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MELATONIN ABLATE ASTROGLIOSIS IN STATUS EPILEPTICUS MODEL: AN ADJUVANT TO ANTICONVULSANT THERAPY

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ABSTRACT

Seizure is one of the frequently occurring neurologic condition in African countries including Nigeria. Advances in the regulatory mechanisms of the circadian rhythm and astrocytes involvement in the detoxification of chemicals have provided beneficial insights in the management of diseases. This study investigates the role of melatonin (a known circadian regulator) in the co-administration of anticonvulsants and melatonin in the treatment of status epilepticus. Status epilepticus model was induced chemically using Isoniazid (INH, 300mg/kg, i.o, single dose), at the post-induction the rats were treated with carbamezapine (CBZ), melatonin (MT), and co-treatment (CBZ + MT), then sodium valproate (VPA) and melatonin (MT) monotherapy, and co-treatment (VPA + MT). The saline and INH untreated served as the controls. The immunoperoxidase method using GFAP was use to highlight astrocytes morphologic expressions in the prefrontal and temporal cortices. The untreated revealed marked astrogliosis, mild astrocytic retraction were seen in the monotherapy treated section particularly with melatonin. However, following melatonin co-treatment with CBZ and VPA in either cortex, it revealed prominent astrocytes with retracted features. Melatonin enhanced the therapeutic efficacy of the anticonvulsants, a vital role of an adjuvant in the management of epilepsy.

KEYWORDS: GFAP, Anticonvulsant, Melatonin, Seizure, Circadian clock, Astrogliosis.

INTRODUCTION

Seizure is one of the frequently occurring neurologic condition in African countries including Nigeria (1). It cause changes in awareness, behavior and associated with accumulating brain damage and neurological deficits (2). The temporal and frontal lobes of the brain are the most frequently affected due to impaired GABA synthesis by GABAnergic neurons as in epilepsy. The familiar features involve complex pathophysiology of neuronal plasticity, imbalance between gamma aminobutyric acid (GABAnergic) inhibitory effect, glutamatergic (excitatory) neurotransmitter system and ion exchange dysfunction (3-5). Preclinical studies have explored different animal models of seizure which include the pilocarpine model, kainite, pentylenetetrazol (PTZ) and Isoniazid which mimics the status epilepticus (4, 6-7).

Experimentally induced seizure using Isoniazid can mimic symptoms of status epilepticus (SE) which produces a characteristic repetitive convulsive episode, metabolic acidosis, respiratory depression and death (6). This is principally due to the inactivation and/or inhibition the production of Vitamin B6 (Pyridoxine) when bound to the receptor site of glutamic acid decarboxylase enzyme, thereby inhibiting the conversion of glutamatergic neurotransmitter (excitatory) and the production GABA by GABAnergic neurons. Consequently, hyper polarization and inhibition of postsynaptic neuron target leads to seizure (4,8). The seizure is putatively attenuated with the administration of pyridoxine while, unresponsive to the usual anticonvulsants (4,8,9).

The most frequently used therapy in the management of status epilepticus are anticonvulsants. The challenge with anticonvulsants including carbamezapine (CBZ) and sodium valproate(VPA) is the non responsiveness in refractory cases and even in the seizure that are not secondary to Isoniazid toxicity. What remained to be explored is the influence of the circadian rhythm or the biological block in the pathogenesis and enhancement of the therapeutic efficacies of anticonvulsants. Circadian rhythm is a phasic pattern in an animals influenced by light and dark changes which in turn regulate many biological events such as timing of hormone release and aetiology of some diseases (10-11). This biological

pattern (circadian) also modulates the therapeutic interaction of certain drugs and the human system in health, disease and has become a useful tool for improving treatment outcome (12). Evidence has shown that epileptic patients also tend to exhibit abnormal circadian rhythms in several physiological processes (12-13). The use of the tradition anticonvulsant monotherapy may not be efficient and would require an adjuvant therapy. In addition, the alteration of circadian rhythm during epileptic seizure provides an insight to regulate the sleep-wake cycle in order to resolve the seizure as with other neurological disorders (14). For instance, administration of different doses of anticonvulsants to relieve seizure and appropriate timing of the drug intake to specific periods of the sleep-wake cycle enhances the efficacy (12). Hence, therapeutic modulation of the circadian rhythm may help to improve patient's condition.

In evaluating or correcting the interference of circadian rhythm in other diseases conditions, melatonin and cortisol have been used to achieve stable shift in the biological clock which provided beneficial effect in the treatment of epilepsy (11,14-15). Convulsive seizures exhibit an opposite circadian ryhythmicity in both animal and humans (13).Melatonin is a hormone secreted by the epiphysis cerebri (pineal body) in the brain and possesses modulatory properties on the central nervous system (CNS) and regulates circadian rhythms (13,16). Exogenously derived melatonin has antioxidant, anti-inflammatory, anxiolytic, anticonvulsant and neuroprotective activities (11,13). Thus, with optimization of circadian rhythm there is tendency to abort diseases and modulate therapeutic intervention to achieve a better treatment option with improved and optimal care rate for disease like epilepsy. This study seeks to assess the potential role of co-treatments of seizure/epilepsy with melatonin and anticonvulsants such as carbamazapine and sodium valproate. It particularly uses astrocytes credential in chemical detoxification and brain metabolism.

MATERIALS AND METHODS

Drugs and Chemicals

Isoniazid (Isonamede, India), Carbamazapine (Tegretol, Novartis, India) and Melatonin (n-Acetyl-5-Methoxytryptamine, Puritan's Pride) were purchased from registered pharmacy premises. The isoniazid (INH) solution was constituted by dissolving 300mg in 10ml of distilled water at room temperature.

Experimental Procedures

Twenty-eight (28) Wistar rats were used for the study.The animals were procured from animal husbandry of ESUT College of Medicine (ESUCOM). The rats housed in the same facility for the period of the study under a 12-h/12-h light/dark cycle with feed and water *ad libitum.* Twenty -four(24) rats were induced seizure using isoniazid overdose as previously described by Elizabeth et al and Finbarrs-Bello et al.(4,5). Four (4) rats were used as controls which received 0.1ml normal saline and 300mg/kg/bw Isoniazid single dose orally, for treatment post-induction animals received as follows: 200mg/kg/bw CBZ, 3mg/kg/bw MT, 200mg/kg/bw VPA, Co-treatments 200mg/kg/bw CBZ +3mg/kg/bw MT and Co- treatment (200mg/kg/bw VPA +3mg/kg/bw MT). All the animals including the control were euthanatized on the first day post treatment under ether anesthesia and aortic perfusion was performed by 4% buffered paraformaldye. The temporal and frontal lobes were dissected and processing.

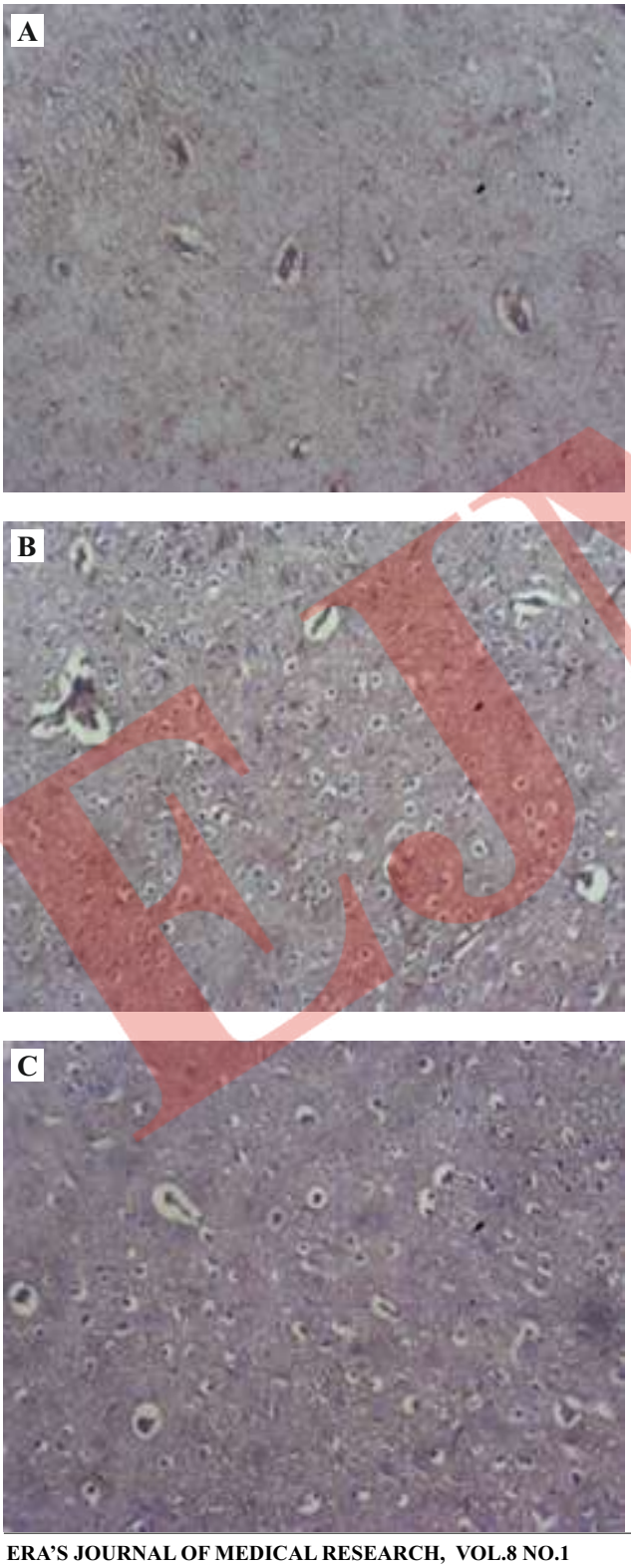
Tissue Processing and Immunohistochemistry

The method of Olopade et al (17) was adopted with slight modification. The tissue was sectioned manually at 10um thickness with rotary microtome.Astrocytes were labelled astrocytes were labelled using glial fibrillary acidic protein (GFAP) (Novocastra, LEICA Germany). Blockade of Endogenous peroxidase activity carried out using 0.3% H_2O_2. After washing, the sections were pre-incubated for 1 h at room temperature in the appropriate normal serum before incubation in primary antibodies overnight at 4°C. The sections were then rinsed and incubated in secondary antibodies at1:200 dilution for 2 h at room temperature, and then reacted in avidin biotin complex solution (Novocastra, LEICA Germany) for 1.5 h using 3-3-diaminobenzidine (DAB) as chromogen. The sections were then mounted on slides, dried, dehydrated, cleared and coverslipped with Entellan (Merck, Darmstadt, Germany). The slides were interpreted and photographed. Star shaped cells with specific dark brown colors in the cytoplasm or nuclei depending on the antigenic sites are considered to be positive. The haematoxylin stained cells without this form are scored negative. Nonspecific binding/brown artifacts on cells and connective tissue were disregarded.

RESULT

Seizure induction was achieved with single dose of Isoniazid although 2 deaths were recorded given a percentage survival of 90%. The GFAP immunohistochemical staining showed positive immunoreactivity in control, non treated seizure group and the treated groups. However, the control revealed astrocytes with normal morphology and evenly distributed. Following induction astrocytes became reactive with hypertrophic cell bodies and prominent processes in the Non-treated seizure. There were mild

retractions in the astrocytes morphology following treatment with melatonin, Carbamazapine and sodium valproate in but the temporal and prefrontal cortices respectively. Co-treatments with Melatonin with either Carbamazapine or sodium valproate in the temporal and prefrontal cortices revealed features consistent with retracted astrocytes.



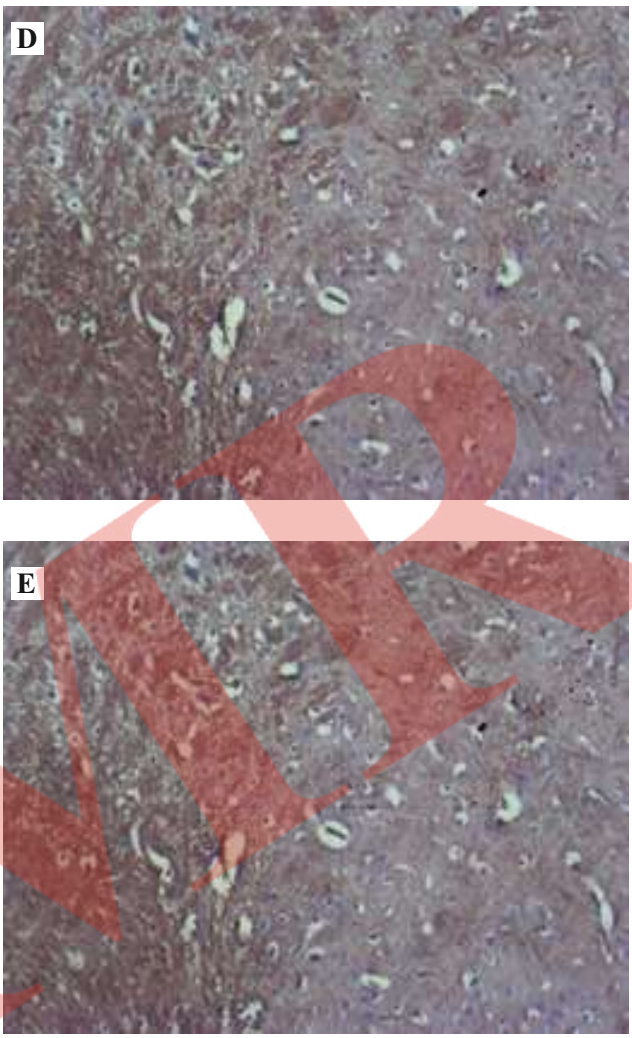
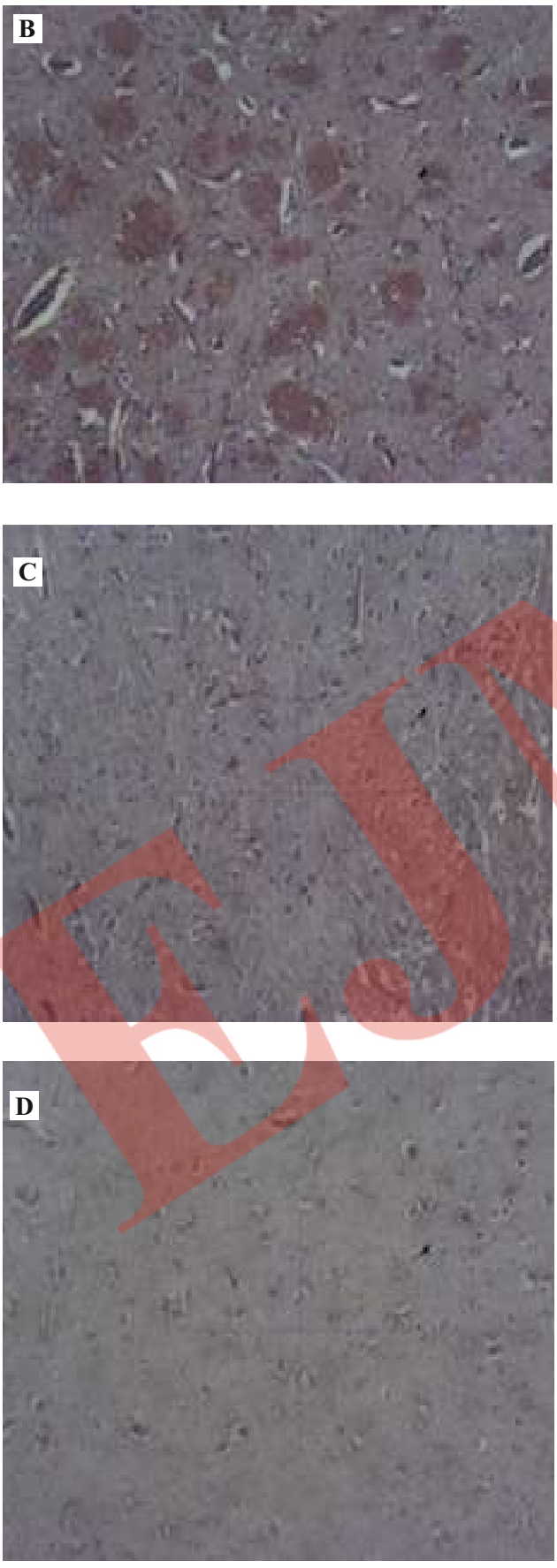


Fig. 1: Image of GFAP- immunoreactive astrocytes in the temporal lobe control rat (A), untreated seizure rat model (B),CBZ treated rat model (C), Melatonin treated rat model(D) Carbamazapine/ melatonin co-treated rat model (E). GFAP. x400.



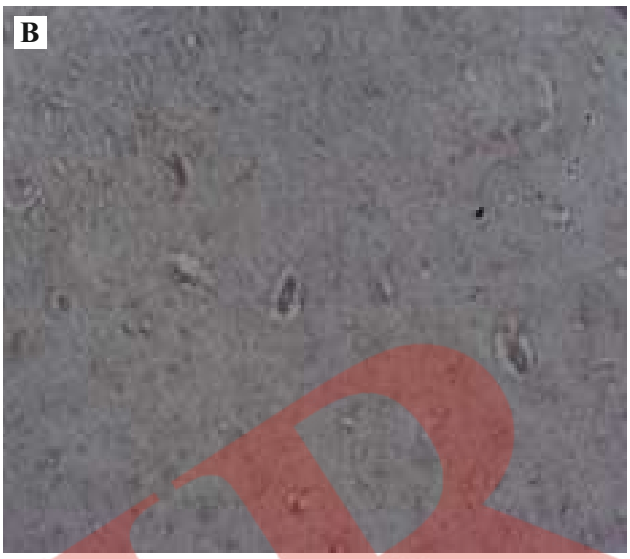


Fig 2: Image of GFAP- immunoreactive astrocytes in the prefrontal lobe negative control rat (A), untreated seizure rat model (B), VPA treated rat model (C), Melatonin treated rat model(D), VPA+ melatonin co-treated rat model (E). GFAP. x400.

DISCUSSIONS

Metabolic interaction and signalling amongst brain cells specifically neurons and glia cells are key for neuronal protection against chemical attack (18). Astrocytes have been previously described as essential component of cerebral defence system (19). This study demonstrated the role of astrocytes in accessing the efficacy of the treatments particularly the combination of anticonvulsant and melatonin. Astrocytic activation (Astrogliosis) was detected in the seizure group which depicts a consistent pattern of astrocytes morphological expression following neurotoxicity (20). These activated astrocytes were found to be reduced or mild after the treatment with melatonin in the temporal and prefrontal cortices, as well as treatment with carbamazapine in temporal and valproate in prefrontal cortex. However, Co-treatment with anticonvulsants revealed highly retracted astrocytes; the above effect was equally observed in both the temporal and prefrontal cortices. Therefore, it implied astrocytes responded to the metabolic milieu of the neuron following treatment and provided neuroprotection. This can be attributable to the diverse roles of melatonin in the body and of particular the regulation of biological clock, antioxidant and inflammatory activities (11).

Often reactive astrocytes have notable morphology in various models of recurrent seizure including Isoniazid model, in epileptic tissue appearance of reactive astrocytes tend to foster and prevent seizure development across diverse of neural pathways (21).

These involve morphological and biochemical changes in astrocytes and generate new astrocytes from stem cells (18,19,22). Numerous studies have also reported calcium evoked glutamate release in astrocytes that causes depolarization in neighbouring neurons (22-23). The depolarization instigates the state of hyperpolarization earlier seen in seizure.

Studies have affirmed that Isoniazid- induce seizure is unresponsive to anticonvulsant therapies (6,9). In this study, activated astrocytes were ablated by co-treatment of anticonvulsants (carbamazapine and sodium valproate) with melatonin in the temporal and prefrontal cortices respectively. This activity was credited to the effect of melatonin such as the antioxidant, anti inflammatory and specifically the modulatory effect on the circadian rhythm.

CONCLUSION

The mild astrocytic retraction following the carbamazapine and valproate treatments was expected since INH induced seizure is unresponsive to conventional anticonvulsants. The study has demonstrated the potential of melatonin in enhancing the therapeutic efficacy of anticonvulsants and modulation of circadian rhythm, a vital role of an adjuvant in the management of epilepsy.

Ethical Statement

The experiment was carried out with the ethical permission of Enugu State University of Science and Technology Animal Care and Use Research Ethics Committee. The research was conducted according to the institutional guidelines for the Use and Care of Laboratory Animals.

Conflict of Interest

The authors declare that they have no competing interest.

Author contributions

FBE and ACG performed the experiments and wrote the first draft of the manuscript; FBE designed the study and supervised it, EAE participate the technical aspect and finalized the manuscript draft. All authors read and approved the final manuscript.

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