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EFFECT OF GENTAMICIN AND PROTECTIVE ROLE OF VITAMIN A ON SPERMATOGENESIS OF ALBINO RATS

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ABSTRACT

The process of gametogenesis in the male occurs within the seminiferous tubules of the testes, resulting in the production of sperm. Spermatogenesis begins with the spermatogonia. The factors that can interfere with spermatogenesis are: Drug treatment, chemotherapy, toxins and environmental. These factors generate the harmful products (oxidants) and affect sperm normal production. Epididymal sperm count, % sperm motility, sperm abnormalities, changes in number of germ cells per tubular cross section at stage VII of spermatogenesis, testicular markers like Testosterone, antioxidant status was evaluated by Catalase and Hydroxyl radical (OH^-) in all three groups. Histopathological Examination was done. Sperm count was higher in Group1 than II and III. Sperm motility was considerably reduced in the gentamicin group compared to that of the control group.). The testicular activities of Catalase was considerably ($p < 0.05$) lowered in gentamicin exposed animals in comparison with the controls indicating the impaired testicular antioxidant defense against ROS, which facilitated oxidative stress induction. Testicular androgenic enzymes and plasma testosterone showed significant variation among the three groups. Gentamicin administration led to the increased oxidative stress which caused a considerable reduction in sperm count and sperm movement. When gentamicin was administrated simultaneously with vitamin A respectively the sperm count level, sperm motility, SOD, LDH, SDH, and testosterone significantly elevated, indicating the protective effect of vitamin A.

Keywords: Spermatogenesis, Gentamycin, Antioxidant.

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INTRODUCTION

Spermatogenesis

The process of gametogenesis in the male occurs within the seminiferous tubules of the testes, resulting in the production of sperm. Spermatogenesis begins with the spermatogonia. Some spermatogonia remain near the basement membrane of the seminiferous tubule in an undifferentiated state to serve as a reservoir of cells for future cell division and subsequent sperm production. The rest of the spermatogonia loose contact with the basement membrane, squeeze through the tight junctions of the blood–testis barrier, undergo developmental changes, and differentiate into primary spermatocytes.

The spermatogenesis process takes 65-75 days [1]. Spermatogenic activity requires an adequate concentration of testosterone and androgen which are produced by the Leydig cells, when they are stimulated by luteinizing hormone (LH) [2].

Factors effecting spermatogenesis:

Infertility is one of the major health problems in couple's life, approximately 30% of cases of infertility are due to a male sex. The other factors that can interfere with spermatogenesis are: Drug treatment, chemotherapy, toxins and environmental. These factors generate the harmful products (oxidants) and affect sperm normal production. The routinely used the antibiotic amino glycosides (Neomycin, Streptomycin and Gentamicin) and fluoro quinolones (ofloxacin) to treat bacterial infections occurring former to in vitro fertilization**.**

Gentamicin:

Gentamicin is synthetic antibacterial agent belonging to the family of amino glycoside antibiotics with a very broad spectrum against of microbial pathogens, especially gram negative and urinary tract infectious diseases which have good effect in diseases treatment world-wide [3].

Effect of Oxidative stress

Oxidative stress is described as impairment of equilibrium between pro oxidant and antioxidant systems resulting in excess free radicals or decreased effective concentration of antioxidants or both [4]. Free radical is defined as a species that contains one or more unpaired electrons in its outer orbital, which renders it considerable degree of reactivity [5,6]. Oxidative stress (OS), results from accumulation of excessive reactive species (RS). RS can be produced in large amounts macrophages, neutrophils and also by spermatozoa and other cell types under pathologic conditions. Antioxidant is defined as any substance that delays, prevent or removes oxidative damage to a target molecule [7].

The generation of pro-oxidants in the form of RS is effectively kept in check by the various levels of antioxidant defense [8, 9]. The complex antioxidant defense system depends on the dietary (exogenous) intake as well as the endogenous production [10]. The different types of antioxidant are antioxidant enzymes (Superoxide dismutase, catalase). Anti-oxidative proteins (Hemoglobin, Ceruloplasmin) Small molecular weight compounds (Ascorbic acid,Tocopherols) and others (Ubiquinone, flavonoids) [7,11].

Vitamin A:

A group of unsaturated nutritional organic compound that includes Retinol, Retinal and Retinoic acid and several provitamin A caretenoids (mostly β Carotene. Vitamin A has multiple functions [12]. It's important for growth and development for the maintenance of immune system and good vision [13] All forms of vitamin A have a beta-ionone ring to which an isoprenoid chain is attached, called a *retinyl group*. Both structural features are essential for vitamin activity [14]. Antioxidant status is a critical tool for assessing redox status, which is defined as the balance between oxidants (free radicals and other reactive species) and antioxidants [15, 16].

MATERIALS AND METHODS

The study was performed on male Albino Wistar rats (250±10g). Animals were obtained from the Animal House, Mamata Medical College, and Khammam, India. Experimental animals were used after obtaining prior permission and handled according to the Institutional Animal Ethics Committee (285/CPCSEA) as regulated by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

The experimental animals were divided into three groups of six rats each as follows:

Group I: Control rats were given normal saline (2ml/kg body weight, i.p.), followed by oil vehicle for 14 days.

Group II: Rats were given 80 mg gentamicin /kg for 14 days

Group III: Rats were injected with gentamicin as in group II and concomitantly supplemented with Vitamin A (Vit-A) (1000 IU/kg/day, i.p.) for 14 days.

Sample collection

All the animals were anesthetized with ether after the 14 days experimental period (i.e., on the 15th day) and laparotomy was conducted. Samples of blood, testes, epididymis, seminal vesicles, and ventral prostate were removed, cleared of adhering connective tissue, and weighed. The analysis of sperm parameters and biochemical investigations was performed in all these tissues. The testicular tissue was homogenized in icecold 0.1M Tris-HCl buffer (pH 7.4) and centrifugation was performed at 10,000g in 4°C for 10 min to collect the supernatant. Epididymal sperm count, % sperm motility, sperm abnormalities, changes in number of germ cells per tubular cross section at stage VII of spermatogenesis, testicular markers like Testosterone, antioxidant status was evaluated by Catalase and Hydroxyl radical (OH^-) in all three groups.

Histopathological Examination

Tissue biopsies were fixed in 10% formaline, subjected to dehydration with increasing concentrations of ethanol and then embedded with paraffin wax. Following dehydration and embedding, histological sections were cut (5-7 µm) with rotary microtome and the paraffin was washed off with three xylene baths, followed by three isopropanol baths, and rehydration. Finally, the sections were stained with Hematoxylin and Eosin (H & E) and examined microscopically under 400x. The tissue sections were analysed under light microscopy by a blinded pathologist.

Evaluation of Biochemical Parameters

Assessment of Testicular Antioxidants and Oxidants

The testicular tissue was homogenized in ice-cold 0.1M Tris-HCl buffer (pH 7.4) and centrifuged at 10,000 3 g in 4°C for 10 min and the supernatant was collected. The protein content of the preparation was calculated by BCA Kit (Pierce) using BSA as a standard and then used for the following biochemical analysis.

Assay of CAT

In the ultraviolet range H_2O_2 shows a continual increase in absorption with decreasing wavelength. The decomposition of H_2O_2 can be correctly followed by monitoring the decrease in absorbance at 240 nm for 60 s in spectrophotometer. The change in absorbance is the indicator of the Catalase activity and was expressed as nmol/mg protein/s. The extinction coefficient of H_2O_2 at 240 nm is 40 M^{-1} cm^{-1} [17].

Assay of ROS

Hydrogen peroxide was quantified by the slightly modified method [18] In brief, to the assay mixture containing 0.1 ml KCl (1.13M), 0.1 ml potassium phosphate (150mM), 0.05 ml $MgCl_2$ (60mM), 0.05 ml EDTA (8mM), 0.1 ml Tris-HCl (200mM, pH 7.4), 0.1 ml of 1mM acetylated ferrocytochrome c, and 0.1 ml cell extract were added, and the oxidation of ferrocytochrome c, which gives the measure of H_2O_2 production, was measured at 550 nm in a spectrophotometer. The H_2O_2 content of the sample was expressed as micromole per minute per milligram protein.

RESULTS

Sperm count was higher in Group1 than II and III. Sperm motility was considerably reduced in the gentamicin group compared to that of the control group. This increased motility value shows the effectiveness of the combination therapy in maintaining sperm motility in rats administered with gentamicin (Table 1). When gentamicin was administered along with Vitamin A, respectively the % sperm abnormalities level was significantly ($p<0.05$) reduced (Table 2). A significant $P<0.05$ decrease in the numbers of Asg, pLSc, mPSc and 7Sd at stage VII of the seminiferous epithelium cycle was examined after gentamicin administration when compared to the controls simultaneous administration of Vitamin A with gentamicin significantly increased the numbers of Asg, pLSc, mPSc and 7Sd in comparison with gentamicin treated rats (Table 3). The testicular activities of Catalase was considerably ($p < 0.05$) lowered in gentamicin exposed animals in comparison with the controls indicating the impaired testicular antioxidant defense against ROS, which facilitated oxidative stress induction.(Table 4). A significant increase in the generation of testicular hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) due to gentamicin exposure with respect to the controls, showing the testicular ROS generations and induction of oxidative stress(Table 5).

Testicular androgenic enzymes and plasma testosterone showed significant variation among the three groups (Table 6).

Histopathological investigations

a) Testis

In the histopathological staining of control rat testis, arrow indicated well differentiated germ cells with respect to spermatogonia including spermatid and sperm. The centre lumen showed the presence of matured sperm cells. In the gentamicin treated rats testis, circle indicated coagulative necrosis and arrow showed loss of seminiferous tubule, thereby substantiating the toxicity profile of gentamicin on testicular tissue. Vitamin A administration resulted in normal spermatagonia (circle) and densely populated sperms, indicating spermatogenesis (arrow), against gentamicin toxicitiy.

Table 1. Effects of Gentamicin, Vitamin A on epididymal sperm count and % sperm motility

Group	Epididymal sperm count (million/g cauda epididymis)	% Sperm Motility
I	273.17±12.7	72.0 ± 2.6
II	129± 6.8	51.0 ± 2.9
III	242.17 ± 13	58.5 ± 1.9

Table 2. Effects of Gentamicin, Vitamin A on the head, tail and total sperm abnormalities

Group	Sperm abnormalities (%)		
	Head	Tail	Total
I	2.05 ± 0.12	3.78 ± 0.15	5.83±0.25
II	6.01± 0.14	8.83 ± 0.22	14.83±0.24
III	2.93 ± 0.29	4.98 ± 0.26	7.91±0.46

Table 3. Changes in number of germ cells per tubular cross section at stage VII of spermatogenesis following Gentamicin treatment, Vitamin A supplementation

Group	Asg	pLSc	mPSc	7Sd
I	1.54±0.07	17.2±0.93	19.1±0.86	68.4±1.2
II	0.83±0.05	7.9±0.54	10±0.33	44.4±1.4
III	1.18±0.05	12.5±0.58	14.2±0.80	55.7±0.88

Table 4. Effects of Gentamicin, Vitamin A on the activities of testicular antioxidant enzymes

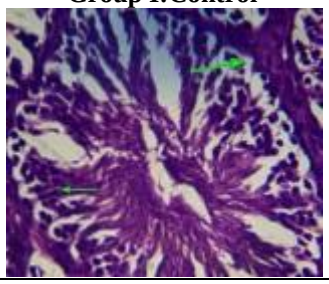
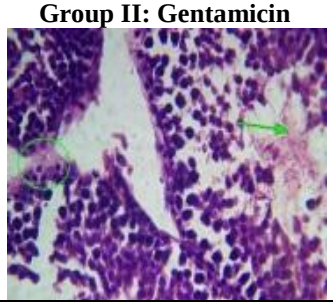
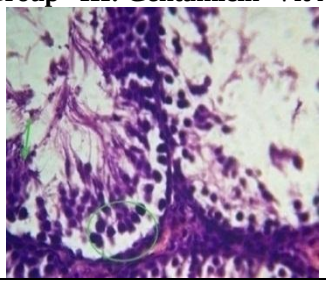
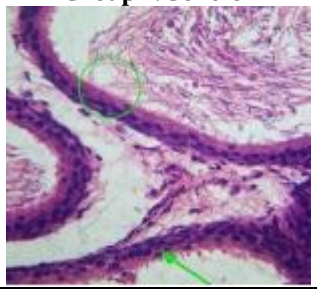
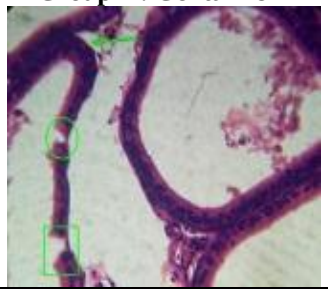
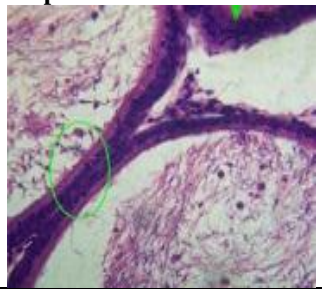
Group	**CAT** (µmol H_2O_2 consumed/min/mg protein)
I	15.9 ± 0.82
II	9.0±0.84
III	13.1±0.85,

Table 5. Effects of Gentamicin, Vitamin A on the activities of oxidation products

Group	**Hydrogen peroxide (H_2O_2)** (µmol/min/mg of protein)
I	1.7 ± 0.25
II	5.6±0.41
III	5.0±0.73

Table 6. Effects of Gentamicin, Vitamin A on the activities of key testicular androgenic enzymes and plasma testosterone

Group	**Δ^5, 3β-HSD** (Units mg tissue/h)	**17β-HSD** (Units/mg tissue/h)	**Testosterone**
I	21.6 ± 1.8	19.7 ± 1.7	3.7 ± 0.17
II	13.8±0.82	12.3±0.97	1.5±0.30
III	17.7±0.73	15.8±0.85	3.1±0.31

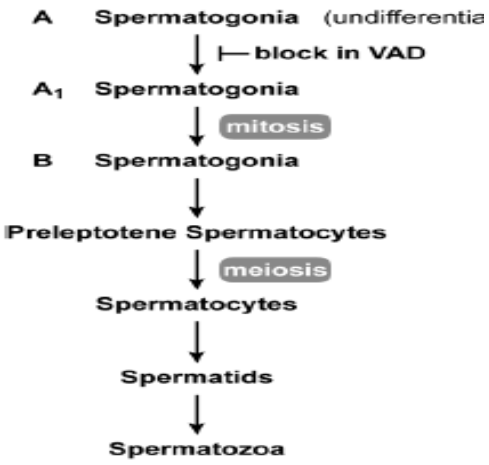
Group I:Control	Group II: Gentamicin	Group –III: Gentamicin+Vit A
		
Group I:Control	**Group II: Gentamicin**	**Group –III: Gentamicin+Vit A**
		

b) Epididymis

The tissue of control animals showed pseudo stratified epithelium with dense population of sperm in centre tubular lumen (circle) and normal architecture of pseudo stratified epithelium (arrow) in epididymis. Gentamicin intoxicated rat tissue displayed epithelial degeneration (square and circle) and vacuolization of epithelial cells (arrow). Administration of Vit A showed normal epithelium with active sertoli cells thereby indicating that the epididymal architecture is preserved

DISCUSSION

Vitamin A is essential for male reproduction. The combined actions of FSH, testosterone, and RA are essential for normal mammalian spermatogenesis. FSH acts on Sertoli cells and may affect spermatogonial populations. RA acts on both Sertoli cells and germ cells and pushes undifferentiated spermatogonia into the differentiation pathway and, eventually, meiotic prophase.Testosterone also acts on Sertoli cells and is necessary for round spermatid formation. Spermatogenesis occurs in the epithelium seminiferous tubules of testis tubules from puberty through adulthood. Undifferentiated (A-type) spermatogonia at the base of the seminiferous epithelium divide mitotically until they enter the differentiation pathway to become A1 spermatogonia. A1 spermatogonia undergo division to A1–A4 and finally B spermatogonia. B spermatogonia split to produce preleptotene (primary) spermatocytes that migrate away from the base of the seminiferous tubule to undergo meiosis. During the first meiotic divison secondary spermatocytes are produced, and after the second meiotic division, spermatids (haploid cells) commence the differentiation process (spermiogenesis) to spermatozoa.



Spermatogenesis in the adult

The testicular activities of H_2O_2 significantly ($p < 0.05$) decreased in gentamicin exposed animals in comparison with the controls indicating the suppressed testicular antioxidant defense against ROS, which facilitates the induction of oxidative stress. The resultant oxidant stress may lead to an increase in germ cell apoptosis and subsequent hypo spermatogenesis. This may result in changes in the dynamics of testicular micro vascular blood flow, endocrine signaling, and germ cell apoptosis. Oxidative stress, therefore, becomes a major and the most probable finding associated with male infertility. Reactive oxygen species (ROS) are very reactive molecules ranked as free radicals owing to the presence of one unpaired electron such as a superoxide ion (O^{-}_{2}), nitrogen oxide (NO) and hydroxyl radical (HO─), administration of this antioxidants with gentamicin was also able to counterbalance the negative effect of gentamicin on sperm count. Scavenging of these free radicals during spermatogenesis by means of antioxidants could provide a promising approach to suppress such damage. Vitamin A deficiency (VAD), the epithelia of the epididymis, prostate, and seminal vesicle is replaced with stratified squamous keratinizing epithelium, and spermatogenesis is arrested [19]. Later work revealed that in the VAD rat testes, undifferentiated spermatogonia, Sertoli cells and a small number of preleptotene Spermatocytes remain (20,21,22), while in the mouse, spermatogenesis is arrested at the spermatogonia stage [23]. Upon addition of vitamin A, spermatogenesis can be reinstituted by stimulating A to A1 Spermatogonial differentiation in a coordinated manner (24, 23).

Recent work supports the conclusion that the vitamin A metabolite, RA, is required both for adult male Spermatogonial differentiation (transition to A1) and the entry into meiosis (25, 26,; 27], Van Pelt and de Rooij found that a large dose of RA (5 mg) administered by injection twice a week, when combined with a RA-containing diet, supported the development of spermatocytes, and their successive development into spermatids in VAD rats supporting that the active form of vitamin A in male reproduction is RA [28]. A CYP26-mediated catabolic barrier comprised of peritubular myoid cells environs the seminiferous tubule, and may prevent RA in the general circulation from reaching cells in the interior of the tubule, thus explaining the requirement of such high doses of exogenous RA [29 ,30]. Within the normal tubule, the Sertoli cell is assumed to generate RA by the action of *Raldh1* (31; 32), and possibly *Raldh2* [31]. *Raldh2* is also found in late pachytene and diplotene spermatocytes, and early stage spermatids.

CONCLUSION

Gentamicin administration led to the increased oxidative stress which caused a considerable reduction in sperm count and sperm movement. When this dose of gentamicin was administrated simultaneously with vitamin A respectively the sperm count level, sperm motility, SOD, LDH, SDH, and testosterone significantly elevated, indicating the protective effect of vitamin A.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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