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 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 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 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.

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%). The nutritional imbalance factor is one of the causes of reproductive system health problems besides exercise. obesity, caffeine. age, 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 antioxidant system and oxidative stress can to impaired development Ωf spermatogenesis, abortion, disorders, and cancer in children.² congenital

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. ^{2,3,4}

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.5

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. ^{6,7} The research by Maria (2003) shows that the extract of Pasak Bumi can increase spermatogenesis in mice. ⁸ 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

- 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.

3. 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 ρ <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	ρ Value between the study groups
М	3.5 ± 0.50	
P1	4.63 ± 0.48	 ρ Value between M & P1 groups (0.005) ρ Value between M & P2 groups (0.015) ρ Value between M & P3 groups (0.247) ρ Value between M & P4 groups (0.090)
P2	4.25 ± 0.79	
P3	3.79 ± 0.56	
P4	4.29 ± 1.06	p value betileen in a 1 4 gloups (0.000)

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	ρ Value between M & P1 groups
P2	398.29 ± 101.67	ρ Value between M & P2 groups (0.002) ρ Value between M & P3 groups (0.693)
P3	240.00 ± 24.87	ρ Value between M & P3 groups (0.093) ρ Value between M & P4 groups (0.026)
P4	278.33 ± 34.79	

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) P value between M & P2 groups (0.037) P value between M &P3 groups (0.015) P value between M & P4 groups (0.001)
P2	8.13 ± 2.21	
P3	8.42 ± 1.86	
P4	8.46 ± 1.21	

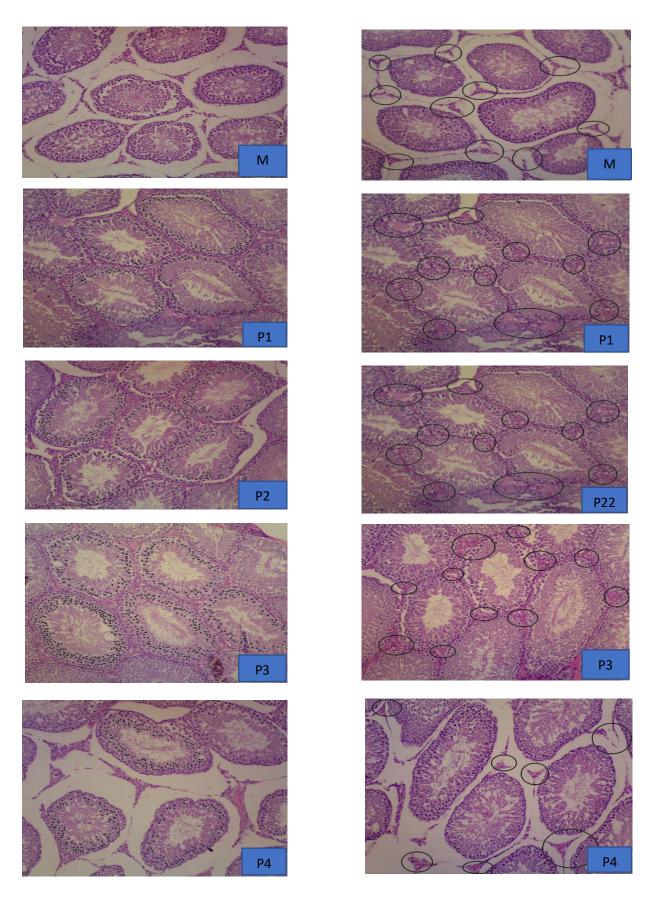


Figure 1. Microscopic morphology of testes (M-P4), black circles (leydig cells), black dots (primary spermatocytes); HE staining; Magnification of 100 x and 400 x, Optilab camera; Image Raster, light microcope

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 arginine, leucine, proline, and glutamine), play an important role in activating cell signaling and metabolic pathways necessary for protein synthesis and cystoskeletal remodeling 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. 10 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 b-oxidation, fatty acids are the main energy substrate for the mother's liver, skeletal muscle, heart, and kidneys.¹

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 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) pate 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.1

Factors that can cause changes in the balance between the antioxidant system and oxidative stress can lead to developmental spermatogenesis, damage 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. 13 Maternal malnutrition (70 days of gestation to term) is reported to reduce the number of Sertoli cells in the testes of newborn sheep. 14 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.

Oxidative stress is the abundance of reactive oxygen species (ROS) or a deficiency of antioxidants¹⁶. The imbalance produced by ROS causes cell damage. The detrimental effects of spermatozoa damage have been known since the 80s. 16 However, excessive numbers are pathophysiological and lead to damage and even apoptosis. 17,18 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. 19,20 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. ^{6,7} Likewise, research conducted by Maria (2003) showed that the Pasak Bumi extract of increased spermatogenesis in mice 8. 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, aprodiacic, and antimalarial effects. 21,22,23,24,25 It can be concluded that giving Pasak Bumi extract as a supplement improve can 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.

ACKNOWLEDGMENTS

Financial support for this study was provided by a grant from Lambung Mangkurat University.

REFERENCES

- Depkes RI. Data and Information on Indonesia's Health Profile 2017; Depkes; 2018.
- Muzi-Filho H, Bezerra CG, Souza AM, Boldrini LC, Takiya CM, Oliveira FL, et al. Undernutrition affects cell survival, oxidative stress, Ca2+ handling and signaling pathways in vas deferens, crippling reproductive capacity. PLoS One. 2013; 8(7): e69682.
- Herbert DC. Growth patterns and hormonal profile of male rats with protein-calorie malnutrition. Anat Rec. 1980; 197(3): 339– 54
- 4. Carvalho M, Mateus L, Afonso F, Van Harten S, Cardoso LA, Redmer DA, et al. Testicular angiogenic activity in response to food restriction in rabbits. Reproduction 2016; 20-5.
- Russell, LD, Ettlin RA, SinhaHikim, AP; Clegg ED. Histological and histopathological evaluation of the testis. Clearwater. Cache River Press; 1990.
- Rosida L. Pengaruh Pemberian Ekstrak akar Pasak Bumi (Eurycoma longifolia Jack.) Per Oral Terhadap Jumlah Sel Spermatogenik, Sel Sertroli dan Sel Leydig pada Mencit (mus musculus) Jantan Strain [penelitian eksperimental Laboratoris Laporan Penelitian]. Swiss: GDLHUB Pasca Sarjana Universitas Airlangga. Surabaya; 2013.
- 7. Hestianah EP. Gambaran Spermatogenesis Tubulus semineferus Mencit (Mus Muculus) Jantan Strain Swiss Setelah Pemberian Ekstrak Metanol Akar Pasak Bumi (Eurycoma longifolia Jack.) Per Oral Penelitian eksperimental laboratoris [laporan penelitian]. Surabaya: JIPTUNAIR Fakultas Veterinarr UNAIR; 2004.
- 8. Maria N. Pengaruh Akar Pasak Bumi (Eurycoma longifolia Jack) terhadap Spermatogenesis dan Kualitas Spermatozoa Mencit [skripsi]. Yogyakarta: Jurusan Biologi Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Gadjah Mada Yogyakarta; 2003.
- Bazer FW, Wu G, Spencer TE, Johnson GA, Burghardt RC, and Bayless K. Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. Mol Human Reprod. 2010; 16: 135-52.
- Wallace JM, Luther JS, Milne JS, Aitken RP, Redmer DA Rey- nolds LP, and Hay WW. Nutritional modulation of adolescent

- pregnancy outcome. A review. Placenta 2006; 27: 61-68.
- Burdge GC and Lillycrop KA. Nutrition, epigenetics, and developmental plasticity: Implications for understanding human disease. Annu Rev Nutr. 2010; 30: 315– 39
- 12. Wu G. Amino acids: Metabolism, functions, and nutrition. Amino Acids. 2009; 37: 1–17.
- 13. Omar A.H. Ahmed-Fari, Maha Nasr, Rania F. Ahmed and Rofanda M. Bakeer. Beneficial effects of curcumin nanoemulsion on spermatogenesis and reproductive performance in male rats protein deficient diet model: under enhancement of sperm motility. conservancy of testicular tissue integrity, cell energy and seminal plasma amino Journal of Biomedical acids content. Science. 2017: 24:66 DOI 10.1186/s12929-017-0373-5.
- 14. Aitken J, Fisher H. Reactive oxygen species generation and human spermatozoa: The balance of benefit and risk. Bioessays 1994; 16: 259–67. [CrossRef].
- 15. Rae MT, Kyle CE, Miller DW, Hammond AJ, Brooks AN, Rhind SM: The Effects Of Undernutrition, In Utero, on Reproductive Function In Adult Male And Female Sheep. Anim Reprod Sci. 2002, 72: 63-71.
- Agarwal A, Rana, M Qiu, E. AlBunni, H Bui AD, Henkel R. Role of oxidative stress, infection and inflammation in male infertility. Andrologia. 2018; 50: e13126. [CrossRef].
- Aitken RJ, Clarkson JS. Cellular basis of defective sperm function and its

- association with the genesis of reactive oxygen species by human spermatozoa. J. Reprod. Fertil. 1987; 81: 459–69.
- Smits R, Mackenzie-Proctor, R.; Fleischer, K.; Showell, M.G. Antioxidants in fertility: Impact on male and female reproductive outcomes. Fertil. Steril. 2010; 110: 578–80.
- 19. WG Goreja and A. Tongkat, Tree That Cures a Hundred Diseases. New York: Amazing Herbs Press; 2004.
- 20. Bhat, A. A. Karim. Tongkat Ali (Eurycoma longifolia Jack): a review on its ethnobotany and pharmacological importance. Fitoterapia. 81.
- 21. Farouk AE, Benafri A. Antibacterial activity of Eurycoma longifolia Jack. A Malaysian medicinal plant. Saudi Med J. 2007; 28: 1422-4.
- 22. Kuo PC, Damu AG, Lee KH, Wu TS. Cytotoxic and antimalarial constituents from the roots of Eurycoma longifolia. Bioorg Med Chem. 2004; 12: 537-44.
- 23. Ang HH, Lee KL, Kiyoshi M. Sexual arousal in sexually sluggish old male rats after oral administration of Eurycoma longifolia Jack. J Basic Clin Physiol Pharmacol. 2004; 15: 303-9.
- Chan KL, Choo CY, Abdullah NR, Ismail Z. Antiplasmodial studies of Eurycoma longifolia Jack using the lactate dehydrogenase assay of Plasmodium falciparum. J Ethnopharmacol. 2004; 92: 223-7.
- Sholikhah EN, Wijayanti MA, Nurani LH, Mustofa. Stage specificity of pasak bumi root (Eurycoma longifolia Jack) isolate on Plasmodium falciparum cycles. Med J Malaysia. 2008; 63 Suppl A:98-9.