

# Antioxidant Effects of Methylcobalamin on Cerebellar Granule Cells (A 6 Week Quantitative Study in Albino Rats)

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## ABSTRACT

**Objective:** As there is deficient text on the regenerative capacity of Methylcobalamin on neuronal tissue therefore this original study was carried out to document its effects on granule cell degraded by lithium carbonate.

**Study Design:** Observational Experimental study

**Place and Duration of Study:** This study was conducted at the Animal House of Basic Medical Sciences Institute (BMSI) JPMC, Karachi from 1st June and ended at 14th July 2013.

**Materials and Methods:** 15 male albino rats were selected weighing 195-200 grams and divided according to the treatment duration of the research was 6 weeks. Group A had 5 animals on lab diet, Group B consisted of 5 animals on lithium carbonate. Lithium carbonate (Adamjee pharmaceuticals) was given 25 mg/kg/day in saline once every day for six weeks. Group C had 5 animals on Injection Methylcobalamin (Amson vaccines and pharmaceuticals) 200 µg/kg/day / intraperitoneally for 6 weeks.

**Results:** Group C showed restoration of the number of granule cells

**Conclusion:** Our present study proved that Methylcobalamin plays a vital role as an antioxidant vitamin

**Key Words:** Antioxidant, Granule cell, Scavenger, Superoxide radical

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## INTRODUCTION

Neuronal cell stabilizer Methylcobalamin<sup>1</sup> discovered more than seventy years ago<sup>2</sup> is available in active form of cobalamin a vitamin essential for many cellular functions like DNA synthesis.<sup>3</sup> It augments the vascular endothelial cell metabolism and antioxidant capacity of nerves.<sup>4</sup>

Vitamin B12 decreases the chances of central nervous system degeneration<sup>5</sup> and it is a potent scavenger of superoxide radicals.<sup>6</sup>

The beneficial effects of vitamin B12 are well documented and its role is appreciated in reversal of cerebellar disease.<sup>7</sup>

Cerebellum is considered as a focal structure responsible for major Central nervous system disorders,<sup>8</sup> it is the major part of hindbrain<sup>9</sup>, consisting of two cerebellar hemispheres each hemisphere comprises a white and gray matter.

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The gray matter has three layers the outer molecular layer middle, Purkinje cell layer and innermost is the granule cell layer,<sup>10</sup> and it consists of 99 percent of granule cells.<sup>11</sup>

Lithium destroys cerebellar granule cells.<sup>12</sup> which leads to irreversible cerebellar damage.<sup>13</sup>

## MATERIALS AND METHODS

MY experimental research was conducted at Basic Medical sciences institute JPMC Karachi for a period of six weeks. Fifteen male albino rats weighing 190-200 grams were kept in the animal house for six weeks. The selected albino rats were randomized into three groups. Group A was the control on lab diet, Group B received Lithium carbonate taken from Adamjee Pharmaceuticals at a dose of 25 mg/kg/day<sup>14</sup> in saline solution once every day for six weeks, Injection Methylcobalamin 200mcg/kg/day IP<sup>15</sup> for six weeks. The albinos were fed laboratory chow and then they were decapitated. The brain was then removed and hindbrain was identified, then the cerebellum after removal from the surrounding structures was preserved granule in haematoxylin and eosin and the other half in formal thionin. Section 4 microns thick were made and stained for micrometry under light microscope. My results of granule cells count were done with counting reticule for all three groups. Analysis of the data was completed by using student T test. Results were expressed as mean SEM p<0.001 was considered statistically highly significant and calculations then

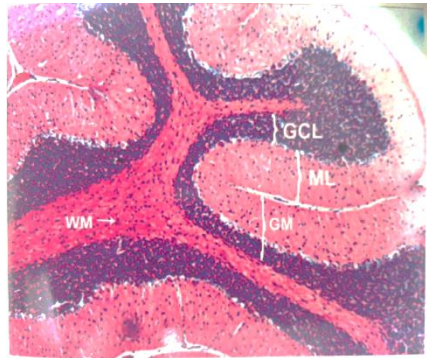
were documented by using computer software SPSS version 16.

**RESULTS**

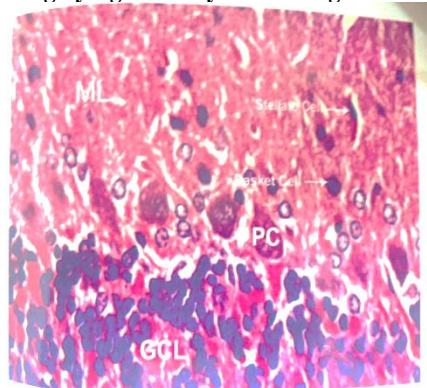
The granule cells count of Group C at 6 weeks was highly significantly increased as compared to that of in group B in which the granule cells count were highly significantly decreased  $p < .001$  as compared with Group A and Group C.

**Table No.1: Mean values of the cerebellar Granule cells count (cells/ $\mu\text{m}^2$ ) among various groups**

Major Groups	No. of Subjects	6 <sup>th</sup> Week		P-Value
		Mean	SEM	
A2 Normal Diet (ND)	5	68.4	0.19	0.001
B 2ND + Lithium Carbonate	5	49.2	0.23	0.001
C2 ND + Lithium + Inj. Methy	5	65.4	0.14	0.001



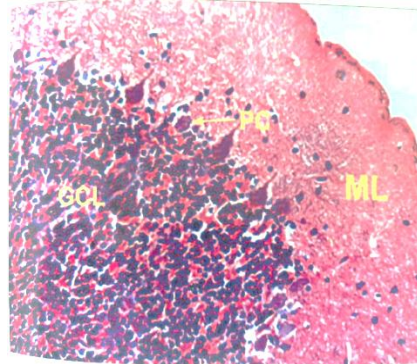
**Figure No.1: Haematoxylin and eosin stained 4µ section of cerebellar cortex of Group A 2 (animals on lab diet) shows a highly significantly increased granule cells in GCL**



**Figure No.2: Haematoxylin and eosin stained 4µ sections of Group B2 (animals on lab diet +Lithium carbonate) shows highly significant decreased granule cell count and apoptotic granule cells**

A highly significantly increased granule cells count was observed in Group A at six weeks. A highly significantly decreased granule cells count was

documented in animals of Group B treated with Lithium carbonate at six weeks. The Group C showed a highly significantly increased granule cells count as the animals were injected with Methylcobalamin



**Figure No.3: Haematoxylin and eosin stained 4µsections of Group C2 shows a highly significantly (animals on lithium carbonate and methylcobalamin) increased granule cells count and normal morphology of granule cells**

**DISCUSSION**

Cerebellum is the key stone for co-ordination, muscle tone and equilibrium. It consists of two cerebellar hemispheres. The cerebellar hemispheres has three well defined cerebellar cortex which are outer Molecular layer, the middle Purkinje cell layer, the innermost is the Granule cells layer.<sup>16</sup>

The innermost Granule cells are the largest group of cerebellar neuronal cells. The Granule cells in the innermost layer of cerebellar cortex form the thickest neuronal layer of the cortex and are essential for motor learning. They have a small cell body with few dendrites, receiving input from a single mossy fiber.<sup>17</sup>

My research showed a paucity of granule cells count in Lithium treated group B as my findings are in agreement with Yousafani<sup>18</sup> (et al 2020) they in their study have proved that lithium causes cellular oxidative stress due to lysosomal membrane leakage.

Lysosomal disruption enhances reactive oxygen species (ROS). This ROS causes mitochondrial damage leading to apoptotic or cell death.<sup>19</sup>

Granule cell degradation<sup>20</sup> may be due to increment of Caspases 3 causing the neuronal nucleus catastrophe and death.

Wang and Xu<sup>21</sup> have documented in their study that Methylcobalamin is a scavenger of reactive oxygen species which decreases intracellular oxidative stress and neuronal cell apoptosis, the same is in accordance with our experimental research as we found an increase of granule cells count in group C in which my animals ingested lithium carbonate and they were injected with Methylcobalamin. This may be due to the fact that vitamin B12 treatment obviously decreased the amount of Caspases in neurons, resulting in decreased cell death. Our research proved that the vitamin B12-treated

group C had attenuated Lithium induced neuronal cell apoptosis.

## CONCLUSION

Our study proved the beneficial effects of Methylcobalamin decreased the toxic effects of light metals like lithium carbonate on cerebellar cortex. This study plays a role model for neurologists to prescribe Methylcobalamin frequently in cerebellar neuronal diseases.

### Author's Contribution:

Concept & Design of Study: Tazeen Kohari  
 Drafting: Tazeen Kohari  
 Data Analysis: Tazeen Kohari  
 Revisiting Critically: Tazeen Kohari  
 Final Approval of version: Tazeen Kohari

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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