Effect of vitamin E on serum MDA and antioxidant enzymes in traumatic brain injury-induced rats

ABSTRACT

Objective: Traumatic brain injury (TBI) represents one of the major causes of mortality and disability in the world. This study was designed to investigate the role of antioxidants in the treatment of induced TBI in albino rats.

Methodology: Adult albino rats were induced with TBI by the weight-drop method. Rats were grouped into three groups of eight rats each. Group I served as a traumatized-treated group (TT), Group II served as a non-traumatized, non-treated group (TNT), and group III served as normal control group. The treatment group received 67.5 mg/kg of vitamin E (VE). Treatment started 30 minutes after the trauma and continued for 21 days. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) in serum tissue were assayed to evaluate oxidative stress (OS).

Results: The treated group showed a significant ($p < 0.05$) increase in the activities of antioxidant enzymes (SOD, CAT GPx) and a significant ($p<0.05$) decrease in the concentration of MDA compared to the TNT group.

Conclusion: Conclusively, these promising results suggest that the use of antioxidant VE may be a useful neuroprotective strategy in the treatment of TBI.

Key words; Oxidative stress, Traumatic brain injury, Antioxidant, Malondialdehyde

1. INTRODUCTION

Traumatic brain injury (TBI) is characterized by alterations in brain function or immediate physical damage to brain tissue caused by an external force, followed by subsequent biochemical processes that can worsen the injury [1]. The severity of the damage isn't always immediately apparent and depends on various factors, including whether the external force was accidental or intentional, a direct impact, or acceleration, or deceleration, or the result of blast forces, and whether it involved penetration or not. Every year, around 70 million individuals suffer from TBI, with over 8 million people suffering from TBI-related disabilities [2]. In the United States alone, around 2.3 million cases of TBI occur yearly [3], with over 64,000 of these cases resulting in mortality, equating to nearly 176 TBI-related deaths every day [4]. Unfortunately, TBI is more common in developing nations [5]. The degree of brain injury can vary from minor to severe, and even seemingly mild injuries can lead to life-threatening complications. Projections for the prevalence of TBI in Africa are substantial, with an estimated 6 to 14 million new cases expected by 2050 [6]. Road traffic accidents (RTA) are the major cause of head and spinal cord injuries in Africa, with head injuries being the most common among all injuries in Nigeria [7]. RTA is responsible for 80% of all injuries in Nigeria alone [8], with an annual incidence rate of 2710/100,000 [7]. The high occurrence in Nigeria is thought to be related to a lack of adherence to safety norms as well as a poor road network [9]. Concussions, penetrating injuries, closed head injuries, skull fractures, hematomas, lacerations, anoxia, contusions, and diffuse axonal injuries (DAI) may result from the impact exerted by external forces on the brain [10]. This impairs the normal cellular function of the brain and may be present in all injury severality levels (mild, moderate, and severe) [11].

Based on the mechanism of injury, TBI is classified into two categories: primary injury, which occurs immediately after impact, and secondary injury, which is derived from cellular and biochemical changes that occur hours or days after trauma [12]. TBI severity is determined by primary injuries [13], which alter the structural integrity of the brain tissue, resulting in vascular and parenchymal damage, intracerebral haemorrhage, and axonal shearing [14].

Secondary injuries aggravate the damage caused by TBI [13], through a cascade of mechanisms that leads to long-term or life-long difficulties [14] and even mortality [15]. Mechanisms such as hypoxia, glutamate excitotoxicity, disruption of the blood-brain barrier (BBB), calcium overload, mitochondrial dysfunction, and neuro-inflammation all contribute to cell death via necrosis or apoptosis [15, 16,]. Of all these mechanisms, TBI is believed to be chiefly caused by the interplay between glutamate excitotoxicity, $Ca2^{+}$ excess, and oxidative stress, [12] with oxidative stress being the major cause of neuronal cell death.

One of the well-studied and established aspects of secondary injury to brain tissue is the generation of free radicals following TBI [17]. “A free radical is any chemical species capable of independent existence with one or more unpaired electrons which is responsible for its reactivity” [18]. “Reactive oxygen species (ROS) and reactive nitrogen species (RNS) comprise both free radicals and compounds that can

decompose to generate free radicals. ROS and RNS are frequently produced after TBI through numerous mechanisms. These reactive species are involved in the pathogenesis of TBI by worsening other secondary injury mechanisms and stimulating oxidative stress" [19].

"Oxidative stress has been identified as a potential contributor to the pathogenesis of acute central nervous system injury. After brain injury, the spontaneous generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to tissue damage via various cellular and molecular pathways. Radicals can cause damage to lipids, proteins, and nucleic acids (e.g. DNA), resulting in oxidative stress and subsequent cell death" [17]. "Mammalian cells possess intracellular or endogenous antioxidants such as superoxide dismutase, catalase, or glutathione peroxidase, in order to protect the cells against excessive levels of free radicals" [20].

"Antioxidants are chemicals that prevent the oxidation of other chemicals. They protect the key cell components by counteracting the damaging effect of free radicals" [21].

"The first and most important line of antioxidant defense systems against ROS is the enzymatic antioxidant (SOD, CAT, GPx)" [22]. "While these endogenous antioxidants increase their level of activity after TBI, the magnitude of the increase in free radicals compromises the antioxidant system's ability to neutralize the adverse effects of free radicals" [17].

"However, exogenous addition of compounds with antioxidant properties, such as vitamins, minerals (selenium, zinc), or other compounds like albumin, can provide additional protection" [23]. "These natural antioxidants or other compounds that can neutralize free radicals may be of central importance in the prevention of oxidative stress. Vitamin E, a potent peroxyl radical scavenger, is a chain-breaking antioxidant that prevents the propagation of free radical damage in biological membranes" [24, 25]. "Vitamin E has a potent function in improving the immune system, stress, and disease resistance" [25]. The aim of this research work is to validate the neurochemical role of vitamin E in TBI.

2. MATERIALS AND METHODS

2.1 Animals

All the experimental rats were apparently healthy albino rats weighing 200-250 g.They were obtained from the Animal House of the Biological Sciences Department, University of Maiduguri, Nigeria, for this study. The rats were allowed to acclimatize to the research laboratory conditions 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*.

2.2 Experimental Design

The experimental animals were randomly divided into three groups. Group I (induced & treated with VE), Group II-traumatized but not treated (TNT), and Group III normal control group (that is non-traumatized and non-treated (NTNT). The treatment lasted for 21 days. This work was approved by the board of the University of Maiduguri after meeting national and international standards. Care of animals used was in accordance with institution guidelines.

2.4 Induction of TBI

Head injury was induced in the entire experimental group except in the negative control group by the weight-drop method using an acceleration impact device from Marmarou [26].

2.5 Sample collection

The rats were anesthetized using chloroform in a glass jar, and blood was collected by cardiac puncture, and serum was harvested for biochemical analysis.

2.6 Analysis of oxidative Stress

Oxidative stress markers were assayed in the serum tissue. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and lipid peroxidation byproduct malondialdehyde (MDA) were assayed using Cayman's Assay Kits, with batch numbers 706002 for SOD, 707002 for CAT, 703102 for GPx, and 700870 for MDA. The manufacturer's instructions were carefully followed.

2.7 Statistical Analysis

Results were analyze using the statistical package SPSS version 22. Results were expressed as means ± SD. The data were analyzed by one-way analysis of variance (ANOVA). If the F values were significant, the Tukey post-hoc test was used to compare groups.

3. RESULTS

3.1. The Effect of Supplementation of TBI Rats with Vitamin E on the Activity of Serum SOD

Figure 1 shows the result of serum SOD level in antioxidant-treated groups. The result indicated that TBI caused a significant ($P<0.05$) decrease in the activity of the enzyme. Supplementation of the antioxidant at 67.5 mg/kg body weight (BW) increased the SOD activity significantly ($P<0.05$).

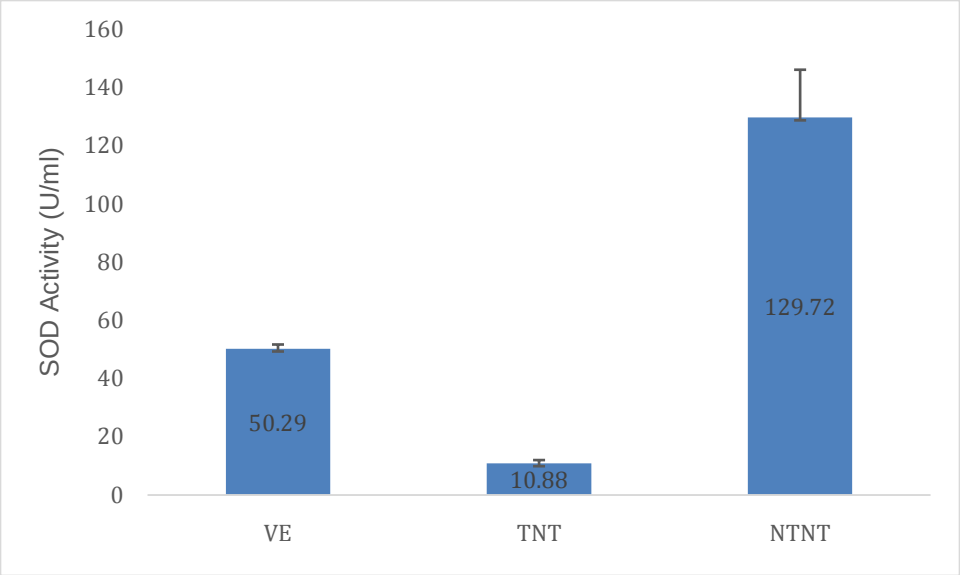

Figure 1: Effects of VE on the Activity of Superoxide dismutase in blood

*SOD: Superoxide dismutase, TNT: Traumatized non- treated, NTNT: Non-traumatized non- treated, VE: Vitamin E *(p<0.05).. n=8, one-way analysis of variance (ANOVA), Tukey post-hoc's test.*

3.2. The Effect of Supplementation with Vitamin E on the Activity of Serum CAT

Figure 2 shows the outcome of supplementation with VE on the activity of CAT in TBI rats. The result indicated that TBI caused a significant (P<0.05) decrease in the activity of the enzyme. Supplementation of the antioxidant at 67.5 mg/kg body weight increased the activity significantly (P<0.05).

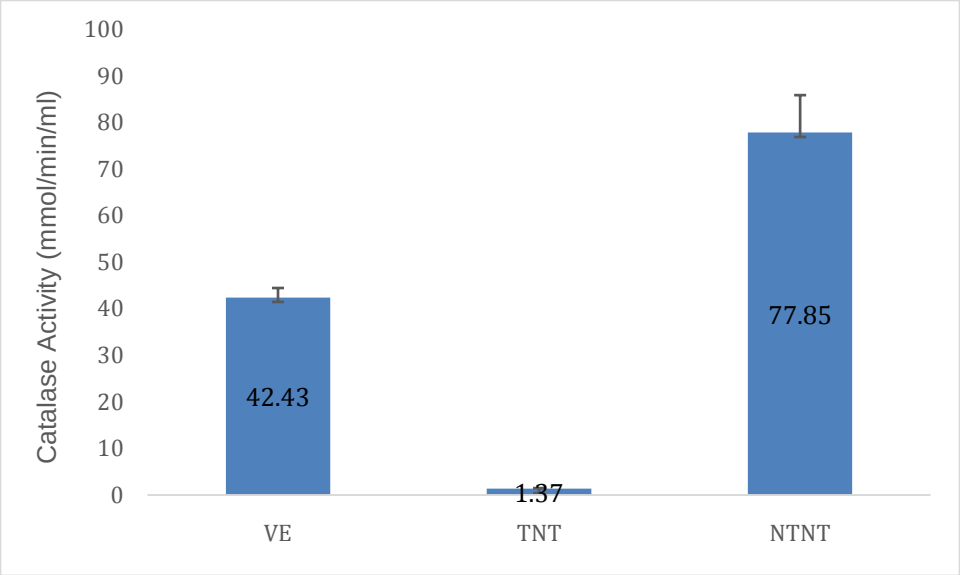

Figure 2: Effects of VE on the Activity of CAT in blood

CAT: Catalase, TNT: Traumatized non- treated, NTNT: Non-traumatized non-treated, VE: vitamin E. (p<0.05) (p<0.05). n=8, one-way analysis of variance (ANOVA), Tukey post-hoc's test.*

3.2. The Effect of Supplementation with VE on the Activity of Serum GPx

The effects of VE on the activity of GPx were presented in Fig. 3. The results showed that TBI caused a significant ($P<0.05$) decrease in the activity of the enzyme. Administration of the antioxidant at 67.5 mg/kg BW, significantly ($P<0.05$) increased the activity.

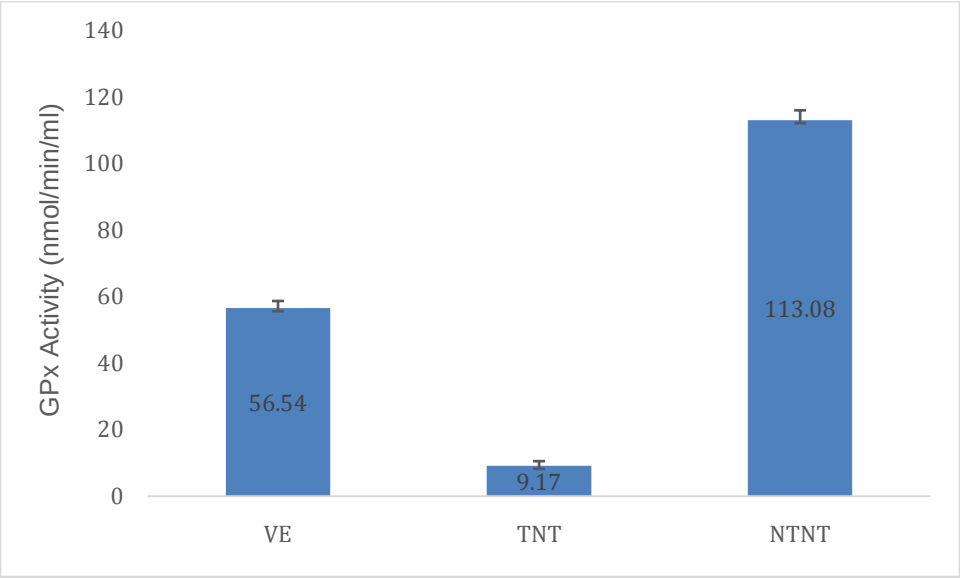

Figure 3: Effects of VE on the Activity of GPX in blood

GPX: Glutathion peroxidase, TNT: Traumatized non- treated, NTNT: Non-traumatized non- treated, VE: vitamin E ($p<0.05$). n=8, one-way analysis of variance (ANOVA), Tukey post-hoc's test.

3.3. The Effect of Supplementation with VE on the Serum Concentration of MDA

Figure 4 shows the effects of LMWA on lipid peroxidation. The results indicated that TBI caused a significant ($P<0.05$) increase in the concentration of MDA in the TNT group. After supplementation with VE at 67.5mg/kg, the concentration of MDA decreased significantly ($P>0.05$).

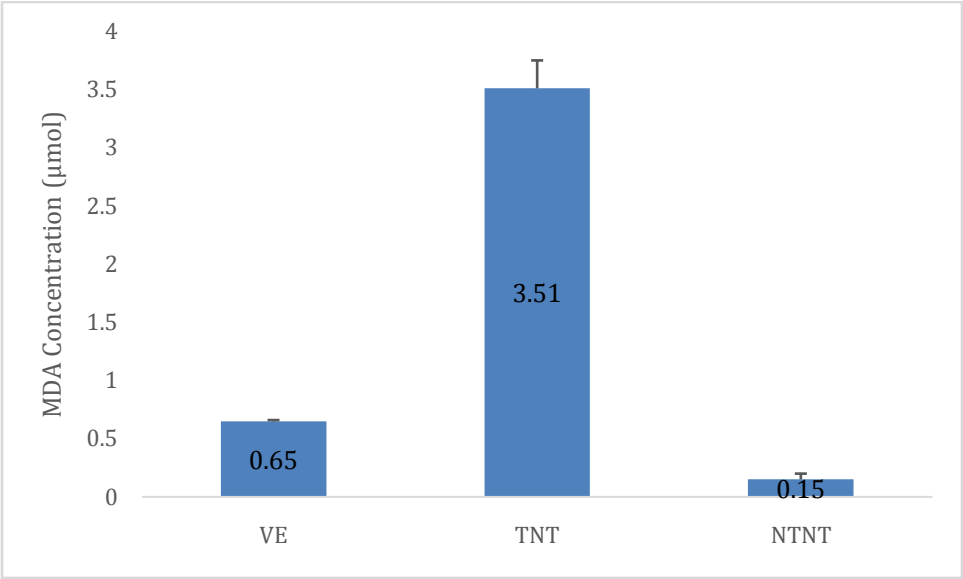

Figure 4: Effects of VE on the Concentration of MDA in the blood of experimental rats

MDA: Malondialdehyd, TNT: Traumatized non treated, NTNT: Non-traumatized non- treated, VE: vitamin E. ($p<0.05$). n=8, one-way analysis of variance (ANOVA), Tukey post-hoc's test.

4. DISCUSSION

Oxidative stress in this study was evaluated by measuring the levels of SOD, CAT, GPx, and MDA as indicators of enzymatic antioxidant activity and lipid peroxidation respectively. A significant decrease was observed in the activities of SOD, CAT, and GPx, and an increase in the concentration of MDA in the serum tissue of TNT rats as compared to NTNT rats (Figure 1-4). This, suggests the occurrence of OS due to the induced TBI. Supplementation with V E ameliorated the induced OS (Figure 1-4).

Treatment of TBI-induced rats with 67.5 mg/kg of VE indicated a significant increase ($P< 0.05$) in the activities of SOD, CAT, and GPx and decreased concentration of MDA in the serum tissue of the treated group compared to the TNT group (Figure 1 – 4). This might be due to the ability of VE to quench free radicals and reduce their oxidative activity on lipids, proteins, and nucleic acid which leads to the suppression of the antioxidant system and accumulation of MDA.

It can also be due to the regeneration of vitamin E and glutathione which are very effective against ROS [27]. This might be attributed to the characteristics of vitamin E as the most relevant chain-breaking antioxidant and abundance in cells and mitochondria membrane where ROS are also generated and their dysfunction causes excessive release of free radicals. Therefore it may have acted by inhibiting lipid

peroxidation and OS in these important sites of free radical generation as reported by Inci and colleagues [28]. Jean-MARC [29], reported that vitamin E is a lipid-soluble antioxidant that prevents the formation of lipid peroxide. Also, the modifying effect of vitamin E on OS pathways and improving neurological outcomes, have been reported in many animal studies [28]. It is also known that apart from its direct effect on ROS, vitamin E can react with various antioxidants such as vitamin C, GSH, and bring about synergistic activity. In return, these antioxidants regenerate vitamin E thereby potentiating its effect [30].

The findings of this work revealed that the group treated with VE had significantly ($P<0.05$) increased activities of the antioxidant enzymes and significantly ($P<0.05$) decreased levels of MDA compared to the TNT counterpart (Figure 1– 4).

CONCLUSION

In conclusion, after TBI induction, a significant decrease was observed in antioxidant enzymes activities, with a concomitant increase in MDA levels indicating the occurrence of oxidative stress. Oxidative stress is the strategic pathogenic mechanism of secondary injury that results in neuronal degeneration and functional deficits. Mitigating this damaging process is neuroprotective and restores neuronal function. The antioxidant given have indicated its neuroprotective and neurorestorative potential by boosting the antioxidant capacity and inhibiting lipid peroxidation. These promising results suggest that vitamin E may be useful in the management of TBI.

ETHICAL APPROVAL

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.*

This work was approved by the board of the University of Maiduguri after meeting national and international standards. The care of animals used was in accordance with institution guidelines

REFERENCES

1. Williamson, C. Traumatic Brain Injury: Epidemiology, classification, and pathophysiology. *UpToDate*. 2023. Available at https://www.uptodate.com/contents/traumatic-brain-injury-epidemiology-classification-and-pathology Accessed March 20 2023
2. Wiles, M. D., Braganza, M., Edwards, H., Krause, E., Jackson, J. and Tait, F. Management of traumatic brain injury in the non-neurosurgical intensive care unit: a narrative review of current evidence. *Anaesthesia.* 2023; 1-11. doi:10.1111/anae.15898
3. Centers for Disease Control and Prevention (CDC), U.S. Department of Health and Human Services. Surveillance Report of Traumatic Brain Injury-related Emergency Department Visits, Hospitalizations, and Deaths. 2014. Available at: https://www.cdc.gov/traumaticbraininjury/get_the_facts.html.
4. Centers for Disease Control and Prevention (CDC). National Center for Health Statistics: Mortality data on CDC WONDER. 2023 Available at: https://wonder.cdc.gov/mcd.html.
5. Adegboyega, G., Zolo, Y., Sebopelo, L. A., Dalle, D. U., Dada, O. E., Mbangtang, C. B., Tetinou, F., Kanmounye, U. S. and Alalade, A. F. The Burden of Traumatic Brain Injury in Sub-Saharan Africa: A Scoping Review, *World Neurosurgery.* 2021. https://doi.org/10.1016/j.wneu.2021.09.021.
6. 10. Ahmed, S., Venigalla, H., Mekala, H. M., Dar, S., Hassan, M. and Ayub, S. Traumatic brain injury and neuropsychiatric complications. *Indian Journal of Psychological Medicine*. 2017; 39 (**2**):114-21
7. Wong, J. C., Linnd, K. A., Shinoharad, R. T. and Mateen, F. J. Traumatic brain injury in Africa in 2050: a modeling study*. European Journal of Neurology*. 2016; **23**: 382–386
8. Ogunleye, O. O., Shuaibu, I. S. and Obanife, H. O. Epidemiological Patterns of Head Injury in Bauchi, North-Eastern Nigeria. *Acta Scientific Neurology.* 2021; **4**(**5**): 27-32
9. Suleiman, N., Bilbis, L. S., Saidu, Y., Nasiru, J. I., Dallatu, M. K., Sahabi, S. M., Ngaski A. A., Garba, B., Yakubu, A. S. and Bulama, I. Effect Of Some Low Molecular Mass Antioxidants In The Management Of Traumatic Brain Injury In Albino Rats. *International Journal of Current Research.* 2015; **7**(**7**):18492-18499.
10. Bulama, I., Suleiman, N., Suleiman, M., Bilbis, L. S. and Saidu Y. Effect Of Mannitol And Uric Acid On Lipid Peroxidation And Level Of Creatine Kinase In Albino Rats Induced With Traumatic Brain Injury. *European Journal Of Pharmaceutical And Medical Research*. 2016; **3**(**8**): 179-183.
11. Capizzi, A., Woo, J., and Verduzco-Gutierrez, M. Traumatic Brain Injury: An Overview of Epidemiology, Pathophysiology, and Medical Management. *Medical Clinics of North America*. 2020; **104**: 213–238.
12. Owoeye, O. 1., Awoyemi, F. O. 1., Imosemi, I. O. 1., Atiba, F. A. 1. and Malomo, A.,O. 2019.

13. Dai, W., Wang, H., Fang, J., Zhu, Y., Zhou, J., Wang, X., Zhou, Y. and Zhou, M. Curcumin provides neuroprotection in models of traumatic brain injury via the Nrf2-ARE signaling pathway. *Brain Research Bulletin.* 2018; 1-18. https://doi.org/10.1016/j.brainresbull.2018.03.020
14. Mondello, S., Thelin, E. P., Shaw, G., Salzet, M., Visalli, C., Cizkova, D., kobeissy, F. and Buki, A. Extracellular vesicles: pathogenetic, diagnostic and therapeutic value in traumatic brain injury. *Expert Review of Proteomics.* 2018. DOI: 10.1080/14789450.2018.1464914
15. Bulama, I., Suleiman, N., Bello, A., Abbas, A. Y., Nasiru, J. I., Saidu, Y., Chiroma, S. M., Aris, M. M. M., Norma, M. T. C., Waziri, A. and Bilbis, L. S. Antioxidant-based neuroprotective effect of dimethylsulfoxide aainst induced traumatic brain injury in rats model. *Frontiers in Pharmacology.* 2022; **13**: 998179
16. Farkhondeh, T., Samarghandian, S., Roshanravan, B. and Peivasteh-roudsari, L. Impact of Curcumin on Traumatic Brain Injury and Involved Molecular Signaling Pathways. *Recent Patents on Food, Nutrition & Agriculture.* 2019; **10**: 00-00.
17. Ma, M. W., Wang, J., Zhang, Q., Wang, R., Dhandapani, K. M., Vadlamudi, R. K., & Brann, D. W. NADPH oxidase in brain injury and neurodegenerative disorders. *Molecular Neurodegeneration*, 2017; *12*(1), 7. https://doi.org/10.1186/s13024-017-0150-7.
18. Jyoti, A., Mishra, N., & Dhas, Y. Ageing: Consequences of excessive free radicals and inflammation. *Current Science*, 2016; *111*(11), 1787–1793. https://doi.org/10.18520/cs/v111/i11/1787-1793.
19. Bruschetta, G., Impellizzeri, D., Campolo, M., & Casili, G. FeTPPS Reduces Secondary Damage and Improves Neurobehavioral Functions after Traumatic Brain Injury, 2017; *11*(February), 1–13. https://doi.org/10.3389/fnins.2017.00006.
20. Venegoni, W., Shen, Q., Thimmesch, A. R., Bell, M., Hiebert, J. B., & Pierce, J. D. The use of antioxidants in the treatment of traumatic brain injury. *Journal of Advanced Nursing*, 2017; *73*(6), 1331–1338. https://doi.org/10.1111/jan.13259.
21. Szwajgier, D., Borowiec, K., & Pustelniak, K. The Neuroprotective Effects of Phenolic Acids: Molecular Mechanism of Action. *Nutrients*, 2017; *9*(5), 477. https://doi.org/10.3390/nu9050477.
22. Shahim, P. *Blood Biomarkers for Traumatic Brain Injury*. 2015.
23. Arteaga, O., Álvarez, A., Revuelta, M., Santaolalla, F., Urtasun, A., & Hilario, E. Role of antioxidants in neonatal hypoxic–ischemic brain injury: New therapeutic approaches. *International Journal of Molecular Sciences*, 2017; *18*(2). https://doi.org/10.3390/ijms18020265.
24. Evans HM, Bishop KS. On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science, 1922; 56:650-I.
25. Burton GW. Joyce A. Ingold KU. Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? Arch Biochem Biophys 1983; 221:281-90.

26. Marmarou A, Foda MA, van den Brink W, Campbell J, Kita H, Demetriadou K. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. *J Neurosurg.* 1994; 80:291–300.
27. Heidi, G., Eleonora, B., Leif, S., Mogens, A. Antioxidant synergism between fruit juice and α-tocopherol. A comparison between high phenolic black chokeberry (Aronia melanocarpa) and high ascorbic blackcurrant (Ribes nigrum). *Eur Food Res Technol*, 2008; *226*(4):737–743.
28. Inci, S., Ozcan, O., & Kilinic, K. Time–level relationship for lipid peroxidation and the protective effect of a-tocopherol in experimental mild and severe brain injury. *Neurosurgery*, 1998;*43:* 330–335.
29. Jean-MARC, Z. Vitamin E An overview of Major Research Directi. *Mol Asp Med.*, 2007; *28:*400–422.
30. Rani, K. Role of Antioxidants in Prevention of Diseases. *Journal of Applied Biotechnology & Bioengineering*, 2017; *4*(1): 4–5. https://doi.org/10.15406/jabb.2017.04.00091