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Original Article

Protective Effects of Vitamin C and E in Gentamicin induced Testicular Toxicity in Albino Mice

Anila Shah Bukhari[1], Maqbool Illahi[2], and Anwar Ul Haque[3]

[1]Department of Anatomy, Khyber Girls Medical College, Peshawar, [2]Department of Anatomy, Northwest School of Medicine, Peshawar, [3]Department of Pathology, Northwest School of Medicine, Peshawar

ABSTRACT

Background: Gentamicin is a versatile antibiotic used in wide range of infective diseases including fertilization treatment when there is high leukocyte count in semen. Its therapeutic applications are however hampered due to its nephrotoxicity and ototoxicity. It is also known to cause testicular damage and may lead to infertility. It has been claimed that Vitamin C and E restores sperm motility and fertility in case of gentamicin induced testicular toxicity.

Objective: To assess the preventive effect of Vitamin C and E on gentamicin induced testicular toxicity

Methods: 30 adult albino male mice were selected and divided into following groups; Group A: control group with no gentamicin and no vitamins. Group B: Only intraperitoneal gentamicin with no vitamins, Group C1: 25 mg intraperitoneal gentamicin with oral 100 ml/kg Vitamin C & E, Group C2: 25 mg intraperitoneal gentamicin with intraperitoneal 100ml/kg Vitamin C & E, Group D1: 50 mg intraperitoneal gentamicin with oral 100 ml/kg Vitamin C & E, Group D2: 50 mg intraperitoneal gentamicin with intraperitoneal 100ml/kg Vitamin C & E. 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 effects of vitamin C & E.

Conclusion: Vitamin C and Vitamin E have protective effects on gentamicin induced testicular toxicity in albino mice.

Keywords: Gentamicin, Vitamin C, Vitamin E, 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 fertilization treatment used prior to bacterial infection specially 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]

CORRESPONDENCE AUTHOR
Dr. Anila Shah Bukhari
Email: anilashahbukhari@yahoo.com

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]

The present study analyzed the effects of gentamicin on seminiferous tubules, interstitium and germ cells in the mice. Described gentamicin induced seminiferous changes includeatrophy, 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]

Vitamin C and E have powerful antioxidant and androgenic activities.[7,11] As a powerful antioxidant Vitamin C and E may prevent and mitigate generation of free radicals likes OH ion and singlet Oxygen.[7, 12,13]

Adverse effects of aminoglycosides on testes:
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]

Aims and Objectives

The aim of the study was to study the

1. Morphological changes in testis of mice induced by gentamicin.
2. Prevention of morphological changes by combined Vitamin C & E.

Methods

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

This was an experimental study, spanned over 6 months. Mice were bought from Veterinary Research Institute Peshawar. Sample size was 30.Calculated dose was given to each mouse according to the dosage schedule.

Inclusion criteria; Healthy male mice, aged between 6-8 weeks.

Exclusion criteria; Female mice and sick mice.

The mice weighed 30-50 grams and were aged between 6-8 weeks. They were kept in VRI Peshawar in animal house under suitable condition 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**; This was free of drugs i.e no gentamicin and no vitamins given in this group **2. Experimental groups= Group B**; Only intraperitoneal 50mg OD gentamicin given to observe the adverse effects of intraperitoneally in in testes In groups C1,C2,D1 and D2 different doses of gentamicin used i.e. 25mg and 50 mg intraperitoneally OD while vitamin C and vitamin E was used in equal dose in two different routes i.e. oral and intraperitoneally OD. **Group C1**; 25 mg intraperitoneal gentamicin along with oral 100 ml/kg Vitamin C & E, **Group C2**; 25 mg intraperitoneal gentamicin along with intraperitoneal 100ml/kg Vitamin C & E, **Group D1**; 50 mg intraperitoneal gentamicin along with oral 100 ml/kg Vitamin C & E, **Group D2**; 50 mg intraperitoneal gentamicin along with intraperitoneal 100 ml/kg Vitamin C & E. 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.

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. They were sacrificed after completion of experimental duration. Testes were removed and kept in 10% buffer formalin in separately labeled bottles. Then slides were made by using hematoxylin and eosin stain.

Results

Gross Parameters

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 which is free of drugs. 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

Microscopic parameters; All slides were studied under light microscope.

Group A (control group); This group is neither given gentamicin nor vitamins. The mice were sacrificed after two weeks as expected no abnormality was found**. Thick** tunica albuginea comprised of thick

collagen. Many seminiferous epithelium were present back to back within the intervening stroma. (Fig.1) Seminiferous tubules had spermatogonia in the most peripheral region which gradually transformed into stories talk about itspermatozoa in orderly fashion towards the center. (Fig 2) Epididymis contains fully mature spermatozoa with very long tails. (Fig.3) Leydig cells were not prominent. They lied in between the seminiferous tubules.

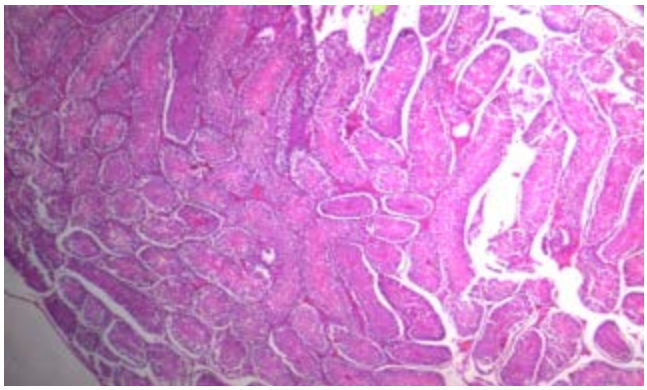

Figure 1: Many seminiferous tubules, full of spermatogenic cells (H & E x 40)

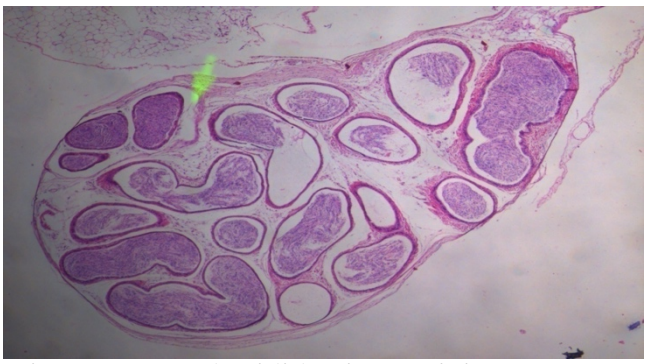

Figure 2: Normal epididymis containing numerous spermatozoa withlong tails (H & E x 40)

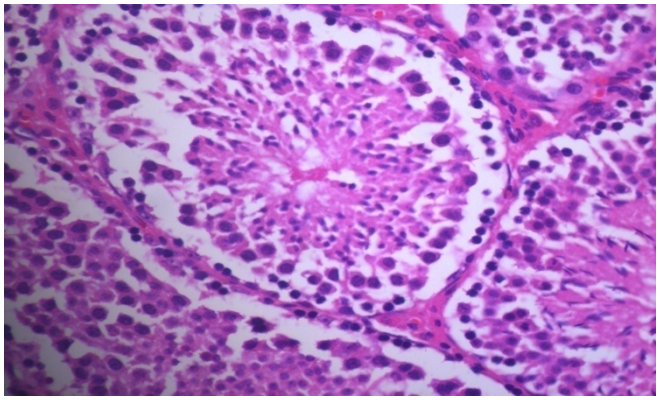

Figure 3: Seminiferous tubules showing normal spermatogenesis (H& E x 400)

Group B: (0nly Gentamicin with no vitamins); Tunica albuginea wall was congested. **(Fig.4)** Capsule wall was peeled off. The nuclei were pale. Coalescent vacuoles were present between spermatogonia and basement membrane. These vacuoles indeed 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.5)** Necrosis was present**. (Fig.6)** The spermatocytes were dis-cohesive. 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.9)** Apoptotic cells were observed in the periphery near spermatogonia in seminiferous tubules. They were very small round bodies. Epididymis had frequent hemorrhages. **(Fig.8)** There were many macrophages engulfing RBC's. **(Fig.7)**

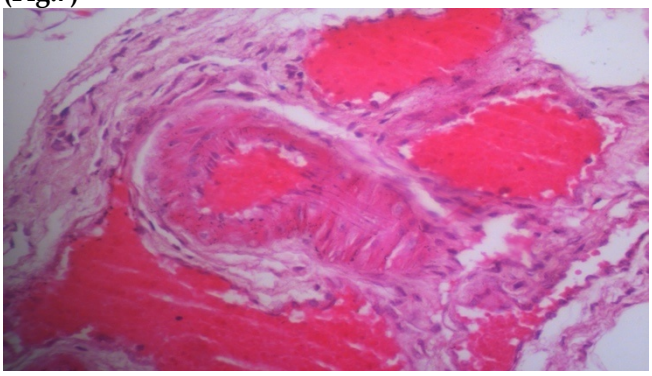

Figure4: Marked vascular congestion in the periphery of epididymis. (H&E x 400)

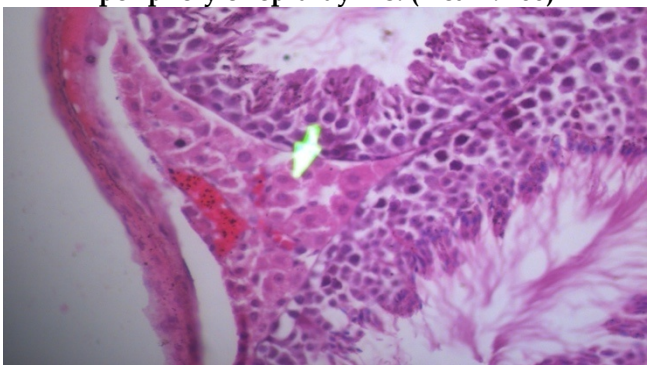

Figure 5: Prominent Leydig cells in a triangular shaped area sandwiched between adjacent seminiferous tubules and present prominent vacuoles. (H &E x 400)

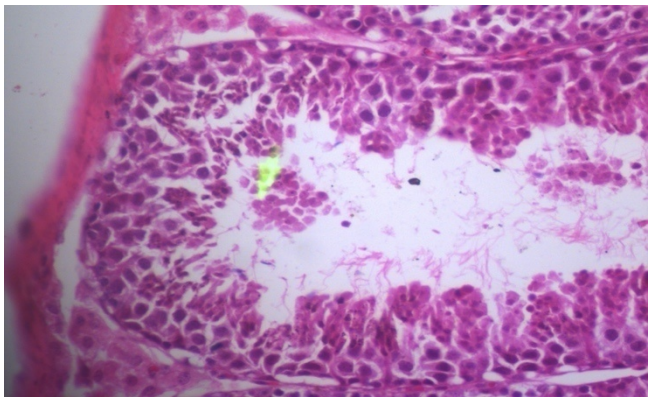

Figure 6: Necrosed spermatocytes in the center of seminiferous tubules. (H &E x 400)

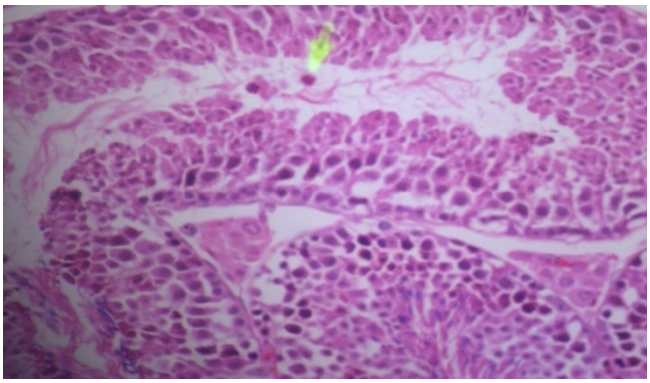

Figure 8: Macrophages, prominent vacuoles in the periphery, leydig cells and atrophy of the seminiferous tubules. (H &E x 400)

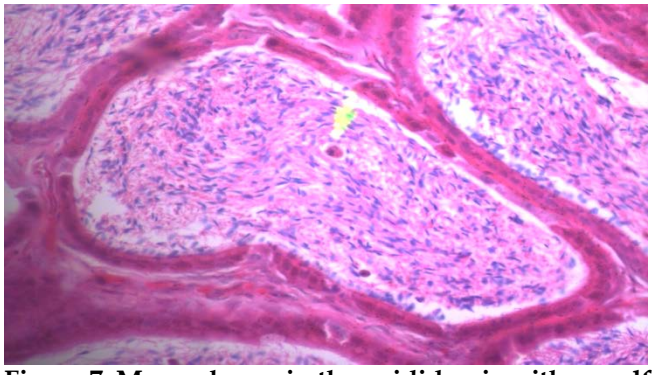

Figure 7: Macrophages in the epididymis with engulf RBC's. (H &E x 400)

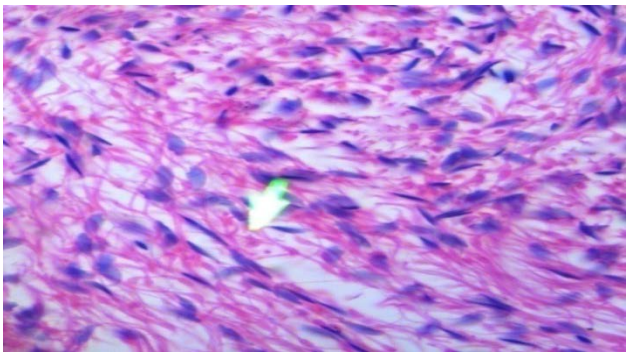

Figure 9: A spermatozoa tails had knots, macrophages in epididymis. (H &E x 400)

MICROSCOPIC PARAMETERS

Table 2. Changes in various cells in different groups

Groups	Tunica Albuginea		Leydig cells		Sertoli cells		Seminiferous tubules	
	Normal	Distorted	Not swollen	swollen	Normal	Number increased	Not congested	congested
Group A	yes	no	yes	no	yes	no	yes	no
Group B	no	yes	no	yes	no	yes	no	yes
GroupC1	yes	no	yes	no	yes	no	yes	no
GroupC2	yes	no	yes	no	yes	no	yes	no
GroupD1	yes	no	yes	no	yes	no	yes	no
GroupD2	yes	no	yes	no	yes	no	yes	no

Table 3. Presence or absence of different cell in various groups

Groups	Types of germ cells							
	spermatogonia		spermatocytes		Spermatids		spermatozoa	
	Vacuoles		pyknosis		Shape		number	
	Not present	present	Not present	present	Notchanged	Changed	normal	decreased
Group A	Yes	No	Yes	No	Yes	No	Yes	No
Group B	No	Yes	No	Yes	No	Yes	No	Yes
Group C1	Yes	No	Yes	No	Yes	No	Yes	No
Group C2	Yes	No	Yes	No	Yes	No	Yes	No
Group D1	Yes	No	Yes	No	Yes	No	Yes	No
Group D2	Yes	No	Yes	No	Yes	No	Yes	No

Table 4. Degenerative changes in various groups

Groups	**Degenerative changes**									
	Septal congestion		**Hemorrhages**		**Macrophages with RBCs**		**Apoptosis**		**Necrosis of germ cells**	
	Yes	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
Group A	-	No	-	No	-	No	-	No	-	No
Group B	Yes	-	Yes	-	Yes	-	Yes	-	Yes	-
Group C1	-	No	-	No	-	No	-	No	-	No
Group C2	-	No	-	No	-	No	-	No	-	No
Group D1	-	No	-	No	-	No	-	No	-	No
Group D2	-	No	-	No	-	No	-	No	-	No

Discussion

Gentamicin is a common use 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 are now becomes limited because it causes nephrotoxicity and ototoxicity, beside that it also causes severe damage to the testes which leads to infertility. In different literatures it is tested widely on rats and mice to see their toxic effects and how to prevent its toxic effects. It mainly causes apoptosis, necrosis and hemorrhges.[4,5]

In this study the effects of Vitamin C and E 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 action of gentamicin, it induces structural and cytotoxic changes in testis by causing oxidative stress in testis and negatively affect spermatozoa and 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] Vitamins C and E have 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 vitamins C & E showed essentially normal morphology proving the efficacy of vitamins C& E in prevention of gentamicin toxic effects. These findings agreed 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 and 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. (Oxidative stress)

In previous studies apoptosis was the 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 vitamin C and vitamin E were given along with the gentamicin.

Conclusion

Vitamin C and E in combination were effective in nullifying the gentamicin induced testicular damage

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CONTRIBUTION OF AUTHORS:

- Anila Shah Bukhari gave idea, conception, study designing, experimental work and planning.
- Maqbool Ilahi contributed in study designing.
- Anwar Ul Haque contributed in study slides, analysis and discussion