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VITAMINS C AND E EFFECTS ON ANTIOXIDANT ENZYMES AND ELECTROLYTES STATUS OF RATS INDUCED WITH ISCHEMIC STROKE

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ABSTRACT

Rapid increase in the production of reactive oxygen species (ROS) immediately after ischemic stroke quickly overwhelm the antioxidant defenses of the body, thereby causing neuronal dysfunction, tissue damage and cell death. This research reports the effects of Vitamins C and E on the antioxidant enzymes and electrolytes status of Wistar rats induced with ischemic stroke (IS). Twenty apparently healthy rats were divided into four groups of five rats each. IS was induced using middle cerebral artery occlusion (MCAO). Vitamins C and Vitamin E at 45mg/kg body weight were orally administered to the rats for two weeks, antioxidant enzymes (catalase (CAT) superoxide dismutase (SOD) and glutathione peroxide (GPX) activities and oxidative stress biomarkers (thiobarbituric acid reactive species (TBARS) concentration, were assessed. Blood glucose and serum electrolyte status were also assessed. IS caused significantly (p<0.05) decrease in the activities of SOD, CAT and GPx and significant increase (P<0.05) in the concentration of TBARS. IS also caused significant increase (P<0.05) in electrolytes and glucose level in the IS rats. Treatment with vitamins C and E resulted in the significant increase (P<0.05) of the activities of CAT, SOD and GPX. Also, there was significant (p<0.05) decrease in the concentration of TBARS. There was significant (P<0.05) decrease in the electrolytes and glucose level of the IS rats following the treatments. Supplementation with Vitamins C and E reduced oxidative stress and its biomarkers in induced ischemic stroke in rats and may be a good therapeutic strategies for the management of IS.

KEY WORDS: Ischemic stroke; Oxidative stress; Antioxidant enzymes; Electrolytes; Vitamins C and E.

INTRODUCTION

Stroke, a reduction in blood flow to the brain, is caused by blockage in a cerebral artery by a clot or embolus (ischemic stroke) or rupture of the blood vessel (hemorrhagic stroke). Both forms of stroke result in damage or death of neurons in the affected brain region, leading to loss of brain function. Estimates of worldwide stroke prevalence (number of individuals with the disease) range from 1.7 to 20 per 1000 individuals (Feigin et al., 2009). Thus, stroke accounts for 9.6% of all deaths in the world (Rosamond et al., 2007), making it the fourth leading cause of death internationally, and accounted for the 5, 8 and 17 percent's leading cause of medical death in University College Ibadan, Lagos University Teaching Hospital and Ogun State University Teaching Hospital, Nigeria respectively (Ojo and Onyegiri, 2017). The pathophysiology of stroke involves

numerous processes that includes: energy failure, excitotoxicity, oxidative stress, disruption of the bloodbrain barrier (BBB), inflammation, necrosis and/or apoptosis (Yiwang et al., 2013). Disorders of serum electrolytes i.e. sodium and potassium are the commonest electrolyte abnormalities found in stroke patients (Kusuda et al., 1989). Electrolyte disturbances such as hyponatraemia, hypernatraemia resulting, increase in brain natriuretic peptide and atrial natriuretic peptide are common in acute phase of stroke (Meenakshi et al., 2017). Serum calcium plays an important role in the pathogenesis of ischemic cell damage. Intracellular accumulation of calcium can leads to neuronal cell damage by triggering cycle of cytotoxic events and apoptotic cell death. Calcium influx into the cell via NMDA receptors leads to delayed cell death and excitotoxicity associated with ischemia (Simond et al.,

2007 and Macdonald et al., 2006) Magnesium deficiency is associated with vasoconstriction and vascular endothelial cell injury. Several studies have demonstrated that hyperglycemia on admission is a common characteristic in acute stroke, involving more than 50% of patients and which affects all stroke subtypes (Scott et al., 1999). High glucose levels on admission have been related to poor outcome independent of age, stroke severity, or stroke subtype (Weir et al., 1997). Glycemia is a continuous physiological parameter that can increase in stressful situations, thereby exerting a deleterious effect on stroke outcome due to sustained increased levels within the first hours from stroke onset (Baird et al., 2003). The objective of the present study was to evaluate the effects of Vitamins C and E on the antioxidant enzymes and electrolytes status of rats induced with ischemic stroke (IS).

MATERIALS AND METHOD MATERIALS

Experimental animal

Twenty apparently healthy rats of Wistar strain weighing between 180-200g was obtained from the Animal house

of the Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria. The Ethics Committee of the Department of Veterinary Physiology and Biochemistry approved the animal experiment with ethical clearance number VPB/EC/17/16. The rats were allowed to acclimatize to the research laboratory condition and were randomly divided into four groups of five rats each. The experimental rats were subjected to a 12hours light/12hours dark schedule. The rats will be fed with grower's mash of vital feed, and were allowed complete access to water *ad-libitum*.

METHOD

Experimental design

The experimental rats were divided into four (4) groups of 5 rats each, group 1 and 2 were induced with ischemic stroke and treated with vitamin C and vitamin E respectively. Group 3 rats were induced with ischemic stroke and were not treated, while group 4 rats were left without stroke induction and without treatment as indicated in the table below.

Group	Treatment
Ι	Stroke induced and treated with vitamin C
II	Stroke induced and treated with vitamin E
III	Stroke induced and not treated
IV	Non stroke induced non treated

Ischemic stroke induction

Middle Cerebral Artery Occlusion (MCAO) method of Spratt et al. (2006) was used with some modification. Ischemic stroke (IS) was induced by occluding the MCA in Wistar rats. Ketamine and Xylazine at the doses of 80mg/kg and 5mg/kg body weight respectively were used to anaesthized 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 siliconcoated 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 nonabsorbable 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 were regulated or maintained at normal rate of 330-480 beats per minute and 35.9-37.5°C respectively.

Blood sample collection

After two weeks of the antioxidant supplementation to the IS rats, blood samples were collected through cardiac puncture. The rats were anaesthetized using gas anesthetic agent chloroform in a glass jar. 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 plane tubes, spun and stored.

BIOCHEMICAL ANALYSIS

Estimation of catalase activity

This was assayed using Cayman's Catalase Assay Kit's following the manufacturer's instruction.

Principle

The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2 . The formaldehyde produced was measured spectrophotometrically with 4- amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. Purpald specifically forms a bicyclic hetrocycle with aldehydes, which upon oxidation changes from colourless to a purple coloured complex that measured at 540 nm (Johansson and Borg 1988).

Procedure

Three wells were designated as sample, standard and control. To each well, 100 μ l of assay buffer and 30 μ l of methanol were added. To standard well, 20 μ l of prepared standard was added and to sample well 20 μ l of

serum/ brain tissue homogenate was added, $20 \ \mu l$ of $H_2 O_2$ was added to each well to initiate the reaction. The plate was covered with lid and incubated on a shaker for 20 minutes at room temperature. Then to each well, $30 \ \mu l$ of potassium hydroxide was added to terminate the reaction and $30 \ \mu l$ of purpald was then added. The plate was covered once again and incubated for 10 minutes at room temperature on a shaker. To each well, $10 \ \mu l$ of potassium periodate was added, covered and incubated for 5 minutes on a shaker once again.

The absorbance was read at 540 nm using Rayto (RT 2100C) plate reader. The average and the corrected absorbances of each standard and sample were calculated. The corrected absorbance of standards was ploted as a function of final formaldehyde concentration (μ M). The formaldehyde concentration of the samples was calculated using the equation obtained from the linear regression of the standard curve substituting corrected absorbance values for each sample. One unit is defined as the amount of enzyme that will cause the formation of 1.0 nmol of formaldehyde per minute at 25°C.

Formaldehyde (
$$\mu$$
M) = [Sample absorbance - (y intercept)] $\times \frac{0.17 \text{ ml}}{0.02 \text{ ml}}$

(sample absorbance – is the absorbance of the samle, y intercept – is the intercept on y axis, 0.17/0.02 ml - is a factor for converting from U/ml in well to U/ml in 20 µl added to 170 µl well volume).

CAT activity of the sample is then calculated using the equation below

CAT Activity (nmol/min/ml) =
$$\frac{\mu M \text{ of Sample}}{20 \text{ min}} \times \text{dilution factor}$$

(20 min - is the reaction time, dilution factor - is the factor used in calculating the amount of diluent used in diluting the samples)

Estimation of superoxide dismutase (SOD) activity

This was assayed using Cayman's Superoxide Dismutase Assay Kit's, following the manufacturer's instructions.

Principle

This assay utilizes a tetrazolium salt for the detection of superoxide radicals generated by the reaction between xanthine oxidase and hypoxanthine (Marklund, 1980).

Procedure

Two wells were designated as standard and sample. To each well 200 μ l of the diluted radical detector (tetrazolium salt) was added then 10 μ l of prepared standard to the standard well and 10 μ l of serum/ brain tissue homogenate to the sample wells. 20 μ l of diluted xanthine oxidase was added to both standard and sample wells to initiate the reaction. The plate was shaken for a few seconds to mix and covered with cover plate. The plate was then incubated on a shaker at room temperature for 20 minutes and absorbance was read at 460 nm using Rayto (RT 2100C) plate reader. The average absorbance

of each sample and standard were calculated. To get the linearized rate, the absorbance of the first standard is divided by itselfs and all other standards and samples absorbance. The linearized SOD standard rate (LR) was plotted as a function of final SOD activity. SOD activity was calculated using the equation below obtained from the linear regression of the standard curve substituting the linear rized rate (LR) for each sample. One unit of SOD is defined as the amount of enzyme needed to exhibit 50 % dismutation of the superoxide radical. SOD activity is standardized using the cytochrome c and xanthine oxidase coupled assay.

$$SOD(U/ml) = \left[(Sample LR - Y intercept) \frac{0.23ml}{0.01ml} \right] \times dilution factor$$

(0.23 ml / 0.01 ml is a factor for converting from U/ml in well to U/ml in10 µl added to 230 µl well volume. Sample LR is the linearized sample, y intercept is the intercept on y axis).

Estimation of glutathione peroxidase activity (GPx)

GPX was assayed using Cayman's Glutathione Peroxidase Assay Kit and according to the manufacturer's instructions.

Principle

This assay measures glutathione peroxidase activity indirectly by a coupled reaction with glutathione reductase. Oxidized glutathione, produced upon reduction of hydroperoxide by glutathione peroxidase, is recycled to its reduced state by glutathione reductase and NADPH:

$$\begin{array}{c} \begin{array}{c} \hline Glutathione\ Pexoxidase \\ \hline GSSG + H_2O \\ \hline GSSG + NADPH + H^+ \end{array} \xrightarrow{Glutathione\ reductase} R-O-H + \\ \hline ADP^+ \end{array}$$

The oxidation of NADPH to NADP⁺ is accompanied by a decrease in absorbance at 340 nm (Paglia and Valentine, 1967).

Procedure

Three wells were designated as sample, non-enzymatic and control. To sample wells, 100 μ l of assay buffer, 50 μ l of co-substrate mixture and 20 μ l of serum/ brain tissue homogenate were added. To non-enzymatic wells, 120 μ l of assay buffer and 50 μ l of co-substrate mixture were added and to positive control wells 100 μ l of assay buffer, 50 μ l of co-substrate mixture and 20 μ l of diluted GPx were added.

The reaction was initiated by adding 20 μ l of Cumene hydroperoxide to each well and the plate was carefully shaken for a few second to mix. The absorbance was read at 340 nm using Rayto (RT 2100C) plate reader once every 3 minutes.

$$Abs/min = \frac{Abs \ (time \ 2) - Abs \ (time \ 1)}{Time \ 2 \ (min) - Time \ 1 \ (min)}$$

(Abs in time 2 is the absorbance read at second time, Abs in time 1 is the absorbance read at the first minute, time 2 is the time at which the second absorbance reading was taken, time 1 is the time in which the first absorbance recorded).

GPx activity nmol/min/ml = $\frac{Abs / min}{0.00373 \ \mu M} \times \frac{0.19ml}{0.02ml}$

(Abs/min- change in absorbance per minute, 0.00373 is the extinction coefficient for NADPH, 0.19/ 0.02 ml is a factor for converting from U/ml in well to U/ml in 20 μ l added to 190 μ l well volume).

Estimation of malondialdehyde concentration (MDA) Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was measured using the manufactures instructions.

Principle

The assay is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid, forming an MDA-TBA₂ adduct that absorbs strongly at 535 nm (Niehans and Samuelson, 1968).

Procedure

Into two test tubes labeled sample and standard, 100 μ l of serum/ brain tissue homogenate and standard were added respectively and treated with 100 μ l of TCA

RESULTS

(trichloroacetic acid) reagent. Then 800 μl of color reagent (106 mg thiobarbituric acid, 10 ml TBA-acetic acid solution and 10 ml NaOH) was added to each test tube and vortex.

Tubes were heated in boiling water for one hour and cooled on ice to stop reaction and incubated for ten minutes on ice. After ten minutes, tubes were centrifuge for another ten minutes at 4000 rpm and stabilized at room temperature for 30 minutes. After transferring 200 μ l of the supernatant to the plate absorbance was read at 540 nm using the plate reader. Concentration of MDA was calculated from the standard curve using the formula;

$$MDA(\mu M) = Corrected absorbance - \frac{y intercept}{Slope}$$

(Corrected absorbance – absorbance value of standard minus absorbance value of sample, y intercept – intercept on y axis on the standard curve, slope – slope of the standard curve).

Statistical analysis

Results were expressed and presented as means \pm SD. Data were analyzed by one-way analysis of variance (ANOVA). Microsoft excel version 2010 was used for the analysis.





Fig. I: Effects of Vitamins C and E on SOD Activity of stroke induced rats.



Fig. II: Effects of Vitamins C and E on CAT Activity of stroke induced rats.

Figure I: The effect of management with vitamins C and E on the serum activity of SOD of Stroke induced rats is presented in figure I. The results indicated that IS induced significant (P<0.05) decrease in the activity of SOD. Management with the vitamins C and E however, caused a significant (P<0.05) increase in the activity of SOD.

Figure II: The effect of management with vitamins C and E on the serum activity of CAT of Stroke induced rats is presented in figure II. The results indicated that IS induced significant (P<0.05) decrease in the activity of CAT. Management with the vitamins C and E however, caused a significant (P<0.05) increase in the activity of CAT.



Fig. III: Effects of Vitamins C and E on TBARS Activity of stroke induced rats.





Figure III: The effect of management with vitamins C and E on the serum activity of GPx of Stroke induced rats is presented in figure III. The results indicated that IS induced significant (P<0.05) decrease in the activity of GPx. Management with the vitamins C and E however, caused a significant (P<0.05) increase in the activity of GPx.

Figure IV: The effect of management with vitamins C and E on the serum concentration of MDA of stroke induced rats is presented in figure IV. The results indicated that IS induced significant (P<0.05) decrease in the concentration of MDA. Management with the vitamins C and E however, caused a significant (P<0.05) increase in the concentration of MDA.



Fig. V: Effect of Vitamins E and C on Na level in stroke induced rats.



Fig. VI: Effect of Vitamins C and E on the level of K in stroke induced rats.

Figure V: The effect of management with vitamins C and E on the serum level of sodium of stroke induced rats is presented in figure V. The results indicated that IS induction caused significant (P<0.05) increase in the level of sodium. Management with the vitamins C and E however, caused a significant (P<0.05) decrease in the level of sodium

Figure VI: The effect of management with vitamins C and E on the serum level of potassium of stroke induced rats is presented in figure VI. The results indicated that IS induction caused significant (P<0.05) decrease in the level of potassium. Management with the vitamins C and E however, caused a significant (P<0.05) increase in the level of potassium.



Fig. VII: Effect of Vitamins C and E on Ca2+ in stroke induced rats.



Fig. VIII: Effect of Vitamins C and E on Mg2+ Level in stroke induced rats.

Figure VII: The effect of management with vitamins C and E on the serum level of calcium of stroke induced rats is presented in figure VII. The results indicated that IS induction caused significant (P<0.05) decrease in the level of calcium. Management with the vitamins C and E however, caused a significant (P<0.05) increase in the level of calcium.

Figure VIII: The effect of management with vitamins C and E on the serum level of magnesium of stroke induced rats is presented in figure VII. The results indicated that IS induction caused significant (P<0.05) reduction in the level of magnesium. Management with the vitamins C and E however, caused a significant (P<0.05) increase in the level of magnesium.







Fig. X: Effect of Vitamins C and E on GLU Level in stroke induced rats.

Figure IX: The effect of management with vitamins C and E on the serum level of phosphorus of stroke

induced rats is presented in figure IX. The results indicated that IS induction caused significant (P<0.05)

increase in the level of phosphorus. Management with the vitamins C and E however, caused a significant (P<0.05) increase in the level of phosphorus.

Figure X: The effect of management with vitamins C and E on the serum level of glucose of stroke induced rats is presented in figure X. The results indicated that IS induction caused significant (P<0.05) increase in the level of phosphorus. Management with the vitamins C and E however, caused a significant (P<0.05) increase in the level of glucose.

DISCUSSION

Oxidative stress (OS) has been implicated as one of the major contributor to the pathogenesis of acute central nervous system (CNS) injury and it is found to be responsible for mortality following stroke (6). According to the report of Christensen and Boysen (2002), glucose levels appear to rise after stroke because, in one of the study it was reported that nondiabetic patients demonstrates a rise in median blood glucose level from 5.9 mmol/L at 2.5 h to 6.2 mmol/L at 6 h following stroke (Christensen et al., 2002), a study by Wong et al. (2007) claims that hyperglycemia following stroke is a frequent phenomenon, with up to 50% of the patients having an initial blood glucose of above 6.0-7.0 mmol/L. Electrolyte disruption are commonly found in acute stroke situation. Hypernatremia, hyponatermia and hypokalemia are the commonest types of disturbance usually encountered in stroke (Bandyopadhyay et al., 2017).

In this study, stroke induction caused significant (P<0.05) decrease in serum antioxidant enzymes; SOD, CAT, GPX activities and increased in plasma TBARS concentration. This could be as a result of high production of ROS which might have consumed the endogenous antioxidants (SOD, CAT and GPX) and this is suggestive of the role of oxidative stress in stroke. According to Rodrigo et al. (2011), the study reports that oxidative stress occurs when there is an increase in the concentration of ROS and reactive nitrogen species [RNS] in the steady state. ROS and RNS are families of highly reactive species formed either enzymatically or non-enzymatically in mammalian cells. Oxidative stress arises from an imbalance between the generations of these species due to increased pro-oxidant activity over the antioxidant defence system in the body so that the latter become overwhelmed (Rodrigo et al., 2011). Therefore the significant (P<0.05) increase in TBARS seen in this study is an indicator of oxidative stress that might have resulted from increase in ROS that would have consumed the endogenous antioxidants that is supposed to counterbalance its effect.

Treatment of the IS rats with vitamin C caused significant (P<0.05) increase in antioxidant status and decrease in the thiobarbituric acid reactive species (TBARS) concentration. In a study reported by Cook et al. (2010), vitamin C is found to be more abundant in tissues, where ROS production is more important. Also,

vitamin C can regenerate vitamin E and GSH, which are very potent against ROS, findings of this work are in agreement with the result of Rabec and Pierce (1994), the study reports that ascorbate is highly efficient in trapping free radicals, and preventing them from forming lipid hydroperoxide that can be generated during stroke. Eghwrudjakpor, and Allison, (2010) also reported that ascorbic acid is an efficient free-radical scavenger because it neutralizes or removes the impurities formed by the free radicals, produced during stroke. It was observed in this study that treatment with Vit C significantly increased the activities of SOD, CAT, GPx and it decreased the concentration of TBARS in the treated groups, when compared with the IS induced without treatment group. Karaca et al. (2002) claims that vitamin C is reported to offer an option as a preventive measure in patients undergoing procedures with an increased risk of developing perinterventional brain ischemia, such as carotid coronary artery bypass surgery.

Treatment with vitamin E caused significant (P<0.05) increase in the activities of the enzymes (SOD, CAT, and GPx), and significant (P<0.05) decrease in the concentration of plasma TBARS in this study. This study explains that the result reported in this research could be attributed to relevant chain-breaking antioxidant capacity of vitamin E and its abundance in cells and mitochondria membrane according to the research of Inci et al. (1998). In addition, vitamin E acts directly on ROS, and can also react with various antioxidants such as vitamin C, GSH, β -carotene to bring about synergistic activity.

Furthermore, vitamin E is promising in modifying OS pathways and improving neurological outcome in many animal studies as reported by Inci et al. (1998). Vitamin E is a lipid-soluble antioxidant which prevents the formation of lipid peroxide (Carmen and Oyvind (2001). From this study, supplementation of antioxidants in the IS induced rats were effective as evident by the increase observed in the enzymes activities.

This study reports that ischemic stroke induction caused significant (P<0.05) decrease of Sodium (Na) and Potassium (K) concentrations in the group that was induced with stroke but not treated. The non-stroke non treated group and groups treated with Vitamin C and Vitamin E showed significant (P<0.05) increase in the minerals Sodium (Na) and Potassium (K), this may be due to increase dietary demand of electrolytes (Na and K) in the serum level of IS patients. Disorders like subarachnoid hemorrhage, brain tumors, stroke and meningitis can all cause hyponatremia and hypokalemia, and thereby worsened brain swelling. This could be as a result of altering normal hormonal control of water, potassium and sodium level in the body.

Kahle et al. (2009) reported that for an actual increase in brain volume to occur, additional fluid must be added to the brain's extracellular space. During permanent ischemia, blood sodium rapidly enters the extracellular fluid of the brain, leading to consequences of brain edema. This study may therefore be in line with the study of Kahle et al. (2009) that postulates that higher serum sodium levels may be associated with higher risk and exacerbation of events following brain ischemia.

Ischemic stroke induction caused significant (P<0.05) decrease of Calcium (Ca) and Magnesium (Mg) concentration in the group that was induced with stroke but not treated. This is in line with the report of Bandyopadhyay et al. (2017) the research reports that calcium and magnesium level in stroke patients is low due to continues utilization by neuronal tissues, and this finding could be as a result of high production of ROS which might have altered the body electrolytes (Ca and Mg) and this is suggestive of the role of oxidative stress in stroke. There was significant (P<0.05) increase in Non-stroke non treated group and Vitamin C and Vitamin E treated groups. Elevated serum calcium levels have been associated with better clinical outcomes and smaller cerebral infarct volume, though the role played by calcium is largely unknown. In addition, the response to a lack of oxygen and nutrients caused by ischemia results in a local release of chemicals that can damage brain cells, even beyond the damage that can be expected by ischemia alone as reported by Schellinger et al. (2010). Perhaps the most harmful of these chemicals is glutamate, an amino acid used in very low amounts by brain cells to communicate with each other. However, during stroke, the enormous amount of glutamate released produces an abundance of calcium inside brain cells which in turn causes them a premature death. Magnesium is thought to have the ability to prevent glutamate from causing this flood calcium in the cells, thus protecting them from premature death (Saposnik et al., 2011).

In this study, ischemic stroke induction caused significant (P<0.05) increase of Phosphorus (P) and Glucose (Glu) concentration in the group that was induced with stroke but not treated, while there was significant (P<0.05) decrease in Non-stroke non-treated group. Also the group treated with Vitamin C and Vitamin E showed decrease in the minerals Phosphorus (P) and Glucose (Glu). It is possible that the antioxidants therapies might have blocked the effects of ROS and oxidative stress leading to the utilization of glucose in circulation and thereby reducing the level of phosphorus that is available therein. Findings of this study is in agreement with the result of Rabec and Pierce (1994), the research reported that the union of phosphorus and glucose is highly efficient in trapping free radicals, and preventing them from forming lipid hydroperoxide that can be generated during stroke. So also, high sugar level is a marker of stroke severity because it has been reported by Candelise et al. (1985) that several studies have indicated that patients with diabetes are more likely to die or to have substantial neurological disability after acute stroke than nondiabetic subjects. Also, in another research it was stated that animal models of focal

cerebral ischemia suggested that the type of vessel occlusion, the presence of collateral blood flow, and occurrence of reperfusion were relevant and that hyperglycemia might influence neuronal damage through accentuated tissue acidosis and lactate generation (Breiman et al., 1985). Furthermore, using MR spectroscopy, researchers have demonstrated a mechanistic link between admission hyperglycemia and stroke outcome involving infarct growth through recruitment of penumbral tissue and increased cerebral lactate production (Vancheri et al., 2005).

It has been claimed previously that phosphorus is known to plays an important role in the delivery of oxygen to tissue by regulating the level of 2,3-DPG and ATP in erythrocytes. Phosphorus could have an important role in the brain where oxidation of glucose through the Krebs cycle is necessary for the synthesis of ATP [1 Knochel, 1977]. The association between serum levels of phosphate and stroke has been investigated in studies, with controversial results (Wannamethee et al., 2013, Li et al., 2014 and Aronson et al., 2013).

The relationships between ischemic stroke and phosphorus has not been extensively reviewed in previous publications. Massry (1987) has shown the clinical syndrome of phosphate depletion According to Massry's study on phosphate depletion, the syndrome can lead to disturbances in the central nervous system, hematopoietic system, parathyroid glands, cardiac and renal function, and also in muscles and bones. The low levels of phosphorus affect the organs; a decrease in 2,3diphosphoglycerate in red blood cells leads to tissue hypoxia, mitochondrial energy production decreases due to low tissue content of ATP. Correspondingly, hypoxia in the tissue and energy deficiency in the cells may lead to organ failure (Lichtman et al., 1971). Studies examining acute ischemic stroke and phosphorus levels have produced only a few results. Wannamethee et al. (2013) concluded that their study finds a relationship between raised serum phosphorus levels and increased total mortality of stroke cases. Li et al. (2014) concluded that there is no association between serum level of phosphate and stroke. Aronson et al. (2013) reported that higher phosphorus levels were also associated with increased risk of heart failure, but not the risk of myocardial infarction or stroke.

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