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Lasianthera africana Aqueous Extract Protects the Cerebral Cortex and CA1 Hippocampal Region against Ischemic-reperfusion Insult by Bilateral Common Carotid Artery Occlusion in Adult Male Wistar Rats

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ABSTRACT

Animal models of cerebral ischaemia make a significant contribution to the knowledge of the pathogenesis of stroke and its methods of management. Many treatments have failed to reduce neuronal injury in cerebral ischaemia, prompting the exploration of new treatment models. The purpose of this study was to investigate the antioxidant potential of *Lasianthera africana* aqueous extract (LAAE), which is rich in phenols, flavonoids, and vitamin C, against induced ischemic stroke in adult male albino rats. Forty adult male albino rats were randomly divided into five groups, each with eight rats. The control was group A, which received water only. Group B received 2500 mg/kg of LAAE orally daily for 10 days. Group C had bilateral common carotid artery occlusion (BCCAO) only. Group D received 2500 mg/kg of LAAE orally for 10 days before BCCAO, and Group E underwent BCCAO before receiving 2500 mg/kg of LAAE for 10 days. The study assessed various parameters, namely morphology, neurobehavioral, biochemical, and histological. Biochemical factors assessed were: superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), and reduced glutathione (GSH). *Lasianthera africana* aqueous extract ameliorated certain neurobehavioral impairments and attenuated the decreased activities of SOD, CAT, GSH, and reduced lipid peroxidation, caused by acute cerebral ischaemia. Additionally, it improved BCCAO-induced histopathological alteration in the cerebral cortex and CA1 hippocampal neurons. In conclusion, *L. africana* extract demonstrated neuroprotective effects against acute cerebral ischaemia induced by bilateral common carotid artery occlusion. Notably, its neuroprotective potential was more pronounced when administered before the onset of cerebral ischaemia.

Keywords: *Lasianthera africana*, cerebral ischaemia, stroke, reperfusion, neuroprotective.

INTRODUCTION

Cerebral ischaemia-reperfusion injury is a common pathological feature of various cerebrovascular diseases, including ischaemic stroke, where the sustained reduction and abrupt restoration of blood flow can swiftly lead to irreversible damage in nervous tissue [1,2]. Cerebral ischaemic stroke ranks as the leading global cause of mortality and primarily contributes to disability. It is associated with high mortality and morbidity rates and can result in lasting neurological deficits, including memory and learning impairments [3, 1]. The brain's requirement for more oxygen and its low-level antioxidant makes it highly susceptible to oxidative damage, which is exacerbated during cerebral ischaemia/reperfusion (I/R), leading to the generation of reactive oxygen species (ROS) and resulting in protein oxidation, DNA damage, cell death, and lipid peroxidation in the brain[4]. Reperfusion characterized by the generation of superoxide anion free radicals, hydroxyl free radicals, and nitric oxide (NO) can further lead to lipid peroxidation, inflammation, and cellular apoptosis [5]. The primary mechanism of damage and neurodegeneration in cerebral ischaemia is the activation of various pathways [6] and recent studies on pharmacological agents targeting these pathways (metabolism, apoptosis, immune modulation, angiogenesis) had unsuccessful clinical translation [7]. Free radical scavengers, such as neuroprotective drugs, enhance brain cell protection and boost brain tissue tolerance to ischaemia and hypoxia during acute cerebral infarction [8]. Ample evidence suggests that ROS build-up right after an ischaemic stroke worsens the injury by activating mitochondrial apoptosis and compromising the BBB [8,9]. Thus,

efforts to limit ROS generation are commonly employed to aid cerebral recovery from ischaemic stroke. For instance, certain antioxidants have proven effective in safeguarding the brain against ischaemia/reperfusion-related damage by directly reducing oxidative stress and neuronal cell death through ROS scavenging. Additionally, specific drugs designed to eliminate ROS have shown great promise in protecting the nervous system. Hence, it is logical to seek a therapeutic approach that effectively reduces ROS accumulation to mitigate I/R injury and enhance neurological recovery [10, 11].

Lasianthera africana (P. Beauv) is a recognized vegetable valued for both its nutritional and medicinal qualities. In the Southern Nigerian Ibibio and Annang tribes, it is called Editan, while in the Eastern Nigerian Igbo tribe, it is referred to as Kpurugiza. Meanwhile, the Kpe and Muni tribes of West Cameroon call it Belele and Itebebele, respectively. It has a history of traditional use in treating a range of conditions such as typhoid fever, diarrhoea, candidiasis, ulcers, malaria, and diabetes in folk medicine [12-15]. The plant's fruit serves as a therapeutic choice for individuals dealing with asthma, high blood pressure, and skin ailments, and as a means of binding wounds [13]. The leaf extract has an LD_{50} of 5000 mg/kg and contains various compounds like alkaloids, flavonoids, tannins, terpenes, saponins, cardiac glycosides, and anthraquinones [16,17]. The leaves have been employed for generations as herbal remedies to treat bacterial skin infections gonorrhoea, and abdominal issues [18,19]. Additionally, the leaves are known to have high levels of phenols and flavonoids, exhibiting potent in vitro antioxidant properties [12, 20]. This present research investigated the antioxidant and ameliorative activities of *L. africana* aqueous extract against ischaemic-reperfusion injury in adult male albino rats.

MATERIAL AND METHODS

Plant material and preparation of extract

Twigs of *Lasianthera africana* (P. Beauv) along with leaves were purchased from Akpanadem market in Uyo Local Government area of Akwa Ibom state. Following the removal of stems and thorough leaf washing, the leaves were air-dried at room temperature for 3 weeks. Subsequently, the dried leaves were finely ground using a Marlex Excellent grinder from Mumbai, India. The resulting powder was then sifted through a 0.5 mm pore size to achieve a uniformly fine texture. A total of 200 grams of the powdered leaves were immersed in 1 litre of distilled water for 48 hours. Afterward, filtration was carried out using Whatman's filter paper to obtain the aqueous filtrate. The filtered solution was then evaporated at room temperature until complete dryness, and the concentrated extract was carefully stored in a refrigerator throughout the entire administration period. The toxicity study of aqueous extract of *L. africana* was earlier done and the result reported that it was not toxic even at a dosage of 5000 mg/kg for rat [17]. However, in this study, rats were administered 2500 mg/kg body weight orally for 10 days [16, 17].

Ethical approval

Approval for the research project was secured from the Animal Care and Use Research Ethics Committee (UI-ACUREC) at the University of Ibadan, located in Oyo State, Nigeria. The reference number for this approval is UI-ACUREC/App/23029.

Experimental animals

The study involved the use of forty adult male Wistar rats with weights ranging from 180 g to 240 g. The rats were acquired from the animal house of the Department of Veterinary Medicine at the University of Ibadan, located in Oyo State. The rats were randomly allocated into five experimental groups, each consisting of eight rats. Animals within each group were accommodated in transparent plastic cages and given one week to acclimate to the conditions of the animal house before the commencement of the experiment. Unrestricted access to food (Breedwell growers purchased from a feed store at Ajibode road, Ibadan, Oyo state) and water was given to the rats. Softwood shavings were used as their bedding and were changed regularly to maintain a hygienic environment. Animals were grouped and treated as shown in Table 1.

Table 1: Grouping and treatment of experimental animals

S/N	Group	Treatment
1	Control	Tap water
2	LA	2500 mg/kg of LA (single daily dose) for 10 days
3	BCCAO	Bilateral common carotid artery occlusion (BCCAO) only
4	LA+BCCAO	2500 mg/kg of LA for 10 days daily before BCCAO
5.	BCCAO+LA	BCCAO before 2500mg/kg (single dose) of *L. africana* for 10 days.

BCCAO = Bilateral common carotid artery occlusion, LA = *Lasianthera africana*

Procedure for inducing ischaemic brain injury in animals

Ischaemic brain injury was induced in rats following a previously modified method [3, 21]. In brief, the rats were anesthetized using ketamine (100 mg/kg) administered intraperitoneally, and additional doses were given as required. The rats were securely positioned on a dissection board with their heads firmly stabilized. The underside of the rats' necks was shaved and cleansed with a disinfectant, and a midline incision was then made on the neck, extending below the mandible to the manubro-sternal junction. A careful blunt dissection was employed to separate the skin from the underlying fascia and salivary glands. After gently retracting the sternocleidomastoid muscle laterally, the common carotid artery (CCA) was carefully isolated from the surrounding sternohyoid muscle and vagus nerve. A silk suture (3/0) was then passed under the CCA. This procedure was replicated on the opposite side of the neck. The CCA was temporarily blocked for 30 minutes as reported in previous studies [3, 21]. The incision in the skin was closed using interrupted sutures, and ampiclox injection (100 mg/kg body weight) was administered intraperitoneally to prevent bacterial infection. Reperfusion was achieved by carefully releasing the ligature knot, and this state was maintained for 24 hours. Subsequently, the animals were placed back into their cages with fresh bedding and were regularly monitored until they had fully recovered and were clinically stable.

Behavioural studies

Open field analysis: The open field test (OFT) serves as a conventional method to quantify exploratory behaviour and overall activity levels in rodents. It is used to assess the potential sedative, toxic, or stimulating effects of a substance, encompassing more than just the animals' movement patterns [32]. A slightly modified version of the previously reported method was employed in this experiment [23]. The setup comprised a square arena measuring 56 x 56 x 20 cm, constructed from white wood, with the floor divided into 16 squares to distinguish between central and peripheral areas. To initiate the session, each rat was placed individually in the middle of the arena, and their activity was observed for 5 minutes. Subsequently, the rats were returned to their respective cages, and the arena was sanitized with methylated spirit, allowing it to dry before the next test. Behavioural assessments included evaluating Transition (the frequency of rats crossing grid lines with all four paws), Rearing (the frequency of rats standing on their hind legs in the maze), and Grooming (duration of time spent by the animal licking or scratching itself while stationary) [3, 31].

Forelimb Grip Test: The forelimb grip strength test serves as a metric for assessing muscular strength and the coordination of skeletal muscles [31]. In this assessment, rats were subjected to a test where their front paws were located on a horizontally positioned metal wire, which was approximately 7 mm in diameter and positioned at a height of about 1 meter above a designated landing area. The duration for which each rat could maintain its grip on the wire before either falling off or resorting to supporting its forepaws with the hind paws is measured. The test lasts for a total of 60 sec. This test serves as an indicator of the muscular strength exhibited by the animals [3].

Y-maze Test: The Y-maze is a tool that evaluates short-term memory in rodents. It enables researchers to measure spontaneous alternation, a reflection of spatial working memory driven by the natural inclination of rodents to explore new environments [24]. This is a behavioural assessment test employed to measure a rat's inclination to explore a novel environment. Typically, rodents tend to show a preference for investigating a new arm of the maze instead of revisiting a previously explored one. This task employs various brain regions [24]. The Y maze apparatus consists of a Y-shaped compartment measuring 21 x 7 x 15.5 cm, featuring three equally long arms with elevated walls to prevent the animals from escaping. These arms are labelled A, B, and C. During the test procedure, the animals are gently positioned at the central junction of the Y maze, where all three arms meet. An overhead camera is positioned at a distance to record the rat's movements as it explores each arm over a 5-minute testing period. Following each rat's test session, the maze's walls and floor are cleaned with 70% ethanol. After the test, video footage is reviewed, and parameters are calculated for each rat.

Biochemical assays

Determination of lipid peroxidation level: Malondialdehyde (MDA) which is a by-product of lipid peroxidation was quantified using a method reported previously [24]. In this procedure, 0.25 mL of 0.05 M phosphoric acid was added to 0.2 mL of tissue homogenates, followed by the addition of 0.3 mL of 0.2% TBA. All samples were then subjected to 30 minutes of boiling in a water bath. Afterward, the samples were transferred to a cold bath, and 0.4 mL of n-butanol was added to each sample. Following centrifugation at 3500 rmin–1 for 10 minutes using a Thermo Scientific ST16 centrifuge, USA, the MDA content in each sample was determined.

Determination of reduced glutathione (GSH) activity: GSH concentration was assessed using 5,5'-dithiobis (2-nitrobenzoic acid) and a

spectrophotometer. After deprotonating the tissue homogenate in the centrifuge, 0.1 mL of these samples were mixed with 0.1 M phosphate buffers and 0.04% DTNB in a 3.0 mL solution (pH 7.4). The resulting product was measured at 412 nm with a Shimadzu spectrophotometer, UV-1900i, Japan. A standard GSH concentration was used to create a standard curve, enabling the calculation of GSH content expressed in nanomolar protein units [25].

Determination of catalase activity: The assay relies on catalase (CAT) catalysing the reaction between methanol and H_2O_2 to produce formaldehyde. This formaldehyde then reacts with Purpald chromogen to create a purple-coloured complex. In the procedure, cells were treated, and protein lysates were collected following the GPX method. Various formaldehyde concentrations (0-120 μM) and lysate samples were mixed in a sample buffer. The reaction, initiated with H_2O_2, was followed by Purpald chromogen addition and subsequent absorbance measurement at 540 nm using a spectrophotometer (SpectraMaxM2e). The CAT activity was calculated by extrapolation of formaldehyde standard curve [26].

Determination of superoxide dismutase (SOD) activity: A method that involves measuring the inhibition of pyrogallol antioxidants was used to evaluate the SOD activity. A fraction of SOD activity was defined as the protein amount causing a 50% reduction in pyrogallol auto-oxidation. A control without homogenate in Tris–EDTA buffer served to account for non-enzymatic pyrogallol oxidation [27].

Following the behavioural assessments, the animals were humanely euthanized. Subsequently, the brains were collected and preserved.

Haematoxylin and Eosin stain

Rat brains were halved, preserved in formalin (10%), and processed for tissue examination. This involved grossing, dehydration, clearing, embedding in paraffin wax, and microtomy. Sections were spread on a heated water bath, dried, and stained with Haematoxylin and Eosin (H&E). Results displayed blue nuclei and pink cytoplasm under a Leica ICC50 E light microscope (Germany), with images captured using an attached camera [3].

Cresyl violet stain

Cresyl violet stain is a histological technique used to visualize neuronal cells. Tissue sections are deparaffinized, rehydrated, and then immersed in Cresyl Violet solution for 2 minutes. After staining, sections are dehydrated, cleared, and mounted. Neurons appear purple, allowing for the identification and examination of their morphology under a microscope.

Histomorphometric study

Histological images captured with a Leica ICC50 E digital camera (Germany) were then analysed and measurements were made using Image J (Fiji) software.

Pyramidal cell count of *Cornu ammonis* 1 (CA1) and capillary density

For each group, eight randomly chosen photomicrographs of x400 magnification were analyzed to count CA1 pyramidal cells and capillaries in the cerebral cortex. Image J (Fiji) software was employed to calibrate the images and place a one-inch box consistently 0.5 cm away from CA2. The software's cell counter plugin was then used for manual counting of normal pyramidal cells and capillaries in the CA1 and cerebral cortex respectively. The capillary density was subsequently calculated.

Statistical analysis

The results were presented as mean ± SD. Differences between group means were evaluated using a one-way analysis of variance (ANOVA), followed by a Bonferroni post-test using computer software (SPSS). To visually represent the data, graphs were created using GraphPad Prism 9.0 software. A significance level of less than 0.05 (p-value <0.05) was used to determine statistical significance.

RESULTS

General observation

During the experiment, a total of five (5) rats died during the surgical procedure and treatment. Rats in group A (control) were active whereas rats in the BCCAO (group C) were inactive. Also, rats in the LA + BCCAO (group D) and BCCAO + LA (group E) were mildly active. BCCAO only (group C) and BCCAO + LA (group E) displayed general body weakness, reduced food and water intake, and hence, loss in body weight.

Gross morphology

A significant rise in the brain-to-body weight ratio was noticed in the rats subjected to BCCAO, in contrast to the rats in both the control and LA groups as shown in Figure 2. Also, in the body weight difference assessment shown in Table 2, the BCCAO-

only and BCCAO+LA groups experienced a notable decrease in mean body weight.

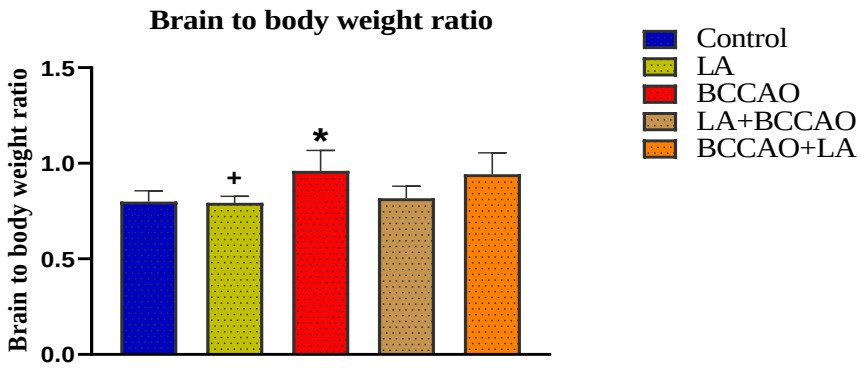

Figure 1: Effect of *Lasianthera africana* and BCCAO on brain-to-body weight ratio. Data are presented as mean±SD (n=5). BCCAO- bilateral common carotid artery occlusion. * significantly different from control ($p<0.05$). + significantly different from BCCAO ($p<0.05$).

Body weight difference

Table 2: Effect of *Lasianthera africana* and bilateral common carotid artery occlusion on body weight difference. Data are presented as mean±SD (n=5).

Groups	Initial body weight (g)	Final body weight (g)	Weight gain/loss (g)	%Weight gain or loss (g)
Control	197	209.75	12.75	6.47
LA	206	220.5	14.5	7.04^{+}
BCCAO	197.5	181.5	-16	-8.1^{*}
LA + BCCAO	194	200.75	6.75	3.48^{+}
BCCAO + LA	201.25	193.25	-8	3.98^{+}

BCCAO- bilateral common carotid artery occlusion. * significantly different from control ($p<0.05$). + significantly different from BCCAO ($p<0.05$).

Behavioural and Forelimb Grip Test

Figure 3 shows a significant decrease ($p<0.05$) in the number of transitions occurring within 5 minutes in the BCCAO, LA+BCCAO, and BCCAO+LA when compared to the control group. Conversely, there was a significant increase ($p<0.05$) in the number of transitions in the LA group in comparison to the BCCAO group. In the same figure, the BCCAO group displayed a significant reduction ($p<0.05$) in animal rearing compared to both the control group and the LA group. However, there was a non-significant increase in rearing behaviour observed in the LA+BCCAO and BCCAO+LA groups when compared to the BCCAO group. Furthermore, grooming activity within a 5-minutes duration showed a significant rise ($p<0.05$) in the BCCAO when compared across the groups. Additionally, there was a significant decrease ($p<0.05$) in grooming activity observed in both the LA and LA+BCCAO groups when compared to the BCCAO+LA group. Figure 3 also shows a notable reduction ($p<0.05$) in the number of alternations observed in both the BCCAO and BCCAO+LA groups in comparison to all other groups. The forelimb grip test result shows a significant decrease ($p<0.05$) in BCCAO+LA group when compared with BCCAO group.

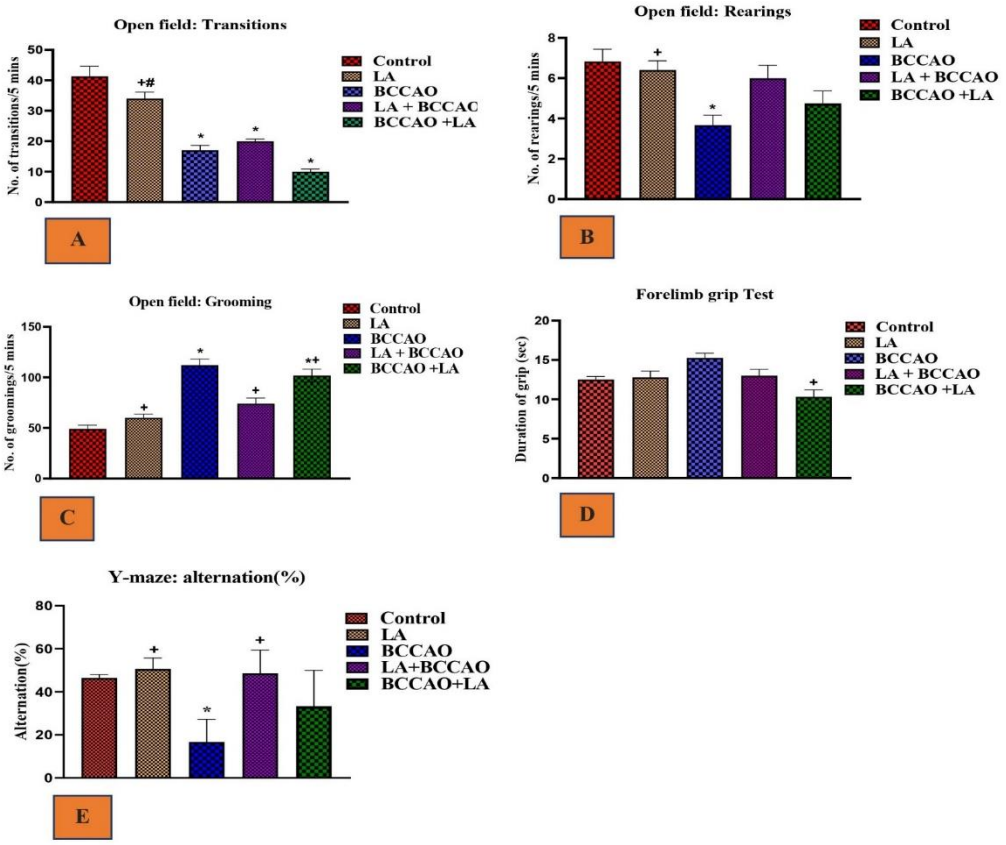

Figure 2: Effects of *Lasianthera africana* and bilateral common carotid artery occlusion on behavioural parameters. Data are presented as mean ± SD (n=5). LA - *Lasianthera Africana* extract, BCCAO- bilateral common carotid artery occlusion. * significantly different from control ($p<0.05$). + significantly different from BCCAO ($p<0.05$).

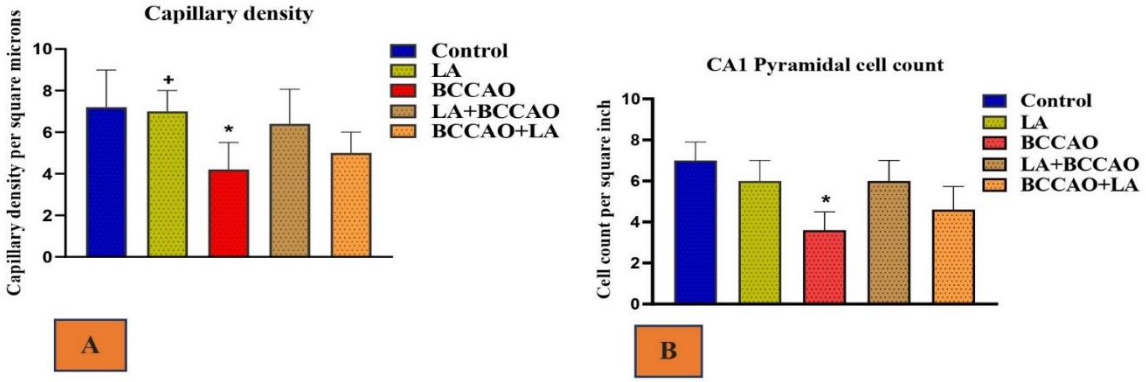

Figure 3: Effects *Lasianthera africana* and bilateral common carotid artery occlusion on cerebral cortex capillary density and CAI pyramidal cells. Data are presented as mean ± SD (n=5). MDA- Malondialdehyde, GSH- reduced glutathione, SOD – superoxide dismutase, CAT – catalase, LA - *Lasianthera Africana* extract, BCCAO- bilateral common carotid artery occlusion. * significantly different from control ($p<0.05$). + significantly different from BCCAO ($p<0.05$).

Biochemical parameters

Table 3 indicates a significant rise ($p<0.05$) in MDA levels within the BCCAO group compared to the control group, LA group, and LA+BCCAO group. Nevertheless, the increase is not statistically significant when compared to the BCCAO+LA group. In the same table, it is evident that there is a significant decrease ($p<0.05$) in the levels of GSH and the activity of SOD within the BCCAO group when compared to the control group and LA group. However, both the LA+BCCAO and BCCAO+LA treatments led to a significant increase and a non-significant increase, respectively, in these parameters when compared to the BCCAO group. Also, the BCCAO group showed a significant decrease ($p<0.05$) in CAT activity compared to the control group, LA group, and LA+BCCAO group. Additionally, the reduction in CAT activity was not statistically significant when compared to the BCCAO+LA group.

Table 3: Effect of *L. africana* and bilateral common carotid artery occlusion on biochemical parameters. Data are presented as mean±SD (n=5).

Group/Test	MDA (µmol)	GSH (mM)	SOD (units/L)	CAT (units/ml)
Control	0.998±0.016	2.479± 0.229	1.394±0.131	3.287±0.37
LA	0.934±0.506^{+}	2.732±0.173^{+}	1.381±0.038^{+}	3.196±0.401^{+}
BCCAO	2.292±0.246*	1.8319±0.109	0.934±0.17*	2.435±0.38*
LA+BCCAO	1.171±0.106^{+}	2.149±0.312	1.344±0.046^{+}	3.113±0.715^{+}
BCCAO+LA	1.459±0.571	2.099±0.4	1.247±0.123^{+}	2.968±0.851

MDA- Malondialdehyde, GSH- reduced glutathione, SOD- superoxide dismutase, CAT- catalase. LA- *Lasianthera africana* extract, BCCAO- bilateral common carotid artery occlusion. *significantly different from control ($p<0.05$). + significantly different from BCCAO ($p<0.05$).

Histological examination of the cerebral cortex and *Cornu ammonis* 1 (CA1) of hippocampal formation

The histological examination of the cerebral cortex in the Control, LA+BCCAO, and BCCAO+LA groups revealed normal cortical neurons, some of which displayed an open chromatin pattern with scattered glial cells within the parenchyma. In contrast, the representative cortical neurons in the BCCAO group exhibited a darkened appearance with signs of pyknosis, indicating cellular death. Similarly, the pyramidal neurons in the CA1 region of the Control, LA+BCCAO, and BCCAO+LA groups displayed large neurons with open chromatin nuclei, while in the BCCAO group, these neurons were dispersed and exhibited pyknotic features (refer to Fig. 5 and 7).

Histomorphometry

Figure 3 presents the quantified data for capillary density in the cerebral cortex and pyramidal cell density in the CA1 region of the rat brain, respectively. The BCCAO group displayed a significant decrease in capillary density compared to the control and LA-only groups. On the other hand, the LA+BCCAO and BCCAO+LA groups exhibited a non-significant increase in capillary density when compared to the BCCAO group. Additionally, there was a significant reduction in pyramidal cell density in the BCCAO group compared to the control group, and this reduction was non-significant when compared to the other treatment groups.

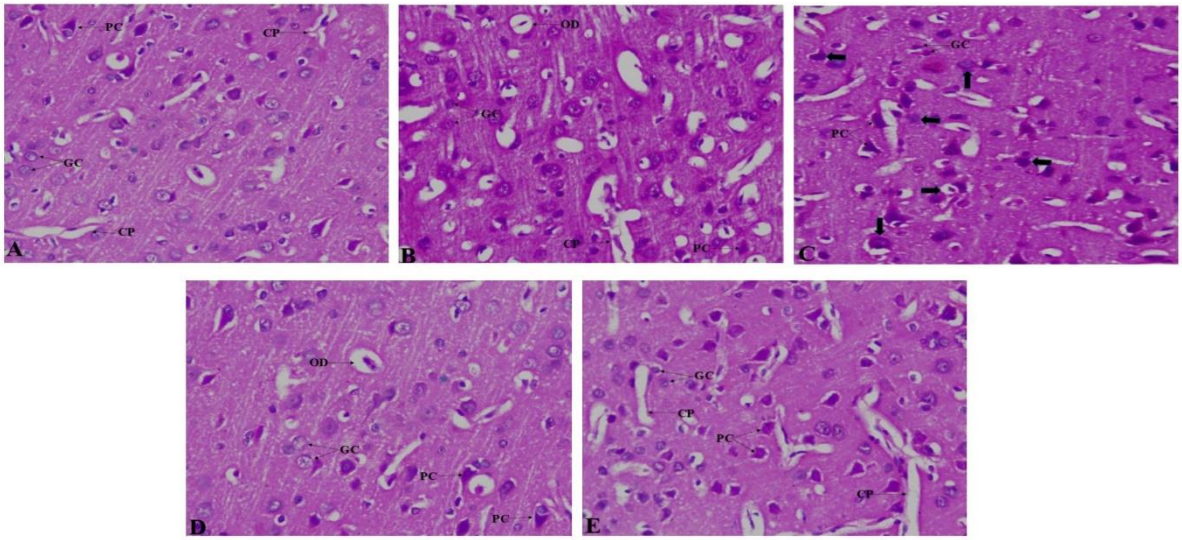

Figure 4: Photomicrographs of the cerebral cortex (internal pyramidal layer) of rats showing A- Control, B- LA, C- BCCAO, D- LA+BCCAO, and E- BCCAO+LA, H&E X 400. OD- oligodendrocyte, GC- granule cell, PC- pyramidal cell and CP- capillary. Black arrows indicate pyknotic neurons, vacuolation, and degenerating neurons in group C (BCCAO group).

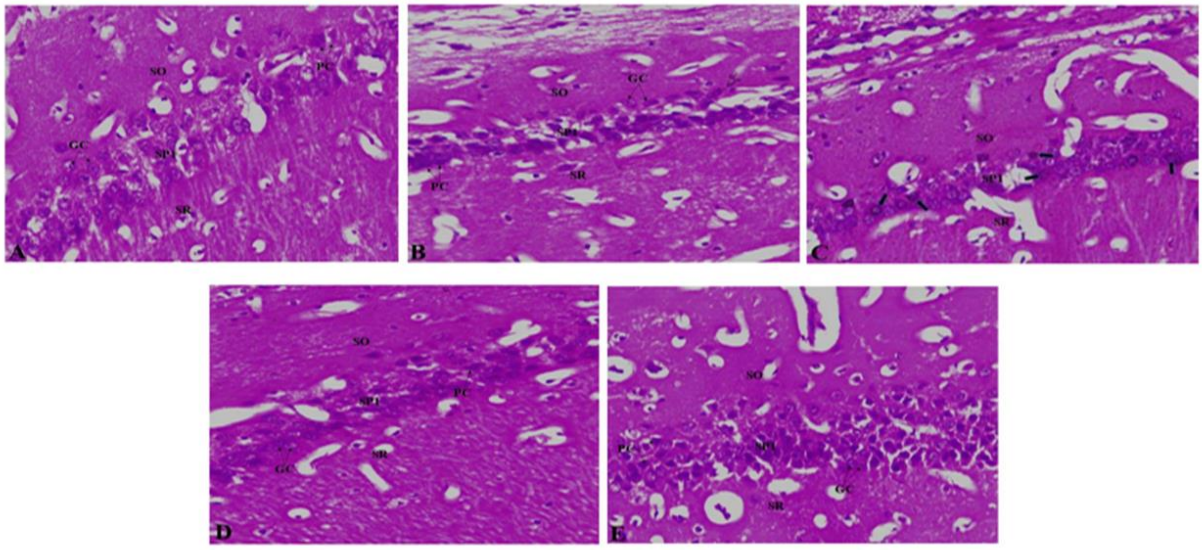

Figure 5: Photomicrographs of the CA1 hippocampal region of rats showing A- Control, B- LA, C- BCCAO, D- LA+BCCAO, and E- BCCAO+LA, H&E X 400. SO- stratum oriens, SR- stratum radiatum, SP1- stratum pyramidal of cornu ammonis 1 (CA1). Black arrows in group C (BCCAO group) indicate pyknotic neurons, vacuolation, and degenerating neurons.

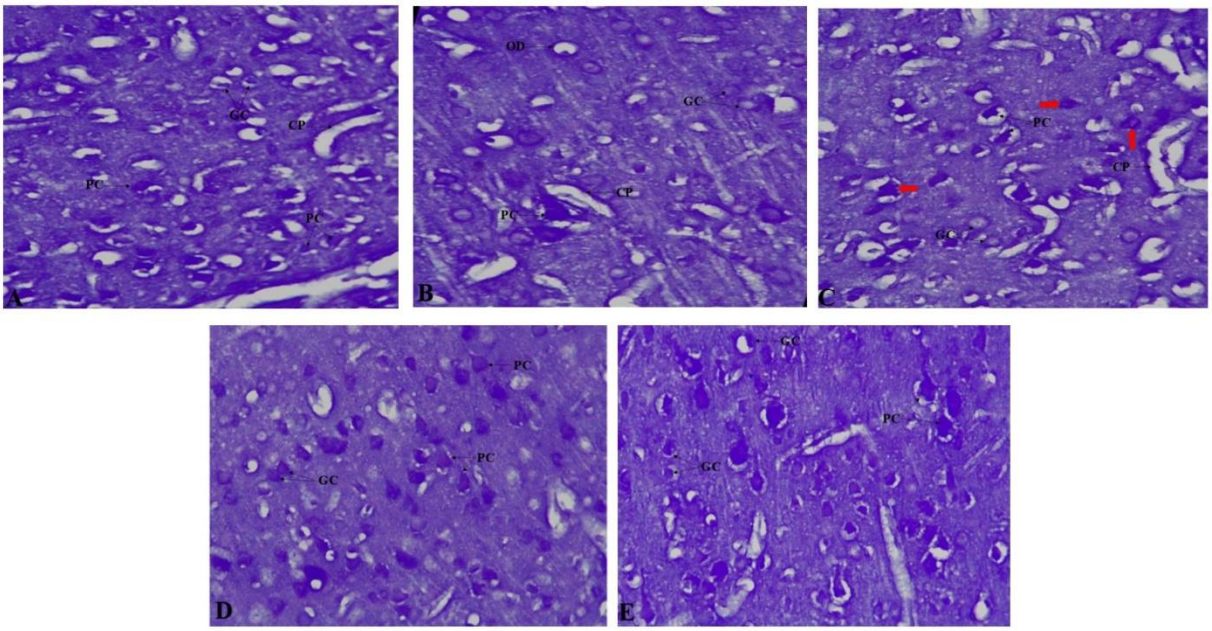

Figure 6: Photomicrographs of the cerebral cortex (internal pyramidal layer) of rats showing A- Control, B- LA, C- BCCAO, D- LA+BCCAO, and E- BCCAO+LA, Cresyl violet X 400. OD- oligodendrocyte, GC- granule cell, PC- pyramidal cell and CP- capillary. Red arrows indicate pyknotic neurons, vacuolation, and degenerating neurons in group C (BCCAO group).

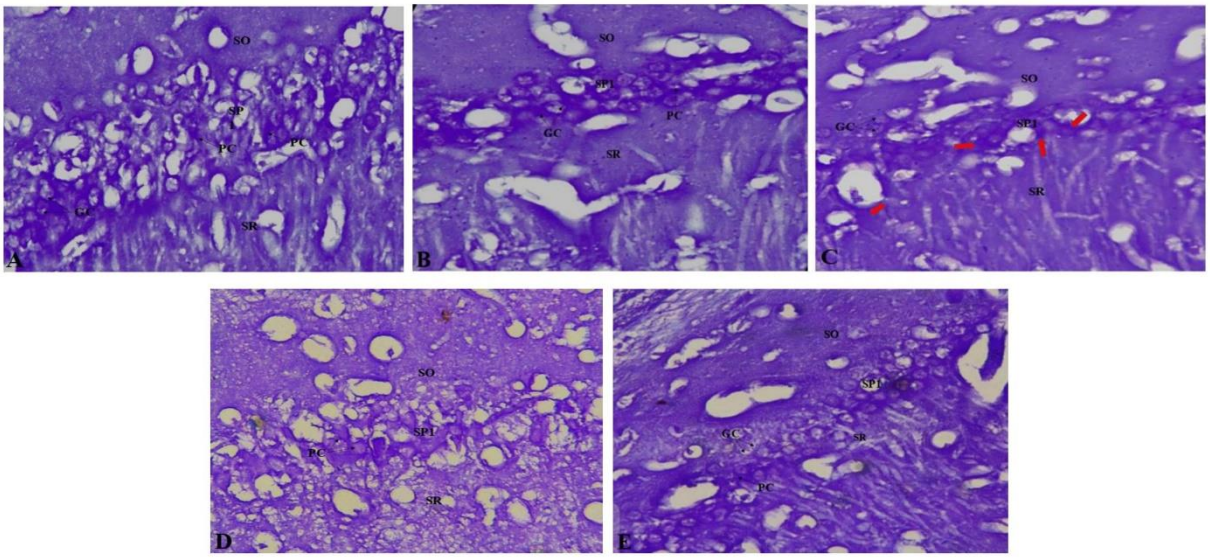

Figure 7: Photomicrographs of the CA1 hippocampal region of rats showing A- Control, B- LA, C- BCCAO, D- LA+BCCAO, and E- BCCAO+LA, Cresyl violet, X 400. SO- stratum oriens, SR- stratum radiatum, SP1- stratum pyramidal of cornu ammonis 1 (CA1). Red arrows in group C (BCCAO group) indicate pyknotic neurons, vacuolation, and degenerating neurons.

DISCUSSION

This research demonstrated that subjecting rats to BCCAO (Bilateral Common Carotid Artery Occlusion) and subsequent reperfusion for twenty-four hours had an impact on the oxidative markers in the rat brain, as well as the structure of the Cornu ammonis 1 region in the hippocampus and the neurons in the cerebral cortex. However, when rats were administered *L. africana* as a pre-treatment or post-treatment these negative effects were ameliorated. The brain-to-body weight ratio can be used as a gauge for determining an organ's condition, a large decline in the ratio's value might be linked to organ or tissue

necrosis, while a marked increase in the ratio's value could be a sign of tissue inflammation [28]. Even if there are no morphological changes, animals subjected to treatment and left untreated may have significant differences in brain weight. Brain-to-body weight analysis is also a crucial endpoint in toxicity investigations for identifying test substance effects that could be dangerous [29]. The result shows a depicting of the brain-to-body weight ratios of the rats. The evaluation of these ratios in the BCCAO group revealed a notable increase compared to both the control group and the group that received LA alone. This may be a possible indication of tissue inflammation, as earlier reported [28]. However, animal strain, age, sex, as well as environmental settings, have all been reported to have an impact on an animal's organ weight [30]. Consistent with the toxicity observations previously reported [17], the oral administration of an aqueous extract from *L. africana* (P. Beauv) did not result in acute toxicity in rats even at doses as high as 2500 mg/kg of body weight. Furthermore, the results indicated a significant reduction in the average difference in body weight among animals in both the BCCAO group and the BCCAO+LA group, in comparison to the other groups. This weight decline could be attributed to the effects of ischaemic injury, which typically led to weakened bodies and decreased food and water consumption in the animals. Based on the result of the behavioural assessment performed. Rats in the BCCAO group demonstrated a negligible increment in their grip strength, a finding that contradicts previous observation [31].

The transition rate within 5 minutes was significantly reduced in the BCCAO, LA+BCCAO, and BCCAO+LA groups compared to the control group. Generally, rats subjected to surgery showed reduced line-crossing behaviour compared to the control and LA groups. Also, rats in the pre-treated group exhibited a higher line-crossing frequency than those in the post-treatment and BCCAO groups. The BCCAO group of animals displayed a notably lower frequency of rearing (vertical movement) compared to the other groups, potentially attributed to central nervous system sedation [33]. Furthermore, the result of significantly higher grooming activity in animals in the BCCAO group when compared with animals in other groups is an indication of anxiety [33, 34]. Interestingly, the grooming activity was notably greater in the post-treated group compared to the pre-treated group, indicating that *L. africana* may have a stronger potential to alleviate anxiety when administered before cerebral ischaemia.

The number of alternations was lower in the BCCAO group compared to the control and LA groups, indicating impaired spatial working memory. Additionally, *L. africana* (P. Beauv) aqueous extract improved spatial working memory more in the pre-treated group than in the post-treated group, as seen in their higher number of alternations.

Free radicals in excess can trigger lipid, protein, and nucleic acid peroxidation, damaging cell membranes, and leading to irreversible changes, and cell death. These events can disrupt microcirculation and increase blood-brain barrier permeability in brain tissue [10]. Our result indicates that the BCCAO rat group had higher malondialdehyde (MDA) levels compared to the other groups, pointing to increased lipid peroxidation caused by cerebral ischaemia-reperfusion injury. Interestingly, *L. africana* reduced lipid peroxidation in both the pre-treated and post-treated groups, with a noteworthy but not statistically significant decrease noticed in the pre-treated group compared to the post-treated group.

Superoxide dismutase (SOD) is a crucial natural antioxidant that inhibits the generation of free radicals [35]. Multiple investigations have demonstrated that SOD, acting as an enzyme that scavenges superoxide radicals, notably diminishes neuronal injury caused by ischaemia in animal models [36]. Our research revealed a significant decrease in SOD activity in the BCCAO rats in comparison to all other groups. Once again, *L. africana* aqueous extract increased SOD activity in both the pre-treated and post-treated ischaemia-reperfusion groups, exhibiting an antioxidant potential. The reduced SOD activity combined with elevated lipid peroxidation in the untreated ischaemia-reperfusion group may suggest brain damage resulting from the build-up of free radicals [3, 10]. Glutathione (GSH) and catalase (CAT) are key components of the brain's natural antioxidant defense, responsible for removing hydrogen peroxide and lipid peroxidase. A decrease in GSH levels can lead to an increase in oxidative stress [37]. GSH is vital for protecting the brain from oxidative stress by scavenging free radicals and inhibiting lipid peroxidation. Its depletion in ischaemic reperfusion injury can result from GSH conversion, reduced production, and mixed disulfide formation [38]. Catalase functions in converting hydrogen peroxide into water and oxygen. When superoxide dismutase increases without a matching rise in catalase, hydrogen peroxide accumulates and turns into damaging hydroxyl radicals in the brain [3]. Our findings align with previous reports [3, 31], showing reduced GSH and CAT activities in the BCCAO rats. However, in both pre-treated and post-treated groups, GSH and CAT activities significantly increased compared to the BCCAO rats, highlighting the potent antioxidant effects of *L. africana* aqueous extract.

Histopathological examinations in our study show minor neuronal alterations in the cerebral cortex of BCCAO rats. In comparison to the control group, BCCAO rats' neurons exhibit slight changes in both morphology and staining patterns, along with noticeable cortical neuron degeneration. The *L.*

africana (P. Beauv) aqueous extract maintains the integrity of forebrain cortical neurons in treated ischaemic groups, confirming its neuroprotective potential. Importantly, pre-treatment provides greater neuron protection than post-treatment in the ischaemic group.

Furthermore, acute BCCAO resulted in ischaemic neuronal changes in the CA1 region of the hippocampus, consistent with prior research [3,37, 39, 40], highlighting the CA1 subfield's susceptibility to ischaemic damage in the brain. Photomicrographs of rat brain tissue showed pyknosis in the hippocampal CA1 pyramidal cells of the BCCAO group. This indicates brain cell death, leading to reduced short-term and spatial working memory, as earlier shown by the number of spontaneous alternations. Pyramidal neuron damage in the CA1 region, seen in the BCCAO animals, was less severe in the pre-treated group than in the post-treated group, as confirmed by photomicrographs. Morphometric testing showed significantly fewer viable CA1 neurons in the BCCAO rats compared to other groups, with a non-significant increase observed in the pre-treated group compared to the post-treated group.

When the mammalian brain encounters hypoxia, it triggers various compensatory mechanisms. One such mechanism involves vascular changes, including alterations in brain capillary density [3, 41]. As previously stated, an increased brain capillary density in animals receiving pre-treatment before vascular occlusion suggests improved capillary survival, enhanced blood flow, and stabilized cerebral energy levels [42]. Histomorphometry analysis revealed a significant decrease in capillary density in untreated ischaemic rats compared to control and LA groups. *L. africana* aqueous extract preserved more brain capillaries in the pre-treated group compared to the post-treated group, though these increases were not statistically significant compared to the BCCAO rats.

Conclusion

The study shows that *Lasianthera africana* aqueous extract demonstrated neuroprotective effects against oxidative stress and neuronal damage caused by bilateral carotid artery occlusion in rats. This may be due to its ability to regulate antioxidant enzyme activities because of its rich polyphenol and alkaloid content as previously reported. However, the extract is more effective when given before ischaemic injury hence preventive than when administered after injury induction.

Conflict of interest

There was no conflict of interest

Contributions

Enobong Obong financed the entire cost of the research, performed the experiment, analysed, and interpreted the data. He also wrote the manuscript. Olatunde Owoeye designed and supervised the experiments.

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