# Effect of vitamin C on testosterone level

*by* Elly Usman3

**Submission date:** 18-Jan-2022 04:22PM (UTC+0800)
**Submission ID:** 1743422369
**File name:** count_and_sperm_morphology_in_gentamicin-induced_Wistar_rats.pdf (205.93K)
**Word count:** 4186
**Character count:** 21399

International Journal of Research in Medical Sciences
*Rahayu I et al. Int J Res Med Sci. 2019 Feb;7(2):451-456*
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20190352

**Original Research Article**

# Effect of vitamin C on testosterone level, sperm count and sperm morphology in gentamicin-induced Wistar rats

**Iis Rahayu[1]*, Elly Usman[2], Mohamad Reza[3]**

[1]Master Program, Graduate School of Biomedical Sciences, Faculty of Medicine, Andalas University, Padang, West Sumatera, Indonesia
[2]Department of Pharmacology, [3]Department of Biology, Faculty of Medicine, Andalas University, Padang, West Sumatera, Indonesia

**Received:** 04 December 2018
**Accepted:** 12 January 2019

***Correspondence:**
Dr. Iis Rahayu,
E-mail: iisrahayu115@gmail.com



## ABSTRACT

**Background:** Gentamicin is an aminoglycoside-class antibiotic that can lead to an increase in ROS and decrease antioxidant reserves that cause destruction of testicular cells that are known to inhibit cell division in testicular germ cells and protein synthesis in the testes as well as affect the production of testosterone that affects the quality of spermatogenesis. Vitamin C as an antioxidant can counteract ROS so that testicular cells can be repaired.
**Methods:** This study was an experimental research with post tests only control group design on 25 male rats, 2-3 aged months, weight 160-240 g. Animals were divided into 5 groups. Negative control group was placed in cage without treatment, positive control given induced gentamicin 5mg/kgbb for the first 10 days, and 3 treatment given induced gentamicin 5mg/kgbb for the first 10 days and at days 11-51 were given vitamin C at a dose of 1.6mg; 3.25mg; 4.5mg. On 52 day, all of wistar rats perform terminated for analyzed the testosterone levels were by ELISA, sperm count was calculated with then haemocytometer observed with digital microscope, morphology of spermatozoa was analyzed by eosin staining and was observed with digital microscope.
**Results:** Results of this study showed that the average of testosteron serum on control negatif group of 3.82ng/ml, control positif 3.73ng/ml, treatment 1 is 9.52ng/ml, treatment 2 is 8.29ng/ml, and traetment 3 is 2.28ng/ml. Sperm count on control negatif 41.68 million/ml, control positif 21.06million/ml, treatment 1 is 52.92 million/ml, treatment 2 is 57.12million/ml, treatment 3 is 80.88 million/ml and sperm morphology on control negative 74.00%, control positif 46.96%, treatment 1 is 58.82%, treatment 2 is 68.68%, and treatment 3 is 78.72%.
**Conclusions:** It is concluded that vitamin C has significant effect on increasing testosteron level and sperm quality in gentamicin-induced wistar rats.

**Keywords:** Gentamicin, Spermatozoa, Testosteron, Vitamin C

## INTRODUCTION

Infertility is a reproductive problem in which there is no pregnancy after 12 months or more in couples who have regular and adequate sexual intercourse without contraception.[1] According to data from WHO there are about 50-80 million couples have infertility in the world. In 2010 infertility prevalence data showed that as many as 48.5 million couples around the world have infertility, especially in developing countries. The highest rates are in Asia and Africa, 4.2%, Nepal 9.1% and South African Republic 6.9%, and male factor causing 50% of infertility.[2]

The underlying causes of male infertility are grouped into 3 groups: pre testicular factors, testicular factors (chromosomal abnormalities, varicocles, radiation, drugs, and systemic diseases), and post testicular factors which

is abnormalities in the reproductive tract including epididymis, vas deferens and ejaculatory duct (coitus disorder and impaired sperm function or motility). In the testicular factors, it is explained that the use of drugs to be one of the triggers of the occurrence of abnormalities or causes of infertility, among which are gentamycin antibiotics.[3,4]

The effect of aminoglycosides is to inhibit protein synthesis and cause misreading in translation of mRNA.[5] Gentamycin has side effects that can lead to increased oxidative stress status by forming free radicals and lipid peroxidation, as well as lowering antioxidant reserves.[6] This causes structural and cytotoxic changes in the testis that may affect the sperm count, mortality and morphology of spermatozoa.[7]

The number of gentamycin use in Padang city can be seen in the research conducted in 2013 about the evaluation of antibiotic usage at a Public Hospital in Padang city. It is known that there is an inaccurate dose of gentamycin as much as 16.66%, and inaccuracy of gentamycin administration duration as much as 23%, this indicates an irrationality of the use of antibiotic drugs.[8]

To reduce the status of oxidative stress, antioxidants is needed. Enzymatic antioxidants consist of superoxide dismutase (SOD), catalase, glutathione peroxide. While non-enzymatic antioxidants consist of vitamin C, vitamin E and carotenoid.[9]

Vitamin C is one of the vitamins that play an important role in the body such as helping the work of certain enzymes or as enzymatic precursors, protecting the food substances from the oxidant process, helping the absorption of food in the intestine, protecting the blood portion that is sensitive to oxidants and protecting vitamin E. Vitamin C is an primary water-soluble antioxidant in blood plasma and cytosol. Vitamin C captures singlet oxygen and reacts quickly with hydroxyl, superoxide, and hydrogen peroxide radicals.[10]

## METHODS

This study was an experimental research with post tests only control group design with an experiment approach. This study on 25 male rats, 2-3 aged months, weight 160-240g. Animals were divided into 5 groups.

- Group 1: Negative control (NC), was placed in a cage without treatment.
- Group 2: Positive control (PC), given induced gentamicin 5mg/kgbb for the first 10 days.
- Group 3: Treatment 1 (T1), given induced gentamicin 5mg/kgbb for the first 10 days and at days 11-51 were given vitamin C at a dose of 1.6mg;
- Group 4: Treatment 2 (T2), given induced gentamicin 5mg/kgbb for the first 10 days and at days 11-51 were given vitamin C at a dose of 2.25mg;
- Group 5: Treatment 3 (T3), given induced gentamicin 5mg/kgbb for the first 10 days and at days 11-51 were given vitamin C at a dose of 4.5mg.

On 52 day, all of wistar rats perform terminated for analyzed the testosterone levels were by Enzyme-Linked Immunosorbent Assay (ELISA) method, measuring instrument with spectrophometer, with the results of measuring in ng/ml and interval scale. sperm count was calculated with then haemocytometer observed with digital microscope and a measuring device with a measurement result of million/ milliliters and ratio scale, morphology of spermatozoa was analyzed by eosin staining and was observed with digital microscope and a counter with the percentage and ratio scale results.

The inclusion criteria in this study were male rats, 2-3 aged months rats, weight 160-240 gram, and exclusion criteria is rats died during the study, rats sick during the study. The data is obtained by normality test with Shapiro-Wilk test then processed with one-way ANOVA parametric test with significance level $p<0.05$.

## RESULTS

Data on testosterone levels, amount and morphology of spermatozoa obtained were tested statistically to assess normality using Shapiro-Wilk test. Meanwhile, to see the effect of Vitamin C on testosterone levels, sperm count and morphology of male rats administered by vitamin C was done by one-way ANOVA test.

**Table 1: Average testosterone levels among groups after treatment.**

| Subject groups | Average testosterone levels (ng/ml) | | |
|---|---|---|---|
| | N | ±SD | P |
| Negative control | 5 | 3.82±4.77 | |
| Positive control | 5 | 3.73±5.01 | 0.019 |
| Treatment 1 | 5 | 9.52±6.47 | |
| Treatment 2 | 5 | 8.29±3.71 | |
| Treatment 3 | 5 | 2.28±3.53 | |

Table 1 shows that the highest testosterone levels were found in the first treatment group (T1) of 9.52±6.47ng/ml while the lowest levels were found in the third treatment group (T3) of 2.28±3.53ng/ml with p value <0.05. It can be concluded that there is difference of average serum testosterone level among five groups. This shows that the administration of gentamycin lowers serum testosterone levels and vitamin C can maintain testicular hormone serum levels in rat. Result of data analysis with one way anova statistical test showed p value = 0.019 ($p<0.05$) where it can be concluded that there is effect of vitamin C administration to rat testosterone levels.

**Table 2: Average sperm count after treatment.**

| Subject groups | Average sperm count (million/ml) | | |
|---|---|---|---|
| | N | ±SD | N |
| Negative control | 5 | 41.68±5.36 | |
| Positive control | 5 | 21.06±5.16 | 0.000 |
| Treatment 1 | 5 | 52.92±9.27 | |
| Treatment 2 | 5 | 57.12±3.67 | |
| Treatment 3 | 5 | 80.88±43.57 | |

Table 2 shows that the highest average sperm count were found in the third treatment groups (T3), 80.88±43.57million/ml, while the lowest were in the positive control group (PC), 21.06±5.16million/ml and p <0.05. It can be concluded that there is difference of average sperm count among five groups. This suggests that administration of gentamycin decreases the rat sperm count and vitamin C can maintain the sperm count. The results of data analysis with one-way ANOVA statistical test showed p value = 0.000 (p≤0.05). It can be concluded that there is effect of vitamin C administration to induced-gentamycin rat sperm count.

**Table 3: Average percent of normal morphology of spermatozoa in each group after treatment.**

| Subject groups | Average spermatozoa (%) | | |
|---|---|---|---|
| | N | Morphology ±SD | P |
| Negative control | 5 | 74.00±12.71 | |
| Positive control | 5 | 46.96±8.28 | 0.005 |
| Treatment 1 | 5 | 58.82±19.11 | |
| Treatment 2 | 5 | 68.68±10.92 | |
| Treatment 3 | 5 | 78.72±7.09 | |

Table 3 shows the highest average percentage of normal morphology spermatozoa in the third treatment group (T3), which is 78.72±7.09% while the lowest average was found in the positive control group (PC), 46.96±8.28%. The results of one way ANOVA statistical test obtained p value = 0.005 (p≤0.05) so it can be concluded, there is effect of vitamin C administration to percentage of normal morphology of spermatozoa in gentamycin-induced rats.

**Table 4: Bonferroni multiple comparison post Hoc test in serum testosterone levels.**

| Groups | Serum testosterone levels degree of significance (p) | | | | |
|---|---|---|---|---|---|
| | NC | PC | T1 | T2 | T3 |
| NC | - | 0.767 | 0.068 | 0.069 | 0.284 |
| PC | 0.767 | - | 0.038* | 0.038* | 0.433 |
| T1 | 0.068 | 0.038* | - | 0.997 | 0.007* |
| T2 | 0.069 | 0.038* | 0.997 | - | 0.007* |
| T3 | 0.284 | 0.433 | 0.007* | 0.007* | - |

Based on the post Hoc Bonferoni test results in Table 4, it can be concluded that not every group showed significant differences between the other groups because p>0.05 was seen in the first treatment group (T1) with negative control (NC) with p value = 0.068 and negative control (NC) with second treatment group (T2) with p value = 0.069, and negative control (NC) with third treatment group (T3) with p value = 0.284 the last negative control (NC) with positive control (PC) with p value = 0.767.

**Table 5: Bonferroni multiple comparison post Hoc test in sperm count.**

| Groups | Sperm Count Degree of Significance (p) | | | | |
|---|---|---|---|---|---|
| | NC | PC | T1 | T2 | T3 |
| NC | - | 0.000* | 0.069 | 0.005* | 0.000* |
| PC | 0.000* | - | 0.000* | 0.000* | 0.000* |
| T1 | 0.069 | 0.000* | - | 1.000 | 0.000* |
| T2 | 0.005* | 0.000* | 1.000 | - | 0.000* |
| T3 | 0.000* | 0.000* | 0.000* | 0.000* | - |

Based on the post hoc Bonferoni test results in Table 5, it can be concluded that not every group showed significant differences between the other groups because p>0.05 was seen in the first treatment group (T1) with negative control (NC) with p value = 0.069 and one treatment group (T1) with second treatment group (T2) with p value = 1.000.

**Table 6: Bonferroni multiple comparison post Hoc test in spermatozoa morphology.**

| Groups | Spermatozoa morphology degree of significance (p) | | | | |
|---|---|---|---|---|---|
| | NC | PC | T1 | T2 | T3 |
| NC | - | 0.025* | 0.666 | 1.000 | 1.000 |
| PC | 0.025* | - | 1.000 | 0.117 | 0.006 |
| T1 | 0.666 | 1.000 | - | 1.000 | 0.193 |
| T2 | 1.000 | 0.117 | 1.000 | - | 1.000 |
| T3 | 1.000 | 0.006* | 0.193 | 1.000 | - |

Based on the result of Bonferoni post Hoc test in Table 6, it can be concluded that not every group shows significant difference between other groups (p>0.05). However, in the NC group with PC (p = 0.025) and PC with T3 (p = 0.006) showed a significant difference where the p value <0.05.

## DISCUSSION

***Effect of vitamin C on testosterone serum levels of gentamycin-induced Wistar rats***

Results of 51-day study showed a decrease in serum testosterone hormone levels in the positive control group compared with the negative control group and decreased in the third treatment group (T3). This suggests that with gentamycin induction can cause a decrease in serum testosterone levels. Statistically using One Way Anova test there was significant result, p = 0.019 (p<0.05) which

mean there is effect of vitamin C administration to serum testosterone hormone level in gentamycin-induced rats.

Gentamycin is known to induce oxidative stress, decrease amounts of antioxidants, lipid peroxidation and histopathological changes in the testis.[15] This decrease in antioxidant activity leads to increased ROS concentrations and oxidative stress, causing damage to leydigh cells. Leydigh cells assisted by luteinizing hormone (LH) play a role to regulate the secretion of testosterone. The damage to leydigh cells leads to decreased concentrations of testosterone hormones.[11]

In addition, gentamycin has a toxic effect on the sperm-producing organ, testis. Gentamycin is known to inhibit cell division in testicular germ cells and to inhibit protein synthesis in testis.[12] In a 2009 study of the effects of aminoglycosides, gentamycin 5mg/kg IP for 14 days on testicular tissue in mice, examined using light and electron microscopy, it was shown that mouse germ cells all which is administered gentamycin drugs underwent testicular germinal cell depletion, testicular germ cell necrosis, particularly in leydig cells and spermatogonium showed abnormal fibroblasts appearance, against abnormal distance between one leydig cell and the previous leydig cells.[5] While, it is known that the testosterone pis roduced by leydig interstitial cells when the testis stimulated by pituitary gland.[13]

The hypothalamus synthesizes a decapeptide, gonadotropin-releasing hormone (GnRH), then GnRH will bind to gonadotropin and stimulate the release of luteinizing hormone (LH) and FSH (in lower degrees) into the body circulation. Luteinizing hormone (LH) will be taken by leydigh cells which will be bound to specific membrane receptors.

This bond will lead to activation of adenylyl cyclase and cAMP formation which eventually leads to the secretion of testosterone. In contrast, elevated testosterone levels inhibit LH from the anterior pituitary through a direct effect on the pituitary or an inhibitory effect on the hypothalamus level, this is more commonly known as hypothalamus feedback mechanism.[14]

In a study conducted by Narayana (2007) states that gentamycin is alleged to increase the status of oxidative stress associated with elevated levels of ROS in the body, decreased amount of antioxidants, lipid peroxidation and histopathological changes in the testis. Thus, here vitamin C acts as one of the antioxidants that will help improve the spermatogenesis and the decrease negative effects on the testis that result in disruption of the testosterone.[15]

The researchers synthesis of the effect of vitamin C administration with a dose of 4.5mg/200gr did not affect the elevated serum testosterone levels in gentamycin-induced rats probably because the dosage was not effective for the improvement of serum testosterone levels, and the number of samples used in this study which is not much so that the possibility for the occurrence of bias from the results of this study is large.

With the average testosterone levels obtained, shows the minimal effects of gentamycin given to the positive control group than the negative control group, this may be due to the biological variation of the experimental animals used by each group, so the possibility of different effects would be larger, and also due to the absence of repetition of treatment in this study. This factors which affect the results of this research.

***Effect of vitamin C to sperm count of gentamycin-induced Wistar rats***

Based on one-way ANOVA test, p value of 0.000 means that there is effect of vitamin C administration to gentamycin induced rat sperm count.

This study found a decrease in the average rat sperm count in the positive control group compared with the negative control group, T1, T2 and T3. This suggests that gentamycin induction can cause a decrease in the sperm count. However, in the third treatment groups (T3) the average sperm count was higher than the first treatment group (T1) and the second treatment group (T2). This can be interpreted that the dose of vitamin C at 4.5mg/200 gr is more effective to increase the number of spermatozoa compared with the dose of 1.6mg/200 gr and dose 2.25mg/200gr. Gentamycin administration in the positive control group results in an increase of Reactive Oxygen Species (ROS) that can lead to a decrease in enzymatic antioxidant activity which in turn causes lipid peroxidation.[16] In increasing ROS conditions there will be lipid peroxidation, and lipid peroxidation in cell membranes may interfere with membrane function, causing irreversible damage to fluidity and membrane elasticity so that it can cause membrane rupture. If this happens continuously it will cause cell death.[17]

The synthesis of the researchers in this study was the serum testosterone levels which showed a decrease in the third treatment groups (T3) but on the examination of the sperm count, showed improved results in the third treatment group (T3). The decreased levels of testosterone in the T3 group is still within the limit of normal serum testosterone levels i.e. with a range of 0.5-11ng.[18] So that the decreases of testosterone that occur in the T3 group does not affect the number of spermatozoa produced because the hormone results are still within normal limits.

***Effect of vitamin C to the sperm morphology in gentamycin-induced Wistar rats***

Based on the one-way ANOVA test, which showed p value = 0.005, which means there is an effect of vitamin C on sperm morphology of gentamycin induced rats. decrease of spermatozoa morphology in positive control group (PC) compared to negative control group (NC),

first treatment group (T1), second treatment group two (T2), and third treatment group (T3). This proves that gentamycin may decrease the morphology of spermatozoa. However, the third treatment group (T3) had the highest average score compared to the other treatment groups. It can be concluded that to affect the morphology of spermatozoa in this study, the effective vitamin C dose to improve spermatozoa is at 4.5mg/200g compared to the dose of 1.6mg/200g and dose 2.25mg/200gr.

Morphological abnormalities of spermatozoa may affect infertility, the normal limit of spermatozoa morphology for fertility success when there is 4% normal morphology.[17] An impaired spermatogenesis process will result in abnormal spermatozoa morphology which can be caused by several factors, one of which is the aminoglycoside antibiotic gentamycin which causes a reduction in antioxidant reserves and causes an increase in ROS due to free radicals. This study showed that there was a significant difference between negative control group (NC) and positive control (PC) Inj gentamycin-induced rats for 10 days. This suggests that gentamycin affects the morphology of male rat spermatozoa.

Gentamycin is known to increase the status of oxidative stress, decrease amount of antioxidants, lipid peroxidation and histopathological changes in testis.[15] This is the reason that gentamycin is cytotoxic especially for testicular organs. Gentamycin administration may lead to the formation of free radicals that cause lipid peroxidation. If the formation of free radicals is not stopped, it will damage the cell membrane and spermatogonium cells mitochondrial cell membrane, sertoli cells, and leydig cells contained in the testis. In addition, free radicals from the side-effects of gentamycin can directly interfere with the function of epididymis as a place of maturation and suppliers of spermatozoa metabolic needs. If the process of maturation and metabolism impaired, then the resulting spermatozoa can be impaired as well.

## CONCLUSION

Administration of vitamin C has an effect to increase the serum testosterone level, sperm count and morphology of gentamycin-induced wistar rats. It is recommended for further research to investigate the effects of gentamycin on other hormones as well as to examine testicular hypothesis and examination of MDA levels to examine the levels of ROS.

## ACKNOWLEDGEMENTS

Authors would like to thank the Head and Staff of Pharmacology Laboratory of Pharmacy Faculty of Andalas University and Biomedical, Department of The Faculty of Medical, Andalas University who gave assistance in treating and examining the samples.

*Funding: No funding sources*
*Conflict of interest: None declared*
*Ethical approval: The study was approved by the Ethical Committee on Animal Study of Faculty of Medical Andalas University (Approval No. 180/KEP/FK/2017)*

## REFERENCES

1. Agarwal A, Prabakaran SA and Said TM. Prevention of oxidative stress injury to sperm. J Androl. 2005;26:654-60.
2. Tanagho, Emil A, Jack W, Mc Aninch. Smith's general urology. New York. The Mc. Graw-Hill Companies; 2008.
3. Angulo C, Maldonado R, Pulgar E, Mancilla H, Cardova A, Villarroel F, et al. Vitamin C and oxidative stress in the seminiferus epithelium. Biol Res. 2011;44 (2):169-80.
4. Istiantoro YH, dan Gan VGH. Penicillin, Cefalosporin and Antibiotics Batalaktam in pharmakologi and therapy. 5th ed. Editor Sulistia G. Ganiswara. Jakarta; 2007.
5. Khaki A, Ghaffari MN, Fathiazad F, Hossinchi J. Ultra-structural study of gentamicin and ofloxacin effect on testis tissue in rats: light and transmision electron microscopy. Afr J Pharm Pharma. 2009;3(4):12-34.
6. Winarsi H. Natural antioxidants and free radicals. Yogyakarta: Kanisius. 2007;5:122-204.
7. Champe, Pamela C, Harvey. Biochemistry. Jakarta. EGC; 2010.
8. Almasdy D, Deswinar, Helen. Evaluation of antibiotic use in a government hospital in the city of Padang. Proceeding of National Seminar Development of Police Science Clinic. 2013;2339-2592:7-15.
9. Keffer JC, Agarwal A, Sabanegh E. Role of antioxidant in the treatment of male infertility. Glickman Urological and Kidney Institute, Clevelnd Clinic, Cleveland, Ohio, USA. International J Urol. 2009;16:449-57.
10. Evans WJ. Vitamin E, vitamin C, and exercise. The Am J Clin Nutri. 2000 Aug 1;72(2):647S-52S.
11. Laurence R, Gesquiere, Patrick O, Onyango, Susan C, Alberts, Altman J. Endocrinology of year-round reproduction in a highly seasonal habitat: environmental variability in testosterone and glucocorticoids in baboon males. Am J Physical Anthropol. 2011;176:144:69.
12. Akondi BR, Akula A, Challa RS. Protective effects of rutin and naringenin on gentamicin induced testikule oxidative stress. College of Pharmaceutical Sciences, Division of Pharmacology, Andhara University, Visakhapatnam, India. Euro J General Med. 2011;8(1):57-64.
13. Marrai, Darawany E, Fadiel AA, Abdel A. Reproductive performance traits as effected by heat stress and its alleviation in sheep. Faculty of Agriculture Zagazig University. 2008;8:209-34.

14. Greenspan AC, Hall JE. Text book of Medical Physiology. USA. Saunder Elsevier; 2014.
15. Kilarkaje N. An aminoglycoside antibiotic gentamicin induces oxidative stress, reduces-antioxidant reserve, and impairs spermatogenesis rats. J Toxicol SCI. 2007;33(1):85-96.
16. Cruz, Lume C, Vieira JS, Nunes A, Castro A. Oxidative stress markers: can they be used to evaluate human sperm quality. Turky J Urol. 2015;41(4):198-207.
17. WHO. Mother or nothing: the agony of infertily. Bull World Health Organ. 2010;88:881-2.
18. Favig EM, Foad O. Serum and plasma levels of total and free testosterone and of sex hormone binding globulins in rats growing in the below sea level environtment of the Jordan valley. J Endo. 2009;5(2):1-6.

**Cite this article as:** Rahayu I, Usman E, Reza M. Effect of vitamin C on testosterone level, sperm count and sperm morphology in gentamicin-induced Wistar rats. Int J Res Med Sci 2019;7:451-6.

# Effect of vitamin C on testosterone level

ORIGINALITY REPORT

| 13% | 13% | 4% | 4% |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | 1library.net<br>Internet Source | 6% |
| 2 | ibbj.org<br>Internet Source | 4% |
| 3 | Submitted to Udayana University<br>Student Paper | 3% |

| | | | |
|---|---|---|---|
| Exclude quotes | On | Exclude matches | < 3% |
| Exclude bibliography | On | | |