
Research Article**Upshot of Gentamicin and role of Antioxidant on Spermatogenesis of Albino rats****Dr. T. Kalpana¹, Dr. P. Sri Lakshmi², Dr. B. Naveen Kumar³**¹Assistant Professor, Department of Anatomy,²Associate professor Biochemistry³Department of Anatomy,

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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. Many factors like Drugs, chemotherapy and toxins can generate harmful effect on spermatogenesis and affect sperm normal production. Gentamycin is a synthetic antibacterial agent belonging to the family of amino glycoside. Antioxidants scavengers of free radicals can ameliorate the severity of the effects of antibiotics. The study was performed on male Albino Wistar rats. Animals were obtained from the Animal House, Mamata Medical College, and Khammam, India. The experimental animals were divided into three groups of six rats each as follows. Group I: In Control rats, normal saline was given followed by oil vehicle for 14 days. Group II: Rats received gentamicin for 14 days. Group III: Rats were injected with gentamicin as in group II and concomitantly supplemented with Coenzyme Q10 for 14 days. The testicular tissue antioxidant, Malondialdehyde (MDA) a product of Lipid per oxidation, sperm count, % sperm motility, sperm abnormality, quantitative analysis of spermatogenesis and testicular markers like testosterone, Lactate dehydrogenase (LDH) and Sorbitol dehydrogenase (SDH) were measured in all three groups. In results compared to the control group, considerable drop in epididymal sperm count was observed in Group I. When this dose of gentamicin was administered together with Coenzyme Q10 respectively the epididymal sperm count level was appreciably increased in Group III. The % sperm motility was higher in Group III than other two groups. The testicular antioxidant enzyme was significantly lowered in group II than controls and Group III. MDA was significantly elevated in group II than controls and Group III. In conclusion the Antioxidant therapy could help improve the spermatogenesis and % of sperm motility, SOD, LDH, SDH, and testosterone and decreased the sperm abnormalities and MDA.

Keywords: Spermatogenesis, Gentamycin, Antioxidant, MDA.

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].

Role of CoQ₁₀

Coenzyme Q (CoQ) also known as ubiquinone is a lipid molecule commonly distributed in nature. In mitochondria, similar to other cellular compartments, it exists both in its oxidized state (ubiquinone) and in its reduced one (ubiquinol). [12].

CoQ is made of benzoquinone moiety with an isoprenoid side chain the length of which is 10 units both in man and various mammals; CoQ₁₀ and reduced CoQ₁₀ (ubiquinol-10). Natural CoQ₁₀ is available as a food supplement and extracted from some microorganisms which synthesize CoQ₁₀, the same which is found in humans and other mammals.

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 [13, 14].

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 (2 ml/kg body weight, i.p.), followed by oil vehicle for 14 days.

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

Group III: Rats were injected with gentamicin as in group II and concomitantly supplemented with Coenzyme Q10 (CoQ10) (800µg/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. The organs such as 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 ice-cold 0.1M Tris-HCl buffer (pH 7.4) and centrifugation was performed at 10,000 g 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, LDH and SDH, antioxidant status was evaluated by MDA and SOD in all three groups.

Evaluation of sperm parameters

Epididymal sperm count

The epididymal sperms were counted using a Neubauer chamber. The complete spermatozoa (head with tail) were counted [15].

Percentage sperm motility

The percentage of sperm motility was measured using a light microscope with heated stage as described [16].

Sperm abnormalities:

Morphologically abnormal spermatozoa expressed as percentage after staining with eosin–nigrosin [17].

Quantitative analysis of spermatogenesis:

Quantitative study of spermatogenesis was performed by counting the relative number of each variety of germ cells at stage VII of the seminiferous cycle, i.e. type-A spermatogonia (Asg), preleptotene spermatocytes (pLSc), mid pachytene spermatocytes (mPSc) and stage 7 spermatids (7Sd). Stage VII spermatogenesis was examined because this stage is highly susceptible to testosterone deficiency and also reflects the final stages of spermatid maturation and thus provides evidence of spermatogenesis as a whole [18].

Evaluation of biochemical parameters

Estimation of SOD by NADPH oxidation method [19]. The MDA level was measured according to the concentration of thiobarbituric acid reactive species [20].

Assay of testicular markers

Testicular concentration of testosterone was measured by immuno enzymatic method by ELISA reader. Testicular LDH and SDH activities were assessed based on the NADH oxidation [18].

RESULTS

Sperm count was higher in Group I than II and III. Sperm motility was considerably reduced in the gentamicin group compared to that of the control group. There was a significant ($p > 0.05$) rise in the sperm motility in all the treated groups when compared with the group receiving only gentamicin. The motility value was found to increase in the group receiving Coenzyme

Q10, along with gentamicin (Table 1). The sperm head and tail abnormalities were found to have risen significantly after gentamicin treatment when compared with the controls. When gentamicin was administered along with Coenzyme Q10 or respectively the % sperm abnormalities level was significantly ($p < 0.05$) reduced from, when co administered along with gentamicin (Table 2). Changes in number of germ cells per tubular cross section at stage VII of spermatogenesis (Table 3). The results showed that, gentamicin treated group showed a significant decrease in LDH and SDH levels compared to that of the control group. The Co administration of Coenzyme Q10 with gentamicin improved the activity of the enzymes LDH, SDH and testosterone levels were lower in group II than group I and controls (Table 4). The testicular activities of SOD considerably ($p < 0.05$) lowered in gentamicin exposed animals in comparison with the controls (Table 5). MDA was higher in group II than I and III (Table 6).

Table 1: Effects of Gentamicin, CoQ10 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	225.83 ± 15.7	62.0 ± 2.6

Table 2: Effects of Gentamicin, CoQ10 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	2.95 ± 0.24	4.92 ± 0.09	7.87±0.23

Table 3: Changes in number of germ cells per tubular cross section at stage VII of spermatogenesis following Gentamicin treatment, CoQ10 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	1.08±0.05	11.8±0.54	14.8±0.54	56.2±0.78

Table 4: Effects of Gentamicin, CoQ10 on the activities of key testicular enzymes and plasma testosterone

Group	LDH (n mol of NADH oxidized/min/mg protein)	SDH (nmol of NADH oxidized/min/mg protein)	Testosterone (ng/ml)
I	16.0±0.7	4.9±0.28	3.7 ± 0.17
II	9.6±1.0	2.6±0.24	1.5±0.30
III	13.8±0.56	4.2±0.40	3.1±0.42

Table 5: Effects of Gentamicin, CoQ10 on the activities of testicular SOD

Group	SOD (Unit/mg protein)
I	8.8±0.55
II	2.6±0.61
III	8.0±0.51

Table 6: Effects of Gentamicin, CoQ10 on MDA

Group	MDA (n mol/ mg protein)
I	2.8 ± 0.13
II	7.8±0.67
III	5.4±0.50

DISCUSSION

The testicular activities of SOD significantly ($p < 0.05$) decreased in gentamicin exposed animals in comparison with the controls indicating the suppressed testicular antioxidant defense against ROS, which facilitated 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. RS changes lipid/protein ratio of membranes by affecting polyunsaturated fatty acids and lipids leads to per oxidation product resulting in the formation of MDA. Scavenging of these free radicals during spermatogenesis by means of antioxidants could provide a promising approach to suppress such damage.

When gentamicin was administrated together with Coenzyme Q₁₀, SOD, was significantly ($p < 0.05$) elevated. This data is in harmony with the earlier study done by [21]. Coenzyme Q₁₀ effectively ameliorated the oxidative-testicular damage. When this dose of gentamicin was administrated together with Coenzyme Q₁₀ the testicular content of MDA, the product of lipid per oxidation of the polyunsaturated fatty acid present in cell membrane, was significantly decreased than group II indicating the decreased testicular RS generations and reduction of oxidative stress.

After many recent studies highlighting the implications of CoQ₁₀ in male infertility, the value of CoQ₁₀ treatment in improved semen quality in men with idiopathic infertility [22]. There was a noteworthy increase in Oxidized and reduced CoQ₁₀ concentration both in seminal plasma and sperm cells, along with sperm motility, after 6 months of therapy with 200 mg/day CoQ₁₀. The elevated levels of CoQ₁₀ and QH₂ (reduced CoQ₁₀) in seminal plasma and sperm cells, the improvement of semen kinetic features and treatment, and the proof of a direct correlation between CoQ₁₀ concentrations and sperm motility strongly support a cause-effect relationship. Comparable results were found by [23]. The Co administration of Coenzyme Q₁₀, along with gentamicin improved the activity of LDH and SDH in testis. The results suggested that co-administration of Coenzyme Q₁₀ plays an important role in the maturation and energy metabolism of Spermatogenic cells and spermatozoa [24]. The levels of testosterone reduced significantly following exposure

to gentamicin. This is probably due to necrosis interstitial cells of testis arise from a reduction in the level of this hormone which is due to oxidative stress. A significant reduction in the numbers of Asg, pLSc, mPSc and 7Sd at stage VII of the seminiferous epithelium cycle was observed after gentamicin treatment when compared to the controls. Co-administration of Coenzyme Q₁₀ significantly increased the numbers of Asg, pLSc, mPSc and 7Sd in comparison with gentamicin treated rats. Similarly, studies were reported by Jana et al [25] are also in line with this report in regard with the Spermatogenic disorder reflected by the decreased number of sperm cells at various stages.

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 Coenzyme Q₁₀ respectively the sperm count level, sperm motility, SOD, LDH, SDH, and testosterone significantly elevated. These observations indicate the protective effect of Coenzyme Q₁₀.

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