Ovozoa 12 No. 2, August 2023 p-ISSN 2302-6464 e-ISSN 2722-967X
Available at https://e-journal.unair.ac.id/OVZ/index

Original article

Effect of ethanolic extract of *Moringa oleifera* leaves on the number of spermatogenic cells and Leydig cells of gentamicin-induced rats

Nailul Ngizzah[1], Wurlina Wurlina[2], Poedji Hastutiek[3], Iwan Sahrial Hamid[4], Eka Pramyrtha Hestianah[5*], Maslichah Mafruchati[5], Lita Rakhma Yustinasari[6]

[1] Pet and Wild Animal Interest Group, Jalan Ir. Soekarno, Surabaya, Indonesia
[2] Division of Veterinary Reproduction, [3] Division of Veterinary Parasitology,
[4] Division of Veterinary Basic Medical Science, [5] Division of Veterinary Anatomy,
Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia
[6] Laboratory of Basic Veterinary Science, Joint Graduate School of Veterinary Medicine,
Yamaguchi University, Yamaguchi, Japan

[*] Corresponding author, e-mail: ekamirta@gmail.com
Open access under CC BY – SA license, DOI: 10.20473/ovz.v12i2.2023.99-106
Received October 18 2022, Revised August 11 2023, Accepted August 12 2023
Published online August 2023

ABSTRACT

This study aimed to determine the effect of the administration of ethanolic extract of *Moringa oleifera* leaves on the number of spermatogenic and Leydig cells of gentamicin-induced rats (*Rattus norvegicus*). This study used 25 white male rats divided randomly into five groups. The rats were injected with Gentamicin 5 mg/kg bw/day subcutaneously for 14 days, except for the negative control group (NCG). During the next 14 days, the rats were given oral moringa leaf extract of 200, 316, and 500 mg/kg bw/day respectively for E200, E316, and E500 groups. The data were analyzed with one-way ANOVA followed by Duncan's test. The results showed that the number of spermatogonia, spermatocytes, and spermatids in NCG was higher ($p < 0.05$) than in the other groups. The number of spermatogonia, spermatocytes, and spermatids in the E316 group was higher ($p < 0.05$) than in PCG. The number of spermatogonia in the E500 group was higher ($p < 0.05$) than in the E316 group, but the number of spermatocytes and spermatids in the E316 group was similar ($p > 0.05$) to the E500 group. The number of PCG Leydig cells was the smallest ($p < 0.05$) compared to the other groups. The administration of Moringa leaves extract in the E200 and E316 groups increased ($p < 0.05$) the number of Leydig cells compared to PCG. The number of Leydig PCG cells in the E500 group was not significantly different ($p > 0.05$) from that of the NCG. It could be concluded that administration of *Moringa oleifera* leaves extract restored the number of spermatogenic and Leydig cells of gentamicin-induced rats.

Keywords: gentamicin, Leydig, moringa, reproductive health, spermatogenic

INTRODUCTION

Aminoglycosides are broad-spectrum antibiotics that can inhibit bacteria from synthesizing protein. One of the groups of aminoglycosides is gentamicin which can be used to treat bacterial infections, including mycobacterium, septicemia, complications of urinary tract infections, endocarditis, peritonitis, and others, especially gram-negative bacteria in humans and animals (Krause *et al*., 2016). The effective dose for treating urinary tract infections is 160 mg/day. Gentamicin is a very effective antibiotic, but its use is limited (Ross *et al*.,

How to cite this article: Ngizzah N, Wurlina W, Hastutiek P, Hestianah EP, Mafruchati M, Hamid IS, Yustinasari LR. 2023. Effect of ethanolic extract of *Moringa oleifera* leaves on the number of spermatogenic cells and Leydig cells of Gentamicin-induced rats. Ovozoa: Journal of Animal Reproduction. 12: 99-106.

2019). It can cause toxicity to non-targeted tissues by producing reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS can bind to phospholipids in cell membranes which causes damage to the structure and function of cell membranes (Gamaan *et al*., 2023).

Moringa leaves contain antioxidants, including flavonoids, vitamin C, and vitamin E (Vergara-Jimenez *et al*., 2017). Moringa leaves contain the most antioxidants among other plant parts. The antioxidant content can reduce lipid peroxidation levels and normalize antioxidant enzyme levels (Peñalver *et al*., 2022). The effectiveness of ethanolic extract from Moringa leaves for treating the reproductive system due to gentamicin induction has yet to be studied. Therefore, it is necessary to determine the effect of Moringa leaves extract on the male reproductive system, especially on the number of spermatogenic and Leydig cells in rats (*Rattus norvegicus*).

MATERIALS AND METHODS

This study used male rats (*Rattus norvegicus*) aged 2-3 months with a weight range of 150-200 g with a total of 25 rats, gentamicin, 96% ethanol, 0.5% Na-CMC, and Moringa leaves ethanolic extract, and pellet rats feed, drinking water and husks. This study used Erlenmeyer flasks, filters, rotary evaporators, rat cages, sonde needles, Styrofoam boards, nails, scalpels, clamps, tweezers, scissors, syringes, tissue storage pots, cameras, and a light microscope (Monocular L-301).

Extraction of Moringa leaves

Moringa leaves samples were taken from Moringa trees that grow in Pare, Kediri. The Moringa leaves were washed, and then the Moringa leaves were air-dried on clean white paper. The dried moringa leaves are then crushed using a grinding machine. Extraction were carried out by soaking Moringa leaves powder in 96% ethanol solvent. Maceration was carried out for 3 × 24 hours and stirred every day, then filtered to obtain the form of macerate. The macerate was put into the rotary evaporator at 40°C with a speed of 50 rpm until all the solvent and water content of the Moringa leaves were separated so that a concentrated ethanol extract of Moringa leaves was obtained (Mardatillah *et al*., 2022).

Treatment

This research proposal was approved by the Experimental Animal Research Ethics Commission No. 1.KE.013.01.2020. Mice were adapted to the cage for a week, then randomly divided into five groups, negative control group (NCG), positive control group (PCG), treated groups with Moringa leaves extract 200, 316, and 500 mg/kg bw/day (E200, E316, and E500). The dose of gentamicin was 5 mg/kg bw for 14 days subcutaneously, based on a study by Mardatillah *et al*. (2022). The dose of the ethanolic extract of Moringa leaves was taken based on research from Ogunsola *et al*. (2017).

In the negative control group (NCG), mice were injected with 0.5 mL of aquadest subcutaneously once a day for 14 days, followed by 0.5 mL of 0.5% Na-CMC orally once a day for the next 14 days. In the positive control group (PCG), rats were injected with gentamicin 5 mg/kg bw/day subcutaneously for 14 days, followed by the administration of 0.5 mL of 0.5% Na-CMC orally once a day for the next 14 days. In the E200 group rats, gentamicin 5 mg/kg bw was injected subcutaneously for 14 days, followed by administration of 0.5 mL of Moringa leaf extract solution 200 mg/kg bw/day in 0.5% Na-CMC. In the E316 group, rats were injected subcutaneously with gentamicin 5 mg/kg bw for 14 days. This was followed by the administration of 0.5 mL of Moringa leaf extract solution 316 mg/kg bw/day in 0.5% Na-CMC. The E500 group rats were injected with gentamicin 5 mg/kg bw subcutaneously for 14 days. This was followed by administration of 0.5 mL of Moringa leaf extract solution 500 mg/kg bw/day in 0.5% Na-CMC. After the treatment, all rats were euthanized using ketamine at 70 mg/kg bw. The rat's abdominal wall was opened

How to cite this article: Ngizzah N, Wurlina W, Hastutiek P, Hestianah EP, Mafruchati M, Hamid IS, Yustinasari LR. 2023. Effect of ethanolic extract of *Moringa oleifera* leaves on the number of spermatogenic cells and Leydig cells of Gentamicin-induced rats. Ovozoa: Journal of Animal Reproduction. 12: 99-106.

to take the testicles and put in a tissue storage pot containing 10% formalin. Histological slides were prepared using Hematoxylin-Eosin staining (Mardatillah *et al*., 2022).

Spermatogenic cells count

Each testicular histological slide was observed under a light microscope with 400x magnification. Spermatogenic cells (spermatogonia, spermatocytes, spermatids) count in one field of view is conducted by differentiating the types of spermatogenic cells. Observations were made by counting the number of each type of spermatogenic cells in five fields of view, and then averaged.

Spermatogonia cells are characterized by a spherical shape near the basement membrane, oval nuclei with smooth chromatin, and this membrane is thin. Spermatocyst cells consist of primary spermatocytes and secondary spermatocytes. Primary spermatocytes have the largest size among other gamete cells, heterochromatic nuclei, and are located between the basement membrane and the tubular lumen. Secondary spermatocytes look similar to primary spermatocytes, dividing into spermatids quickly, so they are rarely observed. Spermatid cells are characterized by a round shape, more diminutive than spermatocytes, and round nuclei are pale and bright (Nishimura and L'Hernault, 2017).

Leydig cell counts

The number of Leydig cells in the seminiferous tubule interstitium was counted under a light microscope with a magnification of 400x. Observations were made by counting the number of Leydig cells in five fields of view, then averaged. Leydig cells are round with eosinophilic granular cytoplasm that gather to form polyhedral interstitial testes (Aladamat and Tadi, 2022).

Data analysis

Data analysis used Statistical Product and Service Solution (SPSS) computer software version 21. The One-way Analysis of Variance was followed by Duncan's test if the data distribution was normal and homogeneous or Tukey's test if the data distribution was normal and not homogeneous to know the differences between each group at the 95% confidence level.

RESULTS

Spermatogenic cells (spermatogonia, spermatocytes, spermatids) were arranged in layers according to their stage of development from the basement membrane towards the lumen (Figure 1). Differences in the number of Leydig cells can be seen in the interstitial between the seminiferous tubules (Figure 2).

Table 1 Number of spermatogenic and Leydig cells in gentamicin-induced rats (*Rattus norvegicus*), given oral administration of Moringa leaves extract

	spermatogonium	spermatosit	spermatid	Leydig
NCG	60.76 ± 8.35 ^{c}	133.44 ± 23.17 ^{c}	157.84 ± 32.99 ^{b}	11.08 ± 1.97 ^{d}
PCG	46.12 ± 6.41 ^{a}	73.76 ± 8.19 ^{a}	103.80 ± 32.99 ^{a}	5.24 ± 1.45 ^{a}
E200	46.00 ± 8.28 ^{a}	79.64 ± 16.00 ^{a}	90.68 ± 24.05 ^{a}	6.92 ± 1.25 ^{b}
E316	56.20 ± 7.89 ^{b}	107.44 ± 11.29 ^{b}	146.28 ± 46.62 ^{b}	9.52 ± 2.40 ^{c}
E500	66.84 ± 6.49 ^{d}	111.56 ± 40.88 ^{b}	145.04 ± 35.24 ^{b}	10.40 ± 2.32 ^{cd}

Different superscripts in the same column indicate a significant difference ($p < 0.05$) between treatments; NCG: negative control group, rats were not induced with gentamicin nor given Moringa leaves extract; PCG: positive control group, rats were injected with gentamicin without being given Moringa leaf extract; E200, E316, and E500: rats were injected with gentamicin and given Moringa leaf extract of 200, 316, 500 mg/kg bw/day respectively; gentamicin were injected subcutaneously at 5 mg/kg bw/day for 14 days; Moringa leaves extract was administered orally daily for the following 14 days

How to cite this article: Ngizzah N, Wurlina W, Hastutiek P, Hestianah EP, Mafruchati M, Hamid IS, Yustinasari LR. 2023. Effect of ethanolic extract of *Moringa oleifera* leaves on the number of spermatogenic cells and Leydig cells of Gentamicin-induced rats. Ovozoa: Journal of Animal Reproduction. 12: 99-106.

The number of spermatogonia cells, spermatocytes, and spermatids in NCG was higher ($p < 0.05$) than in PCG, E200, E316, and E500. The number of spermatogonia cells, spermatocytes, and spermatids in the E200 group was not significantly different ($p > 0.05$) from the PCG group. In the E316 group, the number of spermatogonia, spermatocytes, and spermatids was higher ($p < 0.05$) than in PCG. The number of spermatogonia in the E500 group was higher ($p < 0.05$) than in the E316 group. However, the number of spermatocytes and spermatids in the E316 group was not significantly different ($p > 0.05$) from that in the E500 group.

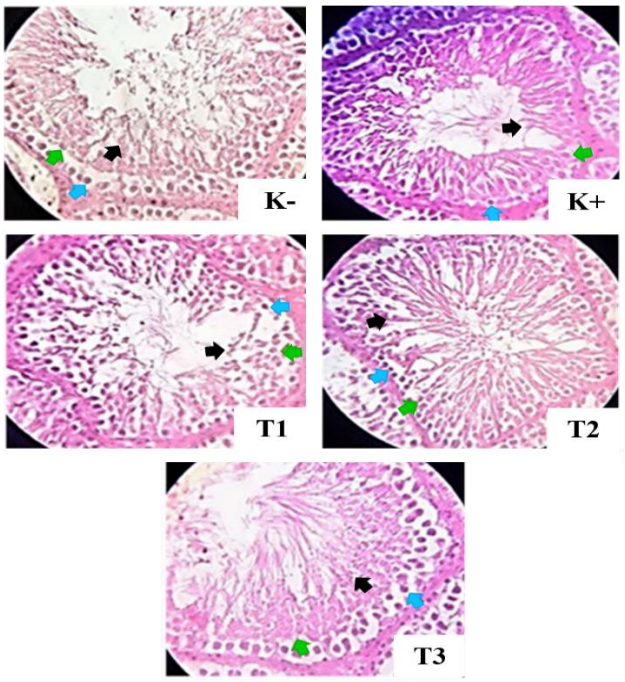

Figure 1 Microscopic view of the cross-section of the seminiferous tubules in the NCG, PCG, E200, E316, and E500 groups, with Hematoxylin-Eosin staining, observed using a light microscope (monocular microscope L-301) at 400x magnification; blue arrows indicate spermatogonia cells, green arrows indicate spermatocytes, and black arrows indicate spermatid cells.

Number of spermatogenic cells

The number of spermatogonia cells, spermatocytes, and spermatids in NCG was higher ($p < 0.05$) than in PCG, E200, E316, and E500. The number of spermatogonia cells, spermatocytes, and spermatids in the E200 group was not significantly different ($p > 0.05$) from the PCG group. In the E316 group, the number of spermatogonia, spermatocytes, and spermatids was higher ($p < 0.05$) than in PCG. The number of spermatogonia in the E500 group was higher ($p < 0.05$) than in the E316 group. However, the number of spermatocytes and spermatids in the E316 group was not significantly different ($p > 0.05$) from that in the E500 group.

Leydig cell count

The number of PCG Leydig cells was the least ($p < 0.05$) compared to the other groups. The administration of Moringa leaves extract with increasing doses in the E200 and E316 groups increased ($p < 0.05$) the number of Leydig cells compared to PCG. The number of Leydig PCG cells in the E500 group was not significantly different ($p > 0.05$) from that of the NCG.

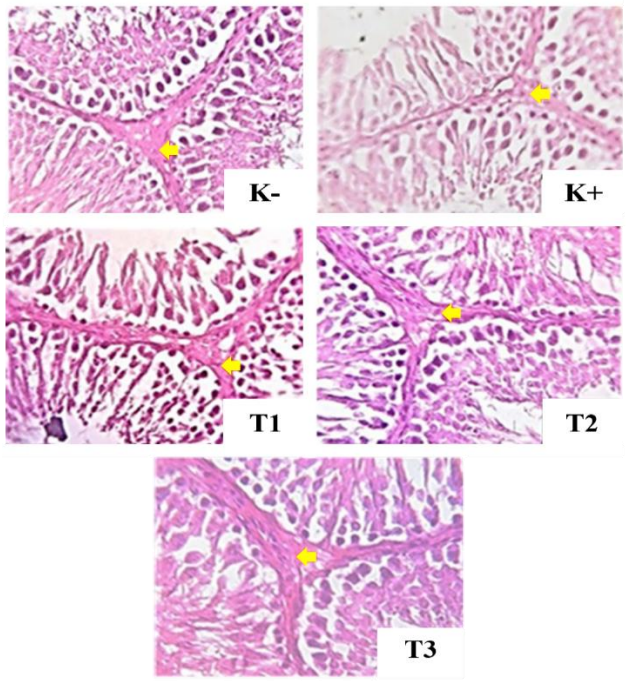

Figure 2 Microscopic view of the cross-section of the seminiferous tubules in the NCG, PCG, E200, E316, and E500 groups, with Hematoxylin-Eosin staining, observed using a light microscope (monocular microscope L-301) at 400x magnification. The red arrows indicate Leydig cells.

How to cite this article: Ngizzah N, Wurlina W, Hastutiek P, Hestianah EP, Mafruchati M, Hamid IS, Yustinasari LR. 2023. Effect of ethanolic extract of *Moringa oleifera* leaves on the number of spermatogenic cells and Leydig cells of Gentamicin-induced rats. Ovozoa: Journal of Animal Reproduction. 12: 99-106.

DISCUSSION

The results showed that the number of spermatogenic and Leydig cells in rats (*Rattus norvegicus*) given gentamicin was lower than in rats not given gentamicin. Injection of gentamicin at a dose of 5 mg/kg bw for 14 days has reduced the number of spermatogenic and Leydig cells. This is in line with a study conducted by Aly (2019), which found that gentamicin administration resulted in a decrease in the number of spermatogenic cells.

Gentamicin can trigger excessive oxidation reactions (Chaves and Tadi, 2023). Oxidative reactions produce unstable molecules that can damage cells and their organelles (Martemucci *et al*., 2022). These molecules are called free radicals, which can cause an imbalance between antioxidant enzymes and free radicals. The imbalance between free radicals and antioxidant enzymes can cause oxidative stress (Vona *et al*., 2021). The reaction between free radicals and cell membranes is followed by a cascade of apoptosis, decreasing the number of spermatogenic and Leydig cells (Asadi *et al*., 2017).

Gentamicin injected subcutaneously circulates systemically and causes lipid peroxidation in all cells, including cells in the hypothalamic-pituitary endocrine axis and testes. Spermatogenesis requires the complex action of various hormones (Aly, 2019). Follicle Stimulating Hormone (FSH) and Luitenizing Hormone (LH) are glycoprotein hormones secreted by the anterior pituitary which act directly on the testes in spermatogenesis. FSH receptors are present in Sertoli cells. FSH works synergistically with testosterone in spermatogenesis (Orlowski and Sarao, 2023). LH receptors are present in Leydig cells. LH stimulates the Leydig cells to produce testosterone (Shah *et al*., 2021). Gentamicin can inhibit the secretion of the testosterone, which plays a vital role in spermatogenesis (Nedresky and Singh, 2023). Testosterone is required for four roles during spermatogenesis, including maintaining the blood-testes barrier, meiosis, Sertoli-spermatid adhesion, and sperm release (Grande *et al*., 2022). The decrease in spermatogenic cells is caused by low levels of FSH, LH, and testosterone due to high oxidative stress. Low testosterone levels because gentamicin inhibits impulses in the anterior pituitary's LH secretion process, so Leydig cells cannot produce testosterone (Ajayi *et al*., 2020).

The ethanol extract of Moringa leaves (*Moringa oleifera*) ccould improve the number of spermatogenic and Leydig cells. The PCG group had fewer spermatogenic and Leydig cells than the E316 and E500 groups because the rats in PCG were not given any Moringa leaves extract after being induced by gentamicin. Gentamicin treatment without antioxidants induced testicular toxicity (Taha *et al*., 2022). The E200 group had spermatogenic cells that were not significantly different from the PCG group. This could be caused by insufficient antioxidants to scavenge the free radicals formed due to gentamicin induction. This situation resulted in a lack of ability of the testes to produce spermatogenic cells and Leydig cells after being induced by gentamicin. The E316 and E500 groups had significantly different results from the PCG group, which could show that the testes could produce spermatogenic cells and Leydig cells again after being induced by gentamicin. The average spermatocytes, spermatids, and Leydig cells in the E316 and E500 groups had similar results. This also happened in the study of Gunawati *et al*. (2019). The dose of 316 mg/kg bw/day is optimal because it can improve the number of spermatogenic cells with results that are not much different from a dose of 500 mg/kg bw/day. The number of spermatogonia cells, spermatocyte cells, and spermatid cells influence each other because spermatogonia cells are cells that can proliferate and eventually become sperm cells (Griswold, 2015).

Moringa leaves contain antioxidants, including vitamins C and E, and flavonoids. Vitamin C is an essential water-soluble micronutrient. Vitamin C, as an exogenous antioxidant, can reduce free radicals directly by donating electrons to form more stable molecules (Gopalakrishnan *et al*., 2016). This

How to cite this article: Ngizzah N, Wurlina W, Hastutiek P, Hestianah EP, Mafruchati M, Hamid IS, Yustinasari LR. 2023. Effect of ethanolic extract of *Moringa oleifera* leaves on the number of spermatogenic cells and Leydig cells of Gentamicin-induced rats. Ovozoa: Journal of Animal Reproduction. 12: 99-106.

can prevent the occurrence of chain oxidation reactions which can prevent the occurrence of lipid peroxidation and damage to DNA (Sikder *et al*., 2013). Vitamin C can protect germ cells from oxidative stress. Vitamin C can pass through Sertoli cells via passive or facilitated diffusion (Sun *et al*., 2019). Vitamin E is a fat-soluble micronutrient. Vitamin E, as an exogenous antioxidant, can work synergistically with vitamin C in stabilizing formed free radicals (Hasanin *et al*., 2018).

Flavonoids belong to a class of secondary metabolites produced by a group of plant polyphenols (Roy *et al*., 2022). Flavonoids have an excellent effect on spermatogenesis, marked by increased sperm count, viability, and motility compared to the group not given flavonoids and a decrease in malonaldehyde levels (Ye *et al*., 2020). Flavonoids prevent damage to cells due to free radicals in various ways, first, by reducing free radicals directly. Flavonoids are oxidized by free radicals to produce molecules that are more stable and less reactive. Second, flavonoids can inhibit enzymes producing free radicals, including xanthine oxidase, cyclo-oxygenase, lipoxygenase, and phosphoinositide 3-kinase. Third, flavonoids can increase antioxidant activity endogenously by helping the work of antioxidant enzymes, one of which is superoxide dismutase (Panche *et al*., 2016).

Moringa leaf extract scavenged free radicals because it contains flavonoids, vitamins C and E (Hegazi and Elebshany, 2019), which affect the increase in the activity of the hypothalamus release GnRH to stimulate the pituitary gland to release FSH and LH levels (Mohlala *et al*., 2023). The increase in LH levels affects the number of Leydig cells, followed by higher production of testosterone (Zirkin *et al*., 2018). Testosterone facilitated Sertoli and germ cells' attachment (Wang *et al*., 2022). FSH, LH, and testosterone can stimulate the growth and secretory function of the male reproductive organs. Therefore, an increase in these hormones causes an increase in the number of spermatogenic cells (Oduwole *et al*., 2021).

This study proves that gentamicin adversely affects spermatogenic cells and Leydig cells. Therefore, in using gentamicin we must be more careful. Moringa leaf extract was proven to overcome the adverse effects of gentamicin on spermatogenic cells and Leydig cells. It is necessary to conduct a future study on using Moringa leaf extract on other aminoglycoside antibiotics.

CONCLUSION

Based on this study, the ethanolic extract of *Moringa oleifera* leaves improved the spermatogenic cell count of rats (*Rattus norvegicus*) induced by gentamicin.

ACKNOWLEDGEMENT

The authors thank Pak Pardi for technical support.

AUTHOR'S CONTRIBUTIONS

Nailul Ngizzah (NN), Wurlina Wurlina (WW), Poedji Hastutiek (PH), Eka Pramyrtha Hestianah (EPH), Maslichah Mafruchati (MM), Iwan Sahrial Hamid (ISH), Lita Rakhma Yustinasari (LRY).

NN, WW, PH: compiled ideas, designed the framework, acquisition, analysis, and interpretation of data, and manuscript drafting. EPH, MM, ISH, and LYS: critically read and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors have no competing interests in this study with other parties.

FUNDING INFORMATION

This study was fully funded by the authors.

REFERENCES

Ajayi AF, Akhigbe RE. 2020. The physiology of male reproduction: Impact of drugs and their abuse on male fertility. Andrologia 52: e13672.

How to cite this article: Ngizzah N, Wurlina W, Hastutiek P, Hestianah EP, Mafruchati M, Hamid IS, Yustinasari LR. 2023. Effect of ethanolic extract of *Moringa oleifera* leaves on the number of spermatogenic cells and Leydig cells of Gentamicin-induced rats. Ovozoa: Journal of Animal Reproduction. 12: 99-106.

Aladamat N, Tadi P. 2022. Histology, Leydig Cells. In: StatPearls [Internet]. StatPearls Publishing; USA. Updated 2022 Nov 14.

Aly H. 2019. Testicular toxicity of gentamicin in adult rats: Ameliorative effect of lycopene. Hum Exp Toxicol. 38: 1302-13.

Asadi N, Bahmani M, Kheradmand A, Rafieian-Kopaei M. T2017. The impact of oxidative stress on testicular function and the role of antioxidants in improving it: A review. J Clin Diagn Res. 11: IE01-IE05.

Chaves BJ, Tadi P. 2023. Gentamicin. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. USA. Updated 2023 Apr 10].

Gamaan MA, Zaky HS, Ahmed HI. 2023. Gentamicin-induced nephrotoxicity: A mechanistic approach. Azhar Int J Pharm Med Sci. 3: 11-9.

Gopalakrishnan L, Doriya K, Kumar DS. 2016. *Moringa oleifera*: A review on nutritive importance and its medicinal application. Food science and human wellness. 5: 49-56.

Grande G, Barrachina F, Soler-Ventura A, Jodar M, Mancini F, Marana R, Chiloiro S, Pontecorvi A, Oliva R, Milardi D. 2022. The role of testosterone in spermatogenesis: lessons from proteome profiling of human spermatozoa in testosterone deficiency. Front Endocrinol. 13: 852661.

Griswold MD. 2015. Spermatogenesis: The commitment to meiosis. Physiol Rev. 96: 1-17.

Gunawati LS, Berata IK, Setiasih NLE. 2019. Histopathological structure of Wistar rats testicle with excessive physical activities that given kelor leaf extract. Indon Med Vet. 8: 637-46.

Hasanin NA, Sayed NM, Ghoneim FM, Al-Sherief SA. 2018. Histological and ultrastructure study of testes of acrylamide exposed adult male albino rat and evaluation of the posible effect of vitamin E intake. J Mic Ultras. 6: 23-34.

Hegazi MAM, Elebshany IAK. 2019. Ameliorative effect of *Moringa oleifera* on oxidative stress in male albino rat brain promotes by aluminium exposure. Nat Sci. 17: 92-100.

Krause KM, Serio AW, Kane TR, Connolly LE. 2016. Aminoglycosides: An overview. Cold Spring Harb Perspect Med. 6: a027029.

Mardatillah M, Wurlina W, Yudaniayanti IS, Plumeriastuti H, Primarizky H, Hamid IS. 2022. *Moringa oleifera*leaf extract restored the diameter and thickness of the seminiferous tubules of rat (*Rattus norvegicus*) injected with gentamicin. Ovozoa : J Anim Reprod. 11: 15-21.

Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG. 2022. Free radical properties, source and targets, antioxidant consumption and health. Oxygen 2: 48-78.

Mohlala K, Offor U, Monageng E, Takalani NB, Opuwari CS. 2023. Overview of the effects of *Moringa oleifera* leaf extract on oxidative stress and male infertility: A review. Appl Sci. 13: 4387.

Nedresky D, Singh G. 2023. Physiology, Luteinizing Hormone. In: StatPearls [Internet]. StatPearls Publishing. USA. Updated 2022 Sep 26.

Nishimura H, L'Hernault SW. 2017. Spermatogenesis. Curr Biol. 27: R988-R994.

Oduwole OO, Huhtaniemi IT, Misrahi M. 2021. The roles of Luteinizing Hormone, Follicle-Stimulating Hormone and Testosterone in spermatogenesis and folliculogenesis Revisited. Int J Mol Sci. 22: 12735.

Ogunsola OA, Joshua O, Sunday FO, Nwobi NL, Falugi B, Akinbola AS. 2017. Moringa plants parts consumption had effects on reproductive functions in male and female rat models. J Dent Med Sci. 16: 82-6.

Orlowski M, Sarao MS. 2023. Physiology, Follicle Stimulating Hormone. In: StatPearls [Internet]. StatPearls Publishing. USA. Updated 2023 May 1.

Panche AN, Diwan AD, Chandra SR. 2016. Flavonoids: an overview. J Nut Sci. 5: 1-15.

Peñalver R, Martínez-Zamora L, Lorenzo JM, Ros G, Nieto G. 2022. Nutritional and antioxidant properties of *Moringa*

How to cite this article: Ngizzah N, Wurlina W, Hastutiek P, Hestianah EP, Mafruchati M, Hamid IS, Yustinasari LR. 2023. Effect of ethanolic extract of *Moringa oleifera* leaves on the number of spermatogenic cells and Leydig cells of Gentamicin-induced rats. Ovozoa: Journal of Animal Reproduction. 12: 99-106.

oleifera leaves in functional foods. Foods 11: 1107.

Ross JDC, Brittain C, Cole M, Dewsnap C, Harding J, Hepburn T, Jackson L, Keogh M, Lawrence T, Montgomery AA, Roberts TE, Sprange K, Tan W, Thandi S, White J, Wilson J, Duley L. 2019. G-ToG trial team. Gentamicin compared with ceftriaxone for the treatment of gonorrhoea (G-ToG): a randomised non-inferiority trial. Lancet 393: 2511-20.

Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, Alshahrani MY, Islam S, Islam MR. 2022. Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. Biomed Res Int. 2022: 5445291.

Shah W, Khan R, Shah B, Khan A, Dil S, Liu W, Wen J, Jiang X. 2021. The molecular mechanism of sex hormones on Sertoli cell development and proliferation. Front Endocrinol. 12: 648141.

Sikder K, Sinha M, Das N, KR Das DKR, Datta S, Dey S. 2013. *Moringa oleifera* leaf extract prevents in vitro oxidative DNA damage. Asian J Pharm Clin Res. 6: 159-63.

Sun J, Yin B, Tang S, Zhang X, Xu J, Bao E. 2019. Vitamin C mitigates heat damage by reducing oxidative stress, inducing HSP expression in TM4 Sertoli cells. Mol Reprod Dev. 86: 673-85.

Taha M, Elazab ST, Saati AA, Ahmed GS, Baokbah TAS, Fathy K, El-Shenbaby I, Abdelbagi O, Hassan MAE, Ibrahim MM, Badawy AM. 2022. Zamzam water ameliorates gentamicin-induced testicular toxicity in a rat model via targeting sperm parameters, testicular tissue oxidative insult, inflammation, apoptosis, and pituitary-gonadal axis. Toxics 11: 2.

Vergara-Jimenez M, Almatrafi MM, Fernandez ML. 2017. Bioactive components in *Moringa oleifera* leaves protect against chronic disease. antioxidants. 6:91.

Vona R, Pallotta L, Cappelletti M, Severi C, Matarrese P. 2021. The impact of oxidative stress in human pathology: Focus on gastrointestinal disorders. Antioxidants 10: 201.

Wang JM, Li ZF, Yang WX. 2022. What does androgen receptor signaling pathway in Sertoli cells during normal spermatogenesis tell us? Front Endocrinol (Lausanne) 13: 838858.

Ye RJ, Yang JM, Hai DM, Liu N, Ma L, Lan XB, Niu JG, Zheng P, Yu JQ. 2020. Interplay between male reproductive system dysfunction and the therapeutic effect of flavonoids. Fitoterapia. 147: 104756.

Zanaria R, Kamaluddin MT, Theodorus T. 2017. Efektivitas ekstrak etanol daun salam (*Eugenia polyantha*) terhadap GLTU di jaringan adiposa dan kadar gula darah puasa pada tikus putih jantan. J Biomed Fak Ked Univ Sriwijaya. 3: 145-52.

Zirkin BR, Papadopoulos V. 2018. Leydig cells: formation, function, and regulation. Biol Reprod. 99: 101-11.

How to cite this article: Ngizzah N, Wurlina W, Hastutiek P, Hestianah EP, Mafruchati M, Hamid IS, Yustinasari LR. 2023. Effect of ethanolic extract of *Moringa oleifera* leaves on the number of spermatogenic cells and Leydig cells of Gentamicin-induced rats. Ovozoa: Journal of Animal Reproduction. 12: 99-106.

