Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 12 (1)

Effects of *Bryophyllum pinnatum* aqueous leaf extract on the Cerebral Cortex of Ischaemia induced stroke in albino Rat.

[1]Okoseimiema SC, [1]Fawehinmi HB and [2]Ihunwo AI
[1]Department of Anatomy, Faculty of Basic Medical Sciences University of Port Harcourt, Nigeria.
[2]School of Anatomical Sciences, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa.

Corresponding Author: Okoseimiema SC
E-Mail: sonny.okoseimiema@uniport.edu; +2348065841579

ABSTRACT
This study was carried out to investigate the effects of aqueous leaf extract of *Bryophyllum pinnatum* on the cytoarchitecture of the cerebral cortex, following administration of extract and Ischaemia induction. Fourty healthy male albino wistar rats were used for this study. The animals were grouped into four groups and ten(10) animals per group. The groups were: general control group, Ischemic control, 200mg/kg and 400mg/kg *Bryophyllum pinnatum group. Aqueous leaf extract of Bryophyllum pinnatum was administered intraperitoneally for 21 days before induction of stroke. The result of the study in day 1 post Ischaemia showed* a normal cytoachitecture of brain tissue and neuronal cells(N) with normal nucleus in the cerebral Cortex for the general Control group. For the Ischaemic control group, it was observed, that, there was hypoperfussion induced marked congestion of blood vessels(HCBV), excessive degeneration of neuronal cells(DN), excessive vaculation(EV) ; pyknosis and necrosis of neurons. Pretreatment with 200mg/kg and 400mg/kg *Bryophillum pinnatum*, showed a mild congestion of blood vessels(MC), Mild degeneration of neurons (MD) and mild vacuolation(MV) which was brought to near normal by the seventh day, post Ischaemia. There was a significant reversal of the conjestion of blood vessels , reversal of necrotic degeneration of neurons in the 200mg/kg and 400mg/kg *Bryophillum pinnatum* pretreated group, when compared to the Ischaemic control group. *Previous phytochemical studies have shown that Bryophylum Pinnatum leaf extracts contains* Tannins, Flavonoids and Phenols which enhances antioxidant, anti-inflammatory *activities.* The action of the chemical compounds; flavonoids, phenols, along with antioxidants, vitamins and enzymes may have contributed to the neuroprotective effect of *Bryophyllum pinnatum* after ischaemia. Knowledge gained from this work will be useful to the Neuroscientist, Anatomist and Ethnomedicinal researchers.

Key words: Anatomist, *Bryophyllum pinnatum,* Neuroprotection, Neuroscientist, and Ethnomedicinal researchers.

INTRODUCTION
Stroke remains one of the leading causes of death and disability worldwide[1,2]. Partial paralysis and lack of coordination of movement are motor and sensory deficits associated with stroke[3]. Cognitive deficits, memory and learning challenges in a stroke patient can occur when higher cortical brain areas are affected. Stroke is the third leading cause of death in the world[4] . It causes a Social, economic and medical problem in the world. One of the commonest risk factor of stroke in Nigeria is hypertension, nicotine abuse and age[5] . Ischemic stroke occurs more frequently in about eighty percent (80%) of all stroke cases, while hemorrhagic stroke accounts for the remaining twenty percent of all stroke cases[6]. The incidence of stroke mortality is greater in blacks than Caucasians[4]. The existing methods and approaches of the epidemiology of stroke in Africa has been reviewed[7]. Wahab[8] , stated that because of epidemiological transition, Nigeria as a nation, is likely to spend more of her resources in managing stroke because of the increasing prevalence of stroke and cardiovascular diseases. Kelechi *et al.*[9], stated in their study, that the prevalence of stroke was higher in males than in females in Anambra State, Nigeria. The prevalence of stroke in Nigeria is 1.14 per 1000 and the fatality rate for 30-days is fourty percent[8]. There is poor funding of high quality stroke research in Nigeria and there is a need for effective stroke treatment in Nigeria[8]. Despite the availability of orthodox medicine, many people living in Nigeria rely heavily on herbal medicine for the management of various diseases and heal injuries[10]. *Bryophyllum pinnatum(BP)* belongs to the family Crassulaceae and it is commonly used in the ethnomedical practices. It is found in the tropical Africa, America, India and Australia[11]. In West Africa, *Bryophyllum pinnatum(BP)* is found in tropical and subtropical areas. The Yoruba ethnic group calls it Abamoda/eru-odundun; the Igbo's refer to it as OdaaOpue; the Edo people calls it Alupu; while the hausa's refered to it as Sutura, ShukaHalinka or Karan Masallachi[12,13]. *Bryophyllum pinnatum is called Mbukiba Diri in Okrika.*

Bryophyllum pinnatum (BP) is used in Okrika, in Rivers State for the management of hypertension, stroke, and several ailments. Literatures regarding the effect of aqueous leaf extract of Bryophyllum pinnatum on the cerebral cortex of ischemia-induced stroke in albino rat

are scarce. This is the driving force behind this research. The aim of this study is to investigate the effects of aqueous leaf extract of *Bryophyllum pinnatum* on the cerebral Cortex of Ischaemia induced stroke in male albino rat.

MATERIALS AND METHODS

Fresh leaves of *Bryophyllum pinnatum* were collected from the Botanical garden of the University of Port-Harcourt and were identified and authenticated in the Department of Plant Science and Biotechnology , University of Port Harcourt. Fresh leaves of *Bryophyllum pinnatum* were washed thoroughly under tap water. 1Kg of the leaves were macerated using Sanyo Blender. The leaf extracts were then strained with a muslin cloth and then freeze dried. The powdered samples were stored at 4°C and used within seven weeks after production.

Fourty healthy male albino wistar rats were used for this study. The animals were bred and housed in the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt. The animals were grouped into ten(10) animals per group as shown in the experimental design. The animals were kept and nurtured under laboratory conditions, temperature, humidity, and light and were allowed free access to food and water ad libitum.

The animals were grouped as follows:

- Group one: General control; No stroke was induced. An administrative equivalence of 0.15 ml of distilled water was intraperitoneally administered.
- Group two: Ischemic control; stroke was induced in this group after final administration of distilled water. An administrative equivalence of 0.15 ml of distilled water was intraperitoneally administered.
- Group three: Before Ischemia, was treated with 200mg/kg (standard dose) of aqueous leaf extract of *Bryophyllum Pinnatum,* intraperitoneally.
- Group four: Before ischemia, was treated with 400mg/kg(standard dose) of aqueous leaf extract of *Bryophyllum Pinnatum,* intraperitoneally.

The administration was done for three weeks. After this, followed the induction of transient cerebral ischemia.

Induction of Transient Cerebral Ischemia: Transient focal cerebral ischemia (15 min) was induced using a four vessel occlusion method with slight modification[14,15]. Rats were anesthetized with diethyl ether for some seconds and removed from the desiccator when it becomes unconscious, after which the animals were pinned in its fore and hind paws on a dissecting board using thumb nail. A ventral incision of about 12cm was made at the neck region of the animal and underlying fascia and muscles flapped. After this, the right and left common carotid arteries were carefully identified and a silk suture thread was firmly tied around the common carotid artery and occlude the artery for 15minutes. The carotid clasps were tightened to produce occlusion. After this fifteen minutes of occlusion, the arteries were untied for reperfusion to take place. This causes an ischaemic stroke in the brain of the rat. The Skin incision was sutured immediately after ischemic reperfusion with stitches via the aid of a suture needle.

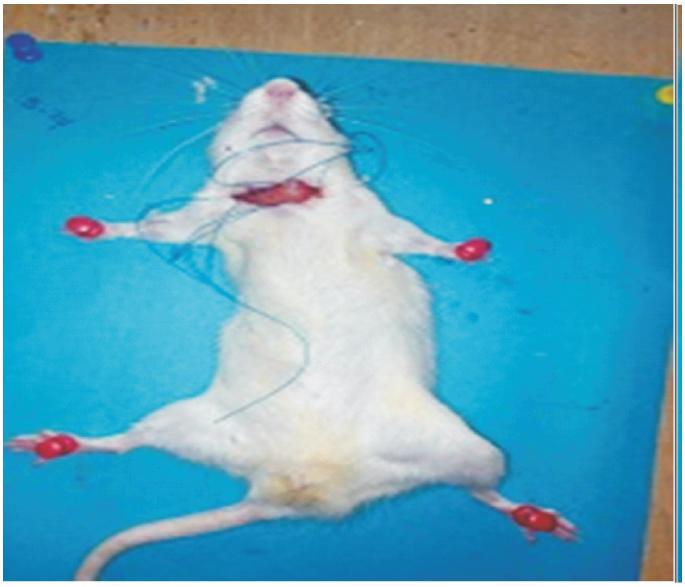

Plate 1: Occlusion of common Carotid artery.

After surgical procedures rats, were maintained for another seven days (7days) under proper post-operative care. The animals were euthanized on day1, 3 and day7, after the induction of stroke for histological studies. Coronal sections from all the group of animals were fixed and slices of 4-5mm thickness of brain sections were embedded in paraffin blocks. Brain sections of 4-6m thickness were stained in hematoxylin and eosin

RESULTS

The results of the H &E routine histological Examination of the cerebral Cortex of Group 1, 2,3 & 4 for day 1 post Ischaemia are shown in Plate 2. Group 1 represents the general control group. It was observed that there were intact neurons with large nuclei and prominent nucleoli. Group 2 represents the Ischaemic Control group. It was observed that, there was hypoperfussion induced marked congestion of blood vessels(HCBV), excessive degeneration of neurons(DN), there was excessive vacuolation(EV), Pyknosis(P), Karyorrhexis and necrosis of neurons. Group 3 represents the 200mg/kg *Bryophyllum pinnatum* administered group. The result showed a Mild degeneration of neurons (MD) , mild vacuolation(MV) and mild congestion of blood vessels(MV). Group 4 represents the 400mg/kg *Bryophyllum pinnatum* administered group. The result showed a Mild degeneration of neurons (MD) , mild vacuolation(MV) and mild congestion of blood vessels(MV). Plate 3 showed the H &E routine histological Examination of the cerebral Cortex of Group 1, 2,3 &4 for day 3 post Ischaemia. It was observed that, there were intact neurons in general control group. There were degeneration of neurons, excessive vacuolation and necrosis in the Ischaemic control group. It was observed that, there was mild degeneration of neurons, mild vacuolation and in the200mg/kg and 400mg/kg *Bryophyllum pinnatum* treated groups.

Plate 4 showed the H &E routine histological Examination of the cerebral Cortex of Group 1, 2,3 &4 for day 7 post Ischaemia. It was observed that, there were intact neurons in general control group. There were degeneration of neurons, excessive vacuolation and necrosis in the Ischaemic control group. It was observed that, there was a reversal of neuronal tissue degeneration to near normal in the200mg/kg and 400mg/kg *Bryophyllum pinnatum* treated groups.

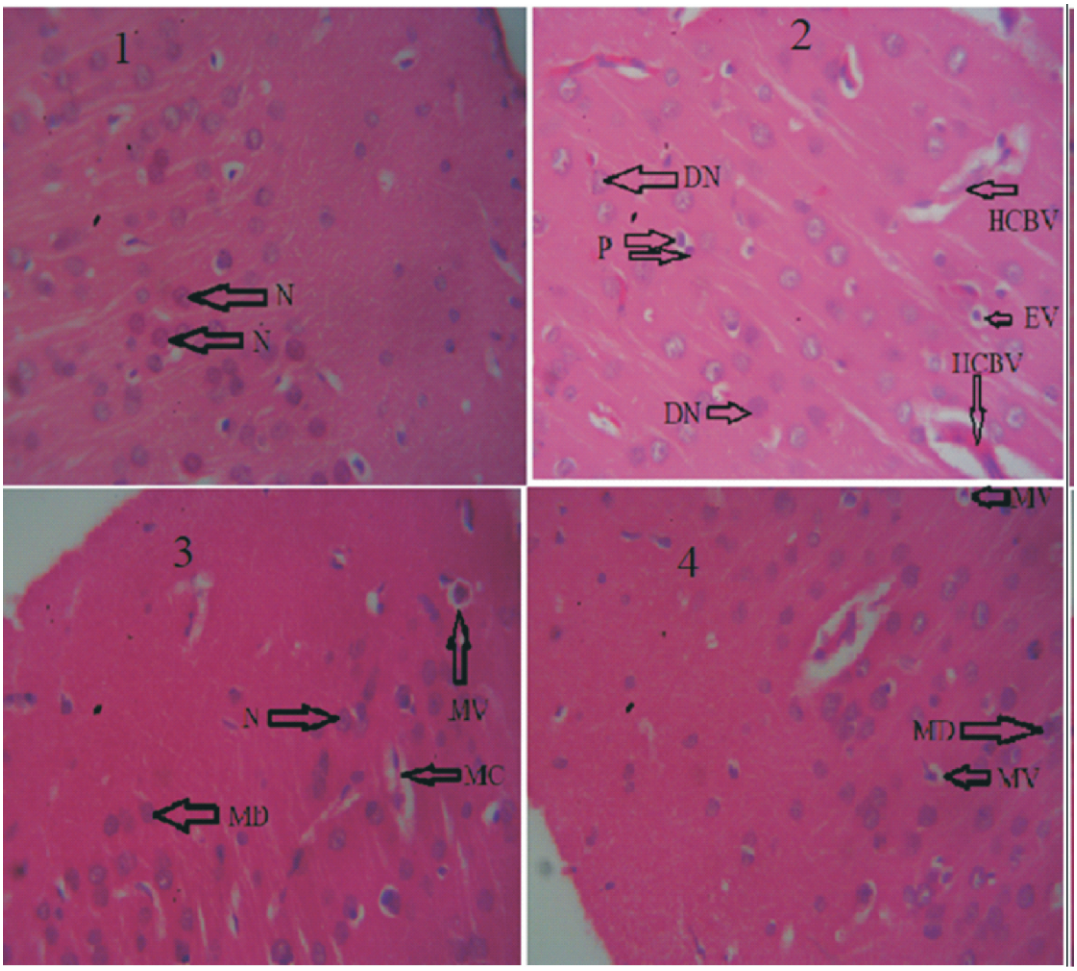

Plate 2: photomicrograph of H&E stain of the cerebral cortex of group 1(General control), group 2(Ischaemic Control) Group3 (200mg/kg BP group) , Group4 (400mg/kg BP group) for day 1 post ischaemia. Magnification:×400. (N- intact neurons, HCBV-hypoperfussion induced marked congestion of blood vessels, DN-excessive degeneration of neurons, EV-excessive vaculation, P-Pyknosis, MD- Mild degeneration of neuronal cells, MV-mild vacuolation)

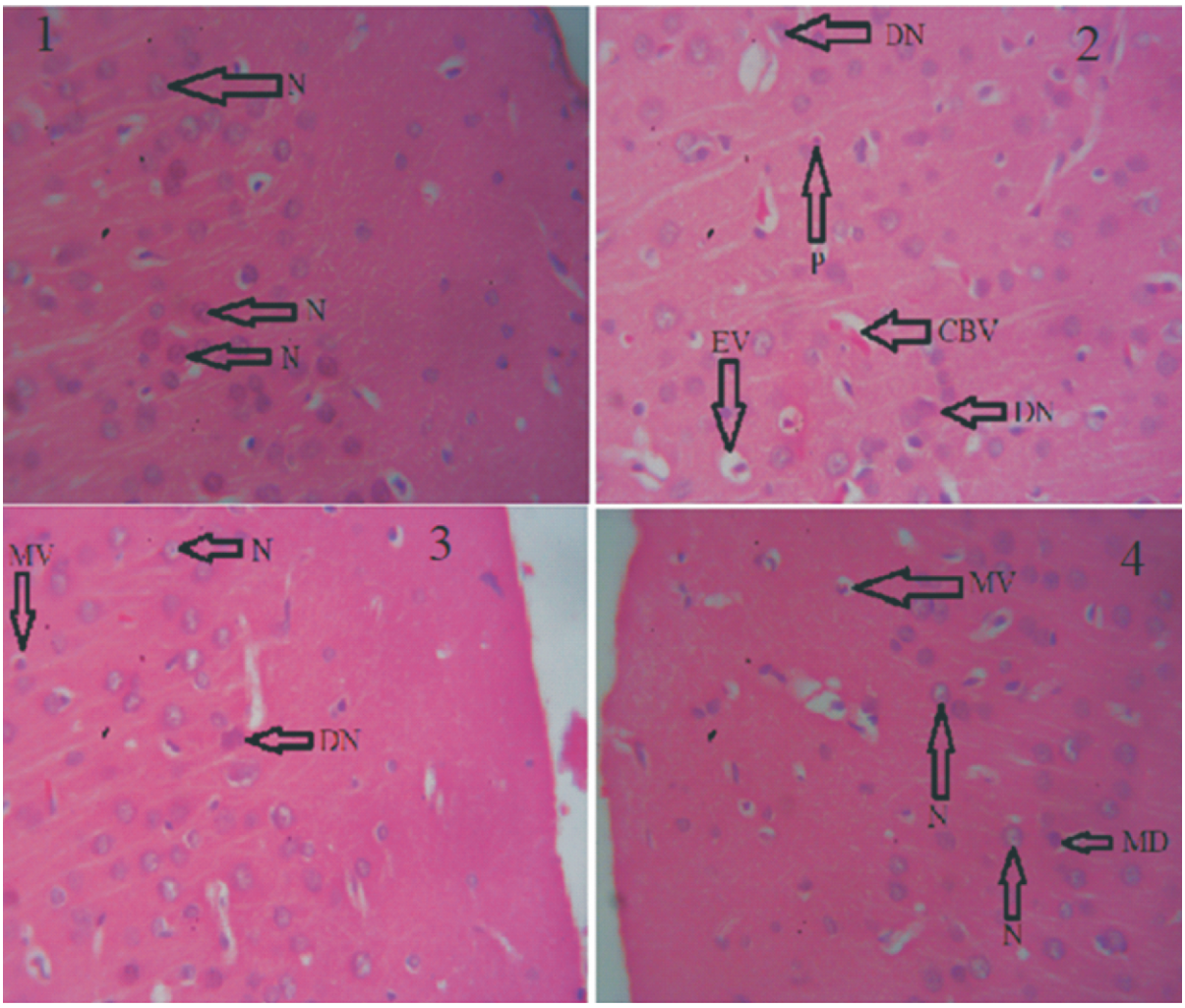

Plate 3: Photomicrograph of H&E stain of the cerebral cortex of group 1(General control), group 2(Ischaemic Control) Group3 (200mg/kg BP group), Group4 (400mg/kg BP group) for day 3 post ischaemia. Magnification:×400. (N- intact neurons; CBV- congestion of blood vessels, DN-excessive degeneration of neuron, EV-excessive vaculation, P-Pyknosis, MD- Mild degeneration of neuronal cells, MV-mild vacuolation.

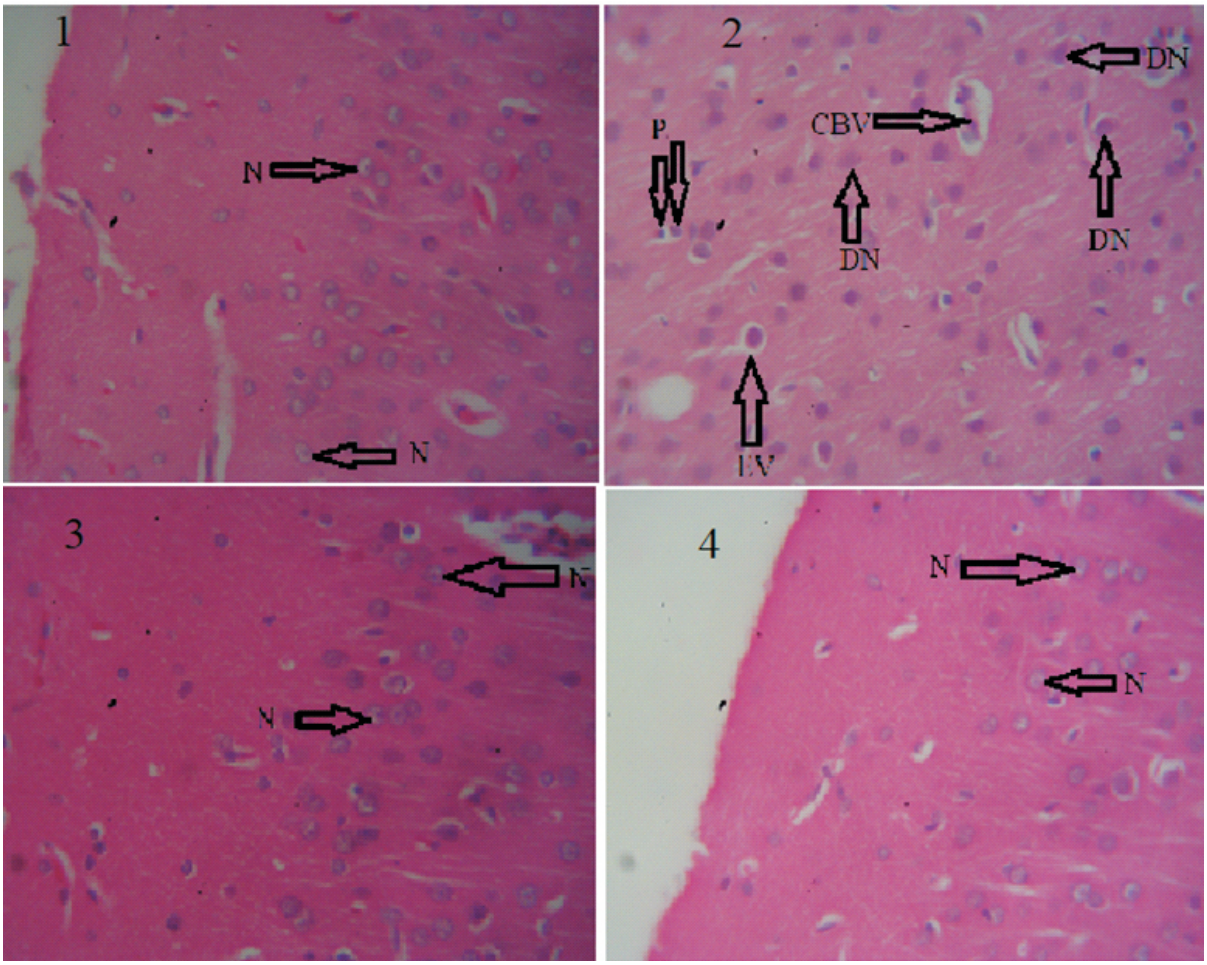

Plate 4: Photomicrograph of H&E stain of the cerebral cortex of group 1(General control), group 2(Ischaemic Control) Group3 (200mg/kg BP group) , Group4 (400mg/kg BP group) for day 7 post ischaemia. Magnification:×400. (N- intact neurons; CBV- congestion of blood vessels, DN- degeneration of neuron, EV- excessive vaculation, P-Pyknosis,).

DISCUSSION

Cerebral ischemia causes cerebral cell death, resulting in neuronal deficit symptoms and cerebral infarction[16]. Numerous reports indicate the involvement of oxidative stress in focal cerebral ischemia[17,18,19]. Ischemic cascade is initiated by energy failure which quickly leads to dysfunction of energy-dependent ion transport pumps (like Na+-K+ATPase) and depolarization of neurons and glia[20,21]. Na+-K+ATPase, is responsible for the maintenance of neuronal excitability and the control of cellular volume in the central nervous system[21]. It is an important parameter to investigate stroke-induced brain damage. In the present study, the rats that were sacrificed on day 1 post Ischaenia, the cytoarchitecture of the General Control group showed a normal cytoachitecture of brain tissue and neuronal cells(N) with normal nucleus in the cerebral Cortex. For the Ischaemic control group, it was observed, that, there was hypoperfussion induced marked congestion of blood vessels(HCBV), excessive degeneration of neuronal cells(DN), excessive vaculation(EV) , Pyknosis, karyorrhexis and necrosis of neural cells. Pretreatment with 200mg/kg and 400mg/kg *Bryophillum pinnatum*, showed a mild congestion of blood vessels(MC), Mild degeneration of neural cells (MD) and mild vacuolation(MV) which was brought to near normal by the seventh day, post Ischaemia. There was a significant reversal of the conjestion of blood vessels , reversal of necrotic degeneration of neuronal cells in the 200mg/kg and 400mg/kg *Bryophillum pinnatum* pretreated group, when compared to the Ischaemic control group on day 7, post ischaemia.

Search for agents providing protection against Ischaemia and enhancing anti-oxidant enzyme defense system is a rational approach for therapy of cerebrovascular ailments. Natural products (i.e. medicinal plants) with intrinsic antioxidant property constitute an ideal choice for maximum therapeutic effects with minimal risk of iatrogenic adverse effects. Some natural products have been used by different authors which enhanced protective effect on middle cerebral artery occlusion (MCAO) induced reperfusion injury due to their

antioxidant property[22,23,24,25,19]. Natural products (i.e. medicinal plants) with intrinsic antioxidant property constitute an ideal choice for maximum therapeutic effects with minimal risk of adverse effects[18]. The phytochemical screening of *Bryophylum Pinnatum* showed the presence of Alkaloid, Saponin, Tannin, Flavonoid, Phenol, Anthraquinone, triterpenoid and Carbohydrates[26]. Tannins and flavonoids are well known to have antioxidant properties, anti-inflammatory and antiproliferative activity[27]. Simonyi[28], suggested the provision of adequate protection against neurodegenerative changes associated with cerebral ischemia by the antioxidant, anti-inflammatory, and antiproliferative activities of plant polyphenols, flavonoids and saponins.

Previous studies have demonstrated the antioxidant effect of *Bryophyllum Pinnatum*[29]. Thus, the administration of Aqueous leaf extract of *Bryophyllum Pinnatum* twenty one days prior to simulation of ischaemia exhibited a cerebroprotective effect in the present study. It is important to note that flavonoids may help provide protection against these diseases by contributing, along with antioxidants, vitamins and enzymes, to the total antioxidant defense system of the human body. This may be responsible for neuroprotection and significant decrease in necrosis of the tissues of the cerebral cortex in the treatment groups[30].

CONCLUSSION

This study has provided a reference data for researchers. It will be useful to the Anatomist, neuroscientist and the ethnomedicinal researchers.

REFERENCES

1. Huuskonen, M. T., Tuo, Q. Z., Loppi, S., Dhungana, H., Korhonen, P., McInnes, L. E., *et al.* The copper bis(thiosemicarbazone) complex cuII(atsm) is protective against cerebral ischemia through modulation of the inflammatory milieu. *Neurotherapeutics Journal,* 2017; *14*, 519–532.
2. Mijajlović, M. D., Aleksić, V. M., Šternić, N. M., Mirković, M. M., & Bornstein, N. M. Role of prediabetes in stroke. *Neuropsychiatr. Dis. Treat.* 2017; *13,* 259–267.
3. Bouet, V., Freret T., Toutain, J., Divoux, D., Boulourard, M., & Schumann-Bard, P. Sensorimotor and cognitive deficits after transient middle cerebral artery occlusion in the mouse. *Experimental Neurology Journal*, 2007;*203*, 555–567.
4. Bertha Ekeh, Adesola Ogunniyi, Emmanuel Isamade & Udeme Ekrikpo. Stroke mortality and its predictors in a Nigerian Teaching Hospital. *African Health Science Journal,*2015*;15*(1), 74–81.
5. Ekeh, B. C., Ogunniyi, A. O.,& Isamade, E. I. Stroke risk factors among patients in a Nigerian Teaching Hospital. *J Med Trop.* 2013; *15*(1),33–36.
6. Liu, Z., Ran, Y., Huang, S., Wen, S., Zhang, W., Liu, X., Ji, Z., Geng, X., Ji, X.,& Du, H., *et al.* Curcumin Protects against Ischemic Stroke by Titrating Microglia/Macrophage Polarization. *Front. Aging Neuroscience,* 2017*; 9*, 233.
7. Mayowa Owolabi, Paul Olowoyo, Femi Popoola, Daniel Lackland, Carolyn Jenkins, Oyedunni Arulogun, Rufus Akinyemi, Odunayo Akinyemi, Onoja Akpa, Olanrewaju Olaniyan, Ezinne Uvere, Issa Kehinde, Anbesaw Selassie, Mulugeta Gebregziabher, Raelle Tagge, & Bruce Ovbiagele. The epidemiology of stroke in Africa: A systematic review of existing methods and new approaches. J Clin Hypertens (Greenwich), 2018; 20(1), 47–55.
8. Wahab, K.W. The burden of stroke in Nigeria. *International Journal of Stroke*, 2008;*3*(4),290-292.
9. Kelechi O. Enwereji, Maduaburochukwu C. Nwosu, Adesola Ogunniyi, Paul O. Nwani, Azuoma L Asomugha & Ezinna E. Enwereji.Epidemiology of stroke in a rural community in Southeastern Nigeria. *Vascular Health and Risk Management Journal,* 2014; *10*, 375-388.
10. Okwu, D.E. & Josiah, C..Evaluation of the chemical composition of *Bryophyllum Pinnatum. Journal of Science,* 2006*; 6,*30-37.
11. Devbhuti, D., Gupta, K. K.,& Devbhuti, P. Studies on antitumour activity of *Bryophyllum calycinumSalisb.* against Ehrlich ascites carcinoma in Swiss albino mice. *Journal of PharmaSci Technology,* 2012; *2*(1),31 –33.
12. Quazi, M., Sayyed, N., Siraj, S., Pravin, G., & Amol, C. Phytochemical analysis of chloroform extract of roots of *Kalanchoe pinnata* by HPLC and GCMS. *International Journal of Pharmaceutical Sciences and Research,* 2011*; 2*(7), 1693 –1699.
13. Yahaya Mobmi Musa, Omar G. Pam & Hafsat Buba Biri. Extraction, phytochemical screening and the antimicrobial activity of the Methanol Extract of *Bryophyllum pinnatum* leaves against Candida Albicans and Salmonelatyphi. *Journal of Applied Chemistry,* 2015; *8*(4),4-6.
14. Pulsinelli William, A., Levy David, E. & Duffy Thomas, E. (1982). Regional cerebral blood flow and glucose metabolism following transient forebrain ischemia. *Annals of Neurology Journal,* 11, 499–509.
15. Lee, D., Park, J., Yoon, J., Kim, M.Y., Choi H.Y., Kim, H. Neuroprotective effects of Elentherococcus senticocus bark on transcient global cerebral ischaemia in rats. *J Ethnopharmacol,* 2012; *139*:6-11.
16. Park, S.J., Nam, K.W., Lee, H.J., Cho, E.Y., Koo, U., & Mar, W. Neuroprotective effects of an alkaloid free ethyl acetate extract from the root of Sophora flavescens Ait. against focal cerebral ischemia in rats. *J. Phytomedicine,* 2009; 16(11)1042-51.
17. Shah, Z.A., Gilani, R.A., Sharma, P., & Vohora,

S.B. Cerebroprotective effect of Korean ginseng tea against global and focal models of ischemia in rats. *J Ethnopharmacol,* 2005*;101*:299–307.

18. Thiyagarajan, M., & Sharma, S.S. Neuroprotective effect of Curcumin in middle cerebral artery occlusion induced focal cerebral ischemia in rats. *Life Sci. Journal,* 2005; *74*:969–985.
19. Zang, C., & Yang, J. Protective effects of isoliquiritigenin in transient middle cerebral artery occlusion induced focal cerebral ischemia in rats. *Pharmacol Res,* 2006; *55*:303–309.
20. Martin, R.L., Lloyd, H.G., & Cowan, A.I. The early events of oxygen and glucose deprivation: setting the scene for neuronal deathfi *Trends Neurosci,* 1994*;17*:.251– 257.
21. Streck, E.L., Feier, G., & Burigo, M. Effects of electroconvulsive seizures on Na+-K+ATPase activity in the rat hippocampus. *Neurosci Lett,* 2006*; 404*:254-257.
22. Hosseinzadeh, H., Parvadeh, S., Nassiri, M., Hamid, R., & Sadgnia, Z.T.Effect of Thymoquinone and Nigella Sativa Seed oil on lipid peroxidation level during global cerebral ischemia reperfusion injury in rat hippocampus, *Phyto Med,* 2007; *14*(9)621–627.
23. Shukla, B.A., Mandavia, R.D., Barvaliya, J.M., Baxi ,N.S., & Tripathi, R.C. Evaluation of anti-urolithiatic effect of aqueous extract of Bryophyllum pinnatum (Lam.) leaves using ethylene glycol-induced renal calculi. *Avicenna J Phytomed,* 2014; *4*: 151-159.
24. Thaakur, S., & Sravanthi, R. Neuroprotective effect of Spirulina in cerebral ischemia–reperfusion injury in rats. *J Neuro Trans,* 2010; *117*(9) 1083-1091.
25. Guptha, R., Manjeet, S., & Ajay, S. Neuroprotective effect of antioxidants on ischemia reperfusion induced cerebral injury. *Pharmacological research,* 2003; *48*(2) 209-215.
26. Aprioku, J. S., & Igbe I.Effects of Aqueous Bryophyllum pinnatum Leaf Extract on Hematological, Renal and Sperm Indices in Wistar Rats. *Indian J Pharm Science,* 2017; *79(4)*:521-526
27. Ishikawa, Y., Sugiyama, H., Stylianou, E., & Kitamura, M. Bioflavonoid quercetin inhibits interleukin-1induced transcriptional expression of monocyte chemoattractant protein-1 in glomerular cells via suppression of nuclear factor-B, *J Am Soc Nephrol,* 1999*; 10*:2290–2296.
28. Simonyi, A., Wang, Q., Miller, R.L., Yusof, M., Shelat, P.B., Sun, A.Y., & Sun, G.Y. Polyphenols in cerebral ischemia: Novel targets for neuroprotection.*Journal of Molecular Neurobiology,* 2005; *31*, 135-48.
29. Asiedu-Gyekye, I. J., Antwi, D.A., Bugyei, K.A., Awortwe C., & N'guessan B.B. Vascular effects of an aqueous extract of Kalanchoe Integra Var. Crenata(Andr.) cuf leaves: Reversal of postischaemic dysautoregulation in rat model of cerebral ischaemia. *J Phys Pharm. Adv.* 2013; *3*(4):103-109.
30. Sumitra, M., Manikandan, P., Kumar, D.A., Natarajan, A., Balakrishna, K., Manohar, B.M., & Puvanakrishnan, R. Experimental myocardial necrosis in rats: Role of arjunolic acid on platelet aggregation, coagulation and antioxidant status, *Mol Cell Biochem,* 2001; *277*:135-142.