# Quantitative biochemical studies on the effects of neomycin on central nervous system: An experimental study in albino rats

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## Abstract

The aminoglycoside antibiotics have been the drug of choice for the treatment of tuberculosis and resistant cases of septicemia, but their use has been selective and restricted due to their known toxicities specially the ototoxicity and nephrotoxicity. Besides extensive literature on the various side effects of aminoglycoside antibiotics in general and neomycin in particular, there is paucity of literature regarding the mechanism of toxicity. Although most of the studies highlight peripheral toxicities of neomycin, scientists have not sufficiently studied its central neurotoxicity.Taking these into considerations, the study was planned to know the effects of neomycin on the biochemical parameters (specially, sodium, potassium and calcium) in the central nervous system of albino rats. The present study was carried on 12 healthy, adult rats of either sex weighing 180±10 gm obtained from animal house of JNMC, AMU, Aligarh. Neomycin 100mg/kg body weight was given intramuscularly, every day for 10 days to the experimental animals and equal volume of distilled water, in identical manner to the control animals. The animals were decapitated on 10$^{th}$ day; brain and spinal cord were removed within 30 seconds and blotted on filter paper. The cerebrum, cerebellum, brain stem and spinal cord were separated and weighed to the nearest of milligram on a single pan electrical balance. Tissue 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 and zinc and copper by the method of Donaldson and Pierre. Separate homogenate in distilled water were centrifuged to get the supernatant for the estimation of total proteins in all the four parts by the method of Folin and Crocalteu. It was found that all the three cations i.e. sodium, potassium and calcium showed an increment in different regions of CNS and a zone of inhibition was observed after overnight incubation of CNS homogenate. Although there was a uniform response of different region of CNS for potassium concentration, the sodium was increased in the cerebrum, cerebellum and brain-stem while calcium was only increased in spinal cord. It was concluded that neomycin penetrates the central nervous tissue and central cause of muscular weakness after neomycin intoxication can't be ruled out.

## Introduction

Since their discovery, the aminoglycosidic antibiotics have been the drug of choice for the treatment of tuberculosis and resistant cases of septicemia [1] . But at the same time, their use has been selective and restricted in view of their known toxicities particularly the ototoxicity and nephrotoxicity [2] therefore, considered to be used with caution specially in patients with poor renal function [3].The risk from administrating neomycin is much greater because it can cause deafness in very small doses[3].In view of all this, the parenteral administration of neomycin has been discarded. On the other hand, neomycin is being extensively used in the form of creams, solutions and tablets. Neomycin has also been used intraperitoneally for the treatment of peritonitis.

It is generally believed that during local application, absorption is negligible and hence toxicity should not be expected [3]. Keeping this in view, the clinicians are extensively using neomycin locally, in very large amounts some times, depending upon the size of the wound, e.g. in case of burns. On the other hand,

reports are pouring in about the nephrotoxicity and ototoxicity of neomycin after topical application and irrigation of wounds[4].There are also reports of renal failure, deafness and brain lesions following irrigation of mediastinum with neomycin[5]. Neomycin has proved to be ototoxic even after oral administration [6]. Neomycin is very slowly absorbed by the alimentary tract, but if large doses are given for long periods e.g., for prevention of hepatic coma, the drug may accumulate in blood to toxic level [7].

Several reports on neomycin toxicities are based on animal experiments, e.g. [8] used cats for his studies. [9] had given due importance to neuromuscular blockade as one of the most important toxic effects of neomycin. Their conclusion is based on the reports of [10, 11, and 1213].

In spite of extensive literature on various side effects of aminoglycosidic antibiotics in general and neomycin in particular, very little is known regarding the mechanism of toxicity. Although most of the studies highlight peripheral toxicities of neomycin, scientists have not sufficiently studied its central neurotoxicity. Penetration by neomycin of blood-CSF barrier in adult and blood-brain barrier in neonates (due to its immature nature) makes CNS prone to its toxic effects [14].

Most of the other aminoglycoside antibiotics other than neomycin have been thoroughly investigated in central nervous system in experimental studies [15,16].

Taking into consideration the aforementioned views, the study of neurotoxicity of neomycin after parenteral administration was planned to know the biochemical changes in the central nervous system of albino rats.

## Material and methods

Twelve albino rats weighing about $180 \pm 10$ gm were obtained from the Animal House of the Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh. Animals were divided in two groups (control and experimental) of six rats each and were fed on a diet of gram, green vegetables and tap water ad libitum.

### *Neomycin administration*

Neomycin manufactured by Unichem India Pvt, Ltd., was used in the experiments. The experimental albino rats were injected with neomycin 100 mg/kg body wt., intramuscularly, every day for a period of 10 days. Control group of albino rats were injected with equal volume of distilled water in an identical manner.

### *Removal of brain and spinal cord*
The albino rats were sacrificed by decapitation on the 10th day. The brain and the spinal cord were removed within 30 seconds and blotted on filter paper. The cerebrum, cerebellum, brain stem and the spinal cord were separated and weighed to the nearest of milligram on a single pan electrical balance.

### *Biochemical estimations*
Tissue samples were homogenized and digested in concentrated nitric acid *(100* mg/ml).Homogenates were centrifuged at 3000 rpm for 20 mts. The supernatant solutions thus obtained, were used for the estimation of sodium and potassium by Flame Photometry[17]and calcium levels by the method of [18].The same solution was also used to determine the level of zinc and copper[19].Separate homogenates in distilled water were centrifuged similarly to get the supernatant for the estimation of total proteins in all four parts by the method of [20]. Data were analyzed by using Student's't' test,

### Antibiotic sensitivity test and assay
#### *A). Principle of the test*
The principle includes the preparation of a concentration gradient of the antibiotic in a nutrient medium and the observation of whether or not growth takes place when the medium is seeded with indicator backing and incubated.

#### *B). Cup TEST*
It is a sensitivity test for the presence of antibiotic (Neomycin) in the tissue ( CNS homogenate). Homogenates of the different regions of the CNS in distilled water was placed in circular holes cut with a cork borer in a uniformly seeded thin homogenous layer of Oxford strain of staphylococcus aureus (National Collection of type culture No. 6571) culture plate with nutrient agar. After overnight incubation at 37°C, next day the zone of inhibition was observed.

## Results

It was found that there was generalized decrease in the protein level in the different part of the CNS though it was significant in cerebrum and cerebellum only (Table 1). There was differential response in cationic level in different parts of the CNS. Zinc was significantly increased in cerebrum, brain stem and spinal cord (Table 2). Increment in copper was significant in cerebrum and highly significant in spinal cord (Table 3). Sodium was increased significantly in brain stem and in highly significant manner in cerebrum (Table 4). Potassium was significantly increased in all parts of the CNS (Table 5) but calcium was increased significantly only in spinal cord (Table 4).
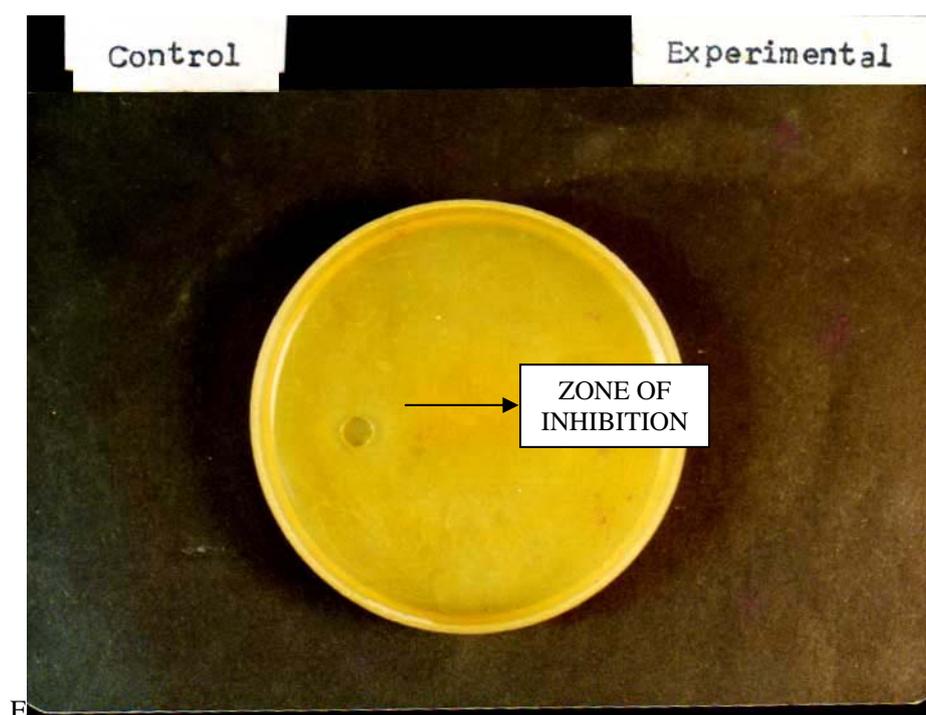
***Figure 1.*** *Nutrient agar plate showing a zone of inhibition.*

**Table 1:**  *Protein values expressed in mg/g*
*Mean ± SD, (N= 6)*

| Part of CNS | Mean ± SE(mean) | | P value |
|---|---|---|---|
| | **Control** | **Experiment** | |
| Cerebrum | 35.65 ±0.92 | 31.34 ± 0.84* | < 0.05 |
| Cerebellum | 34.00 ±1.52 | 23.60 ±0.91*** | < 0.001 |
| Brain stem | 38.20 ±0.12 | 34.60 ±0.98 | - |
| Spinal cord | 38.40 ±0.21 | 34.75 ±0.16 | - |

**Table 2**. *Zinc values expressed in µg/gm,*
 *Mean ± SD (N= 6)*

| Part of CNS | Mean ± SE(mean) | | P value |
|---|---|---|---|
| | **Control** | **Experiment** | |
| Cerebrum | 13.80 ± 0.67 | 30.15 ± 4.53** | < 0.01 |
| Cerebellum | 14.66 ± 0.92 | 13.80 ± 0.72 | - |
| Brain stem | 7.93 ± 1.02 | 13.33 ± 0.53** | < 0.01 |
| Spinal cord | 3.80 ± 0.24 | 11.23 ± 0.88*** | < 0.001 |

**Table 3.** *Copper values expressed in µg/gm*
*Mean ± SD (N= 6)*

| Part of CNS | Mean ± SE(mean) | | P value |
|---|---|---|---|
| | **Control** | **Experiment** | |
| Cerebrum | 3.38 ± 0.25 | 6.35 ± 0.85* | < 0.05 |
| Cerebellum | 6.03 ± 1.24 | 4.65 ± 0.84 | - |
| Brain stem | 3.06 ± 0.07 | 3.33 ± 0.53 | |
| Spinal cord | 3.20 ±0.12 | 2.06 ± 0.10*** | < 0.001 |

**Table 4.** *Sodium values expressed in μg/g*
*Mean ± SD (N= 6)*

| Part of CNS | Mean ± SE(mean) | | P value |
| --- | --- | --- | --- |
| | **Control** | **Experiment** | |
| Cerebrum | 0.07 ± 0.01 | 0.14 ± 0.01*** | < 0.001 |
| Cerebellum | 0.09 ± 0.01 | 0.10 ± 0.01 | - |
| Brain stem | 0.08 ± 0.01 | 0.10 ± 0.01* | < 0.05 |
| Spinal cord | 0.08 ± 0.01 | 0.07 ± 1.92 | - |

**Table 5**. *Potassium values expressed in mEq/g*
*Mean ± SD, (N= 6*

| Part of CNS | Mean ± SE(mean) | | P value |
| --- | --- | --- | --- |
| | **Control** | **Experiment** | |
| Cerebrum | 0.03 ± 0.00 | 0.03 ± 0.00* | < 0.05 |
| Cerebellum | 0.03 ± 0.00 | 0.03 ± 0.00* | < 0.05 |
| Brain stem | 0.04 ± 1.38 | 0.03 ± 1.15* | < 0.05 |
| Spinal cord | 0.02 ± 0.42 | 0.02 ± 1.91* | < 0.05 |

**Table 6.** *Calciun values expressed in mEq/gm*
*Mean ± SD (N= 6)*

| Part of CNS | Mean ± SE(mean) | | P value |
| --- | --- | --- | --- |
| | **Control** | **Experiment** | |
| Cerebrum | 0.28 ± 0.02 | 0.19 ± 0.03 | - |
| Cerebellum | 0.20 ± 0.02 | 0.18 ± 0.01 | - |
| Brain stem | 0.16 ± 0.01 | 0.15 ± 0.01 | - |
| Spinal cord | 0.18 ± 0.01 | 0.34 ± 0.03** | < 0.01 |

## Discussion

Aminoglycosides are known to have some effects on mammalian protein synthesis [21]. In our experiment the protein was significantly reduced in the cerebrum and cerebellum of the experimental animals; thus providing support to the aforementioned statement. Previous reports suggest some alteration in the protein metabolism during the central neurotoxicity of aminoglycoside antibiotic; resulting in the decreased protein content of spinal fluid [22].Studies indicated that carbohydrate metabolism and energy utilization are affected by the aminoglycosides [23] and therefore the enzymes (proteins) concerned might be influenced by these antibiotics. Interestingly, the proteins were not affected in brain-stem and the spinal cord of the experimental animals. Such differential effect of aminoglycosides on the central nervous system has been reported earlier also [15].

The basis of aminoglycoside mammalian toxicity is a disturbance of divalent cations cellular haemostasis [24]. Alterations, therefore observed, in the levels of zinc and copper in different regions of CNS after neomycin intoxication has some relevance. Among the trace elements in the CNS, zinc and copper are of special interest, since they have an interesting regional distribution[19].In our observations zinc showed a higher concentration as compared to copper in different regions of CNS of rats. Similar findings are observed by [16].

An increment of the zinc and copper levels of brain of guinea pig was found by some workers after gentamicin administration [16]. Very much similar are present findings. Levels of zinc and copper in the spinal cord after aminoglycoside toxicity are not cited in previous literature. In present experiment the zinc was increased whereas the copper was reduced in the aforementioned region. This might be due to the disturbance of cellular homeostasis of divalent cations as reported earlier (24). Moreover in cerebrum the copper level was increased after neomycin intoxication. Such differential responses of different parts of CNS have been reported earlier [25] and [15].

It is difficult to speculate on the basis of the changes in zinc and copper levels the damage or effects that would occur in the CNS. However, there are studies available to show that zinc and copper alterations to have disturbing

effects on the embryo [26]. Smart, T.G and Constantini, A have discussed the effects of zinc on neurotransmitters, while Friedrickson, C. J et al have emphasized the toxic effect of zinc on nerve cells following a disturbance of zinc levels[27] and [28], reconsolidating the disturbance in the divalent cations cellular homeostasis as the basis of aminoglycoside mammalian toxocicity.

All the three cations i.e. sodium, potassium and calcium showed an increment in different regions of CNS after neomycin intoxication. Although there was a generalized increase in the concentration of potassium in the different parts of the CNS, the sodium was altered only in the cerebrum and the brain stem, while the calcium was affected in the spinal cord exclusively.

The changes in aforementioned cation levels after streptomycin and kanamycin treatment in rats have been reported earlier [29]. In their reports, kanamycin affected only sodium concentration and that only in cerebellum; streptomycin has influenced all the three cations. Walz et al have described light-dependent repetitive calcium spikes induced by the extracellular application of neomycin in honeybee drone photoreceptor [30]. Although both sodium and potassium are important during impulse transmissions [31], [32], sodium seems to be most important cation involved in the generation of action potential [31] as well as prorogation [33]. Taking into consideration therefore the alterations in sodium ions, the neomycin shows more affinity for cerebrum and brain stem, while streptomycin shows more affinity for cerebellum and spinal cord [29]. This preferential effect of the streptomycin and neomycin on the CNS is exactly according to their nature. Streptomycin is known to produce predominantly vestibular effects, while neomycin primarily affects auditory functions [34]

Calcium is essential for the integrity of the nervous system where it has a major influence on the excitability of this tissue [35]. Taking this into consideration an increase in the calcium concentration in the spinal cord after systemic neomycin intoxication in present study seems to be meaningful as it might affect the excitability by disturbing the divalent cation homeostasis in the spinal cord [24]. Although specific literature is lacking to support the findings, previous results suggest that the neuronal damage in the hippocampus was associated with the marked increase in the amount of calcium deposits [36]. Lithium sulphate produced increase in the brain calcium levels [37]. Acute uraemia was also associated with increased calcium in brain [38].In our study both calcium and zinc was increased in the spinal cord. Interestingly the role of disturbed zinc level on calcium has been emphasized [39].It provides a hint that is still to be proven whether it is zinc affected first and consequently affecting the calcium or both are affected simultaneously by the neomycin. Therefore, one may consider the possibility of a cen-

tral cause of muscular weakness after neomycin intoxication in addition to known curare like effect of aminoglycoside [40, 41, and 42] on the neuromuscular junction.

## Conclusion

The following conclusions are derived on the basis of inhibition test of CNS homogenate and disturbance in the divalent cations cellular homeostasis.

1. Neomycin penetrates the central nervous tissue.
2. Central neurotoxicity of neomycin may also contribute to the muscular weakness along with peripheral toxicity (already reported by earlier workers, reference of the same has been given).

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