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The Effect of Tamarilo Katarrung (*Chypomandra betaceae*) Extract Changes on Histology Description of The Testis of White Rats (*Rattus norvegicus L*) Wistar Strains Induced By Gentamicin

Nur Alif Bahmid[a]*

[a]Veterinary Medicine Study Program, Faculty of Medicine, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10 Makassar 90245

*corresponding author: aliefbahmid02@gmail.com

Abstract

Gentamicin is an antibiotic from the aminoglycoside group that works by inhibiting protein synthesis but has side effects such as nephrotoxicity and ototoxicity and can induce oxidative stress in the testes. Oxidative stress is when the number of free radicals increases or the amount of antioxidants decreases. Oxidative stress induction in the testes has a degenerative effect on the testes, so the normal function and structure are disturbed. Dutch eggplant is one of the natural antioxidants that have the potential to protect cells from damage caused by oxidative stress. The utilization of this fruit extract has been proven to have secondary metabolic components in the form of phenols, flavonoids, anthocyanins, and carotenoids, which are antioxidants. This study aimed to examine the effect of tamarillo katarrung extract on the histology of the testes of gentamicin-induced Wistar rats. The research was conducted at the Integrated Laboratory of Animal Clinic Education, Hasanuddin University, Makassar. The sample used in this study amounted to 20 white rats of the Wistar strain, which were divided into four groups. The negative control group (K-) was untreated rats, and the positive control group (K+) was given gentamicin 20mg/kg BW induction for 11 days. The first treatment group (P1) was given tamarillo katarrung extract 200mg/kg BW for 14 days and gentamicin 20mg/kg BW for 11 days, while the second treatment (P2) was given tamarillo katarrung extract 200mg/kg BW for 14 days and gentamicin 20mg/kg BW for 11 days. The results showed a significant difference ($p<0.05$) between groups K+ and K-, P1 and P2. The histological description of the rat testes with the Johnson score shows that the tamarillo katarrung extract can defend testicular tissue from damage due to oxidative stress with the most optimal dose of 200 mg/kg BW

Keywords: Antioxidant, gentamicin, rat, tamarillo katarrung

Introduction

Tamarillo Katarrung or commonly called the Dutch eggplant belongs to the family Solanaceae, genus Chyphomandra and species Chypomandra betaceae (Cavaniles). Sent is a leading horticultural commodity in North Toraja Regency, South Sulawesi (Djufry *et al.,* 2016). Dutch eggplant extract as an antioxidant has been known in previous studies that its flavonoid content can become a radical scavenger, overcome cell membrane damage, and bind reactive electrons in it so that oxidative chains can be prevented. The effect of the pro-oxidant activity or free radicals can be eliminated by the Dutch eggplant antioxidants so that the contents of the testes (spermatogenic cells) are not affected by these free radicals (Khaerunnisa *et al.,* 2019).

Antioxidants are compounds that are needed by the body to prevent oxidative stress. The imbalance between the number of free radicals and antioxidants is known as oxidative stress. Free radicals are compounds that contain one or more unpaired electrons in their orbitals so that they can oxidize surrounding molecules due to their very reactive nature (Werdhasari, 2014). Antioxidants work as inhibitors against free radicals by reacting and forming stable molecules with free radicals to protect body cells from their harmful effects (Khaira, 2010).

Gentamicin is an antibiotic from the aminoglycoside group that inhibits protein synthesis. Gentamicin has side effects such as nephrotoxicity and ototoxicity and can induce oxidative stress in the testes (Soraya *et al.,* 2020). Oxidative stress is when the number of free radicals increases or the amount of antioxidants decreases. The presence of oxidative stress induction in the testes has a degenerative effect on the testes, so the normal function and structure are disturbed (Elsawah *et al.,* 2020). The testes are organs that carry out spermatogenesis, or the formation of spermatozoa, and regulate reproductive hormones in male animals. In the testes, high saturated fatty acid content and antioxidant reserves can be found in small quantities so that the testes are susceptible to free radical attack (Malini *et al.,* 2020).

This study aimed to determine the effect of Tamarilo Katarrung (Chypomandra betaceae) extract on changes in the histology of the testes of white rats (Rattus Norvegicus L) Wistar strain induced by gentamicin and assessed using the Johnsen Score criteria.

Meterials and Methods

Types of Research

This study used an experimental research design with Post Test Only a Control Group Design to know the effect of Dutch eggplant extract on the histological picture of the rat testes induced by gentamicin. The total samples were 20 Wistar rats with a weight ranging from 150-250 gr. The research was conducted at the Integrated Laboratory of the Veterinary Medicine Study Program, Faculty of Medicine, Hasanuddin University.

Making Tamarilo Katarrung Extract

Tamarillo Katarrung fruit (Chypomandra betaceae) was washed, cut into pieces, and dried using an herbs dryer. Dutch eggplant that has been dried and then blended until it becomes a fine powder. This powder is soaked with 70% ethanol for 3 x 24 hours. The filtrate from the immersion was then collected and evaporated using a rotary vacuum

evaporator to obtain a pure extract from the tamarillo katarrung fruit (Masbintoro *et al.,* 2017).

Administration of Extracts, Treatment, and Examination

Rats were first acclimatized for seven days to adjust to the new environment. Experimental animals were divided into four groups, namely the positive control group (K+), the negative control group (K-), and two treatment groups (P1 and P2). Groups P1 and P2 were each given 200 mg/kg BW and 300 mg/kg BW of Dutch eggplant extract for 14 days. On day 3, all groups were induced with gentamicin intraperitoneally as much as 20mg/kg BW at 2 hours after treatment, except the negative control group. Necropsy was performed on the 15th day, starting from the euthanasia process by injecting high doses of ketamine and xylazine. The testicular organs were then taken, and histological preparations were made with Hematoxylin-Eosin staining. Statistical analysis was performed using One-way ANOVA to determine the optimal dose, followed by LSD post-hoc test with a 95% confidence level (Riris *et al.,* 2020). Observation and assessment of testicular histology using the Johnsen-score criteria with a score of 1-10 can be seen in Table 1.

Table. Johnsen score criteria (Adelati et al., 2016).

Score	Score
10	Normal tubular epithelium, complete spermatogenesis, open tubular lumen, spermatozoa cells ≥ 10
9	Damaged tubular epithelium, closed tubular lumen, spermatozoa cells ≥ 10
8	spermatozoa cells < 10
7	Spermatozoa cells 0, spermatid cells ≥ 10
6	Spermatozoa cells 0, spermatid cells < 10
5	Spermatozoa cells and spermatid cells 0, spermatocyte cells ≥ 5
4	Spermatozoa and spermatid cells 0, spermatocytes < 5
3	Spermatogenic cells consist only of spermatogonia cells
2	Spermatogenic cells 0, only Sertoli cells
1	No cells at all in the tubules

Results and Discussion

The results showed a change in histology in the group given gentamicin. The increase in the Johnsen score in all groups receiving tamarillo katarrung extract is presented in Table 2. Table 2 shows a change in the histology of the Wistar rat testes in the control and treatment groups. The P2 group treated with tamarillo katarrung 200mg/kg and gentamicin induction had the highest score with a Johnsen score of 7.6 1.14 than the K-, K+, and P2 groups. The lowest value was shown by the K+ group in the treatment only induced by gentamicin, with a Johnsen score of 5.6 1.8. This indicates that the stages of administering gentamicin (K+) were impaired compared to the K-, P1, and P2 groups.

Data analysis using a one-way ANOVA test showed a significant difference ($p<0.05$)

between groups. The dose differences were determined in each group by post-hoc LSD test. The results of the post-hoc LSD test showed that the K+ group was significantly different ($p<0.05$) from the K-, P1, and P2 groups. Observation of the histology of the rat testes shows the tabulation of the Johnsen score for each group of rats as follows:

Table 2. Analysis of the histology of the rat testes

Group	Number of samples	Result
Negative control (K-)	5	7.0 ± 1.22^{a}
Positive control (K+)	5	5.6 ± 1.82^{b}
Treatment 1 (P1)	5	7.6 ± 1.14^{a}
Treatment 2 (P2)	5	7.2 ± 1.30^{a}

Superscript $^{a, b}$ in the column, shows a significant difference $p<0.05$

Based on the results of histology preparations for each group that has been observed at 400x magnification, it can be seen that there are differences between the control group and the treatment group, which is presented in Figure 1.

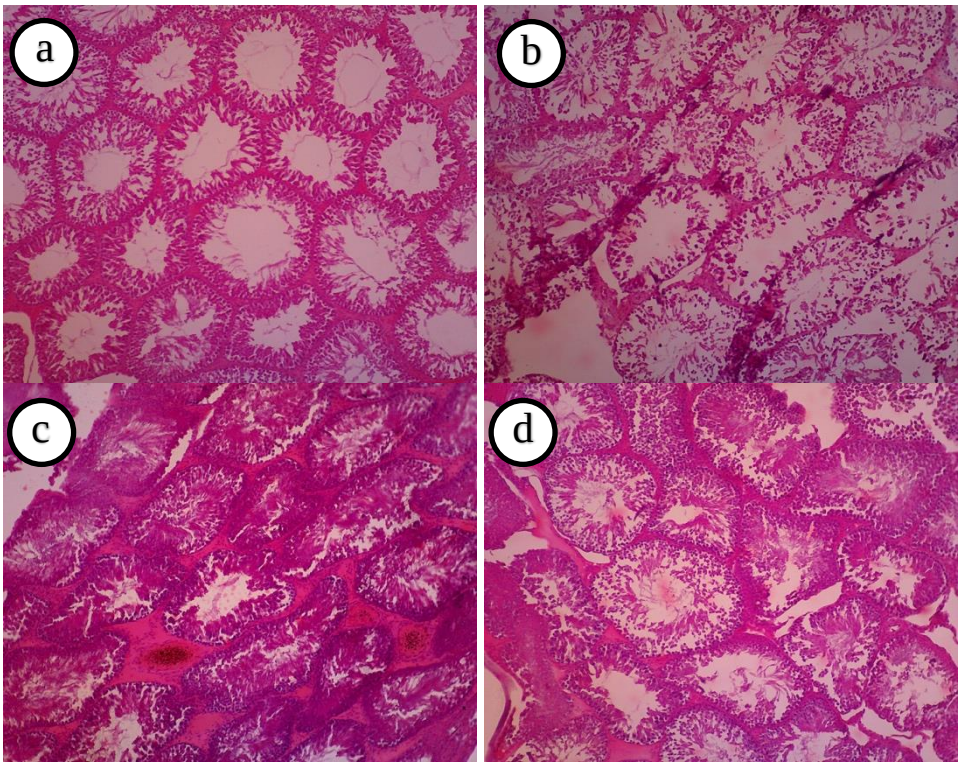

Figure 1. Histological preparations of testicular tissue (a) K- (b) K+ (c) P1 (d) P2

The results of histology preparations, as shown in Figure 1, showed that the K+ group with only gentamicin administration saw quite a lot of germ cell damage with wider and irregular cavities in the seminiferous tubules. Groups P1 and P2, with the administration of tamarillo katarrung and gentamicin extracts, showed that the germ cells in the seminiferous tubules were denser and more regular. They resembled the K- group without treatment. This follows research conducted by Riris *et al.* (2020) that the seminiferous tubules in the testes of mice that received Solanum betaceum extract had a neater and denser shape and an increase in Leydig cells.

Induction of gentamicin with 20mg/kg BW for 11 days in the K+ group caused oxidative stress conditions, disrupting spermatogenesis. Excessive ROS (Reactive Oxygen Species) under conditions of oxidative stress can damage testicular tissue and the membranes of spermatozoa cells and inhibit the secretion of LH (Luteinizing Hormone) and lead to reduced testosterone production by Leydig cells (Ikhtiar, 2019). Testosterone is an important hormone that plays a role in keeping germ cells in the seminiferous tubule epithelium and preventing apoptosis. With reduced testosterone production, testicular organs can be damaged, such as spermatogenic cells, released from the seminiferous tubules. These spermatogenic cells do not arrange until debris from cells is released and accumulates in the central area of the lumen. In a study conducted by Elsawah *et al.* (2020) using a therapeutic dose of gentamicin in rats of 18.25 mg/kg BW and a double dose of 36.5 mg/kg BW once a day for 14 days showed various degenerative disorders of the seminar tubular epithelium accompanied by incomplete spermatogenesis and the absence of spermatozoa in the degenerated seminiferous tubule lumen.

The treatment group showed a higher Johnsen score than the control group. This is because the administration of tamarillo katarrung extract contains several compounds known to be antioxidants. They can have a positive impact as an antidote to free radicals and can maintain testicular tissue. The results of research by Hasan dan Bakar (2013) stated that Solanum betaceum fruit contains phenols, flavonoids, anthocyanins, and carotenoids, which have antioxidant activity. Antioxidants are chemical compounds that can donate one or more electrons to free radicals so that these free radicals can be quenched (Al-omary *et al.,* 2018). Antioxidants react with free radicals by reducing the oxygen concentration, preventing the formation of reactive singlet oxygen, preventing the initiation of the first chain and capturing primary radicals, breaking the hydroperoxide chain, and decomposing the primary radical products into non-radical compounds (Astuti, 2014).

Giving tamarillo katarrung extract at a dose of 300 mg/kg BW showed a decrease in the Johnsen score compared to 200 mg/kg BW. This shows that higher doses do not guarantee better testicular tissue. Various antioxidant doses also show an inverse relationship between antioxidant activity and concentration. (Hasan dan Bakar, 2013) The antioxidant activity decreases the higher the antioxidant concentration. This is because small antioxidant activity results in denser molecules, so the electrons from these antioxidants cannot react with free radicals. The oxidation rate can be affected by the increased concentration of antioxidants. Other studies also mention that giving high concentrations of antioxidants can be pro-oxidants. (Orororo *et al.,* 2018)

Antioxidants become effective as a protective measure when given at the right target, time, and duration. It is also stated that some evidence finds that antioxidant mechanisms in balancing free radicals are influenced by appropriate antioxidant supplementation. Administration of antioxidants can interfere with cellular communication and increase the risk of disease when antioxidant levels in the body are too high, and ROS in the body are too low. Under certain conditions, such as exposure to lead acetate, it can increase ROS in the body, thus requiring additional exogenous antioxidants. Exogenous antioxidants' role is to support endogenous antioxidants' role in preventing oxidative stress so that cell resistance will be well maintained. Giving Dutch eggplant extract can balance the levels of ROS and antioxidants because of the high anthocyanin content. This shows that tamarillo katarrung extract acts as an exogenous antioxidant, explicitly playing a role in breaking free radical chains to maintain testicular tissue protected by antioxidant mechanisms in tamarillo katarrung extract (Riris *et al.,* 2020).

Conclusion

This study concludes that the extract of tamarillo katarrung (Chypomandra betaceae) has the potential as a protective agent by preventing germ cell damage in rat testicular tissue due to oxidative stress induced by high doses of gentamicin. This study showed changes in the histology of the testes marked by differences in the Jhonsen score in each group. The group with the best testicular histology in sequence were P1, P2, K-, and K+. The effective dose of tamarillo katarrung extract is 200mg/kg BW.

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