Research Output Journal of Public Health and Medicine 4(3):52-59, 2024

ROJPHM

ISSN ONLINE: 1115-9715
ISSN PRINT: 1115-6147

https://rojournals.org/roj-public-health-and-medicine/

https://doi.org/10.59298/ROJPHM/2024/435259

Neuroprotective Effect of Cholecalciferol on Acute Traumatic Brain Injury

[1,2,3]Ozor, Ignatius Ikemefuna; [1,2]Ebuoh, Maryann Chiamaka; [1]Ogbo, F. Ovie; [1,2]Ugwu, Valentine Ifebuchechukwu and [1]Okpala, Onyedikachukwu Precious

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, State University of Medical and Applied sciences (SUMAS), Igbo-Eno, Enugu State, Nigeria.
[2]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, Enugu State University of Science and Technology (ESUT), Enugu, Nigeria.
[3]Department of Surgery, Faculty of Clinical Medicine, College of Medicine, Enugu State University of Science and Technology, Enugu, Nigeria
Corresponding author: Ebuoh, Maryann Chiamaka (maryann.ebuoh@sumas.edu.ng +2348149560255)

ABSTRACT

Traumatic brain injury (TBI) results from external mechanical forces causing temporary or permanent impairments in cognitive, physical, and psychosocial functions. Cholecalciferol, a hormone with secosteroidal, neuroactive, and neurosteroidal properties, has demonstrated potential neuroprotective effects, including axon regeneration, increased axonal diameter, and improved sensory responses to metabolic stimulation. This study evaluated the histological changes in the prefrontal cortex of TBI-induced Wistar rats treated with Cholecalciferol. Forty-two Wistar rats (180–230g) were randomized into four groups, each with 12 rats, further divided into sub-groups treated or untreated with Cholecalciferol. TBI was induced using weights of 100g, 200g, and 300g. Brain tissues were harvested at 6 hours, 24 hours, and 3 days post-injury and processed using paraffin embedding and Hematoxylin-Eosin staining. Histological analyses showed normal neuronal distribution in the control group, while untreated TBI groups exhibited perinuclear halo and degenerative plaques. Cholecalciferol-treated sub-groups demonstrated varying degrees of glial cell activation, post-traumatic regeneration, and reduced tissue scarring, with significant improvements observed at higher injury severities. Findings suggest that Cholecalciferol promotes histological recovery in TBI by enhancing neuronal integrity and regeneration.

Keywords: Cholecalciferol, Traumatic brain injury, Neuroprotection, Histology

INTRODUCTION

Traumatic brain injury (TBI) is a significant public health issue and one of the leading causes of morbidity, disability, and mortality across all age groups (1–5). Annually, over 50 million individuals worldwide suffer from TBIs, highlighting its widespread impact (1). By 2005, approximately 3.17 million TBI survivors experienced various post-traumatic complications, including neurological and psychosocial impairments, as well as long-term disabilities (6–9). TBI can be broadly categorized into two types: closed head injuries and penetrating injuries. Clinical manifestations of TBI often include prolonged coma, headaches, nausea, aphasia, seizures, amnesia, and behavioral abnormalities such as aggression and anxiety (6, 10). These symptoms can occur within seconds to minutes after the injury but may persist for months or even years in some cases (6, 10). Penetrating TBI results from the entry of a foreign body through the skull and dura mater into the brain parenchyma. Like closed head injuries, penetrating TBI leads to focal tissue damage, intracranial hemorrhage, cerebral edema, and ischemia. Additionally, the introduction of high-velocity projectiles can cause tissue cavitation, further exacerbating the extent of injury (11). Cholecalciferol, commonly referred to as vitamin D3, is synthesized in the skin following exposure to UV-B light. It is also found in certain foods and available as a dietary supplement (12). Vitamin D belongs to a group of fat-soluble secosteroids responsible for enhancing the intestinal absorption of magnesium,

phosphate, and calcium, all of which are vital for maintaining overall health (13).Of particular relevance to TBI is **osteopontin (OPN)**, a glycoprotein associated with vitamin D. OPN has demonstrated significant neuroprotective effects in ischemic injuries affecting the brain and other organs, making vitamin D a promising candidate for mitigating TBI-related complications (14).

MATERIALS AND METHODS

The protocol for the conduct of the study was reviewed and approved by the Faculty of basic medical sciences Research Ethical Committee of Enugu State University, College of Medicine ESUCOM/FBMS/ETR/2024/004.

Experimental Design

The rats were randomized into four (4) groups of 12 rats in each group. Seven sub-groups of six rats in each. Group A rats were induced with head injury. Group B rats were induced with 100g weight. Sub-group b1 will not be treated with drug. Sub-group b2 will be treated with drug. Group C rats were induced with 200g weight. Sub-group c1 were not treated with drug. Sub-group c2 were treated with drug. Group D rats were induced with 300g weight. Sub-group d1 were not treated with drug. Sub-group d2 were treated with drug.

TBI Induction

Marmarou's weight drop apparatus was used to carry out the induction of traumatic brain injury. It comprises of a long cylindrical plastic guide of about 2.2cm (diameter), 1.5m which was attached to a clamp stand. A clear transparent U-shaped plastic stage covered with foil was placed underneath the guide tube. A fishing line was attached to metal masses of different weight, the line was passed through the plastic guide tube and attached to the clamp stand. The masses was left hanging freely at about 2.5cm about the tin foil.

The rats was placed in an isoflourane chamber and lightly anesthetized until they become unconscious. The rat was placed face down on the tin foil, with their head placed directly in the path of the falling weight. A rat was returned back to the isoflourane chamber if it begins to move before being placed on the tin foil. The Allen key was pulled allowing the weight to fall vertically through the plastic guide tube and hit the rat on the head. Immediately the rats was removed, topical lidocaine (analgesic) was applied on its head to relieve pain. The procedure was carried out for each group with their corresponding weights.

Anaesthetizing the animal

Syringe and ketamine were used. The animals were injected 1ml of ketamine on the thigh to make them unconscious. Pinch response method was used to determine the depth of the anaesthesia. The animals were unresponsive before dissection was done.

Sacrifice/Organ Harvest

A rat were selected from each group at 6hours, 24hours, 3 days post trauma. Before sacrificing, the final weight of the animal were taken. The animals were placed vertically in anatomically supine position on the dissecting table. The hands and foots were pinned for easy dissection. A vertical incision on the thoracic and abdominal region was made with a scalpel. Normal saline was passed through the apex of the heart for easy circulation for ten minutes. 10% formaldehyde was fixed for twenty minutes to prevent autolysis and putrefaction. The cranium of the rats were dissected, the brain harvested and stored in the organ tube containing formalin.

Histology Analysis

The harvested brains were processed using routine paraffin technique (Geoffrey Rolls, 2022). Staining was done using Hematoxylin and Eosin counterstaining method. Magnification for snapping: X300.

Camera used for snapping**:** AMSCOPE 3.7 (5.0Megapixel)

Scale bar**:** 0.4\8μm.

RESULTS

Group A

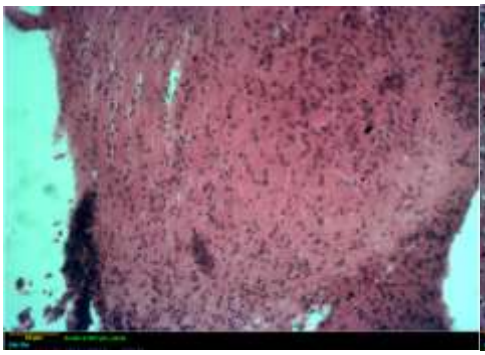

Plate A1: Representative Photomicrograph of cortex microstructure showing the prefrontal cortex microstructure normal neuronal cell distribution. **H & E.**

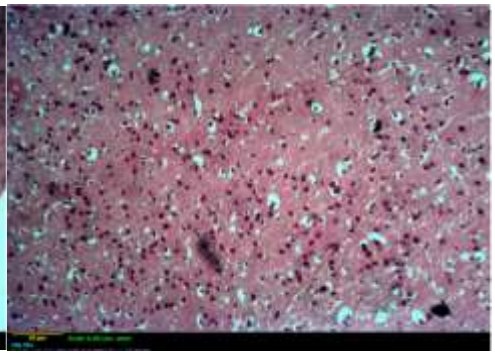

Plate A2: Representative Photomicrograph of the prefrontal showing normal neuronal cell distribution. **H & E.**

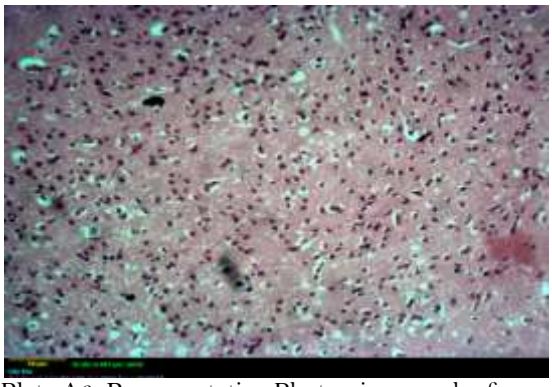

Plate A3: Representative Photomicrograph of the prefrontal cortex microstructure showing normal neuronal cell distribution. **H & E.**

Group B

Plate B1: Representative Photomicrograph of the prefrontal cortex microstructure Showing severe glial cell activation. **H & E.** showing normal neuronal distribution with

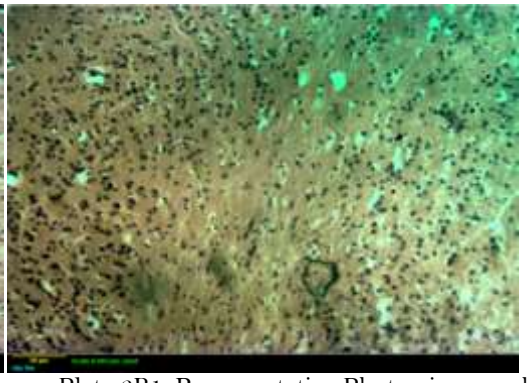

Plate 2B1: Representative Photomicrograph of the prefrontal cortex microstructure

mild glial cell activation. **H & E.**

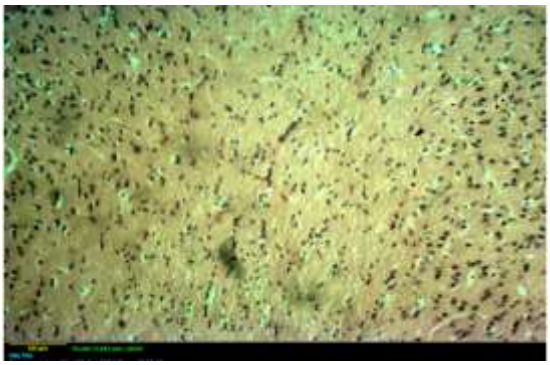

Plate B2: Representative Photomicrograph of the prefrontal cortex microstructure showing normal neuronal distribution with mild glial cell activation **H & E**

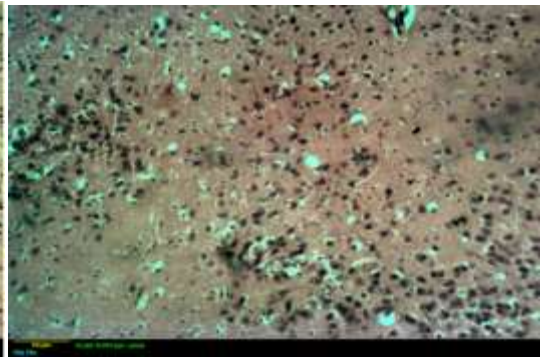

Plate 2B2 : Representative Photomicrograph of the prefrontal cortex microstructure showing moderate glial cell activation. **H & E**

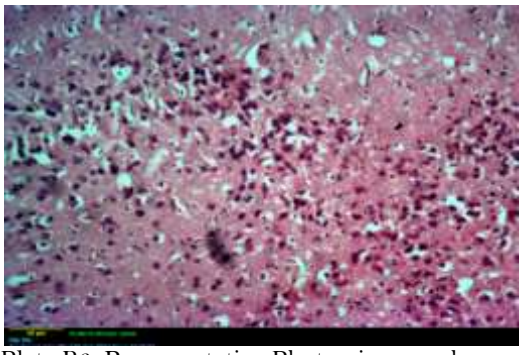

Plate B3: Representative Photomicrograph of the prefrontal cortex microstructure neuronal distribution prominent glial cell activation. **H & E.**

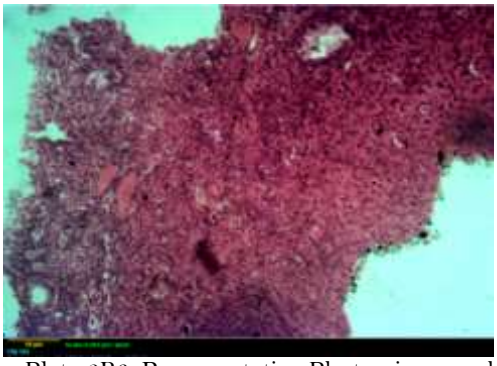

Plate 2B3: Representative Photomicrograph of the prefrontal cortex microstructure showing normal with moderate glial cell activation. **H & E.**

Group C

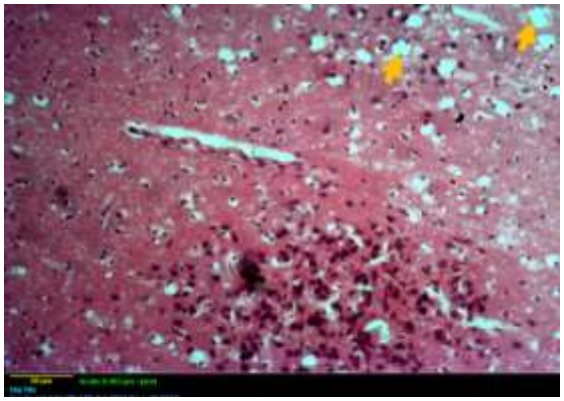

Plate C1: Representative Photomicrograph of the prefrontal cortex microstructure showing perinuclear spotty halo (arrow). **H & E**

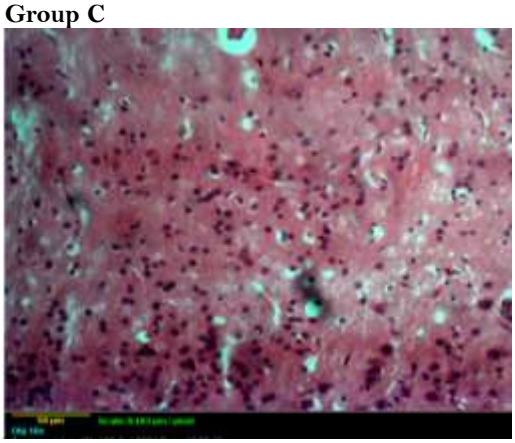

Plate 2C1: Representative Photomicrograph of The prefrontal cortex showing activated glial cells. **H& E.**

.

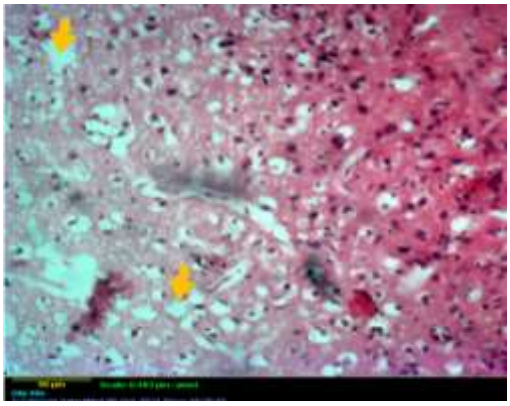

Plate C2: Representative Photomicrograph of the prefrontal cortex showing post traumatic regenerative perinuclear halo(arrow). **H & E.**

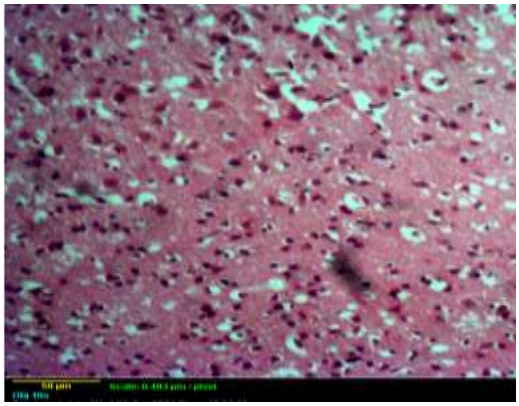

Plate 2C2: Representative Photomicrograph of the prefrontal cortex microstructure showing cell activation with focal areas of tissue. **H & E.**

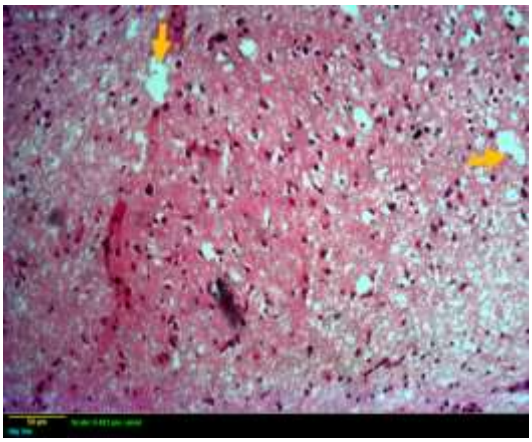

Plate C3: Representative Photomicrograph of the prefrontal cortex microstructure showing spotty perinuclear halo (arrow). **H & E.**

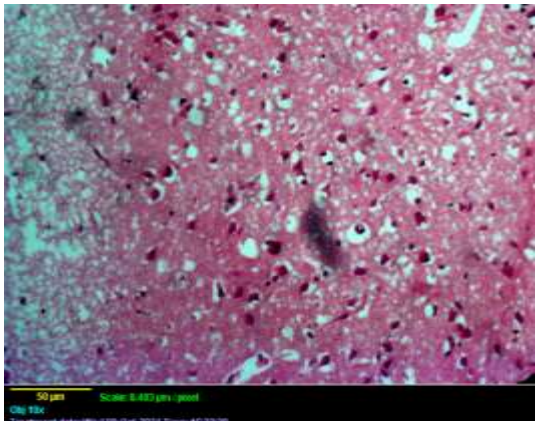

Plate 2C2: Representative Photomicrograph of the prefrontal cortex microstructure showing normal neuronal distribution. **H & E.**

Group D

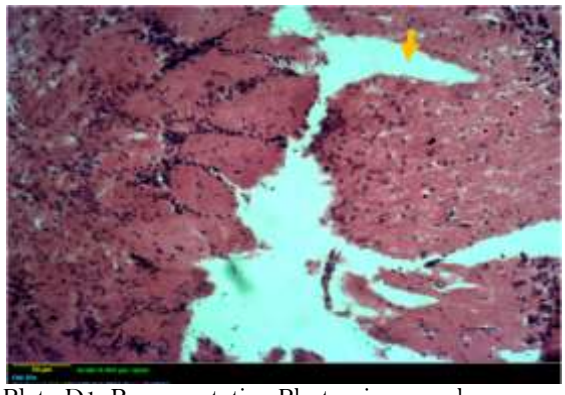

Plate D1: Representative Photomicrograph of the prefrontal cortex microstructure showing degenerated plaques (arrow) **H & E.**

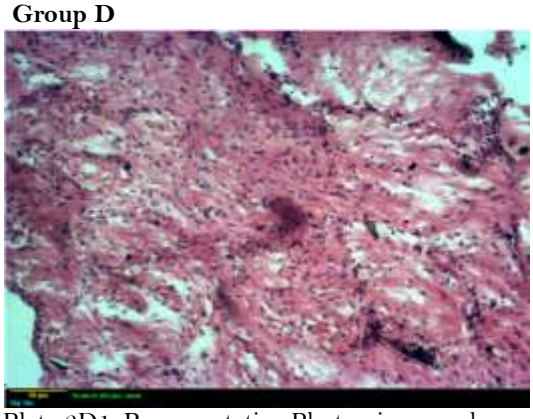

Plate 2D1: Representative Photomicrograph of the prefrontal cortex microstructure showing moderate glial cell proliferation. . and moderate glial cell activation. **H & E.**

Plate D2: Representative Photomicrograph of the prefrontal cortex microstructure showing severe pigmentation leading to reactivity (arrow) with activated glial cells. **H & E.**

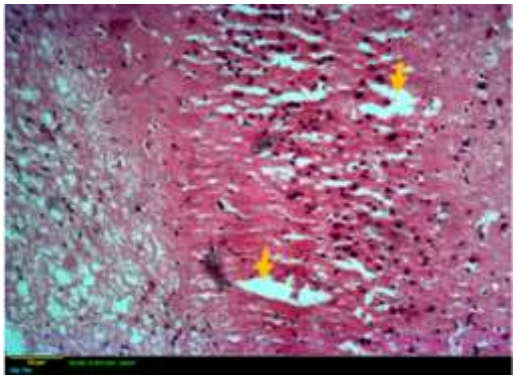

Plate 2D2: Representative Photomicrograph of the prefrontal cortex microstructure showing tissue scarring due to cerebrovascular tissue degeneration and neuronal cell death. **H & E.**

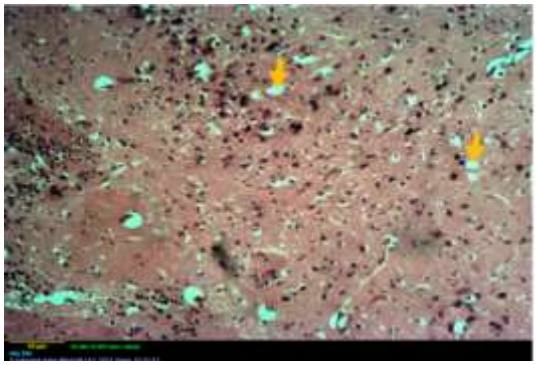

Plate D3: Representative Photomicrograph of the prefrontal cortex microstructure showing focal perinuclear spotty halo (arrow). cerebrovascular reactivity (arrow) with activated glial cells. **H & E.**

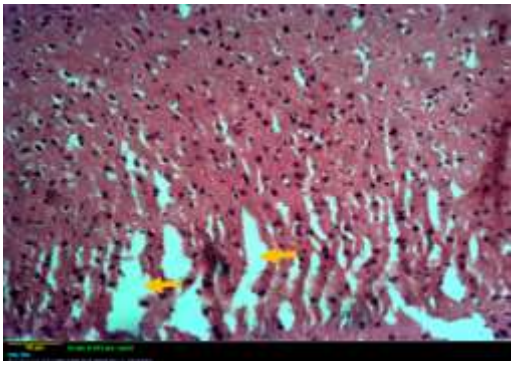

Plate 2D3: Representative Photomicrograph of the prefrontal cortex microstructure showing severe tissue scarring due to **H & E.**

DISCUSSION

Traumatic brain injury (TBI) progresses in two distinct phases. **Primary TBI** occurs immediately upon impact, while **secondary TBI** manifests seconds to minutes later and is characterized by neuroinflammation, oxidative stress, and increased intracranial pressure due to brain edema (15). Effective TBI treatment often focuses on mitigating secondary damage, as this significantly reduces morbidity and mortality (16). Identifying novel therapeutic targets for cellular injury holds promise in minimizing disability from nervous system trauma. This study assessed the impact of cholecalciferol on the secondary phase of TBI. Histological analysis of untreated sub-groups B, C, and D revealed abnormal neuronal cell distribution in the prefrontal cortex, marked by glial cell activation, perinuclear spotty halos, degenerative plaques, and neuronal cell death. In contrast, sub-group B1 exhibited normal neuronal distribution, likely due to the reduced severity of trauma in this group. Mild to moderate glial cell activation observed in group B suggests that the 100g weight caused minimal brain tissue damage, or that cholecalciferol successfully bypassed the blood-brain barrier (BBB). This observation aligns with findings that the extent of neurological damage depends on the size, speed, route, and force of the external object penetrating the brain (11). The presence of vitamin D hormone (VDH) in the central nervous system (CNS) relies on both passive and active transport across the BBB (17). Perinuclear spotty halos in untreated sub-group C suggest cerebral vessel leakage, leading to blood and cerebrospinal fluid (CSF) loss. This condition resulted in hematomas, cerebral edema, obstructive hydrocephalus, hypoxia, and potential infection, consistent with evidence-based TBI guidelines (18). In contrast, treated sub-group C showed regenerative tissue and normal neuronal distribution, demonstrating the neuroprotective role of cholecalciferol. The findings are supported by studies highlighting the significance of vitamin D3 in maintaining general health and the neuroprotective effects of osteopontin (OPN), a glycoprotein associated with vitamin D3, in ischemic injuries (14).Untreated sub-group D

exhibited degenerated plaques and neuronal cell death, whereas treated sub-group D showed moderate glial cell proliferation and severe tissue scarring due to cerebrovascular reactivity with activated glial cells. These outcomes align with studies by Tang-Schomer et al. (2010), which identified Wallerian degeneration as a hallmark of diffuse axonal injury (DAI) occurring within minutes (4). Furthermore, the treated sub-group D results correspond with findings by Fawcett and Asher (1999), which reported that astrocytic processes intermingle with oligodendrocytes, meningeal cells, microglia, and fibroblasts to form scar-like structures. These structures, while impeding axonal regeneration, are critical to understanding TBI recovery mechanisms (19).

CONCLUSION

There is a significant change in brain histology following treatment of TBI with cholecalcifrol. However, Cholecalciferol has neuroprotective effects on acute TBI.

REFERENCES

1. Maas, A. I., Murray, G., Henney, H., III., Kassem, N., Legrand, V., Mangelus, M., et al.. (2008). Efficacy and safety of dexanabinol in severe traumatic brain injury: results of a phase III randomised, placebo-controlled, clinical trial. Lancet Neurol. 5, 38–45. 10.1016/s1474-4422(05)70253-2
2. Aminmansour, B., Nikbakht, H., Ghorbani, A., Rezvani, M., Rahmani, P., Torkashvand, M., Nourian, M., Moradi, M. (2012). Comparison of the administration of progesterone versus progesterone and vitamin D in improvement of outcomes in patients with traumatic brain injury: a randomized clinical trial with placebo group. Adv Biomed Res;1:1–5.
3. Hua, F., Reiss, J.I., Tang, H., Wang, J., Fowler, X., Sayeed, I. (Apr, 2012). Progesterone and low-dose vitamin D hormone treatment enhances sparing of memory following traumatic brain injury. Horm Behav. 61(4):642–51.
4. Tang-Schomer, M. D., Patel, A. R., Baas, P. W., Smith, D. H. (2010). Mechanical breaking of microtubules in axons during dynamic stretch injury underlies delayed elasticity, microtubule disassembly and axon degeneration. FASEB J. 24, 1401–1410. 10.1096/fj.09-142844.
5. **Geoffrey, Rolls**. (7th April, **2022**). Artifacts in Histological and Cytological Preparations
6. Bruns, J., Jr., Hauser, W. A. (2003). The epidemiology of traumatic brain injury: a review. Epilepsia 44, 2–10. 10.1046/j.1528-1157.44.s10.3.x.
7. Dewan, M. C., Rattani, A., Gupta, S., Baticulon, R. E., Hung, Y. C., Punchak, M., et al.. (2018). Estimating the global incidence of traumatic brain injury. J. Neurosurg. 130, 1039–1408. 10.3171/2017.10.JNS17352.
8. Zaloshnja, E., Miller, T., Langlois, J. A., Selassie A. W. (2008). Prevalence of long-term disability from traumatic brain injury in the civilian population of the United States, 2005. J. Head Trauma Rehabil. 23, 394–400. 10.1097/01.htr.0000341435.52004.
9. Bazarian, J. J., Cernak, I., Noble-Haeusslein, L., Potolicchio, S., Temkin, N. (2009). Long-term neurologic outcomes after traumatic brain injury. J. Head Trauma Rehabil. 24, 439–451. 10.1097/htr.0b013e3181c15600.
10. Andriessen, T. M., Jacobs, B., Vos, P. E. (2010). Clinical characteristics and pathophysiological mechanisms of focal and diffuse traumatic brain injury. J. Cell. Mol. Med. 14, 2381–2392. 10.
11. Black, K. L., Hanks, R. A., Wood, D. L., Zafonte, R. D., Cullen, N., Cifu, D. X., et al.. (2002). Blunt versus penetrating violent traumatic brain injury: frequency and factors associated with secondary conditions and complications. J. Head Trauma Rehabil. 17, 489–496. 10.1097/00001199-200212000-00001.
12. Coulston, A.M., Boushey, C., Ferruzzi, M. (2013). *Nutrition in the Prevention and Treatment of Disease*. Academic Press. p. 818. *ISBN 9780123918840. Archived* from the original on 30 December 2016. *Retrieved 29 December 2016*.
13. Lee, J.M., Jeong, S.W., Kim, M.Y., Park, J.B., Kim, M.S. (Jun, 2019). The effect of vitamin D supplementation in patients with acute traumatic brain injury. World Neurosurg.126:e1421–6.
14. Enkhjargal, B., McBride, D.W., Manaenko, A., Reis, C., Sakai, Y., Tang, J. (Jul, 2017). Intranasal administration of vitamin D attenuates blood–brain barrier disruption through endogenous upregulation of osteopontin and activation of CD44/P-gp glycosylation signaling after subarachnoid hemorrhage in rats. J Cereb Blood Flow Metab. 37(7):2555–66.
15. Niu, X., Zheng, S., Liu, H., Li, S. (2018). Protective effects of taurine against inflammation, apoptosis, and oxidative stress in brain injury. Mol Med Rep. 18(5):4516–4522. doi: 10.3892/mmr.2018.9465.
16. Korkmaz, Dilmen. O., Akçıl, E.F., Tunalı, Y. (2015). ıntensive care treatment in traumatic brain ınjury. Turk J Anaesth Reanim. 43:1–6. doi: 10.5152/TJAR.2014.26680.
17. Lawrence, D.W., Sharma, B. (16 May, 2016). A review of the neuroprotective role of vitamin D in traumatic brain injury with implications for supplementation post-concussion. Brain Inj.30(8):960–8.

18. Marion. (2006). Evidence-based prehospital management of severe traumatic brain injury: a comparative analysis of current clinical practice guidelines. 18(2):265-73. doi: 10.3109/10903127.2013.856506
19. Fawcett, J. W., Asher, R. A. (1999). The glial scar and central nervous system repair. Brain Res. Bull. 49, 377–391. 10.1016/s0361-9230(99)00072-6.

CITE AS: Ozor, Ignatius Ikemefuna; Ebuoh, Maryann Chiamaka; Ogbo, F. Ovie; Ugwu, Valentine Ifebuchechukwu and Okpala, Onyedikachukwu Precious (2024). Neuroprotective Effect of Cholecalciferol on Acute Traumatic Brain Injury. Research Output Journal of Public Health and Medicine 4(3):52-59. https://doi.org/10.59298/ROJPHM/2024/435259