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**Original Article**

# Effect of Ginger Extract on Gentamycin Induced Testicular Atrophy in Albino Mice

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## ABSTRACT

**Background:** Gentamicin is a broad spectrum antibiotic used in wide range of infective diseases including infertility cases of high leukocyte count in semen. Its therapeutic applications are however hampered due to its nephrotoxicity and ototoxicity. It is known to cause testicular damage and may lead to infertility. It has been claimed that Ginger Extract restores sperm motility and fertility in case of gentamicin induced testicular toxicity.

**Objective:** To assess the preventive effect of Ginger Extract on gentamicin induced testicular toxicity

**Methods:** 50 adult albino male mice were selected and divided into following groups; Group A: control group. Group B: Only intraperitoneal gentamicin with no Ginger extract, Group C1: 25 mg/kg intraperitoneal gentamicin with oral 100 ml/kg Ginger extract, Group C2: 25 mg/kg intraperitoneal gentamicin with intraperitoneal 100ml/kg Ginger extract, Group D1: 50 mg/kg intraperitoneal gentamicin with oral 100 ml/kg Ginger extract, Group D2: 50 mg/kg intraperitoneal gentamicin with intraperitoneal 100ml/kg Ginger extract. All albino mice were sacrificed after 2 weeks. Testes were preserved in formalin; histological slides were prepared and examined under light microscope. Data was recorded on a Performa and analyzed by SPSS version 20 and t-test was applied for weights of mice and testes.

**Results:** Group A, C and D showed normal testicular morphology while Group B showed decreased diameter of seminiferous tubules, congestion of blood vessels, necrosis, occasional apoptosis, reduced sperm counts and distorted tunica albuginea with a p value of <0.001confirming protective and reversal effects of experimental work.

**Conclusion:** Ginger extract has protective effects on gentamicin induced testicular toxicity in albino mice.

**Key Words**: Gentamicin, experimental work, albino mice testes, testicular atrophy

## Introduction

Gentamicin belongs to the aminoglycoside group.[1]It works against both gram negative and gram positive organisms and commonly used as broad spectrum antibiotic in urinary tract infection, infective endocarditis, pelvic inflammatory disease, meningitis, pneumonia, sepsis and in cases of infertility prior to bacterial infection when there is high leukocyte count in semen.[2,3,7] Adverse effects are nephrotoxicity, ototoxicity, neuromuscular paralysis and infertility due to testicular toxicity.[4,5]

Aminoglycosides act as irreversible inhibitors of protein synthesis by interfering with initiation of peptide formation, mRNA misreading and helping in the breakup of polysomes into nonfunctional monosomes[5]. It can be administered through intravenous, intramuscular, intra-peritoneal routes and topical application.[2,3]

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The present study analyzed the effects of gentamicin on seminiferous tubules, interstitial and germ cells in the mice. Described gentamicin induced seminiferous changes include atrophy, sloughing cytoplasm, vacuolation, gaps formation, nuclear pyknosis and decreased spermatocyte count. Recent studies suggest that due to oxidative stress gentamicin induces structural and cytotoxic changes and adversely affect spermatogenesis.[8,9]

Ginger extract has powerful antioxidant and androgenic activities.[7,11]As a powerful antioxidant Ginger extract may prevent and mitigate generation of free radicals likes OH ion and singlet Oxygen.[7, 12,13]

Assisted reproductive techniques (ARTs) are being used worldwide and antibiotics especially aminoglycosides (e.g. gentamicin and neomycin) and fluoroquinolones (e.g. ciprofloxacin, ofloxacin) are frequently used in ARTs and many bacterial diseases however the aminoglycosides and fluoroquinolones may cause infertility due to apoptosis, decreased number of sperms and their motility.[14,15]

**Objectives:**

Our objective was to study the

1. Morphological changes in testis of mice induced by gentamicin.
2. Reversal of morphological changes by Ginger extract

## Methods

Approval was obtained by Advanced Study and Research Board of Khyber Medical University (KMU). The study was carried out at Veterinary Research Institute(VRI) Peshawar, Pakistan

This was an experimental study, spanned over 7 months. Mice were bought from Veterinary Research Institute Peshawar. Sample size was 50. Calculated dose was given to each mouse according to the dosage schedule. Healthy male mice, aged between 6-8 weeks were included. Sick mice were excluded from the study. The mice weighed 30-50 grams and were aged between 6-8 weeks. They were kept in VRI Peshawar in animal house under suitable conditions and fed on special feed which was made commercially. Every day distilled water, food and bedding was changed. Mice were divided into groups and kept in different labeled cages.

The mice were divided into following groups; **1**.**Control Group= Group A**; no gentamicin OR Ginger extract given in this group. **2. Experimental groups= Group B**; Only intraperitoneal 50mg/kg OD gentamicin given to observe the adverse effects of gentamicin in testes. In groups C1,C2,D1 and D2 different doses of gentamicin were used i.e 25mg/kg and 50 mg/kg intraperitoneal OD while Ginger extract was used in equal dose in two different routes i.e oral and intraperitoneal OD. **Group C1**; 25 mg/kg intraperitoneal gentamicin along with oral 100 ml/kg Ginger extract, **Group C2**; 25 mg/kg intraperitoneal gentamicin along with intraperitoneal 100ml/kg Ginger extract, **Group D1**; 50 mg/kg intraperitoneal gentamicin along with oral 100 ml/kg Ginger extract, **Group D2**; 50 mg/kg intraperitoneal gentamicin along with intraperitoneal 100 ml/kg. All mice were closely observed for any change in behavior and weight. The initial weight of mice was recorded prior to the experiment then it was compared after experiment with the final weight of mice. All albino mice were sacrificed after 2 weeks. Testes were removed and kept in 10% buffer formalin in separately labeled bottles. Then slides were made by using hematoxylin and eosin stain and examined under light microscope. Data was recorded on a Performa and analyzed by SPSS version 20 and t-test was applied for weights of mice and testes.

## Results

Weights of the mice in experimental groups which was given gentamicin decreased. Mice were sacrificed and testes were removed and compared with the control group A. There were no changes in color and shape of all the testes, however the weights of mice of gentamicin only group were significantly reduced. (Table 1)

**Table 1. Initial and final weight of mice before and after treatment**

| Groups | Initial weight of mice before treatment | Final weight of mice after treatment | P value |
|---|---|---|---|
| Group A | (29gm to 32gm) | (31 gm to 34 gm) | 0.621 |
| Group B: | (30gm to 33gm) | (21gm to 24gm) | Significant0.00 |
| Group C1 | (31gm to 32gm) | (27gm to 29gm) | Significant <.001 |
| Group C2 | (28gm to 32gm) | (25gm to 30gm) | Significant<.001 |
| Group D1 | (30gm to 32gm) | (29gm to 31gm) | Significant<.001 |
| Group D2 | (31gm to 33gm) | (28gm to 32gm) | Significant<.001 |

**Group A (control group: No Gentamicin / Ginger extracts); Thick** tunica albuginea comprised of thick collagen. Many seminiferous epitheliums were present back to back within the intervening stroma. Seminiferous tubules had spermatogonia in the most peripheral region which gradually transformed into spermatozoa in orderly fashion towards the center. Epididymis contains fully mature spermatozoa with very long tails. Leydig cells were not prominent. They lied in between the seminiferous tubules**.(Fig. 1)**

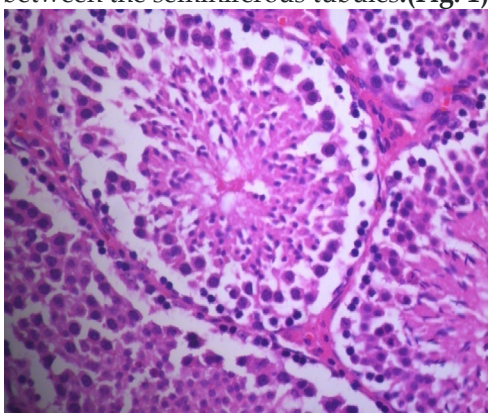

**Figure 1: Seminiferous tubules showing normal spermatogenesis (H & E x 400)**

**Group B:** (only Gentamicin with no Ginger extracts); Tunica albuginea wall was congested. **(Fig.2)** Capsule wall was peeled off. The nuclei were pale. Coalescent vacuoles were present between spermatogonia and basement membrane. These vacuoles were initially intracytoplasmic which pushed the nucleus to one side. Degeneration was prominent in peripheral seminiferous tubules. There was marked congestion through out in the testis including the interstitial septa between the adjacent seminiferous tubules as well as in the epididymis leading to hemorrhages containing macrophages. Leydig cells showed prominent degenerative changes. **(Fig.3) Necrosis was present. (Fig.4)**The spermatocytes were discohesive. They were prominently shrunken and small with pale nuclei. They had ghost like appearance. There were marked decreased in the number of spermatozoa. Most of their tails showed knots towards the end perhaps affect their motility. **(Fig.5)** Apoptotic cells were observed in the periphery near spermatogonia in seminiferous tubules. They were very small round bodies.
Epididymis had frequent hemorrhages. **(Fig.6)** There were many macrophages engulfing RBC's.

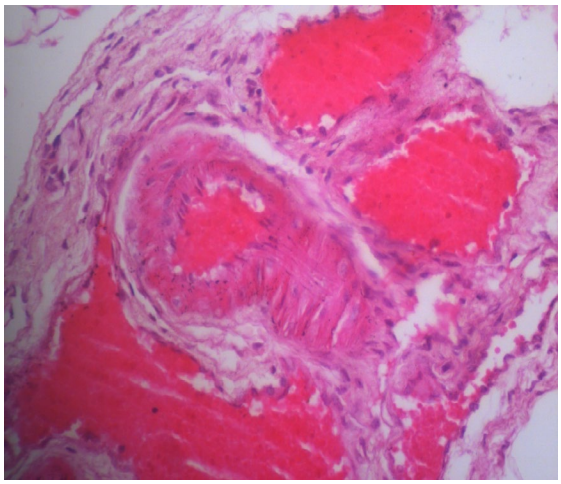

**Figure 2: Marked vascular congestion in the periphery of epididymis. (H &E x 400)**

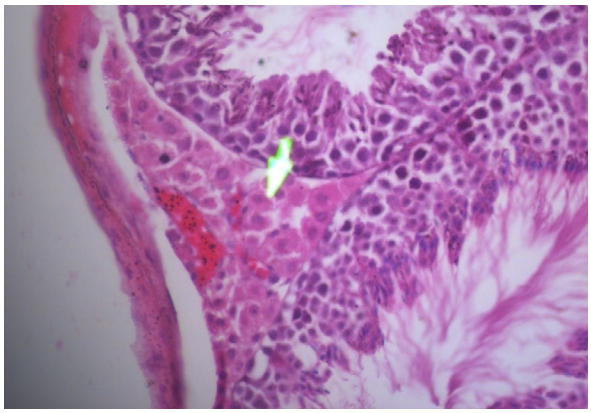

**Figure 3: Prominent leydig cells in a triangular shaped area sandwiched between adjacent seminiferous tubules and also present prominent vacuoles. (H &E x 400)**

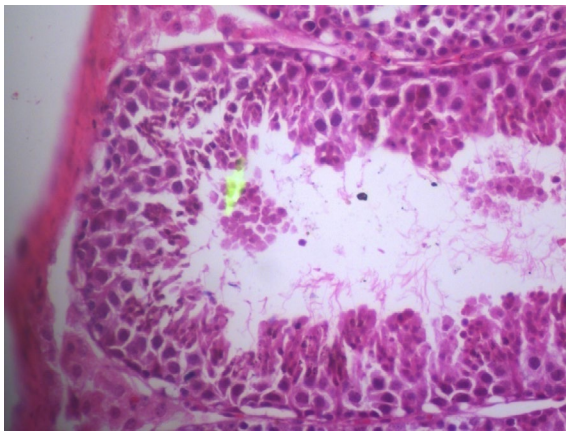

**Figure 4: Necrosed spermatocytes in the center of seminiferous tubules. (H &E x 400)**

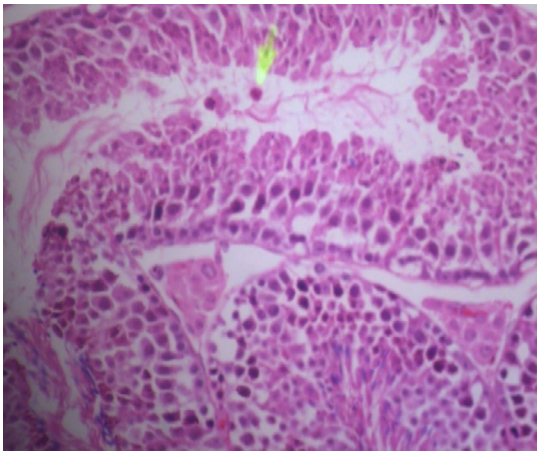

**Figure 5: Macrophages, prominent vacuoles in the periphery, leydig cells and atrophy of the seminiferous tubules. (H &E x 400)**

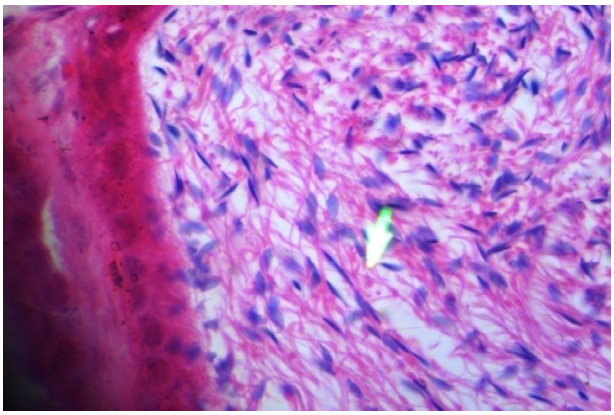

**Figure 6: A spermatozoa tails had knots, macrophages in epididymis. (H &E x 400)**

## Discussion

Gentamicin is a commonly used antibiotic and belongs to aminoglycoside group.[1] Clinically it is used for diseases like sepsis, pneumonia, urinary tract infection, infective endocarditis, pelvic inflammatory disease, meningitis and in fertility treatment.[2,3] Its therapeutic applications have now became limited because it causes nephrotoxicity and ototoxicity, beside that it also causes severe damage to the testes which leads to infertility.. It mainly causes apoptosis, necrosis and hemorrhges.[4,5]

In this study the effects of Ginger extract were investigated for the possible protective action against infertility caused by gentamicin in albino mice.

The present study is directed upon analyzing the effects of Gentamicin on testicular structure and germ cells parameters in the mice. Structural changes due to Gentamicin includes sloughing, vacuolization and gaps formation in the seminiferous epithelium, atrophic changes and nuclear pyknosis as demonstrated by tubular shrinkage in a few tubules as indicated by decreased Seminiferous tubules and Seminiferous epithelium.[6,7] Dose of gentamicin is 5-6 mg/kg/day given in three equal divided doses.[5] Recent studies suggests that due to free radical formation and lipid peroxidation, it induces structural and cytotoxic changes in testis by causing oxidative stress in testis and negatively affect spermatozoa and causing a decline in sperm count, decrease in motility and structural changes in spermatozoa.[8,9] Apoptosis in testis was formerly attributed to be the effect of ciprofloxacin neomycin and streptomycin; however recent studies suggest gentamicin and ofloxacin to have similar effect.[10] Ginger extract has powerful antioxidant activities.[11] Fertility parameters are improved by increasing antioxidant enzymes that work as a protective defense against oxidative stress.[12,13]

Our results showed prominent histological changes including atrophy of the seminiferous tubules, congestion, hemorrhages, apoptosis, necrosis, vacuoles formation, reduced sperm counts and distorted tunica albuginea due to gentamicin. The groups which was given gentamicin and Ginger extract showed essentially normal morphology proving the efficacy of Ginger extract in prevention of gentamicin toxic effects. These findings were in agreement with Zahedi, Afshin, et al who also observed that gentamicin leads to infertility by causing apoptosis. Fertility parameters are improved by increasing antioxidant enzymes, work as a protective defense against oxidative stress.[13] This study also co-relates with the study of Arash khaki, Sanati E and Nikmanesh M in which parameters like germ cell apoptosis is caused by gentamicin the aminoglycosides.[14,15] Our study in addition also showed marked congestion which at time led to frank hemorrhages key role, inducing apoptosis, atrophy, prominent leydig cells, necrosis due to diminished oxygen supply.

In previous studies apoptosis was the main focus, however in the current study we identified some additional features like weight of mice which was markedly decreased, shrunken germ cells with ghost like appearance containing pale nuclei. Large numbers of vacuoles were present in the germinal epithelium. The sertoli cells were markedly swollen and the leydig cells were prominent due to atrophy of seminiferous tubules. These all effects were well protected when Ginger extract were given along with the gentamicin.

## Conclusion

Ginger extract is effective in nullifying the gentamicin induced testicular damage

**Conflict of Interest:** Authors declare no conflict of interest.

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| HISTORY | |
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| CONTRIBUTION OF AUTHORS | |
|---|---|
| **Author** | **Contribution** |
| Anila Shah Bukhari | A |
| Faran Shah | B |
| Sana Shah | C |

**KEY FOR CONTRIBUTION OF AUTHORS:**

A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion