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Antioxidative Strategy in Traumatic Brain Injury: Role of Low-Molecular-Weight Antioxidants

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Abstract

Objective*: Traumatic brain injury (TBI) is a major cause of mortality and disability worldwide. This study was designed to investigate the beneficial and neuroprotective role of some Low-Molecular-Weight antioxidants (LMWA) in the treatment of TBI in albino rats.*

Methods*: TBI was induced in adult albino rats using the weight-drop method. A total of 70 Rats was used and were divided into 12 treatment groups, a traumatized non-treated group (TNT) and a Non-traumatized non-treated group (NTNT). There were 5 rats per group. Each of the treatment groups received 22.5 or 45 mg/kg of dimethyl sulfoxide (DMSO), Alpha Lipoic acid (ALA), Uric acid (UA), vitamin C (VC), vitamin E (VE), or Mannitol. Treatment was started 30 min after the trauma and continued for 21 days.*

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To evaluate the functional outcomes, the modified neurological severity score (mNSS) was calculated. The antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)], and malondialdehyde (MDA) were assayed to evaluate oxidative stress (OS).

***Results**: At 7 days post-TBI, the antioxidant-treated groups exhibited significant ($p<0.05$) improvements in neurological scores compared to the traumatized non-treated group (TNT). The treated groups showed a significant ($p<0.05$) increase in the activities of antioxidant enzymes (SOD, CAT and GPx) and a significant ($p<0.05$) decrease in the concentration of MDA compared with the TNT group.*

***Conclusion**: These promising results suggest that the use of low-molecular-weight antioxidants may be a useful neuroprotective strategy in the treatment of TBI. However, further studies should investigate the molecular mechanisms of these antioxidants on TBI pathophysiology and functional outcome.*

***Key words**: Traumatic brain injury, Oxidative stress, Low-Molecular-Weight Antioxidants, Malondialdehyde, Neurological score*

Introduction

Traumatic brain injury (TBI) is characterized as an alteration of cerebral function or abrupt mechanical impairment of brain tissue caused by an external force, subsequently leading to biochemical cascades that may exacerbate the injury (1). Though the extent of damage is not always immediately apparent, the severity of brain damage can vary depending on the type of external force applied and may range from minor to critical (2). Life threatening complications can develop even after mild injuries (3). Mortality rates in developed nations are observed to range between 20-30%, while developing nations can experience rates as high as 90% (4). The socioeconomic impacts of TBI are of noteworthy importance, as it constitutes one of the foremost causes of mortality and morbidity among individuals in the young adult demographic (5). The cause of TBI involves primary and secondary injury processes leading to neurodegenerative and disabling effects. Primary injury can be simple or complex but leads to a series of molecular events resulting in secondary injury.

These events include depolarization, ionic homeostasis perturbations, neurotransmitter release, mitochondrial dysfunction, inflammation, and free radical release, contributing to chronic neurodegeneration and neurological impairment (6).

The production of free radicals after TBI is a well-researched aspect of secondary brain injury (7). A free radical is a chemical entity that possesses one or more unpaired electrons, and this unpaired electron is accountable for its reactivity (8). Reactive oxygen species (ROS) and reactive nitrogen species (RNS), which consist of both free radicals and decomposable compounds that can generate free radicals, are commonly produced after TBI through various mechanisms and play a role in the pathogenesis of TBI by exacerbating secondary injury mechanisms and inducing OS (9). The role of OS in acute central nervous system injury is significant, as the generation of ROS and RNS after brain injury leads to tissue damage and cell death via various pathways (7). Mammalian cells have inherent antioxidant mechanisms, namely superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which function to shield the cells from an overabundance of free radicals (10).

Antioxidants refer to chemical compounds which serve to inhibit the oxidation of other chemicals. These substances play a pivotal role in safeguarding essential cell components by neutralizing the detrimental impact of free radicals (11). The first and paramount mechanism of antioxidant defense systems to counteract ROS involves the utilization of enzymatic antioxidants such as SOD, CAT, and GPX (12). When a TBI occurs, the body's endogenous antioxidants experience an increase in activity level. However, the sheer intensity of free radicals overwhelms the antioxidant system's ability to counteract the detrimental impact of these molecules (13). Exogenous administration of compounds possessing antioxidant properties, such as vitamins, minerals (selenium, zinc), or albumin, can offer supplementary safeguarding measures (14). Natural antioxidants and other compounds that can counteract free radicals are crucial for preventing OS, indicating that augmenting the endogenous antioxidant defense mechanism may be neuroprotective following injury (15,16). Neuroprotection is vital in the treatment of TBI to prevent or reverse secondary brain injury and avoid further neurological decline caused by the release of toxic free radicals and neuronal necrosis (17). Neuroprotective agents that can limit secondary tissue loss and/or improve behavioral outcomes can be of therapeutic value in acute brain injury (18). However, as of the present moment, the

quest to establish efficacious neuroprotective agents has yet to yield any fruitful results. Nevertheless, the pressing need to continue said quest for neuroprotective agents that are truly effective remains paramount (15). The aim of this research work is to validate the beneficial and neuroprotective role of some Low-Molecular-Weight antioxidants in TBI.

Materials and Methods

Animals

All the experimental rats were apparently healthy albino rats weighing 200-250g. They were obtained from the Animal House of the Biological Sciences Department, Usmanu Danfodiyo University, Sokoto, Nigeria for this study. The rats were allowed to acclimatize to the research laboratory condition and were subjected to a 12-hours light/12-hour dark schedule. The rats were fed with growers' mash of vital® feed and allowed to clean drinking water *ad libitum.*

Experimental Design

Number of rats used in this work was 70 which were randomly divided into 12 treated groups, 1 positive control group to be traumatized non-treated (TNT), and 1 normal control group that is non-traumatized non-treated (NTNT). Each group has five rats. Rats in the treatment groups were induced with TBI and treated with six different antioxidants (in two different doses) for 21 days. Table 1 below shows the groupings of the work. This work was approved by the board of postgraduate school of Usman Danfodiyo University Sokoto after meeting national and international standard care of animals used was in accordance with institution guidelines.

Induction of TBI

Head injury was induced in the entire experimental animals except in the negative control group by weight drop method using an acceleration impact device according to Mamarou et al. (19).

Neurological Assessment

Animal were examined and scored with a modified neurological severity score (mNSS) by the modified method of Scallert *et al.* (20). The composite score of 18 points was comprised of five distinct components, namely, consciousness and respiration, cranial nerve function, motor function, sensory function, and coordination. A total of 18 assessments were conducted to evaluate these functions, wherein one point was allocated for the failure to perform a task, while zero points were given for success. The scores ranged from zero for healthy, uninjured animals to a maximum of

18, indicating severe neurological dysfunction with a failure in all tasks. The mNSS, immediately after the trauma, is indicative of the initial severity of injury. Following the initial evaluation of mNSS, the rats were assigned to treatment groups, which were evenly distributed to ensure homogenous grouping.

Sample collection

The rats were subjected to anesthesia via chloroform contained within a glass jar, and subsequent to this, blood was collected through the process of cardiac puncture and the serum was then obtained. The extraction of the brain from the skull was carried out for the purpose of histopathological and biochemical examination.

Table 1: The design of the experiment

Group	Trauma induction	Treatment	Dose
1	Yes	Vitamin E	22.5 mg/kg
2	Yes	Vitamin E	45 mg/kg
3	Yes	Vitamin C	22.5 mg/kg
4	Yes	Vitamin C	45 mg/kg
5	Yes	uric acid	22.5 mg/kg
6	Yes	Uric acid	45 mg/kg
7	Yes	DMSO	22.5 mg/kg
8	Yes	DMSO	45 mg/kg
9	Yes	α- Lipoic acid	22.5 mg/kg
10	Yes	α- Lipoic acid	45 mg/kg
11	Yes	Mannitol	22.5 mg/kg
12	Yes	Mannitol	45 mg/kg
TNT	Yes	NO	0 mg/kg
NTNT	NO	NO	0 mg/kg

Key; TNT – traumatized non-treated, NTNT- non-traumatized non-treated

Analysis of Oxidative Stress

In the present investigation, markers of oxidative stress were examined in the serum and brain tissues of rats. The assessment of antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), as well as the evaluation of lipid peroxidation byproduct malondialdehyde (MDA), were performed using Cayman's Assay Kits with the following batch numbers: 706002 for SOD, 707002 for CAT, 703102 for GPX, and 700870 for MDA. The SOD assay utilizes a tetrazolium salt to detect the superoxide radicals generated by the reaction between hypoxanthine and xanthine oxidase (XO). Briefly, Two wells were designated as standard and sample. To each well 200µl of the diluted radical detector, 10µl each of prepared standard to the serum were added to the standard well and sample well respectively. Twenty microliter (20µl) of diluted xanthine oxidase was added to both standard and sample wells to initiate the reaction. The plate was shaken for a few seconds and covered with cover plate. The plate was then incubated on a shaker at room temperature for 20 minutes and absorbance was read at 450nm using Rayto RT 2100C plate reader (21). CAT is determined by the reaction of catalase with methanol in the presence of an optimal concentration of H2O2. The resulting formaldehyde is analyzed spectrophotometrically using 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. Briefly, Three wells were designated as sample, standard and control. To each well, 100µl of assay buffer and 30µl of methanol were added. To standard well, 20µl of prepared standard (Formaldehyde Standards) were added and to sample well 20µl of serum were added. 20µl of H2O2 were added to each well to initiate the reaction. The plate was covered with lid and incubated on a shaker for 20 minutes at room temperature. To each well, 30µl of potassium hydroxide were added to terminate the reaction and 30µl of purpald were then added. The plate was covered once again and incubated for 10 minutes at room temperature on a shaker. Once again, to each well, 10µl of potassium periodate were added, covered and incubated for 5 minutes on a shaker. The absorbance was read at 540nm using Rayto RT 2100C plate reader (22). GPx activity is measured indirectly through a coupled reaction with glutathione reductase, which allows for the assessment of glutathione peroxidase activity. The reduction of hydroperoxides by glutathione peroxidase leads to the formation of oxidized glutathione, which is subsequently regenerated to its reduced state by glutathione reductase and NADPH. Briefly, three wells were designated as

sample, non-enzymatic and positive control. To sample well, 100µl of assay buffer, 50µl of co-substrate mixture and 20µl of serum were added. To non-enzymatic well, 120µl of assay buffer and 50µl of co-substrate mixture were added and to positive control well 100µl of assay buffer, 50µl of co-substrate mixture and 20µl of diluted GPx were added. The reaction was initiated by adding 20µl of cumenehydroperoxide to each well and the plate was carefully shaken for a few seconds. The absorbance was read at 340nm using Rayto RT 2100C plate reader (23). The MDA assay is based on the reaction between thiobarbituric acid and MDA, resulting in the formation of an MDA-TBA2 adduct with strong absorption at 535nm. Briefly, Into two test tubes labeled sample and standard, 100µl of serum/ brain tissue homogenate and standard were added respectively and treated with 100µl of TCA (trichloroacetic acid) reagent. Then 800 µl of color reagent (106 mg thiobarbituric acid, 10 ml TBA-acetic acid solution and 10 ml NaOH) was added to each test tube and vortex. Tubes were heated in boiling water for one hour and cooled on ice to stop reaction and incubated for ten minutes on ice. After ten minutes, tubes were centrifuge for another ten minutes at 4000 rpm and stabilized at room temperature for 30minutes. After transferring 200 µl of the supernatant to the plate absorbance was read at 540 nm using the plate reader (24).

Statistical Analysis

The statistical software package GraphPad Prism 9 was utilized to conduct an analysis of the results. The data was presented as means with standard deviation. The statistical method of one-way analysis of variance (ANOVA) was employed to analyze the data. In the event that the F values were deemed significant, the Tukey post-hoc test was utilized to compare the groups.

Results

This work evaluated the possible therapeutic potentials of Vitamin C, Vitamin E, α-Lipoic acid, Uric acid, DMSO, and Mannitol in rat model of TBI.

Neurological assessment

Fig. 1 shows the outcome of the neurological assessment (mNSS) of all the experimental groups. No neurological changes were observed in the NTNT rats. There were noteworthy improvements in the neurological response in the rats treated with the antioxidants as indicated by their mNSS. The TNT group did not show significant improvement in their neurological score.

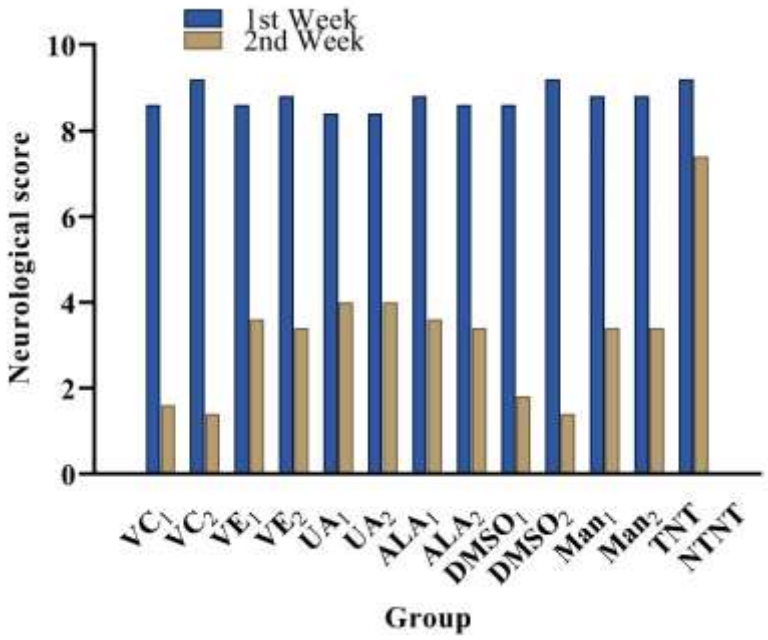

Fig. 1 Modified Neurological Severity Score of Rats; VC_1. – Vitamin C 22.5 mg/kg, VC_2- Vitamin C 45 mg/kg, VE_1-Vitamin E 22.5 mg/kg, VE_2- Vitamin E 45 mg/kg, UA_1- Uric acid 22.5 mg/kg, UA_2- Uric acid 45 mg/kg, ALA_1 – α-Lipoic acid 22.5 mg/kg, ALA_2- α-Lipoic acid 45 mg/kg, $DMSO_1$ – dimethyl sulfoxide 22.5 mg/kg $DMSO_2$ – 45 mg/kg, Man_1- Mannitol 22.5 mg/kg, Man_2 – Mannitol 45 mg/kg, TNT- traumatized non-treated, NTNT – Non traumatized non-treated

The Effect of Supplementation of TBI Rats with LMWA on the Activity of Serum SOD

Fig. 2 depicts the outcomes of the serum SOD level in groups treated with antioxidants. The findings suggest that TBI incurred a noteworthy decline ($p<0.05$) in the enzyme's activity. However, administering antioxidants at 22.5mg/kg and 45mg/kg increased the SOD activity significantly ($p<0.05$). The antioxidants increased SOD activity in a dose-dependent manner. The results indicate that DMSO and Vitamin C exhibited significantly ($p<0.05$) greater efficacy on the enzyme activity than all other antioxidants.

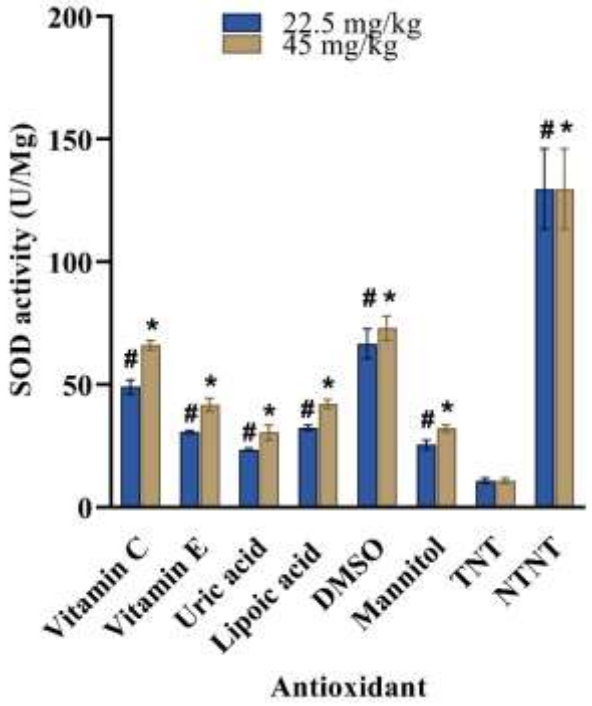

Fig. 2 Effects of LMWA on the Activity of SOD in Serum; SOD- Superoxide dismutase, DMSO- Dimethyl sulfoxide, TNT- Traumatized non- treated, NTN- Non-traumatized non- treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of Supplementation with LMWA on the Activity of Serum CAT

Fig. 3 displays the results of administering LMWA supplements on CAT activity in TBI rats. The findings reveal that the enzyme's activity significantly decreased ($p<0.05$) due to TBI.

However, administering antioxidants at 22.5 and 45 mg/kg doses significantly ($p<0.05$) increased the enzyme's activity. Furthermore, the activity displayed a dose-dependent pattern, with the exception of Vitamin E, Lipoic acid, and mannitol groups, which exhibited a similar effect. Vitamin C and DMSO produced significantly higher effects on the enzyme activity compared to the other antioxidants.

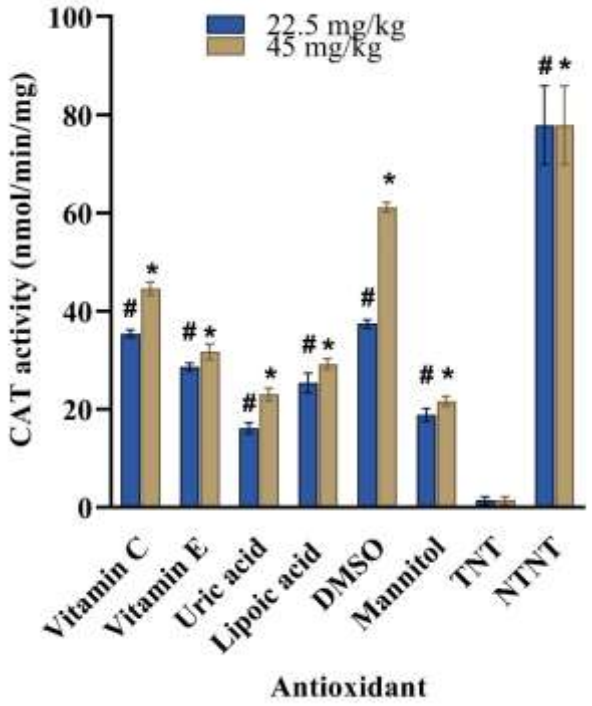

Fig. 3 Effects of LMWA on the Activity of CAT in Serum; CAT- Catalase, DMSO- Dimethylsulfoxide, TNT- Traumatized non-treated, NTN- Non-traumatized non-treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of Supplementation with LMWA on the Activity of Serum GPx

The effects of LMWA on the activity of GPx in serum was presented in Fig. 4. The results showed that TBI caused significant ($p<0.05$) decrease in the activity of the enzyme. Administration of the antioxidant at 22.5 and 45 mg/kg, significantly ($p<0.05$) increased the activity in a concentration dependent manner. The result also indicated that DMSO and Vitamin C had significantly higher effects compared to the remaining treatment while DMSO had significantly higher effect than Vitamin C.

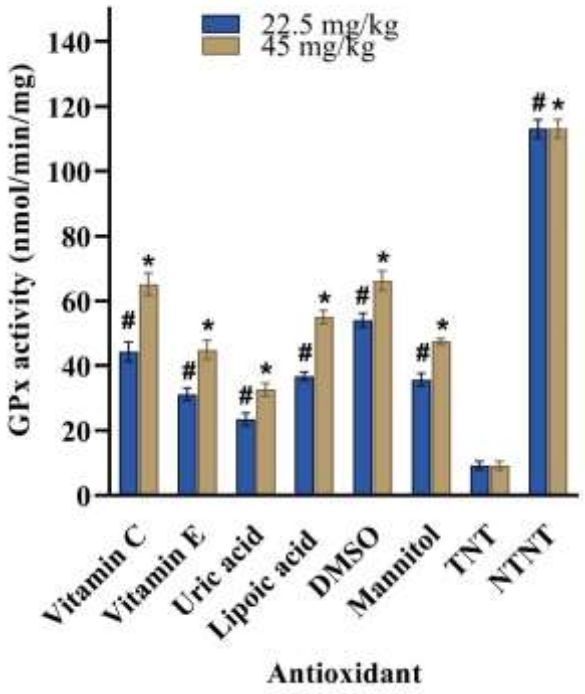

Fig. 4 Effects of LMWA on the Activity of GPX in Serum; GPX- Glutathione peroxidase, DMSO- Dimethylsulfoxide, TNT- Traumatized non-treated, NTN- Non-traumatized non-treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of LMWA Supplementation on the Serum MDA Level

Fig. 5 shows the effects of LMWA on lipid peroxidation. The results indicated that TBI caused significant ($p<0.05$) increase in the concentration of MDA in the TNT group. After supplementation with the antioxidants in two different doses, the concentration of MDA decreased significantly ($p>0.05$) in all the treated groups in a concentration dependent manner. Vitamin C and DMSO at 22.5 mg/kg had no significant difference between their effects on MDA but both had significantly higher effect compared to all other groups. At 45 mg/kg DMSO had higher effect than Vitamin C which also had higher effect compared to the rest.

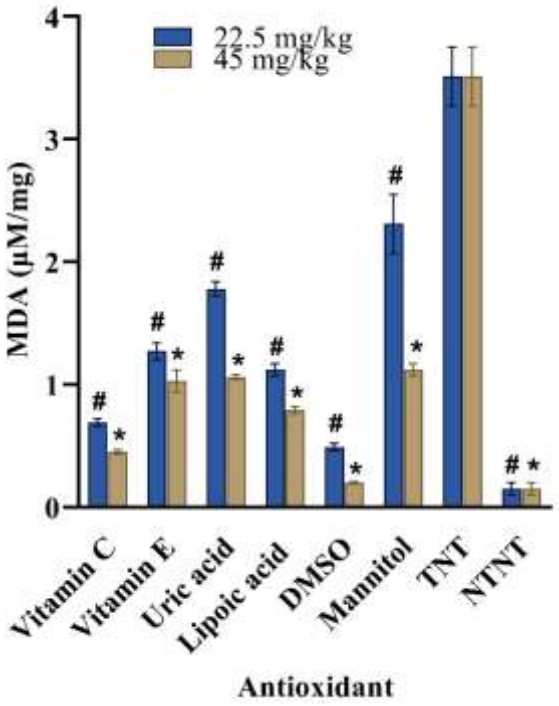

Fig. 5 Effects of LMWA on the Level of MDA in the Serum of experimental rats; MDA- Malondialdehyde, DMSO- Dimethyl sulfoxide, TNT- Traumatized non-treated, NTN- Non-traumatized non-treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of LMWA Supplementation on the Brain Tissue Activity of SOD

The results presented in Fig. 6 shows the activity of SOD in brain tissue of TBI rats treated with LMWA. The result indicated that TBI caused significant ($p<0.05$) decrease in the activity of the enzyme. Supplementation significantly ($p<0.05$) increased the activity except 22.5 mg/kg of Uric acid and mannitol. Comparison between the groups indicated that the highest activity was observed in DMSO and Vitamin C treated groups.

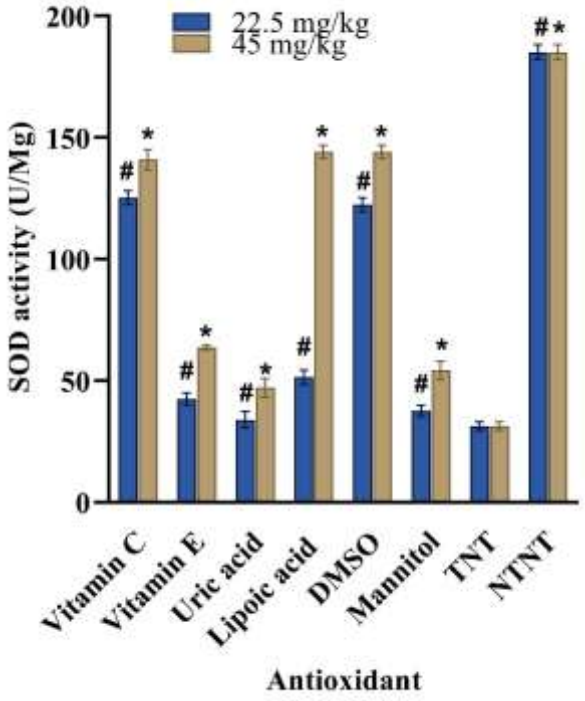

Fig. 6 Effects of LMWA on the SOD Activity in Brain Tissue; SOD- Superoxide dismutase, DMSO- Dimethyl sulfoxide, TNT- Traumatized non- treated, NTN- Non-traumatized non-treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of Supplementation with LMWA on the Brain Tissue Activity of CAT

Fig. 7 shows the result of the effect of LMWA on brain tissue level of CAT. The result indicated that TBI caused significant ($p<0.05$) decrease in the activity of the enzyme.

Supplementation of the antioxidants (22.5 and 45 mg/kg) increased the activity significantly ($p<0.05$) except in the groups treated with 22.5mg/kg of Uric acid and mannitol. The activity increased in a dose dependent manner. The enzyme activity in Vitamin C group is not statistically different from the DMSO group.

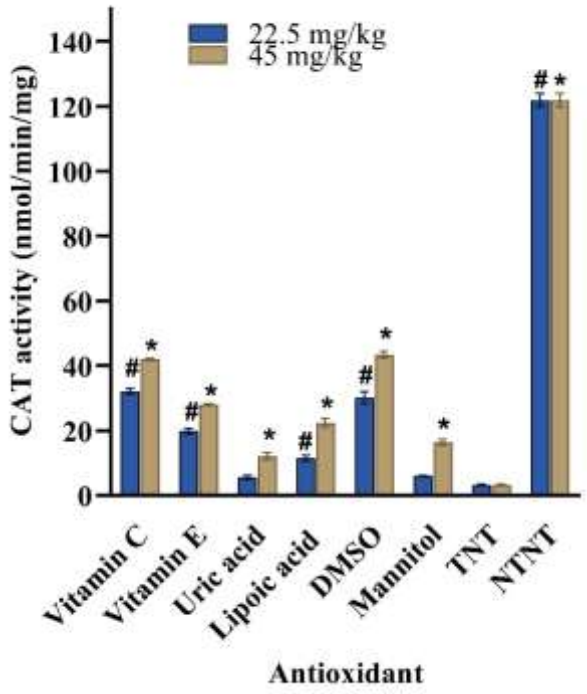

Fig. 7 Effects of LMWA on the CAT Activity in Brain Tissue; CAT- catalase, DMSO- Dimethyl sulfoxide, TNT- Traumatized non-treated, NTNT non traumatized non-treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of Supplementation with LMWA on the Brain Tissue Activity of GPX

The results in Fig. 8 showed that TBI caused significant ($p<0.05$) decrease in the activity of the enzyme while administration at 22.5 mg/kg and 45 mg/kg BW of the antioxidants significantly ($p<0.05$) increased the activity in a dose-dependent manner except in uric acid. At 22.5 mg/kg, Vitamin C and DMSO had similar increasing effect on the enzyme activity while Lipoic acid compared to DMSO had significantly lower effect. Vitamin E, Uric acid and mannitol at the dose of 22.5 mg/kg showed statistically similar effects on the enzyme activity. Supplementation at 45 mg/kg indicated that Vitamin E and Lipoic acid have no significant difference between their effects on GPX activity.

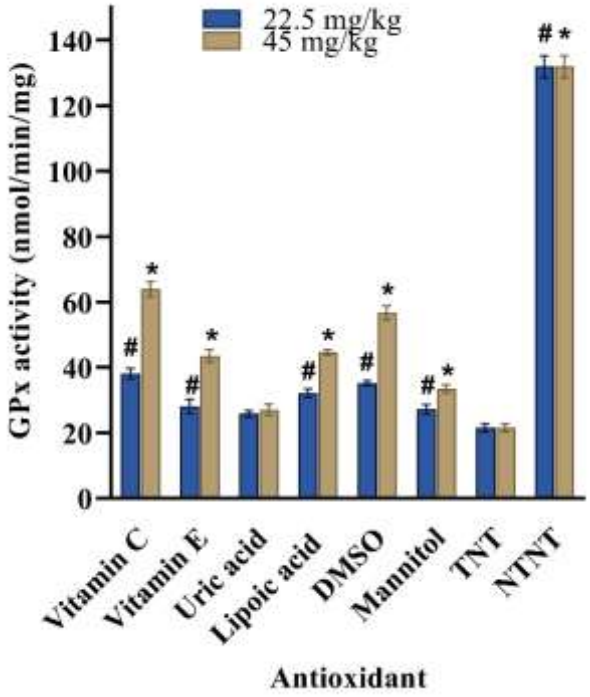

Fig. 8 Effects of LMWA on the GPX Activity in Brain Tissue; GPX- Glutathione peroxidase, DMSO- Dimethyl sulfoxide, TNT- Traumatized non-treated, NTNT- Non traumatized non-treated. Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

The Effect of Supplementation with LMWA on Brain Tissue MDA Level

The results in Fig. 9 indicated that TBI caused significant ($p<0.05$) increase in the level of MDA in the brain of TNT rats. After administration of the antioxidants in two different doses, the concentration of MDA decreased significantly ($p<0.05$) in all the treated groups in a concentration dependent manner when compared to TNT group. Vitamin C and DMSO showed higher effects than all the treatment groups.

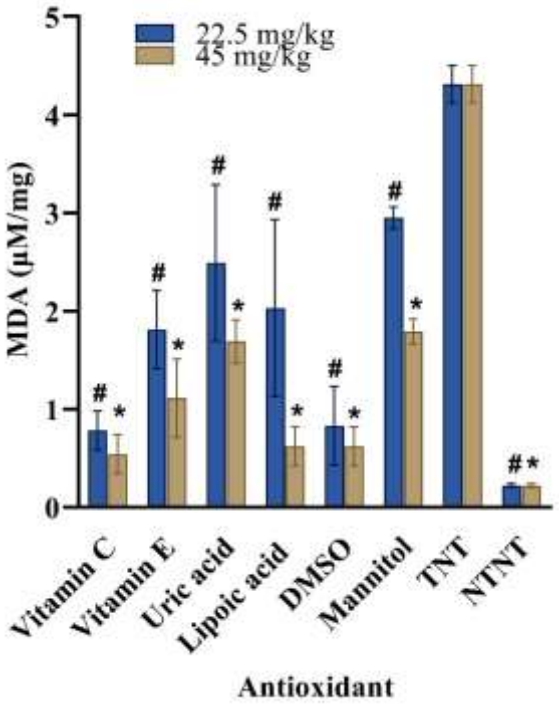

Fig. 9 Effects of LMWA on the Level of MDA in Brain Tissue; MDA- Malondialdehyde, DMSO- Dimethyl sulfoxide, TNT- Traumatized non-treated, NTNT- Non traumatized non-treated Values are significantly different ($^{\#}p<0.05$) compared to TNT at 22.5 mg/kg and ($^{*}p<0.05$) compared to TNT at 45 mg/kg

Discussion

In this study, the neurological assessment was conducted to include only animals with moderate scores (mNSS of 8-10) to ensure homogeneity in injury distribution and trauma level after induction (Fig.1). The results of the assessment indicated significant improvement in all treated groups (from an average score of 8-9 to 4-1), whereas the untreated group showed only minimal improvement (from an average score of 9 to 7). The mitigating effects of antioxidants on oxidative damage may be linked to the observed reestablishment of neurological function in the groups receiving treatment. This is due to the fact that TBI induces both structural damage and functional impairments through OS, which consequently impacts neurological function (25).

In this study, the assessment of OS was conducted by means of quantifying the levels of SOD, CAT, GPx, and MDA, which serve as indicators of enzymatic antioxidant activity and lipid peroxidation. Our findings indicated a substantial reduction in the activities of SOD, CAT, and GPx, and a corresponding increase in the concentration of MDA in both the serum and brain tissues of TNT rats, in comparison to NTNT rats (refer to Fig. 2-9). Such observations suggest that the induced TBI

leads to oxidative stress. However, the administration of vitamin C, vitamin E, α-Lipoic acid, Uric acid, DMSO, and Mannitol, in two different doses, showed an alleviation of the induced OS in a dose-dependent manner (refer to Fig. 2-9).

Treatment vitamin C resulted in a noteworthy increase ($p< 0.05$) in the activities of SOD, CAT and GPx, and a reduction in the concentration of MDA in the serum and brain tissues of the treated groups, in comparison to the TNT group (refer Fig. 2-9). These effects were observed to be dose-dependent. It is plausible that these benefits are attributed to the potential of vitamin C to counteract free radicals and mitigate their oxidative activity on lipids, proteins, and nucleic acids, which in turn leads to the suppression of the antioxidant system and the accumulation of MDA. It may also be attributed to the revitalization of vitamin E and glutathione by vitamin C, which exhibit remarkable efficacy against ROS as indicated by Denniss et al. (26). In this study, it was also observed that vitamin C exhibited a higher degree of antioxidant effect, second only to DMSO. This finding may be attributable to its robust scavenging and reduction potential, as well as it's ability to enhance various substances that possess antioxidant properties, such as α-Lipoic acid, vitamin E, Uric acid and other mild antioxidants.

The administration of vitamin E at doses of 22.5 and 45 mg/kg to rats with TBI led to a significant increase ($p<0.05$) in the activities of SOD, CAT, and GPx in both serum and brain tissues, while there was a significant decrease ($p<0.05$) in the concentration of MDA in these tissues, as observed in Fig. 2–9. Moreover, this outcome was dose-dependent, which could be attributed to the particular characteristics of vitamin E as a highly relevant chain-breaking antioxidant that is abundantly present in cells and mitochondria membranes, where the generation of ROS also occurs, and their dysfunction results in the excessive release of free radicals. Thus, it is possible that its mode of action was through the inhibition of lipid peroxidation and OS in these vital locations of free radical production, as indicated by Inci et al. (27). Ehizuelen et al. (28) has indicated that vitamin E, a lipid-soluble antioxidant, effectively averts the formation of lipid peroxide. It is also widely recognized that, aside from its direct impact on ROS, vitamin E can interact with a range of antioxidants such as vitamin C, GSH, and β-carotene to potentiate synergistic activity. As a result, these antioxidants facilitate the regeneration of vitamin E, which in turn enhances its therapeutic efficacy (29).

The outcomes of this research demonstrated that groups administered with Uric acid

exhibited a significant ($p<0.05$) elevation in the activities of SOD, CAT, GPx, and a significant ($p<0.05$) reduction in the level of MDA in serum and brain tissue when compared to their TNT counterpart (as illustrated in Fig. 2–4). This effect was found to increase in a dose-dependent manner. This can be attributed to the ability of uric acid to prevent OS through the scavenging of free radicals and chelating metal ions, which promote the development of free radicals, as indicated by Watanabe et al. (30). The decline in MDA caused by Uric acid can be attributed to its inhibitory role against lipid peroxidation and inflammatory reaction. The latter can result in increased ROS levels and tissue oxidation. According to Jagroop et al. (31), urate has been found to be effective in preventing lipid peroxidation, whereas its anti-inflammatory effect has been reported by Hooper et al. (32).

The application of α-Lipoic acid resulted in a notable enhancement of the diminished levels of SOD, CAT, and GPx caused by free radicals as compared to the TNT group (Fig. 2-9). Additionally, the use of α-Lipoic acid led to a meaningful decrease in MDA levels among the treated groups in contrast to the TNT group, with a dose-dependent effect observed wherein higher doses yielded greater efficacy. Notably, these outcomes are potentially attributed to the chelation of metal ions by α-Lipoic acid, which in turn impedes the metal-dependent production of free radicals, as supported by Patwa et al. (33). The ability of α-Lipoic acid to recycle vitamin C and vitamin E, both potent free radical scavengers as reported by Jones et al. (34) and Packer et al. (35), may be responsible for its observed effects. Furthermore, it has been demonstrated that α-Lipoic acid metabolites possess anti-inflammatory properties, according to Mei and You-wen's study (36). When pro-inflammatory cells and substances are mobilized to the site of injury, they trigger the release of more ROS, which can lead to OS. Modulating this reaction can help to mitigate the negative effects of OS.

In the present study, DMSO administration resulted in a significant ($p< 0.05$) elevation of antioxidant enzymes (SOD, CAT, GPx) levels in both serum and brain tissue of treated rats, when compared to the TNT group (Fig. 2-9) in a does-dependent manner. Additionally, DMSO exhibited a decreasing effect on the MDA level, with the concentration being reduced to the level observed in the NTNT rats at a dose of 45 mg/kg in serum. The observed effects of DMSO could be attributed to its ability to scavenge free radicals (37), which might have prevented the accumulation of free radicals that consumed the enzymatic

antioxidants and reduce their levels. As a result, DMSO's scavenging effect could have minimized the chain reaction oxidation of lipids by free radicals.

In the present study, the administration of mannitol resulted in a significant ($p< 0.05$) increase in the serum levels of SOD, CAT, and GPx, along with a decrease in the serum concentration of MDA, in a dose-dependent manner in the treated groups compared to the TNT group (Fig. 2-9). These beneficial effects were also observed in brain tissue. The observed antioxidant effect of mannitol can be attributed to its free radical scavenging properties. This is supported by previous findings by Kalemci et al. (38) who demonstrated the free radical scavenging effect of mannitol. Mannitol finds extensive usage in the reduction of cerebral edema during TBI. The accumulation of water in the brain results in an increase in intracranial pressure, which leads to a decrease in cerebral blood flow, thereby worsening tissue damage as a consequence of brain ischemia. Hence, the anti-edema effect of mannitol might have contributed to its antioxidant effect observed in this study (39).

The findings that were obtained from the present study indicate that the Low-Molecular-Weight antioxidants that were examined exhibited promising and beneficial effects concerning neuroprotection, as well as the modulation of Oxidative Stress (OS), thereby ameliorating neurological deficits in the experimental rats. Additional studies must be conducted to investigate the molecular mechanisms of these antioxidants concerning TBI pathophysiology and functional outcomes.

Conclusion

To conclude, after TBI induction, a remarkable decrease in antioxidant enzyme activities was observed, accompanied by an increase in MDA level, indicating the occurrence of oxidative stress. The administered antioxidants demonstrated their neuroprotective and neurorestorative potential by enhancing the antioxidant capacity, inhibiting lipid peroxidation, and improving the neurological deficits. These encouraging outcomes suggest that low-molecular-weight antioxidants may hold potential in the management of TBI.

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Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Ethical approval

This study was performed in line with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving animals. Approval was granted by the Ethics Committee of Usman Danfodiyo University Sokoto. Animal care was done by following institutional guidelines.

Author Contribution

Lawal Suleiman Bilbis and Ibrahim Bulama contributed to the conception. Material preparation, investigation and data collection were performed by Ibrahim Bulama. Nasiru Suleiman curated the data. Yusuf Saidu and Yusuf Yakubu provide supervision. Umar Faruk Saidu performed the formal analysis and prepared Figures 1-9; Abdullahi Yahaya Abbas, Umar Faruk Saidu, and Nasiru Ismail Jinjiri wrote the first draft of the manuscript. Umar Faruk Saidu and Ibrahim Bulama wrote the final version of the manuscript. All authors contributed to manuscript revision, read and approved the final manuscript.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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