http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS

Pakistan Journal of Biological Sciences

ISSN 1028-8880

DOI: 10.3923/pjbs.2023.360.370

Research Article

Effect of Moringa Leaf Extract (*Moringa oleifera* Lam.) on the Diameter of the Primary and Secondary Follicles in Female Mice (*Mus musculus*)

[1]Amiruddin, [1]Lili Darlian, [2]Sriyana Herman, [1]Mayang Sari and [3]Rusli

[1]Department of Biology Education, Halu Oleo University, Kota Kendari, Sulawesi Tenggara 93232, Indonesia
[2]Department of Reproductive Health, University of Megarezky Makassar, Kota Makassar, Sulawesi Selatan 90234, Indonesia
[3]Department of Nutrition, Makassar State University, Kota Makassar, Sulawesi Selatan 90222, Indonesia

Abstract

Background and Objective: Infertility is still a phenomenon in the community, so consuming Moringa leaves (*Moringa oleifera* Lam.) is expected to increase fertility. This study aimed to determine the effect of Moringa leaf extract (*Moringa oleifera* Lam.) on the diameter of the primary and secondary follicles in female mice (*Mus musculus*). **Materials and Methods:** This study was an experiment using a Completely Randomized Design (CRD). The population of this study was 45 mice and samples were obtained by a simple random sampling technique from as many as 24 mice with the following criteria: Weight 20-25 g, 2-3 months old, female and in good health. Data analysis was performed through the ANOVA Test with a confidence level of $\alpha = 0.05$ and further tested for the least significant difference (LSD). **Results:** Moringa leaf extract significantly positively affects the diameter of primary and secondary follicles in female mice ($p<0.05$). The average primary follicle diameter was P_0 (92.65 µm), P_1 (124.92 µm), P_2 (150.72 µm), P_3 (175.68 µm) and the average secondary follicle diameter was control (157.17 µm), P_1 (171.33 µm), P_2 (204.57 µm), P_3 (211.11 µm). Giving Moringa leaf extract (*Moringa oleifera* Lam.) significantly increases the diameter of mice's primary and secondary follicles due to the presence of vitamin E in Moringa leaf extract (*Moringa oleifera* Lam.). **Conclusion:** This can stimulate granulosa cells to secrete the hormone estrogen, causing an increase in the diameter of the primary and secondary follicles.

Key words: *Moringa oleifera* Lam., follicles, granulosa cells, antioxidants, histopathological

Citation: Amiruddin, L. Darlian, S. Herman, M. Sari and Rusli, 2023. Effect of Moringa leaf extract (*Moringa oleifera* Lam.) on the diameter of the primary and secondary follicles in female mice (*Mus musculus*). Pak. J. Biol. Sci., 26: 360-370.

Corresponding Author: Amiruddin, Department of Biology Education, Halu Oleo University, Kota Kendari, Sulawesi Tenggara 93232, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indonesia is an archipelagic country with various biodiversity. It has been utilized by Indonesian for a long time until now, such as plants, which are rich in antioxidants, one of which is *Moringa oleifera* Lam.[1]. Several types of plants containing substances such as steroids, alkaloids, bioflavonoids, triterpenoids and xanthones were tested on mice and affected the function of the reproductive organs both morphologically and histologically[2]. According to Lestari *et al.*[3] Moringa leaves (*Moringa oleifera* Lam.) have shown many benefits as antihypertensive, antihyperlipidemic, antiulcer, hepatoprotective, antibacterial, antifungal, antitumor, anticancer, antifertility drugs and various benefits. The benefits of Moringa leaves as an anti-inflammatory, hepatitis, facilitating urination, anti-allergic and used as antimicrobial, antifungal, antihypertensive, anticancer and antitumor. Moringa leaves (*Moringa oleifera* Lam.) can be utilized as food supplements[4-6].

Based on the results of research conducted by Narulita *et al.*[7] stated that the most nutritious part of Moringa is the Moringa leaves. Moringa leaves are predicted to increase the size of the ovaries and optimize their work functions. Moringa leaf (*Moringa oleifera* Lam.) is a plant containing phenolic compounds such as alkaloids, tannins, saponins and flavonoids are efficacious as antioxidants containing β-sitosterol 90 mg g^{-1}, total phenolic 8 μg mL^{-1} and flavonoids 27 μg mL^{-1}. One of the most prominent contents of Moringa leaves is quercetin and kaempferol, including a group of flavonoids[8-10]. The ovary is a vital reproductive organ. The functions of the ovaries include maintaining the number of corpuses luteum and producing oocytes and female reproductive hormones. Optimization of ovarian function is achieved by increasing the number of follicles supported by good ovarian morphology[11]. The quality produced by the ovaries is influenced by nutrition, which is one of the factors that affect the growth and development of follicles[12]. Folliculogenesis is the process of maturing follicles in the ovarian cortex[13,14]. This process describes the change from a small primordial to a large preovulatory follicle. The ovaries have many immature primordial follicles from birth, containing immature primary oocytes. Original follicles undergo changes in their histological and physiological characteristics, forming both tertiary and antral follicles. This process depends on different types of hormones that determine the rate of folliculogenesis and oogenesis, which ends with ovulation or other atresia of the follicle[15,16]. It is consistent with Pramesti *et al.*[17] and Margiana *et al.*[18] which state that giving soybean extract (*Glycine max*) to female mice (*Mus musculus)* can increase the number of ovarian follicles. The highest average follicle count was achieved in treatment 3 (P_3) with a dose of 0.015 mg kg^{-1} b.wt. The higher the apoptosis number, the more atresia of the follicles occurs and they do not develop into mature follicles, which does not lead to ovulation. Failure to ovulate can lead to infertility[3].

The ovary undergoes some morphological changes in the reproductive organs[19,20]. Diet is one of several factors that affect it. Nutrients with high nutrient content enlarge the ovaries and thus ensure good optimization of ovarian function. The size of the ovaries can serve as a benchmark for determining fertility because the size significantly influences the quality of reproduction and thus, the generation's success[21,22]. The ovaries have follicles that contain large numbers of egg cells. The follicle undergoes growth and development called folliculogenesis. The ovaries that develop into egg follicles go through four stages: Primary, secondary, tertiary and de Graaf[23,24]. The ovaries' follicle-stimulating hormone (FSH) level affects follicle growth, allowing the primary and secondary follicles to develop properly[25,26]. The occurrence of inhibition of FSH secretion averages that the level of FSH in the follicle is low. High levels of FSH are not required to develop primary and secondary follicles[27,28]. However, the use of high levels of FSH is necessary for the development of de Graaf follicles. Moringa leaves contain ascorbic acid, a powerful antioxidant that fights free radicals, protecting them from damage and allowing the pituitary gland to produce hormones such as FSH and LH normally[29-31]. Therefore, an increase in these hormones leads to an increase in cell count. Thus, administration of Moringa leaf extract at a dose of 300 mg kg^{-1} b.wt., cannot inhibit the development of spermatogenic cells. In this case, it is shown that the higher the doses of Moringa leaf extract administered, the higher the number of increased spermatogenic cells produced.

Understanding the effect of Moringa extract on follicle diameter can provide insight into mechanisms that can modulate hormone levels and improve hormone balance. This study aimed to determine the effect of *Moringa oleifera* Lam. leaf extract on the diameter of mice's primary and secondary follicles (*Mus musculus*). In addition, *Moringa oleifera* Lam. is used as a safe herbal medicine with Moringa leaf extract (*Moringa oleifera* Lam.).

MATERIALS AND METHODS

Study area: This study was an experimental (experimental laboratory) using a Completely Randomized Design (CRD). This study was conducted for 4 months (April to July, 2023) at the Laboratory of the Department of Biology, Faculty of Teacher Training and Education, Halu Oleo University, Kendari, Southeast Sulawesi.

Study population: The population in this study was 45 female mice (*Mus musculus*). The sample of this study was obtained by simple random sampling technique as many as 24 mice with the sample criteria of weight 20-25 g, age 2-3 months, female and in good health. The mice were obtained from mice's breeders in the city of Kendari. The samples were selected at random. Random sampling was done by numbering the mice, which was then performed using a lottery method to select the mice to be sampled.

Experimental procedure

Animal test preparation: Mice were acclimatized for 1 week, kept in rectangular cages of $38\times27\times13$ cm covered with wire. The pedestal in the cage was wood shavings that are changed twice a week. During maintenance, the mice were given an intake of 6 g of pellets (the feed is compacted and collected through a mechanical process)/day and water *ad libitum* through a drinking bottle.

Moringa leaf extract production: The Moringa leaves used were sourced from moringa farmers in the Andonohu Area in Kendari, Southeast Sulawesi Indonesia. Moringa leaves were dried in an oven for 12 hrs at a temperature of 50°C. The dried Moringa leaves were blended until becoming powder. The dry powder was measured at 1000 g, added 96% ethanol as much as 3 L, then the extraction process was carried out by the maceration method for 24 hrs. The extract was filtered, then the Moringa leaf filtrate was concentrated using a tube evaporator.

Treatments: Moringa leaf extract was administered in the morning (07.00 am) with different doses, such as P_1 = 300 mg kg^{-1} b.wt., P_2 = 400 mg kg^{-1} b.wt. and P_3 = 500 mg kg^{-1} b.wt., the extract was administered for 14 days and on day 15, the female mice were dissected to remove her ovaries.

Histology of preparations: The ovary taken was put in histological preparations, then fixed with Bouin's solution for approximately 2 days and then washed with 70% alcohol (1×60 min). The next process was dehydration using 90% alcohol solution for one night, transferred to 96% alcohol and absolute alcohol for 1×60 min, respectively. After the dehydration process was complete, it was followed by a purification process, soaking the ovarian organs using a toluol solution for one night, then paraffin infiltration was carried out by immersing the ovarian organs into a mixture of toluol and paraffin in a ratio of 1:1 (30 min) followed by pure paraffin I, II and III for 45 min, respectively. The next process is embedding, implanting the ovarian organ into paraffin, then the position is set in the direction of transverse cutting and allowed to freeze and form a block to be cut with a microtome. Then the paraffin block was mounted on a holder, placed on a microtome, cut 6 µm thick and formed a ribbon. From the slices, the best one was selected, then placed on a slide with Mayer's albumin smear and put in a slide warmer for 24 hrs to make the attachment stronger.

Staining and mounting, the sequence of action was: (a) Deparaffinization, the slide containing the slices of the ovary was dipped in xylol solution until the paraffin was dissolved (1 min), then dried on filter paper, (b) Hydration, the dried object glass was put into alcohol with decreasing concentrations starting from absolute alcohol, 96, 90, 80, 70, 60, 50, 40 and 30% aquades one minute each, (c) Placed in a staining jar containing Hematoxylin Ehrlich (7 min) and ashed with running water (10 min), (d) Put in 30, 50, 60 and 70% alcohol each for 1 min, then put into eosin-Y (10 min), rinsed with 70, 80, 90 and 96% alcohol and absolute alcohol for 1 min each and (e) Preparation was put in a xylol solution (15 min), then dried on filter paper, mounted with *Canada balsam* and covered with a cover slip. Subsequently, observations were made under a microscope (LED Light Microscope: S-40 series biological microscope with magnification 40-400$\times$) to see and calculate the diameter of the primary and secondary follicles in each treatment group. Histological observations (diameter of the primary and secondary follicles) were conducted at the Development Laboratory of the Biology Education Department, Faculty of Teacher Training and Education, Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia.

Statistical analysis: The primary and secondary follicle diameter data results were analyzed using ANOVA to test the hypothesis of the effect of *Moringa oleifera* Lam. extract at different doses using inferential analysis using the IBM SPSS Statistic 20 application program:

- **Data normality test:** The data normality test serves as a prerequisite for performing parametric tests. The data normality test aims to determine whether the data is normally distributed. The basis for decision-making are: (1) If the significance value is < (= 0.05), H_0 is rejected and H_1 is accepted, which averages the data are not normally distributed and (2) If the significance value is > (= 0.05), H_0 is accepted and H_1 is rejected, which averages the data are normally distributed

- **Data homogeneity test:** The data homogeneity test using the Levene Test determines whether the data obtained is homogeneous. The basis for decision-making are: (1) If the significance value is < (= 0.05), H_0 is rejected and H_1 is accepted, which averages the data variant is not homogeneous and (2) If the significance value is > (= 0.05), H_0 is accepted and H_1 is rejected, averaging the data variant is homogeneous
- **Hypothesis test:** The hypothesis test uses the parametric Analysis of Variance Test (ANOVA) with a confidence level of 95% (= 0.05). The basis for decision-making are: (1) If the significance value is < (= 0.05), H_0 is rejected and H_1 is accepted, which averages that *Moringa oleifera* Lam. leaf extract influences the diameter of the primary and secondary follicles (*Mus musculus*) and (2) If the Significance value is > (= 0.05), H_0 is accepted and H_1 is rejected, which Averages *Moringa oleifera* Lam. leaf extract does not influence the diameter of the primary and secondary follicles (*Mus musculus*)

If the hypothesis test results are accepted, Duncan's further test can be performed. Duncan's further test was performed to classify the results of the difference in average between each treatment group. The Duncan's Test can compare average follow-up test scores between groups, which is denser than the Tukey LSD test.

RESULTS

Descriptive analysis: Based on the microscopic analysis the average number of the Graafian follicles diameters after administration of Moringa leaf extract (*Moringa oleifera* Lam.) was given in Table 1 and 2.

Based on Table 1 and 2, the comparison diagram of the average diameters of the primary and secondary follicles in mice (*Mus musculus*) can be seen in Fig. 1.

Moringa leaf extract (*Moringa oleifera* Lam.) with various different doses, namely 300, 400 and 500 mg kg^{-1} b.wt., can increase the number of primary and secondary follicle diameters as shown in Fig. 1.

The analysis of Fig. 2-5 reveales a discernible disparity in the sizes of primary and secondary follicles. The increased number of primary and secondary follicle diameters compared to the control treatment (P_1) showed that the P_4 treatment with a 500 mg/BB dose displayed the greatest disparity. Conversely, the control treatment (P_1) displayed the lowest average number of primary and secondary follicle diameters. The highest observed increase in the diameter of primary and secondary follicles was observed in the P_4 treatment group, which received a dosage of 500 mg/BB. This finding indicates that the administration of *Moringa oleifera* Lam. leaf extract had a statistically significant impact on the follicular diameter. Furthermore, the results suggested that higher dosages of the

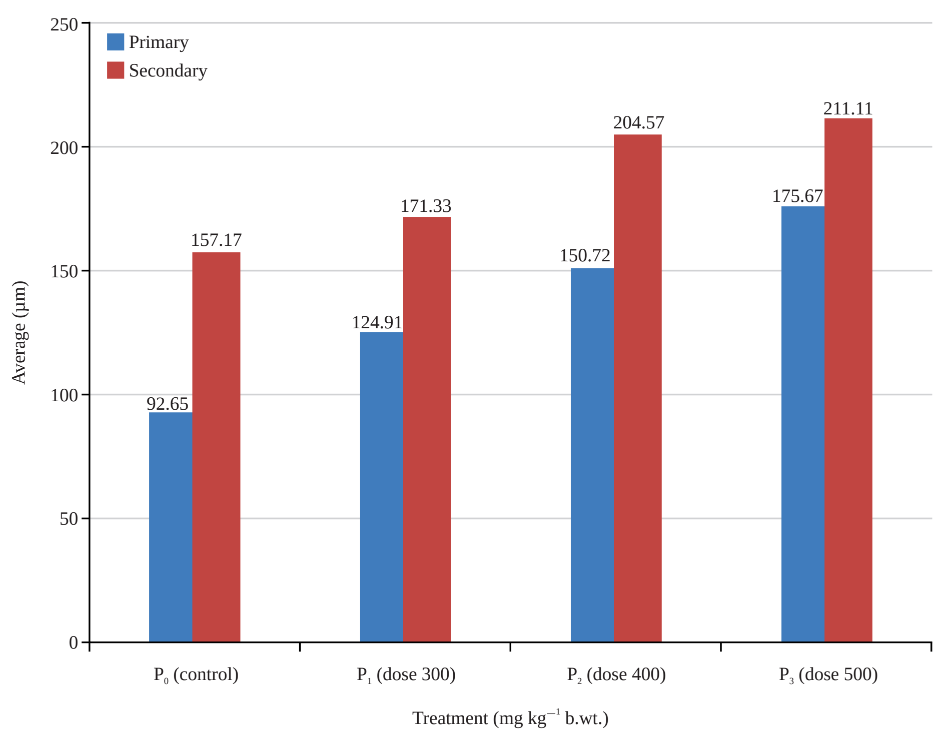

Fig. 1: Graph of average diameter of the primary and secondary follicle in mice after administration of moringa leaf extract

Table 1: Average diameter of primary follicle in mice (*Mus musculus*)

Treatment	Repeat measurement (µm) 1	2	3	4	5	6	Average (µm)
P_0	91.39	86.02	95.72	95.69	100.00	87.09	92.65
P_1	117.20	122.58	123.66	120.43	131.18	134.41	124.91
P_2	136.56	160.22	143.01	150.54	149.46	164.52	150.72
P_3	159.15	211.83	167.74	158.06	162.37	194.88	175.67

P_0: Control, P_1: Dose 300 mg kg^{-1} b.wt., P_2: Dose 400 mg kg^{-1} b.wt. and P_3: Dose 500 mg kg^{-1} b.wt.

Table 2: Average diameter of secondary follicle in mice (*Mus musculus*)

Treatment	Repeat measurement (µm) 1	2	3	4	5	6	Average (µm)
P_0	194.62	33.33	143.01	150.54	133.33	188.17	157.17
P_1	181.72	187.09	174.19	177.42	168.82	138.71	171.33
P_2	187.09	219.36	172.57	212.90	200.00	235.48	204.57
P_3	173.12	211.83	202.15	225.81	230.11	223.66	211.11

P_0: Control, P_1: Dose 300 mg kg^{-1} b.wt., P_2: Dose 400 mg kg^{-1} b.wt. and P_3: Dose 500 mg kg^{-1} b.wt.

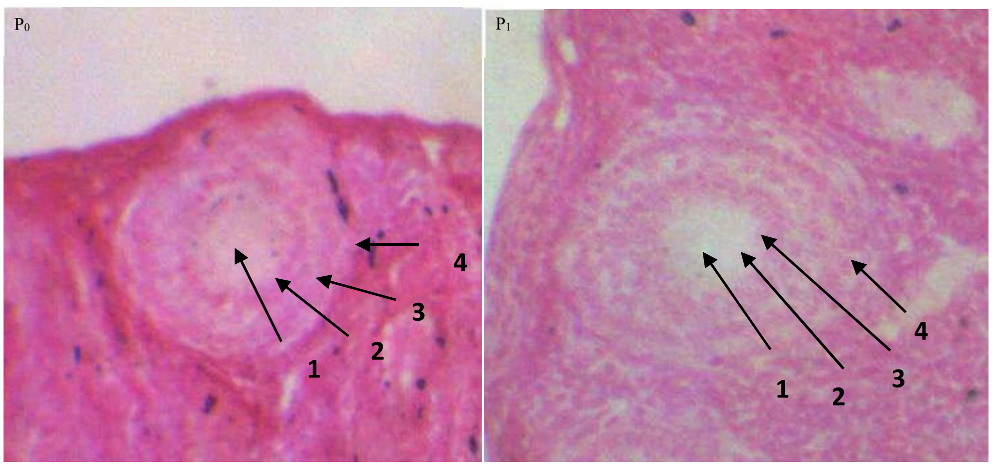

Fig. 2: Primary follicle histology (magnification 400×) for P_0 (control) and P_1 (dose 300 mg kg^{-1} b.wt.)

Information number: (1) Oocyte, (2) Granulosa cells, (3) Pellucida zone and (4) Follicle cell

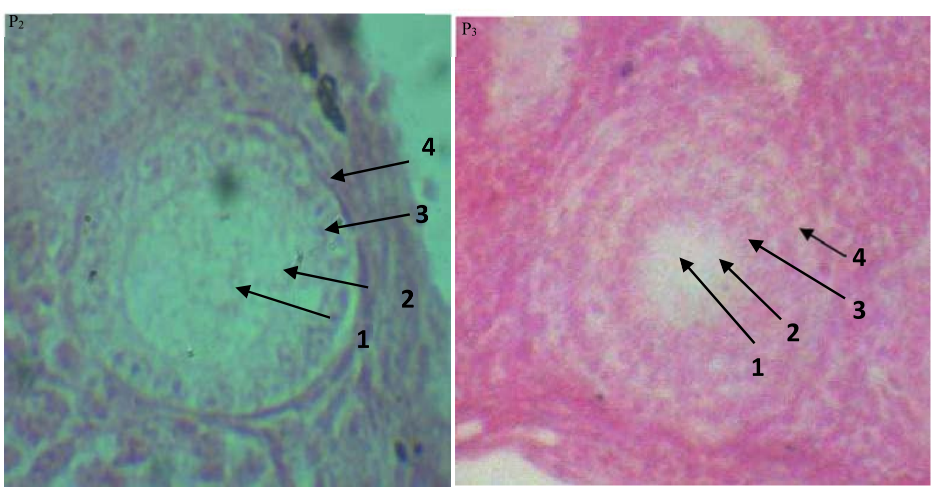

Fig. 3: Primary follicle histology (magnification 400×) for P_2 (dose 400 mg kg^{-1} b.wt.) and P_3 (dose 500 mg kg^{-1} b.wt.)

Information number: (1) Oocyte, (2) Granulosa cells, (3) Pellucida zone and (4) Follicle cell extract corresponded to greater increases in the diameter of both primary and secondary follicles. Administration of Moringa leaf extract at a dose of 300 mg per body weight during intense physical exertion induces alterations in the histopathological architecture. The administration of Moringa leaf extract (*Moringa oleifera* Lam.) has been found to promote the growth of ovarian follicles, increasing follicle diameter. The study results indicated a significant disparity between the control and treatment groups. This discrepancy suggested that as the dosage administered increases, there is a corresponding increase in the diameter of the ovarian follicle, specifically one of its dimensions. The mice being referred to can be categorized into two groups: Primary and secondary.

Statistic analysis

Primary follicle diameter: Table 3 shows that *Moringa oleifera* Lam. leaf extract with various different doses has a significantly positive effect on the diameter of the primary follicle ($p<0.05$) or has a significant difference in each treatment.

Table 4 show that *Moringa oleifera* Lam. leaf extract with various different doses has a significantly positive effect on primary follicle diameter, especially in the treatment group (P_3) with a dose of 500 mg kg^{-1} b.wt. The results of the BNT test analysis measuring primary follicle diameter in mice provide interesting data. The data obtained from groups P_0, P_1, P_2 and P_3 showed a progressive increase in the size of the primary follicle diameter. In the early stages, the P_0 group had a Average primary follicle diameter of 92.65. Then, in the P_1 group, the diameter of the primary follicle increased to 124.92, indicating a significant improvement over the previous stage. In the P_2 group, this increase continued with a average primary follicle diameter of 150.72, in the P_3 group, the primary follicle diameter reached 175.68. These data suggest an organized and consistent evolution in primary follicle size and mice development. However, the analysis also showed that the BNT test result in the P_0-P_3 group was 104.88. This value does not follow the observed trend of increasing primary follicle diameter. This may indicate that other factors besides body size influence the primary follicle's health or development. The possibility of environmental influences or internal factors affecting the activity of the hydrogenase enzyme reflected in the BNT Test results requires further investigation. In general, the results of the BNT Test analysis that measured the diameter of the primary follicles in mice show that even though the size of the primary follicles is getting bigger, the factors that affect their development and health are complicated.

Secondary follicle diameter: The hypothesis test in this study uses the parametric Analysis of Variance (ANOVA) Test seen in Table 5.

Table 3: ANOVA Test for measuring the diameter of primary follicle

Group	Sum of square	df	Average square	F	α
Between group	22753.556	3	7584.519	44.533	0.000
Within group	3406.258	20	170.313		
Total	26159.814	23			

F: Regression coefficient and α: Significance

Table 4: BNT Test for measuring the diameter of primary follicle

		Diameter of primary follicle				
		Subset for alpha = 0.05				
Treatment group	N	1	2	3	4	Symbol
P_0	6	92.65				a
P_1	6		124.92			b
P_2	6			150.72		c
P_3	6				175.68	d
			BNT 0.05			104.88

N: Sample and BNT: Boston naming test

Table 5: ANOVA Test for measuring the diameter of secondary follicle

Group	Sum of square	df	Average square	F	α
Between groups	12131.500	3	4043.833	8.032	0.001
Within groups	10069.425	20	503.471		
Total	12131.500	3	4043.833	8.032	0.001

F: Regression coefficient and α: Significance

Table 6: BNT Test for measuring the diameter of secondary follicle

		Diameter of primary follicle				
		Subset for alpha = 0.05				
Treatment group	N	1	2	3	4	Symbol
P_0	6	157.17				a
P_1	6		171.33			b
P_2	6			204.57		c
P_3	6				211.11	d
			BNT 0.05			179.45

N: Sample and BNT: Boston naming Test

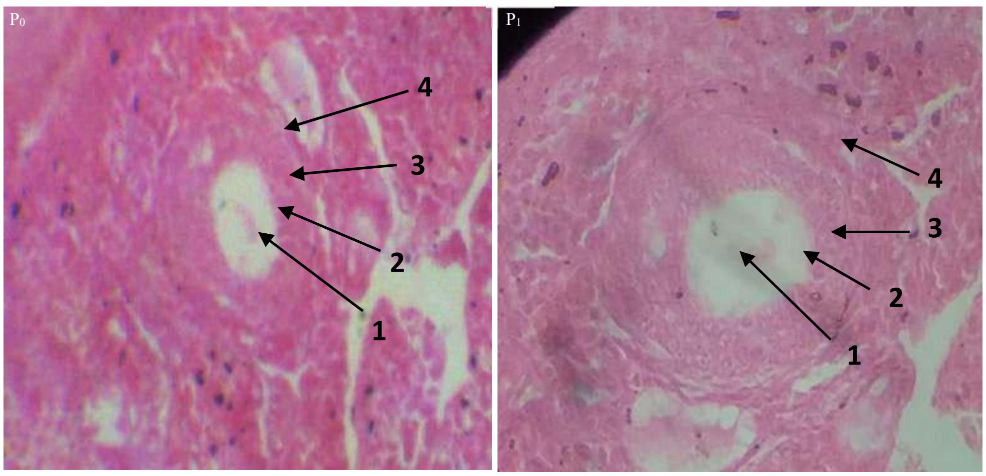

Fig. 4: Secondary follicle histology (magnification 400×) for P_0 (control) and P_1 (dose 300 mg kg^{-1} b.wt.)
Information number: (1) Oocyte, (2) Granulosa cells, (3) Pellucida zone and (4) Follicle cell

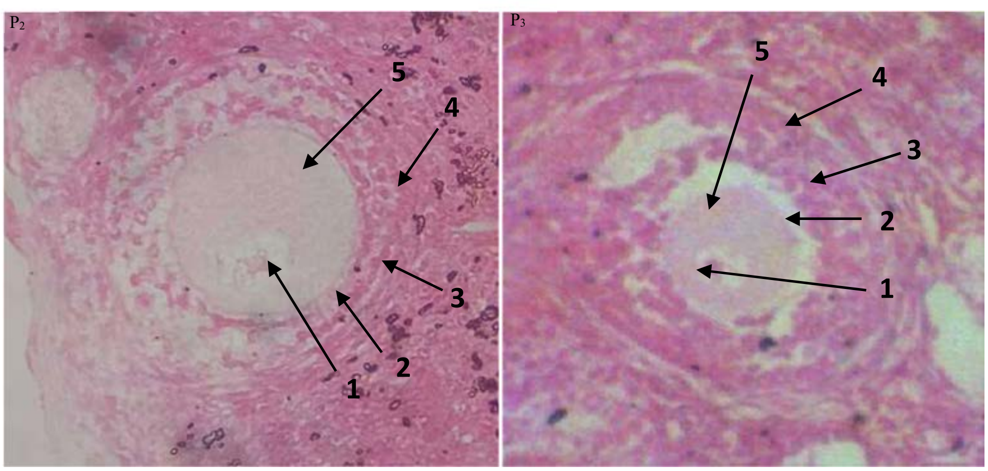

Fig. 5: Secondary follicle histology (magnification 400×) for P_2 (dose 400 mg kg^{-1} b.wt.) and P_3 (dose 500 mg kg^{-1} b.wt.)
Information number: (1) Oocyte, (2) Granulosa cells, (3) Pellucida zone, (4) Follicle cell and (5) Follicular fluid

Table 5 identificate that *Moringa oleifera* Lam. leaf extract with various different doses has a significantly positive effect on secondary follicle diameter ($p<0.05$ or have significant differences in each treatment).

Based on the results of further tests in Table 6, it shows that *Moringa oleifera* Lam. leaf extract with various different doses has a significantly positive effect on secondary follicle diameter, especially in the treatment group (P_3) with a dose of

500 mg kg^{-1} b.wt. The results of the ANOVA Test analysis (analysis of variance) with a 95% confidence level showed that there was a significant difference between the control group and the treatment group (p<0.005) in the diameter of the primary and secondary follicles. The results obtained were $F_{count}>F_{table}$, which indicated a difference or had different effects between the control and treatment groups. The results of the BNT Test, which measured the number of secondary follicles in mice, have helped us learn a lot about how these things develop. The results obtained from the various stages, specifically P_0, P_1, P_2 and P_3, have revealed a constant growth trend in the size of secondary follicles. During the early phase, the secondary follicles within group P_0 exhibited an average diameter of 157.17. The magnitude of this dimension experienced a substantial increase as the various stages of development advanced. Within group P_1, there was a notable increase in the size of the follicles, reaching a value of 171.33. This observation suggests significant growth occurring within a defined period. The trend persisted in groups P_2 and P_3 when the average diameter of the follicles reached 204.57 and 211.11, respectively. Results underscore the systematic and coordinated process of mice's secondary follicle formation. The BNT Test yielded a remarkable outcome, indicating a value of 179.45 for secondary follicles. This finding underscores the importance of metabolic processes within the specific development context. The difference between the estimated follicle sizes (P_0-P_3) and the higher value suggests that the BNT test may measure more than just basic physical dimensions regarding follicle health. The BNT Test's value resides in its ability to accurately depict the metabolic dynamics of secondary follicles, potentially providing valuable insights into the underlying biochemical mechanisms governing their growth and maturation.

DISCUSSION

The results of microscopy observations based on the histology of the mice's ovary (*Mus musculus*) showed several other follicles. The ovarian follicles measured in this study were the diameters of the primary and secondary follicles. The diversity indicates the development of follicles in the ovary. The stages of follicular development start from the primordial, primary, secondary and tertiary follicles to the Graafian. One of the hormonal disorders in follicular development will cause the primary and secondary follicles not to form, so ovulation will not occur. Failure to ovulate can impair reproduction rates[32]. Alfian *et al.*[33] reported that the data that can be tested for distribution patterns and homogeneity are data with normal and homogeneous distribution patterns tested with the ANOVA Test to determine differences between the control and treatment groups. If the ANOVA test shows differences between the groups, try using the Duncan Test further. The results of hypothesis testing using the ANOVA test (analysis of variance) with a 95% confidence level showed a difference between the control and treatment groups, where the highest dose with a positive effect on increasing the diameter of primary and secondary follicles was a dose of 500 mg b.wt., followed by 400 mg b.wt. and 300 mg b.wt. It was in line with Dasopang *et al.*[34], which states that there is an effect of treatment and differences between groups, so the Duncan's Test continued to show a significant difference between the control and treatment groups.

In Fig. 2-5 the number of primary and secondary follicle diameters is different. The highest difference was seen in the treatment (P_3) with a 500 mg b.wt., dose. It was known that the number of primary and secondary follicle diameters was increasing compared to the control group and the average number of primary and secondary follicle diameters was the lowest in the treatment (P_0). The result showed that moringa leaf extract (*Moringa oleifera* Lam.) significantly affected the diameter of the primary and secondary follicles. The more doses given, the higher the diameter of the primary and secondary follicles. The more doses given, the higher the diameter of the primary and secondary follicles. It was consistent with the research by Alioes *et al.*[35] states that giving moringa leaf extract at a dose of 300 mg b.wt., after excessive physical activity causes changes in histopathological structure.

The development of ovarian follicles by giving moringa leaf extract (*Moringa oleifera* Lam.) can increase the follicle's diameter. So, the results showed a difference between the control group and the treatment group, which averages that the higher the dose is given, the more the diameter of the ovarian follicles increases, one of which is the diameter of the primary and secondary follicles of the mice. It was in line with the research by Garcia *et al.*[36] and Dou *et al.*[37] states that there is an effect on the diameter of the primary follicles of the mice, showing increased results between the treatment group and the control group. and each treatment group showed a decrease in the diameter of the primary follicle of mice along with an increase in treatment dose, influenced by several factors such as light, temperature and feed quality, these factors can affect the ability of mice (*Mus musculus*) to reach their genetic potential to grow and reproduce. This condition is related to the shape of the ovaries. Therefore, the feed factor of the mice in biomedical experiments significantly affects the quality of the experimental results[38,39].

Giving moringa leaf extract (*Moringa oleifera* Lam.) is good because it contains good nutrients for health, one of which is vitamin E, which can improve various biological functions, including anti-fertility. One source of potential exogenous antioxidants to ward off free radicals and increase the diameter of the ovarian follicles is moringa leaves. It was in line with research by Jamshidi-Kia *et al.*[40] and Lobo *et al.*[41], which stated that antioxidants were chemical substances that can help protect the body from damage to cells by free radicals. Moringa leaves contain 46 potent antioxidants, which protect the body against the damaging effects of free radicals by neutralizing them before they damage cells and cause disease. One of the antioxidant compounds in moringa leaves is vitamin E, which stimulates the process of steroidogenesis and the anterior pituitary gland to secrete steroid hormones and initiate folliculogenesis events in the ovaries.

This research proves that moringa leaf extract (*Moringa oleifera* Lam.) positively affects ovarian follicle development. For this reason, consuming it will boost the growth of the follicles. It has a good impact on female fertility and the more follicles increase, the more granulosa cells increase in number and will secrete the estrogen hormone. The high estrogen hormone will stimulate the LH hormone to enter the next stage, the ovulation stage. It was in line with Tran and Hinds[42] that the number of follicles can increase after giving Moringa leaf extract, so the more groups of mice given moringa leaf extract at a dose of 500 mg kg^{-1} b.wt., the more ovarian follicles can increase. It averages that moringa leaf extract can work well to improve the development of follicles. Pramesti *et al.*[17] stated that the development of primary and secondary follicles occurs due to the administration of soy leaf extract, which contains phytoestrogens. Thus, it shows an increase in the number of ovarian follicles in mice (*Mus musculus*). This research provides many benefits: (1) This study can explain the impact of moringa leaf extract on the development and growth of primary and secondary follicles in female rats. This information may have implications for understanding the reproductive health benefits of moringa extract in women, (2) The research findings may contribute to understanding of the potential effects of moringa leaf extract on fertility and ovulation. If the extract is found to positively influence follicular development, that may indicate a potential role for Moringa in enhancing fertility, (3) Moringa leaf extract contains various bioactive compounds that have been reported to have hormone-regulating properties.

Results findings provide the following suggestions for future research variability assessment in bioactive compounds across diverse sources of Moringa leaf extracts. Establishing a standardized extract composition will be imperative to guarantee consistent outcomes and the ability to replicate the findings. Therefore, for the continuity of the development of this research, it is very important to analyze this variable.

CONCLUSION

The analysis results concluded that Moringa leaf extract (*Moringa oleifera* Lam.) positively affects ovarian follicle development and boosts the follicles' growth. Then It has a good impact on female fertility and the more follicles increase, the more granulosa cells increase in number and will secrete the estrogen hormone. The high estrogen hormone will stimulate the LH hormone to enter the next stage, the ovulation stage. After that, *Moringa oleifera* Lam. leaf extract has a significantly positive effect on increasing the diameter of the primary and secondary follicles of mice due to the high content of vitamin E in *Moringa oleifera* Lam., thus stimulating the granulosa cells to secrete more estrogen hormone, which directly affects increasing the diameter of the primary and secondary follicles.

SIGNIFICANCE STATEMENT

In this particular study, the focus is on evaluating the effects of moringa leaf extract specifically on the diameter of primary and secondary follicles in female mice. Follicles are structures within the ovaries that contain immature eggs. The size of these follicles can be an important indicator of ovarian health and fertility. Understanding the impact of Moringa leaf extract on follicle diameter in female mice can provide insights into its potential effects on reproductive health. If the extract shows a significant effect on follicle size, it could suggest possible implications for fertility, reproductive disorders, or hormonal regulation.

ACKNOWLEDGMENT

We are very grateful to the experts for their appropriate and the author would like thanks to the Halu Oleo University for support in this research.

REFERENCES

1. Ridwan, Hamim, Suharsono, N. Hidayati and I. Gunawan, 2021. Drumstick (*Moringa oleifera*) variation in biomass and total flavonoid content in Indonesia. Biodiversitas, 22: 493-500.

2. Aisyah, S.S. and A. Gassing, 2016. The effect of moringa (*Moringa oleifera*) stem bark extract on conception rate of mice (*Mus musculus*) ICR males. Biogenesis, 4: 58-63.
3. Lestari, Y.D., H. Hendarto and Widjiati, 2018. Effect of ethanol extract of moringa (*Moringa oleifera* Lam.) leaves on apoptosis of granulosa cells in mice (*Mus musculus*) endometriosis model. Jurnal Biosains Pascasarjana, 20: 1-8.
4. Liu, R., J. Liu, Q. Huang, S. Liu and Y. Jiang, 2022. *Moringa oleifera*: A systematic review of its botany, traditional uses, phytochemistry, pharmacology and toxicity. J. Pharm. Pharmacol., 74: 296-320.
5. Avilés-Gaxiola, S., J. León-Félix, Y.B. Jiménez-Nevárez, M.A. Angulo-Escalante, R. Ramos-Payán, J. Colado-Velázquez and J.B. Heredia, 2021. Antioxidant and anti-inflammatory properties of novel peptides from *Moringa oleifera* Lam. leaves. South Afr. J. Bot., 141: 466-473.
6. Rode, S.B., A. Dadmal and H.V. Salankar, 2022. Natures gold (*Moringa oleifera*): Miracle properties. Cureus, Vol. 14. 10.7759/cureus.26640.
7. Narulita, E., J. Prihatin, K. Anam and F.A.R.H. Oktavia, 2017. Changes in estradiol levels and uterine histology in female mice (*Mus musculus*) with synthetic progesterone induction. Biosfera, 34: 117-122.
8. Islam, Z., S.M.R. Islam, F. Hossen, K. Mahtab-ul-Islam, M.R. Hasan and R. Karim, 2021. *Moringa oleifera* is a prominent source of nutrients with potential health benefits. Int. J. Food Sci., Vol. 2021. 10.1155/2021/6627265.
9. Patil, S.V., B.V. Mohite, K.R. Marathe, N.S. Salunkhe, V. Marathe and V.S. Patil, 2022. Moringa tree, gift of nature: A review on nutritional and industrial potential. Curr. Pharmacol. Rep., 8: 262-280.
10. Anuragi, H., R.K. Singhal, Y. Tanveer, H. Yasmin and A. Srijan *et al*., 2022. The primacy of moringa (*Moringa oleifera* Lam.) in boosting nutrition status and immunity defence amidst the covid-19 catastrophe: A perspective. Phyton-Int. J. Exp. Bot., 91: 1831-1858.
11. Ramadhani, S.A., I. Supriatna, N.W.K. Karja and A. Winarto, 2017. Folliculogenesis control in ovarian with the cottonseed extract. Jurnal Sain Veteriner, 35: 71-80.
12. Balumbi, M., F. Fachruddin and M. Risman, 2021. Ovarian morphometry after giving *Moringa oleifera* Lam leaf extract. Acta Vet. Indones., 9: 44-52.
13. Aerts, J.M.J. and P.E.J. Bols, 2010. Ovarian follicular dynamics: A review with emphasis on the bovine species: Part I: Folliculogenesis and pre antral follicle development. Reprod. Domest. Anim., 45: 171-179.
14. Cortvrindt, R. and J. Smitz, 2001. *In vitro* follicle growth: Achievements in mammalian species. Reprod. Domest. Anim., 36: 3-9.
15. Scaramuzzi, R.J., D.T. Baird, B.K. Campbell, M.A. Driancourt and J. Dupont *et al*., 2011. Regulation of folliculogenesis and the determination of ovulation rate in ruminants. Reprod. Fertil. Dev., 23: 444-467.
16. Monniaux, D., 2016. Driving folliculogenesis by the oocyte-somatic cell dialog: Lessons from genetic models. Theriogenology, 86: 41-53.
17. Pramesti, N.A., T.I. Restiadi, A. Yudhana, T. Hernawati, I.S. Hamid and M.T.El. Purnama, 2018. The effect of soybean extract (*Glycine max*) ontotal growth of ovarian follicles in mice (*Mus musculus*) [In Indonesian]. J. Med. Vet., 1: 120-127.
18. Margiana, R., S.W. Lestari, P. Narulita, A.A. Jusuf and K. Ima, 2023. Dietary soybean (*Glycine max* (L.) Merr.) improved the ZP2 expression in female swiss mice. JBRA Assist. Reprod., 27: 147-155.
19. di Cosmo, A., C. di Cristo and M. Paolucci, 2001. Sex steroid hormone fluctuations and morphological changes of the reproductive system of the female of *Octopus vulgaris* throughout the annual cycle. J. Exp. Zool., 289: 33-47.
20. Bertolin, K. and B.D. Murphy, 2014. Reproductive Tract Changes During the Mouse Estrous Cycle. In: The Guide to Investigation of Mouse Pregnancy, Croy, B.A., A.T. Yamada, F.J. DeMayo and S.L. Adamson (Eds.), Academic Press, Cambridge, Massachusetts, ISBN: 9780123944450, pp: 85-94.
21. LeBlanc, S., 2010. Assessing the association of the level of milk production with reproductive performance in dairy cattle. J. Reprod. Dev., 56: S1-S7.
22. Robeck, T.R., K.J. Steinman, S. Gearhart, T.R. Reidarson, J.F. McBain and S.L. Monfort, 2004. Reproductive physiology and development of artificial insemination technology in killer whales (*Orcinus orca*). Biol. Reprod., 71: 650-660.
23. Man, L., N.L. Guahmich, E. Kallinos, L. Park and B. Caiazza *et al*., 2021. Exogenous insulin-like growth factor 1 accelerates growth and maturation of follicles in human cortical xenografts and increases ovarian output in mice. F&S Sci., 2: 237-247.
24. Utami, I.N., E.Y. Yuniwarti and T.R. Saraswati, 2018. Effect of red fruit oil on ovarian follicles development in rat exposed to cigarette smoke. Biosaintifika: J. Biol. Biol. Educ., 10: 402-408.
25. Wright, C.S., O. Hovatta, R. Margara, G. Trew, R.M.L. Winston, S. Franks and K. Hardy, 1999. Effects of follicle-stimulating hormone and serum substitution on the *in-vitro* growth of human ovarian follicles. Hum. Reprod., 14: 1555-1562.
26. Meduri, G., P. Touraine, I. Beau, O. Lahuna and A. Desroches *et al*., 2003. Delayed puberty and primary amenorrhea associated with a novel mutation of the human follicle-stimulating hormone receptor: Clinical, histological, and molecular studies. J. Clin. Endocrinol. Metab., 88: 3491-3498.
27. Gong, J.G., B.K. Campbell, T.A. Bramley, C.G. Gutierrez, A.R. Peters and R. Webb, 1996. Suppression in the secretion of follicle-stimulating hormone and luteinizing hormone, and ovarian follicle development in heifers continuously infused with a gonadotropin-releasing hormone agonist. Biol. Reprod., 55: 68-74.

28. Ying, S.Y., 1988. Inhibins, activins, and follistatins: Gonadal proteins modulating the secretion of follicle-stimulating hormone. Endocr. Rev., 9: 267-293.
29. Peñalver, R., L. Martínez-Zamora, J.M. Lorenzo, G. Ros and G. Nieto, 2022. Nutritional and antioxidant properties of *Moringa oleifera* leaves in functional foods. Foods, Vol. 11. 10.3390/foods11081107.
30. Hadi, F.S., A. Amir and Desmiwarti, 2022. The effect of *Moringa oleifera* leaf water extract on the number of ovarian follicles and the enzyme levels of superoxide dismutase of *Rattus norvegicus* rats exposed to depot medroxyprogesterone acetate (DMPA). Int. J. Sci. Healthcare Res., 7: 166-178.
31. Alwan, M.S. and B.N. Al-Okialy, 2019. Effect of alpha lipoic acid on some reproductive hormones in adult male Wister rats exposed to hydrogen peroxide. Kufa J. Vet. Med. Sci., 10: 79-87.
32. Ningtyas, N.S.I., 2017. The effect of administration of red fruit (*Pandanus conoideus* Lam.) oil on the histopathology of de graaf follicles in mice (*Mus musculus*) infertile model [In Indonesian]. J. Sangkareang Mataram, 3: 36-38.
33. Alfian, M.A.J., A.J. Sitasiwi and M.A. Djaelani, 2017. Antifertility effects of water extract of papaya seeds (*Carica papaya* L.) on the number and diameter of follicles of de graaf female mice (*Mus musculus*). J. Pro-Life, 5: 476-486.
34. Dasopang, E.S., J. Nasution, A. Suharyanto, R.H. Chandra and I. Pratama, 2021. Hispathology and effectiveness of wound healing cream karo oil herbal extract on male mice in North Sumatra, Indonesia. Nveo-Nat. Volatiles Essent. Oils J., 8: 2769-2777.
35. Alioes, Y., E.A. Zain and Z.D. Rofinda, 2022. The effect of petai (*Parkia speciosa* Hassk) seed extract and glimepiride on blood sugar levels of alloxan-induced mice (*Mus musculus*). IOP Conf. Ser.: Earth Environ. Sci., Vol. 1097. 10.1088/1755-1315/1097/1/012069.
36. Garcia, D.N., T.D. Saccon, J. Pradiee, J.A.A. Rincón and K.R.S. Andrade *et al*., 2019. Effect of caloric restriction and rapamycin on ovarian aging in mice. GeroScience, 41: 395-408.
37. Dou, X., Y. Sun, J. Li, J. Zhang and D. Hao *et al*., 2017. Short-term rapamycin treatment increases ovarian lifespan in young and middle-aged female mice. Aging Cell, 16: 825-836.
38. Huang, H., F. Wei, S. Qiu, B. Xing and J. Hou, 2023. Polystyrene microplastics trigger adiposity in mice by remodeling gut microbiota and boosting fatty acid synthesis. Sci. Total Environ., Vol. 890. 10.1016/j.scitotenv.2023.164297.
39. Gao, Y.Q., L. Ge, Z. Han, X. Hao and M.L. Zhang *et al*., 2020. Oral administration of olaquindox negatively affects oocytes quality and reproductive ability in female mice. Ecotoxicol. Environ. Saf., Vol. 201. 10.1016/j.ecoenv.2020.110826.
40. Jamshidi-Kia, F., J.P. Wibowo, M. Elachouri, R. Masumi, A. Salehifard-Jouneghani, Z. Abolhasanzadeh and Z. Lorigooini, 2020. Battle between plants as antioxidants with free radicals in human body. J. Herbmed Pharmacol., 9: 191-199.
41. Lobo, V., A. Patil, A. Phatak and N. Chandra, 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn. Rev., 4: 118-126.
42. Tran, T.T. and L.A. Hinds, 2013. Fertility control of rodent pests: A review of the inhibitory effects of plant extracts on ovarian function. Pest Manage. Sci., 69: 342-354.