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Growth and Development of Mouse Secondary Spermatocytes (*Mouse muscle*) After Giving Extra Kepel Fruit (*Stelechocarpus burahol*)

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Abstract: Indonesia has a tropical climate is rich in flora diversity. Many medicinal plants, including the Kepel (Stelechocarpus burahol), are herbal medicines. Kepel Fruit (*Stelechocarpus burahol*) contains secondary metabolites of alkaloids, flavonoids, polyphenols, saponins, triterpenoids and quinones. Some of these compounds have anti-fertility properties. Antifertility compounds are compounds that can prevent fertility by interfering with several normal reproductive mechanisms, both in men and women. This study aims to analyze the effectiveness of Kepel fruit extract on the growth and development of secondary spermatocytes in mice (*Mouse muscle*). This research uses true experiments and *Posttest-Only Control* with a quantitative approach. The samples used for this research design, both experimental and controlled, were taken randomly. Testing the significance of the treatment effect uses parametric statistics, namely the One-Way ANOVA test at a significance level of 5% (α = 0.05). Then, if there are differences, continue with the LSD further test (*Least Significant Difference*) at a significance level of 5% (α = 0.05). This research design used a Randomized Block Design (RAK) with a total sample of 32 mice (*Mouse muscle*) with 8 repetitions and 3 treatment groups. The growth and development of secondary spermatocytes in mice are determined by counting the number of secondary spermatocytes in the seminiferous tubules of mouse testicular incisions. The results of the one-way Anova test show that the calculated F is 6.41, and the value is sig. (*P.Value*) of 0.002, these results suggest *P.Value* (0.002) < the α value (0.05), which means that the number of secondary spermatocytes is significantly different in each group so that this study can conclude that the administration of Kepel fruit extract (*Stelechocarpus burahol*) can significantly reduce the number of growth and development of mouse secondary spermatocytes (*Mouse muscle*).

Keywords: Kepel Fruit Extract (*Stelechocarpus burahol*); Mice (*Mouse muscle*); Secondary spermatocytes.

Introduction

The population of Indonesia in 2024 will be 281,603,800 people and will be the fourth largest in the world [1]. Indonesia's total population increased by 2,907,600 people from the previous year. One of the population factors that causes Indonesia's population to be so large is that implementing family planning (KB) programs is less than optimal [2,3]. Factor Another reason for the increase in population is that married couples during their fertile period do not participate in family planning programs or are afraid to use contraception because they are worried about side effects on their health. For example, side effects that can result from consuming birth control pills: stomach cramps, more vaginal discharge than usual, spotting or vaginal bleeding outside the cycle menstruation, the amount of menstrual blood is less than usual, and the menstrual cycle becomes irregular, decreased passion sexual (libido), mood swings, headaches, and swollen or painful breasts [3]. Reducing the fertility of married couples during the fertile period needs to be done by using active substances from plants. Kepel Fruit (*Stelechocarpus burahol*) According to research [4], the results of phytochemical screening tests on Kepel fruit pulp simplicia showed the presence of secondary metabolites in the alkaloids, flavonoids, polyphenols, saponins, triterpenoids, and quinones. Some of these compounds have anti-fertility properties. Antifertility is a term used for compounds or substances that can interfere with the reproductive system. Antifertility compounds are compounds that can prevent fertility by interfering with several normal reproductive mechanisms, both in men and women [5].

Research methods

The design used in this research is a true experimental design. The true experimental design used is the Posttest-Only Control Design. The samples used for this research design, both experimental and control, were taken randomly. Testing the significance of the treatment effect uses parametric statistics, namely the One-way ANOVA test. This research design used a Randomized Block Design (RAK) with a total sample of 32 mice (*Mouse muscle*) with 8 repetitions and 3 treatment groups. This research used tools: basins, knives, trays, blenders, analytical balances, glass bottles, rotary evaporators, beakers, scales, mouse cages, mice drinking bottles, section tools, ovens, microscopes, measuring cups, hot plates, dropper pipettes,

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petri dish, refrigerator, microtome, vial, section board, cell phone camera, gavage needle, slide, and cover glass. Meanwhile, the materials used in this research include 0.9% NaCl solution, pellets, water, Kepel fruit extract, 95% ethanol solvent, 30 distilled water, xylol, 10% NBF solution, dmso, hematoxylin, eosin, paraffin, 70% alcohol, 80% alcohol, 90% alcohol, 96% alcohol, absolute alcohol, tissue, cotton buds, entellan adhesive, and gloves.

This research used the following procedures: Maintenance and acclimatisation of mice one week before treatment. In the acclimatisation stage, mice were fed CP551 pellet food and drank water using a mouse drinking bottle. Feeding and drinking are done in the morning and evening. Making Kepel fruit extract, extraction of Kepel fruit is carried out using the maceration method, where the simplicia powder is soaked in 95% ethanol for 3 days with stirring once a day until all parts of the fruit are properly submerged, namely submerged in 95% alcohol liquid as high as 1 cm above the simplicia powder. This mixture is filtered using filter paper to get only the liquid part. Concentrate the resulting mixture filtered with an evaporator to obtain a concentrated extract [6].

The maceration method's purpose is to avoid damage to the active compounds contained in the sample due to heating. The solvent used is 95% ethanol. To attract polar and nonpolar compounds [7]. 1-2 kg of kepel fruit is washed until clean, cut into small pieces, and air-dried at room temperature. Once dry, the kepel fruit pieces are blended to make powder. After that, the smooth kepel fruit was then weighed for dry weight. The dry weight obtained was 62.2628 g. 62.2628 g of kepel fruit powder was put into a glass jar and soaked in 95% ethanol for 3 days. The maceration results are evaporated using an evaporator at a temperature of 55°C. Heating at a low temperature is carried out to maintain the content of thermolabile active compounds. The result of this evaporation is thick kepel fruit extract. The weight of the thick Kepel fruit extract was 10.4036 g, so the yield of Kepel fruit extract obtained was 16.7%. The Kepel fruit extract solution in various doses is made by adding DMSO and distilled water. This research used 32 experimental animals, male Balb/c strain mice aged 8 weeks. The body weight of the mice in this study was 25-40 grams. The experimental animals were divided into 4 groups, namely the control group, treatment group 1 (T1) with a dose of 50 mg/kgBW, treatment group 2 (T2) with a dose of 100 mg/kgBW, and treatment group 3 (T3) with a dose of 150 mg/kgBW. Calculating the Kepel fruit extract solution concentration is done by dissolving 1 gram of thick Kepel fruit extract and then adding distilled water to 100 ml. The concentration of the solution is 1%, which means it contains 10 mg of thick Kepel fruit extract in every 1 liter (10 mg/L). The volume of extract suspension given to mice orally is calculated using the VAO formula (Drug Administration Volume) [7]. They were given water and pellets for control animal treatment and fed 0.9% NaCl. The animals were given pellet food and sufficient drinking water during the experiment. All Kepel fruit extract is administered orally using a gavage needle [8].

Results and Discussion

Table 1. Average Number of Secondary Spermatocytes in Mice Testes (*Mouse muscle*)

Group	Number of Mice	Number of Secondary Spermatocytes (x ± Sd)
Control	8	9.50 ± 3.338^{a}
T1	8	7.13 ± 2.232^{ab}
T2	8	5.75 ± 1.909^{bc}
T3	8	4.50 ± 1.773^{c}

Note: Different letters in the same column indicate significant differences ($P < 0.05$).

Observations and calculations of secondary spermatocytes in each group are presented in Table 1. In accordance with Table 1, it can be read that the average number of secondary spermatocytes in the control group was 9.50 ± 3.338, in the T1 group was 7.13 ± 2.232, in the T2 group was 5.75 ± 1.909, the T3 group was 4.50 ± 1.773. This data shows a decrease in secondary spermatocytes in the three treatment groups.

Table 2. Results of One-Way Variance Analysis of the Number of Secondary Spermatocytes in Mice Testes (*Mouse muscle*)

Diversity Source	DB	JK	KT	F Count	P
Treatment	3	110.09	36.70	6.41	0.002
Error	28	160.37	5.73		
Total	31	270.47			

Table 3. LSD test results regarding the number of secondary spermatocytes in mouse testicles (*Mouse muscle*)
Use LSD (0.05) = 2.45119

	1	2	3	0
1	-	1.375	$2.625^{*)}$	2.378
2	-	-	1.250	$3.75^{*)}$
3	-	-	-	$5.00^{*)}$

Note: The superscript sign indicates a significant difference ($P < 0.05$).

The LSD test in Table 3 shows that there is a significant difference P < a (0.05) between the number of secondary spermatocytes in the control group, and the T2 and T3 groups showed a significant difference ($P < 0.05$), likewise between the T1 group and the T3 group there was a significant difference ($P < 0.05$), but not there was a significant difference between groups T2 and T3 ($P > 0.05$).

Secondary spermatocytes resulting from meiosis I continue the growth and development of primary spermatocytes [9]. The spermatogenesis process is influenced by the hormones FSH (*Follicle Stimulating Hormone*) and LH (*Luteinizing Hormone*) [10]. The anterior part of the pituitary gland produces the gonadotropin hormone that regulates testicular function. *Follicle Stimulating Hormone* is important in regulating testicular function [11]. In Sertoli cells, adenylyl cyclase is stimulated

by the gonadotropin hormone so that the synthesis of cyclic AMP (cAMP) from ATP increases. Furthermore, cAMP stimulates protein kinase and androgen-binding protein (ABP) phosphorylation processes [11]. LH (*Luteinizing Hormone*) plays an important role in regulating testicular function. Under the influence of LH, the Leydig cells synthesize the hormone testosterone. Testosterone and FSH work synergistically to encourage the change of primary spermatocytes into secondary spermatocytes, which enter meiosis II to produce spermatids, followed by spermiogenesis [12]. Thus, the interaction between the hormones testosterone, FSH, and LH controls spermatogenesis. Any disturbance in the interaction of testosterone, FSH, and LH hormones will cause disturbances in spermatogenesis [13]—Kepel (*Stelechocarpus burahol*), including fruit food plants. Kepel contains bioactive compounds such as flavonoids, cyclooxygenase-2 inhibitors, anti hyperuricemic, cytotoxic substances, anticancer, oral deodorant, and phytoestrogen compounds found in the leaves, flowers, flesh of the fruit, fruit seeds, fruit skin, and stem bark of the Kepel fruit. Kepel's active compounds have the potential to be antioxidants, gout medications, oral contraceptives, and natural deodorants. Some of these compounds are classified as antifertility compounds [14].

The research results in Table I show a decrease in the average number of secondary spermatocytes as the dose given increases. A decrease in secondary spermatocytes is possible due to the Kepel fruit extract's active alkaloids, flavonoids, tannins, polyphenols, saponins, triterpenoids, and quinones. According to [15], the alkaloid and tannin content in Kepel fruit extract affects ATP production, disrupting the spermatogenesis process. The alkaloid content in Kepel fruit is toxic to cells, especially cells that require higher energy, such as spermatozoa cells, because it can increase the production of free radicals. An increase in ROS is thought to disrupt the osmotic balance, damaging spermatozoa [15,16].

The results of the one-way Anova test in Table 2 show a calculated F of 6.41 and a sig. (*P.Value*) of 0.002, these results suggest *P.Value* (0.002) < the α value (0.05), which means the number of secondary spermatocytes is significantly different in each group. The effect of Kepel fruit extract on reducing the number of secondary spermatocytes formed may be influenced by its fertility content, namely that it contains alkaloids, flavonoids, polyphenols, saponins, triterpenoids and quinones [5], [17]. According to [17] and [18], the testes are reproductive organs susceptible to free radicals. Hence, these compounds' presence and high levels disrupt spermatogenesis and spermatogenic cell membranes' integrity. Spermatogenic cells have membranes containing double-chain unsaturated fatty acids. The encounter of spermatogenic cells with excess free radicals will cause a lipid peroxidation reaction [18]. Furthermore, by [19], ROS can also cause disturbances in ATP production and apoptosis in cells. Spermatozoa are very sensitive to high levels of ROS. The reproductive system requires ROS in low concentrations so that the reproductive process can take place. ROS are useful for the complex process of proliferation and maturation of spermatozoa embryos in the testicular seminiferous tubules, from diploid spermatogonia through meiosis to mature haploid spermatozoa. The decrease in the number of spermatogenic cells is thought to be due to an increase in excessive metabolism and the production of free radicals, causing spermatogenic cells to become unstable [20], [21].

The efficiency of spermatogenesis depends on the proliferation of spermatogonia and the loss of germ cells during meiosis and spermiogenesis. The alkaloid compounds in Kepel fruit extract are thought to be able to influence ATP production because they are toxic to cells, especially cells that require higher energy, such as in the second meiosis stage. This is thought to result in reduced cell energy to carry out the second meiotic division so that the number of secondary spermatocytes decreases [22]. On the other hand, [23] stated that the content of Kepel fruit extract is saponin, which is an antifertility compound that has a very strong influence on the spermatogenesis process.

The anti-fertility compound content in Kepel fruit extract inhibits spermatogenesis at the differentiation stage of the morphological transformation of spermatids to form spermatozoa. [24] said that the active compounds in Kepel fruit extract may be cytotoxic to Sertoli cells, causing damage to these cells and resulting in degeneration of spermatogenic cells. Sertoli cells play a role in providing nutrition so that when damage to these cells occurs, the provision of nutrition will be disrupted so that spermatogenic cells cannot continue meiosis. According to [21], the active substance content of flavonoids is caused by the mechanism of *blame* negative for the hypothalamus to suppress gonado-releasing factors in the form of FSHRF and LHRF. The same mechanism that suppresses the anterior pituitary gland cannot produce FSH and LH. FSH is a gonadotrophin hormone that has receptors in the seminiferous tubules of the testicles to stimulate spermatogenesis. Meanwhile, LH/ICSH (*Interstitial Cell Stimulating Hormone*) is a gonadotropin hormone with receptors on Leydig cells, stimulating Leydig cells to secrete the hormone testosterone. These two types of hormones, FSH and LH, play an important role in the spermatogenesis process. If these two hormones are not secreted adequately, they will inhibit spermatogenesis [24]. The active compounds contained in Kepel fruit extract can stimulate negative feedback on the hypothalamus-pituitary-testicular axis so that it can cause a decrease in FSH and LH production by the anterior pituitary gland [25]. A decrease in LH synthesis will trigger a decrease in the synthesis of the testosterone hormone by Leydig cells in the testicles. Decreased FSH synthesis will prevent germ cells in the seminiferous tubules in the testes from carrying out spermatogenesis. Decreased FSH levels also affect the performance of Sertoli cells as cells that provide nutrition for spermatogenic cells. Thus, disruption of Sertoli cell and Leydig cell secretion will disrupt spermatogenesis. The active compound in Kepel fruit extract is thought to have antifertility properties [25].

Antifertility compounds, in principle, work in two ways, namely through cytotoxic or cytostatic effects.

Cytotoxic effects are related to the apoptosis of spermatogenic cells. The cytostatic effect is related to inhibiting the development of spermatogenic cells that are actively dividing and inhibiting the metabolic rate of spermatogenic cells by disrupting the balance of the hormonal system [26]. The mechanism for inhibiting spermatogenic cell growth in this study is a decrease in the number of secondary spermatocytes, which is thought to be closely related to the cytotoxic and cytostatic effects of alkaloids, flavonoids, tannins, polyphenols, saponins, triterpenoids and quinones contained in Kepel fruit extract. Apart from that, the mechanism of decreasing the number of spermatogenic cells is thought to be closely related to a decrease in the hormones FSH, LH, and testosterone [27].

Conclusion

Based on the research results, data analysis, and discussion, it can be concluded that giving kepel fruit extract (*Stelechocarpus burial*) can significantly reduce the growth and development of secondary spermatocytes in mice (*Mouse muscle*).

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