The protective role of the Methylcobalamin on Purkinje cell diameter in ir-radiated cerebellum of young albino rats: A morphometric study.

Amatul Sughra [1,*], Nayab Qazi[2], Fahmida Gul[3], Hemant Kumar[4], Bashir Shaikh[5], Pushpa Goswami[6]

ABSTRACT:
Objective: To observe the radioprotective role, if any, of Methylcobalamin on Purkinje cell diameter in irradiated cerebellum of young albino rat.
Methodology: This experiment was carried out at Hamdard University's Department of Anatomy in Karachi from February to May 2024. Fifty newborn albino rats were randomly assigned to five groups: A (control), B, C, D, and E. Each group was subsequently separated into two subgroups based on two and four-week observation intervals. Group B: Received a single dose of 3 Gy gamma radiation on the eighth postnatal day (PND). Group C received the identical irradiation treatment as Group B, followed by intraperitoneal delivery of methylcobalamin (200 µg/kg/day). Group D: treated with methylcobalamin from PND 1 and irradiated on PND 8. Group E received methylcobalamin from PND 1 without irradiation. Brains were dissected, the cerebellum was prepared for histology, and Purkinje cell diameters were measured.
Results: A marked reduction ($p < 0.001$) in Purkinje cell diameter was observed in subgroups B1 and B2 compared to controls A1 and A2. methylcobalamin treatment in irradiated animals (Group C) resulted in significant ($p < 0.05$) recovery of Purkinje cell size compared to Group B. Pre-treatment with methylcobalamin (Group D) produced a moderately significant ($p < 0.01$) improvement. In non-irradiated methylcobalamin-treated rats (Group E), cell diameters increased significantly ($p < 0.05$ in E1 vs A1; $p < 0.001$ in E2 vs A2).
Conclusion: Gamma irradiation caused noticeable cerebellar damage and reduced Purkinje cell size. Methylcobalamin administration mitigated these effects, indicating a potential neuroprotective role against radiation-induced injury.

Key words: Purkinje cells, Methylcobalamin, irradiation, Cerebellum, Albino Rat

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Introduction:

The term “radiation” originates from the Latin word for “ray of light”, that encompasses a spectrum of energies ranging from non-ionizing to ionizing types.[1,2] Ionizing radiation is known to fragment DNA and trigger apoptosis in proliferating cells[3], disrupt membrane permeability, damage organelles, and interfere with cell division.[4]
Radiation-induced cellular injury is primarily driven by oxidative stress, with hydroxyl radicals playing a particularly destructive role. These radicals are extremely reactive molecules capable of causing direct breaks in DNA strands,[5] leading to impaired replication and cellular death. Within the central nervous system (CNS), free radicals can trigger a cascade of damaging events, including excitotoxicity, disruption of normal metabolic pathways, and alterations in intracellular calcium balance. Such disturbances compromise neuronal stability and function.[6] The immature brain is especially vulnerable; the period from birth to approximately postnatal day 18 is regarded as the most radiosensitive developmental window, during which even mild insults can result in long-lasting structural and functional alterations.[7,8]
Although physical measures of radiation protection are well established and effective in reducing unnecessary exposure,[9] there is growing interest in pharmacological strategies to counter oxidative stress–mediated damage.[10] A range of antioxidant compounds, including vitamins A, C, and E, glutathione, melatonin, selenium, and the calcium channel blocker diltiazem, have been shown to exert neuroprotective effects by neutralizing free radicals or enhancing endogenous defense systems.[11-16]
Mecobalamin, an active coenzyme form of vitamin B12, is efficiently transported to nervous tissue and plays a crucial role in DNA synthesis, myelin maintenance, and neuronal repair.[17-19] Adequate vitamin B12 levels are essential for optimal brain function and may help prevent excitotoxic damage.[20,21] Deficiency has been linked to various neuropsychiatric manifestations, such as cognitive decline, memory loss, and mood disturbances, dementia & Alzehimer’s.[22,23]

Objective:

The present study was undertaken to evaluate whether methylcobalamin could mitigate gamma radiation-induced injury to Purkinje cells in the developing cerebellar cortex of young albino rats.

1. Assistant professor; Department of Anatomy. Hamdard University, Karachi.
2. Assistant professor; Department of Anatomy. Indus Medical College Tando Muhammad Khan
3. Assistant professor; Department of Anatomy. Liaquat University of Medical and Health Sciences, Jamshoro
4. Professor, Department of Anatomy. Hamdard University, Karachi
5. Professor of Anatomy, Department of Anatomy. Hamdard University, Karachi
6. Professor of Anatomy; Department of Anatomy. Liaquat University of Medical and Health Sciences, Jamshoro

**=corresponding author* :
Email: dr.amatulsughra@gmail.com

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Methodology:
This experimental work was carried out at the Department of Anatomy, BMSI, JPMC, Karachi. 5-6 young female albino rats (Charles Brooklyn strain) were chosen to serve as the paternal generation. The animals were housed in a controlled environment with a temperature of 21 ± 2 °C and a 12-hour light/dark cycle (lights turned on at 6:00 a.m.). They had free access to lab food and water. These albino rats married at animal house BMSI, JPMC, Karachi. 50 PND (postnatal day) 01 litters were obtained and divided into five groups: A, B, C, D, and E, each consisting of 10 albino litters. Each group was subsequently separated into two subgroups (A1-A2, B1-B2, C1-C2, D1-D2, and E1-E2), each with five litters, based on the duration of treatment (two weeks or four weeks).
Group A: (A1-A2) The control group received no irradiation or treatment.
Group-B: (B1-B2) received a single dose of 3Gy gamma irradiation on PND 08.
Group-C: (C1-C2) got the same irradiation as group B on PND 08, as well as an intraperitoneal injection of methylcobalamin at a dose of 200 mcg/kg body weight daily.
Group-D: (D1-D2) got daily injections of methylcobalamin as group C, beginning on PND 01, then irradiation as group B on PND 08.
Group-E (E1-E2) received methylcobalamin injections from PND 01, just like group D.
The animals were weighed on PNDs 01, 15, and 29 with a computerized electronic balance. At the end of the intervention, the animals were sedated with ether and placed on the dissection board. Their brains were taken, and the cerebellum was separated. The weights of the cerebellum were measured using a computerized electronic balance. The cerebellum was divided into right and left halves at the mid-sagittal plane using a stereoscope, fixed in 10% formal saline, then processed and embedded in paraffin. Sections of 3 µm thick were cut and stained with haematoxylin and eosin stain (H&E). The sections were investigated for thorough morphology and morphometry.
The mean diameter of Purkinje cells (with visible nuclei) was measured in both the long axis (from base to apex) and the horizontal axis (from side to side) using an ocular counting scale under an 8X ocular and a 100X objective light microscope. The student 't' test was used to compare the quantitative changes of irradiated and irradiated + methylcobalamin treated animals to control animals. A p-value of < 0.05 was considered significant.

Results:
As the results we observed were different in each group, these are reported separately for each animal group.
Control group-A: Purkinje cells were very well aligned in monolayer in between outer molecular layer and inner granular layer. The bases of Purkinje cells were facing granular layer and apex and dendrites were extending towards the pial surface in molecular layer. They were well stained with visible nuclei and cytoplasm. Occasional apoptotic cells were seen, which was normal feature of Purkinje cells. The mean values of diameter of Purkinje cells are revealed in table -1. There was insignificant (P>0.05) decrease in the mean values of diameter of Purkinje cells in subgroup A2 in comparison to A1.
Irradiated Group-B: The monolayer of Purkinje cells in subgroups B1 and B2 was distorted and disoriented, and the Purkinje cells were scattered randomly with majority of the cells having abnormal shapes, faintly stained cytoplasm with pyknotic nuclei and vacuoles. Apoptotic figures were high (Figures-1 and 2). The mean values of diameter of Purkinje cells are shown in table 1. There was highly significant (P<0.001) decrease in the mean diameter of Purkinje cells in subgroups B1 - B2 as compared with subgroups A1 - A2.

Table No 1: Values of mean diameter (µm) of Purkinje cells (PCs) in different Groups in young albino rats (mean±SEM)

Groups (n=10)	Sub-groups (n=5)	Treatment given	Mean Diameter (µm) of Purkinje Cell (PCs)	
			2nd Week (PND 15)	4th Week (PND 29)
A	A1	Normal Diet (ND)	12.60 ± 0.225	
	A2			13.04 ± 0.196
B	B1	Irradiation + ND	11.44 ± 0.162	
	B2			11.82 ± 0.218
C	C1	Irradiation + ND + Methylcobalamin	12.06 ± 0.176	
	C2			12.40 ± 0.247
D	D1	Methylcobalamin + ND + Irradiation	12.24 ± 0.176	
	D2			12.78 ± 0.266
E	E1	Methylcobalamin + ND	12.72 ± 0.263	
	E2			13.96 ± 0.252

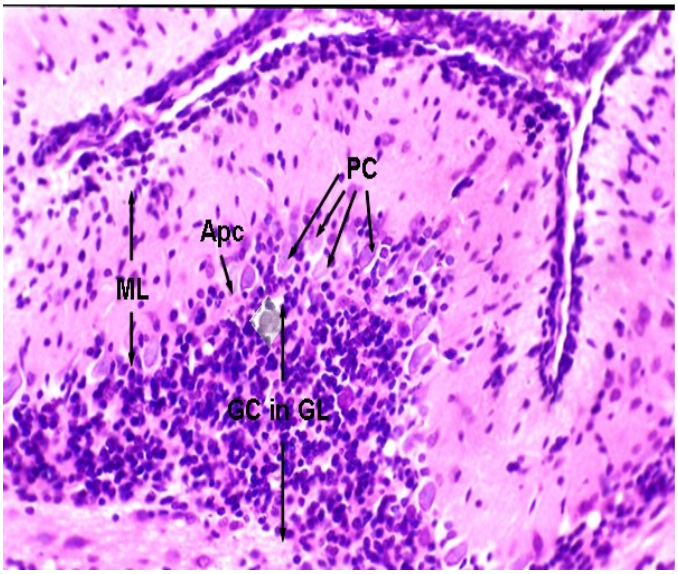

Figure-1
H&E stained, 3µm thick section of cerebellar cortex of irradiated rat, showing distorted monolayer of Purkinje cells (PC), apoptotic Purkinje cell (Apc), molecular layer (ML) and granule cells (GC) in granular layer (GL) after 2 weeks (Photomicrograph x 40).

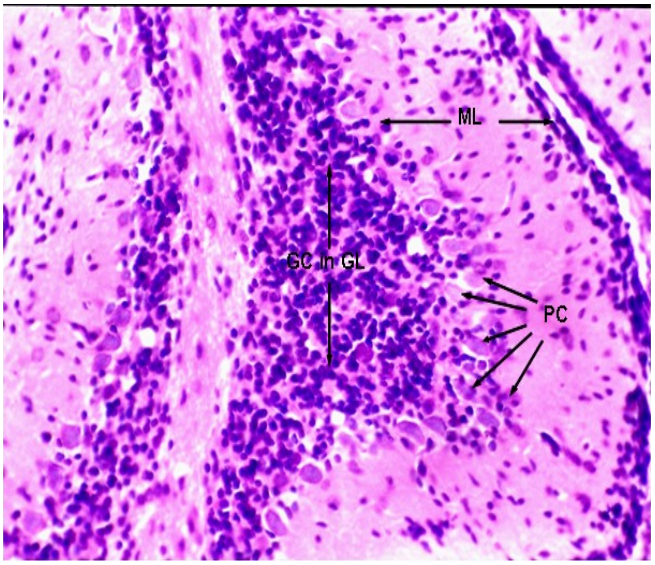

Figure-2
H&E stained, 3µm thick section of cerebellar cortex of irradiated rat, showing distorted monolayer of Purkinje cells (PC), molecular layer (ML) and granule cells (GC) in granular layer (GL) after 4 weeks (Photomicrograph x 40).

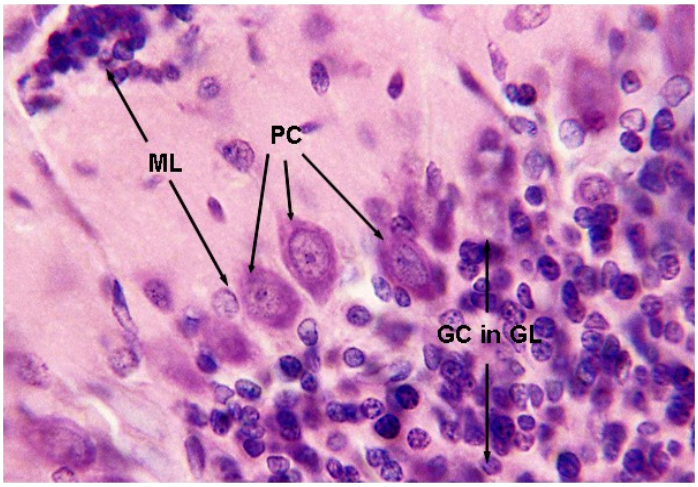

Figure-3
H&E stained, 3µm thick section of cerebellar cortex of irradiation rat, treated with Methycobal, showing reorganized monolayer of Purkinje cells (PC), molecular layer (ML) and granular layer (GL) after 4 weeks (Photomicrograph x 100).

Treated Group-C: The distortion and disorientation of monolayer of Purkinje cells was partly recovered, at many places the Purkinje cells were realigned, well stained cytoplasm with visible nuclei, and at few places they exhibited the picture of apoptosis and necrosis (Figure - 3). The mean values of diameter of Purkinje cells are shown in table -1 There was significant (P<0.05) increase in the mean values of diameter of Purkinje cells in subgroup C2 as compared to C1. There was significant (P<0.05) increase in the mean diameter of Purkinje cells in subgroup C1 as compared with B1. There was significant (P<0.05) increase in the mean diameter of Purkinje cells in subgroup C2 as compared to B2

Treated Group-D: The cytoarchitecture of cerebellar cortex was restored to normal, similar to the control. The majority of Purkinje cells were reorganized and realigned, and had very well stained cytoplasm with very clear and vesicular nuclei. Occasional apoptotic cells were seen in the series of normal Purkinje cells, being the normal feature The mean values of diameter of Purkinje cells are shown in table -1 There was insignificant (P>0.05) increase in the mean values of diameter of Purkinje cells in subgroup D2 as compared to D1. There was moderately significant (P<0.01) increase in the mean diameter of Purkinje cells in subgroups D1 - D2 as compared to subgroups B1 - B2

Treated Group-E: The cytoarchitecture of cerebellar cortex was not disrupted. The Purkinje cells were aligned in the monolayer, and had very well stained cytoplasm with very clear and vesicular nuclei as control. Few apoptotic cells were seen in the series of normal Purkinje cells, being the normal feature. The mean values of diameter of Purkinje cells in subgroups E1 and E2 are shown in table -1. There was moderately significant (P>0.01) increase in the mean values of diameter of Purkinje cells in subgroup E2 as compared with E1. There was significant (P<0.05) increase in the mean diameter of Purkinje cells in subgroup E1 as compared to A1, and moderately significant (P<0.001) increase in the mean diameter of Purkinje cells in subgroup E2 as compared to A2.

Discussion:

Brain tissue is particularly vulnerable to oxidative damage because of its exceptionally high oxygen consumption combined with relatively underdeveloped antioxidant defense systems. This susceptibility means that any imbalance between oxidant production and antioxidant capacity can have pronounced effects on neuronal integrity. Ionizing radiation, as well as various pro-oxidant agents, interact with biological tissues through mechanisms involving secondary ionization. Such interactions generate reactive oxygen species (ROS) that damage essential cellular components.[7,8]

It is well documented that antioxidant and free radical-scavenging compounds play an important role in shielding DNA from the harmful effects of oxidizing radicals. Findings by Kumar et al.[15] further reinforce that antioxidants and free radical scavengers can mitigate the detrimental consequences of radiation exposure. In the present investigation, mecobalamin was selected for evaluation as a radioprotective compound. Previous research by Weiss Weiss JF[16] and Zhang Iqbal et al.[19] has highlighted that numerous plant-derived agents—such as those found in Chinese herbal medicine, Ayurvedic remedies, cruciferous vegetables, green tea, mint, and black plum—contain phytochemicals with strong antioxidant and radioprotective properties.

Macroscopic examination in the current study revealed that the cerebella of methylcobalamin-treated animals closely resembled those of untreated control rats. In contrast, the irradiated Group B showed severe alterations: Purkinje cells were distorted, misaligned, and often displaced into multilayer arrangements. These abnormalities are most likely attributable to irradiation-induced interference with normal cellular growth and DNA integrity. Ionizing radiation is known to produce a variety of DNA lesions, either directly via particle impact or indirectly through oxygen-derived free radicals and secondary products of lipid peroxidation.[8]

Quantitative analysis confirmed that Purkinje cell diameters in irradiated Group B were markedly smaller than those in control Group A. This finding aligns with Enogieru et al.[21] who observed significantly reduced Purkinje cell diameters in irradiated animals compared to controls at both 14th and 25th days post-exposure. Similarly, Edagha et al.[22] documented that Artemether exposure caused neurotoxic

changes in the cerebellum of adult male Wistar rats, including reduced Purkinje cell diameters. Viñas-Noguera[23] also reported reduced nuclear and cytoplasmic dimensions of Purkinje cells in 40-day-old female rats subjected to three weeks of emotional stress.

In the current study, Groups C and D—both treated with methylcobalamin demonstrated larger Purkinje cells than irradiated Group B. This may be explained by methylcobalamin ability to support DNA replication and cellular growth, as noted by Guyton and Hall.[24] These results, however, differ from Kanu,[25] who found no significant difference in Purkinje cell diameter between irradiated rats and those treated with dexamethasone. Furthermore, in Group E, methylcobalamin treatment without irradiation-maintained Purkinje cell diameters near control values, again differing from Kanu's findings, which indicated reduced cell size even with treatment.

Conclusion:

On the basis of current study, it is concluded that irradiation induces cerebellar damage and alters the diameters of purkinji cells. Damage could be minimized by treating with methylcobalamin.

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Author's Contribution	
Amatul Sughra	Concept & design of study.
Amatul Sughra & Nayab Qazi	Methodology and Manuscript Draft
Hemant Kumar & Fehmida Gul	Data Collection and Analysis:
Pushpa & Bashir Shaikh	Final Manuscript writing and Approval of Version