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GENTAMICIN INDUCED BIOCHEMICAL CHANGES IN AUDITORY CORTEX OF ADULT ALBINO RATS

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ABSTRACT

Background*: The aminoglycoside antibiotics have been the drug of choice for the treatment of tuberculosis and resistant cases of septicaemia, but their use has been selective and restricted due to their known toxicities specially the ototoxicity and nephrotoxicity. Aminoglycosides have good activity against many multi-drug resistant Gram negative bacilli. Gentamicin, due to it cost effectiveness has been used in most of the trials as compared to other aminoglycoside. Most studies documented the peripheral toxicities of gentamicin without any concern of central neurotoxicity.* ***Objective****: the study was planned to observe the effects of gentamicin on the biochemical parameters (specially, sodium, potassium and calcium) in auditory cortex of albino rats.* ***Methods****: It was an experimental study. Twenty rats were randomly divided in two groups; Group I (Experimental: n = 10) received intramuscular injection of Gentamicin for twenty one days and Group II (Control: n =10) received normal saline intramuscularly. Tissue samples were taken from the area around lateral sulcus of rat cortex i.e. auditory cortex. The samples were homogenized and digested in concentrated nitric acid (100mg/ml). The supernatant solution was used for estimation of sodium and potassium by Flame Photometry and calcium level by the method of Clark and Collip.* ***Results:*** *The calcium level was increased; the sodium level was decreased while*

*potassium level remained unaltered.**Conclusion:** Alteration in sodium and calcium level demonstrates central neurotoxicity of gentamicin.*

Keywords: Aminoglycosides, Auditory cortex, Nephrotoxicity, Ototoxicity.

1. INTRODUCTION

The aminoglycosidic antibiotics have been the drug of choice for the treatment of tuberculosis and resistant cases of septicaemia, since the time of their discovery (Bowman and Rand, 1980). Gentamicin is a member of aminoglycoside family of antibiotics produced by micrmonospora pupurea. It can be used in different type of infections, including gram positive and gram negative bacteria. In addition, it has significant effect against pseudomonas. Gentamicin works by inhibiting protein synthesis. It binds very strongly to ribosome's (30 S) sub unit and interferes with protein synthesis. Gentamicin enters the cell by binding to negatively charged phospholipids and enters the cytosol via electron transport linked system and thus need oxygen and ATP to enter cytosol and be effective. Therefore, gentamicin antibiotics are effective only in aerobic bacteria. The most common clinical application (either alone or as part of combination therapy) of the gentamicin is in the treatment of serious infections caused by aerobic gram-negative bacilli. Gentamicin has also been used for the treatment of selected staphylococcal and enterococcal infections. Gentamicin is the usual all-purpose agent of choice (Kumana and Yuen, 1994). Gentamicin has been shown to destabilize the outer membrane of Pseudomonas aeruginosa and form holes in the cell wall, independent of its action on ribosomes (Kadurugamuwa *et al.*, 1993). This action of the aminoglycosides may be the most important.

Ototoxicity is the most important adverse effects clinically, and had dominated attempts to rationalize aminoglycoside dosing (Begg and Barclay, 1995). According to Takada *et al.* (1984) disequilibrium and ataxia were main symptoms of vestibulotoxicity. Both acute (reversible) and chronic (irreversible) ototoxicity had been observed. Ganesan *et al.* (1984), described that chronic toxicity was related to aminoglycoside-phosphoinositol binding, leading to altered membrane structure and permeability. According to Kahlmeter and Dahlager (1984), gentamicin toxicity was the most common single known cause of bilateral vestibulopathy. Aminoglycoside antibiotics are known to cross blood brain barrier and whole nervous system is exposed to these drugs. Evidence is available suggesting central neurotoxicity of aminoglycosides (Faruqi and Khan, 1986). Aminoglycosides damage central nervous system by excitotoxic process involving activation of NMDA receptors (Segal *et al.*, 1999) but exact mechanism of CNS toxicity is unknown. Based on the literature, we planned this study to observe Gentamicin induced biochemical changes in auditory cortex of adult albino rat after parenteral administration of drug.

2. MATERIAL AND METHODS

It was an Experimental study. Twenty adult albino rats weighing 130±20 grams were obtained from Central Animal House, J. N Medical College, AMU, Aligarh. They were divided into two groups. Group I: Experimental (n = 10) received an injection of 135 mg/kg body weight of Gentamicin intramuscularly for twenty one days (Gentamicin WHO food Additives series 34, www.inchem.org\documents). Group II: Controls (n = 10) received normal saline in same volume

by intramuscular route for twenty one days. The rats were kept in plastic cages in a room 12:12 light/dark photoperiod, temperature of 20-30C and relative humidity of 50-60%. Ethical approval was sought and received from the Department of Anatomy, JN Medical College, AMU, Aligarh, UP, India.

Samples were obtained from the area around lateral sulcus of rat cortex to observe biochemical changes. The area around the lateral sulcus of rat cerebral cortex is the site of primary auditory area. After obtaining samples from the auditory area of cerebral cortex (control and experimental), tissue samples are weighed on digital weighing pan (error of weighing pan is calculated about 0.022 grams). Accurate weight of the samples has been calculated by deducting standard error from estimated weight. Tissue samples were homogenized and digested in concentrated nitric acid (100 mg/ml). Homogenates were centrifuged at 3000 revolution per minutes (rpm) for twenty minutes. The solutions, thus obtained, were used to estimate sodium, potassium and calcium levels. Values of sodium and potassium were estimated by sodium, potassium calorimetric assay done by ELYTE 2 KIT. Values of calcium were estimated by cresolphthalein complexone method. The data was entered and analysed using SPSS 20.0. Mean ±S.D is given for quantitative likes sodium, potassium and calcium. Two in independent sample t test is applied to compare the various concentrations in control and experimental groups. A p value of <.05 will be considered as statistically significant.

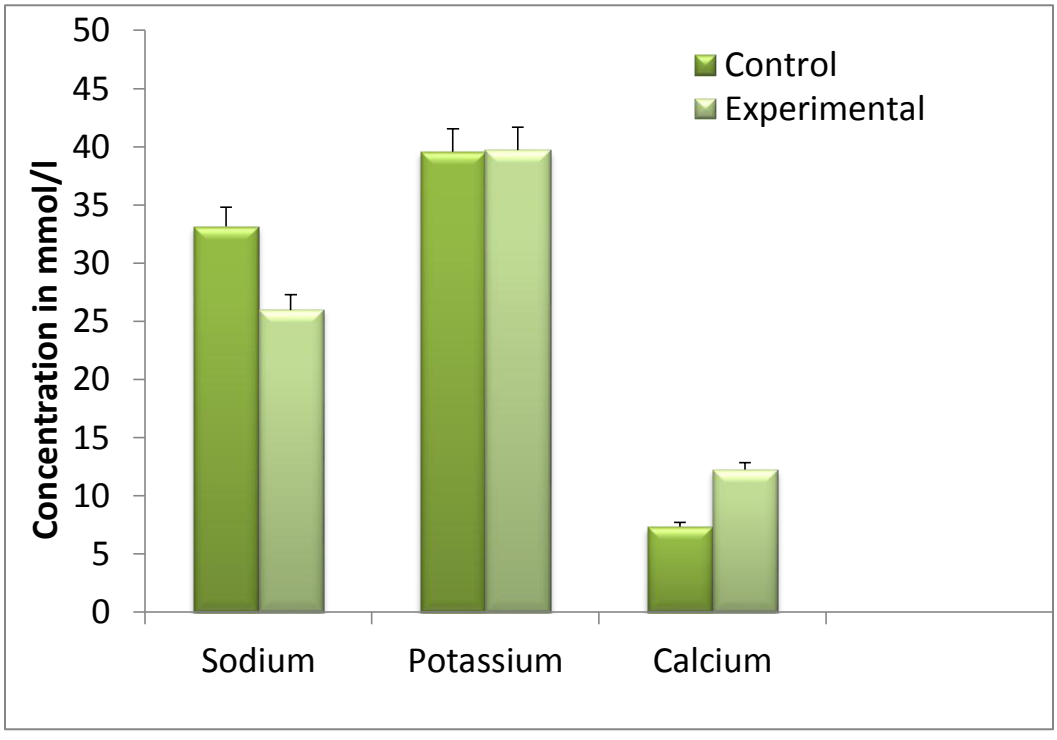
3. RESULTS

The range of sodium concentrations was 24.88 – 34.20 (mmol/l) with a mean concentration of 29.54 $\pm$ 3.78 (mmol/l). The range of potassium concentrations was 37.80 – 40.0 (mmol/l) with a mean concentration of 39.60 $\pm$ 0.55 (mmol/l). The range of calcium concentrations was 6.80 – 12.90 (mmol/l) with a mean concentration of 9.79 $\pm$ 2.56 (mmol/l). Significant difference was observed between sodium concentrations of control and experimental group $p<0.001$, showing that the concentration of sodium was higher in control group as compared to experimental group (33.11$\pm$1.21 VS 25.98$\pm$ 0.72) (mmol/l). Significant difference was also observed between calcium concentrations of control and experimental group $p<0.001$, showing that the concentration of calcium was higher in experimental group as compared to control group (12.26$\pm$0.56 VS 7.33$\pm$0.33) (mmol/l). No significant difference was observed between potassium concentrations in control and experimental group $p=0.584$ (table 1).

Table-1. Concentrations of Sodium, Potassium and Calcium in Control & Experimental Groups

Concentrations	**Mean $\pm$ S.D n= 10 Control**	**Mean $\pm$ S.D n= 10 Experimental**	**p-value**
Sodium	33.11$\pm$1.21	25.98$\pm$ 0.72	<0.001
Potassium	39.56$\pm$0.67	39.70$\pm$0.42	0.584
Calcium	7.33$\pm$0.33	12.26$\pm$0.56	<0.001

Fig-1. Biochemical estimation of concentration of sodium, potassium and calcium in auditory region of brain obtained from control (n =10) and experimental group (n =10) of rats.



4. DISCUSSION

The present study was conducted to observe biochemical effects of gentamicin on auditory cortex of adult albino rat. Findings in the literature are available showing ototoxicity, neuromuscular blockade and neurotoxicity caused by aminoglycosides.

Biochemical findings of present study show significant decrease in mean value of sodium concentration ($p < 0.001$) and significant increase in mean value of calcium concentration ($p < 0.001$) in experimental group receiving gentamicin (Table 1). Potassium concentration was not effected significantly in auditory cortex by gentamicin (Table 1). Faruqi and Khan (1986), conducted study to demonstrate effect of aminoglycosides on central nervous system by estimating sodium, potassium and calcium in different region of CNS. These biochemical results were in conformity with study conducted by Faruqi and Khan (1986) which showed enhanced calcium concentration in cerebrum by aminoglycosidic antibiotics without any significant change in potassium content. In electrophysiological study on free moving cat, Fisenko *et al.* (2003) reported greater sensitivity of aminoglycosidic antibiotics to the auditory cortex as compared to the periphery (cochlea). This study was suggestive of gentamicin neurotoxicity with auditory cortex. Convulsions, encephalopathy, confusion, hallucinations, mental depression and sometimes pleocytosis observed in cerebrospinal fluid of humans, are clinical side effects of gentamicin on central nervous system (Fernando and Jayakodi, 1994).

According to Best and Taylor (1967), both sodium and potassium are important during impulse transmissions but sodium seems to be most important cation involved in the generation of

action potential as well as propagation (Aslam *et al.*, 1990). Calcium is essential for the integrity of the nervous system where it has a major influence on the excitability of this tissue (Peach, 1975). Taking this into consideration an increase in the calcium concentration of auditory cortex of experimental group in present study seems to be meaningful as it might affect the excitability by disturbing the divalent cation homeostasis in the auditory cortex (Kellan *et al.*, 1999).

According to Kellan *et al.* (1999) that glutamate induced neuronal injury involve profound mitochondrial depolarisation that appears to require the synergistic action of calcium and nitric oxide. Neurons exhibiting mitochondrial depolarisation undergo a delayed secondary increase in calcium concentration which progressed to prolonged alteration of calcium homeostasis and ultimately to cell death.

5. CONCLUSION

Exposure of rat to gentamicin for three weeks showed alteration of calcium and sodium concentration at molecular level in auditory cortex. These changes can be explained on the basis of disturbance in cation cellular homeostasis induced by neuronal degeneration.

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