Research Article

Neuroprotective and Haematological Effects of Hydrochlorothiazide in Traumatic Brain Injury: Insights from a Rat Model Study

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ABSTRACT

This study evaluated the effects of hydrochlorothiazide (HCT) on neurological and haematological abnormalities in albino rats induced with traumatic brain injury (TBI). Three groups of five rats each were used: Group 1 received HCT treatment (25 mg/kgbw) for 14 days post-TBI, Group 2 was TBI-induced but not treated, and Group 3 served as the non-traumatic non-treatment control. Neurological score, blood parameters (neutrophils, lymphocytes, monocytes, HB, PCV, WBC, RBC, the neutrophil/lymphocyte ratio, plasma glucose levels), serum electrolytes, and brain histology were assessed. HCT treatment significantly improved neurological scores and adaptability compared to the untreated TBI group. Plasma glucose levels in the treated group were lower in the first three hours post-induction but showed no difference from the untreated group on days two through five. The untreated TBI rats had significantly reduced RBC, HB, and PCV values, while the HCT-treated rats had higher values. The untreated group also showed higher counts of WBC, neutrophils, and lymphocytes, which were lower in the treated group. The neutrophil/lymphocyte ratio increased in the untreated group but decreased in the treated group. Electrolyte imbalances in the untreated group were corrected in the HCT-treated group. Histological analysis revealed more severe brain lesions in the untreated TBI group than in the treated group. In conclusion, HCT therapy reduces the inflammatory response induced by TBI and improves neurological function in albino rats.

Keywords: Traumatic Brain Injury, Hydrochlorothiazide, Electrolytes, Haematology


INTRODUCTION

Traumatic brain injury (TBI) is a multifaceted disorder that is defined by the physiological disruption of neural tissue, typically brought on by piercing accidents, blunt force trauma, or acceleration-deceleration factors (Capizzi et al., 2020). TBI can cause a range of physiological reactions, such as changes in plasma, electrolyte, and acid-base balance, in addition to acute

Bulama et al.
physical damage (Pin-on et al., 2018). Cognitive capacity and long-term medical conditions may be significantly impacted by these abnormalities (Okediran et al., 2021). Maintaining appropriate blood circulation to the brain while minimizing the risks of excess fluid and cerebral oedema is a difficult balancing act when managing osmotic diuretics in TBI patients. This entails choosing the right kind and quantity of fluids, keeping a close eye on the individual's condition, and modifying treatment in response to ongoing evaluations of the patient's health status. For instance, to direct fluid therapy, regular and precise monitoring of hemodynamic variables, ICP, and fluid equilibrium is required. This can demand a lot of resources, including qualified workers and the right tools (Kishen, 2023).

For over 50 years, hydrochlorothiazide, a thiazide-type diuretic, has been utilized in therapeutic settings (Arumugham and Shahin, 2023). The medication is comparatively safe and has been used extensively throughout the world to treat hypertension and recommended as a supplement to corticosteroid, oestrogen therapy, hepatic cirrhosis, and congestive heart failure to relieve oedema (FDA-approved) (Roumelioti et al., 2018). It is further suggested for the treatment of oedema brought on by renal failure (FDA-approved), suitable as an independent or combination treatment for hypertension (approved by the FDA) (Arumugham and Shahin, 2023). Thus, we speculate that HCT may be able to reverse the neuropsychological and haematological changes caused by traumatic brain injury.

MATERIALS AND METHODS

Experimental Animals

Fifteen (15) healthy albino rats (Wister strain) weighing between 200-250g were obtained from the faculty of Veterinary Medicine, University of Maiduguri, Nigeria for this study. The rats were allowed to acclimatize to the research laboratory conditions for two weeks. The rats were fed with a grower's mash of Vital feed ad libitum and were also given water.

Drug Reconstitution and Dosage

Hydrochlorothiazide was obtained from Juvise Pharmaceuticals France. Using the below formula 25mg of hydrochlorothiazide was dissolved in 1ml of distilled water to give a concentration of 25mg/ml. This was used to calculate the volume of the drug to be administered using the below formula:

\[ \text{Volume of Drug} = \frac{\text{Bodyweight} \times \text{Dosage}}{\text{Concentration}} \]

Experimental Design

A total of fifteen (15) seemingly healthy albino rats weighing (200-250g), were randomly divided into three groups 5 rats each (traumatized treated, traumatized not treated and non-traumatized non-treated) rats group respectively.

Group 1: Traumatized treated (TTT)

Group 2: Traumatized not treated (TNT)

Group 3: Non traumatize non treated (NTNT)

Treated daily for two weeks at the dosage of 25mg/kg bw hydrochlorothiazide to group 1

Induction of Traumatic Brain Injury

Head injury was induced in the entire experimental animals except in the negative control group by weight drop method using an acceleration impact device (Marmaru, 1994). The experimental rats were properly restrained and anaesthetized using xylazine and Ketamine at a dose rate of 5mg/kg and 80mg/kg body weight respectively. Once they are unconscious, the head is shaved using a razor blade and cleaned with an antiseptic (Dettol). The skull was exposed by midline incision (1cm) using a scalpel blade and a blade holder and a stainless steel disc measuring 10mm in diameter and 3mm in depth was cemented centrally along the frontal bone. The experimental animals were secured in the prone position on a 10cm deep foam bed. The injury was induced by dropping a one-hundred-and-twenty gram (120g) brass weight from a distance of 1m. The stainless steel disc was immediately removed from the skull; the incised skin was sutured using nylon size 3.0 in a simple interrupted pattern. The animal was allowed to recover in the cage.

Neurological Severity Score Assessment

The score was done as described by Yarnell et al. (2016). Two vacant containers were positioned with approximately 25cm between them, and a linear balance beam was positioned on top of the containers. Subsequently, a rat was positioned at the initial point of the beam to carry out a series of neurobehavioral assessments while accounting for the element of time. These assessments encompassed various tests, namely the general balance test, landing test, sound reflex, tail raise test, drag test, righting reflex, ear reflex, eye reflex, and paw flexion reflex. The findings are documented in the following manner.

0 = rat was able to do each of the above tests successfully without any hindrance.
1 = the rat had some difficulties while undergoing those tests.
2= rats did not respond. The scale has a total of 0 to 20 scores with higher scores indicating an increase in severity.

Sample Collection

At the end of the 14 days treatment period, blood was collected via cardiac puncture after anaesthesia with ketamine and xylazine. The samples were immediately transferred to plane and EDTA sample bottles for
haematology and serum electrolytes determination. Brain tissues were extracted from the skull and fixed in 10% neutral buffered formalin for histology.

**Determination of Packed Cell Volume, Erythrocyte Count, Haemoglobin concentration, Total white blood cell count and Differential leukocyte count**

Using micro-haematocrits and hematocytometry techniques, packed cell volume (PCV), erythrocyte (RBC), and leukocyte counts (WBC) were individually determined for each blood sample (Weiss and Wardrop 2011). The cyanmethemoglobin technique was used for the determination of haemoglobin concentration (Ghelfod et al., 2002).

**Evaluation of Glucose and Plasma Proteins**

Using a digital glucometer and glucometer strip (ACCU CHECK?), the blood glucose level was determined. Briefly, a drop of blood from the tail vain was made onto the glucometer strip appropriately placed in a glucometer and the reading was recorded.

**2.9 Serum electrolytes and total protein determination**

The colorimetric method was used for the determination of Na and bicarbonate ions, method of Tietz and Berger (1995). K ion was determined using Turbidimetric tetraphenylborate (Tietz and Berger 1995) Total protein was determined using the calibration method (Koller et al., 1984).

**2.10 Histology**

Samples of the brain tissue extracted from all the experimental animals were fixed in 10% buffered formalin for 48 hours. the fixed tissues were dehydrated in graded concentrations of alcohol (70%, 80%, 90% and 100%). The tissues were cleared using xylene. Wax-embedded tissues were sectioned at 0.5µm using a microtome knife attached to a microtome the sectioned tissues were mounted on a grease-free, clean glass slide, dried at room temperature and stained with haematoxylin and eosin (H and E) stain. The slides were viewed under a microscope at different magnifications (x10, x20, x40). The Lumper’s technique of ordinal method of scoring was used in scoring the lesions observed in the different groups (Gibson-Corley et al., 2013).

**Data Analyses**

Data obtained were summarized as means ± standard deviations (SD). Differences between means were analysed using analysis of variance (ANOVA; One way) followed by Tukey’s posthoc test and p< 0.05 was considered as statistically significant. Statistical analyses were done using computer software, GraphPad InStat® (2018).

**Ethical Statement and Approval**

All animals were handled according to the ethical procedure approved by the faculty of veterinary medicine committee on animal use and experiment protocol (UDUS/FAREC/AUP-R09/2019).

**RESULTS**

**Effects of HCT on neurological score in TBI rats**

The effects of HCT administration on a neurological score of TBI rats are illustrated in Figure 1. For the neurological score, HCT significantly (p<0.05) decreased the score and improved neurological function in the first week of treatment when compared to TNT rats. Further, the score significantly (p<0.05) decreased in the 2nd week and neurological function improved when compared to the TNT rats (Figure 1).

![Figure 1: Effects of HCT on neurological score in TBI rats](image)

The effects of HCT on glucose level in TBI rats

Figure 2 depicts the effects of HCT administration on glucose levels in mg/dl between HCT treated rats and TNT rats following TBI induction. Glucose levels were taken hourly (1hr, 2hr and 3hrs post TBI) on day one and daily from the 2nd up to the 5th day post trauma. Glucose concentrations were significantly (p<0.0000) decreased for the 1st 2nd and 3rd hours in the HCT group when compared to the TBI non-treated rats. There was no statistically significant difference (p<0.05) between the TNT rats and the HCT treated rats in the glucose levels among the various days (Figure 2).
The effects of HCT administration on RBC, Hb, and PCV in TBI rats

Figure 3 depicts the effects of HCT administration on red blood cells (RBC), haemoglobin (Hb), and packed cell volume (PCV) in TBI rats post injury. We observed that TBI significantly (p<0.0001) decreased RBC in TNT rats when compared to NTNT rats. However, after treatment with HCT, the RBC significantly (p<0.0001) increased in the treated rats when compared with TNT rats (Figure 3a). Similarly, TBI significantly (p<0.0001) decreased Hb level of TNT rats when compared to the NTNT rats. Subsequently, after treatment with HCT, the Hb level increased significantly (p<0.0001) in the treated rats when compared to the TNT rats (Figure 3b). Furthermore, we observed that TBI significantly (p<0.0001) decreased PCV of TNT rats when compared to the NTNT rats. Subsequently, after treatment with HCT, the PCV was increased significantly (p<0.0001) in the treated rats when compared to the TNT rats (Figure 3c).

Figure 3: Effects of HCT on red blood cells, haemoglobin, and packed cell volume in TBI rats. TNT – Traumatized non-treated, NTNT – Non-traumatized non-treated, ns – not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 for ANOVA analysis and Turkey’s Multiple Comparison Test

Bulama et al.
The effects of HCT administration on Total WBC, neutrophil count, lymphocyte count, and neutrophil-lymphocyte count ratio (NLR) in TBI rats

Figure 4 shows the effects of HCT administration on total WBC, neutrophil count, lymphocyte count, and neutrophil-lymphocyte count ratio (NLR) in TBI rats. We observed that TBI significantly (p<0.001) increased WBC in the TNT rats when compared to the NTNT rats. However, following HCT treatment, WBC significantly (p<0.01) decreased in the treated rats when compared to the TNT rats (Figure 4a). Similarly, TBI significantly (p<0.0001) elevated neutrophil counts of TNT rats when compared to the NTNT rats. Subsequently, after treatment with HCT, the neutrophil counts were significantly (p<0.0001) decreased in the treated rats when compared to the TNT rats (Figure 4b). Furthermore, there is no significant difference between the lymphocyte counts of TNT rats and NTNT rats. However, after treatment with HCT, there was a significant (p<0.0001) decrease in lymphocyte counts in the HCT-treated rats when compared with TNT rats (Figure 4c). After further analysis, we found that, TBI significantly (p<0.0001) elevated neutrophil-lymphocyte counts ratio (NLR) in the TNT rats when compared to the NTNT rats. However, when treated with HCT, the NLR was significantly (p<0.0001) decreased in the treated rats when compared with the TNT rats (Figure 4d).

The effects of HCT administration on Total proteins (TP), sodium (Na), and potassium (K) levels in TBI rats

Figure 5 shows the effects of HCT administration on total proteins (TP), sodium (Na), and potassium (K) levels in TBI rats. TBI significantly (p<0.05) elevated TP level in the TNT rats when compared with the NTNT rats. However, after treatment with HCT, TP level was significantly (p<0.05) decreased in the treated rats when compared with the TNT rats (Figure 5a). Furthermore, TBI significantly (p<0.05) increased Na level when compared with the NTNT rats. However, after treatment with HCT, Na level was significantly decreased (p<0.05) in the treated rats when compared with the TNT rats (Figure 5b). Further analysis revealed that TBI significantly (p<0.001) increased K level of TNT rats when compared with NTNT rats. However, after...
treatment, HCT significantly (p<0.0001) decreased K level in the treated rats when compared to TNT rats (Figure 5c).

The effects of HCT administration on bicarbonate (HCO₃⁻) level in TBI rats
The effects of HCT administration on bicarbonate (HCO₃⁻) level in TBI rats is illustrated in Figure 6. After injury induction, TBI was observed to have significantly (p<0.05) decreased HCO₃⁻ level in TNT rats when compared with NTNT rats. However, the HCO₃⁻ levels increased but not statistically significant in the treated rats when compared with TNT rats (Figure 6).

Figure 5: Effects of HCT on Total protein, sodium, and potassium levels in TBI rats. TNT – Traumatized non-treated, NTNT – Non-traumatized non-treated, ns – not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 for ANOVA analysis and Turkey’s Multiple Comparison Test

Figure 6: Effects of HCT on bicarbonate level in TBI rats. TNT – Traumatized non-treated, NTNT – Non-traumatized non-treated, ns – not significant, *p<0.05, **p<0.01, for ANOVA analysis and Turkey’s Multiple Comparison Test
Fig. 7: Photomicrograph of the brain (cerebrum) of rat of normal control group showing normal oligodendroglial cells (arrowheads) and shrunken intensely eosinophilic neurons (red neurons) with undiscernible nucleolus (arrows), H & E, X400

Fig. 8: Photomicrograph of brain (cerebrum) of rat of induced non-treated group showing various karyorrhexic and karyolytic neurons (arrows) and a completely dead neuron (blue arrow) with increased perinuclear halo (arrowhead), H & E, X400
Bulama et al.

DISCUSSION
This research explored how Albino rats responded to hydrochlorothiazide treatment following traumatic brain injury (TBI). The TBI-induced rats showed distinct responses in each haematological parameter after both TBI and hydrochlorothiazide treatment. Inflammatory markers such as differential leukocyte count and total white blood cell count were notably elevated in the TNT group, while red blood cell count, HB and packed cell volume values were reduced. Additionally, a later inflammatory indicator the neutrophil-lymphocyte ratio (NLR) at two weeks was also elevated in the same group. Hydrochlorothiazide appears to mitigate inflammatory responses and anaemia following brain injury.

In this study, we observed significant improvement in neurological severity score for rats in the hydrochlorothiazide treated group. This indicates an improved neurological function. This effect can be attributed to its antioxidant (Ceron et al., 2010) and diuretic properties. Use of diuretics have been associated with improved neurological function (Yasar et al., 2012). This could be through the reduction of cerebral oedema, intracranial pressure and improvement of cerebral blood flow. Antioxidants preserve neurological function by protecting neurons from damage by free radicals after TBI.

The result for glucose level in the treated rats were lower on the first day compared to the TNT rats, but from day 2 to 5, the difference became insignificant. This action can be explained by the fact that hydrochlorothiazide can cause decrease in insulin sensitivity resulting in decrease uptake of glucose hence increase plasma glucose level (Lindholm et al., 2003). This could be the reason for the elevated level of glucose from day 2 to 5 despite significantly lower level on the first day. The lowering of glucose level on the first day might be due to its antioxidant effect because antioxidant activity is associated with hypoglycaemic effect (Munazza et al., 2023).

The results indicated a significant decrease in packed cell volume, haemoglobin, and red blood cell count in the TNT rats compared to the NTNT rats. This suggests anaemia, likely due to ongoing injury processes. Inflammatory mediators can suppress erythropoiesis, and reactive oxygen species released during TBI can lead to red blood cell loss and subsequent anaemia (Bissinger et al., 2018). Previous studies have also reported anaemia in TBI patients and animal models (Salim et al., 2008) (Tango et al., 2009). However, hydrochlorothiazide-treated rats showed a significant increase in packed cell volume, haemoglobin, and red blood cell count compared to TNT rats. Though hydrochlorothiazide may not have direct effect on haematopoiesis, it might have influence this through some indirect mechanism such as its antioxidant effect.

Fig. 9: Photomicrograph of brain (cerebrum) of rat of treated group showing degenerate and dying neurons (arrows), H & E, X400
Another mechanism could be its inhibitory effect on inflammatory mediators as reported by Diniz et al. (1978). This can boost the erythropoiesis which is suppressed during inflammation.

White blood cell count increased significantly in TNT rats compared to NTNT rats, indicating leucocytosis as an inflammatory response. Similarly, Kumar (2012) reported leucocytosis after experimental TBI. This leucocytosis was countered by hydrochlorothiazide treatment, which significantly reduced white blood cell count in the treated rats. The neutrophil count also increased significantly in TNT rats and decreased in hydrochlorothiazide-treated rats, supporting the anti-inflammatory effect of hydrochlorothiazide. Leucocytosis in TBI can be due to increased catecholamine levels and serum cortisol, but hydrochlorothiazide’s inhibitory effect on inflammatory mediators appears to counteract this. Hydrochlorothiazide has been previously evaluated to have an increasing effect on IL10 (Ferreira et al., 2023). Evidence suggests that T cells may not play a significant role in early TBI pathogenesis, but their increase may indicate ongoing tissue damage as they produce pro-inflammatory cytokines. Therefore, the suppression of lymphocytes by hydrochlorothiazide can be attributed to its anti-inflammatory mechanism of inhibiting cytokines production. The increase in neutrophil-lymphocyte ratio (NLR) in TNT rats and its decrease in hydrochlorothiazide-treated rats further illustrate the anti-inflammatory and neuroprotective effects of hydrochlorothiazide. NLR has been correlated with outcomes in TBI patients, suggesting its prognostic value. High NLR has always been linked with poor outcomes in TBI patients. (Siwicka-Gieroba et al., 2019).

The brain is essential for regulating electrolyte balance and acid-base equilibrium in the body. However, brain injury can impair this regulatory function. Our study showed that TNT rats exhibited hypernatremia, hypercalcaemia, and low HCO$_3^-$ levels compared to normal rats, consistent with findings from hospital studies (Kocik et al., 2024; Mwachaka et al., 2020) that these imbalances exacerbate secondary injury processes. TBI can lead to hypothalamic or pituitary dysfunction, causing inadequate ADH release and water loss, resulting in hypernatremia (Pin-on et al., 2018). Hypernatremia may also result from fluid loss due to fever, sweating, or osmotic diuresis linked to hyperglycaemia in the stress response following TBI (Maas et al., 2017). Hyperkalaemia results from the release of potassium from injured cells, including neurons and glial cells, into the extracellular space. Metabolic acidosis, secondary to TBI due to impaired cerebral perfusion and tissue hypoxia, can cause hyperkalaemia (by shifting potassium ions from intracellular to extracellular compartments) and low HCO$_3^-$ levels. Interestingly, hydrochlorothiazide-treated rats showed corrected electrolyte imbalances compared to normal rats. This could be due to hydrochlorothiazide’s diuretic effects. By promoting the excretion of sodium and chloride, hydrochlorothiazide helps correct conditions characterized by fluid overload and hypernatremia. As sodium is excreted, the body tries to maintain a balance by increasing potassium excretion in the urine which results in decreased potassium level. Similarly, increased excretion of chloride can lead to retention of bicarbonate. This may explain why the treated rats have higher HCO$_3^-$ level though not significant compared to the TNT. The antioxidant effects of hydrochlorothiazide may mitigate the oxidative stress and cell damage associated with hypernatremia in the treated group.

TBI can lead to alterations in plasma protein levels due to various mechanisms, including blood-brain barrier disruption, systemic inflammatory responses, and changes in tissue perfusion (Wang et al., 2014). TBI triggers a systemic inflammatory response characterized by the release of pro-inflammatory cytokines and activation of immune cells. This inflammatory cascade can affect plasma protein levels through various mechanisms, including alterations in hepatic protein synthesis, increased vascular permeability in peripheral tissues, and changes in protein turnover rates (Wang et al., 2014). TBI may be associated with haemorrhage and fluid shifts, leading to changes in plasma volume and protein concentration. Haemorrhagic shock or hypovolemia can result in haemoconcentration and elevated plasma protein levels. Similarly, a study by Timaru-Kast et al. (2012) reported alterations in plasma protein profiles in TBI patients, with changes in various acute-phase proteins and inflammatory mediators. In this study, we observed significantly increased serum total protein levels in the TNT rats compared to the NTNT rats. The raised level of total protein is significantly lowered in the hydrochlorothiazide-treated rats. The anti-inflammatory and antioxidant effects of hydrochlorothiazide may explain this.

The TNT group exhibited signs of neurodegeneration and necrosis, including dead neurons, diffuse spongiosis, red shrunken neurons (eosinophilic), and central chromatolysis in the cerebrum, indicating a diffuse brain injury, as described by Andre et al. (2014). Many of these lesions are indicative of the onset of injury, with eosinophilic neurons observed in surviving cases within an hour post-injury (Anderson and Opeskin, 1998). Similarly, shrinkage and pyknosis of dying neurons have been noted as early as 30 minutes after...
injury, persisting for up to a day (Rahaman and Delbigio, 2018). In this study, shrunken neurons were observed two weeks post-injury, suggesting ongoing neurodegeneration, likely due to the continued generation of free radicals and inflammation. However, these lesions were not severe in the group treated with hydrochlorothiazide, possibly due to a restorative or normalization effects resulting from mitigation of the secondary injury processes.

Conclusion
In conclusion, traumatic brain injury can disrupt the delicate balance of electrolytes, acid-base equilibrium, induce anaemia neuro-inflammation and tissue damage leading to a myriad of physiological disturbances. Understanding the underlying mechanisms behind these alterations is essential for effective management and improved patient outcomes, by addressing electrolyte imbalances, anaemia and inflammation promptly, healthcare providers can mitigate secondary brain injury and optimize the recovery process for individuals with TBI. Intervention with hydrochlorothiazide can mitigate these alteration as shown in our findings. However, further investigation is necessary to understand the precise mechanism(s) of action effective monitoring of the use of thiazide diuretic in TBI.

ACKNOWLEDGEMENT
We particularly value the technical assistance provided by the staff at the animal house department of Veterinary Physiology and Biochemistry, University of Maiduguri, Maiduguri, Nigeria.

CONFLICT OF INTEREST
The authors assert that there are no conflicts of interest in the current study.

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