

Research Article

Glial Fibrillary Acidic Protein Expression in the Hippocampal Formation of Mefloquine Induced-Seizured Rats Treated with Aqueous Leaf Extract of *Luffa aegyptiaca* Mill

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Abstract: This study investigates the expression of Glial Fibrillary Acidic Protein and effects of aqueous leaf extract of *Luffa aegyptiaca* Mill on the hippocampus of the brain of Albino Wistar rats with Mefloquine induced seizure. Thirty albino wistar rats (190-250 g) were grouped into 6 groups of 5 rats each. Group 1 was control. Group 2 rats were induced with mefloquine only (4.28 mg/kg). Group 3 were given average dose of luffa extract only (800 mg/kg). Group four rats were induced with mefloquine (4.28 mg/kg) and treated with diazepam (5 mg/kg). Group 5 rats were induced with (4.28 mg/kg) with mefloquine and treated with low dose luffa aegyptiaca mill (400 mg/kg). Group 6 were induced with mefloquine (4.28 mg/kg) and treated with high dose luffa aegyptiaca mill (1200 mg/kg). The rats were then perfused transcardially and sacrificed. Brain sections were analyzed for histological (H&E) and immunohistochemical staining using glial Fibrillary Acidic Protein (GFAP), marker for astrocytes. The histological results showed disruption of pyramidal cells layer in CA3 subfield of hippocampus and regional selectivity of pyramidal cell loss in seized rats indicating induction of seizure with mefloquine. There was some restoration of pyramidal cells with the treated groups but no disruptions in the control group. There was less expression of GFAP positive cells in the control group and treated groups and more expression in the seizure rats. The expression of GFAP positive cells was an indication of different levels of neuroinflammation. The reactive astrocytes being predominant in the seizure group. The present study therefore provides empirical data on GFAP expression in the hippocampus of seizure animal model treated with aqueous leaf extract of luffa.

Keywords: Astrocytes, hippocampus, luffa aegyptiaca mill, mefloquine, neuroinflammation, seizure

INTRODUCTION

World Health Organization (2013) observed that neurons in the central nervous system are particularly prone to injury owing to the wide range of neurological events documented on antimalarial drugs which varies depending on the part of the CNS affected. It was agreed by Peter *et al.* (1998) that astrocytes are neuroprotective neuroglial, involved in healing and recovering of neurons in various nervous system pathology. Injury involving the central nervous system causes the production of astrocytes which act as neuroprotective sheath. They increase in number, fill injury zone, forming glial scar to fill defects left by loss of specialized nervous tissues. They assist in healing and recovery of neurons; such astrocytes are known as reactive astrocytes. In a study by Abbas *et al.* (2004), it

was reported that the presence of reactive astrocytes indicates early sign of cell loss and serve as indicator of pathologic process. Thus, the activity of mefloquine on astrocytes might suggest possible early stages of neuronal deranged activity or loss which could also explain the neurological effects of mefloquine therapy on the hippocampus such as forgetfulness and seizure.

In the hippocampus, the CA3 and CA1 regions are involved and damage has been noted to be greater in ventral as opposed to dorsal hippocampal regions. Interestingly, the highest cholinergic receptor densities are in CA1 and the dentate gyrus, while the region most consistently and severely damaged is CA3 (Clifford *et al.*, 1987). This clearly indicates that the spread of seizure activity beyond the initial focus must entail activation of non-cholinergic pathways (Clifford *et al.*, 1987).

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The increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and developments of several drugs and chemotherapeutics from plants as well as from traditionally used herbal remedies. This practice has gained more grounds because of the ready availability of plants, the insignificant cost of preparation and the desire to avoid the side effects of chemotherapy (Anwar *et al.*, 2007). Although the use of medicinal plants is sometimes associated with superstition and therefore rejected by some people in favour of western medicine (Ojo *et al.*, 2006). Conversely, there are millions of Africans who prefer traditional methods of treatment. The valuable medicinal properties contained in certain plants are not, however, in doubt (Ojo *et al.*, 2006).

Traditionally in Ogoni, Rivers State, Nigeria, the leaf of *Luffa aegyptiaca mill* has been used for the treatment of seizure. It has been demonstrated that *Luffa aegyptiaca mill* can revoke anxiety in rat (David *et al.*, 2016).

The objectives of this study were to elucidate the effect of the leaf extract of this plant on the histology of the hippocampus and to find out if glial fibrillary antigen protein can be expressed and where in the hippocampus, indicating present of reactive astrocytes in a mefloquine seizure-induced rat brain.

MATERIALS AND METHODS

All procedure involving the use of animals in this study was confirmed to the guiding principles for research involving animals as recommended by the declaration of The Research Ethics Committee of Graduate School, University of Port Harcourt, Choba, River State, Nigeria and was approved by the Departmental committee on the use and care of animals in conformity with international acceptable standards.

Thirty Albino Wistar rats of were kept in the propylene cages and maintained at a temperature of about 25°C and were allowed free access to food and water ad libitum. The thirty adult Wistar rats with varying weights was randomly assigned into six groups, namely: control ($n_1 = 5$), Mefloquine induced seizure rats ($n_2 = 5$), rats treated with luffa ($n_3 = 5$), Mefloquine induced rats plus diazepam ($n_4 = 5$), Mefloquine induced seizure rats plus low dose of extract of *Luffa Aegyptiaca* ($n_5 = 5$), Mefloquine induced seizure rats plus high dose of extract of *Luffa Aegyptiaca mill*, ($n_6 = 5$).

The rats were anesthetized with chloform in a petric disc and dissected in the thoracic region to expose the heart. The rats were fixed through trans-cardiac perfusion using 0.9% saline and then 4% paraformaldehyde (PFA) solution. This was done by injecting the needle through the left ventricle of the heart. 10 mL of paraformaldehyde was injected to circulate round the body. The right atrium was cut open

for the fluid to come out. The brains were extracted, post fixed overnight in 4% paraformaldehyde at 4°C overnight and then transferred to 30% sucrose for 2-3 days before embedded in paraffin. Coronal sections of the right hemisphere were cut on a microtome (Hyrax S50 Zeiss, Germany) at 5 μ m. All sections were collected and arranged into 20 parallel series for histological and immunohistochemical staining. Sections were deparaffinized in Xylene for 5 min twice, rehydrates through graded alcohols and washed in running water for 5 minutes. Antigen retrieval of sections in Citrate buffer pH6 was carried out atmicrowaved @med high 720 for 10 minutes and allowed to cool at room temperature for 20 mins. It was then washed in TBS 5 min. Endogenous peroxidase was blocked with 1% H₂O₂ in methanol for 15 min. Washed in TBS pH7.4, 3×5 mins. Incubates with 5% normal horse serum for 1 h. Serum was tapped off slides. Incubates with primary antibody GFAPab 188270 1:2000 (abcam) overnight @4°C. On the secondday, slices were washed in TBS pH7.4, 3×5 mins. Incubates with biotinylated secondary antibody (goat anti- rabbit 1:1000) vector labs BA-2000 for 30 mins, washed in TBS pH7.4, 3×5 mins, incubates with ABC (ABC was prepared 30 mins before use) 30 mins, washed in TBS pH7.4 3×5 mins, incubates with DAB working solution for 5 mins, rinsed in running tap water for 5 mins, counter stained with haematoxylin, hydrated through graded alcohols, cleared in xylene and mount with entellen.

Images of each region considered was recorded with a Zeiss Axiocam HCR digital camera fitted to a zeissaxioskop 2 plus microscope and reversed on an Acer computer by the Axiom software. The positively stained cells were evaluated by three independent investigators on the digital photographs using the NIGH image programme (Scion image).

RESULTS AND DISCUSSION

This study shows positive expression of GFAP in the hippocampus at various regions and also around the hippocampus. Astrogliosis was observed in the mefloquine induced only at CA3 (Fig. 1b), at the dentate region (Fig. 2b) and in the molecular layer (Fig. 3b) indicating neuroinflammation. There was less expression of GFAP in the control group and treated groups (Fig. 1a to 3a). Previous studies have demonstrated widespread of astrocyte in the hippocampus when the animal was induced to create animal model of seizure (Andre *et al.*, 2007) and have additionally suggested that the high expression of GFAP in the damaged area are due to neuronal cell loss (Dube *et al.*, 2001). Neuronal cell lost occur after seizure was created using mefloquine and these cell degenerations was likely caused by apoptosis and

disruption of the pyramidal layer as expressed. This finding is in accordance to the previous animals and clinical studies (O'Brien *et al.*, 1997; Theodore *et al.*, 2001). Widespread microglial activation and persistent astrogliosis in areas with neuronal death was found in pilocarpine induced SE rats. We observed GFAP-positive cells in all the groups. Our findings support the hypothesis that glial cells are activated after SE-a

process that plays an important role in seizure generation (Seifert and Steinhäuser, 2013; Seifert *et al.*, 2006; 2009).

Ekanem *et al.* (2008) induced seizure in adult wistar rats using artequine and mefloquine and found out that mefloquine and artequine induced dose dependent reactive astrocytes formation in the hippocampus. This is in accordance with our findings.

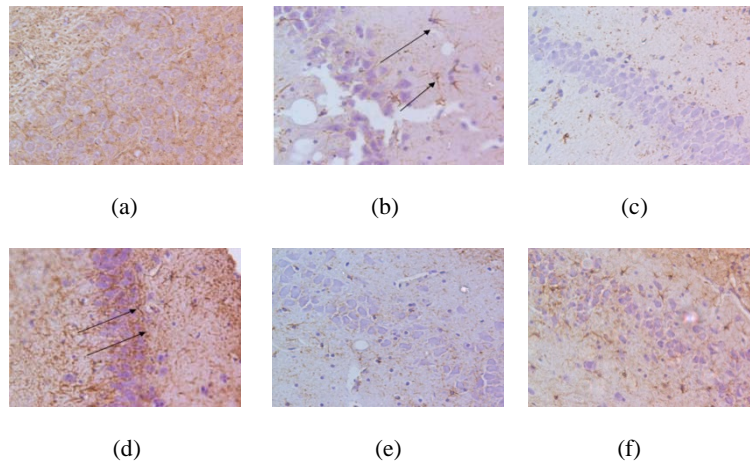


Fig. 1: GFAP expression at CA3 Region of the Hippocampus; (a): Control. No reactive astrocytes; (b): Mefloquine induced. Arrow showing reactive astrocytes and neuroinflammation; (c): Less expression of GFAP in Luffa only; (d): Diazepam treated. Diazepam did not completely ameliorate astrogliosis; (e): Low Dose of Luffa decreases astrogliosis and restore pyramidal cells; (f): High Dose of Luffa also decreases astrogliosis and restore pyramidal cells. Magnification x630.

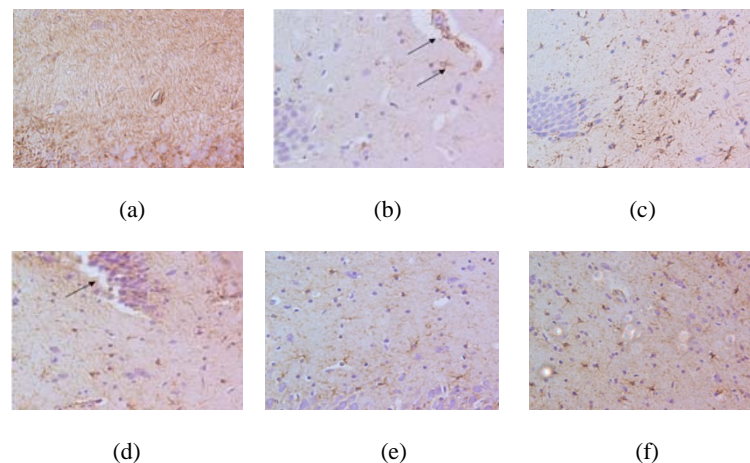
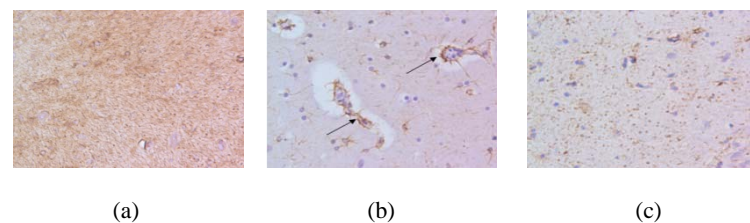


Fig. 2: GFAP expression at the region of Dentate Gyrus and CA1 of the Hippocampus; (a): No reactive astrocytes in the Control; (b): Mefloquine induced. Arrow showing reactive astrocytes and neuroinflammation; (c): No reactive astrocytes in Luffa only; (d): Diazepam reduces astrogliosis but did not ameliorate it and restore pyramidal cells; (e): Low Dose of Luffa decreases astrogliosis and restore pyramidal cells; (f): High Dose of Luffa also decreases astrogliosis and restore pyramidal cells. Magnification X630



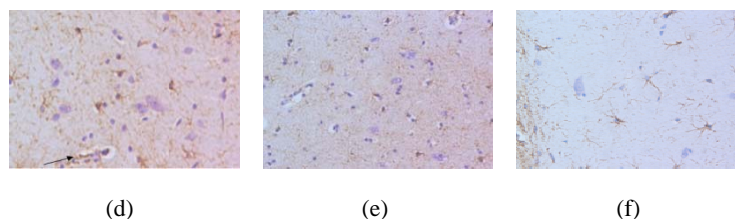


Fig. 3: GFAP at molecular layer of the Hippocampus; (a): No reactive astrocytes in the Control; (b): Mefloquine induced. Arrow showing reactive astrocytes and neuroinflammation; (c): Luffa only. No reactive astrocytes; (d): Diazepam did not ameliorate astroglia; (e): Low Dose of Luffa decreases astroglia and restore pyramidal cells; (f): High Dose of Luffa also decreases astroglia and restore pyramidal cells. Magnification. X630

Udoh *et al.* (2014) in their research on Hippocampal Glial Degenerative Potentials of Mefloquine and Artequin in Adult Wistar Rats showed that the histomorphology of the hippocampal sections of rats revealed large and dense populations of astrocytes and astrocytes' processes, with either loss or reduction in the population of oligodendrocytes. That there was also loss in the population of pyramidal neurons as compared with the control group. Mefloquine and Artequin administration induced dose-dependent reactive astrocytes and astrocytes' processes formation in the hippocampus, proving that it may impair the uptake of neurotransmitter and alter neuronal environment thus altering the hippocampal function. Mefloquine induced dose-related neurological effects in the rat model (Dow *et al.*, 2006).

Olude *et al.* (2015) described astrocyte morphologies and heterogeneity in male African giant rats (AGR; *Cricetomys gambianus*, Waterhouse) across three age groups (five neonates, five juveniles and five adults) using Silver impregnation method and immunohistochemistry against glial fibrillary acidic protein. They found out that Immunopositive cell signaling, cell size and population were least in neonates, followed by adults and juveniles, respectively. In neonates, astrocyte processes were mostly detected within the glia limitans of the mid and hind brain; their cell bodies measuring $32 \pm 4.8 \mu\text{m}$ in diameter against $91 \pm 5.4 \mu\text{m}$ and $75 \pm 1.9 \mu\text{m}$ in juveniles and adults, respectively. Astrocyte heterogeneity in juvenile and adult groups revealed eight subtypes to include fibrous astrocytes chiefly in the corpus callosum and brain stem, protoplasmic astrocytes in the cortex and Dentate Gyrus (DG); radial glia were found along the Olfactory Bulb (OB) and Subventricular Zone (SVZ); velate astrocytes were mainly found in the cerebellum and hippocampus; marginal astrocytes close to the pia mater; Bergmann glia in the molecular layer of the cerebellum; perivascular and periventricular astrocytes in the cortex and third ventricle, respectively. Astrocytes along the periventricular layer of the OB are believed to be part of the radial glia system that transport newly formed cells towards the hippocampus and play roles in neurogenesis migration and homeostasis in the AGR. Therefore, astrocyte heterogeneity was examined across age groups in the

AGR to determine whether age influences astrocytes population in different regions of the AGR brain and discuss possible functional roles (Byanet *et al.*, 2013).

CONCLUSION

Neuronal cell lost occur after seizure was induced using mefloquine and this cell degeneration was caused by apoptosis and disruption of the pyramidal layer as expressed in our results. It was observed from the study that *Luffa aegyptiaca* mill has neuroprotective effect and it causes restoration of the pyramidal cell layer of the hippocampus.

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CONFLICT OF INTEREST

There is no conflict of interest for this research and for the development of this manuscript.

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