

MODULATION OF KEY BIOCHEMICAL MARKERS RELEVANT TO STROKE BY *ANTIARIS AFRICANA* LEAF EXTRACT FOLLOWING CEREBRAL ISCHEMIA/REPERFUSION INJURY

Omotayo B. Ilesanmi^{1,2}, Afolabi C. Akinmoladun^{1,*}, Olanrewaju Sam Olayeriju¹, Ibrahim Olabayode Saliu¹, M. Tolulope Olaleye¹ and Afolabi A. Akindahunsi¹

¹Phytomedicine, Biochemical Pharmacology and Toxicology Laboratories, Department of Biochemistry, School of Sciences, The Federal University of Technology, PMB 704, Akure, Nigeria. ²Department of Biological Sciences, Faculty of Science, Federal University Otuoke, Otuoke, Nigeria.

* Corresponding Author E-mail: acakinmoladun@futa.edu.ng; akinmoladun_afolabi@yahoo.co.uk

Abstract

Background: Oxidative stress plays a significant role in stroke pathogenesis. Hence, plants rich in antioxidant phytochemicals have been suggested as effective remedies for prevention and treatment of stroke and other neurological diseases. *Antiaris africana* Engl. (Moraceae) is traditionally used for the management of brain-related problems but there is paucity of data on its anti-stroke potential.

Materials and Methods: Ischemia/reperfusion injury was induced by a 30 min bilateral common carotid artery occlusion/ 2 h reperfusion (BCCAO/R) in the brain of male Wistar rats. A sham-operated group which was not subjected to BCCAO/R and a group subjected to BCCAO/R without treatment with MEA served as controls. The ameliorative effect of 14 days of pretreatment with 50 mg/kg or 100 mg/kg *A. africana* methanol leaf extract (MEA) on BCCAO/R-mediated alterations to key markers of oxidative stress (malondialdehyde, reduced glutathione, xanthine oxidase, superoxide dismutase, catalase and glutathione peroxidase) and neurochemical disturbances and excitotoxicity (myeloperoxidase, glutamine synthetase, Na⁺/K⁺ ATPase, acetylcholinesterase and tyrosine hydroxylase), was evaluated and compared with the effect produced by treatment with 20 mg/kg quercetin as a reference standard.

Results: Results show that pretreatment with MEA significantly mitigated or reversed BCCAO/R-induced changes in the level or activity of the evaluated biochemical markers of oxidative stress, neurochemical dysfunction and excitotoxicity compared with the BCCAO/R untreated control group ($p < 0.05$). The effect produced by 100 mg/kg MEA was similar to that of the reference standard, quercetin.

Conclusion: These results revealed the neuroprotective potential of *A. africana* in stroke and other ischemia-related pathologies.

Key words: brain ischemia; excitotoxicity; neuroprotection; oxidative stress; phytochemicals; stroke

Introduction

The brain is the centre for coordination of activities of all organs in the body. It consummately relies on glucose as its primary source of energy, and oxygen for complete conversion of glucose to energy. These two molecules are carried via the blood system, and thus a constant flow of blood to the brain is essential for its proper function (Sunil et al., 2011). Cerebral ischemia is the reduction or cessation of blood flow to the brain as a result of occlusion or blockage of arteries conveying the blood which results to stroke and severe damage to the whole body. Mortality and disability from stroke remain among the highest globally (Brouns and De Deyn, 2009; Towfighi and Saver, 2011; Connell et al., 2017).

Ischemia causes depolarization of neurons and upon reperfusion triggers generation of excessive intracellular reactive oxygen species (ROS), calcium overload, excitotoxic cell injury and inflammation, which ultimately lead to irreversible brain injury. The culmination of this intricate cascade of events is cell death via necrosis or apoptosis depending on the intensity and extent of the ischemic episode (Arumugam et al., 2005; Gupta and Gupta, 2017; Reis et al., 2017). Many significant preclinical researches to discover perspicacious treatment that can reduce stroke induced brain damage and deformities have been carried out but the availability of a remarkably guaranteed neuroprotective drug approved for stroke is still lacking (Macrae, 2011; Kalani et al., 2016).

Many drugs originate from plants, thus they have been the focus of search for novel bioactive agents with negligible or zero side-effects in the treatment of diseases. Medicinal plants could be a source of novel phytomedicines

against oxidative stress, excitotoxicity, inflammation and other damaging ischemic cascades and as such could be relevant in the treatment of neurological diseases like stroke and other pathologies whose aetiology involve free radical mechanisms (Akinmoladun et al., 2010).

Antiaris africana Engl. belongs to the family Moraceae. The plant is commonly called “Oro” among the “Yoruba” speaking people of Western Nigeria, “Ojianwu” among the “Ibo” speaking people of Eastern Nigeria and “Farin loko” among the “Hausa” speaking people of Northern Nigeria. In the northern part of Nigeria, it is traditionally used for the management of mental and nervous disorder. Different parts of the plant are reportedly used for the treatment of various ailments ranging from rheumatism, respiratory problem, stomachic pain, syphilis, leprosy, purgative and sore throat to epilepsy (Gill, 1992; Adewuyi et al., 2010). The compounds 3, 39-dimethoxy-49-*O*- β -d-xylopyronosylsuccinic acid, oleanolic acid, strophantidol and ursolic acid with antioxidant and antitumor activities have been isolated from the plant (Kuethe et al., 2009) and a flavonoid-rich fraction of the plant was reported to possess antibacterial activities (Banso and Mann, 2008). In addition, the presence of several phenolics in the methanol leaf extract of the plant has been reported (Omotayo et al., 2016). The present study assessed the ameliorative effect of methanol leaf extract of *Antiaris africana* on bilateral common carotid artery occlusion/reperfusion-induced cerebral injury with a view to ascertaining the anti-stroke potential of the plant.

Materials and Methods

Chemicals and drugs

Reduced glutathione (GSH), adenosine triphosphate (ATP), acetylthiocholine iodide, 6,7-Dimethyl-5,6,7,8-tetrahydropterine (DMTHP), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA), xanthine (2,6-Dihydroxypurine), adrenaline, ammonium molybdate, oxidized nicotinamide adenine dinucleotide (NAD), reduced nicotinamide adenine dinucleotide (NADH), tyrosine, tetramethylbenzidine, L-glutamate, sodium azide (NaN₃) and amino naphthol sulphonic acid (ANSA) were products of Sigma-Aldrich (St. Louis, MO, USA). Other chemicals and reagents used were of analytical grade.

Preparation of plant extract

Antiaris africana leaves were collected from Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria and authenticated at Botany department, University of Ibadan, Ibadan, Nigeria (voucher number 070613M). The leaves were air dried, pulverized and macerated in 80% methanol for 48 h and then filtered. The filtrate was concentrated using a rotary evaporator and further lyophilized to obtain the extract (MEA) used for the study.

Animal grouping and treatment

Male Wistar Albino rats weighing 230-250 g were used. They were provided with food and water *ad libitum*. All animals were acclimatized to laboratory condition for 2 weeks before the commencement of experiment. Guidelines for the care and use of laboratory animals (European Community guidelines (Directive 86/609/EEC of the EU) were strictly adhered to throughout the study. Animals were divided into five groups with eight animals per group and treated as follows: Group I: sham-operated

Group II: administered corn oil (p.o.) and subjected to occlusion of the common carotid arteries and reperfusion (BCCAO/R)

Group III: administered MEA (50 mg/kg p.o.) + BCCAO/R

Group IV: administered MEA (100 mg/kg p.o.) + BCCAO/R

Group V: administered quercetin (20 mg/kg i.p.) as the reference standard + BCCAO/R

Animals were orally administered MEA for 14 days after which surgical procedure for BCCAO/R was performed.

Surgical procedure

Surgical procedure for bilateral common carotid artery occlusion was performed as previously described (Akinmoladun et al., 2015). Briefly, Rats were anaesthetized with 350 mg/kg chloral hydrate (i.p.). A midline incision was made in the neck region and the common carotid arteries were exposed, care being taken to preserve the vagus nerves. The carotid arteries were ligated using cotton threads. With the exception of occlusion of the carotid arteries, surgical procedures in sham-operated animals were the same as those in the BCCAO-operated animals. During surgery, temperature was maintained at 36 - 38°C with a heating lamp. Ischemia was allowed for 30 min, followed by a 2-hour reperfusion initiated by removal of the threads from the arteries. Arteries were visually inspected to confirm cessation and reflow of

blood following ligation and removal of threads, respectively. Following reperfusion, brains were excised and processed for biochemical assays.

Excised brains were quickly rinsed in ice cold 1.15% potassium chloride solution, blotted with filter paper, weighed and homogenized in phosphate buffered saline (pH 7.4). The supernatant used for biochemical estimations was obtained by centrifuging homogenate at 6,000 rpm for 10 min at 4°C.

Biochemical Estimations

Glutamine Synthetase activity

Glutamine synthetase activity was evaluated as per the method previously reported (Sunil et al., 2011). Tris-HCl buffer, 0.2 M glutamate, 0.1 mM ammonium, 3 mM MgCl₂ and 1 mM ATP in a total volume of 700 µl was incubated at 37°C for 5 min. Then 0.1 ml of supernatant was added and the mixture was further incubated at 37°C for 15 min followed by the addition of 0.5 ml of 10% TCA to stop the reaction. The control was prepared by adding 0.1 ml of supernatant after 10% TCA has been added. The reacting mixture was centrifuged at 3500 rpm for 10 min to remove the precipitate and 0.2 ml of supernatant was pipetted into another test tube to which 0.925 ml of distilled water, 0.125 ml of ammonium molybdate and 50 µl of ANSA were added and incubated at 37°C for 10 min. Absorbance was read at 640 nm against a blank using a spectrophotometer. A standard calibration graph was plotted using potassium dihydrogen phosphate in the concentration range of 1.6–8.0 µg.

Na⁺/K⁺ ATPase activity

Assay was carried out as formerly reported (Sunil et al., 2011). Briefly, a reaction mixture containing 184 mM Tris HCl buffer, 600 mM NaCl, 50 mM HCl, 1 mM Na EDTA and 80 mM ATP was incubated at 37°C for 10 min followed by the addition of 25 µl of supernatant and a further incubation at 37°C for 60 min. The reaction was stopped by the addition of 10% TCA. The control was prepared by the addition of supernatant after TCA has been added. The mixture was centrifuged at 3500 rpm for 10 min to remove the precipitate. The liberated inorganic phosphorus was measured as described for glutamine synthetase activity.

Xanthine oxidase (XO) Activity

Assay for XO activity was performed according to Gulec et al. (2006). The reaction mixture consisted of 0.2 ml supernatant of brain, which was incubated for 5 min at 37°C with 0.1 M phosphate buffer (pH 7.4). Then, 0.15 mM xanthine was added to the reaction mixture which was kept at 37°C for 20 min, followed by the addition of 10% TCA and distilled water in a total volume of 4 ml. The mixture was then centrifuged at 1500 g for 10 min and absorbance was read at 290 nm. The enzyme activity was calculated as nanomole uric acid formed/min/mg protein, using a molar extinction coefficient of 12,200 M⁻¹ cm⁻¹.

Myeloperoxidase Activity (MPO)

Evaluation of myeloperoxidase activity was carried out according to a previously published procedure (Eiserich et al., 1998). Brain supernatant (0.2 ml) was added to a solution of tetramethylbenzidine (1.2 mM) and H₂O₂ (100 mM) in NaH₂PO₄ (43 mM, pH 5.4) and absorbance was taken spectrophotometrically at 450 nm.

Tyrosine Hydroxylase activity

This was assayed for as previously described (Shiman et al., 1971; Craine et al., 1972). Briefly, reaction agents were added in the following sequence: 1.5 ml of TRIS buffer, 0.03 ml of tyrosine, 0.05 ml of DMTHP, 0.17 ml of distilled water; mixed and equilibrated at 37°C. Supernatant (0.3 ml) was added and immediately mixed by inversion and the decrease in absorbance was recorded for 5 min to obtain the change in absorbance at 340 nm per minute. The activity was calculated with molar extinction coefficient of 6.22 for β NADH. One unit will form 1.0 nanomole of L-DOPA from tyrosine per minute at pH 7.0 at 37°C.

Acetylcholinesterase activity

This was estimated according to the method developed by Ellman et al. (1961). This is based on the principle of thiol group reacting with DTNB to form yellow colored thionitrobenzoic acid, which was read at 412 nm.

Superoxide Dismutase (SOD) activity

SOD activity was evaluated by the well reported method of Misra and Fridovich (1972) by monitoring absorbance of a mixture containing the brain supernatant, carbonate buffer (0.05 M, pH 10.2) and adrenalin (0.3 mM) for 150 s at 480 nm.

Catalase activity

The activity of catalase was evaluated according to Sinha (1972). This method is based on the disappearance of H₂O₂. The reaction volume contained 0.1 M sodium phosphate buffer (pH 7.4), 0.05 M H₂O₂, and 0.05 ml brain supernatant. Change in absorbance was recorded kinetically at 240 nm. Catalase activity was calculated in terms of mole H₂O₂ consumed/min/mg protein using a molar extinction coefficient of 39.6 M⁻¹ cm⁻¹.

Lipid peroxidation

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to previously published procedures (Ádam-Vizi and Seregi, 1982; Varshney and Kale, 1990). Briefly an aliquot of 0.4 ml of brain supernatant was mixed with 1.6 ml of Tris-KCl buffer to which 0.5 ml 30% TCA was added. Then, 0.5 ml of 0.75% TBA was added and the mixture was placed in a water bath for 45 min at 80°C. This was then cooled and centrifuged at 3000 g. The clear supernatant was collected and absorbance measured against a reference blank at 532 nm. MDA level was calculated using a molar extinction coefficient of 1.56 x 10⁵ M⁻¹cm⁻¹.

Reduced glutathione (GSH) content

GSH content was determined according (Jollow et al., 1974). The reaction is based on the fact that the thiol group of GSH reacts with DTNB to form thionitrobenzoic acid. The supernatant was mixed with 4% sulphosalicylic acid. The mixture was allowed to stand for 5 min and then filtered. Thereafter, 1 ml of filtrate was added to 4.0 ml of the Ellman's reagent. The absorbance was read at 412 nm against a reagent blank.

Glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was evaluated according to the procedure described by Rotruck et al. (1973). A mixture of 0.1 M phosphate buffer (pH 7.4), 10 mM sodium azide, 4 mM GSH, 2.5 mM H₂O₂ and 0.5 ml of brain supernatant was incubated at 37°C for 3 min followed by the addition of TCA (10%) and centrifugation of mixture at 3,000 rpm for 5 min. Two ml of K₂HPO₄ and 1 ml of 0.04% DNTB were added to 1 ml of the supernatant obtained and the absorbance was read at 412 nm against a blank.

Total protein estimation

Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

Statistical analysis

Results were expressed as mean ± standard deviation (SD). All data were analyzed using analysis of variance (ANOVA) followed by Duncan's test. Values of *p*<0.05 were considered significant.

Results

Glutamine synthetase activity

Ischemia followed by reperfusion (BCCAO/R) significantly increased the glutamine synthetase (GS) activity in the brain of animals in comparison to the sham-operated (SO) group (*p*<0.001). Pretreatment with MEA (50 mg/kg) had no

significant effect on brain GS activity. However, group pretreated with 100 mg/kg MEA showed a significant decrease in GS activity compared to BCCAO/R group ($p < 0.001$). Quercetin (20 mg/kg i.p) administered group showed a significant suppression of GS activity as compared to BCCAO/R group (Fig. 1).

Na⁺ K⁺ ATPase activity

The brain Na⁺ K⁺ ATPase activity was significantly reduced in BCCAO/R group compared to SO group ($p < 0.001$). Administration of MEA significantly increased Na⁺/K⁺ ATPase activity compared to the BCCAO/R group ($p < 0.001$). Administration of quercetin also resulted in a significant increase in the enzyme compared to the ischemic control group (Fig. 2).

Xanthine oxidase (XO) and myeloperoxidase (MPO) activities

Xanthine oxidase activity was significantly elevated in the brain of rats subjected to BCCAO/R as compared to the SO group ($p < 0.001$). Pretreatment with MEA significantly suppressed the activity of xanthine oxidase in a dose dependent manner when compared to BCCAO/R group ($p < 0.001$). Quercetin also significantly suppressed the activity of XO in comparison to BCCAO/R group ($p < 0.001$). Significant increase in MPO activity by BCCAO/R in rat brain was observed as compared to SO group ($p < 0.001$). MEA significantly decreased the activity of MPO as compared to BCCAO/R group (Table 1).

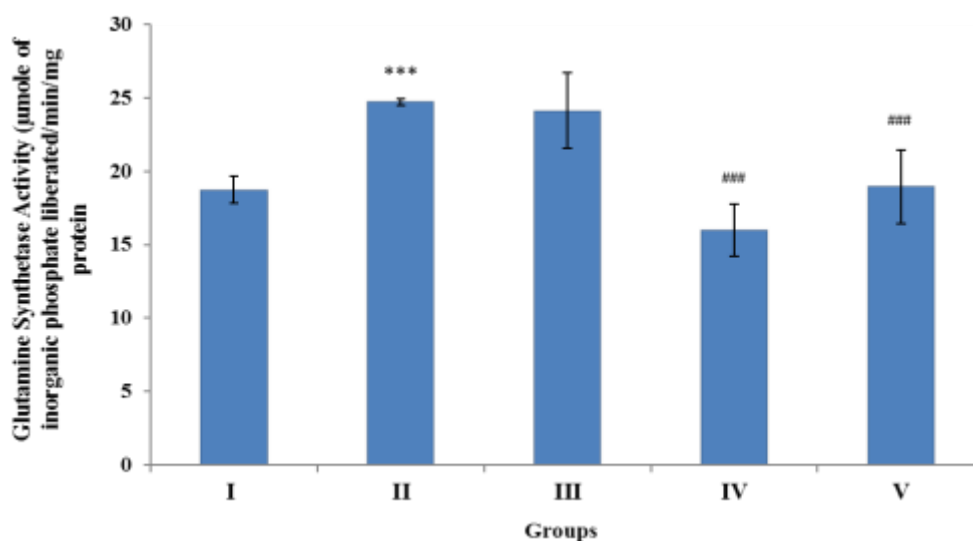


Figure 1: Effect of methanol extract of *Antiaris africana* (MEA) on Glutamine synthetase activity in the brain following bilateral common carotid artery occlusion/reperfusion (BCCAO/R) in rats. Results are expressed as mean \pm S.D. Group I: Sham-operated; Group II: BCCAO/R; Group III: MEA (50 mg/kg) + BCCAO/R; Group IV: MEA (100 mg/kg) + BCCAO/R; Group V: (quercetin (20 mg/kg) + BCCAO/R. ***significantly different from sham ($p < 0.001$), ### significantly different from BCCAO/R ($p < 0.001$).

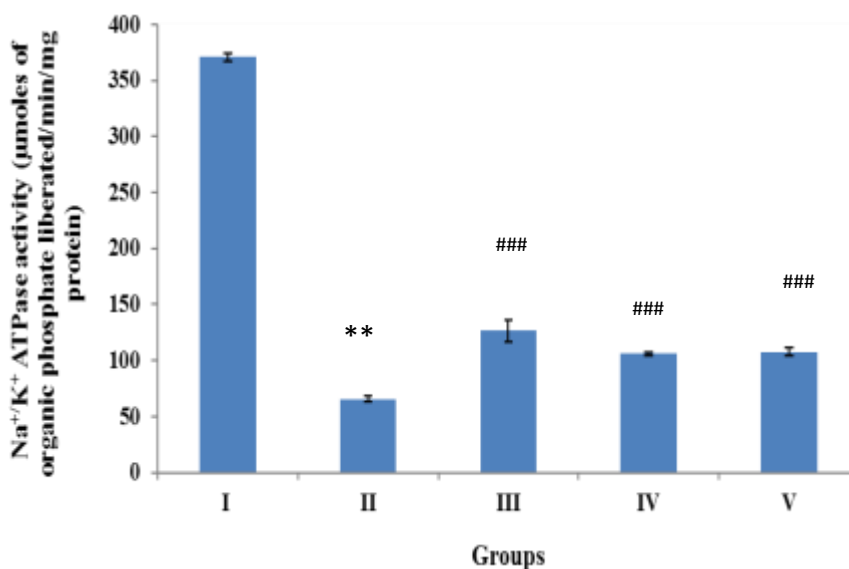


Figure 2: Na⁺/K⁺ ATPase activity in rat brain subjected to bilateral common carotid artery occlusion/reperfusion (BCCAO/R) and pretreated with methanol extract of *Antiaris africana* (MEA). Results are expressed as mean ± S.D. Group I: Sham-operated; Group II: BCCAO/R; Group III: MEA (50 mg/kg) + BCCAO/R; Group IV: MEA (100 mg/kg) + BCCAO/R; Group V: quercetin (20 mg/kg) + BCCAO/R. ***significantly different from sham ($p < 0.001$), ### significantly different from BCCAO/R ($p < 0.001$).

Table 1: Effect of MEA on xanthine oxidase and myeloperoxidase (MPO) activities in the brain following BCCAO/R

Group	Xanthine Oxidase (nmol of uric acid formed/min/mg protein)	MPO (units/mg protein)
Sham	0.08±0.01	0.011±0.003
BCCAO/R	0.16±0.01***	0.036±0.01***
MEA (50 mg/kg) + BCCAO/R	0.10±0.01###	0.025±0.003###
MEA (100 mg/kg) + BCCAO/R	0.06±0.01###	0.022±0.002###
Quercetin + BCCAO/R	0.08±0.01###	0.033±0.004#

Results are expressed as mean ± S.D (n=8). *** $p < 0.001$ versus Sham; # $p < 0.05$ versus BCCAO/R; ### $p < 0.001$ versus BCCAO/R.

Tyrosine Hydroxylase (TH) activity

Significant decrease in the TH activity in the brain of BCCAO/R group was observed in comparison to the SO group. Administration of MEA resulted in a significant amelioration of the decrease in TH activity when compared to BCCAO/R group ($p < 0.001$) (Fig.3).

Acetylcholinesterase activity

The significant decrease in AChE activity in the brain of BCCAO/R groups compared to SO group was ameliorated in 100 mg/kg MEA pretreatment group. Also, quercetin significantly elevated AChE activity compared to BCCAO/R group ($p < 0.001$) (Fig.4).

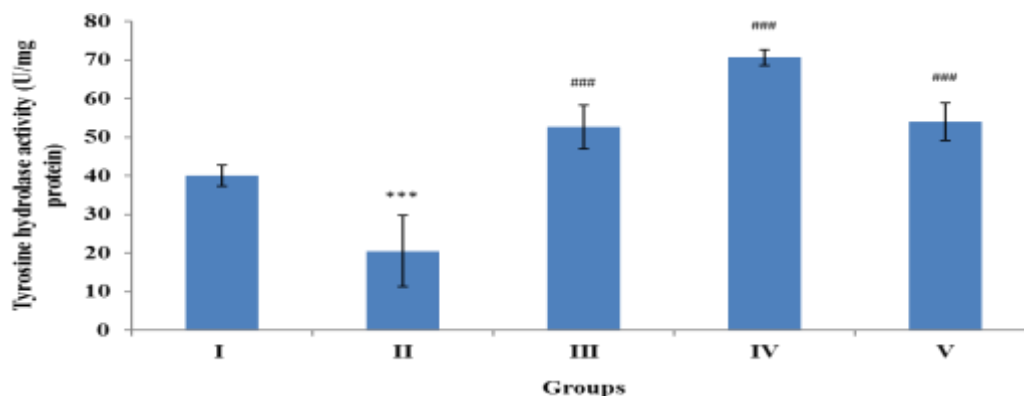


Figure 3: Effect of methanol extract of *Antiaris africana* (MEA) on tyrosine hydroxylase activity in the brain of rats following bilateral common carotid artery occlusion/reperfusion (BCCAO/R). Results are expressed as mean \pm S.D. Group **I**: Sham-operated; Group **II**: BCCAO/R; Group **III**: MEA (50 mg/kg) + BCCAO/R; Group **IV**: MEA (100 mg/kg) + BCCAO/R; Group **V**: quercetin (20 mg/kg) + BCCAO/R.

***significantly different from sham ($p < 0.001$), ### significantly different from BCCAO/R ($p < 0.001$).

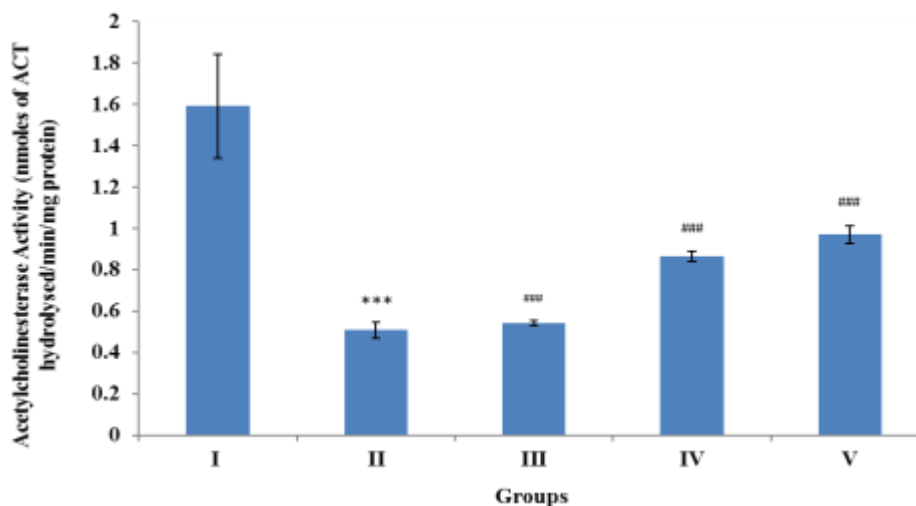


Figure 4: Acetylcholinesterase activity in the brain of rats pretreated with methanol extract of *Antiaris africana* (MEA) and then submitted to bilateral common carotid artery occlusion/reperfusion (BCCAO/R). Results are expressed as mean \pm S.D. Group **I**: Sham-operated; Group **II**: BCCAO/R; Group **III**: MEA (50 mg/kg) + BCCAO/R; Group **IV**: MEA (100 mg/kg) + BCCAO/R; Group **V**: quercetin (20 mg/kg) + BCCAO/R. *** significantly different from sham ($p < 0.001$), ### significantly different from BCCAO/R ($p < 0.001$).

Cerebral antioxidant status

Table 2 revealed that BCCAO/R significantly led to elevated brain MDA levels. MEA treatment resulted in a significant decrease in the MDA level when compared to the BCCAO/R group ($p < 0.05$ at 50 mg/kg and $p < 0.001$ at 100 mg/kg). Also, quercetin significantly suppressed MDA generation compared to BCCAO/R groups ($p < 0.001$). Results for GSH level followed and inverse pattern to that of lipid peroxidation.

The significant decrease in GPx activity occasioned by BCCAO/R was ameliorated by MEA and quercetin pretreatments (Table 3). In addition, there was a significant decrease in SOD activity in rats subjected to BCCAO/R when compared to SO rats ($p < 0.001$) which was mitigated by MEA and quercetin pretreatment ($p < 0.001$). A similar trend was observed for catalase activity.

Table 2: Effect of methanol extract of *Antiaris africana* (MEA) on reduced glutathione (GSH) and lipid peroxidation (MDA) in the brain following BCCAO/R in rats.

Group	GSH (mmol/g protein)	MDA (µmol TBARS/mg protein)
Sham	1.2±0.2	0.072±0.009
BCCAO+R	0.6±0.1***	0.145±0.011***
MEA (50mg/kg) + BCCAO	0.7±0.1 [#]	0.135±0.028 [#]
MEA (100mg/kg) + BCCAO	1.7±0.4###	0.098±0.004 ^{##}
Quercetin (20 mg/kg) + BCCAO	1.6±0.1###	0.079±0.012###

Results are expressed as mean ± SD (n=8). ****p*<0.001 versus Sham; [#]*p*<0.05 versus BCCAO/R; ^{##}*p*<0.01 versus BCCAO/R; ^{###}*p*<0.001 versus BCCAO/R.

Table 3: Effect of methanol extract of *Antiaris africana* (MEA) on antioxidant enzymes in the brain following BCCAO/R in rats.

Group	GPx (µmoles GSH consumed/min/mg protein)	Catalase (Kat.f)	SOD (units/mg protein)
SHAM	14.971±0.255	0.232±0.015	0.0989±0.008
BCCAO +R	11.712±0.394*	0.192±0.009***	0.0443±0.001***
MEA (50mg/kg) + BCCAO	14.910±1.631 [#]	0.234±0.024###	0.064±0.015###
MEA (100mg/kg) + BCCAO	12.893±0.171 [#]	0.207±0.012 ^{##}	0.0071±0.005 ^{###}
Quercetin (20 mg/kg) + BCCAO	12.199±0.546 [#]	0.193±0.007 ^{##}	0.0674±0.008 ^{###}

Results are expressed as mean ± SD (n=8). ****p*<0.001 versus Sham; **p*<0.05 versus Sham; [#]*p*<0.05 versus BCCAO/R; ^{##}*p*<0.01 versus BCCAO/R; ^{###}*p*<0.001 versus BCCAO/R.

Discussion

Ischemia leads to extensive disruption of brain biochemical network which is compounded by reperfusion. BCCAO/R in the present study was characterized by a significant increase in cerebral glutamine synthetase (GS) activity but a decrease in brain Na⁺ K⁺ ATPase activity. Synaptic clearance of glutamate depends to a large extent on glial bound GS and Na⁺ K⁺ ATPase activities (Benarroch, 2005) and the increased activity of GS may be due to glutamate excitotoxicity as a result of ischemia (Ramanathan, 2007). Increase in GS activity alters astrocytes osmoregulation and this may have a secondary effect on energy metabolism (Zwingmann and Butterworth, 2005). Impairment of ATPase action has been implicated in the pathophysiology of cerebral ischemia (Mdzinarishvili et al., 2012). Apart from its role in glutamate clearance, ATPase pump helps to maintain electrolyte and fluid balance across cell membrane. Therefore, impairment of this pump leads to deregulation of ionic gradients across membrane resulting in increased intracellular Na⁺, Ca²⁺ and Cl⁻ concentration and efflux of K⁺. This facilitates increased uptake of water from the extracellular space by the astrocytes which gives rise to cytotoxic brain edema (Khanna et al., 2014).

Increased AChE activity in the ischemia/reperfusion control group is a reflection of the damaging effect of ischemia and reperfusion on cholinergic neurotransmission (Ray et al., 2014). AChE, a membrane-bound enzyme found mainly in the brain, muscles, erythrocytes, lymphocytes, and cholinergic neurons, is considered another valuable therapeutic target for several neurological disorders (Mukherjee et al., 2007; Spanevello et al., 2010; Mekinić et al., 2013; Okoshi et al., 2014). The cholinergic system performs important roles in many functions of both the CNS and peripheral nervous system and acetylcholine (ACh), the main neurotransmitter of the cholinergic system, plays vital functions in processes including movement control and modulation of cerebral blood flow (Parton et al., 2005; Kim and Kim, 2013). The hydrolytic enzyme, cholinesterase, regulates ACh levels by breaking down this neurotransmitter in many tissues. AChE activity is altered in response to redox imbalance and affected by various degree of injury to the organism which can stimulate rapid ACh degradation and consequently, decreased stimulation of ACh receptors leading to negative effects on cholinergic neurotransmission as well promotion of cognitive impairment (Elufioye et al., 2010; Abdalla et al., 2013).

The neurotransmitter, dopamine, is produced from the amino acid, tyrosine, through the catalytic action of tyrosine hydroxylase (TH) which is the rate-limiting enzyme in the pathway. (Daubner and Wang, 2011). The expression of TH is affected by ischemia (Li et al., 2004). In this study, BCCAO/R caused a significant suppression of tyrosine hydroxylase activity. Like acetylcholine, dopamine plays a crucial role in motor function. As a result, its depletion as a result of decreased activity of TH might disturb normal motor function as observed in many stroke patients (Zhang et al., 2010; Magalingam et al., 2014).

Xanthine oxidase (XO) exists primarily as xanthine dehydrogenase (XDH) in normal tissue but is cleaved by a protease and converted to XO during excitotoxic activities. Coupled to this, adenosine triphosphate (ATP) is converted to hypoxanthine, which is highly elevated during ischemia. Hypoxanthine is converted to xanthine by XO with concomitant production of superoxide ion ($O_2^{\cdot-}$ radicals) and uric acid (Parks and Granger, 1983; Granger et al., 1986). Another important activity of XO is the oxidation of xanthine, leading to formation of H_2O_2 and OH^{\cdot} radicals. The BCCAO/R-induced increase in XO activity may be one of the triggers of the increased ROS production and part of the mechanism that links oxidative stress to cerebral ischemic damage.

Inflammation plays a key role in the pathophysiology of ischemic injury (Carrizo et al., 2007; Altermann et al., 2017). Our result shows that BCCAO/R-induced ischemic damage in rats caused a significant increase in myeloperoxidase (MPO) activity. This observation, which supports pro-inflammatory mechanism in ischemic neuronal damage, corroborates similar findings from previous studies (Crack et al., 2006; Akinmoladun et al., 2015). Additionally, there appears to be a strong link between inflammation and tyrosine hydroxylase expression (Ahmed et al., 2011). Inflammation suppresses dopamine production through reduced expression of TH in sympathetic neurons and contribution to local depletion of TH in neuronal cells following cardiac ischemia-reperfusion (Dziennis and Habecker, 2003; Li et al., 2003; Harukuni and Bhardwaj, 2006).

Increase in MDA level and decreased GSH level in the brain of BCCAO/R alongside decreased activities of brain enzymic antioxidants (SOD, catalase and GPx) compared with the SO group are indicative of oxidative stress which is one of the hallmarks of cerebral ischemia (Tulsulkar and Shah, 2013; Akinmoladun et al., 2015; Sood et al., 2016). Neuronal oxidative stress can result from elevated generation of ROS ($O_2^{\cdot-}$ and OH^{\cdot}) leading to further production of other radicals and disruption of the antioxidant system. OH^{\cdot} radical is highly unstable and reacts spontaneously with biomolecules of the cell including lipids, protein and DNA leading to a loss of cellular integrity, enzymatic function and genomic stability (Valko et al., 2007; Parrish et al., 2008). Results obtained from our study gives insight and further corroborates reports from several studies on ROS generation as a cellular event that leads to oxidative neuronal damage in BCCAO/R induced cerebral ischemia (Crack and Taylor, 2005; Saeed et al., 2007; Ghosh et al., 2011).

Excitotoxicity, oxidative stress, inflammation and neurotransmitter dysregulation are key targets in stroke therapy. These processes are underpinned by altered activities of key enzymes such as those investigated in this study which has revealed the possible neuromodulatory, antioxidant and anti-inflammatory properties of *A. africana* via antiexcitotoxic, antioxidant, anti-inflammatory and other mechanistic routes leading to conferment of protection against BCCAO/R insult. Conclusively, this study demonstrated the protective property of *A. africana* leaf extract against cerebral ischemia/reperfusion-induced injury via amelioration of several damaging biochemical cascades of ischemic injury and suggested its potential relevance as a veritable pharmacological intervention against stroke.

References

1. Abdalla, F.H., Cardoso, A.M., Pereira, L.B., Schmatz, R., Gonçalves, J.F., Stefanello, N., Fiorenza, A.M., Gutierrez, J.M., Serres, J.D. da S., Zanini, D., Pimentel, V.C., Vieira, J.M., Schetinger, M.R.C., Morsch, V.M., and Mazzanti, C.M. (2013). Neuroprotective effect of quercetin in ectoenzymes and acetylcholinesterase activities in cerebral cortex synaptosomes of cadmium-exposed rats. *Molecular and Cellular Biochemistry*, 381: 1–8.
2. Ádám-Vizi, V., and Seregi, A. (1982). Receptor independent stimulatory effect of noradrenaline on Na,K-ATPase in rat brain homogenate. Role of lipid peroxidation. *Biochemical Pharmacology*, 31: 2231–2236.
3. Adewuyi, A., Rachapudi, B.N., Prasad, Bhamidipati, V., Rao, S.K., and Oderinde, R.O. (2010). Oil composition, mineral nutrient and fatty acid distribution in the lipid classes of underutilized oils of *Trilepisium madagascariense* and *Antiaris africana* from Nigeria. *Food Research International* 43: 665–670.
4. Ahmed, L.A., Salem, H.A., Attia, A.S., and Agha, A.M. (2011). Pharmacological preconditioning with nicorandil and pioglitazone attenuates myocardial ischemia/reperfusion injury in rats. *European Journal of Pharmacology*, 663: 51–58.
5. Akinmoladun, A.C., Obuotor, E.M., and Farombi, E.O. (2010). Evaluation of Antioxidant and Free Radical Scavenging Capacities of Some Nigerian Indigenous Medicinal Plants. *Journal of Medicinal Food*, 13: 444–451.
6. Akinmoladun, A.C., Akinrinola, B.L., Olaleye, M.T., and Farombi, E.O. (2015). Kolaviron, a Garcinia kola Biflavonoid Complex, Protects Against Ischemia/Reperfusion Injury: Pertinent Mechanistic Insights from

- Biochemical and Physical Evaluations in Rat Brain. *Neurochemical Research*, 40: 777–787.
7. Altermann, C.D., Souza, M.A., Schimidt, H.L., Izaguirry, A.P., Martins, A., Garcia, A., Santos, F.W., and Mello-Carpes, P.B. (2017). Short-term green tea supplementation prevents recognition memory deficits and ameliorates hippocampal oxidative stress induced by different stroke models in rats. *Brain Research Bulletin*, doi: 10.1016/j.brainresbull.2017.03.007 (in press).
 8. Arumugam, T.V., Granger, D.N., and Mattson, M.P. (2005). Stroke and T-cells. *Neuromolecular Medicine*, 7: 229–242.
 9. Banso, A., and Mann, A. (2008). Evaluation of antibacterial properties of flavonoid fraction from *Antiaris africana* (Engl). *Journal of Applied Biosciences*, 12: 665 - 670.
 10. Benarroch, E.E. (2005). Neuron-astrocyte interactions: partnership for normal function and disease in the central nervous system. *Mayo Clinic Proceedings*, 80: 1326–1338.
 11. Brouns, R., and De Deyn, P.P. (2009). The complexity of neurobiological processes in acute ischemic stroke. *Clinical Neurology and Neurosurgery*, 111: 483–495.
 12. Carrizo, G.J., Wu, R., Cui, X., Dwivedi, A.J., Simms, H.H., and Wang, P. (2007). Adrenomedullin and adrenomedullin-binding protein-1 downregulate inflammatory cytokines and attenuate tissue injury after gut ischemia-reperfusion. *Surgery*, 141: 245–253.
 13. Connell, B.J., Saleh, M.C., Rajagopal, D., and Saleh, T.M. (2017). UPEI-400, a conjugate of lipoic acid and scopoletin, mediates neuroprotection in a rat model of ischemia/reperfusion. *Food and Chemical Toxicology*, 100:175-182.
 14. Crack, P.J., and Taylor, J.M. (2005). Reactive oxygen species and the modulation of stroke. *Free Radical Biology & Medicine*, 38: 1433–1444.
 15. Crack, P.J., Taylor, J.M., Ali, U., Mansell, A., and Hertzog, P.J. (2006). Potential contribution of NF-kappaB in neuronal cell death in the glutathione peroxidase-1 knockout mouse in response to ischemia-reperfusion injury. *Stroke; a Journal of Cerebral Circulation*, 37: 1533–1538.
 16. Craine, J.E., Hall, E.S., and Kaufman, S. (1972). The isolation and characterization of dihydropteridine reductase from sheep liver. *Journal of Biological Chemistry*, 247: 6082–6091.
 17. Daubner, S.C., Le, T., and Wang, S. (2011). Tyrosine hydroxylase and regulation of dopamine synthesis. *Archives of Biochemistry and Biophysics*, 508: 1–12.
 18. Dziennis, S., and Habecker, B.A. (2003). Cytokine suppression of dopamine-beta-hydroxylase by extracellular signal-regulated kinase-dependent and -independent pathways. *The Journal of Biological Chemistry*, 278: 15897–15904.
 19. Eiserich, J.P., Hristova, M., Cross, C.E., Jones, A.D., Freeman, B.A., Halliwell, B., and van der Vliet, A. (1998). Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature*, 391: 393–397.
 20. Ellman, G.L., Courtney, K.D., Andres, V., and Feather-Stone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7: 88–95.
 21. Elufioye, T.O., Obuotor, E.M., Sennuga, A.T., Agbedahunsi, J.M., and Adesanya, S.A. (2010). Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some selected Nigerian medicinal plants. *Revista Brasileira de Farmacognosia*, 20: 472–477.
 22. Ghosh, N., Ghosh, R., and Mandal, S.C. (2011). Antioxidant protection: A promising therapeutic intervention in neurodegenerative disease. *Free Radical Research*, 45: 888–905.
 23. Gill, L.S. (1992). *Ethnomedical Uses of Plants in Nigeria*. Uniben Press.
 24. Granger, D.N., Höllwarth, M.E., and Parks, D.A. (1986). Ischemia-reperfusion injury: role of oxygen-derived free radicals. *Acta Physiologica Scandinavica. Supplementum*, 548: 47–63.
 25. Gulec, M., Gurel, A., and Armutcu, F. (2006). Vitamin E protects against oxidative damage caused by formaldehyde in the liver and plasma of rats. *Molecular and Cellular Biochemistry*, 290: 61–67.
 26. Gupta, S., and Gupta, Y.K. (2017). Combination of *Zizyphus jujuba* and silymarin showed better neuroprotective effect as compared to single agent in MCAo-induced focal cerebral ischemia in rats. *Journal of Ethnopharmacology*, 197:118-127.
 27. Harukuni, I., and Bhardwaj, A. (2006). Mechanisms of brain injury after global cerebral ischemia. *Neurologic Clinics*, 24: 1–21.
 28. Jollow, D.J., Mitchell, J.R., Zampaglione, N., and Gillette, J.R. (1974). Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*, 11: 151–169.
 29. Kalani, A., Chaturvedi, P., Kamat, P.K., Maldonado, C., Bauer, P., Joshua, I.G., Tyagi, S.C., and Tyagi, N. (2016). Curcumin-loaded embryonic stem cell exosomes restored neurovascular unit following ischemia-reperfusion injury.

The International Journal of Biochemistry & Cell Biology, 79:360-369.

30. Khanna, A., Kahle, K.T., Walcott, B.P., Gerzanich, V., and Simard, J. M. (2014). Disruption of Ion Homeostasis in the Neurogliovascular Unit Underlies the Pathogenesis of Ischemic Cerebral Edema. *Translational stroke research*;5(1):3-16).
31. Kim, G., and Kim, E. (2013). Effects of treadmill training on limb motor function and acetylcholinesterase activity in rats with stroke. *Journal of Physical Therapy Science*, 25: 1227–1230.
32. Kuete, V., Vouffo, B., Mbaveng, A.T., Vouffo, E.Y., Siagat, R.M., and Dongo, E. (2009). Evaluation of *Antiaris africana* methanol extract and compounds for antioxidant and antitumor activities. *Pharmaceutical Biology*, 47: 1042–1049.
33. Li, W., Knowlton, D., Van Winkle, D.M., and Habecker, B.A. (2004). Infarction alters both the distribution and noradrenergic properties of cardiac sympathetic neurons. *American Journal of Physiology. Heart and Circulatory Physiology*, 286: H2229-2236.
34. Li, W., Knowlton, D., Woodward, W.R., and Habecker, B.A. (2003). Regulation of noradrenergic function by inflammatory cytokines and depolarization. *Journal of Neurochemistry*, 86: 774–783.
35. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein Measurement with Folin Phenol Reagent. *Journal of Biological Chemistry*, 193: 265–275.
36. Macrae, I. (2011). Preclinical stroke research – advantages and disadvantages of the most common rodent models of focal ischaemia. *British Journal of Pharmacology*, 164: 1062–1078.
37. Magalingam, K.B., Radhakrishnan, A., and Haleagrahara, N. (2014). Protective effects of flavonol isoquercitrin, against 6-hydroxy dopamine (6-OHDA) - induced toxicity in PC12 cells. *BMC Research Notes*, 7: 49.
38. Mdzinarishvili, A., Sumbria, R., Lang, D., and Klein, J. (2012). Ginkgo extract EGb761 confers neuroprotection by reduction of glutamate release in ischemic brain. *Journal of Pharmacy & Pharmaceutical Sciences: A Publication of the Canadian Society for Pharmaceutical Sciences, Societe Canadienne Des Sciences Pharmaceutiques*, 15: 94–102.
39. Mekinić, I.G., Burcul, F., Blazević, I., Skroza, D., Kerum, D., and Katalinić, V. (2013). Antioxidative/acetylcholinesterase inhibitory activity of some Asteraceae plants. *Natural Product Communications*, 8: 471–474.
40. Misra, H.P., and Fridovich, I. (1972). The univalent reduction of oxygen by reduced flavins and quinones. *Journal of Biological Chemistry*, 247: 188–192.
41. Mukherjee, P.K., Kumar, V., Mal, M., and Houghton, P.J. (2007). Acetylcholinesterase inhibitors from plants. *Phytomedicine*, 14: 289–300.
42. Okoshi, Y., Tanuma, N., Miyata, R., and Hayashi, M. (2014). Melatonin alterations and brain acetylcholine lesions in sleep disorders in Cockayne syndrome. *Brain and Development*, 36: 907–913.
43. Omotayo, B. I., Olaleye, M. T., Akinmoladun, A.C., and Alawode, T. T. (2016). HPLC quantification of phenolic content and assessment of methanolic extract of *Antiaris africana* for toxicological study. *African Journal of Biotechnology*, 15(9): 320-330.
44. Parks, D.A., and Granger, D.N. (1983). Ischemia-induced vascular changes: role of xanthine oxidase and hydroxyl radicals. *American Journal of Physiology*, 245: G285-289.
45. Parrish, D.C., Gritman, K., Van Winkle, D.M., Woodward, W.R., Bader, M., and Habecker, B.A. (2008). Postinfarct sympathetic hyperactivity differentially stimulates expression of tyrosine hydroxylase and norepinephrine transporter. *American Journal of Physiology. Heart and Circulatory Physiology*, 294: H99–H106. doi:10.1152/ajpheart.00533.2007.
46. Parton, A., Coulthard, E., and Husain, M. (2005). Neuropharmacological modulation of cognitive deficits after brain damage. *Current Opinion in Neurology*, 18: 675–680.
47. Ramanathan, M., Sivakumar, S., Anandvijayakumar, P.R., Saravanababu, C., and Pandian, P.R. (2007). Neuroprotective evaluation of standardized extract of *Centella asiatica* in monosodium glutamate treated rats. *Indian Journal of Experimental Biology*, 45: 425–431.
48. Ray, R.S., Rai, S., and Katyal, A. (2014). Cholinergic receptor blockade by scopolamine and mecamylamine exacerbates global cerebral ischemia induced memory dysfunction in C57BL/6J mice. *Nitric Oxide*, 43:62-73.
49. Reis, C., Akyol, O., Ho, W. M., Araujo, C., Huang, L., Applegate II, R., and Zhang, J. H. (2017). Phase I and Phase II Therapies for Acute Ischemic Stroke: An Update on Currently Studied Drugs in Clinical Research. *BioMedical Research International*, 2017, 4863079.
50. Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., and Hoekstra, W.G. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science (New York, N.Y.)*, 179: 588–590.
51. Saeed, S.A., Shad, K.F., Saleem, T., Javed, F., and Khan, M.U. (2007). Some new prospects in the understanding of the molecular basis of the pathogenesis of stroke. *Experimental Brain Research*, 182: 1–10.
52. Shiman, R., Akino, M., and Kaufman, S. (1971). Solubilization and Partial Purification of Tyrosine Hydroxylase

from Bovine Adrenal Medulla. *Journal of Biological Chemistry*, 246: 1330–1340.

53. Sinha, A.K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry*, 47: 389–394.
54. Sood, A., Kumar, A., Dhawan, D.K., and Sandhir, R. (2016). Propensity of *Withania somnifera* to Attenuate Behavioural, Biochemical, and Histological Alterations in Experimental Model of Stroke. *Cellular and Molecular Neurobiology*, 36(7):1123-38.
55. Spanevello, R.M., Mazzanti, C.M., Bagatini, M., Correa, M., Schmatz, R., Stefanello, N., Thomé, G., Morsch, V.M., Becker, L., Bellé, L., de Oliveira, L., and Schetinger, M.R.C. (2010). Activities of the enzymes that hydrolyze adenine nucleotides in platelets from multiple sclerosis patients. *Journal of Neurology*, 257: 24–30.
56. Sunil, A.G., Kesavanarayanan, K.S., Kalaivani, P., Sathiya, S., Ranju, V., Priya, R.J., Pramila, B., Paul, F.D.S., Venkatesh, J., and Babu, C.S. (2011). Total oligomeric flavonoids of *Cyperus rotundus* ameliorates neurological deficits, excitotoxicity and behavioral alterations induced by cerebral ischemic-reperfusion injury in rats. *Brain Research Bulletin*, 84: 394–405.
57. Towfighi, A., and Saver, J.L. (2011). Stroke declines from third to fourth leading cause of death in the US: historical perspective and challenges ahead. *Stroke*, 42:2351–2355.
58. Tulsulkar, J., and Shah, Z.A. (2013). Ginkgo biloba prevents transient global ischemia-induced delayed hippocampal neuronal death through antioxidant and anti-inflammatory mechanism. *Neurochemistry International*, 62: 189–197.
59. Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry and Cell Biology*, 39: 44–84.
60. Varshney, R., and Kale, R.K. (1990). Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *International Journal of Radiation Biology*, 58: 733–743.
61. Zhang, W., Miao, Y., Zhou, S., Wang, B., Luo, Q., and Qiu, Y. (2010). Involvement of Glutamate Transporter-1 in Neuroprotection against Global Brain Ischemia-Reperfusion Injury Induced by Postconditioning in Rats. *International Journal of Molecular Sciences*, 11: 4407–4416.
62. Zwingmann, C., and Butterworth, R. (2005). An update on the role of brain glutamine synthesis and its relation to cell-specific energy metabolism in the hyperammonemic brain: further studies using NMR spectroscopy. *Neurochemistry International*, 47: 19–30.