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RESEARCH ARTICLE

Effects of *Moringa oleifera* L. Leaves extract on leydig and sertoli cells induced high Temperature on *Rattus norvegicus*

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ABSTRACT:
The aim of this study was to investigate the protective effect of *Moringa oleifera* leaves extract on the number of leydig and sertoli cells on *Rattus norvegicus* due to induced high temperature. Twenty five male rat were divided into five groups, five rat for each group and administered through intragastric gavage with differents treatments for 21 days. The treatment groups were C–(NaCMC 1% 1ml), C+ (NaCMC 1% 1ml + high temperature induction), T1, T2, and T3 (100, 200, and 400mg/kg bw *Moringa oleifera* Lam leaves ethanol extract respectively+high temperature induction). The high temperature induction was 40°C for one hour. The observation was done by examined the histopathological changes on the numbers of Leydig and Sertoli cells. The research showed that *Moringa oleifera* leaves extract could protect the leydig and sertoli cells in rat testes from destructive effect of high temperature induction.

KEYWORDS: *Moringa oleifera,* Leydig, Sertoli, Testes.

INTRODUCTION:

Frozen semen production in Indonesia became a focus of attention in the dairy farm and livestock sector. However, one of the problems is environmental temperature, such as high ambient temperature harms reproductive performances in bull[1]. Heat exposure shown a deleterious impact on reproductive organ, including leydig and sertoli cells[1]. The decreasing numbers of leydig cells could disrupt steroidogenesis process, causing unbalanced testosterone synthesizing and possibly disturb spermatogenesis[2]. Futhermore, degenerative changes of leydig cells cause impaired steroidogenesis and recovered in 140 days after last high temperature exposure. In ultrastructural study reported 14 days hyperthermia cause severe dilated cisterna of the smooth endoplasmic reticulum and swollen mitochondria with degenerated tubular cristae observed in sertoli cells. To maintain this condition at an acceptable level, natural antioxidants, such as vitamins C and E, carotenoids and flavonoids are need in testes[3].

Recently, a lot of attention has been focused on the role of the antioxidative defense system in fighting oxidative stress. Endogenous antioxidants in plants may has an essential role in oxidative damage, possibly preserving the biological function of cells[4,5]. These antioxidants are adequate to prevent oxidative damage by enhancing antioxidant enzymes, which reducing production of free radicals and lipid peroxidations[6].

Moringa oleifera has been known to its protective medicinal properties since ancient times and reported have high levels of multiple natural antioxidants such as polyphenolic (ellagic, chlorogenic, gallic, and ferulic acid), flavonoid that will scavenge the free, radicals activate the antioxidant enzymes and inhibit oxidation. *Moringa oleifera* known as drumstick, horseradish tree, and *kelor* in Indonesian language. The leaves extract is very beneficial and offer important source of vitamin C, proteins, β-carotenes, flavonoids, and phenolic acids[7,8]. This plant also had anti-inflammatory, anti-hypertensive, anti-pyretic, anti-ulcer, anti-diuretic, anti-diabetic[9]. Thus, the aim of this study was to investigate the protective effect of *Moringa oleifera* leaves extract on the number of leydig and sertoli cells on *Rattus norvegicus* due to induced high temperature.

MATERIAL AND METHODS:

Ethical Clearance:

All treatment procedures have been tested through The Animal Care and Use Comittee of Veterinary of Medicine Faculty, Universitas Airlangga (Approval Number: 1.KE.119.07.2019)

Study Design:

This present research was used completely randomized design (CRD) due to the environment and age of rats were homogenized. In this design there was only one source of variability and random effect of treatments. Control groups had undergo the treatment as for C (-) only administered NaCMC 1% 1ml and C (+) rats had administered NaCMC 1% 1ml + incubated for one hour at 40°C. Treatment groups T 1, T2, and T3 given *Moringa oleifera* leaves extract (100, 200, 400mg/kg Bw) and incubated for one hour at 40°C. Rats were adaption for 7 days and treated for 14 days, all treatment were done in five replicates.

Animals:

This study used 25 adult male Wistar rats (200-250g) kept at Universitas Airlangga Laboratory of Experimental Animals, Veterinary Medicine Faculty in a standart animal facility with free access to water and standart rat chow diet.

Evaluation Methods:

the animals were euthanized by cervical dislocation. Leydig cells calculation observed ten areas of views and sertoli cells calculations observed in ten seminiferous tubules. The results of calculating the number of sertoli and leydig cells in each area of view within the tissue samples were calculated. Observation histopathological preparations of rat testes were carried out by microscope at 400x magnitudes.

Data Analysis:

This study was analyzed by using SPSS 21 (SPSS Inc., Chicago, IL), employing one-way analysis of variant (ANOVA) ($p < 0.05$) and followed by Duncan test. Tabulated data were presented as the mean ± standard deviation.

RESULTS AND DISCUSSION:

Histopathology of Leydig cells in rat testes shown in Figure 1. In C(-) group, abundance of normal Leydig cells compared to picnotic Leydig cells. In contrast, C(+) group showed more numbers of picnotic Leydig cells than normal. Histopathological features on T4 group that had given *Moringa oleifera* leaves extract 400 mg/kg bw shown the leydig cells appear to be more normal, moreover picnotic leydig cells were almost invisible. Examination results of normal Leydig cell counted on rat testes in each treatment groups presented in Table 1.

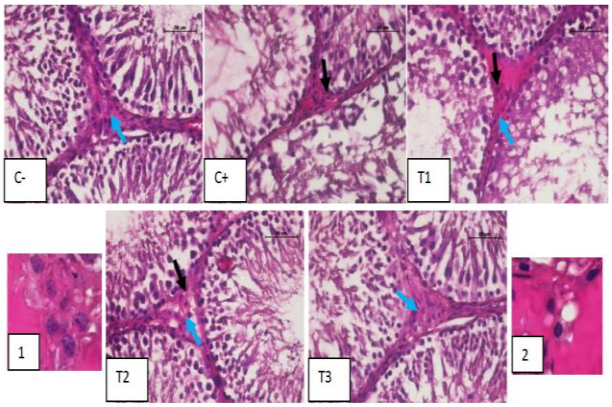

Figure 1. Photomicrographic views on leydig cell in rat testes, C-, C+, T1, T2, and T3 (400x magnifications, H&E); blue arrow showed normal leydig cells and black arrow showed necrotic leydig cells; middle left and right picture (1 and 2) showed the difference between normal and necrotic leydig cells.

Table 1. The number of leydig cells in rat testes on each treatment groups

S. No	Treatment	Number of Leydig Cells (Mean±SD)
1	C+	$5.62^{a} \pm 1.33$
2	C-	$12.26^{b} \pm 1.31$
3	T1	$12.62^{b} \pm 1.78$
4	T2	$14.18^{c} \pm 1.38$
5	T3	$14.34^{c} \pm 1.56$

*Different superscript in the same column indicate significant differences ($p<0.05$)

Histopathology of Sertoli cells in rat testes shown in Figure 2. In C(-) group, abundance of normal Sertoli cells compared to picnotic Sertoli cells. In contrast, C(+) group showed more numbers of picnotic than normal Sertoli cells. Histopathological features on T4 group that had given *Moringa oleifera* leaves extract 400 mg/kg bw shown the sertoli cells appear to be more normal. Moreover, picnotic Sertoli cells were almost invisible. Histopathological features of sertoli cells presented on Figure 4.2. Examination results of normal Sertoli cells counted on rat testes in each treatment groups presented in Table 2.

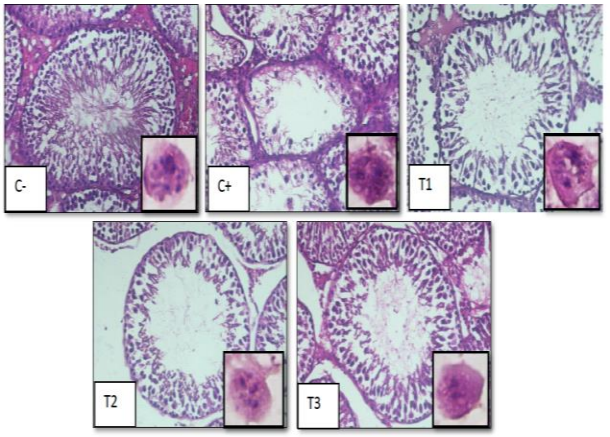

Figure 2. Photomicrographic views on sertoli cells in rat testes, C-, C+, T1, T2 and T3 treatments group (400x magnfication, H&E).

Table 2. The number of sertoli cells in rat testes on each treatment groups

S. No	Treatment	Number of Sertoli Cells (Mean±SD)
1	C+	$9.22^{a} \pm 2.67$
2	C-	$18.90^{d} \pm 1.51$
3	T1	$15.12^{b} \pm 1.79$
4	T2	$15.60^{d} \pm 1.72$
5	T3	$17.76^{c} \pm 1.27$

*Different superscript in the same column indicate significant differences ($p<0.05$)

Mammalian testis are highly suceptible to oxidative stress caused by heat stress. The result of this research shows that negative control (C-) group that only administered NaCMC 1% 1 ml had the significant difference with positive (C+) group that administered NaCMC 1% 1 ml and induced for one hour with high temperature. It revealed the evidence of testes damage by high temperature and proves that high temperature could harm leydig and sertoli cells. High temperature can induces heat stress and excessive formation of reactive oxygen species (ROS). ROS were capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other molecules resulting in cellular damage as well as interferences on cellular function[10,11]. High ambient temperature and humidity harm health and sexual behavior of animals like testicular cells degeneration, reduced percentages of ejaculates and disturbed in the production of sperms and reproductive function[12].

Cells damage induced by increasing level of ROS that might cause an oxidative stress that could damage the testes that consist of high content of polyunsaturated fatty and can leading picnosis in Leydig and Sertoli cells. Previous research has suggested that high concentrations of ROS play an important role in pathophysiology of mammals testes[13]. ROS formation is always accompanied by up-regulation of antioxidant enzyme system, which protects tissue against damage caused by excessive ROS via the scavenging activity of enzymes such as SOD (super oxide dismutase) and GSH-Px (gluthatione peroxidase). Testicular oxidative stress is affected by the balance and this scavenging system[14,15].

Moringa oleifera could prevent the deleterious effect on leydig was also corroborates with previous research that this extract significantly increased male fertility hormone particularly testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone and luteinizing hormone. These results may be due to presence of flavonoids. Flavonoid are well known atioxidants that can ameliorate oxidative stress-related testicular impairment in animal tissues[16,17]. Flavonoid in *Moringa oleifera* absorption is vary, based on chemical structure, molecular weight, glycosylation in every specieces, and esterification in gastrointestinal tract. Flavonoid as quarcetin-4*'*-*O*-glucoside, reached its peak in plasma at 0.7±0.3 hour after absorption[18]. A simplified flavonoid metabolism as it is ingested orally, administration undergo extensive intestinal metabolism, then metabolites to the liver via hepatic portal vein and undergo further mechanism. The liver metabolites can be transported to targeted cells and tissues, excreted to bile and undergo re-circulation, or eliminated via urine and or feces[19,20].

CONCLUSION:

Thus, it is possible to suggest that *Moringa oleifera* Lam leaves extract could protect the number of leydig and sertoli cells in rat (*Rattus novergicus*) testes due to exposed high temperature and the best dose that could preserve the number of Leydig cells due to induced temperature.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

REFERENCES:

1. Das R, Sailo L, Verma N, Bharti P, Saikia J, Imtiwati, Kumar R. Impact of heat stress on health and performance of dairy animals: a review. Veterinary World. 2016; 9(3): 260-268.
2. Ramaswamy S, Weinbauer GF. Endocrine control of spermatogenesis: role of FSH and LH/testosteron. Spermatogenesis. 2014; 4(2): e996025
3. Wang Y, Chen F, Ye L, Zirkin B, Chen H. Steroidogenesis in leydig cells: effects of aging and enviromental factors. Reproduction. 2017; 154(4): 111-122.
4. Mbikay M. Therapeutic potential of *Moringa oleifera* leaves in chronichyperglycemia and dyslipidemia. Front Medical Pharmacology. 2012; 9(3): 6342-6347
5. Fadholly, A, Ansori ANM, Jayanti S, Proboningrat A, Kusala MKJ, Putri N, Rantam FA, Sudjarwo SA. Cyototoxic effect of *Allium cepa* L. extract on human colon cancer (WiDr) cells: in vitro study. Research Journal of Pharmacy and Technology. 2019; 12(7): 3483-3486.
6. Prabsattroo T, Wattanathorn J, Iamsaard S, Sompsat P, Sritagool O, Thukhummee W, Muchimapura S. *Moringa oleifera* extract enhances sexual performances in stressed rats. Journal of Zhejiang University Sciences. 2015; 16(3): 179-190.
7. Tian-yang W, Qing L, Kai-shun B. Bioactive flavonoids in medical plants: structure, activity and biological fate. Asian Journal of Pharmaceutical Sciences. 2018; 13: 12-32.
8. Gopalakrishnan L, Doriya K, Kumar DS. Moringa oleifera: a review on nutritive importance and its medicinal application. Food Science and Human Wellness. 2016; 5: 49-56.
9. Sharma DN, Battacharya L. Role of some antioxidants on mercury chloride induced spermatogenesis in swiss albino mice during pre

pubertal phase of life. Indian Journal of Scientific Reserach. 2012; 1(2): 19-25.
10. Hoque R, Rana S, Nayan SB, Miraz FH, Deb GK, Nahar TN, Habib R, Siddiki MSR. Influence of multiple showering on quality of buffalo semen during hot-humid season. J. Advanced Veterinary and Animal Research. 2018; 5(1): 12-18.
11. Li R, Jia Z, Trush MA. Defining ROS in biology and medicine. React Oxyg Species (Apex). 2016; 1(1): 9-21.
12. Kehrer JP, Klotz LO. Free radicals and related reactive species as mediators of tissue injury and disease: implication for health. Crit ReV Toxicol. 2015; 45(9): 765-798.
13. Liu XY, Zhang SX, Zhang N, Hao CF, Zhuang LL, Huang X. Effects of apigenin on scrotal heat-induced damage in the mice testis, International Journal of Clinical Expert Medicine. 2016; 9(3): 6342-6347.
14. Lubrano V, Balzan S. Enzymatic antioxidant system in vascular inflamation and coronary artery disease. World J Exp Med. 2015; 5(4): 218-224.
15. Asadi N, Bahmani M, Kheradmand A, Rafieian-kopaei M. The impact of oxidative sress on testicular function and the role of antioxidants in improving it: a review. J Clin Diagn Res. 2017; 11(5): 1-5.
16. El-sheikh SM, Khairy MMA, Fadil HA, Abo-elmaaty MA. Ameliorative effect of Moringa oleifera extract on male fertility in paroxwtine treated rats. Zagazig Veterinary Journal. 2016; 44(3): 244-250.
17. Cajuday LA, Pocsidio GL. Effects of Moringa oleifera Lam (Moringaceae) on the reproduction of male mice (Mus musculus). Journal of Medicinal Palnts Research. 2010; 4: 1115-1121.
18. Graefe EU, Wittiq J, Mueller S, Riethling AK, Uehleke B, Drewelow B, Pforte H, Jacobasch G, Derendorf H, Veit M. Pharmacokinetics and bioavailability of quarcetin glycosides in humans. J Clin Pharmacol. 2001; 41(5): 492-499.
19. Thilakarathna SH, Rupasinghe HPV. Flavonoid bioavailability and attemps for bioavailability enhancement. Nutrients. 2013; 5(9): 3367-3387.
20. Zheng M, Sun R, Basu S, Ma Y, Ge S, Yin T, Gao S, Zhang J, Hu M. Disposition of flavonoids via recycling: direct biliary exertion of enterically or extrahepatically derived flavonoid glucuronides. Mol Nutr Food Res. 2016; 60(5): 1006-1019.

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