Histopathological study of apoptotic cerebellar Purkinje neurons after extended Lithium Carbonate Oral Ingestion on Albino Rats.

Tazeen Kohari[1], Raja Faisal Zulfiqar [2,*], Tayyaba Kazmi[3], Roshan Ali Zardari[4], Muhammad Yousuf Khoso[5], Amanullah Khokhar[6].

ABSTRACT:
Objective: To evaluate the purkinje neuronal apoptosis after extended Lithium treatment.
Methodology: This experimental research was conducted at Department of Anatomy, Jinnah Postgraduate Medical Institute Karachi; from 1st of March 2020 to 2nd of May 2020.Twenty-four healthy male albino rats aged 11 to 12 weeks having body weights between 230 to 260 grams were selected and divided into two Groups A & B. The Control group (A) was given lab diet and animals of experiment group (B) were given the same diet as for animals of group A, along with Lithium carbonate tablet for a period of 8 weeks. Animals that had developed disease during the study period were excluded. Results were recorded in both groups. Statistical analysis was performed in both groups on SPSS version -21.
Results: On histological examination of cerebellar cortex there was decreased number of Purkinje neurons after 8 weeks of lithium ingestion. A highly significantly decreased p value < 0.000 was found in B group rodents as compared to that in control Group A.
Conclusion: This histological study had concluded that extended treatment with lithium results in purkinje neuronal damage in rat models.

Keywords: Tentorium cerebelli, Purkinje neurons, Bipolar disorder, Mania, Depression.

Introduction:

A thorough account of the development of the cerebellum has been presented by embryologists. They indicate that in the fifth week, the rhombencephalon, which is a hindbrain vesicle, begins to develop. This structure includes the most caudal brain vesicle, referred to as the myelencephalon, along with the metencephalon, which arises from the isthmus of the rhombencephalon. The walls of the metencephalon give rise to the pons and cerebellum, while the superior section of the fourth ventricle is derived from the cavity of the metencephalon. The cerebellar cortex is organized through the migration of proliferating cells from the mantle layer into the marginal layer.[1] The posterior cranial fossa contains the cerebellum. Tentorium cerebelli, separates it from the cerebrum. The two cerebellar hemispheres are joined by vermis and anterior, posterior, and flocculonodular, are the constituent cerebellarlobes.[2] Cerebellum is easily targeted by neurotoxic agents affecting, all age groups. Researches have proved that Purkinje neurons were the main target of this Cerebello toxic substance taken for prolong period.[3]

1. Associate professor Anatomy. Mohi-uddin- Islamic Medical College. Mirpur Azad Kashmir.
2. Associate Professor Anatomy. RYK Medical College . Abu Dhabi Road near KFUEIT. Rahim Yar Khan, Pakistan.
3. Associate Professor Anatomy. Baqai Medical College Karachi.
4. Senior lecturer/Additional RMO (Anatomy). Sheikh Zayed Medical College & Hospital, Rahim Yar Khan.
5. Assistant Professor Pathology. Al Tibri Medical College. Near Al-Ibrahim Eye Hospital, Old Thana Gadap Town Karachi, Pakistan.
6. Assistant Professor Medicine. Al-Tibri Medical College & Hospital Malir Karachi.

***=corresponding author** :
Email: rajafaisal76@hotmail.com

Lithium is lustrous, white, soft and pharmaceutical industry are producing several of its compounds as it is popularly used in psychiatric disorders.[4] It is absorbed from intestines and reaches blood stream in 3 to 4 hours. Pharmacological half-life is 24 hours and it is 95 percent excreted in urine. The gold standard drug lithium is popular for long term management of mood disorders; mainly used for the prophylaxis of recurrence of manic, hypo-manic, and depressive episodes.[5] Voltage gated sodium channels guards the entry of lithium carbonate and it is transported out of the cell through the electrochemical gradient that moves sodium inside and out of the cell, this action causes a decrease output in action potential. Fall in impulse conduction causes decrease duration of action potential and repolarization phase. This theory suggests that lithium acts to decrease depolarization which results from increased sodium influx the effect of lithium on action potential and after potential suggests that lithium acts to decrease outward current in neurons thereby suppresses action potential exaggeration and for this reason it is used in hyper maniac states or bipolar disorder.[6] bipolar disorder is a critical mental disorder, marked personal and social disability. In hyper manic condition, a strong treatment is usually required; clinically bipolar patients need long-term treatments, to extend the free interval, to stop recurrences, to decrease aggressive symptoms, in patients leading to improvement of family coordination and social demands these are the reasons that and lithium is prescribed in hostile irritable , hypo-manic, conditions.[7] Recent clinical studies have documented that lithium used for 20 years in bipolar disorder caused tremors, ataxia, and cognition failure. Inability to walk, hallucinations and dementia are result of increased serum levels of lithium. Expert management involving fluid diuresis results in resolution of the neural symptoms.[8] Clinicians have shown concerns over the scarcity of the literature on neurological challenges in patient's prescribed lithium therapy for prolonged period of time. The non-availability of therapeutic and medicinal literature on

lithium adverse effects on purkinje neurons compelled us to conduct this present experimental study on the adverse effects of Lithium therapy.

Methodology:

After ethical permission vide letter no: f.1-2/2017/BMSI-E.COMT/ 041/JPMC, study was conducted for a period of was 8 weeks 1st of March 2020 to 2nd of May 2020. Sample size was calculate using Clinstat online sample size calculator, following formula and parameters were applied: [9, 10]

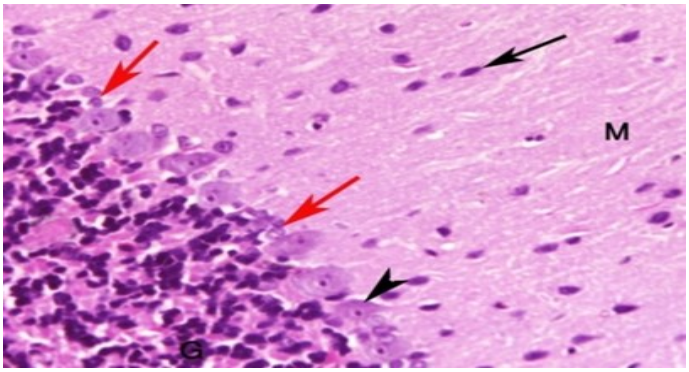
$$= \frac{(r+1)p(1-p)\left(Z_{1-\beta}+Z_{1-\frac{\alpha}{2}}\right)^2}{r(p_1+p_2)^2}$$

(n) = Sample size, r = Ration of case to control, p = Population proportion, p1= Case proportion, p2 = Control proportion, Z1-β = Power of study, Z1-α/2 = Critical value .

For 95% confidence interval (CI) and 80% power of study the sample size of twenty-four (24) was found satisfactory. Twenty-four (24) adults male Wistar albino rats, aged 11-12 weeks and weighed 230-250 grams were selected and divided into two groups A & B. Female, obese, physically inactive animals that had developed signs of disease or felt sick were excluded from this study. At random the rodents were allotted identification numbers. Group A had twelve rodents and Group B contained twelve animals. The Albinos were kept in cages with controlled temperature and lighting. Their diet contained fresh green leafy vegetables, flour pellets and water common for both groups. Both experimental group rodents were acclimatized for a week prior to study in a twelve-hour day night routine cycle. Group A was given only laboratory healthy diet and water and it served as control while the Group B, the experimental group, in addition to lab diet were given Lithium carbonate 20 mg /kg [11] purchased by name of Lithobid mixed in flour pellets. At the end of study period both group animals were sacrificed after giving them ether. After animals of both groups were anesthetized, the cranium was dissected using a straight scissor midline incision was given from backward forward, each half of scalp was grasped by Halsted-mosquito forceps and skull was exposed after mid sagittal incision was made in the scalp. The temporal bones of the skull were exposed after removing temporalis muscle. Cranium was separated from cervical region. Using a straight scissor, the scalp was separated from the underlying tissue. Then cautiously, foramen magnum was grasped and the occipital bone was removed, the cerebellum was identified and removed, then it was placed on dissecting tray. For twenty-four hours cerebellum was fixed in normal saline. Ascending grades of alcohol and xylene were used in the process of clearing cerebellar tissue and then it was infiltrated by paraffin. Blocks of tissue placed in paraffin were prepared and tissues were placed in such a position that transverse sections of cerebellar tissue were obtained and each placed on glass slides. On a rotatory microtome four-micron thick sections were cut and placed on water bath at a temperature of 42 degree centigrade. The drifting sections were then taken from water bath and slides were kept in a slanting position for half hour after they were covered with slide cover. For purpose of permanent fixation, they were kept in oven for 2-3 hours. Staining of the experimental cerebellar tissue was performed with haematoxylin and eosin, the shape of the Purkinje neurons and the Purkinje cells count was documented in both groups.[12] Photography was done for both groups and results were documented.

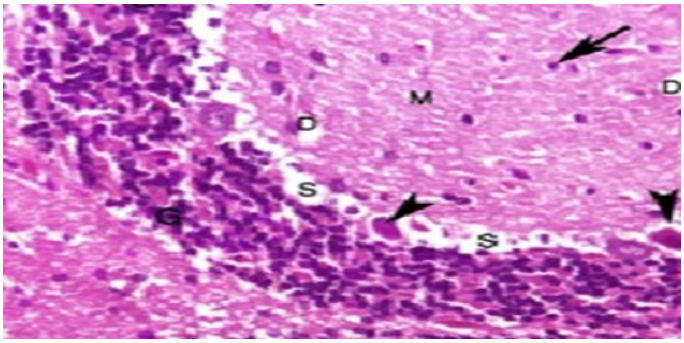
Micrometry: The ocular micrometer and counting reticule were adjusted on the stage micrometer for documentation of the purkinje cells count. The scale of .01mm in length divided into 100 parts so each division measured 10µm was used and placed on microscope stage for calibration There were 100 divisions and the reticule used had 20 squares of the ocular micrometer and calibrations were along both x and y axis, in the right eye the ocular micrometer was placed and the reticule in left eye piece. Calibrations of stage micrometer scale with ocular counting reticule under 100 magnifications. The ocular reticule was placed in left eye piece. One square of ocular reticule in both X and Y axes coincided with two divisions of stage micrometer scale which were equal to 20 µm, so the length of the reticule in both X and Y axes is equal to 20×20=400µm or is equal to 0.4m; therefore the area of reticule equals to 0.40x0.40=0.16mm^2. Micrometry was performed for Purkinje cells count and was noted. The original results of the change measured of decreased purkinje cells count in group B were observed. Readings were taken in both groups and the mean was calculated for each group. The original purkinje cells count were compared and calculated using SPSS version 22.

Results:



(H&E, 400×)

Fig 1. A: Cerebellar cortex of group (A) showing scattered glial cells (black arrow ↑). The cells of the Purkinje cell layer (▲ arrow heads) appear to have vesicular nuclei surrounded by cells of neuroglia (red arrow ↑). molecular layer (M).



(H&E, 400×)

Figure 1-B: Cerebellar cortex of group (B) showing marked hollow spaces (S) with some darkly stained nuclei (▲ arrow heads), (G) indicates shrunken granular layer containing several apoptotic cells containing pyknotic nuclei. Patches of degenerated tissue (D) along with glial cells (black arrow ↑) located in molecular layer (M).

The hematoxylin & eosin-stained sections of cerebellar cortex of control group "A" showed that the purkinje neurons had a normal flask shaped appearance, in comparison group "B" revealed distorted purkinje cell layer with loss of normal histology and apoptosis of purkinje neuronal cells. Karyolytic and pyknotic cells were clearly visible and abundant. **(Picture-1, A & B)**

Figure No 1: A & B.

The mean count of purkinje cells in Group "A" was 28.9 ±0.85 (cells /µm2) in comparison for Group "B" it was 11.4±0.41 (cells /µm2). Statistical analysis using paired sample t test showed a highly significant difference with p value of In comparative analysis a highly significant p-value of 0.001 as shown in table no 1.

Table No 1: Cerebellar purkinje cells count in group A & B. *=paired sample t test.

Groups	Mean purkinje cells count	p-value
A (Control) n=12	28.9 ±0.85 (cells /µm)2	0.000*
B (Treated) n=12	11.4±0.41(cells /µm)2	

Discussion:

In 1870, Dr. Silas Weir Mitchell, a neurologist, recommended lithium bromide, a compound derived from lithium, as a treatment for epilepsy, serving as both an anticonvulsant and hypnotic. The subsequent year, Dr. William A. Hammond, a professor specializing in mental and nervous system diseases at Bellevue Hospital Medical College in New York, became the first to prescribe lithium for mania. Over the years, anecdotal evidence has bolstered the continued use of lithium in medical and psychiatric settings for conditions such as mania, psychosis, depression, and joint inflammation.[13-16] The main effect of lithium is that it transforms intracellular signaling by inhibiting inositol monophosphate, which adversely effects neurotransmission carried out by the secondary messenger system. It causes mutations n genomic expression associated with neurotransmission which leads to a decreased activity of protein kinase C.[17] It's absorbed from gastrointestinal tract and after oral intake it requires 0.25 to 3 hours to reach peak blood concentrations.[18] The drug Lithium is expelled in its original form through the urine. Li+ causes a decline in neurotransmission as it inhibits dopamine and glutamate secretions .Inhibitory transmission is increased by lithium as it increases Gamma amino butyric acid (GABA) receptors activity which results in augmenting Gamma amino butyric acid (GABA) N-methyl D-aspartate (NMDA) receptors and serotonin levels and decreases glutamate and down-regulate NMDA receptors (N-methyl D-aspartate receptors).[19] These actions of lithium causes down regulation of glycogen synthetase kinase 3 and inositol monophosphatase within the signaling pathway. It causes decrease of phosphatidyl inositol 4, 5-biphosphate due to inhibition of the breakdown of inositol monophosphate to inositol; which leads to insufficient quantity of free inositol. The Phosphatidylinositol 4,5-bisphosphate (PIP2) is a predecessor of second messenger's inositol (IP3)inositol trisphosphate and diacylglycerol (DAG) in the cell membrane.[20] Two-second messengers are responsible for diverse damaging effects as researchers have found them to be increased in bipolar disorders (BD).[21, 22]

Histologically cerebellum consists of five types of permanent cells among which purkinje cells provide sole efferent output of the cerebellar cortex and are the central element around which hind brain cerebellar circuit is arranged but they are damaged by Lithium.[23] It was reported that lithium disrupts calcium hemostasis in purkinje cells and leads to calcium hyper excitability causing neuronal death. Neuronal dysfunction like Cerebellar syndrome has been described after acute lithium therapy. Neurodegenerative studies have reported neuronal injury and cellular apoptosis in the cerebellum.[24]

Studies have demonstrated a reduction in the number of purkinje cells due to the blockade of entrance of calcium into purkinje cells causing neuronal nuclear death, the same effects of lithium were discovered by Caballero-Florán RN et al., they had reported that it caused inhibition of glycogen synthetase kinase which leads to purkinje cell loss, the same findings were documented in our study.[6,25]

The prominent clinical side effects caused by lithium are due to Purkinje cell loss as noted by Izchak Kohen and the results of atrophy of purkinje neurons were also detected in our research.[26] The severe cerebellar syndrome is caused by purkinje neuronal loss due to disruption of calcium hemostasis, a finding in agreement with the finding of current study.

Limitations: A larger sample size study will be helpful to reinforce the findings of current study. Electron microscopic evaluation as well as clinical signs and symptoms will provide further information and in-depth analysis.

Conclusion:

Prolonged consumption of lithium can lead to irreversible damage to the cerebellum, primarily due to the apoptosis and subsequent death of Purkinje cells. Consequently, it is essential that lithium be prescribed judiciously, with patients remaining under continuous monitoring.

Conflict of Interest

The authors declare no conflict of interest.

References:

1. Rahimi-Balaei, M. et al.The Embryology and Anatomy of the Cerebellum. In: Marzban, H. (eds) Development of the Cerebellum from Molecular Aspects to Diseases. Contemporary Clinical Neuroscience. Springer, 2023; Cham. https://doi.org/10.1007/978-3-031-23104-9_2.
2. Lara, A., Laureani, F., Morgado, C., Valle, L., Beltran, P., Rojas, D. et al. Latest research on the anatomy and physiology of the cerebellum, Neurology perspectives. 2022; 2(1), 34–46. (www.sciencedirect.com). doi: 10.1016/j.neurop.2021.12.002.
3. Manto M, Perrotta G. Toxic-induced cerebellar syndrome: from the fetal period to the elderly. Handb Clin Neurol. 2018;155:333-352. doi: 10.1016/B978-0-444-64189-2.00022-6, PubMed: 29891070.
4. Chokhawala K, Lee S, Saadabadi A. Lithium. [Updated 2024 Jan 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK519062/4.
5. Brock KE, Elliott ER, Taul AC, Asadipooya A, Bocook

D et al. The Effects of Lithium on Proprioceptive Sensory Function and Nerve Conduction. NeuroSci. 2023 Oct 20;4(4):280-295. doi: 10.3390/neurosci4040023, PubMed: 39484179.

6. Caballero-Florán RN, Nelson AD, Min L, Jenkins PM. Effects of chronic lithium treatment on neuronal excitability and GABAergic transmission in an *Ank3* mutant mouse model. bioRxiv [Preprint]. 2023 Oct 30:2023.10.26.564203. doi: 10.1101/2023.10.26.564203. PMID: 37961630 37961630; PMCID: PMC10634991
7. Nierenberg AA, Agustini B, Köhler-Forsberg O, Cusin C, Katz D, Sylvia LG, Peters A, Berk M. Diagnosis and Treatment of Bipolar Disorder: A Review. JAMA. 2023 Oct 10;330(14):1370-1380. doi: 10.1001/jama.2023.18588, PubMed: 37815563.
8. Kronk NN, Kronk BK, Robbie AT. A Case Report: Lithium-Induced Neurotoxicity, a Differential to Always Consider. Cureus. 2023 Dec 9;15(12):e50225. doi: 10.7759/cureus.50225, PubMed: 38192942, PubMed Central: PMC10773538.
9. Kelley K, Preacher KJ. On effect size. Psychol Methods. 2012 Jun;17(2):137-52. doi: 10.1037/a0028086, PubMed: 22545595.
10. Sadiq, I.Z., Usman, A., Muhammad, A. *et al.* Sample size calculation in biomedical, clinical and biological sciences research. 2024; 5 April J.Umm Al-Qura Univ. Appll. Sci. https://doi.org/10.1007/s43994-024-00153-x
11. Hamstra SI, Roy BD, Tiidus P, MacNeil AJ, Klentrou P, MacPherson REK, Fajardo VA. Beyond its Psychiatric Use: The Benefits of Low-dose Lithium Supplementation. Curr Neuropharmacol. 2023;21(4):891-910. doi: 10.2174/1570159X20666220302151224, PubMed: 35236261, PubMed Central: PMC10227915.
12. Amin SN, Hassan SS, Khashaba AS, Youakim MF, Latif NSA, Rashed LA, Yassa HD. Hippocampal and Cerebellar Changes in Acute Restraint Stress and the Impact of Pretreatment with Ceftriaxone. Brain Sci. 2020 Mar 25;10(4):193. doi: 10.3390/brainsci10040193. Erratum in: Brain Sci. 2024 Sep 18;14(9):933. doi: 10.3390/brainsci10040193, PubMed: 32218213, PubMed Central: PMC7225952.
13. Shorter E. The history of lithium therapy. Bipolar Disord. 2009 Jun;11 Suppl 2(Suppl 2):4-9. doi: 10.1111/j.1399-5618.2009.00706.x. PMID: 19538681; PMCID: PMC3712976.
14. Fountoulakis KN, Young A, Yatham L, Grunze H, Vieta E, Blier P, Moeller HJ, Kasper S. The International College of Neuropsychopharmacology (CINP) Treatment Guidelines for Bipolar Disorder in Adults (CINP-BD-2017), Part 1: Background and Methods of the Development of Guidelines. Int J Neuropsychopharmacol. 2017 Feb 1;20(2):98-120. doi: 10.1093/ijnp/pyw091, PubMed: 27815414, PubMed Central: PMC5408969.
15. Bukhteeva I, Rahman FA, Kendall B, Duncan RE, Quadrilatero J, Pavlov EV, Gingras MJP, Leonenko Z. Effects of lithium isotopes on sodium/lithium cotransport and calcium efflux through the sodium/calcium/lithium exchanger in mitochondria. Front Physiol. 2024 Apr 9;15:1354091. doi: 10.3389/fphys.2024.1354091. PMID: 38655027; PMCID: PMC11036541.
16. Rijal S, Jang SH, Park SJ, Han SK. Lithium Enhances the GABAergic Synaptic Activities on the Hypothalamic Preoptic Area (hPOA) Neurons. Int J Mol Sci. 2021 Apr 9;22(8):3908. doi: 10.3390/ijms22083908, PubMed: 33918982.
17. Yu W, Greenberg ML. Inositol depletion, GSK3 inhibition and bipolar disorder. Future Neurol. 2016 May;11(2):135-148. doi: 10.2217/fnl-2016-0003. Epub 26 April 2016. PubMed: 29339929, PubMed Central: PMC5751514.
18. Ayano, G. Bipolar disorders and lithium: Pharmacokinetics, pharmacodynamics, therapeutic effects and indications of lithium: Review of articles. Austin J Psychiatry Behav Sci. 2016; 3(2): 1053. doi.org/10.3390/ijms22083908.
19. Teleanu RI, Niculescu AG, Roza E, Vladâcenco O, Grumezescu AM, Teleanu DM. Neurotransmitters-Key Factors in Neurological and Neurodegenerative Disorders of the Central Nervous System. Int J Mol Sci. 2022 May 25;23(11):5954. doi: 10.3390/ijms23115954, PubMed: 35682631, PubMed Central: PMC9180936.
20. Saha S, Krishnan H, Raghu P. IMPA1 dependent regulation of phosphatidylinositol 4,5-bisphosphate and calcium signalling by lithium. Life Sci Alliance. 2023 Dec 6;7(2):e202302425. doi: 10.26508/lsa.202302425. PMID: 38056909; PMCID: PMC10700560
21. Kamal ZM, Dutta S, Rahman S, Etando A, Hasan E, Nahar SN, Wan Ahmad Fakuradzi WFS, Sinha S, Haque M, Ahmad R. Therapeutic Application of Lithium in Bipolar Disorders: A Brief Review. Cureus. 2022 Sep 19;14(9):e29332. doi: 10.7759/cureus.29332. PMID: 36159362; PMCID: PMC9484534
22. Hull C, Regehr WG. The Cerebellar Cortex. Annu Rev Neurosci. 2022 Jul 8;45:151-175. doi:10.1146/annurev-neuro-091421-125115, PubMed: 35803588, PubMed Central: PMC10268027.
23. Konieczny K, Detraux J, Bouckaert F. The Syndrome of Irreversible Lithium-Effectuated Neurotoxicity: A Scoping Review. Alpha Psychiatry. 2024 Mar 1;25(2):190-205. doi: 10.5152/alphapsychiatry.2024.231460. PMID: 38798809; PMCID: PMC11117426.
24. Cerqueira AC, Reis MC, Novis FD, Bezerra JM, Magalhães GC, Rozenthal M, Nardi AE. Cerebellar degeneration secondary to acute lithium carbonate intoxication. Arq Neuropsiquiatr. 2008 Sep;66(3A):578-80. doi: 10.1590/s0004-282x2008000400032, PubMed: 18813728.
25. Nelson AD, Caballero-Florán RN, Rodríguez Díaz JC, Hull JM, Yuan Y, Li J et al. Ankyrin-G regulates forebrain connectivity and network synchronization via interaction with GABARAP. Mol Psychiatry. 2020 Nov;25(11):2800-2817. doi: 10.1038/s41380-018-0308-x. Epub 2018 Nov 30. Erratum in: Mol Psychiatry. 2020 Nov;25(11):3106. doi: 10.1038/s41380-019-0361-0. PMID: 30504823; PMCID: PMC6542726.

26. Kohen, I., MD Volume 19 - (2011) Permanent lithium-induced cerebellar toxicity in an elderly patient: Case report and review of the literature. Consultant 360. 2011; 19 (6). Available at https://www.consultant360.com/articles/permanent-lithium-induced-cerebellar-toxicity-elderly-patient-case-report-and-review.