International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

MICROANATOMICAL DEFORMATION AFTER PTZ INDUCED SEIZURE IN MICE BRAIN

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ABSTRACT

Seizure is an abnormal state of brain electrical activity. The causes of seizure are different but seizure can be induced artificially also. Present experiment was conducted on albino mice and seizure was induced by Pentamethyline tetrazole (PTZ). Duration of seizure was maintained more than half an hour. Mice were sacrificed by cervical dislocation and brain was removed. Brain was fixed in Carnoy's fixatives and sectioned at 5 micron thickness. Histological slide was stained in H&E stain. Micro-anatomical deformation of brain was observed in treated animals. Simultaneously control group of mice was maintained in same laboratory conditioned. Loss of cellular architecture in the neocortex I remarkably observed. Excitatory neurotransmitter overloaded in axon terminal may be responsible for neuronal degeneration.

Key words: Brain, Histology, Mouse, PTZ, Seizure.

INTRODUCTION

Epilepsy is a worldwide neurological disorder and a serious problem in developing countries like India [1, 2]. It may appear as a result of variety of causes like interaction of various genetic, environmental and physiological factors, resulting underlying brain dysfunctions. Epilepsy is a neurodegenerative disorder characterized by epileptic seizures predominantly by recurrent and unpredictable interruptions of normal brain function [3]. It affects more than 50 million people worldwide, 5 million of them have seizure more than once per month [4]. Approximately 100 million people have at least one epileptic seizure during their lifetime [5]**.** Rate of epilepsy is highest in the youngest age groups, decreases during childhood, diminishes among adults, and rises again in old age [1]. Indian scenario about the disease is not good; about 5.35 per 1000 population is epileptic [6]. Neuro-degeneration is the progressive loss of structure and function of neurons, including death of neurons and brain tissues, after all, resulting on brain damage. Many neuro-degenerating diseases including Parkinson's, Alzheimer's, and Huntington's occur as a result of neurodegenerative processes. The causes of seizure are although different but this is commonly assumed that seizure may come due to reduced level of the inhibitory neurotransmitter like GABA, serotonin etc. Abnormal activity of GABA receptor may also cause seizure. Artificially seizure can be induced by administering some chemicals for experimental purpose.

PTZ is such type of chemicals although pilocarpine, kainic acid, glutamic acid etc. are other such chemicals that can be considered as artificial seizure inducer. Glutamic acid, caffeine etc. are the constituents of food that can induce seizure. Monosodium glutamate is a food additive can induce of seizure in dose dependent manner. Although short duration single seizure is not harmful for brain but repeated seizure for prolong period harms the brain. It was reported that seizure caused the neural degeneration, formation of reactive astrocytes, prevents the myelination in early life and

demyelination in adult brain. Present investigation is designed to study the histological and histopathological changes of brain after repeated seizure induced by PTZ.

MATERIALS AND METHODS

Experint was conducted on albino mice. Animals were housed in the animal house of Gauhati University. Experimental protocol was approved by the Institutional Animal Ethical Committee of Gauhati University.

Animals: Ten adult albino mice (25–30 g body weight) of both the sex from the animal house of Gauhati University were housed in two groups (each having five) on standard conditions with a 12-hr light–dark cycle (lights from 0700 to 1900) and kept on a laboratory diet and water *ad libitum*. The animals were handled daily to reduce stress due to the manipulation. The first group (control) was administered with normal physiological saline. In the test group PTZ was administered subcutaneously at the dose of 65 mg/ kg of body weight. This dose is sufficient to induce the chronic tonic clonic seizure. Mice were carefully monitored after administration of test compound. After one week of continuous PTZ administration animals were sacrificed and brains were collected. The collected brain samples were fixed in Carnoy's fixative. The fixed tissues were proceeded for paraffin embedded histological sections. Sections were made at 5 µm thickness and stained with H&E using standard procedure.

RESULTS

Behavioral changes of animals after PTZ administration: Seizure is observed after 15-20 minutes of PTZ administration and is characterized by mild myoclonic jerking and straightening of the tail. After 10-15 minutes of seizure, tonic clonic seizure is observed and it takes around 1.15 hr or more time to recover from complete seizure. Tonic clonic seizure is characterized by hind limb extension and severe jerking and jumping of the animal and debriation. In every case it is found to be aggressive.

Histological changes of brain: Leptomeningeal deformation: Cellular damage in meninges is remarkably observed. (Dark arrow tip). Neocortex I region is greatly affected than the deeper layer. Loss of gray matter (arrow) is observed in the neocortex I region of the mice brain. Large numbers of pykonic cells are seen. Large number of reactive astrocytes are observed but nerve cells are less in number in neocortex I region (double headed arrow). Neocortex II /III region of cerebral cortex contain nerve cell with loss of cytoplasm. Separation of neocortex I from rest of the neocortex region is seen (White arrow). Astrocytes become large in size but less eosinophilic material.

DISCUSSION

Pentamethyline tetrazole (PTZ) is a convulsant experimentally used to produce seizure. Different researchers reported that PTZ destabilizes nervous cell membrane to produce convulsion [7, 8, 9]. Pentylenetetrazole (PTZ) has also been reported to produce seizures by inhibiting GABA neurotransmission [10, 11]. GABA is the predominant inhibitory neurotransmitter in the mammalian CNS, and is widely implicated in epilepsy, mediating inhibition of neuronal responsiveness (excitability) and activity by increasing the chloride ion conductance through opening of the chloride-ion channel [12, 13, 14, 15]. It follows therefore that picrotoxin (PCT) and pentylenetetrazole (PTZ), antagonists of GABA receptors close up the channel preventing chloride-ion conductance to induce seizures). Calcium ion plays an important role on neuronal deformation. Entry of calcium ion causes release of different enzymes and free radicals that destroy the neurolemma [16]. PTZ causes the release of excitatory neuro-transmitter upto toxic level that affects the sodium channel. Entry of excess sodium ions may cause the swallowing of nerve terminal and finally disruption of axon terminal may results. Besides, excess excitatory neurotransmitter causes entry of calcium ions inside the neuron and damage the mitochondia and cell membrane. Toxic level of excitatory neurotransmitter causes neurons to quickly overload from too much excitation, releasing toxic chemicals. These substances poison the chemical environment of surrounding cells, initiating degeneration and programmed cell death. Studies have shown that a group of enzymes called matrix metalloproteinases contribute to the toxicity by breaking down proteins that maintain the structure and order of the extracellular environment.

One area of research that shows promisable result is the study of the role of calcium ion influx into the damaged neuron as a cause of cell death and general brain tissue swelling. It is reported that calcium enters nerve cells through damaged channels in the axon's membrane. The excess calcium inside the cell causes the axon to swell and also activates chemicals, called proteases that break down proteins. One family of proteases, the calpains, are especially damaging to nerve cells because they break down proteins that maintain the structure of the axon. Excess calcium

within the cell is also destructive to the cell's mitochondria, structures that produce the cell's energy. Mitochondria soak up excess calcium until they swell and stop functioning. If enough mitochondria are damaged, the nerve cell degenerates. Calcium influx has other damaging effects: it activates destructive enzymes, such as caspases that damage the DNA in the cell and trigger programmed cell death, and it damages sodium channels in the cell membrane, allowing sodium ions to flood the cell as well.

CONCLUSION

It was concluded that oral exposure of PTZ in mice caused seizure that harm the cerebral cortex and meninges. Neocortex I is greatly affected than the remaining part of the brain.

ACKNOWLEDGEMENT

Authors acknowledge the DBT sponsored Institutional Level Biotech Hub, Pub Kamrup College, for giving full facility to perform this experiment. Thankfulness is also expressed in equal sincerity and spirit to the Principal of Pub Kamrup College and Scientist Incharge of IAEC, Department of Zoology, Gauhati University for their help and support to complete this research work.

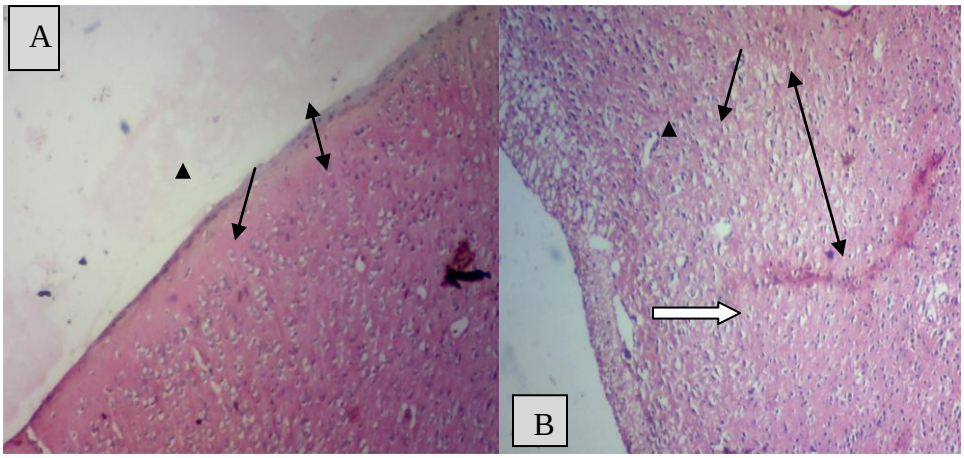

Figure: 1 Histopathological appearance of brain cells. A. Control B. PTZ treated dose (65mg/kg)

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