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# Efficacy of Cardamom Seed Extract against LH, FSH, and Spermatozoa of Obese Rats

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**Keywords:**
Obese, Cardamom, LH, FSH, Spermatozoa

**ABSTRACT**

Infertility to be one in all the real reproductive fitness risks with the improvement of age. Increased weight in guys has been related to a decrease testosterone level, poorer sperm quality, and decreased fertility in comparison to guys of everyday weight. Cardamom incorporates phenols, flavonoids, tannins, saponins, which act as antioxidants in shielding towards unfastened radicals. The aim of the study is To analyze the effectiveness of cardamom seed extract (A. compactum) on LH (luteinizing hormone), FSH (follicle stimulating hormone), spermatozoa levels of obese rats. This research was an experimental study with a post test only control group design conducted at PAU UGM for 6 weeks in Juli 2022. In this study, 30 male wistar rats have been divided into five remedy groups. Based on One Way Anova bivariate statistic, $p < 0.05$, it can be concluded that Cardamom seed extract has a substantial impact on sperm count, motility, and morphology in addition to LH and FSH degrees due to the fact there are substantial differences. Cardamom seeds had a significant effect on the morphology, amount, and motility of spermatozoa, as well as LH and FSH levels in obese rats treated with cardamom seed extract for 14 days.



## 1. INTRODUCTION

Infertility is a major health problem in the world. According to the [1], the infertile couples have been 36% for men and 64% for women. Primary infertility occurred in 17% of couples who have been married for more than 1 year, have no signs of pregnancy, and have never been pregnant at all. WHO estimates that about 50-80 million married couples (1 in 7 couples) have infertility problems, and every year about 2 million infertile couples appear.

The total 270,203,917 population of Indonesia had a total fertile age couple of 39,6055,811 people. The survey of failed pregnancies in couples who have been married for 12 months show that 40% are due to infertility in men, 40% in women, and 10% both, 10% of unknown cause. According to investigate from The [2] in Jakarta, there were 10205 PUS (fertile age couples) who suffered from infertility as many as 520 (5.1%).

Infertility in men is often caused by low sperm motility, lack of sperm count, sperm morphology abnormalities, can be assessed using sperm analysis. One of the factors that affect the quality of spermatozoa is obesity. Obesity is a medical condition where there is excess fat due to an imbalance between food intake and energy expenditure. The prevalence of obesity in Indonesia tends to increase. According to SIRKESNAS 2016, the superiority of obese for the age institution over 18 years is 33.5%. Central Java has obesity prevalence above the national prevalence, which is 6.04% (National Prevalence 5.07%) [3].

Obesity can lead to impaired spermatogenesis. This is due to impaired hormone regulation as a result of an increase in the amount of adipose tissue. [5] in their research showed that obesity affects the decrease in LH (luteinizing hormone) and FSH (follicle stimulating hormone). Impaired regulation of LH and FSH causes an increase in estrogen and a decrease in testosterone which affect spermatogenesis.

Cardamom seeds contain 3-7% essential oil consisting of terpineol, terpinyl acetate, cineol, alpha borneol, and beta camphor which are used as raw materials for the pharmaceutical industry. Cardamom seed essential oil is also used add flavor in the food, medicine, and cosmetic industries. In addition, cardamom has been shown to have analgesic, anti-inflammatory, anti-microbial, antioxidant, and hypolipidemic effects [6]. [7] in his research showed that cardamom has the good ability as an antioxidant. Research on rats showd that cardamom seed extract causing decreased in body weight and testicular weight loss [8]. Cardamom contains phenols, flavonoids, tannins, saponins, which play a role as an antioxidant for protection against free radicals.

Excess food intake can lead to obesity, thus the amount of fat tissue in the body were getting increase, body fat tissue can cause an increase in the number of free radicals, while endogenous antioxidants are unable to bind to all free radicals. Free radicals may be observed withinside the body, one in every of that's Reactive Oxygen Species (ROS). In addition, obesity can interfere with hormone regulation, thereby causing impaired spermatozoa formation. Spermatozoa formation disorders include disturbances in the number, morphology, and motility of spermatozoa. This disorder causes the failure of the spermatozoa to penetrate the ovum, thus causing infertility [4].

Based on this background and no further research on the effectiveness of cardamom seeds (*A. compactum*) on LH, FSH and spermatozoa. The objective of this study is to know more about the effectiveness of cardamom seeds (*A. compactum*) against LH, FSH and spermatozoa of obese wistar rats. This study is expected to provide useful results considering the high prevalence of infertility and obesity in the community.

## 2. MATERIAL AND METHODS

### *2.1 Cardamom Seed Extraction*

Cardamom seed extract was prepared at a dose of 45 mg/kgBW/day dissolved in distilled water toa volume of 1ml for treatment group 1, cardamom seed extract dose of 90 mg/kgBW/day for treatment group 2, and a dose of cardamom seed extract 180 mg/kgBW/day which was dissolved with distilled water to a volume of 1 ml for treatment group 3. Equivalent doses of cardamom ethanol extract from rats to humans with a multiplication factor of 56 in humans and 0.14 in rats. Then, each dose is multiplied by 56 and divided by 0.14 to give humans a dose of 18 g/kgBW/day for 45 mg/kgBW/day, 36 gr/kgBW/day for 90 mg/kgBW/day and 72 g /kgBW/day to 180 mg/kgBW/day.

### *2.2 Experimental Design and Animals*

This study was a true experimental with post-test only of control group design and was carried out at Center for Food and Nutrition Studies (PSPG), Gadjah Mada University in July-August 2022 using 30 Wistar rats

of 6-8 weeks and weighed 200-250 gr. The Ethics Committee of the Faculty of Medicine, Diponegoro University approved this study (No:72/EC/H/FK-UNDIP/VII/2022). The sample was calculated using the Federer method regarding the concept of reduction, replacement, and refinement. Then, 30 white Male wistar rats were given HCD (High Carbohydrate Diet) and HFD (High Fat Diet) for 4 weeks, and assesments taken after 4 weeks. There were 5 groups, randomly as follows: Normal control group (group of mice with normal weight), Negative control group (group of obese mice), Group I (obese rats and were given cardamom extract at a dose of 45 mg/kgBW/day dissolved in 1 ml aquadest for 14 days), Group II (obese rats and were given cardamom extract at a dose of 90 mg/kgBW/day dissolved in 1 ml aquadest for 14 days), Group III (obese rats and were given cardamom extract at a dose of 180 mg/kgBW/day dissolved in 1 ml aquadest for 14 days).

Each treatment was carried out once a day in the morning for 14 days. The treatment for 14 days is based on the duration of a spermatogenic cycle which takes approximately 14 days. On day 15 the rats were killed under chloroform anesthesia, then the cauda epididymis until the ampulla of the vas deferens was cut. After that, sorting and dilution were carried out to obtain rat sperm.

The cut epididymis was placed in a petri dish containing 0.9% physiological NaCl solution. Then the epididymis was massaged using a scalpel handle to remove the spermatozoa. Then the spermatozoa samples were examined for morphology and motility.

***2.3 Hormonal Assessment***

FSH and LH examination of mice begins with taking blood at the tail end. The mice were held on the board, the second examiner took 3 L of blood sample from the tail end using a pipette. Blood samples were diluted in 57 L 0.1 M Phosphate-Buffered Saline (PBS), centrifuged and stored at -20oC for LH and FSH examination by ELISA method.

***2.4 Sperm Analysis***

The amount and morphology of sperm were analysed based on the following steps. Mice sperm were put into a petri dish containing a physiological solution of 0.9% NaCl, then dripped on the slide for about 2 drops. The slide is then dripped with eosin Y and methylene. Furthermore, sperm morphology was observed under a microscope with a magnification of 400 times and the percentage of normal and abnormal morphology was calculated.

The motility of sperm was observed under a microscope and examined at 40x magnification. Observations were made in five fields of view with the following motility criteria:

- A: spermatozoa progress quickly to the front
- B: spermatozoa progress slowly to the front
- C: spermatozoa only move in place
- D: immobile spermatozoa

Spermatozoa motility is expressed in:

- Progressive, if the spermatozoa meet criteria a and b
- Not progressive, if the spermatozoa meet criteria c and d

Data analysis were taken progressive spermatozoa (a+b) from 100 sperm in 5 fields of view. From 100 sperm, the percentage of sperm with progressive motility was calculated using a hand counter.

***2.5 Statistical Analysis***

Data analysis was performed using computerized software. The data was tested for normality with the

Saphiro-Wilk test, then continued with statistical testing. If the results of the Saphiro-Wilk test show normal distribution of data, then the statistical test chosen is ANOVA, whereas if the data is not normally distributed, the statistical test selected is the non-parametric Kruskal-Wallis test. The data is considered meaningful if the p value 0.05. If the test results show that H0 fails to be rejected (no difference between groups), then a post hoc test is not carried out, whereas if there is a difference, a post hoc test will be carried out. The post hoc test carried out depends on the results of the Test of Homogeneity of Variances; if the same variance (significance value > 0.05) is obtained, a post-hoc Bonferoni test will be carried out, whereas if a different variance (significance value <0.05) is obtained, a post-hoc test with Gomes-Howell is performed.

## 3. RESULT AND DISCUSSION

The study was conducted on 30 male Sprague dawley rats, which were divided into 5 groups, namely: normal control group KN, negative control group (K(-)), treatment group 1 given cardamom seed extract 45 mg/KgBW/day, treatment group 2 were given cardamom seed extract 90 mg/KgBW/day, and treatment group 3 was given cardamom seed extract 180 mg/KgBW/day (Figure 1)

### *3.1 Sperm Morphology, Amount, and Motility*

Figure 4. shows the average morphology of sperm along with the results of the normality test. From these data, it shows that the highest mean is group KN (Normal control group), then the lowest average is negative control group (K(-)). The normality and homogeneity test of the data from each group was carried out using the Saphiro-Wilk test because the sample was less than 50, the data obtained were normally distributed with p>0.05, so that further tests were carried out using the One-Way ANOVA test, with the result was p < 0.05 and Levene > 0.05, it can be concluded that there were significant differences in sperm morphology based on the treatment group. To find out the differences between the treatment groups, the test was continued by using the Post Hoc Bonferoni test, because based on the Homogeneity of Variances, the same variance was obtained (significance value > 0.05).

Spermatozoa morphology differences between groups were analyzed using the Bonferroni Post Hoc test. In the results of the analysis, there was a significant difference (p<0.05) in group K(N) (Normal control) against group K(-) (negative control) and group P1 (obesity + cardamom seeds 45 mg/kgBW/ days), all groups K(-) (negative control) against all treatment groups, and in group P1 (obesity + cardamom seeds 45 mg/kgBW/day) ), P2 (obesity + cardamom seeds 90 mg/kgBW/day), against group P3 (obesity + cardamom seeds 180 mg/kgBW/day).

Figure 4. shows the average sperm count and the results of the normality test. From these data, it shows that the highest mean is group KN (Normal control group), then the lowest average is negative control group (K(-)). The normality and homogeneity test of the data from each group was carried out using the Saphiro-Wilk test because the sample was less than 50, the data obtained were normally distributed with p>0.05, so that further tests were carried out using the One-Way ANOVA test. From the results of the One-Way ANOVA test, p value <0.05 and Levene> 0.05, it can be concluded that the number of sperm based on the treatment group there is a significant difference. To find out the differences between the treatment groups, the test was continued by using the Post Hoc Bonferoni test, because based on the Homogeneity of Variances, the same variance was obtained (significance value > 0.05). Differences in the number of spermatozoa between groups were analyzed using the Bonferroni Post Hoc test. In the results of the analysis, there was a significant difference (p<0.05) in the KN group (Normal control) against the K(-) group (Negative control); group P1 (obesity + cardamom seeds 45 mg/kgBW/day); and group P2 (obesity + cardamom seeds 90 mg/kgBW/day), all groups K(-) (negative control) against all treatment groups, and in group P1 (obesity + cardamom seeds 45 mg/kgBW/day), P2 (obesity + cardamom seeds 90 mg/kgBW/day) against group P3 (obesity + cardamom

seeds 180 mg/kgBW/day)

Figure 4. shows the average sperm motility along with the results of the normality test. From these data, it shows that the highest mean is group KN (normal control group), then the lowest average is negative control group (K(-)). The normality and homogeneity test of the data from each group was carried out using the Saphiro-Wilk test because the sample was less than 50, the data obtained were normally distributed with p>0.05, so that further tests were carried out using the One-Way ANOVA test. From the results of the One-Way ANOVA test, p <0.05 and Levene <0.05, it can be concluded that there was a significant difference in sperm motility based on the treatment group. To find out the differences between the treatment groups, the test was continued by using the Post Hoc Games-Howell test, because based on the Homogeneity of Variances, different variances were obtained (significance value <0.05). Differences in spermatozoa motility between groups were analyzed using the Post Hoc Games-Howell test. In the results of the analysis, there was a significant difference (p<0.05) in all groups, but a non-significant difference (p>0.05) were found in the negative control group (K(-)) against the P1, P2, P3 group (obesity + cardamom seeds). 180 mg/kg/day).

Figure 4. shows the average levels of LH along with the results of the normality test. From these data, it shows that the highest mean is group KN (normal control group), then the lowest average is negative control group (K(-)). The normality and homogeneity test of the data from each group was carried out using the Saphiro-Wilk test because the sample was less than 50, the data obtained were normally distributed with p>0.05, so that further tests were carried out using the One-Way ANOVA test. From the results of the One-Way ANOVA test, p <0.05 and Levene <0.05, it can be concluded that there was a significant difference in sperm motility based on the treatment group. To find out the differences between the treatment groups, the test was continued by using the Post Hoc Games-Howell test, because based on the Homogeneity of Variances, different variances were obtained (significance value <0.05). Differences in LH levels between groups were analyzed using the Post Hoc Games-Howell test. In the results of the analysis, there was a significant difference (p<0.05) in all research groups.

Figure 4. shows the average levels of FSH along with the results of the normality test. From these data, it shows that the highest mean is group KN (normal control group), then the lowest average is negative control group (K(-)). The normality and homogeneity test of the data from each group was carried out using the Saphiro-Wilk test because the sample was less than 50, the data obtained were normally distributed with p>0.05, so that further tests were carried out using the One-Way ANOVA test. From the results of the One-Way ANOVA test, p <0.05 and Levene <0.05, it can be concluded that there was a significant difference in sperm motility based on the treatment group. To find out the differences between the treatment groups, the test was continued by using the Post Hoc Games-Howell test, because based on the Homogeneity of Variances, different variances were obtained (significance value <0.05). Differences in LH levels between groups were analyzed using the Post Hoc Games-Howell test. In the results of the analysis, there was a significant difference (p<0.05) in all study groups, but a non-significant difference (p>0.05) were found in the negative control group (K(-)) against P1, P2, P3 group (obesity + seeds). cardamom 180 mg/kgBW/day).

The quality and quantity of spermatozoa are influenced by the regulation of LH and FSH. Luteinizing hormone (LH) which acts as a stimulant of Leydig cell secretion, where Leydig cells function as testosterone producer in men which functions for spermatogenesis process, and Follicle Stimulating Hormone (FSH) serves to stimulate Sertoli cell proliferation. FSH, together with testosterone, is required to maintain normal sperm count and function [32]. Studies have shown that FSH deficiency not only lowers sperm count but also affects sperm quality. In this study, cardamom seed extract had a significant effect on LH and FSH levels. Cardamom contained polyphenols and flavonoids such as lutein, anthocyanins and quercetin as antioxidant

and anti-inflammatory properties [33]. Cardamom powder supplementation prevent dyslipidemia, oxidative stress and liver damage in rats fed in HCHF diet. The high activity of antiinflammatory and antioxidant properties obtained from (−)-epicatechin, vanillin, p-coumaric acid, trans-ferulic acid, ellagic acid of its ethanol extract. According to previous research, the main constituents of cardamom are -terpinyl acetate, -terpineol, 1,8-cineole and limonene, which have potential effects on the metabolic syndrome.

Spermatozoa became one of the variables observed in this study, including their assesments as follows: morphology, number, and motility. According to the results of this study, cardamom seeds had a significant effect ($p<0.05$) on sperm morphology, amount, and motility. This study is in line with [8] that cardamom extract increases testicular mass and body weight in mus musculus albinus rats. The seeds have aphrodisiac substances such as cineole, camphor, borneol and gera-niol. Cineole contained in cardamom is a type of 1,8-cineole and is known to be the most abundant in cardamom seeds. In addition, cardamom extract can minimize cell damage and distortion and stimulate spermatide differentiation.

## 4. CONCLUSION

Cardamom seeds had a significant effect on the number, morphology, and motility of spermatozoa, as well as LH and FSH levels in obese rats treated with cardamom seed extract for 14 days.

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**Table.1** The average of morphology sperm, amount sperm, motility sperm, LH, and FSH

| | Morphology | Amount | Motility | LH | FSH |
|---|---|---|---|---|---|
| KN | 37.87 | 49.32 | 64.15 | 11.99 | 62.79 |
| K(-) | 21.39 | 22.62 | 27.27 | 4.25 | 20.28 |
| P1 | 34.71 | 44.83 | 58.52 | 8.26 | 30.15 |
| P2 | 36.34 | 46.75 | 61.9 | 8.95 | 40.79 |
| P3 | 37.61 | 48.45 | 63.74 | 9.9 | 60.22 |

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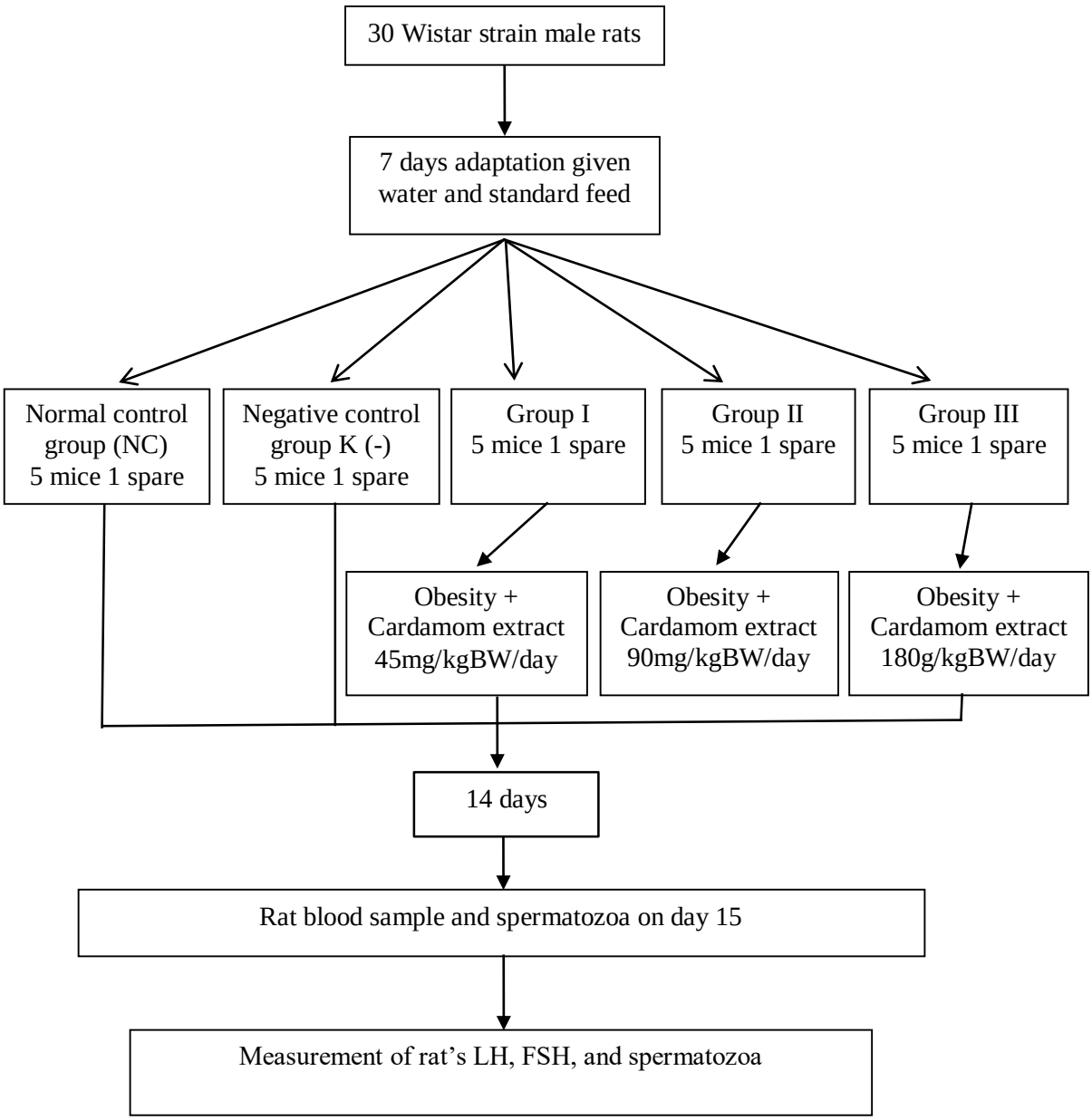


**Figure 1.** Research Flow Diagram

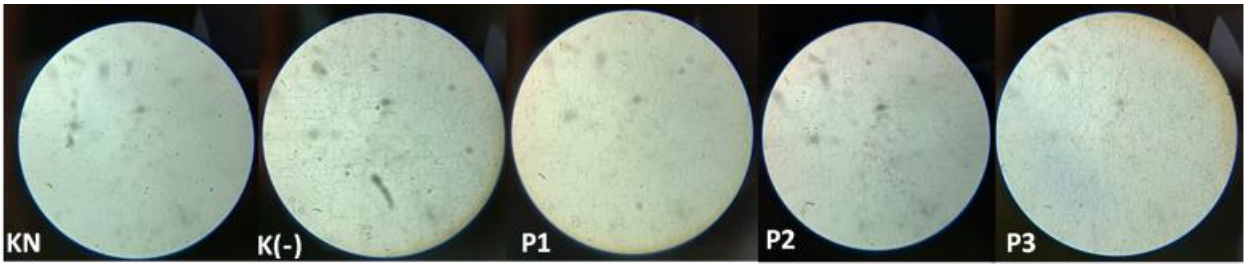


**Figure 2.** Spermatozoa

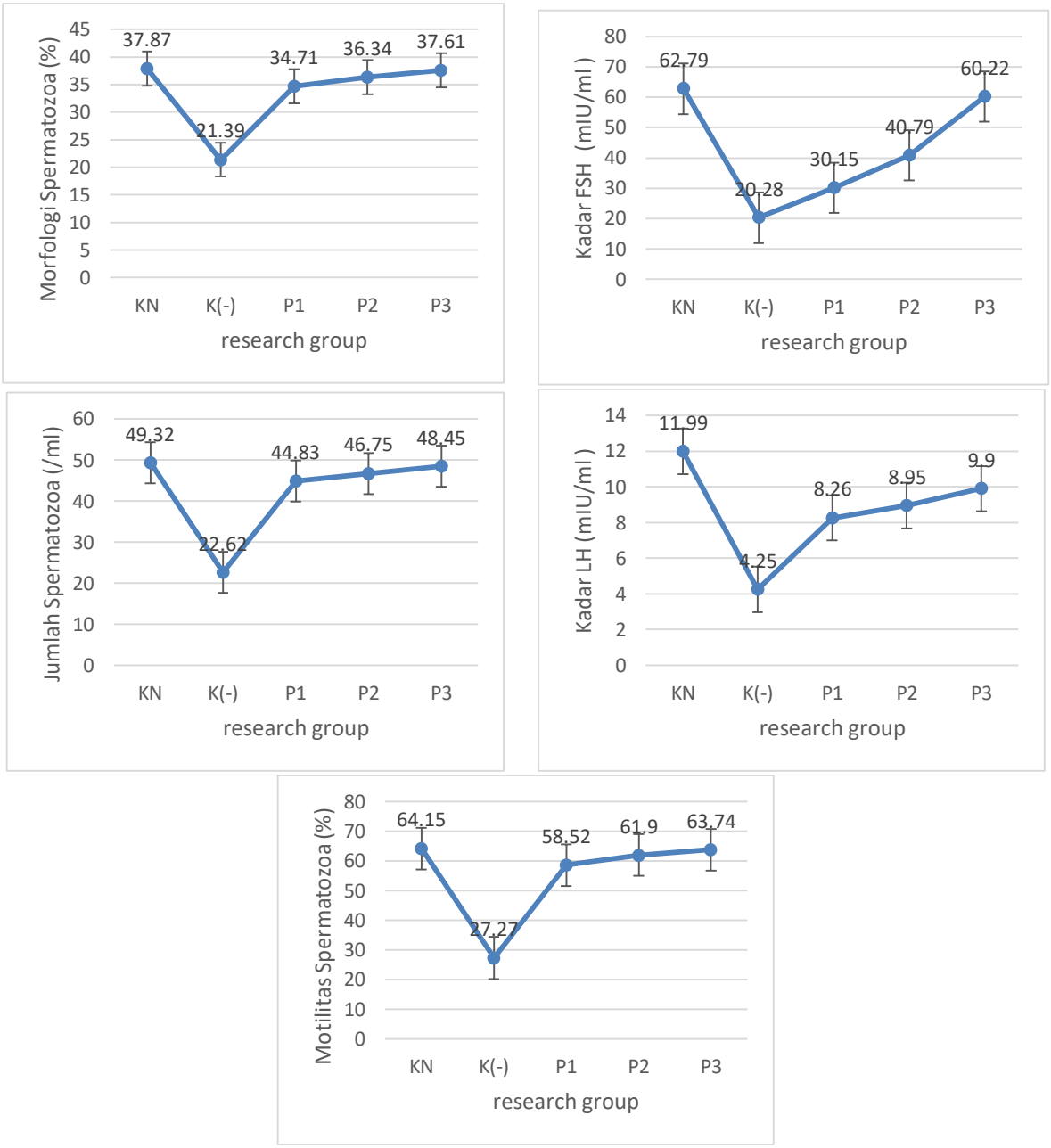


**Figure 3.** Research group with sperm morphology, sperm amount, sperm motility, LH level, and FSH level

A. Morphology

| **Group** | **Mean ± SD** | ***p*** |
|---|---|---|
| KN | 37.84±1.3567 | 0,545* |
| K(-) | 21.39±1.77068 | 0,452* |
| P1 | 34.71±0.7546 | 0,269* |
| P2 | 36.34±0.89945 | 0,437* |
| P3 | 37.61±1.2268 | 0,209* |

Note : * Normal (p > 0,05)

B. Amount sperm

| Group | Mean ± SD | *p* |
|---|---|---|
| KN | 49.32±1.05553 | 0,893* |
| K(-) | 22.62±1.54546 | 0,456* |
| P1 | 44.83±0.82753 | 0,029 |
| P2 | 46.75±0.736953 | 0,623* |
| P3 | 48.45±1.16838 | 0,745* |

Note: * Normal (p > 0,05)

C. Motility sperm

| Group | Mean ± SD | *p* |
|---|---|---|
| KN | 64.15±0.51759 | 0,941* |
| (-) | 27.27±1.92769 | 0,614* |
| P1 | 58.52±0.75114 | 0,383* |
| P2 | 61.90±0.51841 | 0,126* |
| P3 | 63.74±0.42109 | 0,393* |

Note: * Normal (p > 0,05)

D. LH levels

| Kelompok | Mean ± SD | *p* |
|---|---|---|
| KN | 11.99±0.82284 | 0,481* |
| K(-) | 4.25±0.35397 | 0,232* |
| P1 | 8.26±0.22214 | 0,857* |
| P2 | 8.95±0.09391 | 0,576* |
| P3 | 9.90±0.27409 | 0,406* |

Note: * Normal (p > 0,05)

E. FSH Levels

| Group | Mean ± SD | *p* |
|---|---|---|
| KN | 62.79±1.60912 | 0,643* |
| K(-) | 20.28±0.65137 | 0,580* |
| P1 | 30.15±0.58912 | 0,800* |
| P2 | 40.79±1.38249 | 0,409* |
| P3 | 60.22±3.87149 | 0,165* |

Note: * Normal (p > 0,05)

**Figure 4.** Table of normality test of research data