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ORIGINAL ARTICLE

Morphological Study of Gentamicin-Induced Testicular Toxicity and Protective Effects of Zinc in Adult Albino Mice

Anila Shah Bukhari[1], Faran Shah[2], Sana Shah[3], Faridullah Shah[4], Shehla Khatoon[5], Munila Shabnum Khattak[6]

[1] Assistant Professor, Department of Anatomy, Rehman Medical College, Peshawar, Pakistan
[2]General surgery, Lady Reading Hospital, Peshawar, Pakistan.
[3]Gynacologist, Khyber Teaching Hospital, Peshawar, Pakistan
[4] Associate professor, Department of Biochemistry, PIMS, Peshawar, Pakistan
[5]Assistant Professor, Department of Anatomy, KMC, Peshawar, Pakistan
[6]Associate Professor, Department of Anatomy, KMC, Peshawar, Pakistan

Correspondence:
Dr. Anila
Email: bukharianilashah002@gmail.com

Abstract

Background: One of the drugs of the aminoglycoside group, known adverse effects are nephrotoxicity, ototoxicity, and neuromuscular paralysis. Zinc, a transitional metal and best source is meat, fish, whole grain cereals, and dairy products. Zinc protects tissues against free radicals and oxidative stress. Used in fertility, pneumonia, osteoporosis, wound healing, regulating immune function, and decreased risk of senile chronic diseases with no significant side effects.

Objective: Gentamicin effects on testicular structure and sperm parameters in mice. Zinc reversal effects are the focus of the current study.

Material and Methods: An experimental animal-based study done in Veterinary Research Institute Peshawar, for 6 months from June 2019 to December 2019. Fifteen albino mice were collected and sorted into groups. B-1, B-2, C-1 and C-2 were experimental groups, A-1 was control group. Zinc was administered as 1-2mg/kg orally, whereas Gentamicin was administered in two dosages of 50mg/kg intraperitoneally and 75mg/kg. Mice were sacrificed after 2 weeks. Testis was collected, and kept in formalin; slides were made and observed through the light microscope.

Results: Gentamicin-induced structural alterations in experimental groups were sloughing, vacuolation, gaps formation in the somniferous epithelium, atrophic changes, nuclear pyknosis, indicated by tubular shrinkage in a few tubules, decreased somniferous tubules and somniferous epithelium. Zinc nullified these effects when used simultaneously with Gentamicin.

Conclusion: The study evaluated the impact of Gentamicin on the health outcomes of the participants. Additionally, oral supplementation of zinc at 1-2 mg/kg provided a crucial variable that allowed researchers to explore potential protective effects against Gentamicin-induced toxicity. The methodologies reveal pharmacological interactions, and emphasize dosage and administration routes in experimental design, paving the way for future research to improve therapeutic strategies and health outcomes.

Keywords: Zinc, Gentamicin, tests, albino mice.

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Introduction

Gentamicin was discovered in 1963[1,2]. One of the drugs of the aminoglycoside group.14,18. It is made from Bacteria i.e., Micromonospora purpurea[12,18.] Available as a generic medication. It is one of the most crucial medications in the basic health system and has been recognized by the WHO as an essential medication[3]. It is Effective against organisms that are both gram-positive and gram-negative[18]. Aminoglycosides have a hexose ring, which can be streptidine or 2-deoxystreptamine, to which various amino sugar types are joined by glycosidic

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bonds, according to their physical and chemical characteristics[14,18]. There are three ways that aminoglycosides function as permanent inhibitors of protein synthesis: hinder the start of peptide production, misinterpret mRNA, and aid in the disintegration of polysomes into inoperable monosomes.[18].The initial step in the aerobic condition is the process of passive diffusion into the cytoplasm via porin channels across the cell membrane inhibited in an anaerobic condition[14,18]. It is administered intravenously, intramuscularly as well as topical[12,18]. Its half-life in serum ranges from two to three hours to twenty-four to forty-eight hours[18,19]. Broad-spectrum antibiotics are utilized in the treatment of urinary tract infections, infective endocarditic, pelvic inflammatory disease, meningitis, pneumonia, sepsis, and as a prophylactic measure in fertilization treatments, particularly when elevated leukocyte counts are present in semen[1,2.] Known adverse effects are nephrotoxicity, ototoxicity, and neuromuscular paralysis[14,18]. Gentamicin effects on testicular structure and sperm parameters in mice are the focus of the current study. Structural changes brought on by Gentamicin include sloughing, vacuolation, and gap formation in the somniferous epithelium, as well as atrophic changes and nuclear pyknosis, which are indicated by tubular shrinkage in a few tubules and decreased seminiferous tubules and seminiferous epithelium[4]. Gentamicin is administered at a dose of 5–6 mg/kg/day in three equally divided doses[11,18]. According to recent research, the lipid peroxidation action of Gentamicin and the production of free radicals cause oxidative stress in the testis, which damages spermatozoa and results in a drop in sperm count and motility and spermatozoa[5,6]. Ciprofloxacin, neomycin, and streptomycin were formerly thought to cause apoptosis in the testis; however, new research indicates that gentamicin and ofloxacin may have comparable effects[8].

Zinc is a transitional metal and is one of the essential trace elements having biological importance both in plants and animals[5]. The best sources of Zinc are beans, animal meat, nuts, fish, whole grain cereals, other seafood, and dairy products[5,9.] Zinc shields tissues against oxidative stress and free radicals[12]. Zinc is a hepatocellular metallothionein inducer and a necessary mineral for spermatogenesis[12,13]. Zinc protection is mediated by the induction of metallothionein against heavy metal toxicity[9.] Alternatively, it may directly antagonize the toxic effects of heavy metals stabilize cell membranes, and protect lipid peroxidation by free radicals[16]. It is used in fertility, pneumonia, osteoporosis, and wound healing, to regulate immune function, and decrease the risk of age-related chronic diseases[16,17.] It has no significant side effects[17].

Objectives: To investigate the

1. Gentamicin-induced morphological alterations in mouse testicles.
2. Prevention of morphological changes by Zinc in the testis of mice (morphological and histological parameters)

Material and Method:

Study Design: Experimental Study

Study Settings: Peshawar

Study Duration: Six (6) Months. from June 2019 to December 2019 (Weighing 30 to 50 gms).

Sample Size:15 MaleAlbino Mice

Sampling Technique: Simple Randomized Sampling

Sample Selection: Mice must be male to qualify. Mice were six to eight weeks old. Male mice within 6 and older than 8 weeks are excluded. Weight range: <30 to >50 gms.

Data Collection Procedure:

Animals: From the Veterinary Research Institute in Peshawar, 15 male mice were acquired. Weighing 30-50 gms and maintained in the animal house. Food and water were provided. They were acclimatized for one week before the experiment. Maintained in a proper environment in an animal home and fed specialized commercial feed.

Drug: "GENTAMICIN inj. 50-75mg/kg" was used in the experiment.

Experimental design: The mice were housed in distinct cages labeled with the treatment group after being split up into three groups. The numbering of mice in each group was done with the help of a permanent marker by marking tails with it. The animals were weighed before and after the treatments before sacrificial.

The groups were made as follows:

CONTROL GROUP:

Group-A. (3 mice).

EXPERIMENTAL GROUPS: B, C(12 mice)

1. Group B. (6 mice).

B1: Gentamicin given as 50 mg/kg intraperitoneally for 2 weeks with Zinc

Not given

Control Group	Groups in Experiments			
A group	**B Group**		**C group**	
A [3. MICE]	**B-1 [3. MICE]**	**B-2 [3. MICE]**	**C-1 [3. MICE]**	**C-2 [3. MICE]**
Food and Distilled water will be given to each mouse and Sacrificed Normal weight and histological parameters noted	Gentamicin 50 mg/kg I/P injection For 2 weeks Zinc Not given Two weeks later, the mice were sacrificed.	Gentamicin 50 mg/kg I/P injection For 2 weeks Zinc 1-2 mg/kg Orally For 2 weeks Two weeks later, the mice were sacrificed.	Gentamicin 75 mg/kg I/P injection For 2 weeks Zinc Not given Two weeks later, the mice were sacrificed.	Gentamicin 75 mg/kg I/P injection For 2 weeks Zinc 1-2 mg/kg orally For 2 weeks Two weeks later, the mice were sacrificed.

B2: Gentamicin was given as 50 mg/kg intraperitoneally for 2 weeks along with ZINC at a dose of 1-2mg/kg by gastric gavage.

2. Group C. (6 mice).

C1: Gentamicin is given at 75 mg/kg intraperitoneally for 2 weeks. Zinc

Not given

C2: Gentamicin was given as 75mg/kg intraperitoneally for 2 weeks along with ZINC at a dose of 1-2mg/kg by gastric gavage.

LAB PROCEDURE:

Slides are prepared by using Hematoxylin and Eosin stain.

Morphological parameters of testes;

Gross parameters:

- size of testis
- weight of the testis

Histological parameters:

1. Seminiferous tubules luminal diameter
2. Germ cell analysis and evaluation
3. Presence/absence of Sertoli cells
4. Fibrosis, necrosis, Apoptosis, and other degenerative changes in the cell and connective tissue.

Control group A was compared histologically with the experimental groups. (B, C). The protective effects of zinc are observed histologically.

Results:

Mice in experimental groups that received gentamicin had lower weights. Testes were extracted from mice after they were sacrificed, and the results were compared to those of group A, the control. All of the testes' colors and shapes were unchanged, however, the mice in the gentamicin-only group had noticeably lower weights.

(Table 1) The initial and final weight of mice before and after treatment

A group was a control group: no gentamicin / no zinc; Many seminiferous epitheliums were found back-to-back within the intervening stroma, and the tunica albuginea was thick and composed of thick collagen. In the periphery of seminiferous tubules, there were numerous spermatogonia, which progressively systematically changed into spermatozoa as they approached the center. The epididymis contains fully developed spermatozoa with extremely long tails. Leydig cells were located between the seminiferous tubules and were not very noticeable.

B-1 and **C-1** groups were an experimental group with only Gentamicin; crowded wall of Tunica albuginea. The wall of the capsule broke apart, revealing pale nuclei. There were coalescent vacuoles between the basement membrane and spermatogonia. At first, these vacuoles were intracytoplasmic, pushing the nucleus to one side. Degeneration was evident in the peripheral seminiferous tubules, i.e., there was significant congestion throughout the testis, including the interstitial septa between the adjacent seminiferous tubules, as well as in the epididymis, resulting in hemorrhages that contained Leydig cells and macrophages. There was necrosis. Spermatocytes had a ghostly look, were discordant, and were noticeably tiny and shrunken with pale nuclei. There was a noticeable drop in spermatozoa, and the majority of their tails had knots toward the end, which would have affected their motility. Additionally, there were apoptotic

cells in the vicinity of spermatogonia in seminiferous tubules.

B-2, C-2group; their morphology was identical to that of the control group.

Table 1: Initial and final weight of mice before and after treatment

Groups;	Mice's initial weight before treatment	Mice's final weight before treatment	Value of P Significant <.001
A group	(28gm - 32gm)	(30 gm - 34 gm)	0.621
B-1 group	(30gm - 32gm)	(25gm - 29gm)	<.001
B-2 group	(28gm - 32gm)	(27gm - 30gm)	<.001
C-1 group	(29gm - 32gm)	(26gm - 28gm)	<.001
C-2 group	(31gm - 33gm)	(29gm - 30gm)	<.001

Discussion:

A popular antibiotic that is a member of the aminoglycoside category is gentamicin. One is Clinically used to treat conditions such as meningitis, pelvic inflammatory disease, sepsis, pneumonia, urinary tract infections, infective endocarditis, and fertility[2,3]. Its therapeutic uses are currently restricted because it produces ototoxicity and nephrotoxicity, in addition to causing significant testicular damage that results in infertility. It primarily results in hemorrhages, necrosis, and apoptosis[4,5]. The purpose of this study was to examine the potential protective impact of zinc against gentamicin-induced infertility in albino mice. The goal of the current investigation is to examine how Gentamicin affects the mice's testicular structure and germ cell parameters. Sloughing, vacuolization, and gap formation in the seminiferous epithelium are among the structural alterations brought on by gentamicin. Atrophic changes and nuclear pyknosis are also evident, as evidenced by tubular shrinkage in a few tubules, which is a sign of decreased seminiferous tubules and serum epithelium[6,7]. Gentamicin is administered in three equally divided doses at a dose of 5–6 mg/kg/day[5]. According to recent studies, lipid peroxidation and free radical formation cause oxidative stress in the testis, which affects spermatozoa negatively and results in a decrease in sperm count, motility, and structural changes in spermatozoa. This causes structural and cytotoxic changes in the testis.8,9Previously, ciprofloxacin, neomycin, and streptomycin were thought to be responsible for testicular apoptosis; however, new research indicates that gentamicin and ofloxacin may have comparable effects[10].

Zinc possesses potent antioxidant properties[11].To improve fertility indices, antioxidant enzymes that function as a preventative defense against oxidative stress are increased[5,6]. The results of our investigation revealed significant histological alterations, such as gentamicin-induced atrophy of the seminiferous tubules, congestion, hemorrhages, apoptosis, necrosis, vacuole formation, decreased sperm counts, and deformed tunica albuginea. The groups that received zinc and gentamicin had nearly normal morphology, demonstrating zinc's effectiveness in preventing the harmful consequences of gentamicin. These results concurred with those of Narayana K, Kilarkaje N, et al., who also noted that gentamicin causes apoptosis, which results in sterility. Increasing antioxidant enzymes improves fertility indices and serves as a buffer against oxidative stress[4,7]. Additionally, this study is related to those of Arash Khaki, Sanati E., and Nikmanesh M., which found that gentamicin and aminoglycosides cause parameters such as germ cell apoptosis[14,15]. Additionally, this investigation revealed significant congestion, which eventually resulted in frank hemorrhages, apoptosis, atrophy, conspicuous leydig cells, and necrosis because of a reduced oxygen supply. Previously, the primary focus was apoptosis; however, in the new investigation, we discovered several additional traits, such as mice's significantly reduced weight and shriveled germ cells with ghostly appearances and pale nuclei. Within the germinal epithelium, there were a lot of vacuoles. Because of the atrophy of seminiferous tubules, the Leydig cells were visible and the Sertoli cells were noticeably enlarged. When zinc was administered in addition to gentamicin, all of these effects were effectively attenuated.

Conclusion:

Zinc effectively reverses the testicular damage caused by gentamicin. The authors have declared that they have no conflicts of interest. Financial Disclosures & Grant Support: Nonexistent

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