Spermatozoa Quality of Young Male White Rat after Treated with Moringa oleifera Leaf Extract

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Abstract. Spermatogenesis is the process of spermatozoa formation from primitive germinativum cells. There are Leydig (interstitial) cells outside the seminiferous tubules that secrete testosterone hormone into the blood. The quality of spermatozoa is largely determined by the nutrients consumed during the growth period. Moringa leaves contain lots of nutrients, therefore it is often used to improve malnutrition in children. The specific purpose of this study was to determine the quality of spermatozoa of young male rats after treated with Moringa leaf extracts. This study used a randomized complete block design (RCBD) and divided into 4 groups of treatment consisted of 10 young male white rats i.e. control group (K0), treatment group 1 (K1) treated with dose 50 mg/kg, group 2 (K2) treated with dose 75 mg/kg, group 3 (K3) treated with dose 100 mg/kg of Moringa leaf extracts. Parameters observed were sperm quality (sperm count, motility, viability and morphology). The result of this research showed that the treatment of Moringa leaf extracts in the growth period could increase spermatozoa quality at dose 50 and 75 mg/kg body weight (bw) significantly. Moringa leaf extracts at dose 100 mg/kg body weight (bw) could decrease spermatozoa quality significantly (p<0.005). The result proved that the higher doses of Moringa leaf extracts given to young male rats could decrease the quality of spermatozoa.

1. Introduction
A man's fertility determines the end result of reproduction. One component of fertility i.e. quality of spermatozoa, is determined by the intake of nutrients given at the time of growth and development of the baby. The content of nutrients or chemicals in the type of food greatly affect fertility, even one type of food can work differently. Moringa oleifera is a herbal ingredient that is widely used as health herbs and is often used to cope with malnourished infants. However, consuming M. oleifera leaves excessively can cause interference with the kidneys, liver and failure of sperm formation. The treatment of M. oleifera leaf ethanolic extract can damage testicular and epididymis tissues by histological observation. Disruption of epididymis function due to the disruption of epididymis cells and the occurrence of disruption in testicular tissue, especially in germ cells so that interfere the spermatogenesis process [1].

Commercial feeding with M. oleifera leaf meal to 45% in male rabbits can improve reproductive ability i.e. weight of reproductive organs (testes and accessory glands), quality and quantity of spermatozoa (motility, viability, integrity of spermatozoa membrane, number of spermatozoa, spermatozoa morphology), testicular histology (number of seminiferous tubular epithelium, thickness of seminiferous tubule epithelium, seminiferous tubular diameter), testosterone and mating behavior and lowering blood MDA levels [2]. The secondary metabolite group of M. oleifera leaf that has antifertility in males were flavonoids, alkaloids, essential oils and tannins [3]. M. oleifera leaf extract also contain benzyl isothiocyanate, also found in papaya seeds, which is responsible for antifertility activities [4].
There are two different opinions about the impact of \textit{M. oleifera} leaf on the quality of spermatozoa and related studies on the effect of \textit{M. oleifera} leaf extract as malnutrition prevention against reproduction quality on children. This is the background of the need to conduct research on spermatozoa quality of white male rats treated with \textit{M. oleifera} leaf extract during the growth period.

2. Materials and Methods

2.1. Materials

In this research, \textit{M. oleifera} leaves was obtained from Abiansemal Village area, Abiansemal Subdistrict, Badung Regency of Bali Province. The simplicia ingredient in the form of \textit{M. oleifera} leaves were dried by oven at a temperature below 70°C for 15 minutes, then blended and sifted using a 40/60 mesh. The use of dry \textit{M. oleifera} leaf powder was to eliminate the water content of the natural \textit{M. oleifera} leaf that can interfere with the concentration of \textit{M. oleifera} leaf preparations to be given to the animals. Animal models used in this research were male rats (age 4-6 weeks, 50-75 grams of body weight). The doses were converted from human to mice by calculation below.

2.2. Dosage calculation of Moringa leaf extract

Moringa leaf extract dose in human = 1 g/50 kg bw = 0.1 g/kg bw = 100 mg/kg bw. Dosage per kg bw in Mice : 0.1 g/kg bw = X mg/75 g
0.1 g/1000 g = X g/75 g
0.1 g x 75 g = 1000 g
X = 0.0075 = 7.5 mg/75 g

It was determined that the treatment of \textit{M. oleifera} leaf infused for each rat was 2 mL, so the concentration of the infused should be 0.0075 g/2 mL = X g/100 mL = 0.375 g/100 mL.

2.3. Research design

The design used in this study was a randomized block design (RBD) and divided into 4 groups treated with \textit{M. oleifera} leaf with different doses in which each group consisted of 10 male white rats, i.e. control group (K0) which were given distilled water and treatment groups which were given a dose of 50 mg/kg bw \textit{M. oleifera} leaf (K1), a dose of 75 mL/kg bw \textit{M. oleifera} leaf (K2) and a dose of 100 mg/kg bw \textit{M. oleifera} leaf (K3). Each male rats were given 1 mL \textit{M. oleifera} leaf infused orally according to the dose of the treatment for 30 days. The spermatozoa quality variables observed in this study were spermatozoa motility, viability, and morphology. Data were analysed statistically by SPSS 21 software. The statistical tests performed included normality test, homogeneity test, parametric test (One-Way Anova) or non-parametric test (Kruskal Wallis). The result of One-Way ANOVA and Kruskal Wallis test were continued with multiple comparison test of LSD (Least Significant Different) and Chi square Test if the data analysis showed a significant difference (p≤0.05).

3. Result and Discussion

3.1. Result

Based on LSD test, the results of the moringa leaves treatment on the amount and motility of spermatozoa were presented in Table 1. A very significant (p≤0.05) increase of spermatozoa amount was obtained at treatment dose 75 mg/kg bw and very significant decrease (p≤0.05) of spermatozoa amount happened at treatment dose of 100 mg/kg bw compared with control. A very significant increase (p≤0.05) of spermatozoa motility was obtained at treatment dose 50 mg/kg bw and 75 mg/kg bw. However there was no effect at treatment dose 100 mg/kg bw compared to control.

Chi Square test results on viability of spermatozoa after treated with \textit{M. oleifera} leaf were presented in Table 2. From the table it can be seen that treatment dose of 100 mg/kg bw significantly increased (p≤0.05) the percentage of dead spermatozoa compared with control or with treatment dose 50 mg/kg bw and dose 75 mg/kg bw.
| Treatment groups   | Amount of spermatozoa (million) | Motility of spermatozoa (%) |
|--------------------|---------------------------------|----------------------------|
| Control            | 25.40 ± 0.548 b                 | 79.00 ± 0.707 a             |
| Dose 50 mg/kg bw   | 26.40 ± 0.894 bc                | 82.80 ± 1.643 b             |
| Dose 75 mg/kg bw   | 27.20 ± 0.837 c                 | 84.00 ± 1.414 b             |
| Dose 100 mg/kg bw  | 24.20 ± 0.837 a                 | 77.60 ± 0.548 a             |

Different letters in the same column show significantly differences (P<0.05).

Table 2. The viability of spermatozoa (%)

| Treatment groups  | Viability of spermatozoa (%) | Life Rate | Life Percentage | Death Rate | Death Percentage |
|-------------------|------------------------------|-----------|-----------------|------------|------------------|
| Control           | 13.2                         | 86.8 a    | 2.0 a            | 13.2 a     |
| Dose 50 mg/ kg bw | 25.8                         | 89.8 a    | 1.8 a            | 10.2 a     |
| Dose 75 mg/ kg bw | 13.6                         | 62.1 a    | 0.8 a            | 9.9 a      |
| Dose 100 mg/ kg bw| 11.2 *                       | 73.7 *    | 4.0 *            | 26.3 *     |

The * in the same column shows the significantly difference (p <0.05).

The post hoc test (Chi Square test) of *M. oleifera* leaf on the morphology of spermatozoa results were presented in Table 3. It showed that treatment dose of 100 mg/kg bw gave a very significant effect (p<0.05) on the abnormal morphology of spermatozoa compared with control and other treatment doses 50 and 75 mg/kg bw.

Table 3. The morphology of spermatozoa

| Treatment groups  | Morphology (the tail) | Normal | Abnormal |
|-------------------|-----------------------|--------|----------|
|                   | Rate | Percentage | Rate | Percentage |
| Control           | 12.0 | 80.0 a      | 3.0 a | 20.0 a      |
| Dose 50 mg/ kg bw | 14.4 | 88.9 a      | 1.8 a | 11.1 a      |
| Dose 75 mg/ kg bw | 16.8 | 89.4 a      | 2.0 a | 10.6 a      |
| Dose 100 mg/ kg bw| 13.2 *| 72.5 *      | 5.0 * | 27.5 *      |

The * in the same column shows the significantly difference (p =0.05).

The result of statistic test on the spermatozoa quality of male white rats after treated with *M. oleifera* leaf showed that the number of spermatozoa increased significantly (p<0.05) at highest doses (75 mg/kg bw) if it is compared with other groups. This suggests that the content of vitamin C, antioxidants and Zn in *M. oleifera* leaf can increase the number of spermatozoa. The administration of vitamin C affected the number of spermatogenic cells of adult males mice (*Mus musculus* L) induced by monosodium glutamate [5]. The amount of spermatogenic cells of seminiferous tubules in the testes could determine the amount of spermatogenesis that will occur in the testes [6].

*M. oleifera* leaf treatment during the growth period of young male rats increased testes weight insignificantly [7]. The concentration of spermatozoa is influenced by several factors i.e. male sexual maturity, ejaculate volume, shelter interval, feed quality, reproductive health, testes size, age, season, and geographical differences [8]. Treatment with a dose of 100 mg/kg bw caused a significant decrease (p<0.05) on spermatozoa amount compared with the control, doses 50 and 75 mg/kg bw. This result is in line with the research which reported the decrease in spermatozoa concentration caused by the content of benzyl-isothiocyanate, alkaloids and flavonoids inside the 90% ethanolic extract of *M. oleifera* leaf. Another study showed that *M. oleifera* ethanolic leaf extract was a potential antifertility agent, because it damaged the testes and epididymis tissues as seen on histological observation. Epididymal epithelial
cell disruption indicated that epididymal function was impaired and it affected the production of spermatozoa [1].

Table 1 showed the result of spermatozoa mortality of male white rats, there was a significant increase of spermatozoa motility in treatment group after treated with *M. oleifera* leaf at doses 50 and 75 mg/kg bw compared with control and dose 100 mg/kg bw. The increase of spermatozoa motility in both treatments (50 and 75 mg/kg bw) were thought to be due to Zn minerals contained in *M. oleifera* leaf that could increase spermatozoa motility, because it affected the energy synthesis process for spermatozoa motility. This result is supported by the statement that *M. oleifera* leaf as zinc (Zn) mineral source could increase the motility of spermatozoa hyperactivity in Bali, Madura, Brahman, Ongole, Limousin and Simmental cattle [9]. Zn supplementation led to an increase in the percentage of spermatozoa motility [8].

The treatment group with a dose of 100 mg/kg bw decreased spermatozoa motility of male white rat significantly compared to the control, treatment doses 50 and 75 mg/kg bw. An increase number of secondary metabolite contents that serve as antifertility substances that were in line with increased doses of *M. oleifera* leaf could disrupt the spermatogenesis process. This can be proved also by the decrease of the spermatozoa amount. In addition, *M. oleifera* leaf extract also contains benzyl isothiocyanate (also found in papaya seeds) which was responsible for antifertility activities [4].

Table 2. showed the spermatozoa viability parameters of male white rats treated with *M. oleifera* leaf. Treatment dose of 100 mg/kg bw decreased spermatozoa viability significantly compared with control, treatment doses 50 and 75 mg/kg bw. It might be because secondary metabolites in the form of alkaloids and flavonoids were more prevalent at dose 100 mg/kg bw and further gave an impact on spermatogenesis process. Alkaloid compound can suppress the secretion of male reproductive hormone (testosterone) so that it will inhibit spermatogenesis process [10]. The flavonoid compound can inhibit aromatase enzyme, a type of enzyme that catalyzes the conversion of androgens into estrogens, therefore will increase testosterone hormone [3, 10].

Increased testosterone in the blood can initiate a feedback mechanisms on Gonadotropin Releasing Hormone (GnRH) from the hypothalamus, which will inhibit the release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) by the pituitary [11]. A decrease in LH can cause a decrease in spermatozoa viability. This was in line with the research which stated that the decrease in viability can also be caused by the secretion of testosterone hormone by Leydig cells [12]. Testosterone plays an important role in maintaining the survival of spermatozoa inside the epididymis [13].

A decrease secretion of testosterone of white rats will result in a decrease of spermatozoa survival in the epididymis [14]. Treatment doses of 50 and 75 mg/kg bw gave no significant effect on spermatozoa motility compared to control group, might be because the secondary metabolites were still low to affect the motility of spermatozoa. Morphological damage or abnormalities of spermatozoa caused by the treatment of *M. oleifera* leaf on young male rats are presented in Table 3. Treatment dose of 100 mg/kg bw increased spermatozoa abnormalities significantly compared with control, doses of 50 and 75 mg/kg bw. It can be caused by the tannin compounds of *M. oleifera* leaf which were higher in dose 100 mg/kg bw. This tannin content in *M. oleifera* leaf at treatment doses of 50 and 75 mg/kg bw did not affect the percentage of spermatozoa abnormality compared to the control. High tannin content in the treatment doses of 100 mg/kg bw impacted the binding of protein and ionic ions in the spermatozoa membrane so that the tyrosine enzyme and phosphorylation process in the spermatozoa membrane were disrupted and eventually resulted in morphological abnormalities of spermatozoa [15]. This statement is in line with the research which found that spermatozoa abnormalities of Ettawa goat were 10-20% after treated with 10-20% of crude tannins [16].

4. Conclusion

Based on the research, it can be concluded that *M. oleifera* leaf treatments to white male rats during the growth period significantly improved the quality of spermatozoa at treatment doses 50 and 75 mg/kg bw. Besides, treatment dose of 100 mg/kg bw significantly reduced the quality of spermatozoa, it proved that the higher doses of *M. oleifera* leaf infused given orally to young male white rats decreased the quality of spermatozoa.
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