data were then averaged. Normality, homogenity data, mean and deviation standard also significant difference were calculated by SPSS application. Significant difference set on p>0,05.

RESULT AND DISCUSSION

Based on Table 1 showed that short administration of VPA decreased CL (2.0 vs 7.0 control, p<0,05) and increased FA (18.5 vs 1.0 control). These data are confirmed by histopathological images (Figure 1). But Fenugreek seeds extract administration increased CL and mitigate the effect of valproid acid (9.5/13.0; p=0,04/0,01) along with the increase in the extract dose (200 mg/dl). This study differ

Table 1: Number of follicles in the different experimental groups (N = 7).

	Control (K)	P (VPA)	P (VPA + 100)	P (VPA + 200)	Anova (P)	Post Hoc (LSD)		
						VPA vs Control	VPA vs (VPA + 100)	VPA vs (VPA + 200)
Growing follicle	2.00 ± 2.83	0.50±0.71	2.50±0.71	5.50±4.95	0.46	0.632	0.528	0.159
Follicle de graaf	0.50 ± 0.71	0	0.50±0.71	0	0.62	0.374	0.374	1.0
Corpus luteum	7.00 ±0	2.00 ± 0	9.50±4.95	13.00±1.41	0.052	0.124	0.044*	0.013*
Atretic follicle	1.00 ± 0	18.50±3.54	8.00±1.41	14.50±2.12	0.05	0.010*	0.090	0.140

*significant, p<0.05

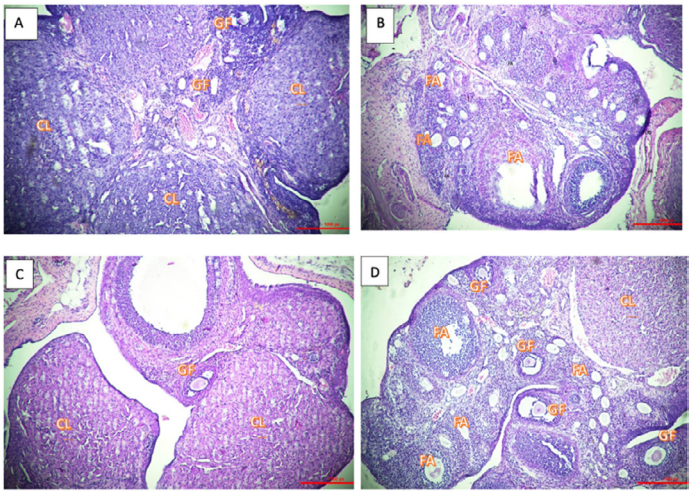

Figure 1: Histopathological images with H.E staining viewed at 200x magnification using a light microscope. (A) The control group shows several growing follicle (GF) and corpus luteum (CL, (B) the VPA group shows many atretic follicle (FA), (C) the VPA+100 shows several growing follicle (GF) and corpus luteum, (D) the VPA+200 shows several growing follicle (GF), corpus luteum (CL) and several atretic follicle (FA).

from Ibrahim *et al.* (2019), which showed that valproate acid consumption for chronic on 90 days just giving impact on folliculogenesis (Ibrahim *et al.*, 2019).

Normal estrus cycle in rats is an average of 4-5 days. The estrous cycle is closely related to the follicular phase, the ovarian phase, and the uterine phase. The histological appearance of the uterus adapts to folliculogenesis and ovarian maturation in preparation for pregnancy. The estrus cycle is divided into four phases. The first phase is the proestrus phase which lasts an average of 14 hours, the second phase is the estrus phase which lasts an average of 24-48 hours, the third phase is the metestrus phase which lasts an average of 6-8 hours and the fourth phase is the diestrus phase which lasts an average of 48-72 hours. The proestrus phase is the follicular development phase in the ovaries for the ovum maturation process (Ajayi, 2020). Thus, the histological features of the ovaries range from primary follicles, growing follicles, Graafian follicles, to the corpus luteum. During the proestrus phase, FSH levels decrease drastically, causing LH levels to peak, causing granulosa and theca cells to produce estradiol and progesterone, and activating meiosis II of the ovum until meiosis II metaphase II. If fertilization occurs, the ovum will complete its second metaphase, maintaining the corpus luteum and sustaining uterine proestrus. However, if fertilization does not occur, the corpus luteum will become a corpus albicans. While Atretic follicle is a primary or secondary follicle that does not grow and develop (Hernandez-Medrano and Belmpa, 2024).

The estrus cycle, folliculogenesis, and oogenesis involve not only the endocrine hormone gonadotrophin, but also several paracrine processes originating from granulosa cells and theca cells of the ovary. As granulosa cells proliferate into several layers, the zona pellucida and theca cells also form. This phase is called follicle growth. Theca cells are divided into theca interna and theca externa cells. Theca interna cells possess vascularization that is beneficial for steroidogenesis, namely for estrogen synthesis, FSH and LH receptor synthesis, members of the TGFβ superfamily, and the Insulin-Like Growth Factor (IGF) system. The IGF system consists of IGFs (I and II), IGF-receptor I, IGF Binding Protein (IGFBP), and enzymes that control IGFBP. IGF1 and IGF2 bind to IGF-receptor 1 on granulosa cells to activate granulosa cell proliferation, activate sensitivity to FSH, and activate steroidogenesis in theca interna cells. IGF activation activates a protein cascade in granulosa cells, namely mTOR1 (mammalian Target of Rapamycin Complex-1). mTOR-1 activates the phosphatidylinositol-3-kinase (PI3k/Akt) pathway, which promotes ovum development, resulting in increased ovum volume (Hernandez-Medrano and Belmpa, 2024).

Based on that theory, a review conducted by Okanovic and Zivanovic (2016) stated that several mechanisms by which valproic acid can influence gynecological disorders for 90 days: First valproic acid can modulate epigenetics by inhibiting the enzyme Histone deacetylase (HDAC) thereby influencing the expression of several proteins and hormones, second, valproic acid increases the concentration of leptin by adipose cells which influences the neuroendocrine axis in the hypothalamus, and third, valproic acid can increase blood sugar concentration due to the insulin resistance effect of valproic acid (Okanovic and

Zivanovic, 2016).

Fenugreek seed extract contains 3 major ingredients: Alkaloids, steroidal saponins and flavonoids, all three of which have been studied to affect body metabolism. In this study, administration of fenugreek seed extract containing steroidal saponin caused follicle maturation in the proestrus phase: the presence of growing follicles characterized by the proliferation of granular cells and theca cells accompanied by the presence of ovum meiosis in them, the formation of de graff follicles characterized by follicles with fluid in the middle called the antrum, ovum surrounded by zona pellucida and follicle cells protruding into the antrum called comulus ooferus, the formation of corpus luteum characterized by the growth of theca cells in the inner layer and the formation of granulosa cells in the outer layer (Ross and Pawlina, 2016). This shows that steroidal saponin has a structure similar to sex hormones, namely estrogen hormones that can affect the hypothalamus- pituitary- ovarian axis. Exogenous estrogen hormones from fenugreek seed extract increase their concentration in the blood so that it has implications in the proestrus phase accompanied by follicle development as in the control. Saponins and alkaloids function to increase insulin sensitivity. This means the protein cascade, starting with the insulin receptor, mTOR1, and PI3K/Akt, is active. Activation of these components increases granulosa cell proliferation, enhances theca interna cell steroidogenesis, and activates ovum development (Patil *et al*., 2009; Zhou and Xu, 2022; Hernandez-Medrano and Belmpa, 2024).

The flavonoid content in the fenugreek seed extract is also useful for folliculogenesis because it increases the expression of the anti-free radical glutathione hormone and reduces the production of reactive oxygen species (ROS) which can be formed from glucose and fatty acid metabolism. This is very useful because ROS can extend the proestrus phase because the primary follicle cannot grow into other follicle cells (Charles *et al*., 2021).

The study's limitation include the failure to examine the estrus phase at baseline, vaginal smears, vaginal histology, and the failure to examine the expression of TGFβ, IGF1, IGF2, and IGF receptor-1 using either immunohistochemistry or molecular analysis.

CONCLUSION

This study shows that fenugreek seed extract can be consumed by female rats exposed to valproic acid because it can repair the side effects of valproic acid on Folliculogenesis which given on short time (10 days).

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NOVELTY STATEMENT

Acute consumption of valproic acid can disrupt the estrous cycle because it interferes with folliculogenesis, but this can be prevented by consuming fenugreek seed extract at the same time.

AUTHOR'S CONTRIBUTION

AKD and VTH carried out the histopathology staining and evaluate the result. AKD and BP participated in study design, the collection of tissue section, and statistical analysis. WW drafted the manuscript. All authors read and approved the final manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Ajayi AF (2020). Staging of the estrous cycle and induction of estrus in experimental rodents: An update. Fertil. Res. Pract., 3: 1–15. https://doi.org/10.1186/s40738-020-00074-3

Awonuga AO, Camp OG, Abu-Soud HM (2023). A review of nitric oxide and oxidative stress in typical ovulatory women and in the pathogenesis of ovulatory dysfunction in PCOS. Reprod. Biol. Endocrinol., 21(1): 1–15. https://doi.org/10.1186/s12958-023-01159-6

Carli M, Francesco W, Giovanna G, Sergio P, Shivakumar K, Francesca V, Cristina V, Carla P, Simone T, Biancamaria L, Roberto M, Marco S (2023). Pharmacological strategies for bipolar disorders in acute phases and chronic management with a special focus on lithium, valproic acid, and atypical antipsychotics. Curr. Neuropharmacol., 21(4): 935–950. https://doi.org/10.2174/1570159X21666230224102318

Charles CN, Weleh II, Green IK (2021). Fleurya Aestuans promotes oogenesis and ovulatory function in wistar rats by shortening the estrus cycle. World Journal of Pharmaceutical Research,10(13): 1951–1966.

Gupta A, R. Gupta, B. Lal (2001). Effect of *Trigonella foenum* graesum (fenugreek) seeds on Glycaemic control and insulin resistence in type 2 diabetes mellitus: A double blind placebo controlled study. Journal Assoc Physicians India, Vol 49 : 1057-1061.

Hernandez-Medrano J, Belmpa M (2024). Folikulogenesis and oogenesis. In: Mastering clinical embryology. 1st edn. CRC Press, pp. 7. https://doi.org/10.1201/9781003269502-11

Ibrahim IH, Adel MA, Rehab HE (2019). Chronic valproate treatment influences folliculogenesis and reproductive hormones with possible ameliorating role for folic acid in adult albino rats. Acta Histochem., Elsevier, 121(7): 776–783. https://doi.org/10.1016/j.acthis.2019.07.003

Khorshidian N, Mojtaba YA, Masoumeh A, Abolfazl AM, Amir MM (2016). Review article fenugreek: Potential applications as a functional food and nutraceutical. Nutrition and Food Science Research. 3(1): 5–16.

Lavle N, Shukla P, Panchal A (2016). Role of flavonoids and saponins in the treatment of diabetes mellitus. Journal of Pharmaceutical Science and Bioscientific Research. 6(2271): 535–541.

Masters GA Julie H, Lulu X, Christine MU, Tiffany AM, Jean YK, Nancy B (2022). Prevalence of bipolar disorder in perinatal women: A systematic review and meta-analysis. J. Clin. Psych., 83(5). https://doi.org/10.4088/JCP.21r14045

Okanovic M, Zivanovic O (2016). Valproate, bipolar disorder and polycystic ovarian syndrome. In: Seminar for phisicians, pp. 6.

Patil HN, Patil PB, Tote MV, Mutha SS, Bhosale AV (2009). Antidiabetic effects of fenugreek alkaliod extract in alloxan. Induced Hyperglycemic Rats, 1(3): 588–597.

Ross MH, Pawlina W (2016). Histology: A text and atlas with correlated cell and molecular biology. Seventh ed. Wolters Kluwer.

Tamura K, Hideo H, Yoshihiro M, Yutaka S, Hiroshi K (1997). Inhibitory effect of a new steroidal saponin, OSW-1, on ovarian functions in rats, pp. 1796–1802. https://doi.org/10.1038/sj.bjp.0701309

Zhou Y, Xu B (2022). New insights into anti-diabetes effects and molecular mechanisms of dietary saponins. https://doi.org/10.1080/10408398.2022.2101425