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## The Potential of Pasak Bumi as a Food Supplement to Improve Spermatogenesis in Mice Model of Malnutrition

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### ABSTRACT

Malnutrition causes disruption of spermatogenesis in children. Pasak Bumi<sup>4</sup> has the potential to increase spermatogenesis due to malnutrition. Aimed of research is to proved Pasak Bumi as a food supplement to improve spermatogenesis disorders due to malnutrition in malnourished rats. The research method used analytical observational method by observing the histological preparations of the testes of malnourished rats. Observations using a binocular microscope were analyzed with Optilab Camera and Image Raster software. There are 5<sup>6</sup> groups of experimental: M: malnutrition without intervention; P1: malnutrition + standard feed + PB 7.5 mg/kgBW; P2: malnutrition + standard feed + PB 15 mg/kgBW; P3: malnutrition + standard feed + PB 22.5 mg/kgBW; P4: malnutrition + standard feed + PB 30 mg/kgBW; Research parameters were number of 2<sup>2</sup> seminiferous tubules, primary spermatocytes and Leydig cells. Data analysis used the Anova One way test with a confidence level of 95%. The results showed number of seminiferous tubules, primary spermatocytes and Leydig cells in the M group was less than the P1-P4 group. This suggests malnutrition causes disruption of spermatogenesis. Conclusion the administration of Pasak Bumi extract at various doses increased spermatogenesis in malnourished.

<sup>4</sup>**Keywords:** Pasak Bumi, malnutrition, seminiferous tubules, primary spermatocytes, Leydig cells

## INTRODUCTION

South Kalimantan was ranked 10th in the national level in 2017 for cases of malnutrition and malnutrition at the age of 0-23 months with a percentage of cases 3.60% (national numbers 3.50%) and the number of cases of malnutrition 12.90% (national numbers 11.30%).<sup>1</sup> The nutritional imbalance factor is one of the causes of reproductive system health problems besides age, exercise, obesity, caffeine, the temperature in the scrotum of clothing, hot water, and even cell phones. This has an impact on spermatogenic cell quality and DNA damage caused by reactive oxygen species (ROS). Malnutrition due to protein deficiency also interferes with the synthesis of enzymes that act as antioxidants, causing antioxidant deficiency and oxidative stress in the organs. Changes in the balance between the antioxidant system and oxidative stress can lead to impaired development of spermatogenesis, abortion, congenital disorders, and cancer in children.<sup>2</sup>

Nutrition plays a very important role in the process of growth and development of the reproductive system. There is a lot of new research evidence in studies on animal and human populations showing that the maturation process of the reproductive system and its function is heavily influenced by malnutrition. Clinically, malnutrition is known in several forms, including malnutrition, mild to moderate protein deficiency and/or lack of energy which is the most frequent cause of disruption in the maturation process and reproductive system function.<sup>2,3,4</sup>

The testes as the main organ of male reproduction are very important to maintain the physiological environment in order to produce sufficient spermatogenic cells and hormones for male fertility. The testes consist of seminiferous tubule components (TS) which contain elements of spermatogenic cells, supporting cells and interstitial cells. If the testicles are damaged anatomically, for example, due to the impact of malnutrition in childhood, the process of forming spermatogenesis and the production of the hormone testosterone will be disrupted, so the risk of the tendency to experience infertility will increase. Anatomical abnormalities can be a decrease in the number of spermatogenic cells, Sertoli cells and Leydig cells, or a decrease in the number of seminiferous tubules.<sup>5</sup>

South Kalimantan has various potential natural resources to overcome the problem of spermatogenesis. Research shows that giving Pasak Bumi (*Eurycoma longifolia* Jack) originating from South Kalimantan can increase the hormones testosterone, FSH and LH and

their effect on increasing spermatogenesis by increasing the number of spermatogenic cells, Sertoli cells, and Leydig cells in mice.<sup>6,7</sup> The research by Maria (2003) shows that the extract of Pasak Bumi can increase spermatogenesis in mice.<sup>8</sup> Thus, it is assumed that Pasak Bumi can also be developed as a supplement to increase spermatogenesis after malnutrition. This research has a beneficial value for society to overcome the problems and impacts of malnutrition, especially on male reproductive disorders.

## METHOD

The research method used was analytic observational research. This study observed research samples from testicular histological preparations of malnourished rats under a microscope. The testes used as histological preparations for HE staining (done at BVET Banjarbaru) were derived from the testes of malnourished mice whose treatment was carried out in the Biochemistry Laboratory FK ULM. For the observation process using a binocular microscope, and as a research observation tool using Image Raster and Optilab Camera computer software (done at the Histology Laboratory FK ULM). The total number of mice used was 32 male rats (based on Federer's formula). The experimental animal group was divided into 5 groups, namely:

- M: malnutrition without other intervention<sup>6</sup>  
 P1: malnutrition + standard feed + PB 7.5 mg/kgBW  
 P2: malnutrition + standard feed + PB 15 mg/kgBW  
 P3: malnutrition + standard feed + PB 22.5 mg/kgBW  
 P4: malnutrition + standard feed + PB 30 mg/kgBW

The research variables studied were:

1. Pasak Bumi as a food supplement (independent variable)
2. Number of seminiferous tubules, primary spermatocytes and Leydig cells (dependent variable)

Observation through a light microscope using a 100x and 400x magnification lens. The data collection process was carried out based on the variables measured in this study, namely:

1. Method of counting the number of seminiferous tubules. The TS is calculated in 5 fields of view at 100 x magnification and the average was taken.
2. Method of calculating the number of primary spermatocytes. The TS was calculated in 5

fields of view at 400 x magnification and the average was taken.

- Method of counting the number of interstitial cells (Leydig cells). The number of Leydig cells in the interstitial tissue was counted in 5 visual fields with a magnification of 100x and average was taken.

Data were analyzed using parametric test analysis with one way Anova test to determine the differences in histopathological features

between control data and treatment data. This study used a confidence interval of 95% and a significance value of  $p < 0.05$ .

#### RESULTS AND DISCUSSIONS

Based on the results of research on testicular organs in nutritional model mice (M) and malnutrition model mice that were given Pasak Bumi (P) as a food supplement, the following results were obtained:

Table 1. The mean thickness of the seminiferous tubule layer and the P value between the study groups

Groups	The Mean Thickness of seminiferous tubule layer	p Value between the study groups
M	3.5 ± 0.50	
P1	4.63 ± 0.48	p Value between M & P1 groups (0.005)
P2	4.25 ± 0.79	p Value between M & P2 groups (0.015)
P3	3.79 ± 0.56	p Value between M & P3 groups (0.247)
P4	4.29 ± 1.06	p Value between M & P4 groups (0.090)

Table 2. The Average number of primary spermatocytes and P values between study groups

Groups	The Average number of primary spermatocytes	P values between study groups
M	243.83 ± 20.65	
P1	271.70 ± 28.49	p Value between M & P1 groups
P2	398.29 ± 101.67	p Value between M & P2 groups (0.002)
P3	240.00 ± 24.87	p Value between M & P3 groups (0.693)
P4	278.33 ± 34.79	p Value between M & P4 groups (0.026)

Table 3. The Average number of Leydig cells and P value between study groups

Groups	The Average number of Leydig cells	P value between study groups
M	6.12 ± 0.47	
P1	7.37 ± 0.86	P value between M & P1 groups (0.006)
P2	8.13 ± 2.21	P value between M & P2 groups (0.037)
P3	8.42 ± 1.86	P value between M & P3 groups (0.015)
P4	8.46 ± 1.21	P value between M & P4 groups (0.001)



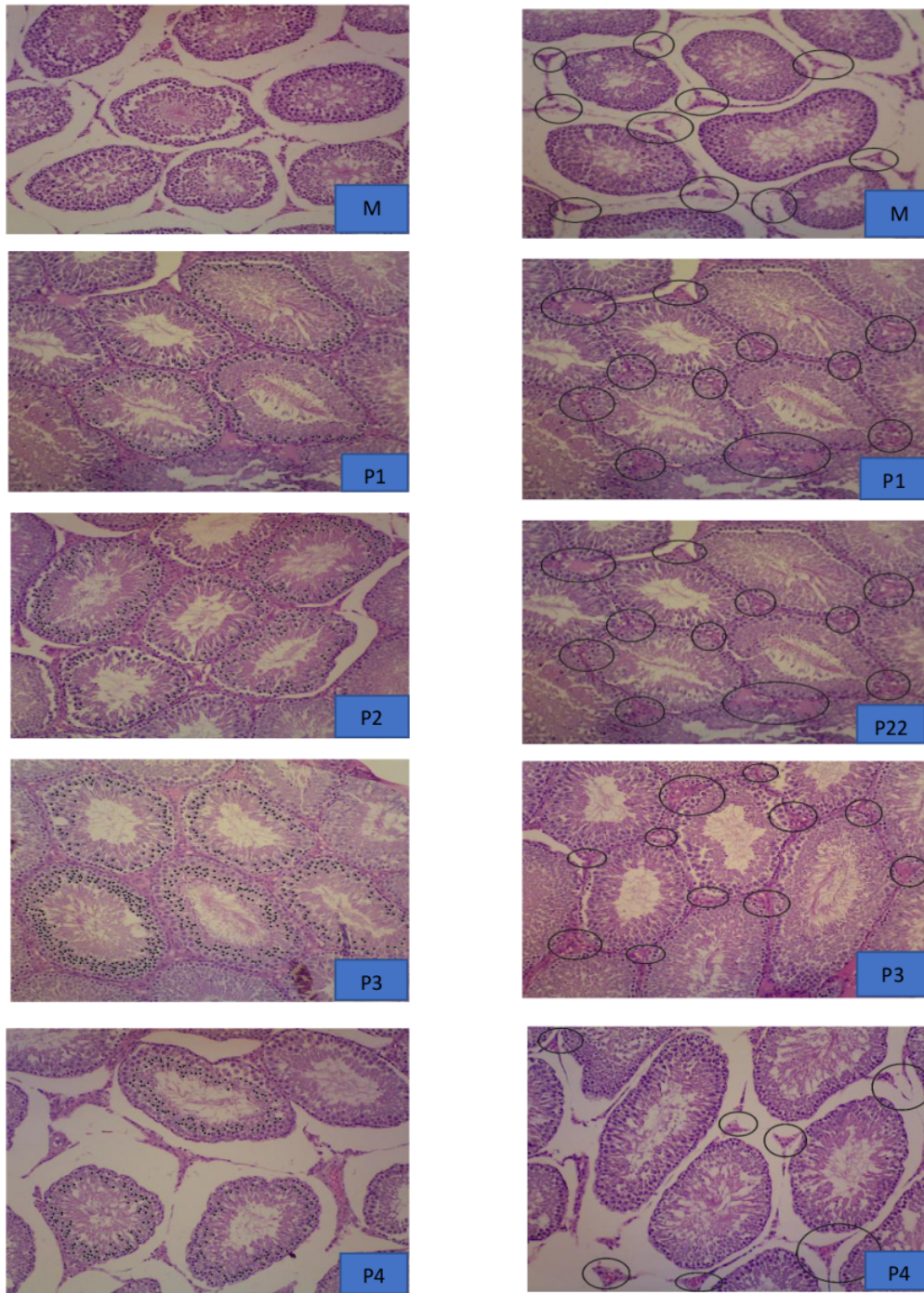


Figure 1. Microscopic morphology of testes (M-P4), black circles (leydig cells), black dots (primary spermatocytes); HE staining; Magnification of 100 x and 400 x, Optilab camera; Image Raster, light microscope

The conception process (embryo/fetus and associated membranes) requires water, amino acids, fats, carbohydrates, minerals, and vitamins for growth and development. Prior to placentation, the embryo receives nutrients from uterine secretions and oxygen from the surrounding environment. These secretions, including glucose and amino acids (eg, arginine, leucine, proline, and glutamine), play an important role in activating cell signaling and metabolic pathways necessary for protein synthesis and cytoskeletal modeling in the conceptus. Lack of nutrition during this period or the inability of conception to respond to nutrition can result in abnormal development or even death of conception.

The placenta transports nutrients, respiratory gases and their metabolic products between the maternal and fetal circulation. Dietary macronutrients (glucose, amino acids, and fatty acids) are the main source of substrate energy during fetal growth.<sup>10</sup> In pregnant women and their developing fetuses, glucose is the main source of energy for red blood cells, brain, retinal cells, and renal medullary cells, and other cells. Meanwhile, the heart of the mother and fetus uses glucose and lactate. In addition, the small intestines of the mother and fetus oxidize glutamate, aspartate and glutamine to meet most of their energy needs. Through  $\beta$ -oxidation, fatty acids are the main energy substrate for the mother's liver, skeletal muscle, heart, and kidneys.<sup>11</sup>

Amino acids are also needed to function not only as building blocks of protein, but also as important precursors for the synthesis of many physiologically important molecules, including hormones, small peptides (for example, glutathione), neurotransmitters, nitric oxide, creatine, carnitine, and polyamines. In addition, through multiple cell signaling pathways, amino acids regulate major metabolic pathways essential for human health, growth, development and reproduction. In particular, several amino acids (histidine, methionine, glycine, serine) participate in the metabolism of one-carbon unit, which is essential for DNA synthesis, as well as cell growth and development. So that if there is a deficiency of one element, especially protein, it will cause significant changes in the formation and function of organs during the embryo period, including the testes.<sup>12</sup>

Factors that can cause changes in the balance between the antioxidant system and oxidative stress can lead to developmental damage to spermatogenesis, abortion, congenital abnormalities, and cancers of the reproductive organs in children. Malnutrition is one of the important causes of disorders in the

development of the reproductive system. Many research reports on this have been done and the results show a link between protein-calorie malnutrition in men with several dangerous health complications including the reproductive system. A study in a group of experimental animals that experienced protein deficiency for 75 days showed tubular atrophy with extensive degeneration of testicular tissue, it was seen that the seminiferous tubules atrophy were marked by the depletion of germ cells, decreased numbers of spermatogonia cells and Sertoli cells.<sup>13</sup> Maternal malnutrition (70 days of gestation to term) is reported to reduce the number of Sertoli cells in the testes of newborn sheep.<sup>14</sup> Male sheep are malnourished during the first 95 days of pregnancy, which also show an effect on the reproductive system in the form of a decrease in ovulation rate.<sup>15</sup>

Oxidative stress is the abundance of reactive oxygen species (ROS) or a deficiency of antioxidants.<sup>16</sup> The imbalance produced by ROS causes cell damage. The detrimental effects of spermatozoa damage have been known since the 50s-80s.<sup>16</sup> However, excessive numbers are pathophysiological and lead to DNA damage and even apoptosis.<sup>17,18</sup>

Endogenous or exogenous factors can cause high ROS levels. The effect of endogenous or exogenous factors, especially malnutrition, is evident from the results of this study, the number of seminiferous tubules (Figure 1; Table 1), the number of primary spermatocytes (Figure 1; Table 2) and Leydig cells (Figure 1; Table 3) were insufficient in the treatment group triggers a high ROS level. Due to a decrease in the number of seminiferous tubules, primary spermatocytes and Leydig cells will trigger infertility disorders because the seminiferous tubules function as gametocyte cells for the continuation of the spermatogenesis process, while Leydig cells act as a producer of the hormone testosterone which plays a role in the fertility process in men or men. If there is a disturbance in the two structures, it will result in infertility conditions in the male reproductive system.

Many natural substances are known to have the potential to increase oxidative stress in organs such as the testes. One of the natural ingredients based on local wisdom in South Kalimantan is *Eurycoma longifolia* Jack (EL). Pasak Bumi herb found in Southeast Asia has been widely used in traditional medicine. This plant belongs to the Simaroubaceae family and is known locally as 'Tongkat Ali' or 'Pasak Bumi' in Malaysia and Indonesia.

The results of this study also indicate that malnutrition can be improved by providing Pasak Bumi as a dietary supplement (Figure 1;



Tables 1, 2 and 3). The administration of Pasak Bumi leads to an increase in the number of seminiferous tubules, primary spermatocytes and Leydig cells at various dose levels in the malnourished mouse model compared to the malnutrition group. Of the 5 doses of Pasak Bumi used as a dietary supplement, the dose of 15 mg / kgBW showed a significantly higher level than the other doses in terms of an increase in the number of seminiferous tubules, primary spermatocytes and Leydig cells. This shows that this dose is the optimal dose of Pasak Bumi to improve the state of spermatogenesis in a malnourished mouse model.

EL (Simaroubaceae family) is one of the traditional medicinal ingredients, the roots have been proven to have medicinal properties, which are rich in various bioactive compounds (eurycomaoside, eurycolactone, eurycomalactone, eurycomanone, and Pasak Bumi), including alkaloids and quassinoids.<sup>19,20</sup> Research at the test animal level shows that the root of Pasak Bumi increased the hormones testosterone, FSH and LH and their effect on increasing spermatogenesis by increasing the number of spermatogenic cells, Sertoli cells, and Leydig cells in mice.<sup>6,7</sup> Likewise, research conducted by Maria (2003) showed that the extract of Pasak Bumi increased spermatogenesis in mice.<sup>8</sup> Previous animal studies have strengthened this conclusion by observing that EL is a potential stimulator of male sexual character in Sprague-Dawley mice. This natural compound also has several effects on hormones, providing clear evidence of its androgenic action. EL has been shown to have anticancer, antibacterial, cytotoxic, aprodic, and antimalarial effects.<sup>21,22,23,24,25</sup> It can be concluded that giving Pasak Bumi extract as a dietary supplement can improve spermatogenesis conditions in poor nutritional conditions.

#### CONCLUSION

Based on the results and discussion above, it can be concluded that Pasak Bumi as a food supplement has the potential to increase the spermatogenesis process that occurs due to malnutrition in children and the recommended dose of Pasak Bumi as a dietary supplement in malnutrition conditions in young rats is 15 mg/kgBW because it has more potential optimal to improve the conditions of spermatogenesis.

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