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Effects of Cholecalciferol on the White Blood Cell Parameters following Traumatic Brain Injury Using Rat Model

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ABSTRACT

Traumatic brain injury (TBI) is a significant cause of illness, disability, and mortality across all age groups. Every year, almost 50 million individuals worldwide suffer from TBI. By 2005, around 3.17 million TBI survivors were dealing with long-term repercussions, such as neurological, psychological, and irreversible disability. Vitamin D may modulate the immune system, according to research, since its metabolites and receptors (VDR) are generated and expressed in white blood cells. This research is to evaluate the impact of cholecalciferol on white blood cell parameters after TBI. A total of 42 adult Wistar rats weighing between 180 and 230g were randomly split into four groups (A, B, C, and D) of 12 rats each, with additional subgroups of six rats. Group A functioned as the control group, and no brain damage occurred. Group B was subjected to brain damage with a 100g weight; sub-group B1 got no therapy, whilst sub-group B2 received cholecalciferol. Group C was given a 200g weight; sub-group C1 went untreated, whereas sub-group C2 got therapy. Group D was given a 300g weight; sub-group D1 was left untreated, while sub-group D2 received cholecalciferol. The brain damage were caused by the Marmarou's weight-drop equipment. Blood samples were taken from all rats one week before TBI induction to establish baseline values, as well as after 6, 24, and 72 hours after the shock. These samples were examined at a haematology lab to determine differential white blood cell counts. White blood cell counts and differentials did not alter significantly after cholecalciferol treatment. Following a TBI, cholecalciferol has no substantial impact on white blood cells or their parameters.

Keywords: Traumatic brain injury, Cholecalciferol, White Blood Cell, metabolites and receptors

INTRODUCTION

Traumatic brain injury (TBI) is defined as brain damage caused by an external mechanical force [1]. It may cause temporary or chronic deficits in cognitive, physical, and psychosocial functioning [2]. TBI is the leading cause of mortality and disability among those under 45 years old [3]. It causes 10 million deaths and hospitalisations worldwide each year, and affects around 57 million people. TBI is mostly caused by falls, motor vehicle collisions, traffic-related incidents, hits with objects, and physical attacks [4]. Cholecalciferol, a steroid hormone, has effects that go beyond its usual function in bone health and calcium homeostasis [5], [6], and [7]. It is absorbed by food, supplements, or skin synthesis in response to ultraviolet-B (UVB) exposure. Cholecalciferol deficiency is a common

health problem, and it has been associated to inflammatory and infectious illnesses [8]. According to research, taking cholecalciferol supplements may help minimise the chance of developing autoimmune illnesses [9].

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/2025/002

Experimental Design

Forty-two adult Wistar rats were randomly split into four groups of twelve. These groups were subsequently split into seven sub-groups, each with six rats. Group A functioned as the control group, and no brain damage occurred. Group B underwent TBI with a 100g weight; sub-group B1 got no therapy, whereas sub-group B2 was given cholecalciferol. Group C was subjected to a 200g weight; sub-group C1 went untreated, while sub-group C2 got therapy. Group D received TBI with a 300g weight; sub-group D1 was untreated, whereas sub-group D2 received cholecalciferol.

TBI Induction

Traumatic brain injury was induced using Marmarou's weight-drop apparatus. This device consists of a 2.2 cm diameter, 1.5 m long cylindrical plastic guide tube secured to a clamp stand. A U-shaped clear plastic stage covered with foil was positioned beneath the guide tube. Metal weights of varying sizes were suspended from a fishing line, which passed through the guide tube and was attached to the clamp stand. The weights were positioned approximately 2.5 cm above the foil. Each rat was lightly anesthetized using isoflurane in a chamber until unconscious. The unconscious rat was then placed face-down on the foil with its head aligned under the falling weight. If a rat began to move before the procedure, it was returned to the isoflurane chamber for further anesthesia. The weight was released by pulling an Allen key, causing it to fall vertically through the guide tube and strike the rat's head. Immediately after impact, topical lidocaine was applied to the injury site to minimize pain. This procedure was performed on each group with their designated weights.

Blood Collection

Blood samples were collected from all rats one week before TBI induction to establish baseline values. Blood collection was performed using a capillary tube inserted into the medial canthus of the eye, allowing blood to flow into pre-labeled EDTA bottles. The samples were thoroughly mixed to prevent coagulation. Following TBI induction, additional blood samples were collected at 6, 24, and 72 hours post-trauma using the same procedure. These samples were sent to the hematology laboratory for differential white blood cell analysis.

Data Analysis

The collected data were analyzed using Statistical Product and Service Solutions (SPSS) software, version 25.0 (Chicago, USA). Results were expressed as mean ± standard error of the mean (SEM). Multiple group comparisons were performed using analysis of variance (ANOVA), and statistical significance was defined as $p < 0.05$.

RESULTS

Table 1: Descriptive and ANOVA result of baseline WBC parameters

Groups	WBC	N %	L %	E %	B %	M %
A	9540.00± 370.94	49.40 ± 6.11	45.40 ± 1.39	2.00 ± 0.63	0.00	3.00 ± 0.95
B	11990.91± 908.9	54.91±2.38	40.27 ± 6.09	1.82 ± 0.33	0.00	2.36 ± 0.45
C	9376.92±805. 92	56.15 ± 2.38	40.08 ± 2.75	2.00 ± 0.20	0.00	2.54 ± 0.33
D	9650.00± 751.92	55.00 ± 2.11	40.50 ± 2.11	1.75 ± 0.25	0.00	2.58 ± 0.36
Total	10178.05± 441.4	54.66± 1.39	40.90 ± 1.37	1.88 ± 0.14	0.00	2.56 ± 0.22
F stat	2.28	0.70	0.48	0.18		0.23
P value	0.10	0.56	0.70	0.91		0.87

Table 1: shows the baseline WBC parameters, reported as mean ± standard error of mean (SEM). WBC is higher in B compared to A,C and D. N% is higher in B,C and D compared to A. L% is higher in A compared to B,C and D. E% is slightly higher in A and C compared to B and D. M% is slightly higher in A compared to B,C and D. However, There is no significant difference ($P>0.05$, ANOVA) in mean WBC, N%, L%, E%, B% and M% among the groups A, B, C, and D.

Table 2: Descriptive and ANOVA result of WBC parameters collected after 6 hours

Groups	WBC	N %	L %	E %	B %	M %
A	70.67 ±7.69	68.67 ± 0.67	30.67 ± 0.67	0.67 ± 0.67	0.00	0.00
B1	68.00 ±5.00	64.00 ± 4.20	36.00 ± 4.50	0.00 ± 0.75	0.00	0.00
B2	68.00 ± 4.2	54.00 ± 4.50	46.00 ±3.34	0.00 ±1.20	0.00	0.00
C1	60.00 ± 3.72	70.00 ± 5.33	30.00 ±5.00	0.00 ± 2.10	0.00	0.00
C2	74.00 ± 8.00	64.00 ± 6.00	35.00 ±7.00	1.00 ±1.00	0.00	0.00
D1	94.00 ± 5.50	70.00 ± 2.70	30.00 ±3.35	0.00 ±1.50	0.00	0.00
D2	66.00 ± 2.00	64.00 ± 2.00	34.00 ± 4.00	2.00 ± 2.00	0.00	0.00
Total	72.20 ± 3.48	65.00 ± 1.74	34.20 ± 1.92	0.80 ± 0.44	0.00	0.00
F stat	0.98	1.85	1.20	0.31		
P value	0.52	0.29	0.44	0.88		

Table 2 shows WBC parameters after 6 hours, reported as mean ± standard error of mean (SEM). WBC and L% are lower in A (negative control group) compared to the traumatized groups B, C and D while N% and E% are higher in A (negative control group) compared to the traumatized groups B, C and D. WBC and N% is higher in traumatized untreated sub-groups of B, C and D (B1, C1 and D1) compared to traumatized treated sub-groups of B, C and D (B2, C2 and D2). L% and E% are lower in traumatized untreated sub-groups of B, C and D (B1, C1 and D1) compared to traumatized treated sub-groups of B, C and D (B2, C2 and D2). However, There is no significant difference (P>0.05, ANOVA) in mean WBC, N%, L%, E%, B% and M% between the groups B, C, and D compared to group A and between the traumatized treated and untreated sub-groups.

Table 3: Descriptive and ANOVA result of WBC parameters after 24 hours

Groups	WBC	N %	L %	E %	B %	M %
A	74.00± 1.00	68.00±0.50	32.00±0.92	0.00±0.43	0.00	0.00
B1	68.00±3.44	70.00± 2.72	30.00±2.85	0.00±0.50	0.00	0.00
B2	70.00± 2.00	60.00± 4.00	39.00± 1.00	1.00± 1.00	0.00	0.00
C1	71.00± 3.00	65.00± 1.00	34.00± 0.00	1.00± 1.00	0.00	0.00
C2	80.00± 2.00	69.00± 1.00	30.00± 0.00	1.00± 1.00	0.00	0.00
D1	64.00± 3.50	58.00± 3.54	42.00±1.72	0.00± 1.34	0.00	0.00
D2	76.00± 2.00	61.00± 5.00	39.00 ±3.00	0.00± 0.00	0.00	0.00
Total	72.73± 1.62	64.18± 1.58	35.27 ±1.65	0.55± 0.28	0.00	0.00
F stat	3.94	1.47	1.32	0.30		
P value	0.10	0.37	0.41	0.91		

Table 3 shows the WBC parameters after 24 hours, reported as mean ± standard error of mean (SEM). WBC and E% are higher in A (negative control group) compared to the traumatized groups B, C and D while L% and N% are lower in A (negative control group) compared to the traumatized groups B, C and D. WBC and N% is higher in traumatized untreated sub-groups of B, C and D (B1, C1 and D1) compared to traumatized treated sub-groups of B, C and D (B2, C2 and D2). L% and E% are slightly lower in traumatized untreated sub-groups of B, C and D (B1, C1 and D1) compared to traumatized treated sub-groups of B, C and D (B2, C2 and D2). However, There is no significant difference (P>0.05, ANOVA) in mean WBC, N%, L%, E%, B% and M% between the groups B, C, and D compared to group A and between the traumatized treated and untreated sub-groups.

Table 4: Descriptive and ANOVA result of WBC parameters after 72 hours

Groups	WBC	N %	L %	E %	B %	M %
A	68.00± 5.00	54.00± 2.45	46.00± 2.07	0.00± 0.33	0.00	0.00
B1	85.00± 7.00	67.00± 3.00	32.00 ± 2.00	1.00± 1.00	0.00	0.00
B2	86.00± 6.40	64.00±1.50	36.00± 1.75	0.00± 0.00	0.00	0.00
C1	81.00± 7.00	66.00± 0.00	33.00 ± 1.00	1.00± 1.00	0.00	0.00
C2	88.00± 3.06	68.00± 2.00	32.00 ± 2.00	0.00± 0.00	0.00	0.00
D1	88.00± 4.00	69.00± 1.00	30.00± 2.00	1.00± 1.00	0.00	0.00
D2	81.00± 3.00	65.00± 1.00	33.00± 1.00	2.00± 2.00	0.00	0.00
Total	83.69± 2.09	65.85± 1.21	33.39 ± 1.25	0.77± 0.36	0.00	0.00
F stat	1.25	3.95	4.52	0.45		
P value	0.40	0.06	0.04	0.82		

Table 4 shows WBC parameters after 72 hours, reported as mean ± standard error of mean (SEM). WBC, N% and E% are lower in A (negative control group) compared to the traumatized groups B, C and D while L% is higher in A (negative control group) compared to the traumatized groups B, C and D. WBC, L% and E% are slightly lower in traumatized untreated sub-groups of B, C and D (B1, C1 and D1) compared to traumatized treated sub-groups of B,C and D (B2, C2 and D2). N% is significantly higher in traumatized untreated sub-groups of B, C and D (B1, C1 and D1) compared to traumatized treated sub-groups of B, C and D (B2, C2 and D2). However, There is no significant difference (P>0.05, ANOVA) in mean WBC, N%, L% , E%, B% and M% between the groups B, C, and D compared to group A and between the traumatized treated and untreated sub-groups.

DISCUSSION

Traumatic Brain Injury (TBI) disrupts normal brain function and leads to various pathological changes [10]. Cholecalciferol has been reported to reduce the inflammatory response following TBI [11]. Inflammatory processes, such as those triggered by TBI, typically result in elevated white blood cell counts [12], which was observed across all injured groups in this study. However, treatment with cholecalciferol did not produce any significant differences in white blood cell parameters between the untraumatized (negative control group) and traumatized groups. And between the traumatized untreated and treated sub-groups at different time intervals.

These findings are consistent with the studies conducted by [13] and [14], which also concluded that cholecalciferol supplementation does not significantly alter white blood cell counts. However, the results of this study contradict the findings of [15], which reported that cholecalciferol supplementation improved hematological parameters and reduced leukocyte migration into the peritoneal and pulmonary cavities in alloxan-diabetic mice. This discrepancy in outcomes may be attributed to differences in toxicity levels or experimental conditions.

CONCLUSION

This study shows that there is no significant effect of cholecalciferol on the white blood parameters following Traumatic brain injury.

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