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Short communication

Hippocampal Astrogliotic Reduction in Scopolamine Hydrobromide-Induced Alzheimer's Cognitive Dysfunction Wistar Rats Following Administration of Aqueous Extract of *Telfairia occidentalis* (Hook F.) Seeds

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Summary: Astrocytes are small star-shaped glial cells that maintain normal human brain physiology including secretion of several active compounds and the formation of blood brain barrier. Reactive astrocytes support regenerating axons and also actuate some genes responsible for the induction of synapse formation. In this study, the effect of aqueous extract of *Telfairia occidentalis* seeds on hippocampal astrogliosis was done using scopolamine-induced Alzheimer's type cognitive dysfunction Wistar rats. Thirty Wistar rats of 6 weeks of age (180-200g) were randomly grouped into five designated A, B, C, D and E. Each group contained six rats. Alzheimer's type cognitive dysfunction was induced in groups B to E by administering intraperitoneally, 1 mg/kg body weight of scopolamine for seven days before Donepezil and the aqueous extract of *Telfairia occidentalis* seeds for fourteen days. Twenty-four hours after the last administration, the animals were sacrificed; their brain tissues perfused and stained with glial fibrillary acidic protein (GFAP) dye. Results revealed prominently stained astrocytes with their processes intact (group A). Some densely stained numerous *astrogliosis* with hypertrophied fibres were noticed in group B. Group C demonstrated prominent astrocytes with hypertrophied fibres; group D, moderately stained *astrogliosis* with hypertrophied fibres while group E showed numerous astrocytes with prominent nuclei and hypertrophied fibres. In conclusion, there was a reduced hippocampal *astrogliosis* mostly in group D treated with *Telfairia occidentalis* which may have neutralized oxidative stress and enhanced learning and memory in the Wistar rats of the present study.

Keywords: *Alzheimer, Astrogliosis, Hippocampus, Scopolamine hydrobromide, T. Occidentalis seeds, Wistar rats*

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INTRODUCTION

Alzheimer's disease (AD) is a chronic neuro-degrading disorder with cognitive and recall impairment, chaperoned by intracellular neurofibriliary entangles and extracellular amyloid plaques (Zheng *et al*., 2007). The global occurrence of nervous disease among individuals > 60 years is between 5-7% (Prince *et al.*, 2013). A study reported that AD is the most frequent type of dementia before blood vessel dementia (Akter *et al.*, 2012). More so, there are light and discord information on the prevalence of dementia and its subtypes in the sub-Saharan Africa (Prince *et al.*, 2013) with far-reaching implications on public health policies. While some studies revealed a reduced occurrence of dementia in the sub-Saharan Africa (Yusuf *et al.*, 2011; Prince *et al.*, 2013), others reported similar occurrence compared to those in the western world (Paddick *et al*., 2013). Research also revealed obvious difference in the occurrence of AD in men and women with two third of Alzheimer's disease patients being women (Alzheimer's Association, 2017). In 2010, the global economic implication of dementia was estimated to be eight hundred and eighteen billion dollars which may reach one trillion dollars in 2018 (Prince *et al.*, 2016).

Due to high cost of management and treatment of AD, the use of plants such as pumpkin seeds (*Telfairia Occidentalis*) as an alternative became paramount to scientists. Pumpkin seeds are very common in most parts of Nigeria. The plant is characterised with pharmacological activities such as antioxidants, antidiabetic, antibacterial, anti-inflammatory and antifungal effects (Nkosi *et al*., 2006). Pumpkin seeds have been documented to possess neuroprotective effects and enhanced learning and memory (Eru *et al*., 2020; 2021).

The endogenous (glutathione secreted by the neuronal cells) and exogenous antioxidants from plants such as *T. occidentalis* seed help to neutralize the redundant free radicals, defend the cell from poisonous substances and also, chip in to avert disease (Pharm-Huy *et al.*, 2008). The free antioxidant property of *T. Occidentalis* seed may provide safer option, hence, the necessity to probe the outcome of aqueous extract of *T. occidentalis* seeds on hippocampal astrogliosis using scopolamine hydrobromide-induced Alzheimer's type cognitive dysfunction Wistar rats.

MATERIALS AND METHODS

Ethical consideration: Ethical approval was obtained from the Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Calabar, Nigeria (Approval number: FAREC-FBMS 042ANA3719) in line with the principles of laboratory animal care (NIH publication NO. 85-23, revised 1985) as well as specific National laws applied.

Animals: A total of thirty adult female and male Wistar rats of 6 weeks of age (180-200g) were bought from the University of Calabar animal farm, kept in animal room in the Department of Anatomical Sciences, for two weeks under standard conditions of temperature (27ºC – 30ºC) for acclimatization. The animals were fed with raw chow manufactured by the Agro Feed Mill Nigeria Ltd, Calabar and allowed access to drinking water. After acclimatization, the experimental rats were randomly grouped into five, each containing six rats designated A, B, C, D and E.

Plant extract preparation: Fresh *Telfairia occidentalis* seeds were obtained from the Watt market, Calabar, Cross River State, Nigeria. The fresh *Telfairia occidentalis* seeds were identified, authenticated and registered with voucher number: HERB/BOT/UCC/322 in the Department of Botany, University of Calabar, Calabar. The seeds were removed from shell, washed to free debris, chopped into smaller pieces and air-dried in the laboratory. The dried samples were blended into powder using blender (with model number Bravo3JARS Mixer grinder) with 1600 g of the powder seed soaked in 1000 mls distilled water for twenty-four hours. The mixture was then filtered using chess cloth and the Whatman No.1 filter paper. The solution was obtained and concentrated to a syrupy residue at 40ºC-50ºC using man-made thermostatic water bath (with model number F.NR: 1508.0271) and kept in a cool dry place for use during administration.

Induction of Alzheimer's type cognitive dysfunction: Alzheimer's type cognitive dysfunction was induced to the rats in groups B, C, D and E through intraperitoneal injection of 1.0 mg/kg body weight of scopolamine hydrobromide (SHB) for seven days.

Determination of LD_{50}: The LD_{50} of aqueous extract of *Telfairia occidentalis* seeds was established to be > 7000 mg/kg according to Lorke's method. The dose of aqueous extract administration was determined using 12.5% (875 mg/kg) and 25% (1750 mg/kg) of the established LD_{50} (7000 mg/kg body weight of aqueous extract of *Telfairia occidentalis* seeds).

Plant extract and Donepezil administration: Group A served as the negative control and received animal feed with water *ad libitum*; group B served as the positive control and received 1.0 mg/kg body weight of SHB only; group C received 1.0 mg/kg body weight of SHB and 1.0 mg/kg body weight of Donepezil, group D received 1.0 mg/kg body weight of SHB and 875 mg/kg body weight of aqueous *Telfairia occidentalis* seeds while group E received 1.0 mg/kg body weight of SHB and 1750 mg/kg body weight of aqueous *Telfairia occidentalis* seeds for fourteen days.

Tissue processing and staining procedure: Twenty-four hours after the last administration, the animals were sacrificed with their brain tissues perfused and processed immunohistochemically. Serial paraffin sections of 5 µm thick were deparaffinised and dehydrated. The endogenous peroxidise activity was blocked with 0.05% hydrogen peroxide for 30 minutes. The slides were washed for 5 minutes in phosphate-buffered saline at a pH of 7.4. Sections were later placed in a 0.01M citrate buffer (pH 6) in a microwave for 5 minutes. The slides were incubated in 1% Bovine serum albumin for 30 minutes at 37ºC. Two drops of antibodies were applied to the sections and then incubated for 90 minutes at room temperature. Glial fibrillary acidic protein (GFAP) was then applied to the sections. The anti-mouse immunoglobulins conjugated to a peroxidise-labelled dextran polymer. The slides were incubated in 3,3'-diaminobenzidine for 15 minutes. The slides were counterstained with Mayer's haematoxylin and then dehydrated, cleared, mounted with dibutylphthalate polystyrene xylene (DPX) and observed under light microscope.

RESULTS

Sections of the hippocampus of adult Wistar rats in the negative control (group A) revealed prominently stained astrocytes and their processes (Plate 1A) while group B treated with 1mg/kg body weight of SHB showed densely stained numerous astrogliosis with hypertrophied fibres (Plate 1B). Group C treated with 1mg/kg body weight of SHB and Donepezil showed prominent astrocyte with hypertrophied fibres (Plate 1C), group D treated with 1mg/kg body weight of SHB and 875 mg/kg of *Telfairia occidentalis* revealed moderately stained astrogliosis with hypertrophied fibres (Plate 1D) while group E treated with 1mg/kg body weight of SBB and 1750 mg/kg of *Telfairia occidentalis* revealed numerous astrocytes with prominent nuclei and hypertrophied fibres (Plate 1E).

Immunohistochemical staining showed few of the GFAP positive astrocytes in the hippocampus of the negative control rats (Plate 1). In contrast, reactive astrocytes (astrogliosis) and hypertrophied fibres were markedly increased in the hippocampus of the SHB treated group (Plate 2).

DISCUSSION

The result of this study is in line with Anderson *et al.*, (2016) who reported considerable proliferation of astrocytes seen sequel to trauma when the reactive response produced a protective scar around the injury. Reactive astrocytes from other groups treated with Donepezil and *T. occidentalis* seeds were markedly increased in the hippocampus (plates 3-5) when compared with the negative control (plate 1) but less reactive when compared with the positive control treated with SHB (plate 2).

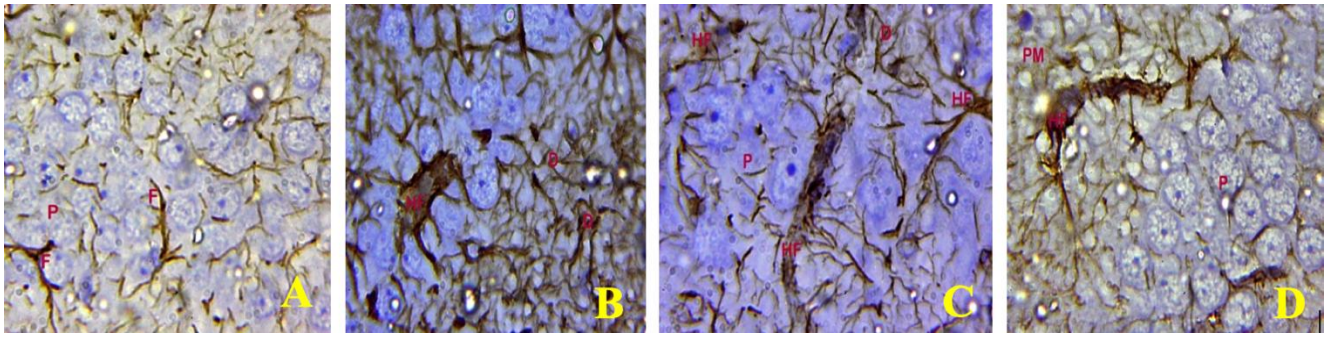

Plate 1:

A. Photomicrograph of a section of the hippocampus negative control (group A) showing prominently stained fine astrocyte processes and fibres.

B. Photomicrograph of a section of the hippocampus positive control (group B) treated with 1mg/kg body weight of SHB showing numerous astrocytes with prominently stained extensive processes and interdigitations in addition to astrocyte proliferation (astrogliosis).

C. Photomicrograph of a section of hippocampus (group C) treated with 1mg/kg body weight of SHB and Donepezil showing astrocytes with prominently stained interdigitating and hypertrophic fibre.

D. Photomicrograph of a section of hippocampus (group D) treated with 1mg/kg body weight of SHB and 875 mg/kg body weight of *Telfairia occidentalis* showing astrocytes with moderately stained and hypertrophic fibres coupled with astrocytes proliferation (astrogliosis).

E. Photomicrograph of a section of hippocampus (group E) treated with 1mg/kg body weight of SHB and 1750 mg/kg body weight of *Telfairia occidentalis* showing numerous astrocytes with prominent nuclei and extensive processes with hypertrophic fibres.

Astrocytes are characteristic star-shaped cells in the brain and spinal cord (Verkhratsky and Butt, 2013). In reaction to destruction inflicted on the central nervous system, astrocytes alter from their normal quiescence state to a so-called reactive state. This process of reactive gliosis is noted by morphological variations (hypertrophy), functional changes and profound increase in the expression of astrocytes specific intermediate filament and the GFAP (Middeldop and Hol, 2011). In this research, a tremendous rise in the GFAP-positive astrocytes was observed in SHB treated group (plate 2) compared to the control animals (plate 1). Immunohistochemical results from this study showed reduced expression of the GFAP positive reactive astrocytes in groups C, D and E (plates 3-5). Reactive astrocytes accompany every acute injury and chronic neurological disease that exists in two different states of activation; A_1 and A_2. Reactive astrocytes while providing trophic support to the generating axons (Anderson *et al.*, 2016), can also inhibit axon regeneration (Silver and Miller, 2004). However, the consumption of antioxidants through diets and supplements is expected to remove reactive oxygen species from the living system and provide health benefits (Zhang *et al.*, 2011) as *T. occidentalis* has been documented to have antioxidants and minerals that aid normal brain function. The reduction in GFAP expression cells in these treated groups may be attributed to the antioxidant potentials of the *T. occidentalis* seeds hence, resulting to cellular regenerations. In conclusion, hippocampal astrogliosis induced by SHB was observed to be reduced mostly in group D following the administration of aqueous extract of *Telfairia occidentalis* seeds. The extract may neutralize oxidative stress, and increase cellular regeneration with enhanced learning and memory in Wistar rats.

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