Effect of Antioxidant Vitamins and Minerals Supplementation on SOD, GPX, and CAT Induced Acute Ischaemic Stroke Albino Rats

ABSTRACT

Introduction: Stroke and its complications are major health problems in developing countries including Nigeria.Stroke, a reduction in blood flow to the brain, is caused by blockage in a cerebral artery by a clot or embolus (ischaemic stroke IS) or rupture of the blood vessel (hemorrhagic stroke).Ischemic stroke causes substantial disability, consumption of resources and death. Whenever there is an increased in free radical (reactive oxygen species ROS or reactive nitrogen species RNS) which occurs during ischemic stroke overwhelmed antioxidant leading to oxidative stress. Treatment using recombinant tissue plasminogen activator –rtPA is prohibitively expensive and not routinely stoke by pharmaceutical outlets but Aspirin, and antioxidant vitamins and minerals are relatively cheap and available.
Aim: This studyaimed to evaluate the effects of antioxidant vitamins and minerals supplementation on MDA, SOD, GPX, and CAT induced acute ischaemic stroke albino rats.

Methodology: The study evaluated the effects of antioxidant vitamins and minerals supplementation on SOD, GPX, and CAT induced acute ischaemic stroke albino rats. Forty albino rats of both sexes were randomly divided into eight groups of five rats each. First group non acute ischaemic stroke rats served as control, while the second group received conventional treatment (CT) of acute ischaemic stroke (AIS) of Aspirin 75mg/kg, the third received conventional treatment of Aspirin with recommended daily allowance (RDA) antioxidant minerals (Zn(15mg/kg), Cu(2mg/kg), and Se(60ug/kg), the fourth group received conventional treatment of Aspirin with recommended daily allowance of antioxidant vitamins (A(2500iu/kg), C(60mg/kg), E(12iu/kg) respectively, the fifth group received conventional treatment of Aspirin with 1.5 recommended daily allowance 22.5mg/kg, 4mg/kg, and 90ug/kg, of minerals Zn, Cu, and Se respectively, while group sixth received conventional treatment of Aspirin with 1.5 recommended daily allowance 3750iu, 90mg/kg, 18mg/kg of vitamins A, C, and E respectively, the seventh group received conventional treatment of Aspirin with both recommended daily allowance of antioxidant minerals and vitamins while eighth group received conventional treatment of Aspirin with both 1.5 minerals and 1.5 vitamins. The oral administration starts immediately when the rat recovered from anaesthesia of Middle cerebral Artery Occlusion (MCAO) within 4 hours and continues daily for 14 days. On the last day, the rats were anaesthetized, and blood sample was collected for biochemical analysis.
Results: The results showed a remarkable increased in MDA in all the groups when compared with the control group indicating the possibility an increased in the production of ROS and free radicals produced after induction of the ischemic stroke. This led to depletion of the enzymatic antioxidants such as SOD, CAT, and GPX. These changes were highest in conventional treatment group of oral administration of Aspirin only and there was significant ($p \leq 0.05$) decrease in MDA levels in group 3, 4, 5, 6, 7, and 8 where oral administration of Aspirin with antioxidant vitamins only, or Aspirin + antioxidant mineral only or Aspirin + combination of antioxidant vitamins and minerals .However, in group 2 the antioxidant enzymes SOD, CAT, and GPX were remarkably reduced below control group because Aspirin (Acetyl salicylic acid

ASA), a commonly used pharmaceutical agent, even at therapeutic doses couple with ischaemic stroke can induce oxidative stress, decreases the levels of SOD, GPX, and CAT and increases ROS, which occur together with mitochondrial dysfunction.
A significant ($p<0.05$) increase in the activities of antioxidant enzymes was observed in all treatment groups in treatment dose manner.
Conclusion: The study suggest that vitamins A, C, and E, in combination (as antioxidants vitamins), minerals Zn, Cu, and Se in combination (as antioxidants minerals) or combinations of vitamins and minerals ameliorate oxidative markers in a concentration dependent manner and confirmed the relevance of antioxidants in management of ischaemic stroke.

Key words: Acute, Ischaemic, Stroke, Antioxidant Vitamins and Minerals, Malondialdehyde (MDA), Superoxide dismutase(SOD), Catalase (CAT), and Glutathione Peroxidase(GPX) andalbinorats.

1.0 INTRODUCTION

"Stroke and its complications are major health problems in developing countries including Nigeria. It could be a major cause of death or disability especially when only clinical assessment is relied upon for diagnosis" (1).
"Stroke is defined as rapidly developing focal (or global) disturbance of cerebral function, including cerebral infarction, intra-cerebral haemorrhage, and subarachnoid haemorrhage" (2). "It usually occurs with one or more clinical signs, lasting for more than 24 hours or leading to death, with no apparent cause other than it being of vascular origin" (3). Stroke, a reduction in blood flow to the brain, is caused by blockage in a cerebral artery by a clot or embolus (ischaemic stroke IS) or rupture of the blood vessel (hemorrhagic stroke). Both forms of stroke result in damage and death of neurons in the affected brain region, leading to loss of brain function.
" Globally, 84.4% of all strokes are ischaemic and 15.6% are haemorrhagic" (4). "According to The Global Burden of Diseases, Injuries, and Risk Factors Study, there are 5.5 million stroke deaths annually around the world and it is the second leading cause of death globally" (5). "Stroke-related morbidity remains high. Most of these stroke deaths and disability are found in the developing countries. The deaths and disability in these countries account for as much as 87% of all the stroke deaths" (6, 7).
"Hospital based studies in Nigeria documented that stroke is the leading reason for neurological admissions, accounting for over 60% of presenting cases" (8, 9). Ogun *et al*.(10) in a 10year retrospective review, found that stroke accounted for 2.4% of all patients presenting at the emergency room (8, 10). In 2017, stroke was the ninth leading cause of death in Nigeria of all ages, rising from the 10th leading cause in the 2007 data (11, 12).
"Oxidative stress is the result of an imbalance between free radicals and antioxidants" (13). "Cells can be damaged by free radicals that are considered to play a main role in the aging process and diseases devineof defense against the detrimental effects of free radical damage, and it is essential to maintain optimal health via different mechanisms of action. Types of antioxidants range from those generated endogenously by the body cells, to exogenous agents such as dietary supplements. Antioxidant insufficiency can be developed as a result of decreased antioxidant intake, synthesis of endogenous enzymes, or increased antioxidant utilization. To

maintain optimal body function, antioxidant supplementation has become an increasingly popular practice through improving free radical protection" (14).

"During normal metabolic functions, highly reactive compounds called free radicals are generated in the body; however, they may also be introduced from the environment. These molecules are inherently unstable as they possess lone pair of electrons and hence become highly reactive. They react with cellular molecules such as proteins, lipids and carbohydrates, and denature them. As a result of this, vital cellular structures and functions are lost and ultimately resulting in various pathological conditions" (15, 16).

"Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. They act by reducing the energy of the free radicals or by giving up some of their electrons for their use, thereby causing them to become stable. In addition, they may also interrupt with the oxidizing chain reaction to minimize the damage caused by free radicals. For the past decade, countless studies have been devoted to the beneficial effects of antioxidant enzymes. It has been found that a substantial link exists between free radicals and more than sixty different health conditions, including the aging process, cancer, diabetes, Alzheimer's disease, strokes, heart attacks and atherosclerosis. By reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements, our body's potential to reduce the risk of free radical related health problems is made more palpable" (16, 17). "Antioxidant enzymes are, therefore, absolutely critical for maintaining optimal cellular and systemic health and wellbeing" (15). "The ability of the cell to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that can become part of potentially damaging molecules commonly called free radical or reactive oxygen species (ROS). About 5% or more of the inhaled O_2 is converted to ROS such as superoxide, hydrogen peroxide, and hydroxyl radicals by univalent reduction of O_2"(15). "Thus cells under aerobic condition are always threatened with the insult of ROS, which however are efficiently taken care of by the highly powerful antioxidant systems of the cell without any untoward effect. This antioxidant system includes, antioxidant enzymes (e.g., superoxide dismutase SOD, glutathione peroxidase GPx, catalase CAT, and reductase, etc.), nutrient-derived antioxidants (e.g., ascorbic acid, tocopherols and tocotrienols, carotenoids, glutathione and lipoic acid), metal binding proteins (e.g., ferritin, lactoferrin, albumin, and ceruloplasmin) and numerous other antioxidant phytonutrients present in a wide variety of plant foods. Whenever the balance between ROS production and antioxidant defense is lost, 'oxidative stress' results which through a series of events deregulates the cellular functions leading to various pathological conditions" (18, 19, 20).

"Enzymatic antioxidants consist of SOD, CAT, GPx and Trx system. Enzymatic antioxidants have more effective protective effects against active and massive oxidative attack due to the ability to decompose ROS (21, 22). Therefore, this set of antioxidants play important roles in disease conditions including acute hyperoxia injury, radiation injury, lung transplantation and inflammation" (22).

2.0 Materials and Methods

2.1 Chemicals and Reagents

All chemicals and reagents were of analytical grade and imported from USA: Electronic Science Kits, 15W. Commercial Ave.Addison, IL 60101-Call;+1(630)345-3450 through Centre for Advance Medical Research and Training (CAMRET) UDU, Sokoto for genes and other analysis. While Zinc supplement was purchased from Alkun Pharmacy and Store, Opposite FMC

Main Gate Jalingo, Taraba State, Copper and Selenium supplements were purchased from HI-MEDIX Pharmacy and Stores, Plot 492, Cadastral Zone, New Aminu Kano by FERMA, Opposite Wakissa, Abuja, and Vitamins A, C, and E were purchased at Zumunci Pharmacy 11 LTD, El-Sudais Road, Opposite Sultan Maccido Institute Main Gate, Sokoto State.

2.2Animals and treatment

Forty (40) apparently healthy albino rats, weighing between 160 - 180g were obtained from the Animal Care Center, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The animals were housed in rat cages, bedded with sawdust and fed with standard pelletized growers feed (Vital feed, Jos, Nigeria). All rats were provided free access to water *adlibitum* and fed for two weeks for acclimatization.

The rats were randomly grouped into 8 of 5 rats per group (i.e 3 male + 2 female or vice visa) as follows:

Group 1: Non acute ischaemic stroke as control

Group 2: Conventional AIS treatment of Aspirin 75mg/kg only

Group 3: Conventional AIS treatment of Aspirin with supplementation of recommended daily allowance (RDA) of the antioxidant minerals mixture: Zn(15mg/kg), Cu(2mg/kg), and Se(60ug/kg).

Group 4: Conventional AIS treatment of Aspirinwith supplementation of recommended daily allowance of the antioxidant vitamins mixture: .A (2500iu/kg), C (60mg/kg), and E (12iu/kg).

Group 5: Conventional AIS treatment of Aspirinwith supplementation with a mixture of 1.5 folds RDA of antioxidant minerals. Zn(22.5mg/kg), Cu(4mg/kg), and Se(90ug/kg).

Group 6: Conventional AIS treatment of Aspirin with supplementation mixture of 1.5 folds antioxidant vitamins. A(3750iu/kg), C(90mg/kg), and E(18iu/kg) respectively.

Group 7: Conventional AIS treatmentof Aspirin with supplementationmixtures of recommended daily allowance (RDA) of the antioxidant minerals and vitamins

Group 8: Conventional AIS treatment of Aspirin supplementation with 1.5 folds mixtures ofantioxidant vitamins and minerals.

2.3Ischaemic Stroke induction

Middle Cerebral Artery Occlusion (MCAO) method of Spratt *et al.* (23) was used with some modification. Ischaemic stroke (IS) was induced by occluding the MCA in albino rats. Ketamine and Xylazine at the doses of 80mg/kg and 5mg/kg body weight respectively were used to anaesthesized the rats. The condition was maintained until the end of the occluding period. The neck region of the rats was shaved and scrubbed with savlon, incision was made under sterile condition to gain access to the common carotid artery (CCA). The artery was ligated proximally, a nitch incision was created on the internal carotid artery distally using 25G needle, and an absorbable suture material was inserted through the hole into the artery until resistance was felt. A silicon coated suture material (coating diameter and length 0.35 and 5mm, respectively) was maneuvered through the external and internal carotid arteries to block the MCA. All the incisions made were closed using a non absorbable suture material; nylon. The rats were allowed to recover from anesthesia in the cages. During the surgery, the heart rate was monitored, and rectal temperature was regulated or maintained at normal rate of 330-480 beats per minute and 35.9-37.5ºC respectively (4).

2.4Blood sample collection and processing

After two weeks daily oral administration of the antioxidant vitamins and minerals supplementation to the AIS rats, blood samples were collected after an overnight fast through cardiac puncture on day 14^{th} supplementation and analyzed for MDA, SOD, GPX, and CAT . The rats were anaesthetized using gas anesthetic agent Isoflurane drop in jar/nose cone. After proper anesthesia, the rats were laid on the right lateral recumbency and the needle was inserted between the intercostal muscles to gain access to the heart. Blood was then collected and poured into plain tubes, separated by centrifuging and stored.

2.5Biochemical analysis

Glutathione peroxidase(GPx) activity was measured using enzymatic method described by Yang *et al.* (24). Catalase(CAT) activity was measured using enzymatic method of Sepasi and Moosavimovahedi, (25).Superoxide dismutase(SOD) activity was measured using enzymatic method of Cristiana *et al.* (26).**Malondialdehyde(MDA)** Spectrophotometric measurement of thiobarbituric acid -reactive substances (TBARS) in the TCA-supernatant of sample developed by Qilong et al. (27).

2.6Data Analysis: The experimental data were statistically analyzed using the statistical Package for Social Sciences (SPSS) version 25.0. The data were expressed as mean ± SEM. Multiple comparison was carried out using one-way analysis of variance (ANOVA) method, followed by post hoc Tukey's test. Differences were considered statistically significant at p value less than or equal to 0.05 ($p \leq 0.05$).

3.0 Results

The overall biochemical analysis of MDA, SOD, CAT and GPX is shown on Table 5.

3.1 Effect of Antioxidant Vitamins and Minerals on MDA of Induced Acute Ischaemic Stroke

The results of the effect antioxidant vitamins and minerals on Malondialdehyde (MDA) of induced acute ischaemic stroke in albino Rats was determined and presented in Table 1. The effect of antioxidant vitamins and minerals on MDA concentration was estimated as indices for oxidative stress status of the rats or MDA is an end product formed during degradation of cellular membrane phospholipids due to lipid peroxidation, after is released into extracellular space and the blood (Lorente et al., 2016).

There was significant increased (p0.≤05) of MDA in all the groups when compared with control(0.154± 0.014) and the MDA (nmol/l) was highest in group 2 which received oral administration of Aspirin only with average mean serum of 0.883± 0.016 but other groups which received oral administration of Aspirin + normal dose of antioxidant minerals such as group 3(0.833± 0.001), Aspirin + normal dose of antioxidant vitamins such group 4 (0.841± 0.009), Aspirin + 1.5 antioxidant normal dose of minerals such as group 5 (0.824± 0.001), Aspirin + 1.5 antioxidant vitamin normal dose such as group 6 (0.822± 0.003), Aspirin + normal dose of antioxidant vitamins and minerals such as group 7 (0.815 0.001), Aspirin + 1.5 normal of

antioxidant vitamins and minerals such as group 8 (0.818 0.005). These were significantly reduced ($p \leq 0.05$) when compared with Aspirin only (group 2).

Table 1.Effects of antioxidant vitamins and minerals supplementation on MDA induced acute ischaemic stroke

Group (n = 5)	MDA (nmol/l)
Group 1	0.154 ± 0.014^{a}
Group 2	0.883 ± 0.016^{b}
Group 3	0.833 ± 0.001^{ab}
Group 4	0.841 ± 0.009^{ab}
Group 5	0.824 ± 0.001^{ab}
Group 6	0.822 ± 0.003^{ab}
Group 7	0.815 ± 0.001^{ab}
Group 8	0.818 ± 0.005^{ab}

Data were presented as Mean ±Standard Error of Mean. Mean values with different superscripts on the row differs significantly. **Group1**: Non acute ischaemic stroke as control (C), **Group2**: Conventional treatment(CT) of AISof oral administration of Aspirin only, **Group3**: Conventional treatment of AIS Aspirin + RDA of antioxidant minerals supplementation,**Group4**: Conventional treatment of Aspirin + RDA of antioxidant vitamins supplementation, **Group5**: Conventional treatment of Aspirin + 1.5 antioxidant minerals supplementation, **Group6**: Conventional treatment of AIS Aspirin + 1.5 antioxidantvitamins supplementation, **Group7**: Conventional treatment of AIS Aspirin +combinations of normal dose antioxidant minerals and vitamins, and **Group8**: Conventional treatment of AIS Aspirin + 1.5 antioxidant minerals + 1.5 vitamins. The result was statistically at $p \leq 0.05$.

3.2 Effects of Antioxidant vitamins and minerals on SOD Induced Acute Ischaemic Stroke

The results of the effect of antioxidant vitamins and minerals supplementation on superoxide dismutase (SOD)induced acute ischaemic albino rats serum was determined and presented in Table 2. The effect of antioxidant vitamins and minerals supplementation on SOD(u/ml) concentration was estimated as indices for the oxidative stress status of the rats. Oral administration of Aspirin significantly decreased SOD in group 2 lower than control group1.The mean serum level of SOD (u/ml) in normal control group was(0.319 ± 0.002) and was higher than group 2 (0.062 ± 0.001) which received Aspirin alone while there was significant increased ($p \leq 0.05$) in activity of SOD in all the remaining groups which received oral administration of Aspirin with minerals alone as in group 3, Aspirin with vitamins alone as in group 4, Aspirin with 1.5 dose of minerals as in group 5 , Aspirin with 1.5 dose of vitamins as in group 6, ,Aspirin with vitamins and minerals as in group 7, Aspirin with 1.5 dose of vitamins and minerals as in group 8.

Table 2.Effects of antioxidant vitamins and minerals supplementation on SOD induced acute ischaemic stroke

Group (n = 5)	SOD(u/ml)
Group 1	0.319 ± 0.002^{ac}

Group 2	0.062 ± 0.001^{bc}
Group 3	0.161 ± 0.004^{abc}
Group 4	0.454 ± 0.002^{abc}
Group 5	0.205 ± 0.002^{abc}
Group 6	0.410 ± 0.007^{abc}
Group 7	0.397 ± 0.002^{abc}
Group 8	0.158 ± 0.004^{ac}

Data were presented as Mean ± Standard Error of Mean. Mean values with different superscripts on the row differs significantly. **Group1**: Non acute ischaemic stroke as control (C), **Group2**: Conventional treatment(CT) of AISof oral administration of Aspirin only, **Group3**: Conventional treatment of AIS Aspirin + RDA of antioxidant minerals supplementation,**Group4**: Conventional treatment of Aspirin + RDA of antioxidant vitamins supplementation, **Group5**: Conventional treatment of Aspirin + 1.5 antioxidant minerals supplementation, **Group6**: Conventional treatment of AIS Aspirin + 1.5 antioxidantvitamins supplementation, **Group7**: Conventional treatment of AIS Aspirin +combinations of normal dose antioxidant minerals and vitamins, and **Group8**: Conventional treatment of AIS Aspirin + 1.5 antioxidant minerals + 1.5 vitamins. The result was statistically at $p \leq 0.05$.

3.3 Effects of Antioxidant vitamins and minerals on CAT Induced Acute Ischaemic Stroke

The results of the effect of antioxidant vitamins and minerals supplementation on CATinduced acute ischaemic albino rats serum was determined and presented in Table 3. The effect of antioxidant vitamins and minerals supplementation on CAT concentration was estimated as indices for the oxidative stress status of the rats. Oral administration of Aspirin significantly decreased SOD in group 2 lower than control group1.The mean serum level of CAT (u/ml) in normal control group was (19.522 ± 0.152) and was higher than group 2 (13.016 ± 0.242) which received Aspirin alone while there was significant increased ($p \leq 0.05$) in activity of CAT in all the remaining groups which received oral administration of Aspirin with minerals alone as in group 3, Aspirin with vitamins alone as in group 4, Aspirin with 1.5 dose of minerals as in group 5 , Aspirin with 1.5 dose of vitamins as in group 6, ,Aspirin with vitamins and minerals as in group 7, Aspirin with 1.5 dose of vitamins and minerals as in group 8.

Table 3 Effects of antioxidant vitamins and minerals on CAT induced acute ischaemic stroke

Group (n = 5)	CAT(u/ml)
Group 1	19.522 ± 0.152^{ac}
Group 2	13.016 ± 0.242^{bc}
Group 3	30.100 ± 0.321^{abc}
Group 4	45.994 ± 0.330^{abc}
Group 5	15.536 ± 0.182^{abc}
Group 6	32.938 ± 0.257^{abc}
Group 7	25.210 ± 0.099^{abc}

Group 8	17.432 ± 0.119^{abc}

Data were presented as Mean ± Standard Error of Mean. Mean values with different superscripts on the row differs significantly. **Group1**: Non acute ischaemic stroke as control (C), **Group2**: Conventional treatment(CT) of AISof oral administration of Aspirin only, **Group3**: Conventional treatment of AIS Aspirin + RDA of antioxidant minerals supplementation,**Group4**: Conventional treatment of Aspirin + RDA of antioxidant vitamins supplementation, **Group5**: Conventional treatment of Aspirin + 1.5 antioxidant minerals supplementation, **Group6**: Conventional treatment of AIS Aspirin + 1.5 antioxidantvitamins supplementation, **Group7**: Conventional treatment of AIS Aspirin +combinations of normal dose antioxidant minerals and vitamins, and **Group8**: Conventional treatment of AIS Aspirin + 1.5 antioxidant minerals + 1.5 vitamins. The result was statistically at $p \leq 0.05$.

3.4 Effects of Antioxidant vitamins and minerals on GPX Induced Acute Ischaemic Stroke

The results of the effect of antioxidant vitamins and minerals supplementation on GPXinduced acute ischaemic albino rats serum was determined and presented in Table 4. The effects of antioxidant vitamins and minerals supplementation on Glutathione peroxidase (GPX) concentration was estimated as indices for the oxidative stress status of the rats. Oral administration of Aspirin significantly decreased (u/ml) in group 2 lower than control group1.The mean serum level of GPX (u/ml) in normal control group was (16.356 ± 0.148) and was higher than group 2 (13.360 ± 0.280) which received Aspirin alone while there was significant increased ($p \leq 0.05$) in activity of GPX in all the remaining groups which received oral administration of Aspirin with minerals alone as in group 3, Aspirin with vitamins alone as in group 4, Aspirin with 1.5 dose of minerals as in group 5 , Aspirin with 1.5 dose of vitamins as in group 6, ,Aspirin with vitamins and minerals as in group 7, Aspirin with 1.5 dose of vitamins and minerals as in group 8.

Table 4 Effects of antioxidant vitamins and minerals on GPPX induced acute ischaemic stroke

Group (n = 5)	GPX(u/ml)
Group 1	16.356 ± 0.148^{ac}
Group 2	13.360 ± 0.280^{bc}
Group 3	20.512 ± 0.051^{abc}
Group 4	28.358 ± 0.135^{abc}
Group 5	14.550 ± 0.083^{abc}
Group 6	19.202 ± 0.095^{abc}
Group 7	18.812 ± 0.061^{abc}
Group 8	14.758 ± 0.038^{abc}

Data were presented as Mean ± Standard Error of Mean. Mean values with different superscripts on the row differs significantly. **Group1**: Non acute ischaemic stroke as control (C), **Group2**: Conventional treatment(CT) of AISof oral administration of Aspirin only, **Group3**: Conventional treatment of AIS Aspirin + RDA of antioxidant minerals supplementation,**Group4**: Conventional treatment of Aspirin + RDA of antioxidant vitamins supplementation, **Group5**: Conventional treatment of Aspirin + 1.5 antioxidant minerals supplementation, **Group6**: Conventional treatment of AIS Aspirin + 1.5 antioxidantvitamins supplementation, **Group7**: Conventional treatment of AIS Aspirin +combinations of normal dose antioxidant minerals and vitamins, and result was statistically at $p \leq 0.05$.

Table 5Malondialdehyde (MDA) Concentration and Antioxidant Enzymes Activities of Acute Ischaemic Stroke Induced rats Supplemented with Vitamins and Minerals.

Parameter Group(n=5)	MDA(nmol/ml)	SOD(u/ml)	CAT(u/ml)	GPX(u/ml)
Group 1	0.154 ± 0.014^{a}	0.319 ± 0.002^{ac}	19.522 ± 0.152^{ac}	16.356 ± 0.148^{ac}
Group 2	0.883 ± 0.016^{b}	0.062 ± 0.001^{bc}	13.016 ± 0.242^{bc}	13.360 ± 0.280^{bc}
Group 3	0.833 ± 0.001^{ab}	0.161 ± 0.004^{abc}	30.100 ± 0.321^{abc}	20.512 ± 0.051^{abc}
Group 4	0.841 ± 0.009^{ab}	0.454 ± 0.002^{abc}	45.994 ± 0.330^{abc}	28.358 ± 0.135^{abc}
Group 5	0.824 ± 0.001^{ab}	0.205 ± 0.002^{abc}	15.536 ± 0.182^{abc}	14.550 ± 0.083^{abc}
Group 6	0.822 ± 0.003^{ab}	0.410 ± 0.007^{abc}	32.938 ± 0.257^{abc}	19.202 ± 0.095^{abc}
Group 7	0.815 ± 0.001^{ab}	0.397 ± 0.002^{abc}	25.210 ± 0.099^{abc}	18.812 ± 0.061^{abc}
Group 8	0.818 ± 0.005^{ab}	0.158 ± 0.004^{ac}	17.432 ± 0.119^{abc}	14.758 ± 0.038^{abc}

Values are presented as mean and standard error of mean (SEM), n = 5, MDA: Malondialdehyde, SOD: superoxide dismutase, CAT: catalase, GPX: glutathione peroxidase, **Group1**: Non acute ischaemic stroke as control (C), **Group2**: Conventional treatment(CT) only, **Group3**: Conventional treatment of acute ischaemic stroke (CT) + RDA of antioxidant minerals supplementation (M), **Group4**: CT + RDA of antioxidant vitamins supplementation (V), **Group5**: CT + 1.5 M, **Group6**: CT + 1.5 V, **Group7**: CT + M + V, and **Group8**: CT + 1.5 M + 1.5 V, $^{a}p \leq 0.05$ versus group 2, $^{b}p \leq 0.05$ versus group 1, $^{c}p \leq 0.05$ between and within groups combined.

4.0 DISCUSION

"Transient or permanent interruption of cerebral blood flow by occlusion of a cerebral artery gives rise to an ischaemic stroke leading to irreversible damage or dysfunction to the cells within the affected tissue along with permanent or reversible neurological deficit. Extensive research has identified excitotoxicity, oxidative stress, inflammation and cell death as key contributory pathways underlying lesion progression" (28). "ROS act directly on lipids to ultimately produce aldehydes, dienals or alkanes, such as malondialdehyde (MDA) and 4-hydroxynonenal (4S-HNE). The latter, 4-HNE induces apoptosis following cerebral ischaemia in neurons" (29).

“ROS generation following cerebral ischaemia is the mitochondria, where they exert their most detrimental role in initiation of cell death via cytochrome C (CytC) release” (30, 31).
“The brain is vulnerable to excessive oxidative insults because of its abundant lipid content, high energy requirements, and weak antioxidant capacity. ROS increase susceptibility to neuronal damage and functional deficits, via oxidative changes in the brain in neurodegenerative diseases” (32).
“Patient with AIS are treated using conventional prescribed drug aspirin (ASA) upon admission to the hospital mainly with the purpose of adjusting their blood coagulability and prevent further recurrent ischaemic event” (33). “Clinical trials have shown the benefits early ASA use in a wide range of patients with AIS, especially to prevent recurrent stroke” (34).

From our results, we observed a remarkable increased in MDA in all the groups when compared with control group which agreed with Dawud *etal*(35) that MDA is an index of oxidative stress, its accumulation indicates the presence of excessive free radicals that cause oxidative stress, resulting in cell damage and MDAis an end product formed during degradation of cellular membrane phospholipids due to lipid peroxidation, after is released into extracellular space and the blood (36). Nasiru*etal*. (37) stated that ‘the increased in MDA level could be as a result of an increased in the production of ROS and free radicals produced after induction of the ischemic stroke, leading to the depletion of the enzymatic antioxidants such as SOD, CAT, GPX, thus leading to OS which is a major player in the pathophysiology of neurodegenerative diseases such as ischemic stroke, traumatic brain injury etc. This is in line with the report of Gilgun-Sherki*et al.* (38). “It was highest in conventional treatment group of Aspirin alone but when compared with groups 3, 4, 5, 6, 7, and 8 there was significant decrease in MDA level. The administration of different antioxidant agents (vitamins: E, A, and C, Minerals:Se, Zn, and Cu) has reduced circulating MDA levels in ischaemic stroke. This agreed with the previous work”(39, 40,36).However, in group 2 the antioxidant enzymes SOD, CAT , and GPX were remarkably reduced below control group which was in agreement with Altintas *etal*.(41) ‘that Aspirin (Acetyl salicylic acid ASA), a commonly used pharmaceutical agent, even at therapeutic doses can induce oxidative stress, decreases the levels of SOD, GPX, and CAT’, and increases ROS, which occur together with mitochondrial dysfunction (42, 43).The decreased level of CAT, GPX and SOD activity observed with aspirin exposure in the present study might be attributed to O^{2-} generating ability of aspirin (44) and stroke (45)or it might be due to the overwhelming effect of their activities to mitigate oxidative stress associated with ROS generated from ischaemic stroke (46).
“Also ASA and its metabolite Salicylic Acid (SA) have been reported to have the ability to undergo hydroxylation, generating H_2O_2” (47). It is thus likely that repeated administration of aspirin caused excessive generation of O^{2-} and H_2O_2, resulting in decreased levels of CAT, GPX, and SOD activities. This was in agreement with Shi *etal*. (48) reported in their studies that ASA has much more effective antioxidant on (O^{-2}) radicals than the (OH^-) and H_2O_2 radicals. A significant increased in the activities of antioxidant enzymes was observed following treatment with conventional treatment of Aspirin + minerals supplement as in group 3, conventional treatment of Aspirin + vitamins supplement as in group 4, conventional treatment of Aspirin + 1.5 of minerals supplement as in group 5, conventional treatment of Aspirin +1.5 of vitamins supplement as in group 6, conventional treatment of Aspirin + combination of RDA of minerals and vitamins as in group 7, and conventional treatment of Aspirin + combination of 1.5 of both minerals and vitamins as in group 8for all the groups supplemented. Antioxidant enzyme activities increased significantly across the groups and OS biomarkers MDA concentration

significantly decreased when compared with the antioxidants enzyme activities and MDA concentration of rats in conventional treatment of Aspirin alone(group 2) in a concentration dependent manner. This was in agreement with Nasiru *etal*. (37).

"The positive outcome of the trace element (TE) Zn in group 3, 5, 7, and 8 is that Zn, a redox inactive metal, does not directly interact with ROS but has a crucial role in maintaining redox balance for the antioxidant defense system in various ways in the cell. It increases the activation of antioxidant enzymes SOD, GPx, and CAT,it also acts as a direct cofactor of SOD-1 and SOD-3 and as an indirect cofactor for GPx" (49). "It inhibits important pro-oxidant enzymes such as NADPH oxidase, inducible nitric oxide synthetase (iNOS), and the reduced form of nicotinamide adenine dinucleotide (NMDA) and regulates oxidant production and metal-induced oxidative damage. It is dynamically associated with sulfur in protein cysteine clusters. It mediates the induction of the zinc-binding protein metallothionein which releases the metal under oxidative conditions and acts as Se scavenging oxidant. It is involved in the regulation of glutathione metabolism and the overall protein thiol redox status" (50). "It competes with redox-active transition metals, iron and copper, for certain binding sites. When zinc binds to these sites, copper and iron are forced to undergo hydrolytic polymerization into unreactive structures, thereby prohibiting the catalysis of free radical formation and the initiation of lipid peroxidation" (49, 51).Zn is mainly expressed in the hippocampus, amygdala, cerebral cortex, thalamus, and olfactory cortex in the brain (52) and is stored as free zinc ions (Zn^{2+}) in the presynaptic glutamatergic neurons. "Zn in synaptic vesicles is released with glutamate and acts as a potent extracellular modulator by interacting with many synaptic receptors during synaptic activity"(52).

"Co-treatment with Zn and Se significantly decreased mitochondrial dysfunction, ROS levels, and lipid peroxidation levels, while significantly increasing cognitive performance, SOD, glutathione peroxidase, and catalase activity in the mitochondria of the brain in an Alzheimer's Disease (AD) rat model" (53). "In a double-blind, placebo-controlled trial of zinc supplementation for premenstrual syndrome, sixty women (18–30 years) were randomly assigned to receive either 30mg of zinc gluconate and/or placebo for 12weeks.The zinc-administered group showed beneficial effects on physical and psychological symptoms of premenstrual syndromes, total antioxidant capacity, and brain-derived neurotrophic factor" (54).

"Further, Cu co-administration in this study, increases the activation of antioxidant enzymes which play an important role in the maintenance of cell homeostasis and preservation of life. It display important structural, regulatory, and catalytic functions in different types of proteins, such as enzymes, receptors, and transporters. Cu+ and magnesium are the cofactors for enzymes such as COX and/or supper zinc, SOD, and neuronal Cu enrichment predispose to Cu^{2+}-catalyzed Fenton chemistry and H_2O_2-assisted protein oxidation" (32).

"Finally, one area of increasing interest is the study of the ability of essential trace mineral to modulate the effects of environmental toxicants. In that respect, several studies have shown that selenium (Se) was of fundamental importance to human health because it is important in many biochemicals and physiological processes" (55, 56). "As a constituent of selenoenzyme-GSH-Px, Se plays an antioxidant role, it protects cells against damages by free radicals and permits regeneration of a membrane lipid molecule through reacylation" (55). "It plays an important role in antioxidant defense systems as well as protects the structure and function of proteins, DNA and chromosomes against oxidation injury" (57, 58).

“These TE (Zn, Cu, and Se) increases the activation of antioxidant enzymes (SOD, GPX, and CAT) and also acts as a direct or indirect cofactor of SOD, CAT and GPX exhibit its functionality on group 3, 5 and 8 by increasing the activity of antioxidants enzymes significantly thereby reducing oxidative marker MDA significantly when compared with conventional treatment of Aspirin alone (group 2). Our results revealed decreased in activity of antioxidant enzymes were lower than control group 1 significantly but were mitigated by co-administration of antioxidant minerals Zn, Cu, and Se in groups 3, 5, and 8 significantly when compared with group 2 respectively. This is in line with” (59, 36).
“Taking into consideration the functionality of each vitamin A, C and E, Vitamins A, C, and E posse’s antioxidant properties” (60). So in group 4, 6, 7 and 8 we can suggest that vitamin C contributed in the activities of antioxidants enzyme ascribed to the report by some researchers (61), where they reported that vitamin C is an important antioxidant that has ability to limit oxidative lipid damage in biological systems. It is also found to be a potent water affinity scavenger in biological fluids and tissues (61). Vitamin C is a strong reducing agent and by donating electron(s), thus it neutralizes ROS directly, which always causes oxidative stress by stealing electrons. It was also reported by (61) that, AA is a low molecular weight antioxidant that scavenges the ROS through electron transfer rapidly and prevents lipid peroxidation as reported by Flora and Tandon (61).
“Ascorbate (ascorbic acid, AA), a ubiquitous water-soluble antioxidant and a cofactor for severalenzymes, can inhibit the generation of ROS, directly scavenge ROS/RNS, and repair other oxidized scavengers” (62). “ROS generation is limited by AA through the inhibition of NOX and nNOS. It also helps in the regeneration of α-tocopherol from α-tocopheroxyl radical and repair of glutathione” (32)
“The oxidizing and free radical scavenging activity of AA inside the cell is not limited to the aqueous phase, but also includes protection of membranes and other hydrophobic compartments through interaction with vitamin E” (63). “AA inhibits the oxidative stress triggered by various neurotoxins and protects against ethanol-induced apoptotic neurodegeneration in prenatal rat hippocampal neurons” (64). “OS in stroke, hypoxia, ischaemia, and seizure activity leads to massive glutamate release and subsequent excitotoxicity, a result of over-activation of glutamate receptors” (65). Therefore, AA can protect against glutamate-induced excitotoxicity and neurodegeneration.

Furthermore, it has been demonstrated by several studies that vitamin C forms the first line of antioxidant defense and effectively protects the lipid plasma and lipoproteins against detectable per-oxidative damage under many different types of oxidizing conditions (66). Our result is in agreement with Huang *et al.*(67) a research work that discovered that dehydroascorbic acid; a blood-brain barrier–transportable form of vitamin C, caused dose-dependent increase in post-reperfusion cerebral blood flow, with reductions in the infarct volume, neurological deficit, and mortality. Also, in the studies by (68, 69) it was found that an increased in antioxidant vitamin C intake resulted in a decreased risk of stroke. This is in line with Nasiru *etal*. (32) and Suleiman *etal* (45).

The possible contribution of vitamin E in co-administration of antioxidant vitamins treated group 4, 6, 7 and 8 of this study compared to conventional treatment could be connected to the mode of action of vitamin E, Vitamin E is a major group of lipid-soluble antioxidants called tocopherols and tocotrienols, of which the most biologically active isoform is $\propto$-tocopherol (70, 71). It is a major chain-breaking antioxidant and exists in a low molar ratio compared to unsaturated

phospholipids. The most important function of vitamin E is its antioxidant activity, which protects the integrity of cellular membranes from polyunsaturated fatty acid generated oxygen free radicals and to act as a direct scavenger of superoxide and hydroxyl radicals (72).The antioxidant ability of vitamin E is continuously restored via vitamin E recycling by other antioxidants such as vitamin C, ubiquinols, and thiols (32).

Vitamin E, similar to other radical scavengers/trappers, influences the flux of lipid hydroperoxide (LOOH), which is derived from both spontaneous and enzymatic formation of lipid peroxyl radicals (LOO^{-}) on the cellular membrane (72).The effects of vitamin E on peroxidation activity appear to involve both the radical scavenging mechanism such as the H atom donor activity and a physical interaction with the polyunsaturated lipid substrate. This work agreed with Nasiru *etal*. (32)

Finally, the positive outcome of vitamin A in co-administration in treated group 4, 6, 7 and 8 compared with conventional treatment of Aspirin alone could be Vitamin A Carotenoids, can function directly as antioxidants by quenching ROS through energy transfer (73) . Carotenoids act through several pathways and interact with free radicals in the plasma, mitochondria, and nuclear membranes of cells via electron transfer, hydrogen abstraction, and physical quenching (74). Carotenoids indirectly react with cell signaling cascades, including the nuclear factor elytroid 2 (NF-E2)-related factor 2 (Nrf2), NF-_B, or mitogen-activated protein kinase (MAPK) (75, 76).So vitamins A, C, and E mixtured act as an antioxidant (77). Epidemiological evidence suggests that vitamins A, C, and E are potent antioxidants and may play a protective role in the development of chronic diseases including cardiovascular diseases, diabetes, cancers, stroke, and inflammatory diseases (59) andthe administration of different antioxidant agents (vitamins: E, A, and C, Minerals: Se, Zn, and Cu) has reduced circulating MDA levels and increased in activity SOD, GPX, and CAT in ischaemic stroke (40, 36).

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The co-administration of TE (Zn, Cu, and Se) minerals supplement, vitamins (A, C, E), supplement and co-administration of both minerals and vitamins supplement in addition to conventional treatment of stroke reduced oxidative stress and its biomarkers in induced ischaemic stroke in rats, confirmed the relevance of antioxidants in the treatment of IS.

5.2 RECOMMENDATIONS

1. We suggest that, the effective and efficient functioning of antioxidative enzymes as well as RDA of both antioxidants mineral and vitamins should be co-administer in addition to conventional management of using ASA alone unless otherwise the use of either antioxidant minerals alone or vitamins alone to ameliorate oxidative stress in IS before any alternatives.
2. We suggest further study be carryout on physiological and structural changes that occur in AIS correspond with biochemical improvement.
3. We suggest that, the group with the highest effect (RDA of vitamins+minerals)be subjected to six(6) weeks to a certain the molecular bases of our intervention.

5.3 RESEARCH CONTRIBUTIONS TO KNOWLEDGE

This research study was able to established that Effect of Antioxidant Vitamins and Minerals Supplementation on SOD, GPX, and CAT Induced Acute Ischaemic Stroke Albino Rats has contribute to existing knowledge that:

1. Our findings confirm that medical treatment substantially reduces the risk of early recurrent stroke after transient ischaemic attack (TIA) and minor stroke and identify aspirin (ASA) with co-administration of antioxidants minerals and vitamins as the key intervention. The considerable early benefit from aspirin with co-administration of antioxidants minerals and vitamins warrants public education about self-administration after possible TIA or IS.
2. Our findings discovered that co-administration ASA with RDA antioxidant vitamins and minerals were superior to over-dosage, also with antioxidant vitamins only, minerals only respectively.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)
Author(s) hereby declarethat NO generative.AIl technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

The Ethics Committee of UsmanuDanfodiyo University, Sokoto approved the animal experiment with ethical clearance UDUSOK HREC REGISTRATION NUMBER NHREC/UDU-HREC/25/06/2023 – PGM15

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