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Research Article

Hippocampal Histomorphology and Biochemistry in Rats fed Diets containing Carbamazepine

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ABSTRACT

In this study, the effects of carbamazepine on hippocampal histomorphology, acetylcholinesterase activity, and glutamate concentration in adult male Wistar rats were investigated. Healthy Wistar rats of the male sex weighing between 200g-250g assigned randomly into 4 groups (n= 5) were utilized for this study. Group A received 2 mLs/Kg of distilled water, groups B, C and D received 160mg/kg, 120mg/kg, and 80mg/kg of carbamazepine. Carbamazepine was supplemented in rat chow for the study span of four weeks. The whole brain was harvested the following sacrifice fixed in 10% Neutral Buffered Formalin (NBF) and stained in H&E and Cresyl violet for hippocampal histomorphology and Nissl substances evaluation. Hippocampal Acetylcholinesterase (AChE) activity and concentration of glutamate were biochemically determined. Data obtained was analyzed using one-way ANOVA followed by Student Newman Keul posthoc test. A value of $p<0.05$ was considered significant. Results revealed statistically significant decrease in weight change across all experimental groups ($F = 6.820$, $p = 0.0036$). Relative brain weight ($F = 0.9048$, $P = 0.4803$), activities of glutamate (($F= 0.4545$, $p = 0.7214$) and AChE ($F= 0.4128$, $p = 0.7484$) were not significantly different across group in comparison to control. Group B which received the highest dose of CBZ (160mg/kg) showed widespread pyramidal and granule neuronal degeneration, while group C also showed mild degenerative changes in comparison to the control Based on the data from our study, carbamazepine exerted neuronal degenerating tendencies on the hippocampus is in a dose-dependent manner. This in turn will affect hippocampal-related functions.

Keywords: *Hippocampus; Carbamazepine; Glutamate; Seizures*

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INTRODUCTION

Carbamazepine (CBZ) is an anti-convulsant medication whose mechanism of action is by decreasing abnormal electrical discharge in the brain (Oliva *et al.*, 2012). As an anticonvulsant, it is used in the treatment of various disorders which are and are not limited to bipolar disorders, mood changes, trigeminal neuralgia, and also majorly in epilepsy treatment. Following its discovery in 1953 by Swiss chemist Walter Schindler (Tolou-Ghamari *et al.*, 2013), it has been made available as a generic medication by the World Health Organization as one of the drugs in the List of Essential Medicines (WHO, 2019). Its mechanism of action is by reducing sustained neuronal firing through the blockade of voltage-gated sodium channels. The therapeutic effects of carbamazepine vis-à-vis inhibition of brain neuronal activities are noteworthy. Neurons communicate by electrical signals and careful regulation of this neuronal circuitry is essential for proper brain function. Dysfunction of the neuronal circuitry is known to be a major cause of seizures (Chan *et al.*, 2016). Carbamazepine influences neurotransmitters like Gamma-aminobutyric acid (GABA) and glutamate. It is noteworthy that chronic administration of Carbamazepine is associated with up-regulation of hippocampal $GABA_B$ receptors. This action may be a potential convergent mechanism for mood stabilization. Carbamazepine is involved in the potentiation of GABA receptors made up of α1, β2, and γ2 subunits. This mechanism may contribute to its efficacy in neuropathic pain and bipolar disorder (Ayano, 2016). Alterations in the morphology of different parts of the developing and immature brain following carbamazepine treatment have been extensively studied. This study aims to assess the effects of carbamazepine on hippocampal histomorphology, acetylcholinesterase activity, and glutamate concentration in adult male Wistar rats.

MATERIALS AND METHODS

Ethical procedure: The rats were kept in the animal holding of Afe Babalola University (ABUAD). Experimental protocols were per the guidelines set by the local institutional

research committee. All rats were handled per the guidelines for animal research as detailed in the guidelines for the Care and Use of laboratory animals by the National Academy of Sciences, 2011.

Drug and Reagents: Carzepin (Carbamazepine tablet B.P. 200mg) was procured from Aromokeye & Co. Ltd Pharmaceuticals, Veterinary Livestock drugs & Agrochemical, Ado-Ekiti, Ekiti State. All other reagents used for this study were of analytical grade and purity.

Rat care and Management: Twenty healthy adult male Wistar rats weighing between 200 g-250g were utilized for this study. The rats were procured from the animal holding unit of Afe Babalola University, Ado-Ekiti, and divided into 4 groups (n=5 each). The acclimatization period was for one week under normal jurisdictions of acclimatization and allowed unrestricted access to rodent chow and clean water was provided *ad libitum*. The natural light and dark cycle was maintained during the study period.

Rats in group A received distilled water (2mLs/Kg), group B received 160mg/kg of carbamazepine, group C received 120mg/kg of carbamazepine, group D received 80mg/kg of carbamazepine supplemented in 100g of rat chow for four weeks

Table 1:
Grouping, Dose Regimen, and Schedule

S/N	GROUPS	AGENTS	DOSES	DURATION
1	A	Distilled Water	2mLs/Kg	4 weeks
2	B	Carbamazepine	160mg/kg	4 weeks
3	C	Carbamazepine	120mg/kg	4 weeks
4	D	Carbamazepine	80mg/kg	4 weeks

Determination of Body Weight and Sacrifice of Animals: The rats' body weight and relative brain weight were determined by the formulae:
Relative brain weight was calculated using the formula;

$$\frac{Brain\ weight}{final\ bodyweight}$$

Percentage weight change was calculated using:

$$\frac{Finalweight - Initialweight}{Initialweight} \times 100$$

Rat sacrifice and histological analysis: Following the last day of administration. The rats were sacrificed by cervical dislocation. The whole brain was harvested carefully and weighed using the gallenhamp electronic balance (MP 10001) weighing balance and fixed in 10% NBF for histological studies. Collected blood samples were centrifuged for biochemical analysis.

Biochemical analysis: The whole blood via cardiac puncture collected was centrifuged for biochemical estimation of acetylcholinesterase and glutamate concentration. In the bench centrifuge, each compartment is balanced at the opposite compartment. The machine spun for 15 minutes at 3000 RPM separating the blood cells at the bottom and serum atop the blood.

Acetylcholinesterase (AChE) Assay procedure: AChE activity was determined spectrophotometrically by measuring the rate of hydrolysis of the substrate, ATCI according to the method of Ellman *et al*., (1961). Aliquots of the brain homogenates were diluted with 2.6 ml 0.1 M phosphate buffer (pH 7.4) to a total volume of 3 ml, which contained 100 mM DTNB. The enzyme activity was calculated by extinction co-efficient (E412= 1.36 x 104 M-1 cm-1) for the yellow anion 5-thio-2 nitrobenzoic acid. The AChE and activity was expressed as nmole of ATCI hydrolyzed per min per milligram of protein and for serum ATCI/BuTCI hydrolyzed/ml serum/min

Glutamate Assay Procedure: Estimation of glutamate level was done by preparing brain homogenates in phosphate buffer saline (PBS) according to the method of Adelodun *et al*., (2021). The brain homogenates were centrifuged at 13000 g for 10 minutes to remove all forms of insoluble materials. Supernatants were collected for measurement of glutamate using Enzyme-linked immunosorbent Assay (ELISA) kits by a microplate reader at 450nm. The samples were brought to a final volume of 50µL with glutamate assay buffer. Glutamate levels were determined using a standard curve as described by the kit manufacturer

Histological and Histochemical Staining: The hematoxylin and Eosin method was used to demonstrate the general histoarchitecture of the hippocampus. Cresyl Fast Violet (Vogts') Method for Nissl Substance evaluation

Photomicrography: Olympus binocular microscope was used for taken digital micrographs. A 5.1-megapixel MV550 research camera for microscopes was mounted in one of the oculars. This was connected to a computer running on image capture and analysis software. The system was adjusted to obtain clarity and resolution. Digital images were taken and archived.

Statistics: Data obtained was analyzed using one-way ANOVA, followed by Student Newman-keuls (SNK) test for multiple comparisons. Graph Pad Prism 5 (Version 5.03, Graphpad Inc.) was the statistical package used for data analysis. The significant difference was set at $p<0.05$.

RESULTS

All experimental groups showed significant difference in weight change ($F = 6.820$, $p = 0.0036$) in comparison to the control. *Posthoc* analysis showed that weight of group B rats was significantly lower ($p< 0.05$) than the control group (A) and group D (Fig. 1). There was no significant difference in relative brain weight across all experimental groups ($F = 0.9048$; $P = 0.4803$) (Table 2). Also, there were no significant difference in glutamate and ACHE across all experimental groups ($F= 0.4545$, $p = 0.7214$) (Fig. 2) and ($F= 0.4128$, $p = 0.7484$) (Fig. 3) respectively.

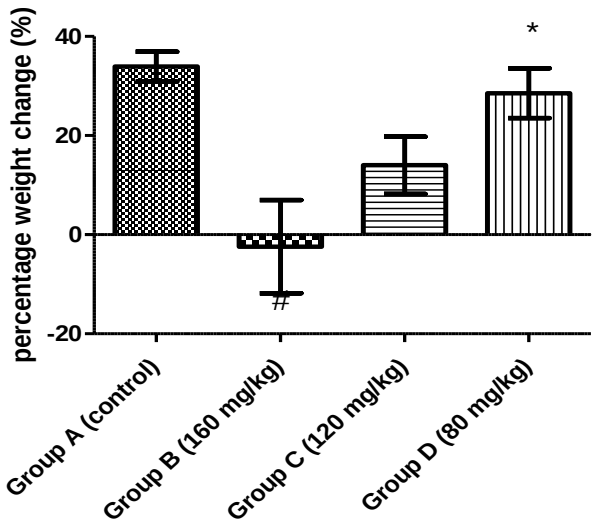

Figure 1:
Bar chart showing the effect of Carbamazepine on body weight change across groups. Values are expressed as % weight change ± SEM. # = relative to control at $p<0.05$; *= relative to group B at $p<0.05$.

Table 2:
The Effect of Carbamazepine on the brain and relative brain weight of Rats

Groups	Absolute Brain Weight (g)	Relative Brain Weight (%)± SEM
A (Control)	1.76 ± 0.05	0.73 ± 0.03
B (Carbamazepine 160mg/kg)	2.03 ± 0.17	0.67 ± 0.07
C (Carbamazepine 120mg/kg)	1.81 ± 0.09	0.63 ± 0.03
D (Carbamazepine 80mg/kg)	1.64 ± 0.06	0.67 ± 0.03

Values are expressed as mean ± SEM. No significant difference across the groups ($p<0.05$)

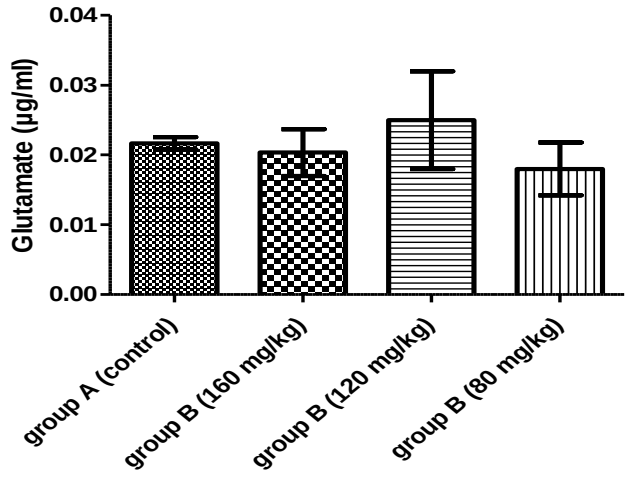

Figure 2:
Bar chart showing the effect of carbamazepine on glutamate levels. Values are expressed as mean ± SEM, no significant difference across the groups ($p<0.05$).

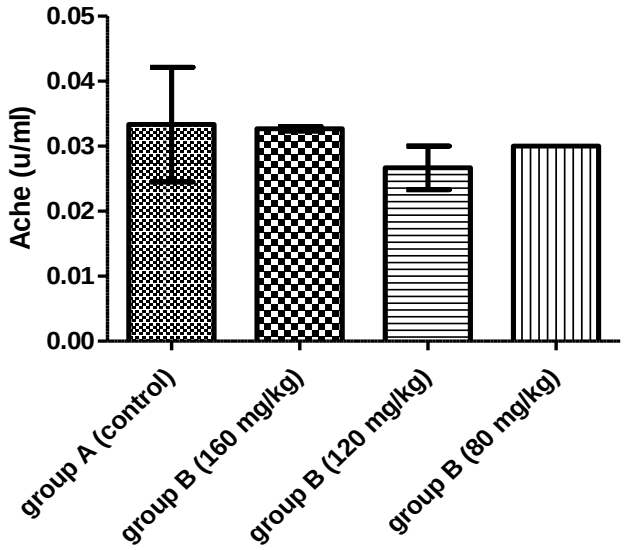

Fig 3: Bar chart showing the effect of carbamazepine on AChE activity. Values are expressed as mean ± SEM. No significant difference across the groups.

Hippocampal Histopathology: Results obtained from histological studies of H and E-stained sections revealed showed distinct cellular arrangement of pyramidal neurons in the *Cornus ammonis* (CA1, 2, and 3) regions and likewise the granule cells of the dentate gyrus in the control group and the group administered the lowest dose of CBZ (group D) (Plate 1A and D). Pyramidal neurons in the CA1, 2, and 3 regions of groups B and C (Plate 1B, and C) showed distortion in the cellular arrangement. This pattern of cellular distortion was mild in group C and more profound in group B which received the highest dose of CBZ. The hippocampal sections were also stained in Cresyl violet to demonstrate the presence of Nissl substances and the result obtained revealed normal granule neurons histomorphology in groups A and D (Plate 4A and D) with distinct cell body and well-delineated cytoarchitecture, while groups B and C (Plate 4B and C) showed granule neurons with degenerative features evident by intensely stained eosinophilic cytoplasm, cytoplasmic fragmentation, and intracellular nuclear material aggregation.

DISCUSSION

This study assessed the effects of carbamazepine on hippocampal histomorphology, acetylcholinesterase activity, and glutamate concentration in adult male Wistar rats.

CBZ possesses strong anticonvulsant properties and its mechanism of action could be by potentiation of GABA receptors, increase in synaptic GABA uptake, and also by interaction with sodium and calcium ion channels (Rogawski *et al*., 2016; Rho, 2017; Sada and Inoue, 2018; Dahlin and Nielsen, 2019). Results obtained from the study showed a significant reduction in the weight of group B animals administered 160 mg/kg of carbamazepine. The reduction in percentage bodyweight of the rats observed in this group could be as a result of the dose administered. There was a drastic weight reduction in this group when compared to other groups.

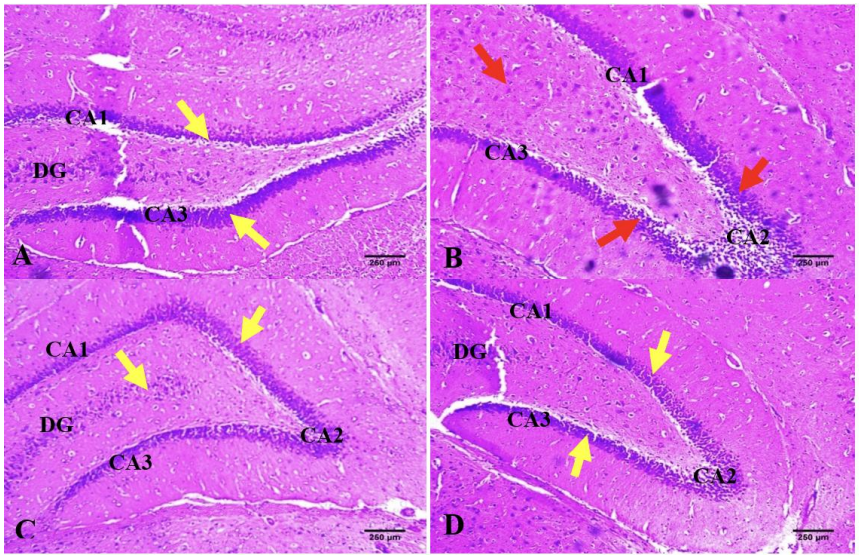

Plate 1
Representative photomicrographs of the hippocampal subfields **CA1**, **CA2**, **CA3,** and the dentate gyrus (**DG**) in group A (Control), group B (160 mg/kg of carbamazepine), group C (120 mg/kg of carbamazepine) and group D (80 mg/kg of carbamazepine). Cells in the control group (A), groups C, and D are in a normal array (yellow arrow) and are characterized by the distinct cellular arrangement of pyramidal neurons in the *Cornus ammonis* region and the granule cells of the dentate gyrus. While cells in group B presented with several pyknotic changes and disorderliness in pyramidal and granular neuronal arrangement (red arrow). Stain: H&E. Scale Bars: **250µm**

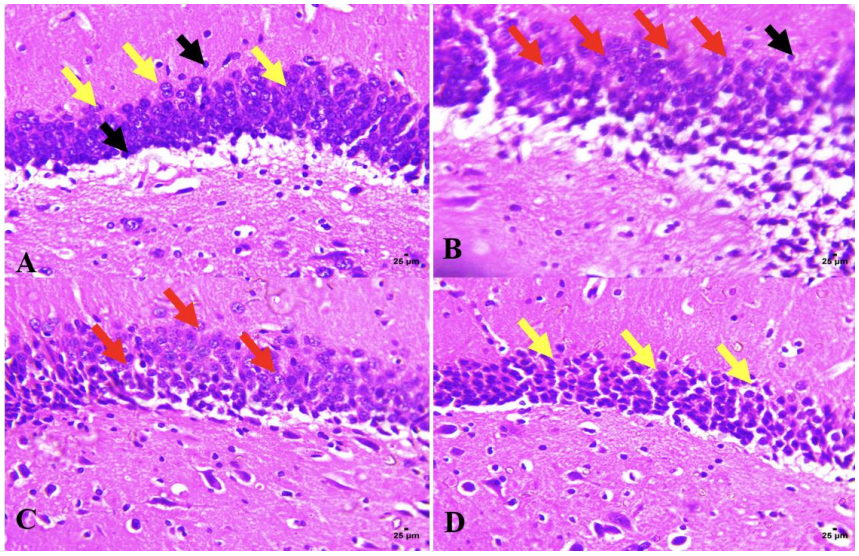

Plate 2
Representative photomicrographs of the Dentate gyrus (**DG**) in group A (Control), group B (160 mg/kg of carbamazepine), group C (120 mg/kg of carbamazepine) and group D (80 mg/kg of carbamazepine). Group (A) and D show the normal morphological and cellular arrangement of granule neurons (yellow arrow) intermixed with few oligodendrocytes (black arrow) in the granular layer, while morphological alteration and cellular degeneration were mild in group B and more profound in C (red arrow). Stain: H&E. Scale Bars: **25µm**

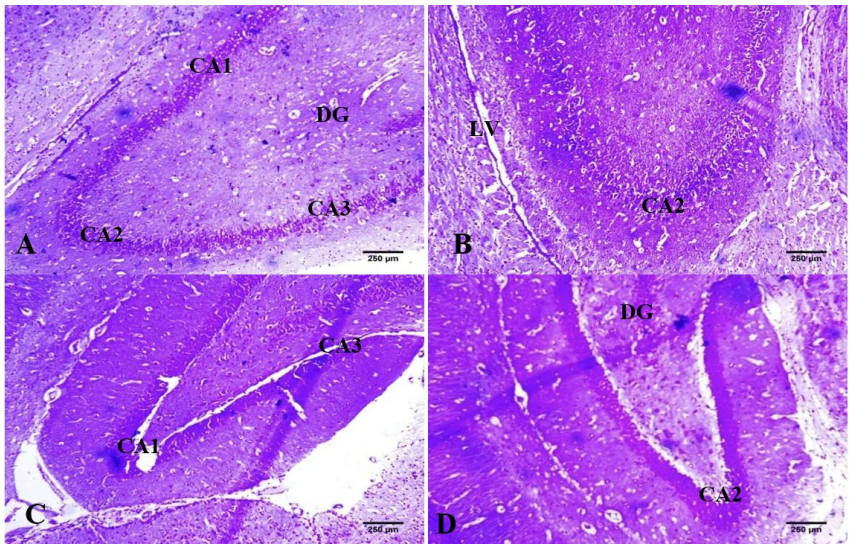

Plate 3
Representative photomicrographs of the hippocampus showing different subfields **CA1**, **CA2,** and **CA3,** the lateral ventricle (**LV**) and the dentate gyrus (**DG**) in group A (Control), group B (160 mg/kg of carbamazepine), group C (120 mg/kg of carbamazepine) and group D (80 mg/kg of carbamazepine). Stain: Cresyl Violet. Scale Bars:**250µm**

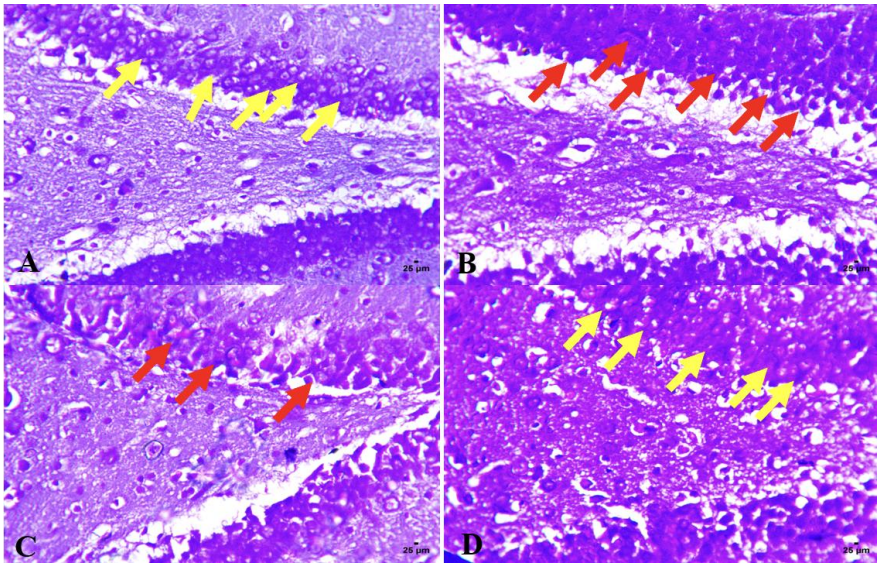

Plate 4
Representative photomicrographs of the dentate gyrus (**DG**) in group A (Control), group B (160 mg/kg of carbamazepine), group C (120 mg/kg of carbamazepine) and group D (80 mg/kg of carbamazepine). Granule neurons in groups A and D shows normal cell body with well-delineated cytoarchitecture, while group B and C shows degenerative features evident by intensely stained eosinophilic cytoplasm, cytoplasmic fragmentation and intracellular nuclear material aggregation (red arrow). Stain: Cresyl Violet. Scale Bars: **25µm**

This present research is in tandem with Gerenuttiet *et al.*, (2008) who reported significant weight reduction in pregnant rats following CBZ administration. However, there was no significant difference in the absolute and relative brain weight of the animals across all groups.

One of the most important excitatory neurotransmitters in the brain is glutamate. It has various roles in both physiological and pathological states. In excessive amounts, it possesses the potentiality of acting as a neurotoxin by activation of proteolytic enzymes and increasing neuronal excitability (Rania and Naglaa, 2016). Normal glutamatergic transmission is dependent on amino acid transporters resident in neurons and glial cells are essential in the regulation of extracellular glutamate levels below potentially excitotoxic concentrations (Rubio *et al.*, 2012; Rao and Sari, 2012; Chi-Castaneda *et al*., 2017). In the present study, glutamate concentration was not significant across the groups. Carbamazepine, an anticonvulsant drug works by decreasing the electrical activity of neurons thereby preventing hypersynchronous neuronal discharge (Rogawski *et al*., 2016). Though seizures were not induced in the experimental rats this would have led to an increase in glutamate concentration as it plays a role in seizure formation. Excessive glutamate excitation without inhibition is a major mechanism in epileptic seizures. This may be a pointer to why this carbamazepine alone did not increase glutamate concentration in any of the experimental groups.

Acetylcholine has several roles it plays in behavior, learning, and memory and several neurodegenerative diseases (Rania and Naglaa, 2016; Bekdash, 2021). In the present study, results showed that administration of carbamazepine in rats did not cause any significant difference in AChE activity in the hippocampus. AChE is an enzyme necessary for the degradation of acetylcholine at the synapse into choline and acetate whose activity terminates at synaptic transmission. AChE is located on the post-synaptic membrane that terminates synaptic transmission by hydrolyzing ACh. The yielded choline is taken up by the presynaptic component for the formation of another Ach in combination with acetyl-CoA (Scarpa *et al*., 2020).

Photomicrography of the H and E section of the control group (figure 4A) revealed distinct cellular arrangement of pyramidal neurons in the *Cornusammonis* (CA1, 2, and 3) regions and likewise the granule cells of the dentate gyrus. Pyramidal neurons in group B (figure 4B), which received the highest dose of CBZ presented with features of degeneration such as pyknotic appearances and distortion in the cellular arrangement. Chronic Carbamazepine administration in Wistar rats has been found to significantly increase neurons showing degenerative features in the hippocampus and striatum (Pahuja *et al*., 2012; Olayemi *et al.*, 2014; Tutka *et al*., 2019). Results obtained from Cresyl violet stained sections revealed normal granule neurons histomorphology in groups A and D (figure 7A and D) with distinct cell bodies and well-delineated cytoarchitecture, while groups B and C (figure 7B and C) showed granule neurons with degenerative features evident by intensely stained eosinophilic cytoplasm, cytoplasmic fragmentation, and intracellular nuclear material aggregation and most of these neurons are poorly stained. The hippocampus is involved in many cognitive processes which involve and are not limited to memory consolidation, learning, spatial memory e.t.c. Morphological alteration in the pyramidal, granule neurons of the hippocampus may affect cognitive processes (Reeta *et al*., 2011; Badurek *et al*., 2020; Ismail *et al*., 2022). The hippocampus also receives input from the entorhinal cortex via the perforant pathway and any disruption in this neuronal network will severely affect hippocampal function.

In conclusion, the present study has demonstrated that the adult rat brain is susceptible to the detrimental effects of carbamazepine administration at a higher dose. While the level of glutamate and the activity of AChE remain unaltered. There was a widespread histomorphological alteration with a high dose of carbamazepine with mild neurodegenerative features in medium, showing that the mechanism by which CBZ causes hippocampal neuronal degeneration is in a dose-dependent fashion and this by extension will impact hippocampal-mediated functions

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