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The Effect of *Averrhoa carambola* (Star Fruit) Aqueous Fruit Extract on the Hippocampal Astrocyte Expression Following Diazepam-Induced Neurotoxicity in Wistar Rats

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ABSTRACT

Neurotoxicity is caused by physical, chemical or biological agents which exert an effect on the structure or function of the nervous system. Drugs of abuse, including Diazepam are common, and have been known to cause neurotoxicity.The hippocampus which plays a critical role in the formation, organization and storage of memory is the most affected structure in several neurological disorders. *Averrhoa carambola* is a plant native to tropical South-East Asia and known for its health protective properties. The study investigated the effect of aqueous *Averrhoa carambola* fruit extract on hippocampal astrocyte expression following Diazepam-induced neurotoxicity in Wistar rats. Twentyfive female rats were divided into five (5) groups. Group A served as the negative control group, while group B served as positive control and received Diazepam (25mg/kg body weight) alone. Groups C, D and E were given Diazepam (25mg/kg BW) + 1000mg*Averrhoa carambola* extract, Diazepam (25mg/kg BW) + 600mg *Averrhoa carambola* extract and Diazepam (25mg/kg BW) + 300mg *Averrhoa carambola* extract respectively for 30 days. Glial fibrillary acidic protein (GFAP) immunoreactivity in the hippocampus displayed immunonegativity for group A rats, while a high level of GFAP-reactive astrocyte expression was observed in groups B, D and E. An increase in the number of astrocytes (hyperplasia) as well as reduced GFAP-reactive astrocyte expression was observed in group C which may be indicative of neuroprotective effects of the aqueous fruit extract. *Averrhoa carambola* aqueous fruit extract can therefore ameliorate Diazepam-induced toxicity of the hippocampus in Wistar rats, and the effects were dose-dependent.

Keywords: *Averrhoa carambola*, Diazepam, Hippocampus, Glial Fibrillary Acidic Protein.

Introduction

The hippocampus is an important part of the brain. It is small in structure and it plays a critical role in the formation, organization and storage of new memories, as well as the connection of certain sensations and emotions to these memories.[1] Since alterations in learning and memory are a common consequence of toxicant exposure, it is possible that the hippocampus would be a target for neurotoxicity. It is believed to be the earliest and most severely affected structure in several neurological disorders.[2,3] Environmental toxicants such as heavy metals and drugs of abuse have been known to inflict damage to the hippocampus.[2] Drug intoxication is always related to a generation of free radicals that result in cellular distortion and alteration of neurotransmitter homeostasis, which seems to be a common unspecific mechanism to toxic responses that may lead to impairment of neuronal function.[4] Diazepam is a benzodiazepine which is a class of psychoactive drugs with a long history of use in the treatment of a wide spectrum of neurological disorders, including anxiety and epilepsy.[5] It is generally effective when used therapeutically, but when abused or engaged in long-term treatment could lead to oxidative stress which can be injurious to the brain.[6] Oxidative stress results from prooxidative/antioxidative imbalance in the body which could lead to tissue damage. [7] The central nervous system is extremely sensitive to free radical damage because of its relatively small antioxidant capacity. The protection provided by fruits and vegetables against neurodegeneration is attributed to various antioxidants contained in these foods.[8] It has been reported that many plants possess an array of non-enzymatic antioxidants that reduce the effect of reactive oxygen species-induced damage which underly many diseases.[9] *Averrhoa carambola* is one of such plants that possess very high antioxidant content and potential. It is a plant native to Southern Asia and is cultivated in many countries around the world. It is generally called star fruit due to its fruit being five-lobed. The fruit is green when small but turns yellow or orange when ripe and is known to possess health promoting compounds, including flavonoids, alkaloids, tanins and saponins.[10] The entire fruit is edible, and of high nutritive value. It is known for its medicinal uses traditionally, especially in the treatment of digestive tract disorders, diabetes mellitus, lowering of blood pressure, as well as anti-cancer, anti-dotal and anti-microbial effects. The anti-oxidant property makes it useful in the management of illnesses, including neurological disorders.[11] In view of these facts, the study investigated the effects of aqueous *Averrhoa carambola* fruit extract on the hippocampus of Diazepam-induced neurotoxicity in female Wistar rats.

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Materials and Methods

Animals

A total of 25 Wistar rats (145-215 g) were acquired from the animal house, College of Medical sciences, University of Calabar. The rats were kept under standard conditions of temperature (27^0C – 30^0C)and fed with rat chow purchased from Agro Feed Mill Nigeria Limited, Calabar and provided with distilled water for drinking.. They acclimatized for three weeks prior to commencement of the experiment. The animals were housed in properly ventilated plastic cages.

Ethical approval

An approval for experimental procedures with registration number 045ANA119 was obtained from the Faculty of Basic Medical Sciences, University of Calabar, Cross River State.

Drug

Diazepam was purchased from a pharmacy in Calabar Municipality, Calabar, Cross River State, Nigeria. Each tablet contained 5 mg of Diazepam.

Plant collection and identification

Fruits of *Averrhoa carambola* were sourced from a farm in Lemna, Calabar Municipality Local Government Area, Cross River State. The fruits were obtained on a daily basis for use during the period of the experiment. The plant was identified and authenticated in the Department of Botany, Faculty of Science, University of Calabar, Cross River State and issued a voucher number Herb/BOT/UCC/085.

Plant extraction

Freshly plucked ripe *Averrhoa carambola* were washed with distilled water and air-dried. They were then chopped into small pieces and blended with an electric blender (Crown star smoothie blender). The blended residue was emptied into a strainer and the aqueous juice extracted through filteration. The extraction was done daily just before administration to the rats in order to preserve the integrity of its bio-constituents.

Acute toxicity test

The LD_{50} of the *Averrhoa carambola* fruit extract was established to be >10.000mg/kg according to Lorke's method. The dosage was determined using 60% (1,000 mg), 40% (600 mg) and 20% (300 mg). No mortality was recorded and it conforms with a study that proved that the LD_{50} value of the aqueous fruit extract is greater than 10ml/kg.[12] In another study, it was observed that the LD_{50} value is greater than 5,000 mg/kg.[13]

Diazepam administration

Diazepam (Valium) tablets (5 mg) were dissolved in distilled water and a stock solution of 100 mL (5 mg tablet to 1 mL of water) was prepared. 25 mg/kg BW of Diazepam solution was then obtained and administered orally. The aqueous *Averrhoa carambola* fruit extract was also administered through the same route. .

Experimental protocol

25 female albino Wistar rats were divided into 5 groups (A to E) of 5 rats each. Group A served as negative control and received only food and water *ad libitum* (no treatment or placebo was given).Group B served as positive control and received 25 mg/kg BW of Diazepam alone; Group C received 25 mg/kg BW of Diazepam + 1000 mg *Averrhoa carambola* fruit extract; Group D received 25 mg/kg BW of Diazepam + 600 mg *Averrhoa carambola* fruit extract while Group E received 25 mg/kg BW of Diazepam + 300mg *Averrhoa carambola* fruit extract. The aqueous fruit extract was administered to the rats 2hrs after Diazepam administration.

Termination of experiment

Twenty-four hours after the last administration, the rats in each group were sacrificed by cervical dislocation. The skulls were opened so that the whole brain was excised. They were fixed in 10% buffered formal saline and later processed to paraffin wax embedding.

Immuno-histochemical study (GFAP): 5µm thickness of serial paraffin sections of the hippocampus from all groups were deparaffinised and rehydrated. The endogenase peroxidase activity was blocked with 0.05% of Hydrogen peroxide in absolute alcohol for 30mins, after which the slides were washed in phosphate-buffered saline (PBS) for 5min. Then in citrate buffer (pH 6) and placed in a microwave for 5mins. The slides were incubated in 1% Bovine Serum Albumin dissolved in PBS for 30 min. Ready-to-use primary antibody was applied to the sections and then incubated for 90 min. The slides were rinsed with PBS and incubated for 60mins with anti-mouse immune-globulins conjugated to a peroxidase-labelled dextran polymer. In order to detect the reaction, the slides were incubated in 3,3-diaminobenzidine for 15 min. The slides were counter-stained with Mayer's Hematoxylin and then dehydrated, cleared and mounted by DPX. GFAP-positive cells appeared brown and nuclei appeared blue.[14,15] The slides were then examined under light microscope and photomicrographs taken.

Results and Discussion

The photomicrograph of GFAP labelled cells in the hippocampus of group A rats (plate 1) displayed an intact hippocampus microstructure with sparse amount of light-brown astrocytes. Plate 2 (group B) showed reactive (immunopositive) astrocytes with prominent cell processes wrapped around injured neurons. They were hypertrophied and increased in number around injured neurons. Plate 3 (group C) displayed increased number of astrocytes (hyperplasia) with conspicuous cytoplasm and processes. Plate 4 (group D) showed reactive astrocytes with prominent processes wrapped around regenerating neurons. The astrocytes displayed hyperplasia. There was less GFAP expression as compared with group B. Group E (plate 5) presented reactive astrocytes with cell processes wrapped around the pyramidal cells. The astrocytes displayed both hypertrophy and hyperplasia. Here the level of GFAP expression is similar to that of group B.

Astrocytes are characteristic star-shaped cells in the brain and spinal cord.[16] They are known to play a critical role in maintaining the structural and physiological integrity of neurons, as well as involvement in all types of brain pathologies.[17] In response to brain injury, astrocytes react by hypertrophy and hyperplasia.' . The reactive astrocytes possess greatly enlarged cytoplasmic processes and produce high amounts of GFAP which is a quantitative histochemical marker for toxicant-mediated injury in the nervous system.[18] Astrocytes have been known to protect injured tissues and cells in various ways.[16] In the present study, plate 1 (group A) revealed sparse amount of light brown astrocytes. A high level of GFAP-reactive astrocyte expression was observed in plate 2 (group B rats) which were treated with Diazepam alone. The GFAP expressed is a specific protein released after any brain injury.[19] The high expression of reactive astrocytes has also been observed in some drug neurotoxicity studies.[20-22] The presence of these reactive astrocytes is indicative of brain insult[23] and the gliosis that developed in group B rats could possibly be caused by the generation of free radicals.[24] It has been stated that long term Diazepam treatment is associated with oxidative tissue damage.[6] The mechanism for the increase in the number of astrocytes and the major decrease in GFAP-reactive astrocyte expression as observed in Plate 3 (group C) is not yet understood but it may be due to the neuroprotective effects of the phenolic compounds and flavonoids in the fruit extract which possesses strong radical scavenging properties.[11] Some studies have illustrated the high antioxidant potential of *Averrhoa carambola* fruit extract and its protection against free radical damage.[11,25,26] Other studies as well have demonstrated its tissue protective effects. [27-31] The high level of GFAP-reactive astrocyte expression as observed in Plate 4 (group D) and plate 5 (group E) may be indicative of reduced neuroprotective action following the administration of lower doses of *Averrhoa carambola* fruit extract.

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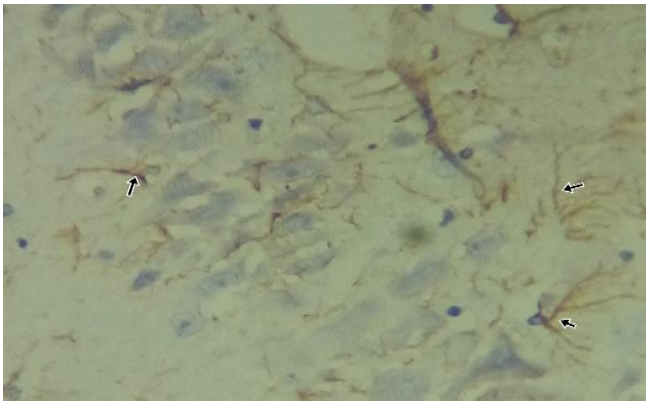

Plate 1: Photomicrograph (X400) of a section of the hippocampus of the negative control group (group A) stained with GFAP, showing sparse amount of light brown astrocytes indicated by the arrows.

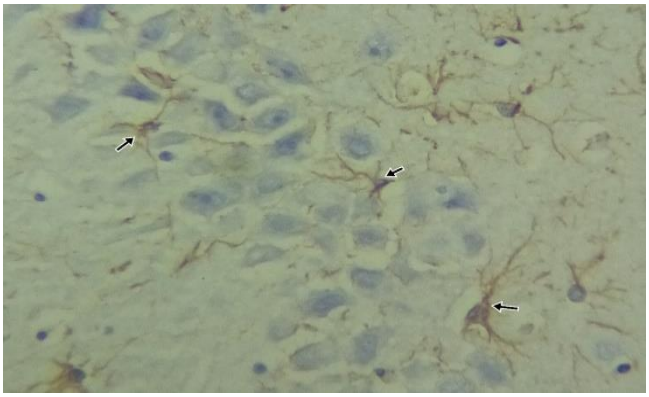

Plate 2: Photomicrograph (X400) of a section of the hippocampus of the positive control group (group B) treated with 25mg/kg BW Diazepam alone. This shows reactive astrocytes as indicated by the arrows. They are hypertrophied

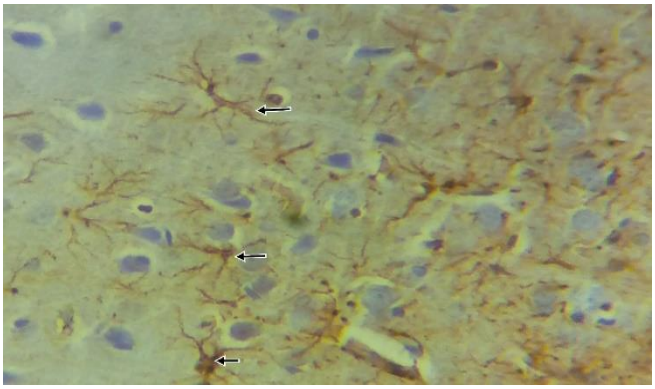

Plate 3: Photomicrograph (X400) of a section of the hippocampus of group C rats treated with 25mg/kg BW Diazepam and 1000mg of *Averrhoa carambola* fruit extract. This section shows increased number of astrocytes (hyperplasia) with conspicuous cytoplasm and processes

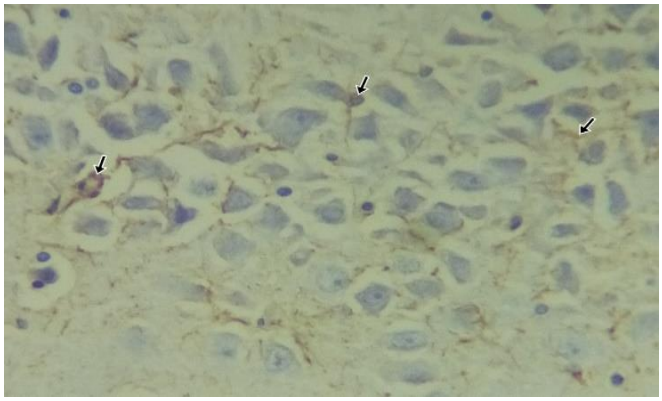

Plate 4: Photomicrograph (X400) of a section of the hippocampus of group D rats treated with 25mg/kg BW Diazepam and 600mg of *Averrhoa carambola* fruit extract. This section shows reactive astrocytes (indicated by arrows) with prominent processes wrapped around regenerating neurons. The astrocytes displays hyperplasia.

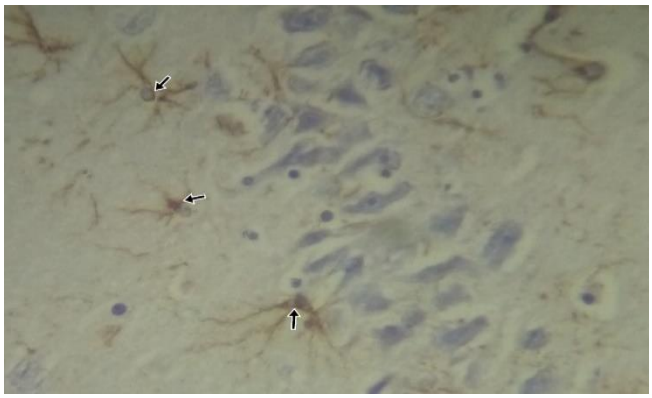

Plate 5: Photomicrograph (X400) of a section of the hippocampus of group E rats treated with 25mg/kg BW Diazepam and 0.3ml of *Averrhoa carambola* fruit extract. This section presents reactive astrocytes (indicated by arrows) with cell processes wrapped around the pyramidal cells. The astrocyte displays both hypertrophy and hyperplasia.

The ameliorative potentials of the aqueous fruit extract may be due to its antioxidant properties which is in line with studies where *T. Occidentalis* has the potential to ameliorate hippocampal microstructure as well as enhance learning and memory in scopolamine hydrobromide-induced Alzheimer's type cognitive dysfunction in rats and this was also attributed to its high antioxidant components.[32,33]

Conclusion

The study revealed ameliorative potentials of Averrhoa carambola fruit extract on the hippocampus of Diazepam-induced neurotoxicity in female Wistar rats.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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