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Original Article

Bilateral Common Carotid Artery Occlusion (BCCAO)-induced Ischemic Stroke causes Cognitive Impairment and Alterations in Histoarchitectural Features of CA1 and CA3 Regions of the Hippocampus

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ABSTRACT

A stroke occurs when there is an alteration in the blood circulation within the brain, resulting in neurological impairments. This study aimed to evaluate the cognitive and histological impairment as a result of bilateral common carotid artery occlusion-induced ischemic stroke.Twelve adult male Wistar rats were divided into two groups (n=6); the Control group was only operated on and administered 2 ml/kg distilled H_2O, while the BCCAO/R group was induced with cerebral ischemia by bilateral common carotid artery occlusion and reperfusion (BCCAO/R). Following the induction of ischemic stroke, the Novel Object Recognition (NOR) test was performed to evaluate learning and memory. After experimentation, the animals were anaesthetized with chloral hydrate (350 mg/kg i.p) and euthanatized. The brains were harvested, and the hippocampi were carefully dissected. Homogenates were prepared from half of the disserted hippocampi to assess the specific activity levels of Acetylcholinesterase. The other halves were preserved in 4% paraformaldehyde. The preserved tissues were processed using routine histological procedures and stained with Haematoxylin and Eosin. The result of this study showed a significant decrease in the discrimination ratio and difference score in the BCCAO/R group when compared to the control group during the NOR test. Hippocampal AchE enzyme activity levels increased significantly ($P<0.05$) in the BCCAO/R group when compared to the control. Histological examination of the CA1 and CA3 regions of the hippocampus in the control group showed normal histoarchitecture of these regions while the BCCAO/R group demonstrated marked neuronal degenerative changes in the CA1 and CA3 regions presenting as karyorrhexis, perineural vacuolation, pyknotic neurons, dark neuron, cytoplasmic vacuolation. BCCAO-induced ischemic stroke induced changes in the cognitive function and histoarchitecture of the CA1 and CA3 regions.

Keywords: Neurological impairment, histopathology, stroke, hippocampus, Wistar rats

INTRODUCTION

The term "stroke," also known as a cardiovascular accident, is derived straight from the word "strike" because it occurs suddenly (Diji and Zhicheng, 2020). It describes a brain injury brought on by an unexpected interruption of the brain's blood flow. The definition of a stroke is "rapidly emerging clinical evidence of focal or global abnormalities in brain function, with symptoms lasting longer than 24 hours or proceeding to death, and with no known explanation other than the vascular origin" (World Health Organization, 2016). Stroke may be classified into either ischemic (embolic or thrombotic) or hemorrhagic stroke that occurs due to (intracranial haemorrhage or subarachnoid bleeding). (Lopez *et al*., 2001; Adnyana *et al*., 2017).

According to Feign *et al.* (2009) stroke is a significant public health issue since it is the top cause of disability and the third leading cause of death

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worldwide, after ischemic heart disease and cancer. More low- and middle-income nations than developed nations are adversely affected by stroke, particularly in Africa (Saqui, 2007; Ezejimofor *et al*., 2017; Owolabi *et al*., 2018). Studies have revealed that stroke mortality rates in Nigeria are quite high, ranging from 21 to 45 per cent in various locations (Desalu *et al*., 2011).

Cerebral ischemia injury is one of the most common causes of mortality and morbidity (Mozaffarian *et al*., 2015; Johnson *et al*., 2019). The second largest cause of mortality worldwide, cerebral stroke accounts for over 800,000 new or recurrent cases and 11.8 per cent of all fatalities worldwide (Naghavi *et al*., 2017; Benjamin *et al*., 2019). Ischemic stroke is the primary focus of cerebral stroke research worldwide since it accounts for 87 per cent of cerebral stroke cases globally, compared to 13 per cent of hemorrhagic instances (Mozaffarian *et al*., 2015, Virani *et al*., 2021).

Cerebral ischemic stroke occurs owing to the inadequate blood supply to a part of the brain (focal) or the whole brain (global) in which blood and oxygen cannot reach parts of the brain supplied by the vessel, causing neurological difficulties, mostly affecting sensory-motor and cognition (Benjamin and Paul-Muntner, 2019). To better understand the pathophysiological mechanisms of stroke, numerous experimental models (both local and global) have been created to replicate these stroke occurrences in humans (Liguz-Lecznar and Kossut, 2013; Fluri *et al*., 2015; Sommer, 2017; Uzdensky, 2017). Bilateral common carotid artery occlusion (BCCAO) is one of the most frequently used techniques for generating cerebral ischemia in animal models, especially in rat models (Durukan and Tatlisumak, 2007). This procedure temporarily closes both common carotid arteries, reducing blood supply to the brain and causing cerebral ischemia (Wang *et al*., 2021). BCCAO-induced cerebral ischemia in rats has been demonstrated to mimic some of the pathophysiological alterations seen in ischemic stroke in people, including oxidative stress, inflammation, and neuronal death (Durukan and Tatlisumak, 2007). This model has been extensively utilized to look into the processes that lead to stroke aetiology and to assess prospective therapy approaches (Li *et al*., 2022).

Researchers have demonstrated that stroke causes damage to the central nervous system (CNS), which results in functional deficits (Cramer, 2008), and that this immune response is made worse by inflammation that is produced by both the innate and adaptive immune systems (Gelderblom *et al*., 2009). Certainly, a stroke can cause cognitive and memory problems, motor dysfunctions, neuronal death, and dementia (Pendlebury *et al*., 2009; Jangg *et al*., 2010).

While BCCAO-induced cerebral ischemia in rats has been extensively used to study the pathophysiology of ischemic stroke, there is a paucity of data on the effects of BCCAO-induced ischemic stroke on cognition and histological changes in the hippocampus. Recent studies have attempted to address this knowledge gap, but there is still a dearth of information. Therefore, this study evaluated the cognitive and histological impairment as a result of bilateral common carotid artery occlusion-induced ischemic stroke.

MATERIALS AND METHOD

Ethical consideration

The Ahmadu Bello University Ethics Committee on Animal Use and Care gave this study's ethical approval, with approval code ABUCAUC/2023/076.

Chemicals

Chloral hydrate, used to anaesthetize the animals during animals euthanization, was manufactured by SAE Manufacturing Specialties Corporation, 86A Bayville Avenue, Bayville NY 11709.

Ketamine and xylazine, used to anaesthetize the animals during surgical procedures, were manufactured by Swiss Parenterals PVT Ltd, Gujarat, India, and 2915 Rocky Mountain Avenue, Suite 400 Loveland, CO 80538 respectively.

Chlorhexidine, used as a disinfectant, was manufactured by Drug Field Pharmacy Shop in Zaria, Kaduna and obtained from Yabo Pharmacy, Zaria, Kaduna State, Nigeria. .

Lidocaine, used as a local anaesthesia, was manufactured by Asset Pharmacy Lagos and obtained from Yabo Pharmacy, Zaria, Kaduna State, Nigeria. .

The rat AChE ELISA kit used in this study was produced by Fine Test Company, Wuhan Fine Biotech Co. LTD, Wuhan, China.

Experimental animals

Before the start of the research, twelve (12) adult male Wistar rats (200-250g) were obtained and kept in the Animal House of the Human Anatomy Department, Faculty of Basic Medical Sciences, Ahmadu Bello University (ABU, Zaria, for one week in clean plastic cages with soft wood shavings as bedding. There was enough food and water available for the rats.

Experimental protocol

The rats were divided into two groups of 6 rats each: The control group was operated without occluding the bilateral common carotid artery and orally administered 2 ml/kg body weight of distilled H_2O

while the BCCAO/R group was induced with cerebral ischemia by occluding the common carotid artery of both sides.

Induction of cerebral ischemia in rats by bilateral common carotid artery occlusion and reperfusion (BCCAO/R) model

The rats were anaesthetized with 80 mg/kg body weight of ketamine and 5 mg/kg body weight of xylazine injected intraperitoneally. Once the rats were unconscious (reached the anaesthetic plane), the fur on their necks was shaved, and cleansed with 70 % ethanol and Chlorhexidine, the rats were thereafter placed in the supine position on the operating table with adhesive tape to hold their paws and tails to the surgical table. The upper margin of the sternum was cut with surgical scissors to create a shallow mid-ventral cervical incision (about 1-1.5 cm long). A pair of ocular forceps was used to delicately relocate the submandibular gland. This made it easier to reach the common carotid artery, which is encased with the vagus nerve in the carotid sheath between the muscles, by exposing the sternohyoid and sternocleidomastoid muscles. The vagus nerves were carefully preserved, the soft tissues were removed, and both the right and left common carotid arteries were carefully separated. The carotid arteries were clamped for 30 minutes using a non-traumatic artery clamp to cause ischemia. Care was taken to prevent bleeding and blood vessel piercing. The clamp was released from both arteries to allow for reperfusion after 30 minutes of cerebral ischemia. Visual inspection of the arteries was done to ensure that the blood flow stopped when the clamp was applied and resumed after it was removed. After cleaning with 70 % ethanol and applying an Bivatracin® an antibiotic powder spray, the skin was sutured and lidocaine was applied as a local anaesthetics and analgesic agent. Except for blocking the carotid arteries, surgical procedures in the control group were identical to those in BCCAO-operated animals. During the operation, the rat's rectal temperature was monitored with a rectal probe and maintained at 37±1 ^{o}C with a thermostat-controlled heating pad to avoid hypothermia. A corneal protectant was applied to each eye to prevent the eys from drying. The animals were returned to their home cages with access to food and water after being kept warm until they had fully recovered from the anaesthetics (Sun *et al*., 2022).

Novel Object Recognition (NOR) test

The Novel Object Recognition (NOR) test is an effective method for evaluating learning and memory in rodents (Ennaceur and Meliani, 1992). There is no requirement for reinforcers because it is based on rodents' innate propensity for exploring new things (Lueptow, 2017). A Plexiglas box (40 x 40 x 40 cm in length, breadth, and height) was used for the test. Three phases make up the task: habituation, training, and testing. On the first day of habituation, the rats were given 20 minutes to freely explore the arena without any object present, to acquaint themselves with the surroundings. On the second day of training, two identical objects were positioned in the arena and the rats were given 10 minutes to investigate them. On the third day of testing, one of the objects was exchanged with a new one, and the rats were placed in the arena for 7 minutes to examine the two objects. Tests 1 and 2 in the current study were carried out 24 and 48 hours after habituation, respectively. The assessment of recognition memory was assessed using the novel object index (NOI), which is the time spent exploring the novel item divided by the time spent exploring the two objects (Chen *et al*., 2021).

Between each trial, the objects were cleaned with a piece of rag soaked in natural rat odour that had been preserved in filthy sawdust from cages housing other rats that were roughly the same age to rule out the chance of fragrance traces remaining on the objects. The objects were washed with a 70 % alcohol solution after each day's testing, dried, and then put back into the filthy sawdust (Braida *et al*., 2011).

The time (in seconds) spent examining the familiar object (TF), the time (in seconds) spent exploring the new object (TN), and the sum of the time (in seconds) spent exploring both things (TF + TN) are among the parameters that were measured. The following equation was used to determine the discrimination index (DI) percentage:

DI (%) = $\frac{TN}{(TN+TF)} * 100\%$ (Khan *et al.,* 2023)

Euthanasia

Following completion of the experiment, the animals were anaesthetized with chloral hydrate (350mg/kg body weight, intraperitoneally). The anaesthetized rats were decapitated and the brains were removed from the calvarium. The harvested whole brains were dissected on ice at the midline into two halves, one half, the hippocampus was surgically extracted and homogenized for biochemical analysis and the other half was fixed in 4 % paraformaldehyde for 24 hours before histological processing.

Acetylcholinesterase (AChE) Activity

ELISA Kit was used. AChE activities were assayed according to the manufacturer's instruction

Histological studies

Light Microscopy

Fixed brains were processed using histological techniques. The processing of the brain slices included fixation, dehydration, clearing, infiltration, and embedding in paraffin wax. Haematoxylin and

eosin were used to assess the overall histology of the hippocampus (CA1 and CA3 regions). The stained sections were inspected under a light microscope by a pathologist who is not a member of the group, and photomicrographs were obtained with an AmScope MD 900 digital microscope camera at a magnification of 250X.

Statistical analysis

IBM Statistics was used to perform an independent sample *t*-test on the results, which were expressed as mean ± standard error (14.0 for the window evaluation version). Statistics were considered significant at values of $p \leq 0.05$.

RESULTS

Novel object recognition (NOR) test

Discrimination index following induction of Ischemia/reperfusion

There was a notable decrease ($P<0.05$) in the discrimination ratio in the BCCAO/R group (induced ischemic/ reperfusion) when compared to the control group (2 ml/kg body weight of distilled water) (Figure 1A).

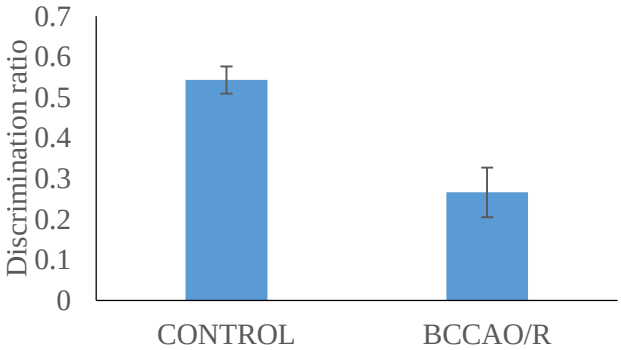

Figure 1A: Discrimination ratio of Wistar rats following induction of Ischemia/reperfusion

Difference score following induction of Ischemia/reperfusion

There was a statistically significant decrease ($P<0.05$) in the difference score in the BCCAO-induced ischemic/ reperfusion group when compared to the control group (2 ml/kg distilled water)(Figure 1B).

Acetylcholinesterase (AchE) activity levels

Hippocampal AchE enzyme activity levels increased significantly ($P<0.05$) in the BCCAO-induced ischemic/ reperfusion group when compared to the control (2 ml/kg distilled water) (Figure 2).

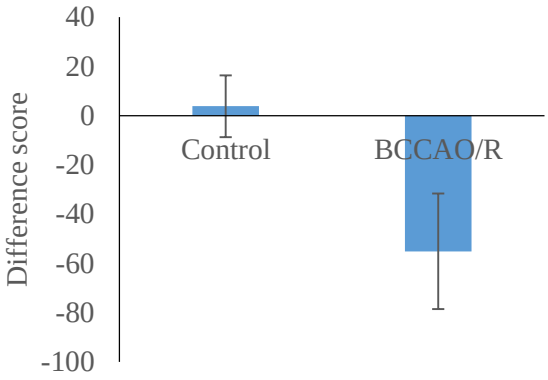

Figure 1B: Difference score of Wistar rats following induction of Ischemia/reperfusion

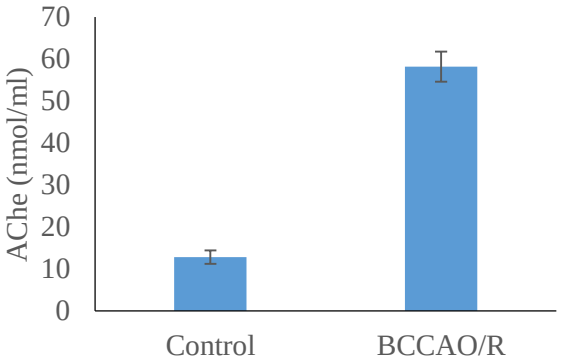

Figure 2: Mean hippocampal acetylcholinesterase (AchE) enzyme activity levels of Wistar rats following induction of Ischemia/reperfusion

Histological (Haematoxylin and Eosin stain) features of the hippocampus (CA1 and CA3 regions)

Histological examination of the hippocampal region CA1 and CA3 in the control group (2 ml/kg distilled water) showed normal histoarchitecture of these regions; the basic pattern of an ordered sheet of neurons (pyramidal and granules cells), whose cell bodies are all packed together. Large and sparse pyramidal cells in the CA3 region, and smaller and closely packed pyramidal cells in the CA1 region (Plate 1A & C). Examination of hippocampal sections of the BCCAO/R group (induced ischemic/ reperfusion) demonstrated marked neuronal degenerative changes in the CA1 and CA3 regions presenting as karyorrhexis, perineural vacuolation, pyknotic neulei dark neuron, cytoplasmic vacuolation (Plate 1B & D).

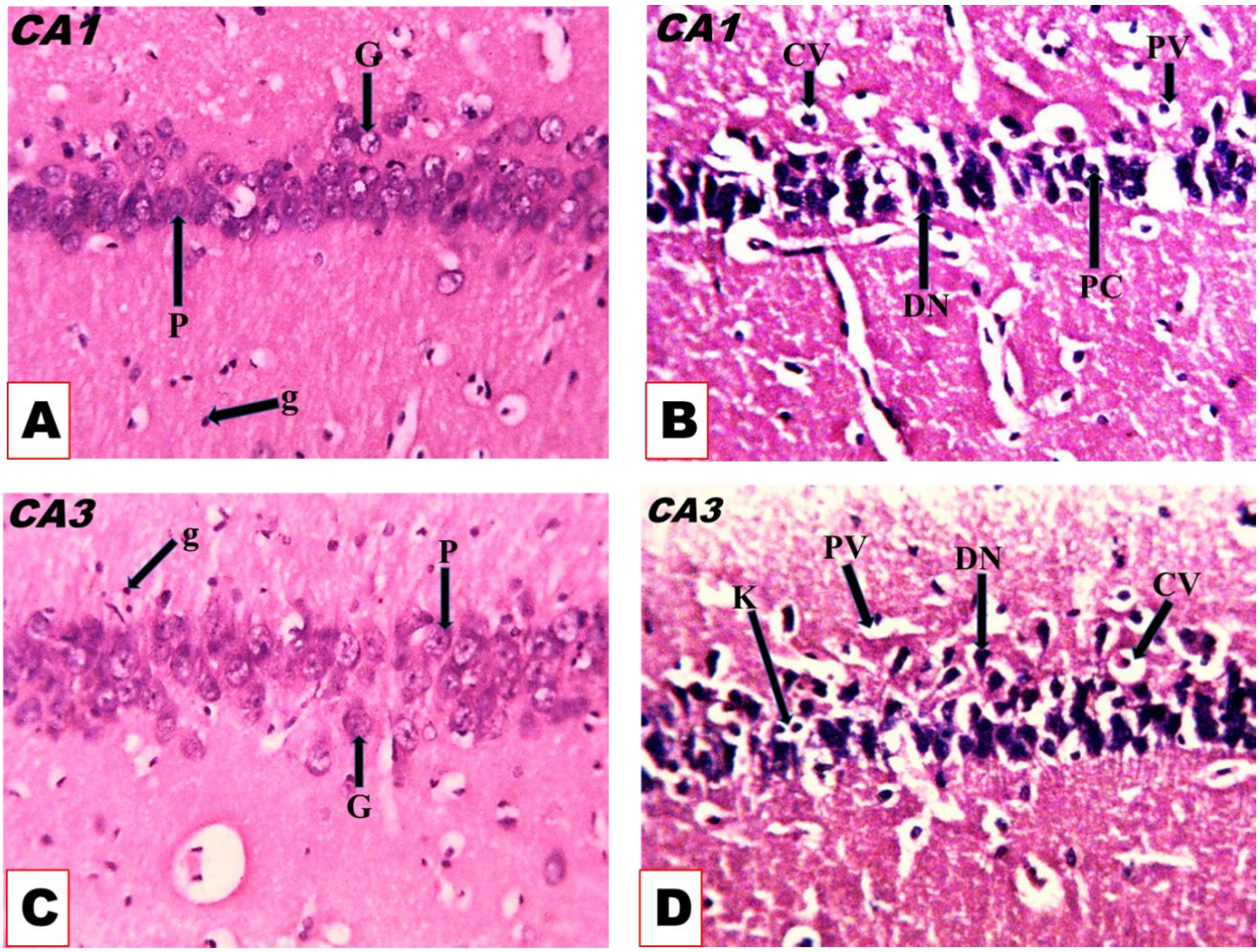

Plate 1: Section of Wistar rat hippocampus (CA1 and CA3) (A) Control group (2 ml/kg distilled water) showing normal histoarchitecture of the CA1. Compact layer of pyramidal cells. (B) BCCAO/R group (induced ischemic/reperfusion) showing marked neuronal degeneration in the histoarchitecture of the CA1. (C) Control group (2 ml/kg distilled water) showing normal histoarchitecture of the CA3. Large and sparse pyramidal cells. (D) BCCAO/R group (induced ischemic/ reperfusion) showing marked neuronal degeneration in the histoarchitecture of the CA3. Pyramidal cell (P); Granule cell (G); Glial cell (g); Pyknotic cell (PC); Karyorrhexis (K); Dark neuron (DN); Cytoplasmic vacuolation (CV); Perineural vacuolation (PV). H and E stain (Mag x250)

DISCUSSION

Ischemic stroke, one of the most severe neurological conditions that causes irreversible declines in motor and cognitive functions, is still a major problem (Pan *et al*., 2017). Following a stroke, up to 80 % of people are at risk of developing cognitive dysfunction, including mild cognitive impairment (Sachdev *et al*., 2014). Memory is the most affected domain, followed by attention and executive function, in terms of cognitive impairments among the more well-established neurobehavioral consequences of brain ischemia (Nys *et al*., 2005; Sachdev *et al*., 2014). In the bilateral common carotid artery occlusion (BCCAO) model of inducing an ischemic stroke, both common carotid arteries are blocked, which results in a general decrease in cerebral blood flow. This decrease in blood flow has been linked to cognitive dysfunction, including problems with recognition memory and spatial memory, as well as neuronal death (Li *et al*., 2016). Researchers have shown that BCCAO-induced ischemic stroke causes problems with recognition memory, and these difficulties may be connected to the inflammation and neuronal damage caused by the stroke (Li *et al*., 2016). Additionally, there are deficiencies in spatial memory in rodents with BCCAO-induced ischemic stroke, raising the possibility that the hippocampus and other brain areas associated with spatial memory have been damaged. In a similar vein, post-mortem investigations have revealed that hippocampus injury may play a significant role in the development of cognitive impairment following ischemia episodes (Pantoni & Garcia, 1997). Memory impairment is specifically linked to CA1 hippocampal injury (Duvernoy, 2005; Bartsch, 2012).

The object recognition task (ORT) was used in this study to evaluate memory impairment in terms of non-spatial learning (recognition memory). Recognition memory grants the capacity to distinguish between unfamiliar and familiar objects, and the functional integrity of the temporal lobe, particularly the hippocampus, is essential for this ability (Kaundal *et al*., 2018). The Wistar rats in the BCCAO/R group showed an impaired preference for the novel object compared to the rats in the control group in the performance of the Novel Object Recognition (NOR) test, indicating deficits in learning and memory capability. This was indicated by a notable decrease in discrimination scores and difference scores in the NOR test.

According to studies, cerebral hypoperfusion, oxidative stress, inflammation, and excitotoxicity are changes that occur after a stroke caused by BCCAO. These alterations can harm neurons and impair the normal operation of brain circuits that control cognitive functions (Yang *et al*., 2019). Long-term cognitive problems may result from stroke's neurodegeneration, synapse loss, and impaired neuroplasticity (Li *et al*., 2016). Similar to this, BCCAO-induced stroke can increase the expression of the neuroinflammatory proteins interleukin-1 (IL-1) and tumour necrosis factor (TNF) in the hippocampus (Yang *et al*., 2019). According to research by Li *et al*. (2016), BCCAO-induced stroke enhanced expression of the excitotoxic NMDA receptor subunit NR2B, which can result in neuronal injury. Studies have also investigated the impact of mitochondrial malfunction and oxidative stress on post-stroke cognitive impairment. For instance, Zhang's (2014) research discovered that BCCAO-induced stroke increased oxidative stress and mitochondrial dysfunction in the hippocampus, which can result in neuronal damage and cognitive deficiencies. The results of this study are in line with a substantial number of other studies that found that ischemic stroke caused by BCCAO impairs many types of memory (Li *et al*., 2016; Truiti *et al*., 2015). In a test of novel object identification, Kaundal *et al*. (2018) found that BCCAO-treated rats were unable to distinguish between familiar and unfamiliar objects. Similarly, Jang *et al*. (2022) used the novel object recognition test to document cognitive impairments in mice seven days after a 20-minute BCCAO-induced ischemic stroke. Several other researchers have also documented memory issues in rodents following BCCAO-induced ischemic stroke utilizing NOR test (Schiavon *et al*., 2014; Wang *et al*., 2021).

Acetylcholine (ACh) is an important component of cell membranes that contributes to their integrity and regulates changes in permeability during synaptic transmission and conduction (Tsakiris *et al*., 2006). It is closely associated with learning and memory, and it undergoes metabolism by the enzyme AChE, resulting in the production of choline and acetyl-CoA. AChE plays a crucial role in maintaining the balance of acetylcholine in the body, making it a significant biomarker for cholinergic nervous system function. The cholinergic system in the brain is essential for cognitive function and the regulation of cerebral circulation (Zhao *et al*., 2015). Studies, both clinical and non-clinical, have shown that reduced synaptic levels of acetylcholine contribute to cognitive impairment in stroke patients (Umukoro *et al*., 2019). Animal studies involving BCCAO-induced stroke have consistently demonstrated increased activity of acetylcholinesterase in all three brain regions, consistent with previous findings (Umukoro *et al*., 2019). Accumulating evidence from animal studies and patients with vascular dementia strongly suggests that impaired cholinergic function may be responsible for the symptoms observed in vascular dementia (Roman, 2005).

The findings of this study demonstrated a significant increase in AChE activity in the hippocampus of the BCCAO-induced ischemic stroke group compared to the control group. The decreased levels of synaptic ACh, which indicate impaired central cholinergic function, have been attributed primarily to the elevated activity of AChE (Gyeyeop & Kim, 2013). Furthermore, reduced concentrations of ACh resulting from increased AChE enzyme activity have been observed in the cerebral tissues of ischemic stroke patients and rats subjected to BCCAO (Ladecola & Anrather, 2011). Studies have indicated that ischemic stroke can lead to heightened acetylcholinesterase activity in the brain. The precise mechanisms underlying this increase are not fully understood; however, several factors may be involved. One potential explanation is the release of oxidative stress-related factors, such as reactive oxygen species (ROS), during ischemia. ROS can activate signalling pathways that promote AChE activity (Rice & Russo-Menna, 1998). Additionally, inflammatory processes occurring after ischemic stroke can influence AChE activity. Inflammation can trigger the release of cytokines, which can modulate the expression and activity of AChE (Bonaventura *et al*., 2008). Numerous studies have reported enhanced AChE activity in the brain following ischemic stroke, suggesting a potential role in the pathophysiology of the condition (Yan, 2019). The findings of this study are consistent with numerous previous studies. Ahmad *et al*. (2021) conducted a study evaluating the cerebro-protective effect of *Ricinus communis* leaves against ischemia-reperfusion injury in rats and reported a significant increase ($p < 0.01$) in acetylcholinesterase (AChE) activity. Furthermore, Polopalli *et al*. (2022) observed a significant elevation in acetylcholinesterase activity

in the cortex ($p < 0.05$), hippocampus ($p < 0.01$), and amygdala ($p < 0.01$) regions of the brain in the BCCAO group compared to the sham control group. Numerous other studies have also reported a significant increase in acetylcholinesterase activity in the brain following BCCAO-induced ischemic stroke (Gaur & Kumar, 2011; Kakkar *et al*., 2013; Ilesanmi *et al*., 2017; Umukoro *et al*., 2018; Bhatia *et al*., 2021; Benedicta *et al*., 2023).

The hippocampus is a brain region that plays a critical role in learning and memory, and its histology is altered in response to BCCAO-induced ischemic stroke. An important feature of global ischemia-reperfusion injury is a substantial delayed apoptotic neuronal death in the hippocampus, generally occurring several days after the event and is most evident in the CA1 (Cornu Ammonis) region of the hippocampus (Bhuiyan *et al*., 2015). Additionally, the CA3 region of the hippocampus and certain areas of the cerebral cortex has been revealed to be damaged due to ischemia-reperfusion injury (Kirino, 2000; Cho *et al*., 2007; Wang *et al*., 2017). The dysregulation in these subsequent regions is due to neuronal loss as well as glial cell dysfunctioning as a result of interrupted oxygen and blood glucose supply (Wang *et al*., 2017). Upon ischemic hypoxia, a series of events are triggered which leads to neuronal damage in different areas of the brain due to disruption of ionic balance, cell swelling, inflammation, excitotoxicity, and apoptosis (Murakami *et al*., 2005; Kim *et al*., 2010; Magnus *et al*., 2012). This may result in the loss of critical brain functions like cognitive capacity, grip strength, and motor coordination. The extent of brain injury will determine whether or not the brain will be able to function (Knowles *et al*., 2016).

Microscopic studies using haematoxylin and eosin revealed a normal histoarchitecture in the CA1 and CA3 regions of the hippocampus in the control group. However, the BCCAO-treated rats demonstrated marked neuronal degenerative changes in the CA1 and CA3 regions presenting as karyorrhexis, perineural vacuolation, pyknotic cell, dark neuron, and cytoplasmic vacuolation. This is an indication that the role of the hippocampus in learning and memory is impeded as shown in the NOR test. The blood flow and oxygen supply to the brain may decrease as a result of the BCCAO-induced ischemic stroke, mismatching the metabolic needs of the brain tissue with the availability of nutrients and oxygen. This may eventually set off a chain of events that contribute to the hippocampus's neuronal damage and cell death by causing energy failure, excitotoxicity, oxidative stress, and inflammation (Dirnagl *et al*., 1999). Apoptotic pathways can also be activated by BCCAO. Caspases are triggered during the programmed cell death process known as apoptosis in response to ischemia injury. Caspases break cellular proteins and ultimately cause cell death. In the hippocampus of rats, BCCAO-induced ischemic stroke significantly increased the expression of caspase-3, demonstrating the activation of apoptotic pathways, according to Biernaskie *et al*. (2004). In addition to the loss of blood flow, oxidative stress, and inflammation are also important mechanisms underlying BCCAO-induced hippocampal damage. Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the ability of the cells to detoxify these species. Tang *et al*. (2019) found that BCCAO-induced ischemic stroke caused a significant increase in the production of ROS in the hippocampus of mice, leading to oxidative stress and hippocampal damage. Inflammation is a response of the brain to injury and involves the activation of immune cells and glial cells. BCCAO-induced ischemic stroke can cause an increase in the expression of inflammatory markers in the hippocampus of rats, leading to inflammation and hippocampal damage (Yu *et al*., 2010). This study is consistent with several other studies that also observed similar findings. Several studies suggest that BCCAO induction of stroke causes significant histological changes in the CA1 and CA3 subfields of the hippocampus, including neuronal loss, apoptosis, pyknosis, necrosis, and reactive gliosis. These changes are accompanied by cognitive deficits in animals (Gao *et al*., 2013; Armstead *et al*., 2019; Li *et al*., 2020; Camargos *et al*., 2020). In conclusion, the result of this study showed that BCCAO-induced ischemic stroke resulted in neurological deficit as indicated in the novel object recognition test and the level of acetylcholinesterase activity. Also, histoarchitectural alterations were observed in the hippocampus's CA1 and CA3 regions of the hippocampus.

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