Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2024; 41(2): 278-285
doi: 10.52142/omujecm.41.2.9

Chemical modification of ascorbic acid to L-ascorbyl-6-palmitate: A novel approach for improved antioxidant therapy in traumatic brain injury

Umar Faruk SAIDU [1,*], Ibrahim BULAMA [2], Ibrahim ABUBAKAR [1], Yusuf ZAYYANA [1], Andrew ONU [1], Suleiman NASIRU [3], Abdullahi Yahaya ABBAS [1], Yusuf SAIDU [1], Lawal Suleman BILBIS [1]

[1]Department of Biochemistry and Molecular Biology, Faculty of Life and Chemical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
[2]Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria
[3]Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Nigeria

Received: 06.02.2024 • **Accepted/Published Online:** 03.04.2024 • **Final Version:** 19.05.2024

Abstract

Oxidative stress, caused by an excessive amount of reactive oxygen species is a major factor in the pathophysiology of complications following traumatic brain injury (TBI). Ascorbic acid, a vital antioxidant, has been employed in TBI therapy, but its instability, limited bioavailability, rapid oxidation, and pro-oxidant effects pose significant limitations. To overcome these drawbacks, the ascorbic acid was chemically modified resulting in a fat-soluble L-ascorbyl-6-palmitate. The effects of L-ascorbyl-6-palmitate on oxidative stress biomarkers in TBI rats were subsequently evaluated. TBI was developed in rats by a weight drop method. The study involved five experimental groups: ascorbic acid group, L-ascorbyl-6-palmitate group, dimethyl sulfoxide group, traumatized non-treated group, and non-traumatized non-treated control group. A total of twenty-five rats were used in the experiment, with five rats in each group (n=5). The levels of malondialdehyde and the activity of antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase were assessed in serum and brain tissue samples. In both serum and brain tissue, ascorbic acid, L-ascorbyl-6-palmitate, and dimethyl sulfoxide showed significant ($P<0.05$) elevation in enzyme activities and reduction in malondialdehyde levels compared to the traumatized non-treated group. Additionally, L-ascorbyl-6-palmitate treatment demonstrated higher antioxidant potential and scavenging ability than ascorbic acid treatment, as evidenced by significantly ($P<0.05$) increased superoxide dismutase, catalase, and glutathione peroxidase activities, and reduced malondialdehyde levels. These findings demonstrate the neuroprotective effects of L-ascorbyl-6-palmitate in managing TBI-induced oxidative stress. Further studies should investigate the underlying molecular mechanisms and long-term effects of L-ascorbyl-6-palmitate treatment on neurological recovery and functional outcomes in TBI, as well as explore its potential synergistic effects with other antioxidants or neuroprotective strategies.

Keywords: traumatic brain injury, antioxidant therapy, oxidative stress, ascorbic acid modification, L-ascorbyl-6-palmitate, antioxidative enzymes

1. Introduction

Traumatic brain injury (TBI), a complicated and multidimensional disorder that is often a consequence of external force application, typically in the form of a violent blow to the cranium, has the potential to inflict permanent or transient damage upon the cognitive, physical, and psychological faculties of an individual (1). TBI poses a significant and pervasive challenge to global health and is a source of considerable social and economic burden (2). TBI is categorized into two distinct types of injury: primary injury and secondary injury. Primary injury, which is sustained at the moment of impact, can be attributed to various forms of physical trauma, including but not limited to skull fractures, coup and contracoup contusions, intracranial hemorrhage, and diffuse axonal injury. Secondary injury, which refers to additional damage to the nervous system, may occur hours or even days after the primary insult. This phenomenon is characterized by a cascade of pathophysiological events that ultimately culminate in the exacerbation of the initial insult. It involves a variety of cellular, molecular, and biochemical mechanisms (3), such as Ischemia, calcium [Ca^{+}] accumulation, oxidative stress, and mitochondrial dysfunction (4, 5).

The increase in the production of reactive oxygen species (ROS), which cause oxidative stress to occur, is of utmost significance in the development and progression of TBI (6). ROS are known to be potent molecules that are capable of inducing detrimental effects on important cellular constituents such as DNA, lipids, and proteins (7). The endogenous antioxidant defense system is capable of safeguarding tissues from oxidative damage by preventing or scavenging ROS formation under normal physiological conditions (8). The development of TBI causes an uncontrollably high level of ROS generation and antioxidant resource depletion (9). It has been demonstrated that, in the aftermath of TBI, vitamins C

*Correspondence: farouk.saidu@gmail.com

and E deplete rapidly in the early minutes and hours, necessitating subsequent supplementation (10). Therefore, the administration of antioxidants as a potential approach may serve as a beneficial tactic in impeding the advancement of injury and enhancing both the likelihood of survival and neurological outcomes.

Ascorbic acid (AA) serves as a primary water-soluble antioxidant in human plasma, which plays an essential role in various enzymatic reactions and demonstrates a proficient scavenging capacity for ROS, thereby protecting the cell from oxidative damage (11). Neurons are found to possess a substantial concentration of ascorbic acid. The brain, which accounts for a quarter of total glucose utilization, exhibits high oxidative metabolism, rendering it reliant on antioxidants for safeguarding against pathological conditions (12). AA has been found to offer significant protection against oxidative damage in the brain through its ability to inhibit ROS production, scavenge existing ROS, and sustain superoxide dismutase (SOD) and catalase (CAT) activities (13).

Nevertheless, AA has limitations such as rapid oxidation, instability, low bioavailability, and pro-oxidant effects. Together with its limited liposolubility, AA's propensity to oxidative and thermal deterioration presents challenges in preserving its physiological value over extended periods, and its ability to penetrate cell membranes (14). Modification by adding lipophilic moieties has led to various derivatives of AA with better antioxidant potential and stability against oxidation (15). In addition, by providing transitional metals, such as iron with electrons, ascorbic acid can act as a pro-oxidant and aid in the production of ROS. This in turn can lead to oxidation of DNA, lipids, or proteins (16).

Although there is mounting evidence that L-ascorbyl-6-palmitate (AP) may alleviate oxidative stress in other studies, this is the first study that we are aware of that looked into how giving AP affected experimental TBI. Thus, there is a paucity of data available on the effect of AP supplementation in traumatic brain injury. Therefore, in the current study, we have modified ascorbic acid to AP, and have assessed the effects of AP in the management of traumatic brain injury.

2. Materials and methods

2.1. Chemicals and Reagents

AA and Dimethyl sulfoxide (DMSO) were procured from Sigma-Aldrich located in Louk, Germany. Meanwhile, CAT, SOD, glutathione peroxidase (GPx), and malondialdehyde (MDA) assay kits obtained from Cayman® Chemical Company hailing from Ann Arbor, USA. Ketamine hydrochloride was sourced from Rotexmedical based in Trittau, Germany. Palmitic acid was acquired from Ashish Nair (MSD Global), whereas Chloroform, Sulfuric acid and Petroleum Ether were obtained from HBD chemical company in China.

2.2. Chemical Modification of Ascorbic Acid to L-ascorbyl-6-palmitate

We synthesized AP, a more potent 6-fatty acid ester of AA, using the methodology presented by Riyaz and colleagues (17). A solution of L-ascorbic acid (16mmol) in 50ml of initially 95-98% sulphuric acid was supplemented with palmitic acid (20mmol). The resulting homogeneous reaction mixture was agitated for a duration of 1 hour at 30^0C, following which it was subjected to an additional 3-30 hour standing period at 25^0C. The reaction mixture was expeditiously added with agitation onto 300 g of fragmented ice, and the mixture was subjected to ether extraction (3 x 5ml). Together, the organic layers were delicately subjected to brine washing (3 x 50ml), and the resultant brine extracts were consolidated and subjected to ether washing (100ml). Together, the ether layers were subjected to desiccation over anhydrous sodium sulphate and allowed to evaporate. The residual solid was subjected to a tripartite hexane extraction (3 x 75ml) and the non-soluble matter was subsequently subjected to desiccation under vacuum conditions. At pH 7.0, the ether-soluble, hexane-insoluble derivatives exhibited 93-96% of L-ascorbic acid's absorbance (γmax=264 nm) and 95-96% of its iodine-reducing capacity. Finally, pure ascorbyl palmitate was obtained by crystallizing the ascorbic acid ester of crude palmitic acid from chloroform.

2.3. The Experimental Design and Animal Treatment

A total of twenty five Wistar albino rats weighing between 180-280g were procured from the animal housing facility of the university's biological sciences department. The rats were given two weeks to acclimate to their new surroundings and were fed with grower's mash of vital feed and clean water before the experiment began. The rats were then split into five groups at random. With each group containing five rats (n=5) as described below.

Group I (AA group): 100 mg/kg AA was administered orally 1 hour after TBI induction once daily for 14 days.

Group II (AP group): 100 mg/kg AP was administered orally 1 hour after TBI induction once daily for 14 days.

Group III (DMSO group): 100 mg/kg DMSO was administered orally 1 hour after TBI induction once daily for 14 days.

Group IV (TNT positive control group): Rats in this group were Traumatized Non-treated. They were induced with TBI and received no treatment.

Group V (NTNT negative control group): Rats in this group were Non-Traumatized Non-treated. They were neither induced with TBI nor received any treatment.

AA was dissolved in normal saline (pH 7.5, 0.1N NaCl), and AP was dissolved in 100% DMSO, which serve as a vehicle. The treatment commenced 1 hour after TBI induction via the oral route once daily for two weeks (14 days).

2.4. Surgery and Induction of TBI in Rats

The Usmanu Danfodiyo University, Sokoto Animal Care and Usage Committee approved the experimental protocol. Prior to the induction of trauma, the animals were administered anesthesia (75 mg/kg Ketamine hydrochloride and 10 mg/kg Xylazine hydrochloride), and were housed in an incubator with a Harvard Rodent ventilator set to room temperature. TBI was generated in the rats using the weight drop injury (WDI) model, as outlined by Mamarou and colleagues (18). In summary, a small midline incision was made to reveal the skull, and to prevent the skull from breaking, a centrally located metallic disk with a diameter of 10 mm and a depth of 3 mm was placed over the skull to distribute the force over a wider surface area. The rats were appropriately positioned on supporting foam that was 10cm deep, and a metal brass weighing 80g was dropped from a distance of 1m to induce injury. After the injury, the metal disk was taken out of the skull, the wound was sutured, and the animals were left to heal in their cages.

2.5. Brain Extraction and Homogenization

The methodology employed was in accordance with the procedures outlined by Rezanejad and colleagues (19). Briefly, the rats were euthanized by decapitation under anesthesia. Micro dissecting scissors were used to open the skin at the mid-line of the head. The incision was made from the roof of the skull to the mid-eye area. After the skin flaps were folded back with the scissors, the skull was incised at the mid-line fissure, while ensuring that the brain tissue remained intact. Curved forceps were used to carefully remove the elevated skull cap while providing a mild pressure. A micro spatula was then used to remove the brain from the skull cavity, following its length from the olfactory lobes to the beginning of the spinal cord. After that, any clots or red blood cells were carefully removed from the brain by gently transferring it to a 60 mm Petri dish and rinsing it with phosphate buffered saline (PBS). After that, the brains were moved to a second Petri dish and sliced into tiny pieces in a 10% PBS solution that contained ice. The slices underwent a 45-minute, 100-cycle sonication. The extract was centrifuged at 1500 rpm for five minutes, and its supernatant was collected in a disposable 1.5 ml container. The brain's MDA levels, SOD, CAT, and GPx activities were measured using the supernatant.

2.6. Blood Sample Collection

Standard test tubes were used to collect blood samples from rats that had undergone cardiac puncture. Then, SOD, CAT, GPx, and MDA levels in the blood were measured using the serum.

2.7. Estimation of Antioxidant Enzyme Activities and Lipid Peroxidation

In the current study, indicators of oxidative stress were assayed in both serum and brain tissues of the rats. The antioxidant enzymes SOD, CAT, and GPX, as well as lipid peroxidation byproduct MDA were assayed using Cayman's Assay Kits, with batch number 706002, 707002, 703102, and 700870 for SOD, CAT, GPx, and MDA respectively. Briefly, the SOD assay uses a tetrazolium salt to identify superoxide radicals generated by the interaction of xanthine oxidase (XO) with hypoxanthine (20). The basis of CAT assay is the reaction of catalase with methanol when H_2O_2 is present at the ideal concentration. Using 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen, the resultant formaldehyde was analyzed spectrophotometrically (21). GPx assay is via the indirect measurement of glutathione peroxidase activity through a coordinated process working in concert with glutathione reductase. The hydroperoxide reduction by glutathione peroxidase yields oxidized glutathione, which is further recycled to its reduced state by glutathione reductase and NADPH (22). Thiobarbituric acid and MDA react to generate an MDA-TBA2 adduct, which has a high absorption at 535 nm. This reaction is the basis for the MDA assay (23).

2.8. Statistical Analysis

The data was statistically analyzed using GraphPad Prism version 9.5.1 (GraphPad Software Inc., San Diego, CA, USA) on Windows. The findings were expressed as means and standard error of the mean (Means ± SE). The Bartlett's test of variance were performed to check the homogeneity of variance in our data. The p-value ($p>0.05$) and standard deviation ($p>0.05$) of the Bartlett's tests were not significant and implied that our data is homogeneously distributed. Furthermore, the De'Agustino-Pearson's, Anderson-Darling's, Sharpiro-Wilk's, and Kolmogorov-Sminov's normality of residuals tests were performed to check for the normality in our data. The p-values for all these normality tests were bigger than the significant threshold of alpha < 0.05 set for the null hypothesis. Therefore, the p-values were not significant ($p>0.05$) and implied passed normality. As a result, One-way analysis of variance (ANOVA) for parametric data was used to identify significant differences between the groups. Since we are comparing every mean with every other mean between the groups, we used Tukey's posthoc test for pairwise multiple comparisons. The significance level was kept at $*p<0.05$ for each analysis.

3. Results

3.1. The impact of AA, AP, and DMSO on MDA levels in the serum and brain tissue of rats with TBI

The outcomes outlined in Fig. 1a and 1b show that TBI significantly elevated the levels of MDA in the serum and brain tissue of TNT rats in comparison to the NTNT rats. However, after being given AA, AP, and DMSO, the MDA levels in the treated groups were significantly decreased when compared to those in the TNT group. In serum, the MDA levels of TNT rats (3.302 ± 0.182) was significantly higher ($F(4, 20) = 75.20$, $p<0.0001$) than the NTNT rats (0.708 ± 0.060). When treated with AA (1.672 ± 0.089), AP (1.144 ± 0.039), and DMSO (1.754 ± 0.133), MDA levels significantly decreased ($F(4, 20) = 75.20$, $p<0.0001$) when compared with TNT rats (3.302 ± 0.182). In addition, the MDA levels in the AP-treated group (1.144 ± 0.039) was significantly lower ($F(4, 20) = 75.20$, p =

0.0265) when compared with AA-treated group (1.672 ± 0.089). Also, the AP-treated group (1.144 ± 0.039) showed significantly lower (F(4, 20) = 75.20, p = 0.0087) MDA levels when compared with DMSO (1.754 ± 0.133) (Fig. 1a). In brain, the MDA levels of TNT rats (2.916 ± 0.060) was significantly higher (F(4, 20) = 90, p<0.0001) than the NTNT rats (0.398 ± 0.104). When treated with AA (1.654 ± 0.118), AP (1.120 ± 0.040), and DMSO (1.588 ± 0.129), MDA levels significantly decreased (F(4, 20) = 90, p<0.0001) when compared with TNT rats (2.916 ± 0.060). Additionally, the MDA levels in the AP-treated group (1.120 ± 0.040) was significantly lower (F(4, 20) = 90, p = 0.0072) when compared with AA-treated group (1.654 ± 0.118). Also, the AP-treated group (1.120 ± 0.040) showed significantly lower (F(4, 20) = 90, p = 0.0207) MDA levels when compared with DMSO (1.588 ± 0.129) (Fig. 1b).

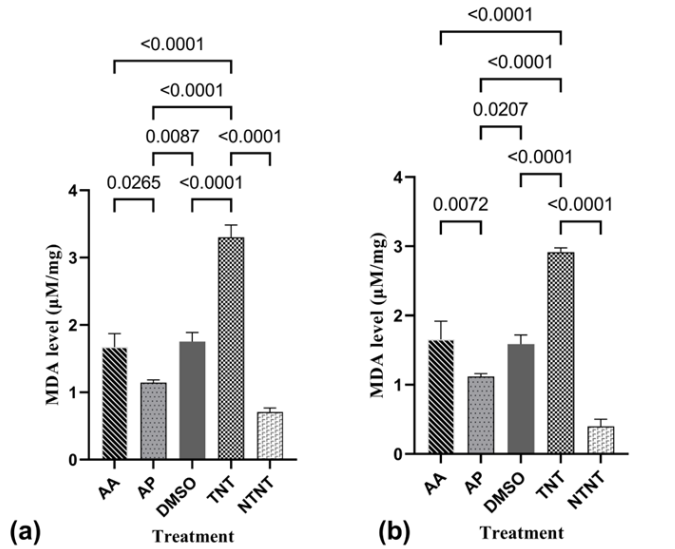

Fig. 1. Effects of AA, AP, and DMSO on MDA levels (**a**) Serum; (**b**) Brain Tissue. MDA- Malondialdehyde, AA- Ascorbic Acid, AP- L-ascorbyl-6-palmitate, DMSO- dimethyl Sulfoxide, TNT- Traumatized Non-treated, NTNT- Non-Traumatized Non-treated. p<0.05, p<0.01, p<0.001, p<0.0001 shows the post hoc Tukey test results of the one-way ANOVA test between the groups

3.2. The impact of AA, AP, and DMSO on SOD activity in the serum and brain tissue of rats with TBI

The outcomes of the administration of AA, AP, and DMSO on SOD activity in the TBI rats are illustrated in Fig. 2a and 2b. When comparing the TNT rats to the NTNT rats, it was found that there was a significant decrease in SOD activity in both the brain tissue and serum. In contrast, it was observed that the SOD activities significantly increased in the AA, AP, and DMSO treated groups when compared to the TNT group. In serum, the SOD activity of TNT rats (1.346 ± 0.198) decreased significantly (F(4, 20) = 147.8, p<0.0001) when compared with NTNT rats (7.852 ± 0.247). When treated with AA (4.504 ± 0.161), AP (6.664 ± 0.193), and DMSO (4.248 ± 0.217)), SOD activities significantly increased (F(4, 20) = 147.8, p<0.0001) in these groups when compared with TNT rats (1.346 ± 0.198). In addition, the SOD activity in the AP-treated group (6.664 ± 0.193) was significantly higher (F(4, 20) = 147.8, p<0.0001) when compared with both AA-treated group (4.504 ± 0.161) and DMSO-treated group (4.248 ± 0.217) (Fig. 2a). In brain, the SOD activity of TNT rats (1.890 ± 0.223) was significantly lower (F(4, 20) = 93.63, p<0.0001) than the NTNT rats (7.608 ± 0.346). When treated with AA (3.768 ± 0.165), AP (6.496 ± 0.206), and DMSO (3.002 ± 0.264), SOD activities significantly increased in these groups when compared with TNT rats (1.890 ± 0.223) with (F(4, 20) = 93.63, p = 0.0003), (F(4, 20) = 93.63, p<0.0001), and (F(4, 20) = 93.63, p = 0.0355) significance difference respectively. Additionally, the SOD activity in the AP-treated group (6.496 ± 0.206) was significantly higher (F(4, 20) = 93.63, p<0.0001) when compared with both AA-treated group (3.768 ± 0.165) and DMSO-treated group (3.002 ± 0.264) (Fig. 2b).

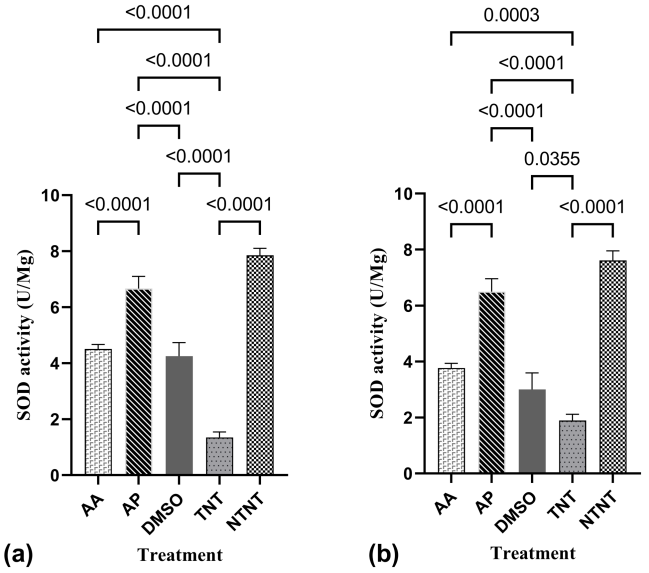

Fig. 2. Effects of AA, AP, and DMSO on SOD activities (a) Serum; (b) Brain Tissue. SOD- Superoxide dismutase, AA- Ascorbic Acid, AP- L-ascorbyl-6-palmitate, DMSO- dimethyl Sulfoxide, TNT- Traumatized Non-treated, NTNT- Non-Traumatized Non-treated. p<0.05, p<0.01, p<0.001, p<0.0001 shows the post hoc Tukey test results of the one-way ANOVA test between the groups

3.3. The impact of AA, AP, and DMSO on CAT activity in the serum and brain tissue of rats with TBI

The outcomes, as depicted in Fig. 3a and 3b, indicated that TBI significantly decreased the activities of catalase in the TNT rats in comparison to the NTNT rats, in both serum and brain tissue. However, it was observed that the CAT activities were significantly increased in the groups treated with AA, AP, and DMSO when compared to the TNT group. In serum, the CAT activity of TNT rats (5.356 ± 0.407) decreased significantly (F(4, 20) = 54.53, p<0.0001) when compared with NTNT rats (40.250 ± 3.699). When treated with AA (18.640 ± 0.795), AP (31.990 ± 1.389), and DMSO (16.750 ± 0.839), CAT activities significantly increased when compared with TNT rats (1.346 ± 0.198) with (F(4, 20) = 54.53, p = 0.0005), (F(4, 20) = 54.53, p<0.0001), and (F(4, 20) = 54.53, p = 0.0025) significance difference respectively. In addition, the CAT activity in the AP-treated group (6.664 ± 0.193) was significantly higher

(F(4, 20) = 54.53, p = 0.0005) when compared with AA-treated group (4.504 ± 0.161) and (F(4, 20) = 54.53, p<0.0001) with DMSO-treated group (4.248 ± 0.217) (Fig. 3a). In brain, the CAT activity of TNT rats (9.100 ± 0.927) was significantly lower than the NTNT rats (44.530 ± 1.639). When treated with AA (22.610 ± 0.588), AP (36.030 ± 1.014), and DMSO (17.660 ± 1.108), CAT activities significantly increased when compared with TNT rats (1.890 ± 0.223) with (F(4, 20) = 164.8, p<0.0001), (F(4, 20) = 164.8, p<0.0001), and (F(4, 20) = 164.8, p = 0.0002) significance difference respectively. Additionally, the CAT activity in the AP-treated group (36.030 ± 1.014) was significantly higher (F(4, 20) = 164.8, p<0.0001) when compared with both AA-treated group (3.768 ± 0.165) and DMSO-treated group (3.002 ± 0.264) (Fig. 3b).

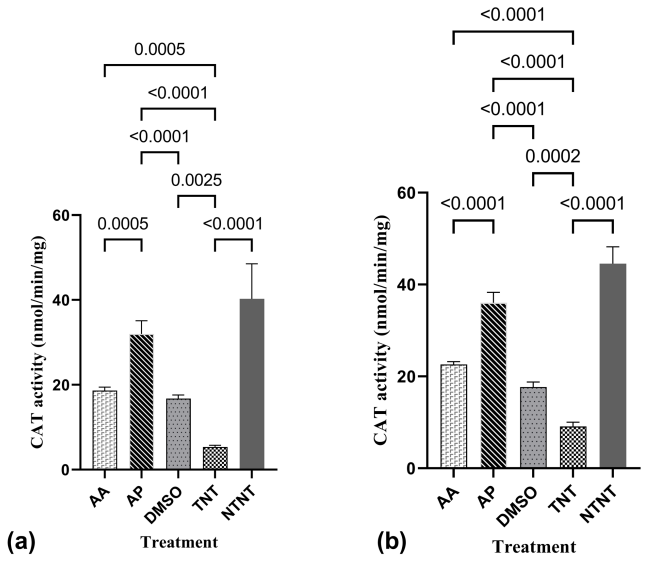

Fig. 3. Effects of AA, AP, and DMSO on CAT activities (a) Serum; (b) Brain Tissue. CAT- Catalase, AA- Ascorbic Acid, AP- L-ascorbyl-6-palmitate, DMSO- dimethyl Sulfoxide, TNT- Traumatized Non-treated, NTNT- Non-Traumatized Non-treated. p<0.05, p<0.01, p<0.001, p<0.0001 shows the post hoc Tukey test results of the one-way ANOVA test between the groups

3.4. The impact of AA, AP, and DMSO on GPx activity in the serum and brain tissue of rats with TBI

The outcomes of the effects of AA, AP, and DMSO administration on GPx activity in TBI rats are depicted in Fig. 4a and 4b. The findings show that TBI induction significantly reduced the GPx activity in the brain tissue and serum of the TNT rats when compared to the NTNT rats. Following therapy, the DMSO, AA, and AP treated groups' GPx activities significantly increased in comparison to the TNT groups. In serum, the GPx activity of TNT rats (11.660 ± 1.013) decreased significantly (F(4, 20) = 118.4, p<0.0001) when compared with NTNT rats (49.550 ± 1.644). When treated with AA (26.410 ± 1.479), AP (43.710 ± 1.762), and DMSO (26.470 ± 0.829), GPx activities significantly increased (F(4, 20) = 118.4, p<0.0001) in these groups when compared with TNT rats (11.660 ± 1.013). In addition, the GPx activity in the AP-treated group (43.710 ± 1.762) was significantly higher (F(4, 20) = 118.4, p<0.0001) when compared with both AA-treated group (26.410 ± 1.479) and DMSO-treated group (26.470 ± 0.829) (Fig. 4a). In brain, the GPx activity of TNT rats (14.980 ± 1.324) was significantly lower (F(4, 20) = 428.1, p<0.0001) than the NTNT rats (80.510 ± 1.357). When treated with AA (49.690 ± 1.299), AP (68.490 ± 1.436), and DMSO (29.870 ± 1.054), CAT activities significantly increased (F(4, 20) = 428.1, p<0.0001) in these groups when compared with TNT rats (14.980 ± 1.324). Additionally, the GPx activity in the AP-treated group (68.490 ± 1.436) was significantly higher (F(4, 20) = 428.1, p<0.0001) when compared with both AA-treated group (49.690 ± 1.299) and DMSO-treated group (29.870 ± 1.054) (Fig. 4b).

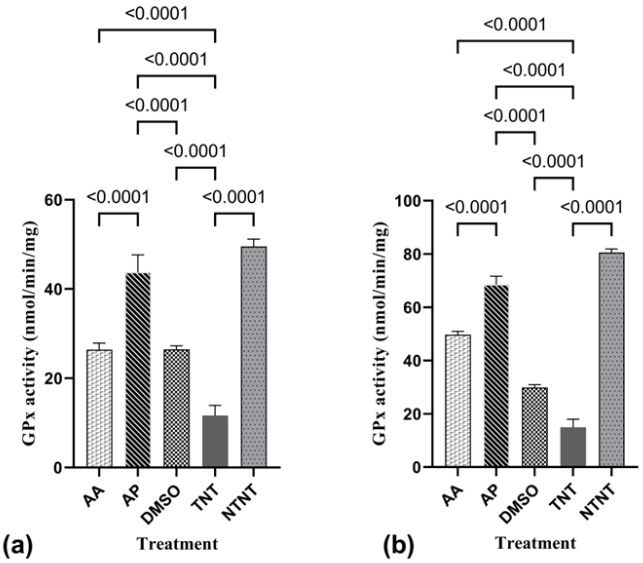

Fig. 4. Effects of AA, AP, and DMSO on GPx activities (a) Serum; (b) Brain Tissue. GPx- Glutathione peroxidase, AA- Ascorbic Acid, AP- L-ascorbyl-6-palmitate, DMSO- dimethyl Sulfoxide, TNT- Traumatized Non-treated, NTNT- Non-Traumatized Non-treated. p<0.05, p<0.01, p<0.001, p<0.0001 shows the post hoc Tukey test results of the one-way ANOVA test between the groups

4. Discussion

The pathogenesis of complications arising from TBI have been linked to oxidative stress (OS), which is caused by the excessive generation of ROS and leads to the depletion of the antioxidant defense system. Since primary brain injuries are essentially irreversible, the focus of TBI treatments has primarily been directed toward secondary brain injury, wherein OS plays a crucial role, and antioxidants exhibit a protective effect.

This study measured the quantity of MDA, an indicator of lipid peroxidation, and the enzymatic antioxidant activities of GPx, CAT, and SOD to determine OS. Our findings reveal that the depletion of antioxidant enzymes and elevation of malondialdehyde level, as seen by a noteworthy (p<0.05) increase in MDA (Fig. 1a, 1b) and a noteworthy (p<0.05) decline in the activities of SOD, CAT, and GPx (Fig. 2a, 2b, 3a, 3b, 4a, 4b) in both serum and brain tissue of TNT rats as compared to NTNT rats, corroborate the significant involvement of oxidative stress in TBI. The present findings

align with those of (24), who posited that the brain generates OS markers such as lipid peroxides and ROS in response to TBI, while protective antioxidant enzymes like SOD, CAT, GPx, GSH, and the GSH/GSSG ratio exhibit reduced activity. Additionally, the administration of antioxidants; AA, AP, and DMSO was observed to mitigate the impact of the induced oxidative stress. This is shown in Fig. 2a, 2b, 3a, 3b, 4a, 4b, where the activities of SOD, CAT, and GPx were significantly higher ($p<0.05$) in the groups treated with AA, AP, and DMSO than in the TNT group. Correspondingly, the levels of MDA (Fig. 1a, 1b) were lowered in the AA, AP, and DMSO treated groups relative to the TNT group, suggesting that these antioxidants could potentially normalize lipid peroxidation.

The observed reduction in MDA concentration and increase in enzymatic activity following AA treatment may be attributed to the inherent reducing properties of AA, which enables it to effectively quench free radicals and harmful oxygen species. These actions serve to safeguard crucial cellular components, including lipids, proteins, and DNA, from oxidative damage. As an exceptional provider of electrons, AA serves as a prime resource for free radicals in search of an electron to restore their stability. In this manner, AA can supply free radicals with electrons, thereby impeding their reactivity (25). Furthermore, the potential amelioration of OS by AA may have been enabled by glutathione and vitamin E recycling, both of which are known to be effective antioxidants. Through the donation of an electron to the vitamin E radical (vitamin-E-O•) and subsequent reduction to vitamin E, the capacity of AA to prevent lipid peroxidation is demonstrated by one-electron reduction of lipid hydroperoxyl radicals (26). In addition, in the context of reducing the impact of OS, it is plausible that AA may have exerted its effects by activating genes that encode proteins involved in antioxidant defense. Bulama et al. (27) conducted a study that revealed the upregulation of antioxidant enzyme genes (SOD, CAT, and GPx) by AA, thereby enhancing the antioxidant defense in an experimental model of TBI. Our findings align with prior research that demonstrated the beneficial effects of AA on TBI-related oxidative damage. Specifically, the works of Bulama et al. (27), Ishaq et al. (10), and Devi et al. (28) all indicate that AA is an effective scavenger of free radicals that inhibits the generation of lipid hydroperoxides, upregulates the expression of antioxidant enzyme genes, and enhances both survival rates and neurological outcomes in experimental TBI. Moreover, Zaidi and colleagues have reported that a reduction in free radical scavenging enzymes, SOD and CAT, coupled with an elevation in MDA levels, can be effectively rectified through the administration of AA (29).

Based on the current investigation, it appears that the antioxidant capacity of AP may be responsible for the significant ($p<0.05$) elevation of SOD, CAT, and GPx activities (Fig. 2a, 2b 3a, 3b, 4a, 4b), as well as the reduction in MDA concentration (Fig. 1a, 1b) observed in AP-administered rats, as opposed to TNT rats. AP has been demonstrated to be a potent scavenger of free radicals, which may confer protection against aging, as well as diseases such as cancer, atherosclerosis, cataracts, and inflammatory disorders (30). The present study observed that AP demonstrated a noteworthy ability to enhance SOD, CAT, and GPx activities while simultaneously reducing MDA concentration, as compared to AA. This superiority of AP over AA might be attributed to its various physical advantages. The amphipathic nature of AP facilitates its absorption into cell membranes, allowing it to remain for a longer duration in comparison to water-soluble AA. Additionally, AP's ability to permeate biological barriers and penetrate neural tissue may enable it to retain its antioxidant action more effectively than AA, thus providing better protection to the lipid membranes of cells. Furthermore, AP's stability also contributes to its greater efficacy than AA, resulting in longer bioavailability and better absorption into tissues (31). The retention of AP in the erythrocyte membrane and its ability to reduce ferric cyanide exhibited superior efficacy to AA in both intact cells and membranes (32). Also, the possible amelioration of OS observed in this study could have been facilitated by AP through the process of recycling the fat-soluble antioxidant α-tocopherol, commonly referred to as vitamin E. The cell's natural defense mechanism against free radical oxidation showed considerable improvement, owing to AP's ability to recycle vitamin E radical to vitamin E inside the membrane (33). The observed long-lasting effect of AP in the brain could be attributed to its capacity to transport ascorbate into neural tissues. A prior investigation by Pokorski et al. (34) indicated that AP administration resulted in significantly higher ascorbate content in the carotid body and cortex, by almost ten-fold, in comparison to ascorbic acid administration. They further emphasized that the lipophilic nature of AP enabled it to effectively traverse biological barriers, thereby satisfying the ascorbate requirements of the tissues, as opposed to the hydrophilic ascorbic acid. This suggests that AP could potentially serve as a superior therapeutic agent, given its ability to effectively transport ascorbate to the brain and sustain its effects over a prolonged duration. Furthermore, there's a chance that AP increased the expression of genes encoding for the antioxidant enzymes SOD, GPx, and CAT like that reported by Bulama and colleagues (27) through the use of AA. Nevertheless, further investigation is necessary to assess the effect of AP on the genes that encode for these antioxidative enzymes, as well as on the Nuclear factor erythroid 2-related factor 2 (Nrf2) transcription factor. The observed protective efficacy of AP in this study could also potentially be attributed to its capacity for chelating transition metals and impeding their pro-oxidant effect in TBI. The Makinen et al. (35) investigation revealed that AP treatment prevents the pro-oxidant effects of iron by chelating it, thus inhibiting the formation of hydroperoxide and accelerating the decomposition of methyl linoleate hydroperoxides. AP has been shown in some research to have beneficial effects when it comes to complications caused by OS. For example, in a

different study, Qian et al. (36) discovered that AP regulated uncontrolled neuroinflammation in brain microglia cells and impeded oxidative stress. Additionally, Yilmaz et al. (37) revealed that AP improved the conversion of reduced glutathione (GSH) from oxidized glutathione (GSSG) through recycling, decreased MDA concentration, and repressed oxidative stress in erythrocytes of diabetic rats.

In the present investigation, we employed DMSO as the carrier for AP. We experimented with different solvents, including water, normal saline, and alcohol, but none of these solvents fully dissolved AP. Subsequently, we tested various concentrations of DMSO and only 100% of DMSO successfully achieved complete dissolution of AP. Moreover, due to the inherent antioxidant properties of DMSO (38), we chose to include a group that was treated with DMSO to assess how its effects differed from those of AP. In our current study, the administration of DMSO alone exhibited a reduction in oxidative stress by diminishing malondialdehyde levels (Fig. 1a, 1b) as well as enhancing the activities of superoxide dismutase, catalase, and glutathione peroxidase (Fig. 2a, 2b, 3a, 3b, 4a, 4b). However, the impact of DMSO alone was comparatively less pronounced when compared to the group treated with AP.

For the first time in literature, this study shows the positive neuroprotective impacts of AP in the management of TBI-induced OS. However, this study has potential limitations. The first limitation is the small sample size which may have reduced the power of the study. The primary limitation to the generalization of these results is, due to limited resources, our study estimated only the SOD, CAT, and GPx enzyme activities and lipid peroxidation, and we were unable to investigate other neurobehavioral assessments, histology, or immunohistochemistry to attribute the “antioxidant potential” of AP to the outcome of neuroprotection in the TBI model. Nevertheless, the findings of this study offer a new avenue for further exploration of L-ascorbyl-6-palmitate (AP) in the management of TBI. Therefore, further studies should investigate the underlying molecular mechanisms and long-term effects of AP treatment on neurological recovery and functional outcomes in TBI models, as well as explore its potential synergistic effects with other antioxidant or neuroprotective strategies.

The current study shows that trauma-induced head injury by weight drop model causes oxidative stress. In a TBI rat model, the chemical modification of ascorbic acid to AP has demonstrated encouraging outcomes in reducing oxidative stress and boosting antioxidant defense. It has been demonstrated that AP is more effective than ascorbic acid, highlighting its possible neuroprotective role. In the continued search for effective antioxidant strategies for the treatment of TBI, these findings provide compelling evidence in support of exploring AP as a potential therapeutic agent for TBI.

Supplementary Materials

The findings of this research have been substantiated by data, which has been duly deposited on Figshare. This data may be accessed through the digital object identifier https://doi.org/10.6084/m9.figshare.23580024

Ethical Statement

The experimental procedure was granted approval by the Animal Care and Usage Committee of Usmanu Danfodiyo University, Sokoto (IACUC Approval number UDUS/FAREC/AUP-R09/2019). The work described in this study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving animals.

Conflict of interest

The authors declare no conflict of interest.

Funding

None to declare.

Acknowledgments

The authors acknowledge the technicians in the chemical lab in the Department of Biochemistry and Molecular Biology at Usmanu Danfodiyo University for their support with the preparation of chemicals and stock solutions. We also appreciate the General laboratory of the College of Veterinary Physiology and Biochemistry in the City Campus of the University for providing space and equipment for the research.

Authors’ contributions

Concept: L.S.B., Y.S., A.Y.A., Design: A.O., S.N., I.B., Data Collection or Processing: U.F.S., I.A., Y.Z., Analysis or Interpretation: U.F.S., Literature Search: U.F.S., I.A., Y.Z., Writing: U.F.S., I.B.

References

1. Orman JAL, Selassie AW, Perdue CL, Thurman DJ, Kraus JF. Surveillance of Traumatic Brain Injury. In: Li G, Baker S, editors. Injury Research. MA: Boston; Springer; 2012, p. 61-85.
2. Sivanandam TM, Thakur MK. Traumatic brain injury: a risk factor for Alzheimer's disease. Neurosci Biobehav Rev. 2012 May;36(5):1376-81.
3. Sahuquillo J, Poca MA, Amoros S. Current aspects of pathophysiology and cell dysfunction after severe head injury. Curr Pharm Des. 2001 Oct;7(15):1475-503.
4. Chan PH. Mitochondria and Neuronal Death/Survival Signaling Pathways in Cerebral Ischemia. Neurochem Res. 2004; 29(11): 1943-1949.
5. Xiong Y, Gu Q, Peterson P, Muizelaar J, Lee C. Mitochondrial Dysfunction and Calcium Perturbation Induced by Traumatic Brain Injury. J Neurotrauma. 1997; 14(1): 23-34.
6. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A. 1993 Sep 1;90(17):7915-22.
7. Hanafy KA, Selim MH. Antioxidant Strategies in Neurocritical Care. Neurotherapeutics. 2011; 9(1): 44-55.
8. He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. Cell Physiol Biochem. 2017; 44(2): 532-553.

9. Pun PBL, Lu J, Moochhala S. Involvement of ROS in BBB dysfunction. Free Radic Research. 2009; 43(4): 348-364.

10. Ishaq GM, Saidu Y, Bilbis LS, Muhammad SA, Jinjir N, Shehu BB. Effects of α-tocopherol and ascorbic acid in the severity and management of traumatic brain injury in albino rats. J Neurosci Rural Pract. 2013 Jul;4(3):292-7.

11. May JM. Vitamin C Transport and Its Role in the Central Nervous System. Subcell Biochem. 2011; 58: 85-103.

12. Harrison FE, May JM. Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. Free Radic Biol Med. 2009; 46(6): 719-730.

13. Santos Ítala MS, Tomé A da R, Saldanha GB, Ferreira PMP, Militão GCG, De Freitas RM. Oxidative Stress in The Hippocampus During Experimental Seizures Can Be Ameliorated with The Antioxidant Ascorbic Acid. Oxid Med Cell Longev. 2009; 2(4): 214-221.

14. Weber V, Coudert P, Rubat C, Duroux E, Leal F, Couquelet J. Antioxidant Properties of Novel Lipophilic Ascorbic Acid Analogues. J Pharm Pharmacol. 2000; 52(5): 523-530.

15. Tripathi R, Singh B, Bisht S, Pandey J. L-Ascorbic Acid in Organic Synthesis: An Overview. Curr Org Chem. 2009; 13(1): 99-122.

16. Bindhumol V, Chitra K, Mathur P. Ascorbic Acid induces reactive oxygen species generation in the liver of male rats. Toxicology. 2003; 188(2): 117-124.

17. Mohamed R, Dharmappa KK, Tarannum S, Jameel NM, Kannum SA, Ashrafulla HS, et al. Chemical modification of ascorbic acid and evaluation of its lipophilic derivatives as inhibitors of secretory phospholipase A2 with anti-inflammatory activity. Mol Cell Biochem. 2010; 345(1): 69-76.

18. Marmarou A, Foda MAA-E, Brink W van den, Campbell J, Kita H, Demetriadou K. A new model of diffuse brain injury in rats. J Neurosurg. 1994; 80(2): 291-300.

19. Rezanejad Keshteli FZ, Parivar K, Joghatayi MT, Beik HA. Study of the differentiation of rat omentum stem cells to nerve cells using brain tissue extract of Wistar rats. International J Cell Mol Biotechnol. 2014; 1-13.

20. Marklund SL. Analysis of extracellular superoxide dismutase in tissue homogenates and extracellular fluids. Methods Enzymol. 1990;186:260-5.

21. Johansson LH, Håkan Borg L. A spectrophotometric method for determination of catalase activity in small tissue samples. Anal Biochem. 1988; 174(1): 331-336.

22. Ursini F, Maiorino M, Gregolin C. The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. Biochim Biophys Acta. 1985 Mar 29;839(1):62-70.

23. Goulart M, Batoréu M, Rodrigues A, Laires A, Rueff J. Lipoperoxidation products and thiol antioxidants in chromium exposed workers. Mutagenesis. 2005; 20(5): 311-315.

24. Rodriguez-Rodriguez A, Egea-Guerrero J, Murillo-Cabezas F, Carrillo-Vico A. Oxidative Stress in Traumatic Brain Injury. Curr Med Chem. 2014; 21(10): 1201-1211.

25. Verma RS, Mehta A, Srivastava N. In vivo chlorpyrifos induced oxidative stress: Attenuation by antioxidant vitamins. Pestic Biochem Physiol. 2007; 88(2): 191-196.

26. Pritam P, Deka R, Bhardwaj A, Srivastava R, Kumar D, Jha AK, Jha NK, Villa C, Jha SK. Antioxidants in Alzheimer's Disease: Current Therapeutic Significance and Future Prospects. Biology. 2022; 11(2): 212.

27. Bulama I, Suleiman N, Bilbis L, Abbas A, Jinjiri N, Saidu Y, et al. Antioxidative and Neurotherapeutic Effect of Ascorbic acid on Albino rats Induced with Traumatic Brain Injury. J Cell Neurosci Oxid Stress. 2020; 12(1): 922-936.

28. Devi SA, Vani R, Subramanyam MVV, Reddy SS, Jeevaratnam K. Intermittent hypobaric hypoxia-induced oxidative stress in rat erythrocytes: protective effects of vitamin E, vitamin C, and carnitine. Cell Biochem Func. 2007; 25(2): 221-231.

29. Zaidi SMKR, Al-Qirim TM, Banu N. Effects of Antioxidant Vitamins on Glutathione Depletion and Lipid Peroxidation Induced by Restraint Stress in the Rat Liver. Drugs R D. 2005; 6(3): 157-165

30. Austria R, Semenzato A, Bettero A. Stability of vitamin C derivatives in solution and topical formulations. J Pharm Biomed Anal. 1997; 15(6): 795-801.

31. Cort WM. Antioxidant activity of tocopherols, ascorbyl palmitate, and ascorbic acid and their mode of action. J Am Oil Chem Soc. 1974; 51(7): 321-325.

32. Ross D, Mendiratta S, Qu Z- chao, Cobb CE, May JM. Ascorbate 6-palmitate protects human erythrocytes from oxidative damage. Free Radic Biol Med. 1999; 26(1): 81-89.

33. May JM, Qu Z-C, Cobb CE. Accessibility and reactivity of ascorbate 6-palmitate bound to erythrocyte membranes. Free Radic Biol Med. 1996; 21(4): 471-480.

34. Pokorski M, Marczak M, Dymecka A, Suchocki P. Ascorbyl palmitate as a carrier of ascorbate into neural tissues. J Biomed Sci. 2003; 10(2): 193-198.

35. Mäkinen M, Kähkönen M, Hopia A. Ascorbic acid and ascorbyl palmitate have only minor effect on the formation and decomposition of methyl linoleate hydroperoxides. Eur Jof Lipid Sci Technol. 2001; 103(10): 683-687.

36. Li Q, Wu Y, Chen XS, Zeng T, Liu LL, Feng ZQ, Liu DY, et al. Ascorbic acid 6-palmitate modulates microglia M1/M2 polarization in lipopolysaccharide-stimulated BV-2 cells via PERK/elF2α mediated endoplasmic reticulum stress. BMC Complement Med Ther. 2022 Nov 18;22(1):302.

37. Yilmaz O, Ozkan Y, Yildirim M, Oztürk AI, Erşan Y. Effects of alpha lipoic acid, ascorbic acid-6-palmitate, and fish oil on the glutathione, malonaldehyde, and fatty acids levels in erythrocytes of streptozotocin induced diabetic male rats. J Cell Biochem. 2002;86(3):530-9.

38. Bulama I, Saidu UF, Suleiman N, Abbas A, Saidu Y, Yakubu Y, et al. Antioxidative Strategy in Traumatic Brain Injury: Role of Low-Molecular-Weight Antioxidants. Exp Appl Med Sci. 2024; 4(4): 573-594.