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Effect of Noni Extract (*Morinda citrifolia* L.) on Spermatozoa Quality of Mice (*Mus musculus* L.)

Pengaruh Pemberian Ekstrak Mengkudu (*Morinda citrifolia* L.) terhadap Kualitas Spermatozoa Mencit (*Mus musculus* L.)

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ABSTRACT

This study aims to see the effect of giving noni extract (*Morinda citrifolia* L.) on the quality of spermatozoa of mice (*Mus musculus* L.). This study was conducted using an experimental method, with a completely randomized design (RAL), 3 treatments with 5 replicates with 15 male mice divided into three treatment groups, namely the control group (P0), the treatment group with a concentration of 0.3 g/BW (P1), and the treatment group with a concentration of 0.6 g/BW (P2) which then the data were analyzed using ANOVA. This research was conducted from March 2 to April 4, 2023. Observations of the effect of giving noni extract were made by making wet preparations of mice sperm and then observing under a microscope. Based on the research conducted, it was found that noni extract had a different effect from each concentration on motility and morphology in sperm. From the research, it can be concluded that the higher the concentration of noni extract given to mice will reduce the quality of sperm in mice However, at a concentration of 0.3 g/BB, it was found that noni had a positive effect on sperm morphology and motility in mice (*Mus musculus* L).

Keywords: Abnormalities, *Morinda citrifolia* L., Motility, *Mus musculus* L., Quality.

ABSTRAK

Penelitian ini bertujuan untuk melihat pengaruh pemberian ekstrak mengkudu (*Morinda citrifolia* L) terhadap kualitas spermatozoa mencit (*Mus musculus* L). Penelitian ini dilakukan dengan menggunakan metode eksperimental, dengan Rancangan Acak Lengkap (RAL), 3 perlakuan dengan 5 ulangan dengan 15 ekor mencit jantan yang dibagi menjadi tiga kelompok perlakuan yaitu kelompok kontrol (P0), kelompok perlakuan dengan konsentrasi 0,3 g/BB (P1), dan kelompok perlakuan dengan konsentrasi 0,6 g/BB (P2) yang kemudian data dianalisis dengan menggunakan ANOVA. Penelitian ini dilaksanakan pada 2 Maret s/d 4 April 2023. Pengamatan pengaruh pemberian ekstrak mengkudu dilakukan dengan membuat preparat basah dari sperma mencit kemudian mengamati di bawah mikroskop. Berdasarkan penelitian yang dilaksanakan didapatkan hasil bahwa ekstrak mengkudu memiliki pengaruh yang berbeda dari setiap konsentrasi terhadap motilitas dan morfologi pada sperma. Dari penelitian dapat disimpulkan bahwa semakin tinggi konsentrasi ekstrak mengkudu yang diberikan kepada mencit maka akan menurunkan kualitas sperma pada mencit. Namun pada konsentrasi 0,3 g/BB didapatkan hasil bahwa mengkudu memberikan pengaruh positif terhadap morfologi dan motilitas sperma pada mencit.

Kata Kunci: Abnormality, *Morinda citrifolia* L., Motilitas, *Mus musculus* L., Kualitas

INTRODUCTION

Indonesia is one of the countries with a market potential for plants as herbal medicines and phytopharmaca has approximately 30,000 species of plants and 940 of them are medicinal plants. Until now it has been utilized by the traditional herbal medicine industry (Sumarni et al., 2019). Noni plant is one of the tropical medicinal plants that can be found in various places. Noni fruit is thought to have the potential to be used as an ingredient to improve the quality of mice semen. This needs to be done to increase the resilience of mice spermatozoa after retention so it is necessary to research to test the effectiveness of noni fruit on the quality of mice spermatozoa. Noni fruit (*Morinda citrifolia* L.) contains many antioxidants, namely carotene, vitamin C,

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xeronin, and proxeronin which can function to counteract free radicals. Vitamin C can strengthen the stability of the protective tissue of the plasma membrane against lipid peroxides, to maintain the quality and fertility of semen. Free radicals can harm sperm motility or the ability of sperm to move (Murcahyana et al., 2016).

The seminiferous tubules are the largest component of the testes. If there is damage or atrophy of the cells making up the seminiferous tubules, there will be a decrease in testicular weight. Testes that have abnormalities will be able to affect the process of spermatogenesis which can affect the decrease in the quality of spermatozoa. radicals cause cell damage, antioxidants are needed which can come from the body itself or from outside. Compounds will be formed from within the body to neutralize, among others, SOD (superoxide dismutase). From the outside, among others, foods contain antioxidants that are used as chain breakers (chain-breaking antioxidants), namely vitamin C, vitamin E, beta carotene, and flavonoids (Kurniati and Dyah, 2019). The process of making liquid semen and frozen semen decreases sperm quality due to oxidative stress which can increase the amount of free radicals. Free radicals can harm sperm motility or the sperm's ability to move. As a result, slow-moving sperm may also be unable to fertilize an egg. The existence of these effects is related to free radicals, for this reason, antioxidants are needed which act as "predators" of free radicals to protect spermatozoa. The highest amount of antioxidants in food comes from fruits and vegetables, one of which is noni. Motility is a progressive movement shown by spermatozoa. Without motility, the spermatozoa will not quickly meet the egg during the fertilization process. The good motility of spermatozoa is one whose movements are straightforward, agile, and fast, with rhythmic tail movements. Factors that influence spermatozoa motility are nutrition, spermatozoa abnormalities, and spermatozoa age (young, mature, or old).

Spermatozoa motility is very important because it is closely related to the ability of spermatozoa in the fertilization process. Motility is the progressive movement of spermatozoa. Motility is useful in bringing together spermatozoa with eggs. The characteristics of normal spermatozoa motility are straightforward, agile, fast, and rhythmic tail movements. Spermatozoa will move slowly when lacking energy, even though the direction remains forward and the tail moves regularly. Motility in spermatozoa is the movement of the tail of spermatozoa. Morphology and viability of spermatozoa have a close relationship because only living spermatozoa can produce energy so that spermatozoa can continue to move. In addition, good spermatozoa morphology will support good spermatozoa motility. When in the epididymis, the sperm maturation process is highly dependent on testosterone levels, decreased levels of testosterone cause abnormal spermatozoa morphology (Indriani et al., 2021). Mass motility can show the individual movement of each spermatozoa. The more active and the more spermatozoa that move, it will look like a cloud that moves wavy and the faster the cement has better quality. The decrease in semen pH causes a decrease in the activity of metabolic enzymes, which results in the energy requirements for sperm motility and survival not being fulfilled. This study used an experimental method using a nested randomized design with a factorial pattern, namely storage time and dose of noni fruit extract. Control solution (P0), as well as 96% ethanol as a basic solution with the addition of 10% (P1) and 20% (P2) noni fruit extract, each treatment was repeated five times. The process of carrying out the research began with the rearing of 15 male mice aged 2.5-3 months with a weight ranging from 20-30 grams for 15 days.

The number of spermatozoa produced is very dependent on the process of spermatogenesis in the seminiferous tubules. If during Spermatogenesis process is disrupted the development of spermatogonia cells will affect the number of spermatozoa formed (Nurhadijah et al., 2018). Spermatozoa will move slowly when lacking energy, even though the direction remains forward and the tail moves regularly. Motility in spermatozoa is the movement of the tail of spermatozoa. Morphology and viability of spermatozoa have a close relationship because only living spermatozoa can produce energy so that spermatozoa can continue to move. In addition, good spermatozoa morphology will support good spermatozoa motility. Spermatozoa abnormalities are all forms of deviation from the morphology of spermatozoa, deviations can occur in several parts of the spermatozoa, in the head the deviation forms include a head that is too big, too small, flat, double, or even without a head, in the middle the deviation forms in the form of folds or indentations, while deviations in the tail are in the form of a coiled tail, broken tail, and double tail (Indriani et al., 2021). Spermatogenesis is the process of proliferation and differentiation of spermatogenic cells into mature spermatozoa so that they can fertilize eggs in the fertilization process. Besides that, the stages that are no less important are the spermatozoa will undergo physiological maturation in the epididymis. Natural antioxidants can protect molecules from cell damage caused by oxidation and can improve sperm quality and increase male reproductive efficiency (Sutyarso et al., 2018). This study aims to determine the effect of noni extract on spermatozoa motility of male mice (*Mus musculus* L.). It is hoped that this research can provide information regarding the potential of noni extract as a traditional medicine that can

improve the quality of sperm in animals and possibly in humans.

MATERIALS AND METHOD

Method

This study aims to determine the effect of giving noni fruit extract on the quality of spermatozoa of male mice in a Completely Randomized Design (CRD) using 15 male mice which were divided into three treatment groups, namely the control group (P0), the treatment group with a concentration of 0.3 g/BB (P1), and the treatment group with a concentration of 0.6 g/BB (P2). The materials used in this study were 15 male mice weighing 25 g aged 3 months, noni fruit extract, a physiological solution, namely 0.9% NaCl, pH indicator, and chloroform.

Making noni extract

The noni fruit used is ripe noni as much as 4 pieces. Then clean the ripe noni fruit and cut the noni fruit into small pieces. Then put it in the oven at 40-60°C for 4 days, until the noni fruit is completely dry and brittle. Then grind the dried noni fruit using a blender until smooth. For the manufacture of noni extract we use the formula: Mice weight = 30 mg; Dosage I = 2 g/BB; Dose II = 4 g/BB. Add 2.5 ml of water to each noni extract, so that each mouse will receive 0.5 ml of noni extract. Noni extract is given once a day

Giving treatment to mice

Before starting the treatment, the mice were acclimated for 8 days with the aim that the mice could adapt to their environment. For 15 days, the mice were still given enough food and water, and the husks were replaced once every 3 days. After 15 days of giving treatment to mice treatment 1 was 0.06 mg/BB and treatment 2 was 0.12g/BB. We used control mice as a comparison for normal spermatozoa, control mice were not treated with noni extract.

Spermatozoa observation

Observation of mouse spermatozoa was carried out by taking the testicles of each mouse, then placing them in a petridish containing a physiological solution (NaCl 0.9%), which functions so that the sperm do not die. After that the testicles are chopped using a setting set, this aims to make the sperm contained in the testes come out. Then take the sperm using a dropper put it in the object glass and cover it using a cover glass. Then observe the morphology and motility of sperm under a microscope. Before observing the morphology and motility of sperm under a microscope, we first measure the pH of the sperm.

Table 1. Guest criteria (sperm movement rate)

Characteristics	Percentage (%)
Very progressive movement, very large waves, and fast indicating 100% motile sperm	100%
The agile progressive movement immediately forms waves with 90% motile sperm	90%
Between 50% -80% of sperm move progressively and produce mass movements	80%
Circular motion, less than 50% motion, and no waves	70%
Spermatozoa movement rotates in place	60%
Movement of immotile or immobile spermatozoa	50%

Sources: Nainggolan et al. (2019)

Data analysis

The data obtained during the experiment was then processed and analyzed using ANOVA.

RESULT AND DISCUSSION

Motility is the progressive movement of spermatozoa. Motility is useful in bringing together spermatozoa with eggs. The characteristics of normal spermatozoa motility are straight forward, agile, fast, and rhythmic tail movements. Several factors affect spermatozoa motility, including nutrition, spermatozoa abnormalities, and the age of young, mature or old spermatozoa) (Syarif et al., 2016). Spermatozoa motility is the quality of spermatozoa movement which includes the type of spermatozoa movement and the speed of movement of spermatozoa. The motility assessment was carried out by counting the spermatozoa that were included in the motile category, namely if the spermatozoa moved quickly straight ahead, moved slowly or not straight, and

moved in place. The results of the assessment are expressed as a percentage indicating the number of motile spermatozoa. A man is considered infertile if he has a percentage of spermatozoa motility of less than 40% or what is known as asthenozoospermia. The back-and-forth movement of the tail (flagella movement) provides sperm motility. This movement is caused by a rhythmic longitudinal gliding movement between the posterior and anterior tubules that form the axoneme. Normal sperm move in a liquid medium at a speed of 1 to 4 mm/minute. The next process after sperm formation is sperm maturation in the epididymis. After forming in the seminiferous tubules, it takes several days for sperm to pass through the 6-meter-long tubules of the epididymis. Sperm that move from the seminiferous tubules and the early part of the epididymis are immature and are unable to fertilize an ovum. However, after the sperm is in the epididymis for 18-24 hours, the sperm will have the ability to motility.

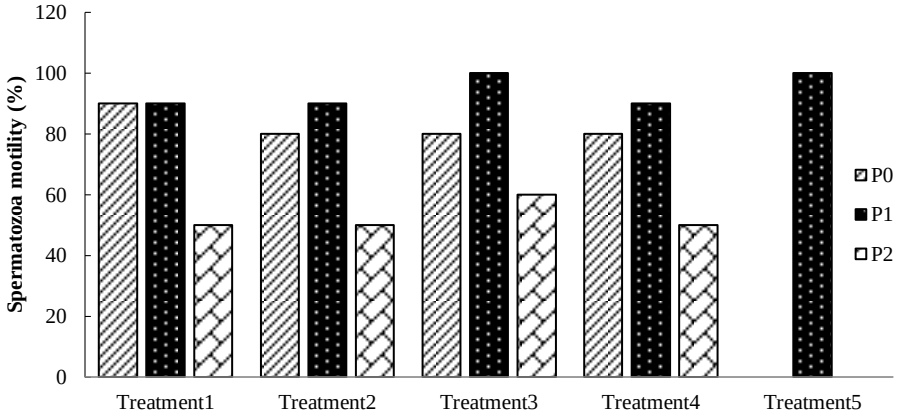

Figure 1. Percentage of mice spermatozoa in each treatment

Deviation from normal spermatozoa is considered an abnormality. Abnormalities can be primary and secondary abnormalities. Primary abnormalities occur in spermatogenesis, including large heads, small heads, double heads, double tails, and rolled tails. Secondary abnormalities occur in the maturation process in the epididymis. Spermatozoa are considered fertile if they have abnormal spermatozoa below 40% (Dillasamola, 2020).

So motility greatly affects the quality of sperm. From the research conducted, different results were obtained at each concentration of noni extract given to mice. The control mice were used as a reference for the first treatment mice (P1) and the second treatment mice (P2). In mice with one repetition, P0 and P1 mice had the same motility, with a motility percentage of 90%. Characterized by agile progressive movement and immediately forms waves with 90% motile sperm. Whereas for P2 mice, the percentage of motility decreased, namely by 50%. Characterized by the movement of immotile or immotile spermatozoa.

In the second repetition of mice, the mice that were given P0 had a motility percentage of 80%. Characterized by progressively moving sperm and producing mass movements. Furthermore, in P1 mice there was an increase in the percentage of motility, namely with a percentage of 90%, characterized by progressive movements that were agile and immediately formed waves with 90% motile sperm. Whereas in P2 mice there was a decrease in the percentage of motility, with a percentage of 50%, characterized by the movement of immotile or immotile spermatozoa.

In three replicate mice, P0 mice had a percentage of 80%. Characterized by progressively moving sperm and producing mass movements. Furthermore, for P1 mice, there was an increase in the percentage of motility, with a percentage of 100%, characterized by very progressive movements, very large waves, and quickly showing 100% motile sperm. Whereas in P2 mice there was a decrease in the percentage of motility, with a percentage of 60%. Characterized by the movement of spermatozoa rotating in place.

In the fourth repetition of mice, for mice, P0 has a percentage of 80%. Characterized by progressively moving sperm and producing mass movements. In P1 mice, there was an increase in the percentage of motility, namely with a percentage of 90%, characterized by progressive movements that were agile and immediately formed waves with 90% motile sperm. Whereas in P2 mice with a percentage of 50%, characterized by immotile or immotile spermatozoa movement.

In mice with four repetitions, there was only one mouse, namely the P1 mouse. This is because the P0 and P2 mice died during the treatment process. The percentage obtained in P1 mice was 100%, characterized by very progressive movements, very large waves, and fast indicating 100% motile sperm. So based on the sperm motility graph in mice, it was found that there was an increase in the graph in P1 mice. Whereas in P2 mice decreased sperm motility. This shows that the administration of noni extract affects sperm motility in mice. The concentration that had a good effect on sperm motility was 0.3 gram/BB, while the noni extract with a concentration of 0.6% had a bad effect on the sperm motility of mice.

The results obtained in this study are in accordance with research conducted by Sabile et al. (2016) which stated that noni fruit contains many antioxidants, namely carotene, vitamin C, xeronin, and proxeronin which can function to counteract free radicals. Vitamin C can strengthen the stability of the protective tissue of the plasma membrane against lipid peroxides, to maintain the quality and fertility of semen. Noni extract supplementation showed a decrease in spermatozoa motility, as the levels of noni fruit extract increased. Noni fruit extract given is high enough so that it is possible to change the pH because the vitamin C contained therein is acidic and also contains antioxidants in large quantities which can act as pro-oxidants that will result in decreased motility.

(a) (b) (c) (d)

Figure 2. Morphology of mouse spermatozoa (a) Normal, (b) Head damaged, (c) No head, (d) No tail

Sperm morphology can affect its motility. The morphological shape of spermatozoa cells affects fertilization, if the number of abnormal spermatozoa is too high, it will reduce fertility. Several forms of irregularities in the morphology of spermatozoa are called spermatozoa abnormalities. Abnormalities occur in several parts such as the head and tail of spermatozoa (Julia et al., 2019). In the research conducted, it was found that the morphology was different between P1 and P2. While P0 is used as a comparison between P1 and P2. In P1, the results showed that the morphology was normal, with healthy sperm having a round head and a long straight tail. Whereas in P2 the results were generally abnormal, characterized by a pin-like head, a crooked tail, and a large and oval head. Higher concentrations can cause sperm morphology in mice.

So, in this study it can be concluded that the administration of noni extract can affect the morphology and motility of mice sperm. Giving noni extract with the right concentration can affect sperm morphology. Where sperm morphology will affect sperm motility. Normal sperm morphology will result in good sperm motility. Meanwhile, if the concentration of noni fruit extract is too high, the sperm morphology of mice will be abnormal. This abnormal sperm morphology will cause a decrease in sperm motility in mice.

CONCLUSION

Based on the research that has been done, it can be concluded that noni fruit extract can affect the sperm motility of mice. Noni fruit extract with a concentration of 0.3 g/BB can increase the sperm motility of mice.

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