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EFFECT OF GRANT LEAF EXTRACT (*MORINGA OLEIFERA* LAM) ON HISTOPATHOLOGICAL FEATUREOF WHITE RAT (*RATTUS NORVEGICUS*) TESTIS EXPOSED HOT TEMPERATURE

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ABSTRACT

The purpose of this study was to determine the benefits of *Moringa oleifera* Lam extracts on the testicular histopathology of male white rats (*Rattus norvegicus*) exposed to hot temperatures. This study used 25 white rats, aged 2-3 months and weighed 200 grams. The white rats were divided into five treatment groups and each group contained five white rats. K- were given 1% CMC Na 1ml, K + was given 1% CMC Na 1 mL and exposed to hot temperatures (40 °C for 60 minutes). The treatment groups P1, P2, and P3 were given *Moringa* leaf extract at doses of 100, 200, and 400 mg / kgbb and exposed to hot temperatures (40°C for 60 minutes). This research was conducted for 14 days. Data were analyzed using the Kruskal-Wallis and Mann-Whitney tests with the SPSS computer program for windows. The results showed that there were significant differences (p <0.05) between treatment groups. The conclusion of this study is the extract of *Moringa* leaf at a dose of 400 mg/kg can maintain the histopathological feature of white mouse testis due to exposure to hot temperatures.

KEY WORDS: Moringa leaf extract, Testis, Hottemperature.

INTRODUCTION

This modern era, the temperature in the world, especially Indonesia, is experiencing global warming. The phenomenon of global warming is due to greenhouse gas emissions due to the carbon cycle and climate response (Meinshausen *et al.*, 2009). According to Febriant i(2009), the average annual air temperature has increased to reach 0.10C per year. Warmer air temperatures can affect body temperature, which can cause oxidative stress, which is an imbalance between higher free radical production compared to antioxidants (Umar *et al.*, 2015).

Free radicals are molecules formed when compounds lose electrons which later become unstable and are also natural products of cell metabolism (Toripah, 2014). Living things naturally have a natural defense system against free radicals, namely endogenous antioxidants which consist of enzymes synthesized by the body such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (Ekambaram *et al.*, 2011). Antioxidants that can not compensate for free radicals, then the reproductive system will be disrupted. Enzymes that function as endogenous antioxidants are not enough to overcome free radicals, therefore we need more exogenous antioxidants (derived from food consumed) to neutralize excess free radicals (Astuti, 2008). The study of Umar *et al*.(2015) shows that the exposure to hot temperatures can cause a decrease in the quality of spermatozoa are because in the process of spermatogenesis, temperature is a factor that influences the process.

Moringa leaves can provide a protective effect by taking free radicals that provide oxidative damage caused by free radicals (Sreelatha and Padma, 2009). Moringa leaf is a plant that has high nutrition and an important source of antioxidants for health (Santos *et al.*, 2012). Moringa leaf extract produces high flavonoids (antioxidants) (Rakesh and Singh, 2010).

MATERIALS AND METHODS

White rats (*Rattus norvegicus*) obtained from the Animal Cage of Try Faculty of Veterinary Medicine, Airlangga University. The white rats used were 25 animals.Materials needed include Moringa leaf extract, 1% CMC Na, 10% NBF solution, alcohol (70%, 80%, 90%, 96%), ketamine, xylazin, liquid paraffin, xylol, Haematoxylin-Eosin dye and rat feed white.

The instrument used is a test animal cage with a size (33x26x15) which is equipped with a mesh cover, husk and drinking place. Artificial incubator with size (90x70x40) equipped with bulb (Philips 100watt), thermostat (W3001), digital LCD Thermometer Hygrometer and zinc plate. Ovens, pumpkin erlenmeyers, rotary evaporators, freeze dryers and measuring cups are used to manufacture Moringa leaf extract. The extract was given using a gastric tube and a catheter tube. Retrieval of testicular organs using surgical support boards, needles, scalpels, clamp arteries, anatomical tweezers, scissors, 1 mL syringes (OneMed), tissue pots. The process of making histopathological preparations using tissue processor (Bavimed), tissue embedding (Tissue-Tek, Sakura), holder / cassete, microtome (Accu-cut SRM, Sakura), Spatula, waterbath (Leica HI1210) and hotplate (Heraeus electronics), staining jar, object glass and cover glass. Observation of histopathological preparations using a light microscope (Nikon E100) and Optilab professional series.

Experimental protocol

This research was carried out in the experimental animal enclosure of the Faculty of Veterinary Medicine, Airlangga University, for 14 days. This study used 25 white rats divided into five treatment groups and five white rats in each group. The research design used was a completely randomized design. Moringa leaf extract is made in the pharmacology laboratory of the Faculty of Veterinary Medicine, Airlangga University. The making of histopathological preparations was carried out in the Veterinary Pathology laboratory of the Faculty of Veterinary Medicine, Airlangga University.

The white rat is adapted first for seven days in a stable environment. After seven days, rats were treated with Moringa leaf extract and exposed to hot temperatures of 400C for 60 minutes for 14 days. According to Sailer *et al*. (1997) and Umar *et al*. (2015) states, giving exposure to hot temperatures of 40^{o}C for 60 minutes resulted in damage to the spermatogenesis process. Provision of heat exposure is done on artificial incubators.

Collection and examination of samples

Retrieval of white rat testicles was carried out on the 15th day. White rat's testicles were put into tissue pots containing 10% NBF solution and allowed to stand for ± 24 hours. Preparation and examination of histopathological preparations carried out in the Veterinary Pathology laboratory of the Faculty of Veterinary Medicine, Airlangga University. Examination of preparations using a light microscope with magnification of 100x and 400x (Safitri and Hariadi, 2019). Seminiferous tubules are assessed using Jhonsen's Scoring (Johnsen, 1970).

Data analysis

Data on testicular histopathological features were analyzed by non-parametric statistical tests using the Kruskal-Wallis test and continued with the Mann-Whitney test to find out the real differences between treatment groups ($p < 0.05$).

RESULTS AND DISCUSSION

Research data on the effect of Moringa leaf extract on the histopathological features of white mouse testes exposed to hot temperatures are presented in Table 1.

Negative control can be an indicator of testicular histology. Histology of white rat testes on negative control was dominated by perfect spermatogenesis, namely tubular epithelial cells, germinal, Sertoli, spermatogonia, spermatocytes, early spermatids, late spermatids and spermatozoa. Perfect spermatogenesis is produced by the process of meiosis in the seminiferous tubules (Griswold, 2016). According to Franka *et al*. (1998)states, one

Table 1. Meanseminiferous tubule score

Treatment group	Tubular Seminiferous Score (Means ± SD)
K(-)	9.54^{e} ± 0.688
K(+)	5.93^{a} ± 1.887
P(1)	6.75^{b} ± 1.598
P(2)	8.29^{c} ± 1.140
P(3)	9.23^{d} ± 0.839

Note: Different superscripts show significant differences ($p < 0.05$)

cycle of spermatogenesis of white rats (*Rattus norvegicus*) lasts for 12.9 days and it goes on continuously.

Histopathological features of the testis in positive control showed damage to most of the assessed seminiferous tubules. This shows that exposure to hot temperatures can cause interference with the process of spermatogenesis, this is in accordance with the statement of Kim *et al*.(2013), namely heat temperatures can affect mitochondrial cell death or DNA damage in germ cells. The following histopathological picture of white rat testis organs from the five treatment groups in Figure 1.

Heat is very closely related to global warming. Hot temperatures can also be caused by environmental effects such as factory heat, combustion, and vehicles, where the engine is the source of heat. Hamerezaee *et al*. (2018) states the assessment of sperm quality among steel industry workers exposed to heat has decreased significantly compared to normal. Exposure to heat can increase the body's metabolic processes and also increase the concentration of ROS (Belhadj Slimen *et al.*, 2016). Exposure to heat also results in disruption of cell function and cell damage, such as apoptosis and necrosis (Pandey, 2012). The process of cell apoptosis due to ROS production occurs mostly in the liver and gonads (Samik and Safitri, 2017). Sperm cells have limited cell cytoplasm and are very susceptible to increased ROS (Li *et al.*, 2015). Liu *et al*. (2016) state that the high ROS will greatly affect the pathophysiological processes of mammalian testes.

The body in hot conditions will go the extra mile to maintain body temperature. Body temperature can be maintained by sweating so that the body temperature remains at normal conditions. Expending body heat increases metabolism in the body. Increased body metabolism makes the body needs ATP as a source of energy. The process of ATP formation in the process of the Kreb cycle in the mitochondria produces ROS (Reczek and Chandel, 2015). The results of Santos' research (2009) also stated that the respiratory chain in the mitochondria can increase ROS production. The ROS produced in mitochondria is H_2O_2 (Sobotta *et al.*, 2015). H_2O_2 can interfere with the Na + ¬ K + pump process and the Ca2 + homeostasis process (Margaritelis *et al.*, 2016). If the Na + - K + pump is disturbed it can cause interference with the cell. According to Cobley *et al*. (2015) states H_2O_2 can be neutralized by antioxidants.

Moringa oleifera Lam extracts are preventively intended to cope with the increase in free radicals. Moringa leaf extract is given 60 minutes before being exposed to heat that the extract at that time has been digested in the body (Kersten and Visser, 1996). Moringa leaves have a high flavonoid content (Coz-Bolaños *et al.*, 2018). According to *Moringa* leaves have high levels of antioxidants and are easily digested. High levels of flavonoids Moringa leaves can bind to free radicals (Nouman *et al.*, 2016). The binding of the antioxidant of Moringa leaves to

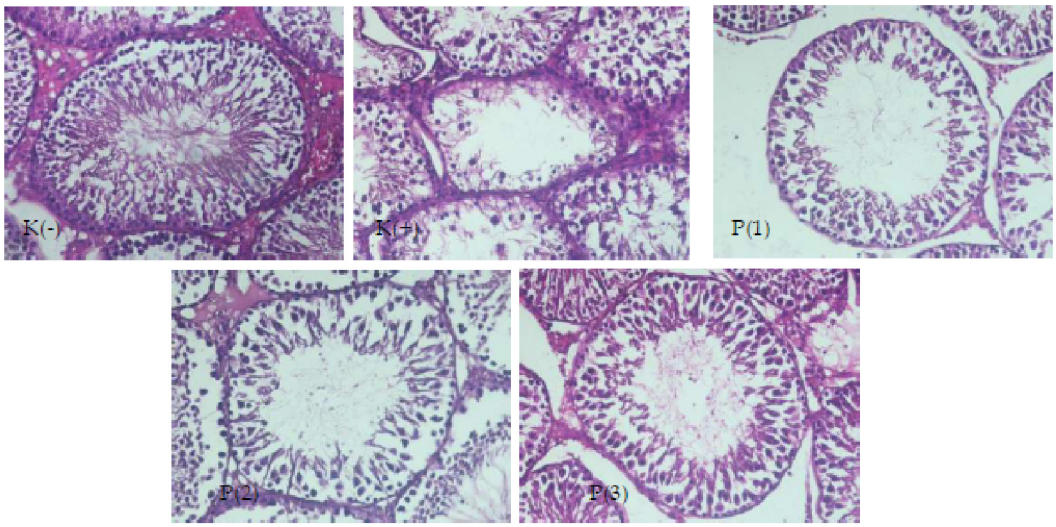

Fig. 1. Histopathological feature of the testes of white rats (*Rattus norvegicus*). (400x magnification, Haematoxilin-Eosin staining).K- shows Scoring 10 (full spermatogenesis), K + shows Scoring 2 (only Sertoli cells and tubular epithelial cells), P1 shows Scoring 5 (no spermatid cells, spermatogenesis to spermatocytes), P2 shows Scoring 7 (spermatogenesis to many phases) early spermatid cells but late spermatids until spermatozoa are absent), and P3 shows Scoring 9 (full spermatogensis) (Johnsen, 1970).

ABTS (2,2-Azinobis 3-ethyl benzothiazoline 6-sulfonic acid) was 93.51 ± 0.19% and DPPH (1,1-diphenyl-2-picrihydrazil) was 58.95 ± 0.3% (Qwele *et al.,* 2013).

The treatment groups P (1), P (2) and P (3) who were given *Moringa oleifera* Lam extracts were proven to be able to maintain testicular histopathology with close to normal indicators (group K (-)) with different results. These results are in line with those delivered by Ajuogu *et al*.(2019) that *Moringa oleifera* Lam powder used as a mixture of rabbit feed can improve reproductive function by increasing the endocrine system and the quality of cement. The difference lies in the dosage of Moringa leaf extract. The treatment group P (1) increased slightly higher than the group K (+) with an average score of 6.75. The mean results showed that spermatogenesis stopped in the initial spermatid phase. The treatment group P (2) also improved than group K (+) with an average score of 8.29. The mean results showed that spermatogenesis stopped in the final spermatid phase. The treatment group P (3) had the closest histopathological feature (group K (-)). P (3) describes the process of perfect spermatogensis and the feature is the same as testicular histology. The process of spermatogenesis is maintained by Moringa leaf extract with an optimal dose of 400 mg/kg. The results of this study are in line with the research of Santos *et al*. (2012) states that the content of Moringa leaves which are rich in antioxidants can maintain the process of spermatogenesis.

CONCLUSION

From the results of the research and discussion above, it can be concluded that the preventive administration of Moringa leaf extract in white rats exposed to 40°C heat for 60 minutes can maintain the histopathological feature of white rat testicular organs efficiently at a dose of 400 mg/KgBW.

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