The protective effect of *Carica papaya* and vitamin E on ischaemic-reperfusion insult of rat brain following bilateral occlusion of common carotid artery

O Owoeye, A Mpetcha and AO Malomo
Department of Anatomy, College of Medicine, University of Ibadan, Ibadan, Nigeria

Abstract

Introduction: Ischaemic stroke is a leading cause of death and neurological disability. Stroke models in animals attempt to mimic the events in human. This study investigated the possible neuroprotective effect of *Carica papaya* leaf aqueous extract (CPAE) and vitamin E on induced ischaemic-reperfusion injury from bilateral common carotid artery occlusion (BCCAO) in rats brain.

Materials and methods: Thirty-five female rats were randomly assigned into one of five groups (n=7): Control (1 mL distilled water); CPAE (500 mg/kg); BCCAO; BCCAO + CPAE (500 mg/kg); BCCAO + VIT E (500 mg/kg). BCCAO was carried out on day 21 for 30 minutes followed by 24 hours of reperfusion, while CPAE was administered daily for 21 days. Behavioural tests were done on day 22 after which rats were euthanized and biochemical and histological changes were assessed.

Results: The BCCAO produced significant ($p<0.05$) elevation in lipid peroxidation and reduced glutathione levels while increasing superoxide dismutase and catalase activities. It also significantly reduced the number of lines crossed, rearing, duration of forelimb grip but increased duration of negative geotaxis. It induced scattered pyknotic neurons in cerebral cortex and pyramidal neurons in the CA1 subfield of the hippocampus. Pretreatment with CPAE and vitamin E improved oxidative, behavioural response and histological alterations of the neurons in both cerebral cortex and CA1 subfield of the hippocampus.

Conclusion: The results support a protective role for CPAE and vitamin E on acute ischaemia/reperfusion injury induced by BCCAO in rats, thus contributing to the continuous search for neuroprotective strategies in stroke.

Keywords: *Bilateral common carotid artery occlusion, ischaemic/reperfusion injury, Carica papaya, oxidative damage, hippocampus, behavioural.*

Abstrait

Introduction : L'Accident Vasculaire Cérébral (AVC) ischémique est l'une des principales causes de décès et d'invalidité neurologique. Les modèles d'AVC chez les animaux tentent d'imiter les événements chez l'homme. Cette étude a examiné l'effet neuroprotecteur possible de l'extrait aqueux de feuille de *Carica pagaya* (CPAE) et de la vitamine E sur les lésions de reperfusion ischémique induites par l'occlusion bilatérale de l'artère carotide commune (BCCAO) dans le cerveau des rats.

Matériels et méthodes : Trente-cinq rats femelles ont été répartis au hasard dans l'un des cinq groupes (n = 7) : contrôle (1 ml d'eau distillée) ; CPAE (500 mg / kg) ; BCCAO ; BCCAO + CPAE (500 mg / kg) ; BCCAO + VIT E (500 mg / kg). La BCCAO a été réalisée au jour 21 pendant 30 minutes, suivies de 24 heures de reperfusion, tandis que la CPAE a été administrée quotidiennement pendant 21 jours. Des tests comportementaux ont été effectués au jour 22, après quoi des rats ont été euthanasiés et les modifications biochimiques et histologiques ont été évaluées.

Résultats : BCCAO a produit une élévation significative ($p <0,05$) de la peroxydation lipidique et une réduction des niveaux de glutathion tout en augmentant les activités de la superoxyde dismutase et de la catalase. Il a également réduit de manière significative le nombre de lignes croisées, l'élevage, la durée de l'adhérence des membres antérieurs, mais a augmenté la durée de la géotaxie négative. Il a induit des neurones pycnotiques dispersés dans le cortex cérébral et des neurones pyramidaux dans le sous-champ CA1 de l'hippocampe. Un prétraitement avec du CPAE et de la vitamine E a amélioré la réponse oxydative, comportementale et les altérations histologiques des neurones dans le cortex cérébral et le sous-champ CA1 de l'hippocampe.

Conclusion : les résultats confirment le rôle protecteur de la CPAE et de la vitamine E dans les lésions d'ischémie / reperfusion aiguë induites par BCCAO chez les rats, contribuant ainsi à la recherche continue de stratégies neuroprotectrices dans les accidents vasculaires cérébraux.

Mots clés : *Occlusion bilatérale de la carotide commune, lésion ischémique / reperfusion, Carica papaya, dommage oxydatif, hippocampe, comportemental*

Correspondence: Dr O. Owoeye, Department of Anatomy, College of Medicine, University of Ibadan, Ibadan, Nigeria. E-mail: owoeye2001@yahoo.com: o.owoeye@mail.ui.edu.ng

Introduction

In humans, ischaemic/reperfusion injury may occur in conditions such as stroke, cardiac arrest, subarachnoid hemorrhage, or head trauma [1]. A stroke, also known as a cerebrovascular accident, is the rapid loss of brain function(s) due to disturbance in the blood supply to the brain. Stroke may be due to ischaemia caused by blockage (thrombosis, arterial embolism) (85%) or haemorrhage (15%) [2, 3]. Tissue damage is observed maximally during reperfusion which is primarily attributed to oxidative injury resulting from the production of oxygen free radicals which exacerbate cerebral ischaemic injury [4]. Oxygen free radicals initiate lipid peroxidation which attack and inflict damage to the macro-cellular components of the cells that are crucial for cell function thus modifying their chemical and histological structures [5].

Consequent upon ischaemic injury, the affected area of the brain cannot function optimally and might result in hemiplegia, inability to understand or formulate speech, or an inability to see one side of the visual field [6]. The incidence of stroke is 254/100,000 population yearly in the United Kingdom (UK), 330/100,000 in Taiwan, and varies between 100 and 300/100,000 in the United State of America (USA) [7]-. In Nigeria, the Report of a Stroke Registry in Ibadan gave the incidence of stroke as 26/100,000 populations in 1977 [8] and in Lagos 1.14/1,000 [9]. In industrialized countries, ischaemic stroke accounted for about 10% - 17% of all deaths [10] and in Nigerian hospitals, it constituted 3.7% of emergency admissions, 8.7% of medical admissions, and 4-17% of medical deaths [11].-

Increasing interest in improving treatment options for ischaemic stroke requires the continuous exploration of new treatments options that may lead to a viable clinical application [12]. There is increasing interest in the neuroprotective potentials of plant natural products with antioxidative activity with the aim of reducing vulnerability of brain tissue to ischaemia since antioxidants curtail the damage caused by reactive oxidative species released during the reperfusion phase of stroke. *Carica papaya*, is a lozenge tropical fruit present in orange-red, yellow-green and yellow-orange hues, with a rich orange pulp. The whole *C. papaya* plant including its leaves, seeds, ripe and unripe fruits, and their juice have been used as traditional medicine [13]. It is a rich source of three vitamins: A, C and E; the minerals: magnesium and potassium; the B vitamin pantothenic acid and folate and fiber and is thus considered a nutraceutical fruit because of its multifaceted medicinal properties [13]. Phytochemically, the plant contains enzymes (papain), carotenoids, alkaloids, monoterpenoids, flavonoids, minerals and vitamins [13].

Vitamin E (α-tocopherol) is a primary membrane bound, lipid-soluble, chain-breaking antioxidant that has been reported to protect against lipid peroxidation-induced tissue damage [14]. Vitamin E pre-treatment has been reported to be beneficial in preventing 2, 2-dichlorovinyl dimethyl phosphate injury [15], formaldehyde-induced tissue damage in rats [14], gamma-radiation injury [16] and phenytoin-induced haematotoxicity and brain oxidative stress [17].

The cerebral cortex is the seat of cognitive functions as well as the control of movement while the hippocampus is involved in memory, learning and spatial cognition [18, 19]. Mammalian brain has been reported to be vulnerable to oxidative stress injury because of its high rate of oxidative metabolic activity, intense production of reactive oxygen species metabolites and relatively low antioxidant capacity [20]. Since oxidative damage has been implicated in ischaemia-reperfusion brain damage, we hypothesized that antioxidant augmentation prior to ischaemic injury should minimize the effect.

The study aimed to investigate the effect of the anti-oxidative properties of *Carica papaya* leaf aqueous extract on induced brain ischaemia by bilateral common carotid artery occlusion in rat brain, using vitamin E as a standard antioxidant.

Materials and methods

Plant material and extract preparation

Fresh, ripe mature fruits of *Carica Papaya* were purchased from Oje market, Ibadan, Nigeria in September, 2014. The fruits were identified and authenticated at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria, as *Carica Papaya* Linn *Caricaceae* and given the Forest Herbarium Identification Number (FHI.110033) where a voucher specimen deposited. The fresh fruits of ripe *Carica Papaya* were peeled, seeds were removed and the pulp then cut into pieces. Five hundred grammes of the fruits were weighed and blended into a beaker and 1.5 L of distilled water added to soak the diced *Carica Papaya* overnight. The juice was filtered using a Whatmann filter paper and concentrated using a rotary evaporator. The filtrate was oven-dried at 40 °C to give a total yield of 162.5 g and a percentage yield of 32.50%. The dried *Carica Papaya* aqueous extract termed (CPAE) was diluted with distilled water and administered orally with syringe and clean intra-gastric gavage at a daily dose of 500 mg/kg for 21 days according to the method of Nayak *et al.* [21].

Ethical approval

The University of Ibadan Ethical Committee's approval with reference number UI-ACUREC/App/2014/002 was obtained and all procedures on animal handling conformed to the acceptable guidelines on the ethical use of animals in research.

Preparation and administration of α-tocopherol (vitamin E)

Each soft gelatin capsule containing 100 mg vitamin E acetate (BIOFEM Pharmaceuticals Nig. Ltd) was neatly and completely aspirated out with the 1 mL insulin syringe. The syringe was thereafter attached to a clean intra-gastric gavage through which each rat was administered orally the measured dose of 500 mg/kg/daily for 21 days [16].

Experimental animals

Thirty-five matured female rats of *Wistar* strain weighing from 150 g – 200 g used for the study were obtained from breeders in College of Medicine Animal House, University of Ibadan. The animals were acclimatized for one week and then randomized into experimental and control groups and housed in plastic cages measuring 39 x 29 x 27 cm with soft wood shavings at room temperature with a 12 hour light/dark cycle. They were fed with standard rat diet (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*.

Experimental design

The 35 female rats were randomly assigned into one of five treatment groups of seven animals per group and then allowed one week to acclimatize to animal house conditions before administration of intervention parameters all through the remaining period of the research. The duration of treatment was twenty-one days. The rats were grouped as in Table 1 below:

Behavioural study

On the 22nd day of the experiment, behavioural study, wire grip test and negative geotaxis test were conducted on all rats:

Open field test

The open field apparatus was constructed of white plywood and measured 72 x 72 cm with 36 cm walls. Blue lines were drawn on the floor with a marker and were visible through the clear Plexiglas floor. The lines divided the floor into sixteen 18 x 18 cm squares. A central square (18 cm x 18 cm) was drawn in the middle of the open field with a central square distinct from the outer locations [22]. Rats were carried to the test room in their home cages and were handled by the base of their tails at all times. Rats were placed into the center of the open field and allowed to explore the apparatus for 5 minutes. After the 5 minute test, rats were returned in their home cages and the open field was cleaned with 70 % ethyl alcohol and permitted to dry between tests. The behaviours scored [23] included: Line Crossing (the frequency with which the rats crossed one of the grid lines with all four paws), Rearing (vertical posture), Stretch Attend Postures (frequency with which the animal demonstrated forward elongation of the head and shoulders followed by retraction to the original position), and Grooming (duration of time the animal spent licking or scratching itself while stationary).

Negative geotaxis

Negative geotaxis was defined as an automatic, stimulus-bound, reflexive response that results in a directional movement with or against the force of gravity. Each rat was subjected to three trials with at least a 2 min rest period between tests.

Wire grip test

Rats were timed for how long they can support their weight holding onto a metal rail suspended between

Table 1: Grouping and treatment of experimental animals.

Group	Treatment
Control	Rat feed and distilled water daily for 21 days
CPAE	500 mg/kg of CPAE daily for 21 days
BCCAO	Distilled water 1 mL daily + BCCAO only on day 21 of experiment
BCCAO+CPAE	500 mg/kg daily of CPAE for 21 days before BCCAO
BCCAO+VIT E	500 mg/kg daily of Vitamin E for 21 days before BCCAO

BCCAO = Bilateral common carotid artery occlusion, CPAE = Carica papaya aqueous extract

two pillars. Each rat was subjected to five trials with at least a 2 min rest period between tests [24].

Surgical procedure for dissection of the rat neck to ligate the common carotid arteries

Rats were transferred to the laboratory at least one hour before surgery and the weight of each rat measured with a Swiss Microwa balance type 7720 on the 22nd day of the experiments before surgery. All the surgical equipments and surgical pad were disinfected with 70% ethanol before the surgery to avoid infection and sepsis. Surgical procedures were performed between 08 - 14 hours in all rats. The surgical technique used in the present study for induction of cerebral ischaemia by BCCAO was adapted from the method of Iwasaki *et al.* [25] with slight modifications. Briefly, the rats were fasted overnight and at onset of surgery they were anaesthetized by an intraperitoneal injection of 100 mg/kg ketamine [26]. Each animal was fixed on a clean dissecting board with pins and their head stabilized on the dissecting board with the aid of plaster. The ventral surface of the rat neck was then cleaned with cotton wool soaked in methylated spirit. A median incision was performed in the skin of the ventral part of the neck of the animal from below the mandible to the manubro-sternal junction and the subcutaneous adipose tissue was dissected avoiding the salivary and the thyroid glands [27].

Using blunt dissection, the salivary glands were lifted to expose the sternomastoid and sternohyoid muscles. The sternomastoid was retracted laterally with a retractor and the common carotid artery freed from its adventitial sheath and the surrounding structures (Sternohyoid, vagus nerve etc) by blunt dissection with non-toothed forceps. The induction of ischaemic phase was performed by ligating the common carotid arteries bilaterally using 3-0 silk sutures for 30 minutes [25]. Skin was closed back by interrupted sutures and Ampiclox injection (100 mg/kg) given intraperitoneally to each animal to prevent infection. Reperfusion was done by loosening the ligatures and releasing the suture after 30 minutes after which the incision site was cleansed with savlon and spirit, dressings applied and rats returned to their cages with fresh beddings for 24 hours at room temperature [28]. On recovery, rats were allowed free access to feed and water.

Sacrifice and dissection

At the end of 24 hours of reperfusion, the animals were euthanized with ketamine (100 mg/kg) and diazepam (10 mg/kg) after which the whole brain was carefully dissected out and removed from the skull. Each brain was divided in a sagittal plane into two halves, rinsed and transferred to appropriate medium for biochemical and histological estimation. For biochemical analysis, the left side brain samples were homogenized in phosphate buffer (pH 7.4) and the resulting homogenate was centrifuged at 4 °C and the supernatant obtained was thereafter used for the biochemical estimations at the Biochemistry Laboratory of Obafemi Awolowo University, Ile-Ife, Nigeria. Brain samples for histology were fixed in neutral buffered formalin until the tissues were processed for wax embedment.

Biochemical Estimations

Determination of Lipid Peroxidation (LPO) products present in the brain samples was estimated by the thiobarbituric acid (TBA) method which measures the malondialdehyde (MDA) reactive products according to published method [29] and expressed as units/mg protein. Glutathione (GSH) estimation was done by the method of Beutler and Kelly [30]. Estimation of Superoxide dismutase (SOD) in the homogenates was done by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at 30 °C using the method described by Misra [31]. Catalase (CAT) activity was determined according to the method of Sinha [32].

Tissue processing, histological studies and histomorphometry

The brain tissue for histological studies was passed through the processes of fixation, dehydration, clearing, infiltration and embedding. Serial sections of 5 microns thick were made using rotary microtome and the slides stained with Haematoxylin and Eosin. They were examined and then evaluated under the light microscope (Olympus 41CX Japan) for histological changes in the cerebral cortex and CA1 subfield of the hippocampus. Photomicrographs were captured using digital camera Sony 14.1 megapixels (Japan). Histomorphometric analyses were done using computerized image analyzer (Apache Open Office Tm4 4.0.0. software version). Using measured squares of the OpenOffice.org.Draw, the density of the cell profile of all neurons and those of dark (pyknotic) neurons of the frontal cerebral cortex and cornu ammonis1 subfield of the hippocampus in each group were obtained and the means calculated. Employing the method of Taveira *et al.* [33], the pyknotic index (PI) was calculated using the equation: PI = pyknotic neurons / total neurons x 100.

Statistical analysis

All data were expressed as means ± standard deviation. One-way analysis of variance (ANOVA) was used to test for differences among all the groups using GraphPad Prism 5.04, 2010 version software, San Diego, CA, USA. A p-value < 0.05 was considered statistically significant.

Results

General observations

Animals tolerated the treatments with *Carica papaya* extract well. During the 24 hours of reperfusion following bilateral common carotid artery occlusion (BCCAO), mortality in the treated rats was: BCCAO group four out of seven (57.2%), BCCAO+ CPAE group three out of seven (42.9%) and BCCAO+VIT E group two out of seven (28.6%). Rats in the BCCAO only and BCCAO+ CPAE groups were sluggish and inactive after recovery from surgery. Some animals which survived surgery adopted a "hunchback" posture, were hyper excitable and reacted to handling with forceful jerks. The animals of BCCAO+VIT E group were more active. There was no significant difference in body weight observed between the control and other groups.

Open field test

Results displayed in Figure 1, showed that BCCAO caused a significant reduction ($p<0.05$) of the frequency of lines crossed and rearings, while increasing that of grooming when compared with control group. In both BCCAO+CPAE and BCCAO+VIT E groups, there was increase in these parameters. However, BCCAO+VIT E significantly ($p<0.05$) elevated these parameters when compared with the BCCAO group.

Forelimb grip strength test and Negative geotaxis,

Figure 2 showed that BCCAO treatment significantly ($p<0.05$) reduced the forelimb grip strength test while increasing the duration of negative geotaxis relative to control, whereas treatment with both BCCAO+ CPAE and BCCAO+VIT E reversed these two parameters when compared with the BCCAO group.

Biochemical analysis results

Table 2 shows that BCCAO caused significant ($p<0.05$) increase in lipid peroxidation when compared with the Control group. The value was significantly ($p<0.05$) reduced in groups BCCAO+ CPAE and BCCAO+VIT E relative to the BCCAO group. The alterations in the GSH, SOD and catalase were not significant

Histological and histomorphometric evaluation of the prefrontal cerebellar cortex and cornu ammonis 1 (CA1) of hippocampal formation.

As shown in Figure 3, the histology of the prefrontal cerebral cortex in the Control, CPAE, BCCAO, BCCAO+ CPAE and BCCAO+VIT E shows cortical neurons whose nuclei exhibit open chromatin and distinct nucleoli. However, some cortical neurons in the BCCAO group were observed to have distinctly dark nuclei scattered among normal neurons. When compared with the control, this was statistically significant ($p<0.05$) although this was

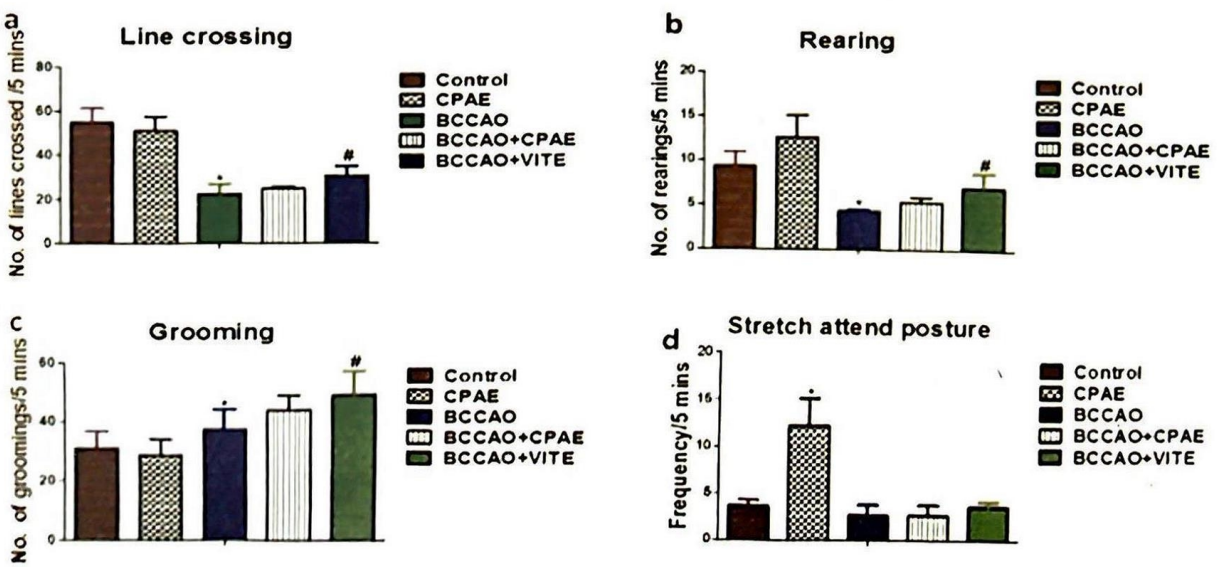

Fig. 1: Histogram showing effects of BCCAO and *Carica papaya* extract on bilateral common carotid occlusion on behavioural parameters. *Values are presented as mean ± SD. CPAE – Carica papaya extract, BCCAO - bilateral common carotid artery occlusion, VIT E- vitamin E. *P<0.05 versus Control; #P<0.05 versus BCCAO.*

Table 2: Effect of BCCAO and *Carica papaya* aqueous extract on lipid peroxidation, glutathione, superoxide dismutase and catalase.

Groups	LPO(μmoles/ protein)	GSH (μmoles/g tissue)	SOD (Unit/mg protein)	CAT(μmole mg H2O2/min/mg protein)
Control	1.077±0.17	0.206±0.08	0.246±0.10	0.127±0.01
CAPE	0.954±0.20	0.209±0.02	0.169±0.10	0.130±0.01
BCCAO	2.105±0.26*	0.198±0.00	0.308±0.08	0.132±0.01
BCCAO+ CPAE	1.324±0.14**	0.201±0.03	0.218±0.08	0.123±0.02
BCCAO+ VIT E	1.052±0.20**	0.205±0.02	0.369±0.06	0.143±0.01

*Values were presented as mean ± SD. CPAE – Carica papaya aqueous extract, BCCAO - bilateral common carotid artery occlusion, VIT E - vitamin E, GSH - Glutathione, SOD - Superoxide dismutase, LPO - lipid peroxidation, CAT - catalase. *P<0.05 versus Control; **P<0.05 versus BCCAO*

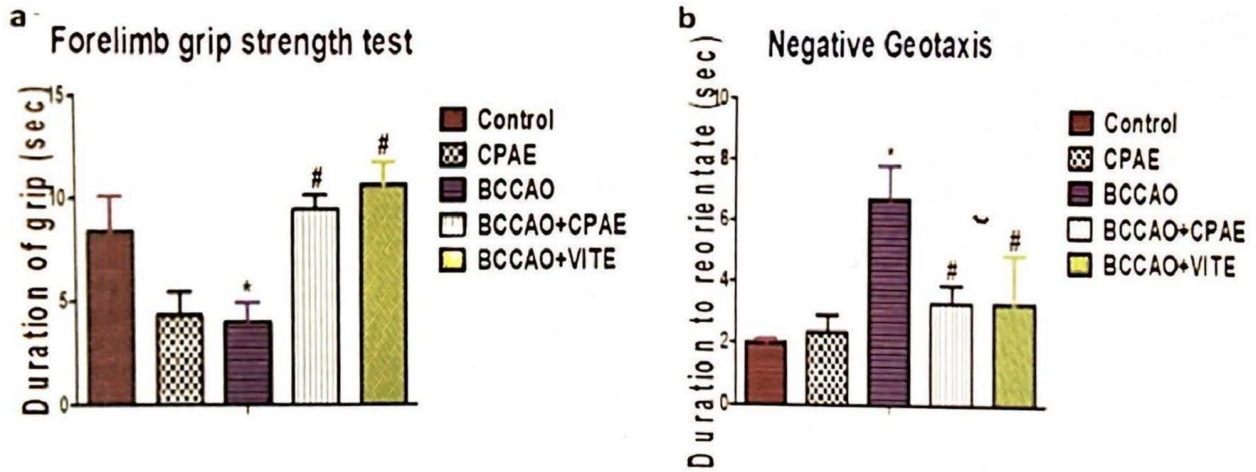

Fig. 2: Histogram showing effects of BCCAO and *Carica papaya* aqueous extract on bilateral common carotid occlusion on forelimb grip strength test and negative geotaxis. *Values are presented as mean ± SD. CPAE – Carica papaya aqueous extract, BCCAO - bilateral common carotid artery occlusion, VIT E- vitamin E. *P<0.05 versus Control; #P<0.05 versus BCCAO*

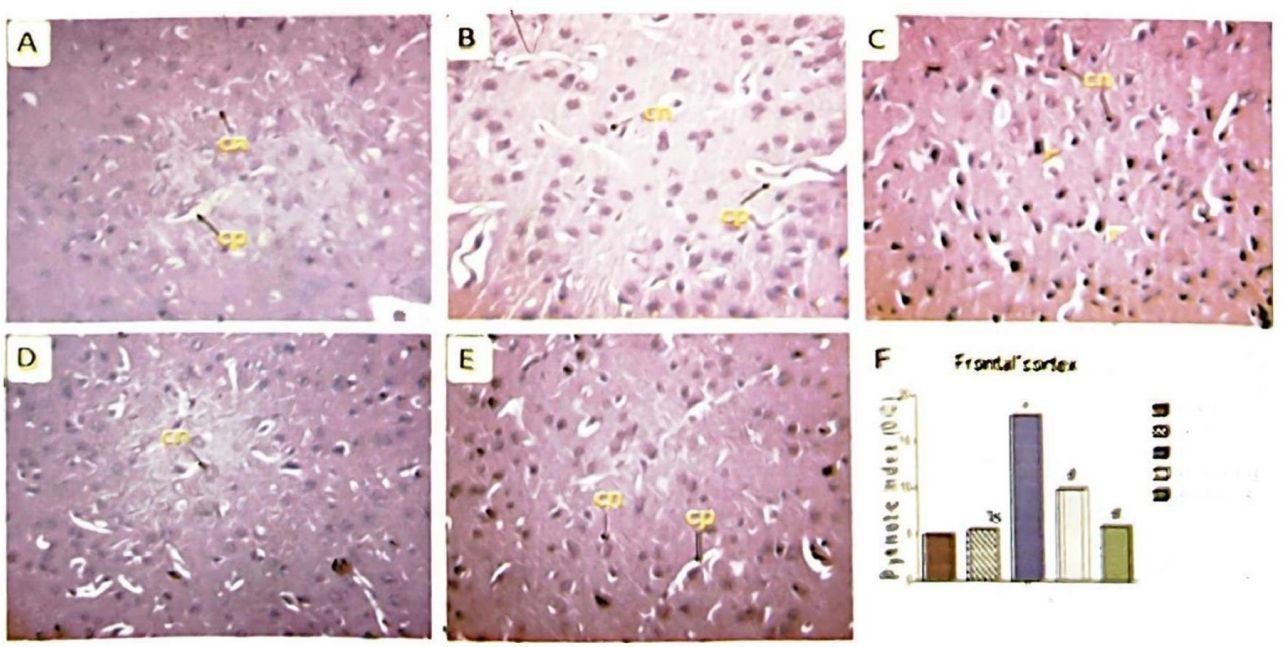

Fig. 3: Representative stained sections of cerebral cortex of rats. (A) Control (B) CPAE (C) BCCAO (D) BCCAO+ CPAE (E) BCCAO+VIT E. *Values were presented as mean ± SD. CPAE – Carica papaya aqueous extract, BCCAO - bilateral common carotid artery occlusion, VIT E- vitamin E. cn, cortical neuron; cp, capillary; yellow arrowhead, dark neuron. H&E. x400. *P<0.05 versus Control; #P<0.05 versus BCCAO.*

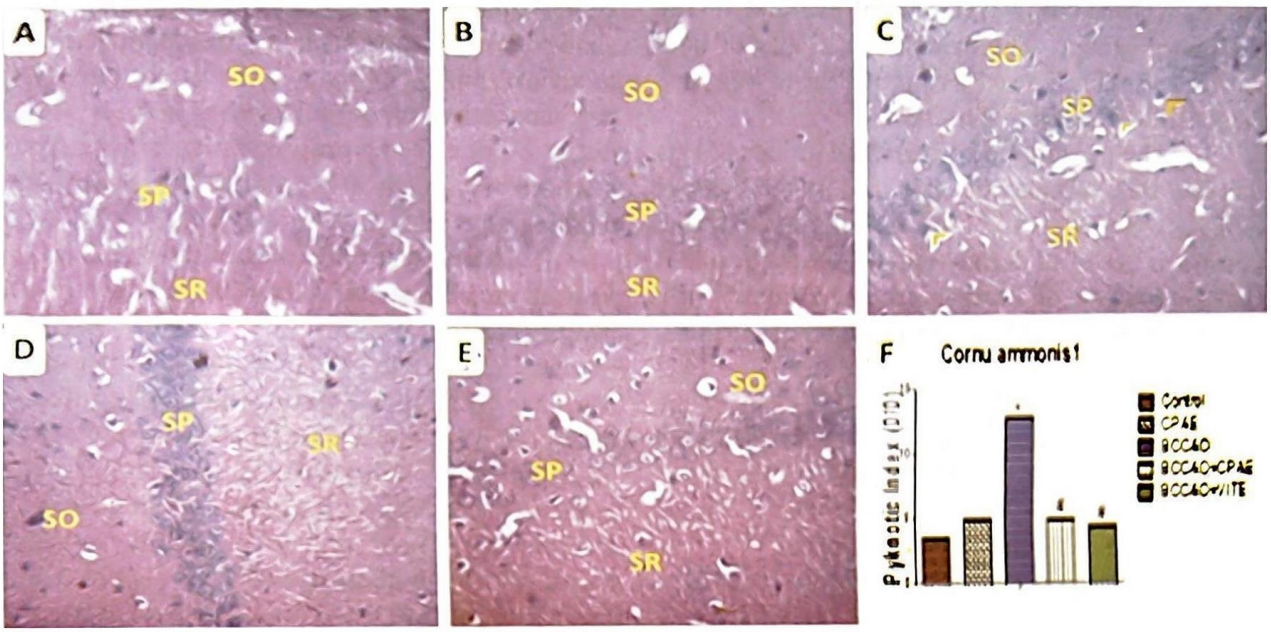

Fig. 4: Representative stained sections of the hippocampus of rats. (A) Control (B) CPAE (C) BCCAO (D) BCCAO+ CPAE (E) BCCAO+VIT E. *Values were presented as mean ± SD. CPAE – Carica papaya aqueous extract, BCCAO - bilateral common carotid artery occlusion, VIT E- vitamin E. SO, stratum oriens, SP, stratum pyramidalis, SR, stratum radiatum, yellow arrowhead, dark neuron. H&E. x400. *P<0.05 versus Control; #P<0.05 versus BCCAO.*

reduced in the pretreated BCCAO+ CPAE and BCCAO+VIT E groups (Fig. 3F).

The photomicrographs of the cornu ammonis1 (CA1) of hippocampus (Figure 4) shows normal layers of the sub-field with open faced pyramidal neurons of the stratum pyramidalis layer in the Control, CPAE, BCCAO, BCCAO+ CPAE and BCCAO+VIT E groups. The neurons of the BCCAO group are observed to exhibit some dark neurons which was quantitatively statistically significant ($p<0.05$) relative to the control. However, this was reduced in the pretreated BCCAO+ CPAE and BCCAO+VIT E groups as shown in Figure 4F.

Discussion

The behavioural and histological effects of *Carica papaya* Linn. aqueous extract (CPAE) and vitamin E (VIT E) on ischaemic-reperfusion insult by bilateral common carotid artery occlusion in rat brain was studied and the results demonstrated the ability of CPAE and VIT E to modulate some of the alterations induced by BCCAO treatment. Experimental models of stroke have been developed in animals in an attempt to mimic the events of human cerebral ischaemia that transient global cerebral ischaemia results in neurological abnormality. Therefore, global cerebral ischaemia of 30 minutes duration followed by reperfusion of 24 hours was employed in the present study [25, 34].

The significant reduction observed in the open field tests namely crossing and rearing, suggested that locomotion and exploratory ability of the rat were affected. Grooming and rearing have been considered as indicators of vertical locomotive activity, an index of alertness [35] and the excitability level of the central nervous system [36] respectively. The reduction in these two parameters of locomotion suggests that the effects of BCCAO reduced the alertness of the rats after recovery from surgery. The ability of CPAE and VIT E to reverse these effects suggests their ameliorative capability. Although BCCAO treatment reduced the stretch attend posture frequency which is an index risk assessment [37], the absence of a significant alteration in rats pretreated with CPAE and VIT E indicated their inability to modulate this parameter. The rats in the BCCAO group demonstrated fear or anxiety as monitored by the significant increase in grooming as reported by Jackson and Turkington [38] and this persisted even in the groups that received pretreatment with CPAE and VIT E. The forelimb grip strength test is an indicator of muscular strength and coordination of skeletal muscles which treatment with BCCAO significantly reduced indicating muscle weakness [39]. The maintenance of posture balance indirectly testing cerebellar coordination as well as vestibular integrity were monitored by the results of negative geotaxis [40]. Rats in the BCCAO group significantly spent longer time to re-orientate

themselves against gravity while coming down the slope, whereas this trend was reversed in the pretreated groups. The modulatory ability of both CPAE and VIT E used as pretreatment was thus demonstrated for both parameters.

The mortality recorded in all groups of rats that had BCCAO may be explained by the findings of Bruce-Keller *et al.* [41] that maximal tissue damage is observed during reperfusion as a result of the production of oxygen free radicals. While Panigrahi et al., [1] reported varying mortality ranging from 26% to 79%, Zhen and Dore [42] reported 11, 25, 38, 80, and 100% depending on the duration of occlusion. The BCCAO lasted for 30 minutes in this experiment followed by 24 hours of reperfusion and this was associated with increased generation of ROS and free radicals [25].

Overwhelming these rats with free radicals as observed by the significant elevation of malondialdehayde, an indicator of lipid peroxidation might be a source of injury. Elevation of lipid peroxidation and reduction of GSH as reported in this study established the involvement of oxidative stress in the animals of BCCAO group [43]. The increases in the SOD and CAT enzymes in the BCCAO group indicated the response of these enzymes to coping with released superoxide ions which SOD converted into hydrogen peroxide and molecular oxygen, while CAT decomposed the hydrogen peroxide into water and diatomic oxygen. SOD is an important endogenous antioxidant that prevents production of free radicals [44].

The ability to reduce lipid peroxidation and thus the oxidative stress as shown by CPAE and VIT E in the pretreated groups demonstrated their antioxidant activity. The lowest mortality recorded in the CPAE+VIT E group is in agreement with the study of Abd-El-Fattah *et al.* [45] whereby vitamin E was shown to have protected against cerebral ischemia, due to its anti-oxidative effects. The high mortality rate recorded in the CPAE pretreated group despite its rich source of three powerful antioxidants: vitamin C, vitamin A and vitamin E might be due to reperfusion injury which these vitamins did not effectively neutralize. Known to be particularly susceptible to oxidative stress is the central nervous system due majorly to the fact that the brain is rich in polyunsaturated fatty acids, relatively low in antioxidants, accumulates redox metal ions, consumes large amount of oxygen and is composed largely of non-mitotic highly differentiated cells that are difficult to repair when damaged [20].

The CPAE pretreated animals showed significantly less lipid peroxides due to ischaemic-reperfusion injury as compared to the untreated control animals group, while vitamin E-treated rats showed lesser degree of lipid peroxidation relative to control group. This suggests that the possibility that the mechanism of protection of brain by CPAE might be due to the anti-oxidative effects of its phenolic compounds especially the flavonoids, vitamins E, A and C.

The presence of scattered pyknotic cortical neurons among the normal neurons in the prefrontal cerebral cortex and selective pyramidal neuronal degeneration in the CA1 field of the hippocampus of rats in the BCCAO group might be the effect of the ischaemia following the reperfusion of the brain after the 30 minutes ischaemia occasioned by the bilateral occlusion of both common carotid arteries in these rats. This is consistent with the previous report that CA1 hippocampal neurons are highly susceptible and vulnerable to ischemia and reperfusion-induced injury [46, 5] and that BCCAO in lower animals lasting for three or more minutes results in selective neurodegeneration of the pyramidal neurons in the hippocampal CA1 subfield [47]. According to the ischaemic cascade of Danton and Dietrich [48], reperfusion, by enhancing production of free radicals, inflammation, and blood-brain barrier breakdown, contributes to cell damage and death.

The effects of this transient ischaemia/ reperfusion was evident in the lipid peroxidation observed and the mortality recorded in all BCCAO treated rats. The effect of cortical neuron death might affect the cerebral functions of cognition and control of voluntary movement. Similarly, death of CA1 pyramidal neurons might disrupt the neural sequences required for effective memory coding and recording of different forms of memory i.e episodic, semantic and spatial secondary to interruption of the trisynaptic pathway of the perforant path which the CA1 neurons of the hippocampus participates in [49].

The damage might alter the Schaffer's collaterals which CA1 neurons receive from the CA3 for onward projection to the subiculum and entorhinal cortex, a neural process that might be disrupted by the death of CA1 neurons [50]. Pretreatment with CPAE and vitamin E for 21 days prior to BCCAO improved behavioural response, reduced lipid peroxidation, increased endogenous enzyme levels of superoxide dismutase, glutathione and catalase. They also attenuated neuronal cell death both in the cerebral cortex and in the CA1 subfield of the hippocampus with a possible result of reduction of the effects of ischaemia on brain. The neuroprotective effect demonstrated in this study by

CPAE and vitamin E might be possibly related to their antioxidant activity.

Conclusion

The overall results of these studies indicate that BCCAO caused alterations in the behavioural, antioxidant and histological parameters of rat brain which pretreatment with CPAE and VIT E ameliorated possibly by their antioxidant activities. The outcome might contribute to the continuous search for neuroprotective strategies in stroke given the tremendous costs it poses on the individual and the society.

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