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Vitamin B Complex Protects Against Maternal Deprivation-Induced Cerebellar Damage in Adolescent Rats

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ABSTRACT

Maternal deprivation during the early neonatal period is linked to many neurodevelopmental disorders. This study was designed to investigate the protective action of vitamin B complex (Vit Bco) on maternal deprivation-induced cerebellar damage in Wistar rats. Pups were divided into four groups: control, which received 50 ml/kg of normal saline from postnatal day (PND) 21-35; Second group received 40 mg/kg of Vit Bco orally from PND 21-35; Third and fourth groups were maternally deprived for 24 h on PND 9, but the fourth group received Vit Bco in addition from PND 21-35. The pups were sacrificed on PND 36 and the cerebellar tissues were harvested and processed for histological and immunohisto-chemical studies, as well as oxidative status using enzymatic markers (superoxide dismutase and catalase). Cerebellar microarchitecture of maternally deprived pups revealed disruption of cortical layers, pyknotic Purkinje cells and activated astrocytes. Superoxide dismutase and catalase activities were significantly reduced in maternally deprived pups when compared with the control and the Vit Bco alone group. Pups that received Vit Bco after maternal deprivation had well-delineated cortical layout, moderately stained Purkinje cells and non-activated astrocytes, while the oxidative enzymes activities were increased compared with pups that did not receive Vit Bco intervention. Conclusively, Vit Bco ameliorates cerebellar histomorphological, neurochemical and oxidative alterations associated with maternal deprivation.

Keywords: *Maternal deprivation, Vitamin B complex, Cerebellar damage, Oxidative stress*

INTRODUCTION

Adverse events in early life have the potential of disrupting the process of neurodevelopment with serious implications on mental health in the later phases of life (Janetsian-Fritz et al. 2018). Maternal deprivation (MD) is an example of such adverse events, and during the period of physical separation of the infant from the mother, the former is deprived of necessary nourishment and emotional attachment (Howard et al. 2011; Marco et al. 2015). Animal studies and human data have proposed that the relationship between the quality of the early environment and emotional response during adulthood appears to be mediated by parental/maternal influences on brain development (Llorente et al. 2010).

Maternal deprivation has both short-term and long-term implications on neurodevelopment, and could predispose the infant to different forms of neuropsychiatric disorders later in life, including schizophrenia (Murray and Fearon 2015; Miragaia et al. 2018). Maternal deprivation in animals has been shown to cause short-term cellular changes in the hippocampus and cerebellum (López-Gallardo et al. 2008; Llorente et al. 2009).

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The cerebellum plays a critical role in higher cortical functions and has important implications in neurodevelopmental neuropsychiatric disorders such as autism and schizophrenia (Fatemi et al. 2008). As observed by Konarski et al. (2005), the cerebellum has a putative role in normal and abnormal mood regulation. Damage to the cerebellum has been implicated in the causation of cognitive and motor dysfunctions (Bolceková et al. 2017), making this brain structure critical in the overall functioning of the central nervous system.

Alteration in the hypothalamic-pituitary-adrenal axis is a primary mechanism linking early life stress to increased mental health problems, although the nature and degree of such alterations depend on the type of stressful condition (Essex et al. 2011; Koss and Gunnar 2017). Previous studies have shown that MD causes oxidative damage to different regions of the brain (Marković et al. 2017; Omotoso et al. 2020a). Emotional stressors favour the generation of reactive oxygen species (ROS), causing a redox imbalance in brain biochemistry (Schiavone et al. 2013).

Vitamin B complex (Vit Bco) is made up of a number of water-soluble B vitamins such as B_1 (thiamine), B_2 (riboflavin), B_3 (nicotinamide), B_5 (pantothenic acid), B_6 (pyridoxine), B_7 (biotin), B_9 (folic acid) and B_{12} (cyanocobalamin): Each of which has distinct functions, and are involved in neurodevelopmental processes and in various aspects of brain function, including energy production and deoxyribonucleic acid/ribonucleic acid synthesis and repair (Kennedy 2016). More than a third of psychiatric admissions have been found to suffer deficiencies in folate or vitamin B_{12} (Reynolds 2006). The basic symptoms of vitamin B_6 deficiency are neurologic, including depression, cognitive decline, dementia, and autonomic dysfunction, while vitamin B_{12} deficiency is often manifested in the form of neurological symptoms preceding the appearance of more typical haematological changes (Reynolds 2006). Due to the absence of a therapeutic cure for these psychiatric conditions, this study aimed to explore the neuroprotective potential of Vit Bco in maternal deprivation model of psychiatric disorder.

MATERIALS AND METHODS

Animal Use and Care

This study was performed in compliance with the Institutional Animal Care and Use Committee (IACUC) guidelines and as approved by the University Ethical Review Committee, University of Ilorin (UERC/ASN/2018/1368), Nigeria. Wistar rats of both sexes with average weight of 148.8±0.62 g (female) and 198.6±1.03 g (male) were obtained and housed in the Animal House Facility of the Faculty of Basic Medical Sciences, University of Ilorin. They were allowed to acclimatize for two weeks and thereafter allowed to mate (a male to two females). All animals had free access to rodent feeds (Livestock & Aqua Feeds Enterprises, Ilorin Nigeria) and water *ad libitum*. At delivery, the pups were weighed and culled to nine per dam. The pups were culled based on their weights, without gender bias.

Maternal Deprivation

The pups were separated temporarily (maternal deprivation) on PND 9 for a period of 24 h (Llorente et al. 2007; Omotoso et al. 2020a): Mothers from deprived groups were removed in the morning (10:00 am), and pups were weighed and left undisturbed in their home cages in the same room, and mothers were returned to their respective cages after 24 h on PND 10.

Vitamin B-Complex Solution Preparation

Tablets of Vit Bco (Dr Meyer's B-Complex High Potency) was obtained from Prime Health Pharmacy, Ilorin: Each tablet contained vitamin B_1 (5 mg), B_2 (2 mg), B_3 (20 mg) and B_6 (2 mg). 40 mg was dissolved in 20 ml of normal saline (0.9% NaCl, pH 7.35) and administered orally at a dose of 40 mg/kg/day (Khan et al. 2008).

Animal Grouping and Administration

The pups were selected without gender bias and grouped into 4 (n=5): First group was Control, given 50 ml/kg of normal saline, second group was Vit Bco group, which received 40 mg/kg/day of Vit Bco orally (Omotoso et al. 2020b), third group was maternal deprivation (MD) group where pups were maternally deprived on PND 9 for 24 h, while pups in the fourth group (MD+VIT Bco) were maternally deprived on PND 9, and also received Vit Bco (40 mg/kg/d) orally. Administration of normal saline and Vit Bco was from PND 21-35.

Tissue Collection and Processing

Two rats per group were used for histopathological studies, while the remaining three rats were used for enzymatic studies. The rats for histopathological studies were anaesthetised on PND 36 with ketamine (5 ml/kg, intramuscularly) and subjected to transcardial perfusion with 0.1 M PBS (pH 7.4) followed by 4% paraformaldehyde (PFA). Cerebellar tissues were excised, weighed and post-fixed in 4% PFA. PFA-fixed tissues underwent tissue processing for histological studies and stained using haematoxylin and eosin (general cerebellar architecture) (Stevens 1982; Fischer et al. 2006) and anti-glial fibrillary acidic protein antibody (GFAP; for immunohistochemical expression of astrocytes) via immunoperoxidase technique.

Immunoperoxidase was carried out on PFA-fixed cerebellar tissue following the method of Goldstein and Watkins (2008), involving antigen retrieval to break the methylene bridges using trypsin. Endoge-

nous peroxidase blocking was done using hydrogen peroxide (0.5-3%), while 5% bovine serum albumin was used to reduce non-specific protein reactions. Tissues were incubated in the primary antibody, anti-GFAP (1:1000), and further incubated in the secondary antibody (goat anti-rabbit, 1:400). Tissue slides were treated with horseradish peroxidase, while colour intensification was done using the chromogen, 3.3ˈ-diaminobenzidine. Slides were counterstained with haematoxylin and differentiated in 1% acid alcohol.

Rats for enzymatic studies were sacrificed by cervical dislocation; the cerebellum was excised, weighed and rinsed in 0.25 M sucrose thrice at 5 min each, and stored in 30% sucrose at 4℃. They were thereafter homogenized in 0.25 M sucrose (1:4, weight/volume) at 4℃ and centrifuged for 15 min at 5000 rpm to obtain the supernatants, which were aspirated into plain labelled glass cuvettes and placed on ice. Superoxide dismutase (KT-60703) and catalase (MBS701713) activities were assayed using spectrophotometric techniques (Sinha et al. 1972; Sun and Zigma 1978).

Statistical Analysis

All quantitative data were analysed using GraphPad® (version 7) software. The results were analysed using one-way analysis of variance with Tukey's multiple comparisons test. Significance was set at 95% confidence interval, and the results were represented as mean with standard error of mean.

RESULTS

Vitamin B Complex Attenuates Weight Loss in Maternally Deprived Wistar Rat's Pups

Following maternal deprivation at PND 9, pups in the MD group consistently reduced body weights when compared with the other groups (Fig. 1). The weights of pups in the control and Vit Bco groups increased sharply from PND 10-31, but gradually till PND 35. Although pups that received Vit Bco alone had slightly less body weights when compared with the control. Pups that received Vit Bco after maternal deprivation recorded higher body weights for the major period of the study compared with pups that did not receive Vit Bco after maternal deprivation.

Vitamin B Complex Counter-Balances Maternal Deprivation-Induced Oxidative Stress

The activities of superoxide dismutase (SOD) and catalase (CAT) were assessed. The activity of SOD was less in maternal deprived pups compared to the control ($p<0.05$) and the Vit Bco groups ($p>0.05$) (Fig. 2). Pups that received Vit Bco intervention after maternal deprivation also showed less SOD activity compared with the control ($p<0.05$) and Vit Bco only groups ($p>0.05$), but a slightly higher SOD activity when compared with those that did not receive the intervention after deprivation ($p>0.05$).

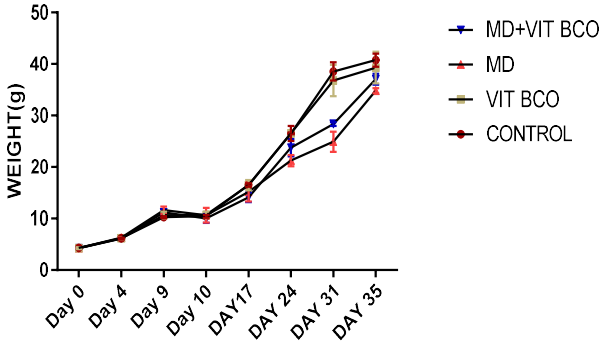

Fig. 1: Pattern of body weights of pups. VIT BCO= vitamin B complex, MD= maternal deprivation, MD + VIT BCO = maternal deprivation + vitamin B complex

The activity of catalase enzyme in the cerebellum (Fig. 3) was higher in the control and Vit Bco-treated groups when compared with the maternally deprived group with or without Vit Bco intervention; although catalase level was higher in Vit Bco-treated pups than the control, the difference was not statistically significant ($p>0.05$). However, maternally deprived pups without Vit Bco intervention showed significantly less catalase activity compared with the Vit Bco-treated pups ($p<0.05$). Pups that received Vit Bco intervention after deprivation increased catalase activity compared with pups that received no Vit Bco after deprivation ($p>0.05$); however, catalase level was lower in the former when compared with the control and Vit Bco alone group ($p>0.05$).

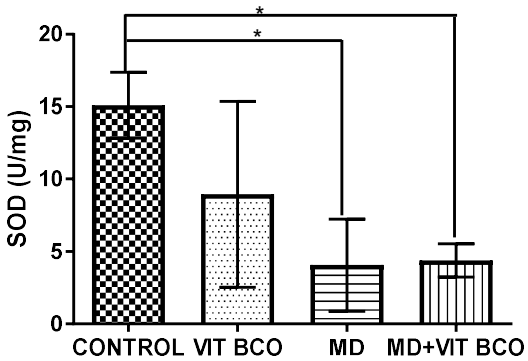

Fig. 2: Activity of superoxide dismutase (SOD) in the cerebellum of pups on PND 36. Significant reduction in maternally deprived (MD) rats compared to the control (* $p<0.05$) and other groups. Pups administered with Vit B complex (VIT BCO) after maternal deprivation (MD + VIT BCO) had slightly higher SOD level compared to the MD group ($p>0.05$). The difference between the control and MD + VIT BCO group was significant (* $p<0.05$).

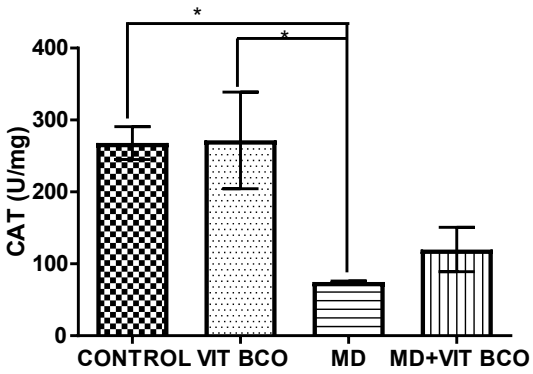

Fig. 3: Activity of catalase (CAT) in the cerebellum of pups. Reduced activity in MD compared with control and Vit Bco group (* p<0.05). Pups administered with Vit B complex (Vit Bco) after deprivation had higher CAT activity compared to MD group (p>0.05). MD= maternal depriva-tion, MD + VIT Bco = maternal deprivation + Vitamin B complex.

Cerebellar Histological Demonstration and Astroglial Expression

General morphological presentation of cerebellar layers in control pups and those treated with Vit Bco were characterized by Purkinje cells with noticeable cell bodies and dendrites deeply projecting into the molecular layers (Fig. 4). The granular layers in these groups consisted of numerous small granular neurons which were well stained. The Purkinje cell layer of the cerebellum of maternally deprived pups appeared distorted with poorly stained and pyknotic Purkinje cells; the granule cells were sparsely distributed within the granular layer. However, in pups that were treated with Vit Bco after maternal deprivation, the Purkinje cell and granular layers appeared normal and similar to the control.

Expression of astrocytes using anti-GFAP antibody (Fig. 5) revealed a sparse population of astrocytes around neurons and between layers, with normal processes in the cerebellar cortex of the control and Vit Bco-treated pups. The cerebellum of maternally deprived pups revealed activated astrocytes and increased astrocytic density compared to other treatment groups. The expression of astroglia within the cerebellar cortex of pups treated with Vit Bco after maternal deprivation appeared similar to those in control and Vit Bco-treated groups.

DISCUSSION

Maternal deprivation during the early postnatal period causes a reduction in the body weights of offspring. As reported by Llorente et al. (2007), the maternally deprived pups showed a significant body weight decrease in adolescence and adulthood. This might be due to the stoppage of breastfeeding during the period of separation from the mother and subsequent alterati-on in the developmental progra-mming of hypothalamic energy regulatory circuits (Remmers and Delemarre-van de Waal 2011).

The oxidative stress occasioned by MD and subsequent ROS generated exerts detrimental effect on cells, thereby damagi-ng the cell structure and macro-molecules, including proteins, lipids and DNA (Shields et al. 2021). According to Zugno et al. (2015), maternal deprivation disrupts mitochondrial energy homeostasis and increases oxidative stress in the rat brain. Such redox imbalance in maternally deprived offspring enhances cell death, contributing to the pathology observed in such cases (Zhang et al. 2002). The current study observed a significant reduction in the level of cerebellar superoxide SOD and catalase activities in maternally deprived rats.

Catalase is the main enzyme responsible for the removal of hydrogen peroxide from normal tissues; by breaking down hydrogen peroxide, it prevents further generation of ROS (Mueller et al. 1997). The reduced level of these enzymes in maternally deprived rats was an indication of increased generation of ROS. When the increased level of free radicals is not checked, it overwhelms the innate body defence mechanisms, resulting in the compromise of cellular integrity and functions (Phaniendra et al. 2015), as shown in the current

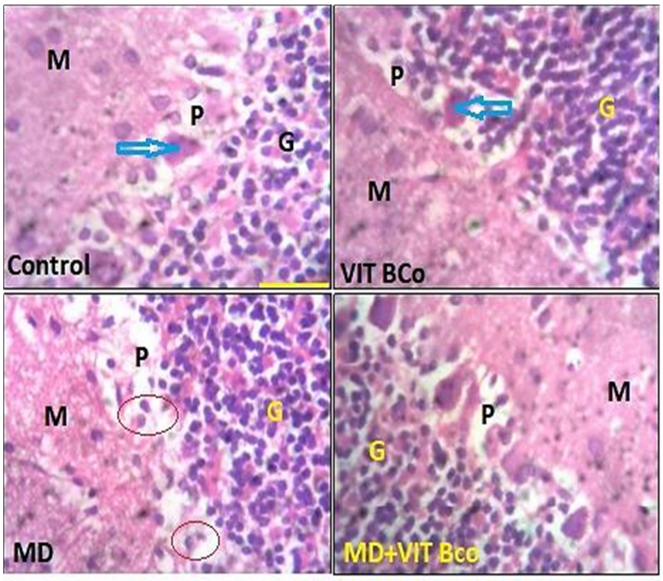

Fig. 4: Representative photomicrographs of the cerebellar cortex of 36 days old pups showing the molecular layer (M), Purkinje cell layer (P) and granular layer (G). The general histology of control and Vit Bco-treated groups showed deeply stained and characteristically normal cellular layering of granule cell layers with well outlined Purkinje cells (arrows) in the Purkinje layer. The Purkinje cell layer in the maternal deprivation group was distorted and the Purkinje cells appeared scanty, poorly stained and pyknotic (red circles), whereas Purkinje cells in pups that received Vit Bco after maternal deprivation were well delineated similar to the control. Haematoxylin & eosin; scale bar: 25µ.

work. Meanwhile, the administration of Vit Bco to maternally deprived rats increased the level of catalase and SOD, underscoring the potential of Vit Bco in alleviating some of the effects of oxidative stress associated with maternal deprivation (Ford et al. 2018). Aside from other mechanisms of action, Vit Bco directly scave-nge ROS, particularly super-oxide and indirectly stimulate ROS scavenging by preserva-tion of glutathione (van de Lagemaat et al. 2019).

Vit B complex are coenzymes involved in many enzymatic processes essential for cellular physiological functioning with a great impact on brain function and neurochemical synthesis (Kennedy 2016). The deficiency of any of these vitamins has been associated with neurologi-cal and psychiatric symptoms (Sturman and Rivlin 1975). Various degrees of alterations were observed in the cytoarch-itecture of the cerebellar cortex of maternally deprived rats, including degeneration of Purki-nje cells and increased expres-sion of activated astrocytes. Earlier findings revealed that maternal deprivation modulates the levels of glial fibrillary acidic protein in the hippocampus, cerebellum and cortex (López-Gallardo et al. 2008; Musholt et al. 2009). Associated with MD-induced oxidative stress is neuroinflammation with subseq-uent release of inflammatory molecules and cytokines, which also contribute to the neuropat-hology in MD (Roque et al. 2016). Astrocytes are key in providing antioxidant support to neural tissues and in the repair of damaged tissues (Baxter and Hardingham 2016). The astro-gliosis associated with MD in the current study is a defence mechanism initiated to limit the effects of oxidative stress and possibly repair damaged neural tissues. However, the introduction of Vit Bco supplement, which is an antioxidant, further enhanced the alleviation process by scavenging free radicals. Hence, the ability of the cerebellum to withstand maternal deprivation-induced damage may be as a result of the Vit Bco intervention which conferred substantial cytoprotection on the cerebellum of rats by preventing astrogliosis and cellular damage.

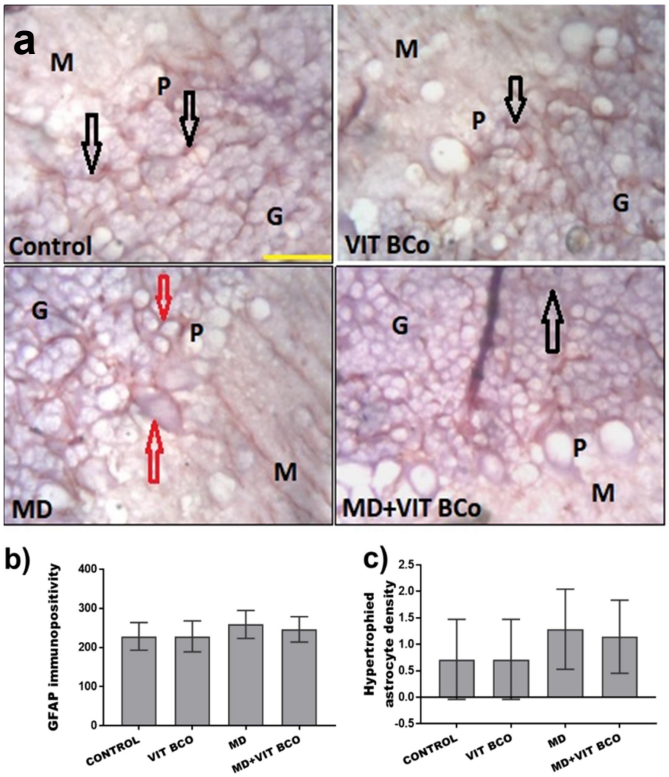

Fig. 5: Representative photomicrographs of the cerebellar cortex of 36 days old pups stained with anti-glial fibrillary acidic protein (GFAP) antibody. GFAP immunopositive cells (black arrows) in the control and Vit Bco-treated group (a) ap-peared sparse around neurons and between cerebellar layers. However, there was increased expression of activated astrocytes (red arrows) within the granule cell layer (G) in the cerebellar layers of maternal deprived pups. Quantitatively, b) showed more GFAP-immunopositivity in maternally deprived (MD) group compared to other groups, while it was slightly reduced in group that received Vit Bco post-deprivation (MD+VIT BCo). In c) highest population of hypertrophied astrocytes was seen in MD pups compared with other groups ($p>0.05$), while those that received VIT BCo post deprivation had slightly lower density compared to maternally deprived (MD) pups ($p>0.05$). P: Purkinje cell layer; M: molecular layer; MD: maternal deprivation; VIT Bco: vitamin B complex. GFAP labelling; scale bar: 25µ.

Conclusion

Vitamin B complex significantly counteracted maternal deprivation-induced neurotoxicity by eliciting significant protective effect on the integrity of neuronal cells in the cerebellum.

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No external funding was received.

Conflict of Interest

None declared.

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Authors' Contributions

Conceptualization: GOO, FAA; Data acquisition: FAA, NYM, AIB; Data analysis or interpretation: FAA, NYM, ITG, GOO; Drafting of the manuscript: FAA, GOO; Critical revision of the manuscript: GOO.

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