# Effects of chronic administration of gabapentin and carbamazepine on the histomorphology of the hippocampus and striatum

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**KEY WORDS**

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**ABSTRACT**

**Background**: Antiepileptic drugs used to avert epileptic seizures necessitate prolonged duration for improved efficacy and could induce some side effects. **Purpose:** The present study investigated the effect of chronic administration of two common antiepileptic drugs – gabapentin and carbamazepine, on the histomorphology of the hippocampus and striatum in adult rats. **Methods**: 25 adult male Wistar rats were grouped randomly into 5 groups. 3 groups were administered either therapeutic doses of gabapentin (16 mg/kg) or carbamazepine (20 mg/kg) or sub-therapeutic dose of gabapentin plus carbamazepine (8 + 10 mg/kg). To confirm anticonvulsant effects, these animals were kindled for seizures at sub-maximal electroshock. Appropriate negative and positive controls were given normal saline. At the end of treatment, brain tissues were obtained and processed for histological procedures. **Results**: The study confirm significant decrease ($P<0.001$) in convulsion parameters tonic flexion, tonic extension and clonic convulsion, between drug treated groups and electroshock control. Histological studies revealed significant increase ($p<0.001$) in neurons showing features of degeneration in the hippocampus, for drug treated groups as compared to normal and electroshock control. Also, drug treatment reduced nissl activity in both hippocampus and striatum. **Conclusion**: Chronic administration of gabapentin and carbamazepine may cause increase in neurodegenerative changes in the adult brain.

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## Introduction

Epilepsy is a chronic neurological disorder characterized by intermittent unprovoked seizures. It affects about 65 million people globally. A seizure is an unusual electrical discharge in the brain that produces alteration in consciousness, sensation, and behavior.[1]

Antiepileptic drugs (AEDs) are used to avert epileptic seizures. They mostly act by interacting with ion channels, neurotransmitter receptors and transporters and metabolic enzymes. They also act by modulation of the bursting properties of neurons, thereby inhibiting the spread of epileptic activity.[2,3]

Existing AEDs necessitate prolonged duration of treatment, mostly via combination therapies that improve efficacy. This has raised concerns over possible adverse effects.[4] AEDs have also been associated with increased risk of congenital and fetal malformations. Exposures to AEDs have been shown to induce adverse morphological changes in the developing nervous system.[3,5] Prenatal exposure to AEDs produce subtle morphological changes in grey matter of the human brain.[6]

Gabapentin [1-(aminomethyl)- cyclohexane acetic acid] (GBP) and Carbamazepine [5H-dibenz [b,f] azepine-5-carboxamide] (CBZ) are amongst the common AEDs used in therapeutic management of epileptic seizures. GBP, a structural analogue of GABA ($\gamma$-aminobutyric acid), is used as an anticonvulsant and analgesic drug. The mechanism by which it acts is still poorly understood.[7] CBZ, on the other hand, is an iminodibenzyl derivative, which is structurally similar to the tricyclic antidepressants. It has been considered as the most frequently prescribed drug for the management of severe forms of epilepsy, with implications for therapeutic benefits for neuropathic pain and psychiatric disorders.[8]

Morphological alterations associated with AEDs have mainly been studied and observed in developing or immature brain. Therefore, the present study has investigated the effect of chronic administration of therapeutic doses of GBP and CBZ, and sub-therapeutic doses of GBP - CBZ combination on the histomorphology of the hippocampus and striatum in adult rats.

## Methods

### *Animal management*

Twenty five adult albino strain male Wistar rats (150–200g) were obtained from the animal house of the College of Health Sciences, Obafemi Awolowo University and used for this study. They were housed in clean plastic cages and provided with standard laboratory chow and water *ad libitum.* All experimental protocols were approved by local institutional research committee. All animals were handled in accordance with the guidelines for animal research as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals by the National Research Council of the National Academy of Sciences, 2011.

### *Drugs and animal treatment*

GBP at 16 mg/kg/day, in comparison between physiological weight in human and body weight of the animals and CBZ at 20 mg/kg/day correspond to the usual anticonvulsive dose in humans which is effective in preventing kindled seizure in Wistar rats.[9–11] Groups A and B rats served as the control and received 0.1 ml/day of normal saline (drugs' vehicle) intraperitoneally (i.p.) for 28 days. Group C received 16 mg/kg body weight (BW) of GBP i.p. for 28 days, Group D received 20 mg/kg BW of CBZ i.p. for 28 days while Group E received sub-therapeutic doses of both GBP 8 mg/kg body weight and CBZ 10 mg/kg body weight (i.p.) for 28 days. GBP and CBZ obtained from Sigma Chemicals, UK.

*Induction of seizures by sub-maximal Electric Shock (SMES)*

To confirm the anticonvulsant effects of these drugs, 24 hours after end of drug administration, animals in groups B, C, D and E were kindled for seizure at sub-maximal electric shock (SMES) at a current of 99 mA, pulse width of 0.5 at 100 Hz for duration of 2 s using electroconvulsometer produced by Ugo Basile. Each animal induced for seizure was observed for the time (in seconds) taken to experience tonic flexion, tonic extension and clonic convulsion.

*Histological studies*

At the end of administration, animals were sacrificed by cervical dislocation and the brain excised. Brain tissues were fixed in 10% formal saline and processed for rapid routine paraffin wax embedding, sectioned on a rotary microtome at 6 $\mu$m thickness, and stained using haematoxylin and eosin (H&E) and Cresyl violet methods described by Drury and Wallington, (1980).[12]

*Photomicrography and image analysis*

Stained sections were observed under a Leica DM750 digital light microscope and digital photomicrographs obtained via attached Leica ICC50 camera. H&E stained photomicrographs were imported into Image J software (NIH-sponsored public domain image analysis software). Neurons showing degenerating features were noted and counted using the Image J cell counter tool.

*Statistical analysis*

Data was expressed as mean±SEM, analyzed using One-way ANOVA, and followed by Student Newman-Keuls (SNK) test for multiple comparisons. GraphPad Prism 5 (Version 5.03, GraphPad Inc.) was the statistical package used for data analysis. Significant difference was set at $p<0.05$.

**Results**

GBP, CBZ, and a combination of both significantly reduced ($p<0.001$) duration of tonic flexion, tonic extension and clonic convulsion compared to SMES control. Also CBZ only significantly reduced ($p<0.001$) these parameters as compared to GBP only, but showed no significant difference when compared to a combination GBP+CBZ (Figure 1)

Histological studies revealed substantial alteration in morphology of the hippocampus (CA3 region) following treatment with GBP, CBZ and a combination GBP+CBZ. These alterations include degenerating features of pyramidal neurons such as the 'classic' eosinophilic appearance with shrunken nuclei as seen with degenerating neurons in H&E stained sections. Another characteristic feature of degenerating neurons, included swelling or vacuolation within the cytoplasm. Histological appearance of the caudate-putamen (CPu) of the striatum did not show much difference between control, SMES and treated groups. Image J count of neurons showing degenerating features in the hippocampus revealed significant increase ($p<0.001$) following treatment with GBP, CBZ and a combination of GBP+CBZ when compared to control and SMES control. Also, GBP+CBZ combination significantly increased number of neurons showing features of degeneration ($p<0.001$), when compared to GBP and CBZ only. No significant difference was observed for count of neurons showing degenerating features in the striatum. (Figure 2 and 3).

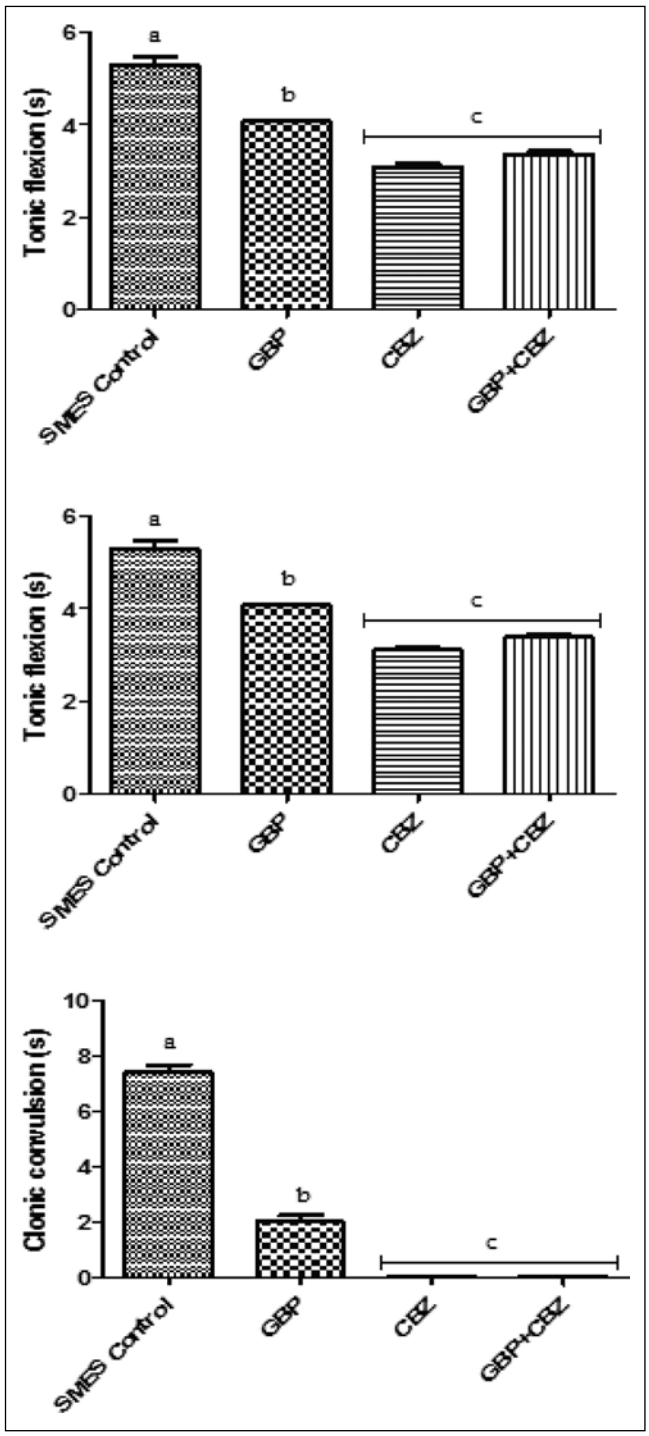


**Fig. 1:** Effects on GBP, CBZ and GBP+CBZ on convulsions. Values are expressed as mean±SEM. Different letters (a,b,c) indicates significant difference at $p<0.001$. One-way ANOVA was used to determine significant difference followed by SNK for multiple comparisons.

Cresyl violet stained sections revealed decrease in staining intensity of nissl substances in the hippocampus and striatum following treatment with GBP and CBZ, when compared to control and SMES control. GBP+CBZ combination further reduced the staining intensity of nissl subtances (Figure 4 and 5, Table 1).

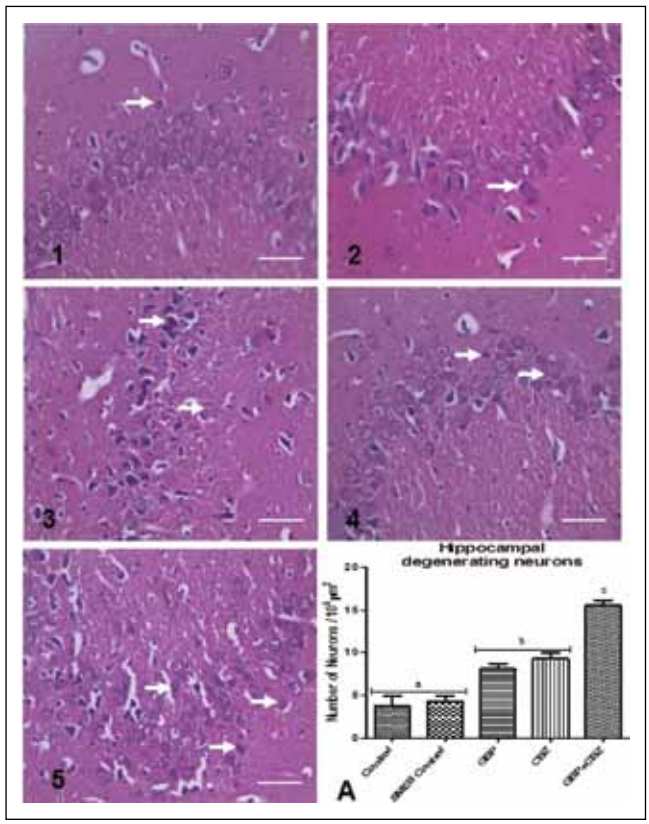


**Fig. 2:** Representative photomicrographs of the hippocampus (CA3) of control, SMES control, GBP, CBZ and GBP+CBZ groups (panels 1–5 respectively). H&E, Scale bars = 50 $\mu$m. Arrows: neurons showing degenerating features such as eosinophilic cytoplasm with shrunken nuclei, swollen or vacuolated cytoplasm. Panel A shows Image J cell count of degenerating neurons; Values are expressed as mean±SEM. Different letters (a,b,c) indicates significant difference at $p<0.001$. One-way ANOVA was used to determine significant difference followed by SNK for multiple comparisons.

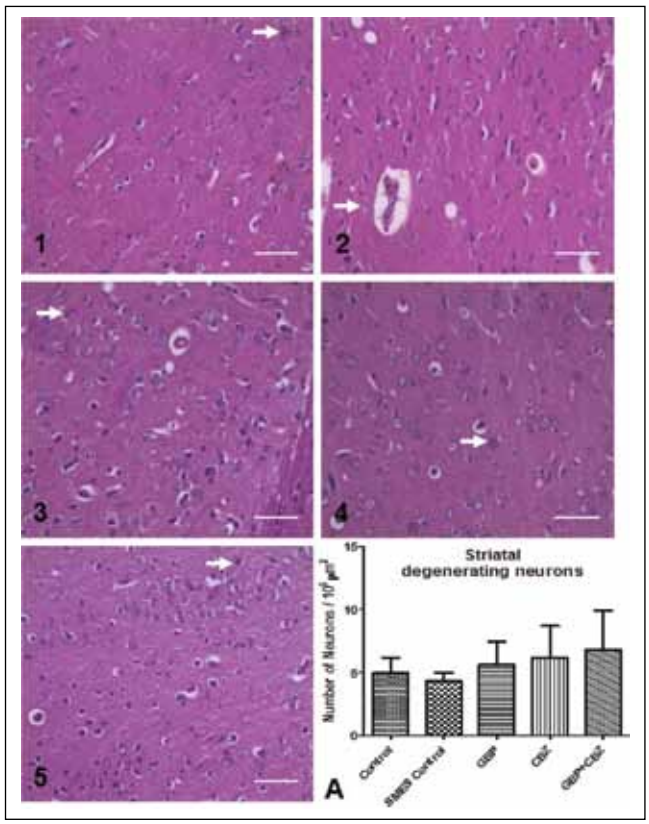


**Fig. 3:** Representative photomicrographs of the striatum (CPu) of control, SMES control, GBP, CBZ and GBP+CBZ groups (panels 1–5 respectively). H&E, Scale bars = 50 $\mu$m. Arrows: neurons showing degenerating features such as eosinophilic cytoplasm with shrunken nuclei, swollen or vacuolated cytoplasm. Panel A shows Image J cell count of degenerating neurons; Values are expressed as mean±SEM. No significant difference ($p<0.05$) was observed between groups. One-way ANOVA was used to determine significant difference.

## Discussion

GBP and CBZ possess strong anticonvulsant properties that have been thought to act through interaction with voltage-sensitive sodium and calcium channels, as well as increase in synaptic GABA and potentiation of GABA receptors.[7,13] The present study shows that both GBP and CBZ significantly inhibit convulsions in rat SMES model of experimentally-induced seizures.

The present study has, however, shown a significant increase in neurons showing degenerating features in the hippocampus but not striatum, following chronic administration GBP and CBZ. A combination of both drugs worsens this effect. This is further confirmed by decrease in nissl substances staining intensity in the hippocampus as well as the striatum following GBP and CBZ only treatment. Further decrease in nissl substances staining is observed following GBP+CBZ administration. Degenerating neurons are known to actually stain very poorly with cresyl violet stain for nissl substances.[14] AEDs have been shown to induce neurotoxicity especially in the developing brain, where they cause neurodegeneration by means of programmed cell death. This occurs at doses and plasma concentrations important for anticonvulsant treatment.[3] Cognitive dysfunctions are major adverse effect observed in adults following exposures to AEDs at earlier stages of development.[3] The hippocampus and striatum are involved in many cognitive processes including long and short term memory, spatial memory, working memory, and affective learning amongst others.[15–18] Hence, cognitive dysfunction may be due to morphological alterations of the neurons within these brain regions. A study has shown that the oldest AED, phenobarbital reduces hippocampal pyramidal and granule neurons following perinatal exposure. Also, phenobarbital disrupts hippocampal cholinergic neurotransmission.[3]

In view of the above, the present study has thus demonstrated that adult brain may also be susceptible to neurodegenerative potentials of AEDs even when exposures occur at adulthood. Though the results suggest that this may be greater in some brain regions than others as only the hippocampus, but not striatum, showed both increased neurodegenerating features and decreased nissl substances activities following chronic treatment with GBP and CBZ. It should also be noted that a combination of both drugs would potentiate neurodegenera-

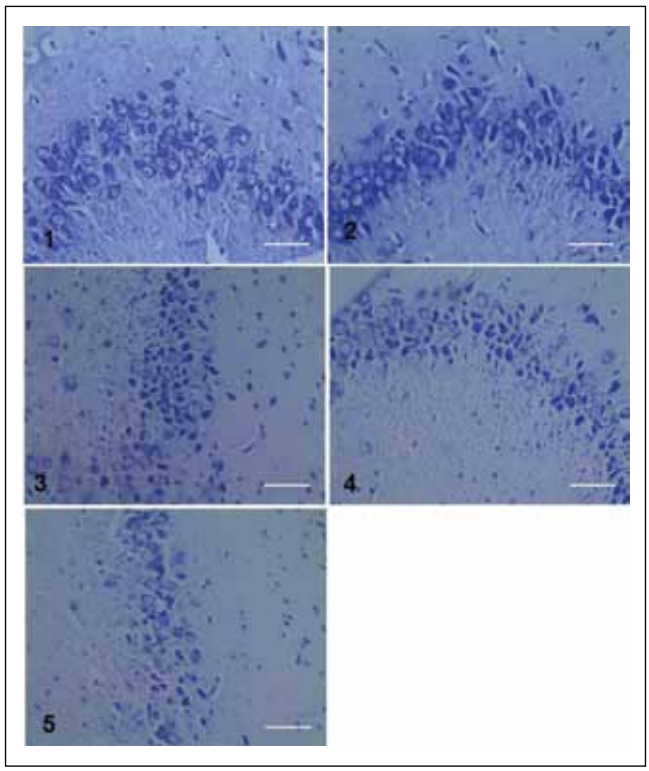


**Fig. 4:** Representative photomicrographs of the hippocampus (CA3) of control, SMES control, GBP, CBZ and GBP+CBZ groups (panels 1–5 respectively). Cresyl violet, Scale bars = 50 $\mu$m. Observe decrease in staining intensity following drug administration.

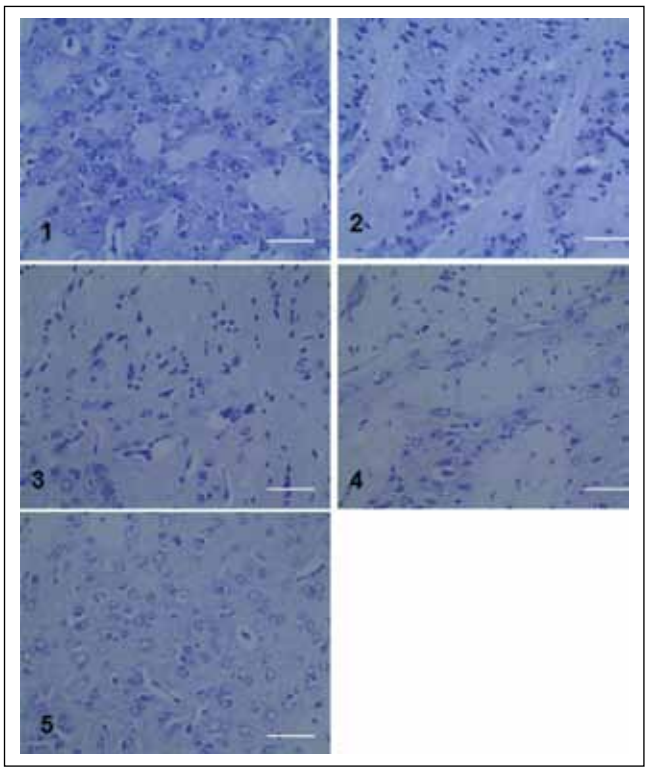


**Fig. 5:** Representative photomicrographs of the striatum (CPu) of control, SMES control, GBP, CBZ and GBP+CBZ groups (panels 1–5 respectively). Cresyl violet, Scale bars = 50 $\mu$m. Observe decrease in staining intensity following drug administration.

**Table 1: Effect of GBP, CBZ and a combination of CBP+CBZ on nissl substances staining intensity**

| | Nissl substances intensity | |
|---|---|---|
| | **Hippocampal** | **Striatum** |
| Control | +++ | +++ |
| SMES control | +++ | +++ |
| GBP | ++ | ++ |
| CBZ | ++ | ++ |
| GBP+CBZ | + – | + – |

Notation: Very intense: +++
Intense: ++
Moderate/mild: + –
Negative: – –

tive effects, considering that most therapeutic management of epileptic seizures involves combined use of different AEDs for prolonged periods.[4]

In conclusion, GBP and CBZ have been shown to possess strong anticonvulsant properties in adult rat models of SMES experimentally- induced seizures, though chronic administration of these drugs may cause neurodegenerative changes in the adult brain.

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