Effect of Insulin Supplementation in Glucose Energy Metabolism of Brain through HIF-1α Gene Expression in the Brains of Pilocarpine Induced Epileptic Rats

A dissertation project

Submitted to

Nirma University

In Partial fulfilment of

Requirement for the Degree of

Masters of Science
In
Biochemistry

Submitted by

Dhriti Shah (12MBC024)
Ganesh Surati (12MBC030)
Richa Raval (12MBC019)

Under the guidance of

Dr. Amee K. Nair

Institute of Science, Nirma University
Ahmedabad-382481, Gujarat, India

APRIL 2014

“In the fond memory of all those innocent lives that had been sacrificed for this project”

DEDICATED TO OUR BELOVED PARENTS

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

"The essence of all beautiful art, all great art is GRATITUDE."

This thesis is the end of our journey for obtaining our M. Sc. Degree and hence, we would hereby like to grab the opportunity to thank all those people who made this project possible and made it an unforgettable experience for us. At the end of our dissertation project, we feel immense pleasure and utmost pride to express our thanks to all those who contributed in number of ways to the success of this study and made it an experience to cherish for the rest of our lives. First and foremost we thank the Almighty.

Foremost we are grateful to ***Prof. (Dr.) Sarat Dalai****, Director, Institute of Science, Nirma University, for providing us all the facilities in the department to carry out our research work. We thank him also for his kind help during the course of our study and his critical evaluation during the project.*

Next, we would like to express our sincere gratitude to our guide, ***Dr. Amee K. Nair*** *under whose guidance, support and encouragement this project became possible. Under her guidance we successfully overcame many difficulties, obstacles and learned a lot. We could never thank her enough for all that she has done for us, not only in the dissertation phase, but throughout our M.Sc. journey. Despite of her ill health, she used to guide us for our project work, review our thesis progress, gave her valuable suggestions and made corrections, without looking to the time. Thank you is too small a word to express our feelings and acknowledge her for all that she did for us. So, we dedicate this work to her with satisfaction.*

We would like to express our deep gratitude and respect to all our faculties - ***Dr. Shalini Rajkumar, Dr. Shiram Seshadri, Dr. Sonal Bakshi, Dr. Mili Das, Dr. Vijay Kothari and Dr. Heena Dave*** *who have continuously encouraged us. We would particularly like to thank* ***Dr. Nasreen Munshi****, who allowed us to use the facility under her guidance and also gave us her valuable suggestions.*

We would also like to thank our PhD scholar, ***Madhavi Joshi*** *for her support and help throughout our dissertation work.*

Along with this we would also like to thank all the PhD Scholars - ***Purvi Zaveri, Palak Patel, Fulesh Kunwar, Parth Rajput, Aditi Mathur, Mahendrapal Singh Rajput, Arpita Maheshwari, Krupali parmar, Kaveri Purandhar, Prashant Jena, Bhumika Prajapati, Suhani Palkhiwala, Manoj***

Acknowledgement

Patidar, Rajesh Parmar, Navin Yadav, Rahul Jog, and Maharshi Pandya *for their gracious support.*

Our sincere thanks go to our Lab Assistants ***Sachinbhai*** *and* ***Shweta di*** *and* ***Hashitbhai*** *and other non-teaching staff of the department who had provided us all the requirements that we need. Also we would like to thank our librarians Jayshree Pandya and Swetal Shukla, who has always helped us to access literature.*

We would like to thank our group mates ***Dhatri****,* ***Rinal****,* ***Gokul,*** ***Krina****,* ***Khushboo****,* ***Pradip*** *and* ***Pooja*** *with whom we started this work. Also would like to thank* ***Yvonne****,* ***Mansi****,* ***Dhwani****,* ***Deeksha****,* ***Ekta****,* ***Jalpa*** *with whom we shared the lab and fun filled environment. And to our friends in NIS who have been with us in our thick and thin – Jenish, Dilip, Vishakha, Shreya, Chinar, Krishna, Shridhar, Hetal, Dipali, Nayana and Shivang.*

Finally, we would like to dedicate this work to our ***Parents.*** *Without their unending support and love from childhood to now, we would never have made this possible. Thank you!!*

Dhriti Shah

Ganesh Surati

Richa Raval

INDEX

INDEX

INDEX

INDEX

List of Tables

List of Tables

List of Figures

List of Figures

List of Figures


ABBREVIATIONS
TM

ABBREVIATIONS

TLE: Temporal Lobe Epilepsy

CNS: Central Nervous System

BBB: Blood Brain Barrier

GLUT: Glucose Transporters

HIF: Hypoxia Inducible Factor

LDH: Lactate Dehydrogenase

NAD: Nicotinamide Adenine Dinucleotide

DNA: Deoxyribonucleic acid

SOD: Superoxide Dismutase

CAT: Catalase

NMDA: N-methyl-D-aspartate

EEG: Electroencephalogram

MTLE: Mesial Temporal Lobe Epilepsy

LTLE: Lateral Temporal Lobe Epilepsy

CA: Cornu Ammonis

DG: Dentate Gyrus

MFS: Mossy Fibre Sprouting

SE: Status Epilepticus

ip: Intra-Peritoneal

sc: Sub-Cutaneous

ATP: Adenosine Tri Phosphate

ADP: Adenosine Di Phosphate

GABA: γ-Amino Butyric Acid

LTD: Long Term Depression

MCT: Mono Carboxylate Transporters

LTP: Long Term Potentiation

PDS: Paroxysmal Depolarizing Shifts

NGF: Nerve Growth Factor

VCI: Vascular Cognitive Impairment

TBI: Traumatic Brain Injury

IGF: Insulin Growth Factor

ROS: Reactive Oxygen Species

GOD: Glucose Oxidase

POD: Peroxidase

RAM: Radial Arm Maze

NOR: Novel Object Recognition


ABSTRACT

ABSTRACT

Epilepsy is a disorder of Brain which is characterized by an enduring predisposition to generate epileptic seizures which lead to an abnormal neural firing. Temporal lobe epilepsy is a most common form of epilepsy which has severe and life threatening consequences and is difficult to control by medication. During the Epileptic condition, Glucose metabolism in body as well as in brain regions is observed to be altered. This abnormal glucose metabolism leads to alterations in glucose concentration in brain during ictal and interictal periods. For the current dissertation project, epilepsy was induced in Adult male Wistar rats by using Pilocarpine (350 mg/kg body weight). Glucose (500mg/kg body weight) and Insulin (0.01 IU/kg body weight) treatment was given to the Epileptic and Control rats. Motor impairment in the Control and the Experimental group of rats was evaluated by means of Wheel Running and Staircase Tests, whereas Memory and Cognitive impairments were evaluated by means of Radial Arm Maze and Novel Object Recognition Tests respectively. Significant results were observed in Epileptic group as compared to the Control group of rats. Activity of LDH was assessed in heart and muscle. Significant increase in LDH activity was obtained in Epileptic group of rats as compared to the Control group. However, for the activity of LDH in Glucose + Insulin treated Control and Epileptic groups, no significance was obtained. Activity of SOD and Catalase was assessed in liver and muscle. Both these enzymes' activity was observed to be significantly decreases in Epileptic rats as compared to the Control. For Gene Expression studies, a good quality of RNA was isolated from cerebral cortex which was used for the preparation of cDNA. A specific primer was designed for HIF 1 α gene, to study its expression. An amplified product of the gene was obtained but significant results were not obtained.

INTRODUCTION

1. Introduction

1.1 Epilepsy

Epilepsy constitutes a common, serious neurological disorder in humans, affecting approximately 60 million people worldwide (Siegel, 2006). Epilepsy is a word derived from the Greek word, which means to "seize" or "take hold of" (Blair, 2011).). It is a complex disorder with diverse clinical characteristics that prelude a singular mechanism (Scharfman, 2007). It is a syndrome of episodic brain dysfunction characterized by recurrent unpredictable, spontaneous seizures (Krishnakumar *et al.,* 2009). It is a disorder of brain characterized by an enduring predisposition to generate epileptic seizures (Fisher and Saul, 2010).

1.2 Temporal Lobe Epilepsy

Temporal lobe epilepsy (TLE) is a term that refers to a condition where seizures are generated in the portion of the brain called the Temporal Lobe. Either the right or the left temporal lobe can be involved, and in rare cases both temporal lobes can be involved in a particular individual (Sucholeiki and Roy, 2005). TLE is the most common type of chronic focal epilepsies in humans (Helmstaedter and Elger, 2009; Blair, 2011). The limbic system gets affected by localisation of seizure particularly in hippocampus, amygdala and entorhinal cortex (Bartolomei *et al.*, 2005).

1.3 Epilepsy and Glucose Metabolism

Although the brain is only 2% of the adult body weight, it accounts for about 20% of the resting metabolic rate. In contrast to other organs, the brain has a limited ability to store energy and is therefore dependent on a continuous supply of its major energy source glucose crossing the Blood Brain Barrier (BBB) (Benton, 2005). Glucose is considered the almost exclusive blood-borne energy substrate utilized by the adult brain to fuel its activity. Most of the energy necessary for brain function is derived from the full oxidation of glucose (Pellerin and Magistretti, 2003).

Glucose uptake and metabolism increases more during epileptic seizures i.e., during the ictal period of epilepsy and immediately after the seizure, than during most other brain activities (Mantis *et al.,* 2004; Natelson *et al.,* 1979). Increased glucose uptake is

responsible for increased cerebral blood to match hyper metabolism which leads to increase lactate build up and increased rate of glycolysis exceeding pyruvate utilization (Scorza *et al.*, 2002). On the other hand, glucose concentration (during fasting) in the period between seizures i.e., during the inter-ictal period tends to be significantly low, especially in the hour before a seizure (Natelson *et al.*, 1979). Thus, glucose has a key role to play in epilepsy.

1.4 Role of Insulin in Brain

Historically, insulin is best known for its role in peripheral glucose homeostasis, and insulin signalling in the brain has received less attention (Ghasemi *et al.*, 2013). The effect of insulin in peripheral tissues is in the stimulation of glucose uptake, oxidation, and storage. However, the effect of insulin on the brain is less well defined (Bingham *et al.*, 2002). But the recent findings show a high concentration of insulin in brain extracts and expression of insulin receptors in CNS tissues have gathered considerable attention over the sources, localization and functions of insulin in the brain (Ghasemi *et al.*, 2013).

Insulin receptors have been demonstrated throughout the human brain, with particularly high concentrations in the hypothalamus, cerebellum, and cortex. For insulin-stimulated glucose metabolism to occur in the brain, insulin, insulin receptors, and insulin –sensitive glucose transporters are required (Hopkins and Williams, 1997). In late 1950s, it was showed that insulin elevates glucose uptake by the spinal cord tissue, and that insulin can stimulate the resting nerve respiration and glucose utilization (Chowers, 1961). Uptake of insulin into the CNS appears to occur via an active trans-endothelial transport across the BBB (Schwartz *et al.*, 1990; Banks *et al.*, 1997).

Various suggested roles of insulin in the CNS includes: its role in growth, development and metabolism and its role as a neuromodulator. Insulin also considered being a link between adaptive feeding and changing energy requirements through its involvement with hypothalamic regulation of feeding behaviour. Hence, it might also be responsible for feeding habits and thus, for weight and energy regulation also (Schulingkamp *et al.*, 2000). Suppression of cell death in the CNS is one of the most important protective effects of insulin that has been studied. It also has protective effects against oxidative stress and ischemia (Ghasemi *et al.*, 2013).

1.5 Epilepsy and Hypoxia

Oxygen delivery to the mammalian brain is under precise and dynamic control so that most of the brain tissue is exposed to the minimal necessary amount of oxygen (Harik *et al.,* 1995). It is commonly accepted that oxygen restrictions and ischemic deprivation exerts marked effects on cognitive functions (Scholey *et al.,* 2001). The brain's capacity to supply and metabolize glucose and oxygen exceeds demand over a wide range of rates (Dienel, 2012). As during the ictal period and up to some-extent during the postictal period, the cerebral metabolism increases very sharply and hence it outstrips the available oxygen supply, and in cases when, body is not able to match this requirement, hypoxic condition prevails, which results in a shift to anaerobic glycolysis and increasing lactate formation by the brain tissue (Beresford *et al.,* 1969)

A possible solution to this apparent paradox is offered by previous work suggesting the existence of a net lactate transfer from glial cells to neurons, known as the lactate shuttle hypothesis (Magistretti *et al.,* 1999).

1.6 HIF-1-α

Hypoxia is a condition of an inadequate oxygen supply to tissues and cells, which can restrict their function (Xiao *et al.,* 2013). Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric transcription factor composed of two subunits: HIF-1α and HIF-1β. It regulates a number of essential pathways in the adaptive responses of cells to hypoxic conditions. The levels and activity of HIF-1α are tightly regulated by cellular O_2 concentrations. It has been shown that HIF-1α regulates the expression of nearly all the enzymes involved in the process of glycolysis. It also regulates GLUT1 and GLUT3 which are involve in cellular glucose uptake. Glucose metabolism also affects the expression of HIF-1α. Reduced levels of HIF-1α in the cells or tissues found from patients with diabetes or from animal models of diabetes (Zhang *et al.,* 2011).

The beneficial effects of HIF-1 arise from the increased expression of HIF-1 which helps in combat oxidative stress, improve blood oxygen and glucose supply, promote glucose metabolism, regulate iron homeostasis, activate the synthesis of dopamine, and block cell death signal pathways (Xiao *et al.,* 2013).

1.7 Lactate Dehydrogenase

Lactate Dehydrogenase (LDH) is an important enzyme for the energy metabolism pathways. It is known that glucose is the major fuel for the brain which gets metabolized for different functions (Dienel, 2012). Most of the times, the cells breakdown this glucose completely, but this requires a lot of oxygen, and if this oxygen is not available, these pathways end-up at glycolysis and further energy generation and other physiological functions does not become possible. This problem is solved by LDH (www.rcsb.org). LDH is the connecting link between the energy generating pathways and the basic substrate glucose, which gets activated when there is a lack of oxygen.

LDH catalyses a reversible redox reaction inter-converting pyruvate to lactate, coupled to NADH and NAD^+ and vice-versa (Beltran *et al.,* 2012). LDH permits organisms to generate temporary oxygen debt energy in the form of accumulated lactate which later gets discharged by re-oxidation of lactate to pyruvate when oxygen becomes available (Markert, 1984). LDH overexpression shows decrease in the glucose oxidation rate at glucose concentrations little more than the control cells, whereas this condition remains unchanged at lower glucose concentrations. Lactate release gets increased in parallel with a decrease in the glucose oxidation rate (Alcazar *et al.,* 2000).

Hence, in epilepsy, when hypoxia prevails during the ictal period, an elevated LDH activity might be observed.

1.8 Antioxidant Enzymes

Animal tissues are constantly trying to cope up with the highly reactive oxygen species, such as superoxide anions, hydroxyl radicals, hydrogen peroxide, and other such free radicals that are generated during various metabolic reactions. Small amount of these radicals have a physiologically important role, but when a large amount of such radicals are generated, it gives rise to oxidative stress (Szymonik-Lesiuk *et al.,* 2003).

Hypoxia and related seizures results in oxidative stress and generation of free radicals that will damage cellular proteins, lipids, and DNA. Seizure-induced mitochondrial superoxide production may mediate seizure-induced neuronal death. The oxidative stress will result into the mitochondrial dysfunction that can make the brain more

susceptible to epileptic seizures. During hypoxia these enzymes will be utilised more as an antioxidant to overcome the free radicals produced due to it (Waldbaum and Patel, 2010).

To fight this oxidative stress, cells develop an enzymatic anti-oxidant pathway against these ROS that are generated during oxidative metabolism. This enzymatic pathway comprises of 2 important steps – 1st is the dismutation of superoxide anion (O^{2-}) to hydrogen peroxide (H_2O_2) and 2nd is the breakdown of this hydrogen peroxide to water (H_2O) and oxygen (O_2) molecules. These are catalysed by enzymes Superoxide Dismutase (SOD) and Catalase (CAT) or Glutathione Peroxidase respectively (Sfar *et al.*, 2013).

1.8.1 Superoxide Dismutase

SOD is the most robust intracellular enzymatic antioxidants (McCord and Fridovich, 1969). Its main role is that it in breaks down the harmful oxygen molecules in cells that might prevent damage to tissues (Buettner *et al.*, 2011).

1.8.2 Catalase

CATs are some of the most efficient enzymes found in the cells. Each CAT molecule catalyses the breakdown of millions Hydrogen Peroxide to water and oxygen molecules every second in order to release oxidative stress (www.rcsb.org). In brain it acts as a powerful reactive oxygen species scavenger and protects the brain from any severe damage caused by them (Armogida M *et al.*, 2011).

"Based on these basic concepts, the aim for this project was to study the effect of Insulin functional regulation on Glucose Energy Metabolism Pathway, Oxidative Stress and Behaviour in pilocarpine induced epileptic rats."

Objectives to achieve our Aim:

1. To induce epilepsy by using Pilocarpine in adult Wistar rats.
2. To check Food and Water intake and change in weight in all experimental group of rats.
3. To carry out behavioural studies to evaluate motor activity, memory and cognition in control and experimental group of rats.
4. To check for the Blood Glucose levels in all groups of Control and experimental rats.
5. To assess the specific activity of LDH, Super oxide dismutase and catalase in control and experimental group of rats.
6. To check the expression of HIF-1α gene in order to assess the Insulin Signalling Pathway in control and experimental group of rats.

LITERATURE REVIEW

2. Literature Review

2.1 Epilepsy

Epilepsy is a chronic nervous disease characterized by fits or seizures occurring at intervals and attended by convulsive motions of muscles and loss of consciousness (Natelson *et al.,* 1979). One another definition that summarizes the various definitions of the previous years that implicate the brain in the phenomenon of epilepsy is, " A symptom of paroxysmal and abnormal discharge in the brain that may be induced by a variety of pathological processes of genetic or acquired origin" (Schmidt and Wilder, 1968).

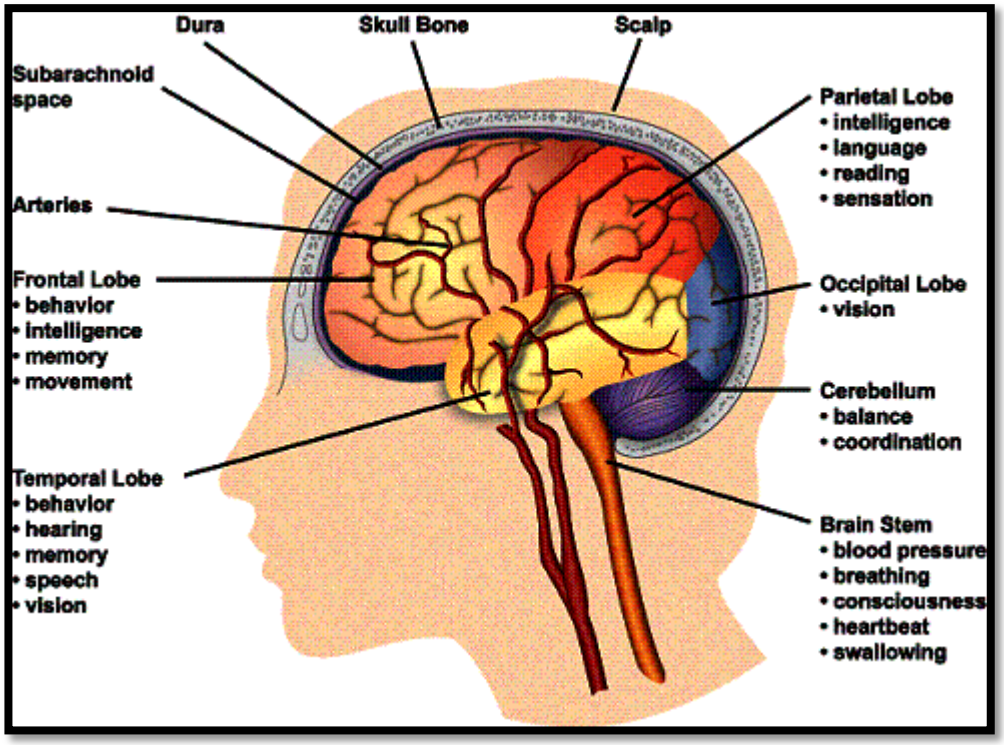
Epilepsy is characterized by recurrent seizures, brief periods of abnormal synchronized neural firing. It can be acquired or inherited (Longstaff, 2002).

2.1.1 Types of Epilepsies

The following are the most common types of Epilepsies:

1. Absence Epilepsy
2. Frontal Lobe Epilepsy
3. Occipital Lobe Epilepsy
4. Parietal Lobe Epilepsy
5. Temporal Lobe Epilepsy
6. Primary Generalized Epilepsy
7. Idiopathic Partial Epilepsy
8. Symptomatic Generalized Epilepsy
9. Progressive Myoclonic Epilepsy
10. Reflex Epilepsy

Figure 1: Regions of Brain and their Functions



(Higashida, 2012)

2.1.2 Epileptogenesis

Epileptogenesis is the process by which a normal brain becomes epileptic (Siegel, 2006). This term refers to the development of the hyper excitable state that predisposes to seizures. N-methyl D-aspartate (NMDA) receptors are necessary for epileptogenesis (Longstaff, 2002).

2.1.3 Seizures

A seizure is the clinical manifestation of an abnormal excessive, hyper synchronous discharge of a population of cortical neurons. A common way to explain seizures in a normal individual is that a disruption has occurred in the normal balance of excitation and inhibition (Scharfman, 2007).

Epileptic seizures are caused by an abnormal, synchronized firing of large populations of neurons that are usually self-limiting (Longstaff, 2002). Seizures can be caused by multiple mechanisms, and often they appear so diverse that no common cause can be expected to apply. However, one principal that is often discussed is that seizures arise when there is a disruption of mechanisms that normally create a balance between excitation and

inhibition. Thus, normally there are controls that keep neurons from excessive potential discharge, but there are also mechanisms that facilitate neuronal firing so the nervous system can function appropriately. Disrupting the mechanisms that inhibit firing or promoting the mechanisms that facilitate excitation can lead to seizures (Scharfman, 2007).

2.1.3.1 Events during Seizures

Seizures result from an electrochemical disorder in the brain. Brain cells use chemical reactions to produce electrical discharges, which causes an excitation or inhibition of other brain cells with its discharge and if the balance of this excitation and inhibition shifts in the direction of excitation, then a seizure results (Fisher and Saul, 2010). And hence, epilepsy is a state of recurrent, unpredictable, spontaneous seizures (Krishnakumar *et al.*, 2009).

2.1.3.2 Stages of Seizures

There are several major phases or stages of seizures. These are:

1. **Pre-ictal or Prodromal Period**: This is the time preceding or before the seizure. It can last from minutes to days, but everyone does not experience this stage and if anyone experiences it, it can be considered as a warning for occurrence of epileptic seizure.
2. **Ictal Period**: This is the actual seizure. During this time there will be actual physical changes in the person's body, which can be and usually is measured using an EEG.
3. **Interictal Period**: This is the time between seizures. As it is known that in epilepsy, there is an occurrence of recurrent seizures; hence interictal period is the phase between two seizures. In TLE, usually many people suffer from emotional disturbances during this period.
4. **Co/interictal Period**: This is the final Phase, the often slow recovery period after a seizure. It can last from minutes to hours, depending upon the type of seizure experienced, the intensity of it, and how long it lasted (Epilepsy Foundation of America, 2009).

2.1.3.3 Classification of Seizures

Table 1: Classification of Seizures

Partial Seizures
- Simple Partial
  - motor
  - somatosensory
  - autonomic
  - psychic
- Complex Partial
  - with focal onset prior to alteration in consciousness
  - without focal onset prior to alteration in consciousness

Generalized Seizures
- Primary Generalized Nonconvulsive
  - absence
- Primary Generalized Convulsive
  - tonic-clonic
  - clonic
  - tonic
  - myoclonic
  - atonic
- Secondary Generalized
  - convulsive
  - nonconvulsive

Status Epilepticus
- Convulsive Generalized
  - primary generalized
  - secondary generalized
- Convulsive Focal
- Nonconvulsive
  - primary generalized (absence)
  - partial with or without secondary generalization (complex partial)

(http://www.ebmedicine.net)

2.2 Temporal Lobe Epilepsy

TLE is a common type of focal (localization – related) epilepsy, which is difficult to control by the use of medications (Chang and Lowenstein, 2003).

TLEs are a group of neurological disorders in which humans and animals experience recurrent epileptic seizures arising from one or both temporal lobes of the brain. Two main types of TLEs are generally recognized, mesial temporal lobe epilepsy (MTLE) which arises in the hippocampus, para hippocampal gyrus and amygdala, and lateral temporal lobe epilepsy (LTLE) which arises in the neocortex. Initial trauma associated with TLE induces complex molecular, biochemical, physiological, and structural changes in the brain

that contribute to epileptogenesis and the subsequent onset of spontaneous and recurrent seizures (Waldbaum and Patel, 2010).

TLE is often associated with mood disorders, particularly depression (Briellmann *et al.,* 2007). Seizure auras occur in many TLE patients and often exhibit features that are relatively specific for TLE (Blair, 2011). The limbic system gets affected by localisation of seizure particularly in hippocampus, amygdala and entorhinal cortex (Bartolomei *et al.,* 2005). Temporal lobe seizures are the most frequent site for the origin of partial seizures (*Blair,* 2011).

2.2.1 Mechanisms underlying TLE

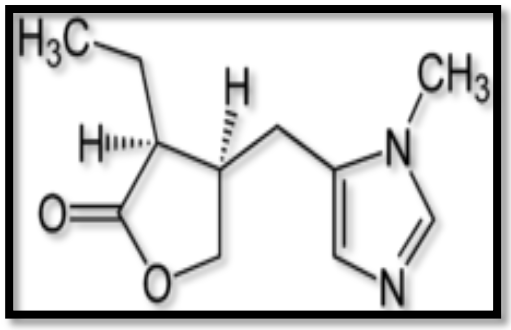
TLE is associated with a specific structural lesion in the hippocampus (Buckmaster, 2004). The hippocampal formation, the major part of the temporal lobe, controls excitability in the neuronal circuitry and is responsive to an epileptic attack (Kuo *et al.,* 2008). Some patients with TLE have tumours or vascular malformations in the temporal lobe. The most common lesion in 70% of the patients is hippocampal sclerosis highlighted by a specific pattern of neuronal loss (Engel *et al.,* 1997). The most vulnerable neurons are in the CA1 region and in the hilus of the dentate gyrus (DG), whereas the least vulnerable neurons are the granule cells in the dentate gyrus and the CA2 pyramidal cells. Lesions also occur on the outside of hippocampus (Buckmaster, 2004).

Mossy fibre sprouting (MFS) is the main characteristic of TLE (Chang and Lowenstein, 2003). Mossy fibres are the axons of granule cells in DG which target to pyramidal cells in CA3 region. After epileptic injury, mossy fibres lose their targets due to massive neuronal death in CA3 and hilus area, and form incorrect synaptic connections on the somatic and dendritic sites of granule cells, causing abnormal neuronal circuits and hyper excitability (Scharfman *et al.,* 2000; Williams *et al.,* 2002). MFS was demonstrated to be highly correlated to the frequencies of recurrent seizures as well as to the degrees of severity of TLE; it was considered an important index to evaluate the severity of TLE in chronic epileptic animal models (Nadler, 2003; Sutula, 2002).

2.3 Models of Epilepsy

2.3.1 Pilocarpine model of Epilepsy

Figure 2: Structural formula of Pilocarpine



(Stadelmann and Sawyers)

Iupac name: (3s, 4r) - 3-ethyl- 4-((1-methyl- 1h-imidazol- 5-yl) methyl) dihydrofuran-2(3h)-one.

Formula: $C_{11}H_{16}N_2O_2$

Mol. Mass: 208.257 g/mol

Half-life: 0.76 hours (5 mg), 1.35 hours (10 mg)

Pilocarpine has been known to cause excessive sweating, excessive salivation, bronchospasm, increased bronchial mucus secretion, bradycardia, vasodilation, brow ache (when used as eye drops) and diarrhoea. It can also result in meiosis when used chronically as an eye drop. Systemic injection of pilocarpine can compromise the BBB allowing pilocarpine to gain access to the brain. This can lead to chronic epilepsy. (www.drugs.com)

The main features of TLE are: (i) the localization of seizure foci in the limbic system, particularly in the hippocampus, entorhinal cortex and amygdala (Bartolomei *et al.*, 2005) (ii) the frequent finding of an "initial precipitating injury" that precedes the appearance of TLE (Mathern *et al.*, 2002); (iii) a seizure-free time interval following the precipitating injury known as "latent period"; and (iv) a unilateral hippocampal lesion leading to atrophy, typically caused by neuronal loss and gliosis.

Some important features of the pilocarpine model are: (i) the induction of acute SE more rapidly than with intraperitoneal (ip) Kainic acid, the other convulsant drug commonly used to reproduce TLE in animals; (ii) the presence of a latent period followed by the appearance of spontaneous recurrent seizures (SRSS, chronic phase) (Leite *et al.*, 1990)

(iii) the occurrence of widespread lesions, some of them localized in the same brain areas affected in TLE patients, and associated with neuronal network reorganization in hippocampal and para-hippocampal regions (for instance, mossy fibre sprouting, interneuron loss and ectopic dentate granule cell proliferation are phenomena shared by TLE patients and pilocarpine-treated animals (Wieser, 2004).

The ability of pilocarpine to induce SE is likely to depend on activation of the M1 muscarinic receptor subtype, since M1 muscarinic receptor knockout rodents do not develop seizures in response to pilocarpine (Hamilton *et al.,* 1997). In addition, pilocarpine-induced SE can be blocked by systemic administration of the muscarinic antagonist Atropine. Once seizures are initiated, however, their maintenance depends on other mechanisms since atropine becomes ineffective (Clifford *et al.,* 1987). Experiments in cultured hippocampal neurons have demonstrated that pilocarpine, acting through muscarinic receptors, causes an imbalance between excitatory and inhibitory transmission resulting in the generation of se (Priel and Albuquerque, 2002). In addition, in vivo micro dialysis studies have revealed that pilocarpine induces an elevation in glutamate levels in the hippocampus following the appearance of seizures. Substantial evidence now supports the suggestion that, following initiation by M1 muscarinic receptors, seizures are maintained by NMDA receptor activation (Smolders *et al.,* 1997).

2.3.2 Other Models of Epilepsy

Two models other than pilocarpine are established to induce epilepsy:

1. The Kainic acid model of experimental epilepsy: When administered systemically or intra-cerebroventricularly in animal models, kainic acid produces an epilepsy syndrome similar to human TLE (Goldstein, 2008).

2. Lithium-pilocarpine model: Pilocarpine (pilo) alone or combined with lithium reproduces most clinical and of neuro-pathological features of human TLE with SE. In adult rats, lithium-pilocarpine treatment leads to SE followed by a latent seizure-free phase of a mean duration of 14 –25 days. During this period, neuronal damage develops mainly in the hippocampus, the hilus of the DG, the piriform and entorhinal cortices, the amygdala, the neocortex and the thalamus (Muller CJ *et al.,* 2009).

2.4 Epilepsy and Glucose Metabolism

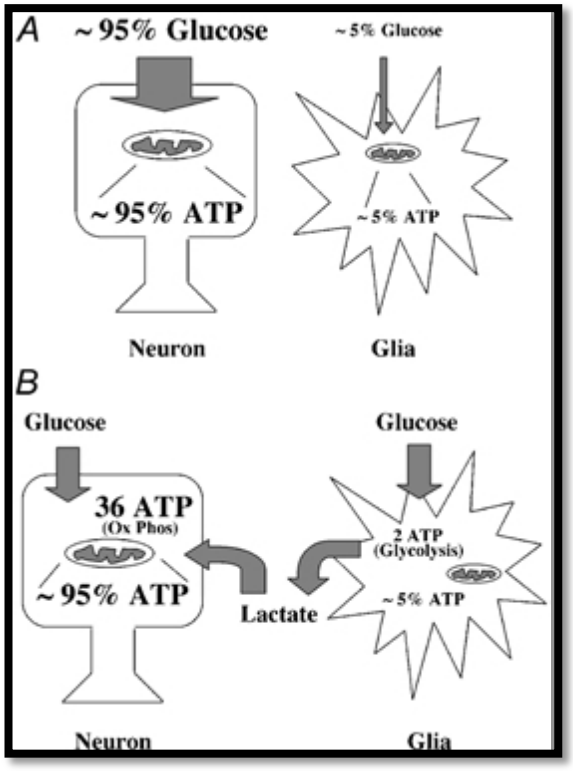
Glucose metabolism in adult human brain is about 30 µmol/100 g/min; the oxygen consumption is approximately equivalent to the glucose utilization. The adult human uses about 110 g glucose each day for brain glucose metabolism. Indeed, in the resting state, approximately 20% of whole-body glucose utilization provides fuel for the substantial energy requirements of the brain (Rayner, 1996).

Glucose is utilized by brain to fuel its activity. It is used as most important blood borne energy substrate. Energy required by the brain for its functioning is derived from the oxidation of the glucose (Pellerin and Magistretti, 2003).

Ketone bodies do enter the brain, in proportion to the degree of ketosis but the fatty acids do not enter the brain because they cannot cross the BBB. In brain major portion of energy (81%) is utilized for neuronal excitatory signalling. Only 5% of energy is utilized for glial cell function and rest is used by neurons (Pellerin and Magistretti, 2003).

Increase in relative glucose uptake was observed in the brain regions during the ictal and early postictal period. High glucose uptake is responsible for increased cerebral blood to match hyper metabolism which leads to increased lactate build up and increased rate of glycolysis exceeding pyruvate utilization (Scorza *et al.,* 2002). Non oxidative metabolism of the glucose will lead to the increase in the ATP production and this ATP will be used as a fuel for the ion exchange in the form of ATPase associated ion pumping and this would increase lactate production. Apart from this pentose shunt pathway flux causes oxidative decarboxylation of glucose without oxygen consumption (Dienel, 2012).

Figure 3: (A) Brain Energy Budget (B) Brain Economy budget with Lactate exchange



(Pellerin and Magistretti, 2003)

Now during the inter ictal period of epilepsy the glucose concentration significantly decreases (Natelson *et al.,* 1979; Cigdem *et al.,* 2010). Low level of glucose leads to the reduction in the ATP/ADP ratio and membrane depolarization or rapid depletion of glycogen stores. If the level of the intracellular ATP falls as low as 10% of previous then there are chances of cell death. Low level of ATP results in the neuronal depolarization leading to the failure of ion exchange. Influx of calcium follows the release of the neurotransmitters, including the high glutamate release. Due to this activation of NMDA and other excitatory neurons will occur. Cell death results if concentrations of intracellular ATP have fallen within 30 to 48 h to very low levels (lower than 10% of the initial value). At the cellular level, depletion of ATP causes neuron depolarisation leading to failure of membrane ion-transport systems. Calcium influx then leads to the release of neurotransmitters, including high quantities of glutamate, which then activates NMDA and other excitatory receptors on other neurons. Repeated depolarization will lead to more glutamate release and may cause glutamate excite-toxicity (Owen and Sunram-Lea, 2011).

Glucose utilization in pilocarpine induced epileptic rats was more compare to control rats. Comparative glucose utilization of different brain area was hippocampus

(50.6%), caudate-putamen (30.6%), fronto-parietal cortex (32.2%), amygdala (31.7%), entorhinal cortex (28.2%), thalamic nucleus (93.5%), pre-tectal area (50.1%) and substantia nigra (50.3%). The observation suggests that these areas contiguously and intensely activated, have a strong tendency to act together as a single functional entity (Scorza *et. al.,* 2002*).*

Substantia nigra responsible for the initiation and propagation of seizure activity within the limbic system becomes activated relatively late in the course of seizures *(*Scorza *et al.,* 2002*).*

2.5 Role of Insulin in Brain

There are various regions of the developing and adult brain that possesses receptors for insulin called as the insulin receptor. Insulin enters the central nervous system through the BBB by receptor-mediated transport to regulate food intake, sympathetic activity and peripheral insulin action through the inhibition of hepatic gluconeogenesis and reproductive endocrinology. Neurotransmitters released by the pre synaptic receptors will saturate the postsynaptic receptors. Therefore the recruitment of functional receptors to postsynaptic domains increase synaptic efficacy (Schwartz *et al.,* 1990; Banks *et al.,* 1997).

On a molecular level, some of the effects of insulin converge with those of the leptin signalling machinery at the point of activation of phosphotidylinositol 3-kinase (PI3K), resulting in the regulation of ATP-dependent potassium channels. Furthermore, insulin inhibits neuronal apoptosis via activation of protein kinase B and it regulates phosphorylation of tau, metabolism of the amyloid precursor protein and clearance of β-amyloid from the brain. These findings indicate that neuronal insulin receptor signalling has a direct role in the link between energy homeostasis, reproduction and the development of neurodegenerative diseases (Plum *et al.*, 2005).

Peripheral insulin crosses the BBB via an active transport mechanism and binds to insulin receptors on neurons and glial cells. Insulin has a catabolic effect; in addition, it influences memory functions by modulating neurotransmitter release and synaptic plasticity (Hirvonen *et al.,* 2011).

It has been reported that insulin will induce rapid translocation of $GABA_A$ receptors from the intracellular compartment to the plasma membrane and enhances the

expression of $GABA_A$ receptors on the dendritic and postsynaptic membranes (Kneussel, 2002; Wan *et al.*, 1997).

Moreover the insulin can promote the internalization of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors from the synaptic membrane of neurons, which causes a long-term depression (LTD) of excitatory synaptic transmission in the hippocampus and cerebellum (Ghasemi *et al.*, 2013).

Also insulin enhances NMDA receptor mediated synaptic transmission at the hippocampal CA1 synapses (Huang *et al.*, 2012).

2.6 Epilepsy and Hypoxia

It is commonly accepted that oxygen restriction and ischemic deprivation exert marked effects on cognitive function. Even transient oxygen deprivation leads to functional but reversible deficits (Owen and Sunram-Lea, 2011).

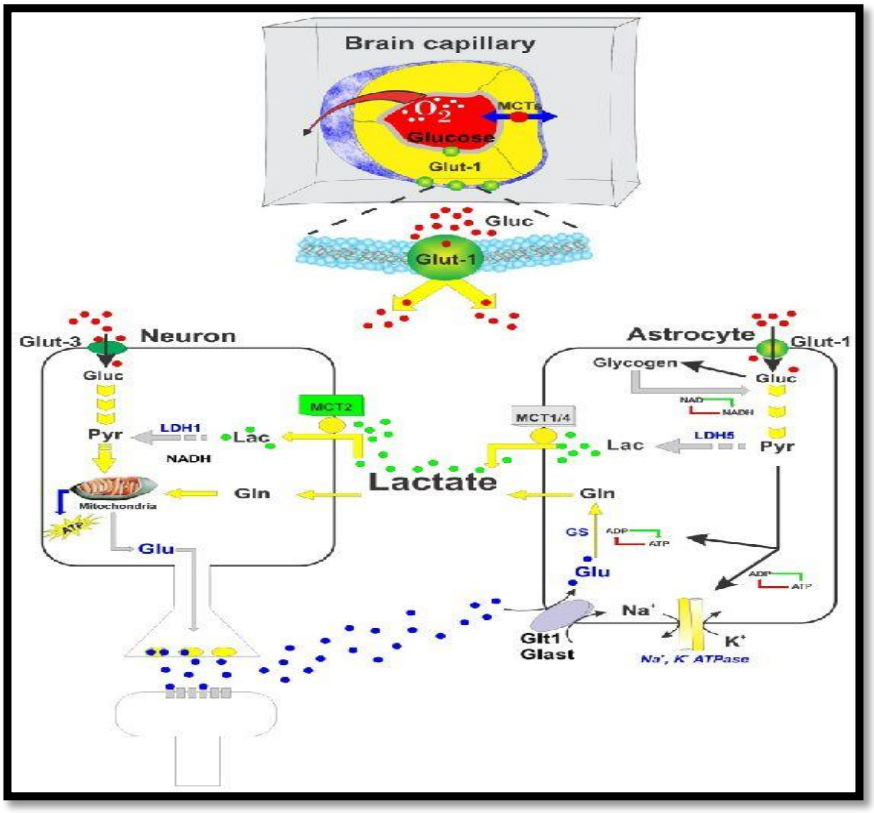
Regional brain tissue oxygen tension is not homogenous. In conditions of mild hypoxia, regional brain oxygen tension is maintained via a compensatory increase in the brain blood flow. However, in conditions of prolonged hypoxia, it induces an increase in the brain capillary density (Harik *et al.*, 1995).

As during the ictal period and post-ictal period, blood glucose transport and utilization is increased, it also leads to the increased oxygen need, which is required for glucose transport. And in cases when, body is not able to match this requirement, hypoxic condition prevails. And if hypoxia prevails, glucose needs also won't be met.

A possible solution to this apparent paradox is offered by previous work suggesting the existence of a net lactate transfer from glial cells to neurons, known as the lactate shuttle hypothesis (Magistretti *et al.*, 1999). Thus, it was postulated that in the central nervous system, astrocytes could respond to increased synaptic activity via glutamate uptake by increasing their rate of aerobic glycolysis, i.e. glucose consumption and lactate production (Pellerin and Magistretti 1994). After its release from astrocytes, lactate would be taken up by neurons via a specific mono carboxylate transporter (MCT) and used as energy substrate. Mounting evidence has been provided indicating that lactate could constitute a significant energy substrate for neurons both in peripheral nerves and in the brain. Moreover, the

existence of a lactate shuttle between cells within the same tissue is not unique to the nervous system since it has been already reported in other tissues (e.g. striated muscle), suggesting that it might be a rather universal concept (Brooks, 2002). Data presented by Véga *et al.*, (2003) strongly indicate that a net transfer of energy substrate must be taking place between Schwann cells and axons, and points at lactate as the likely candidate.

Figure 4: Schematics of neuro-energetics pathway: The astrocyte – neuron lactate shuttle



(Naik *et al.*., 2012)

Glycolysis occurring in glial cells would provide 2 ATP and lead to the production of 2 lactate per glucose consumed. In return, oxidation of 2 lactate molecules by the neurons (or nerve) could potentially provide up to 36 ATP. Interestingly enough, this distribution of ATP production respects the previously established energy budget with ~5 % of the ATP being produced in the glial cell while the rest would be generated from lactate in the neuron (Poitry-Yamate *et al.*, 1995).

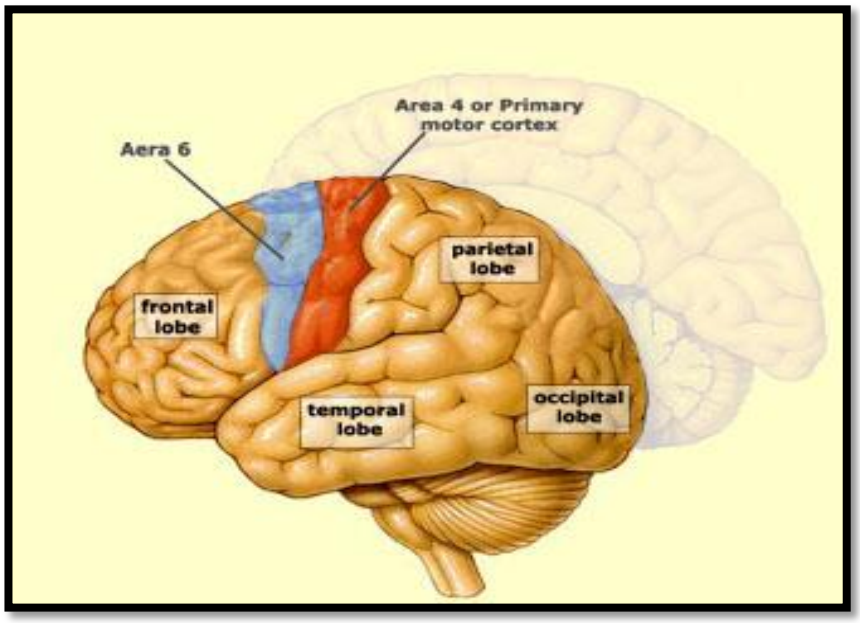
2.7 Epilepsy and Motor impairment

Genetic programs are responsible for building the neuronal circuits during embryonic development and are modified through interactions with the internal and external

environment. Sensory circuits such as sight, touch, hearing, smell, taste bring information to the nervous system, whereas motor circuits send information to muscles and glands. The simplest circuit is a reflex, in which a sensory stimulus directly triggers an immediate motor response. The complex responses occur when the brain integrates information from many brain circuits to generate a response. Simple and complex interactions among neurons, takes place on time scales, ranging from milliseconds to months. The brain is organized to recognize sensations, initiate behaviours, and store and access memories that can last a lifetime (Neuroscience Core Concepts, 2008)

Body's voluntary movements are controlled by the brain. One of the brain areas that are mostly involved in controlling these voluntary movements is the motor cortex.

Figure 5: Regions of Brain



Area 4 – Primary Motor Cortex Area 6 – PMA and SMA

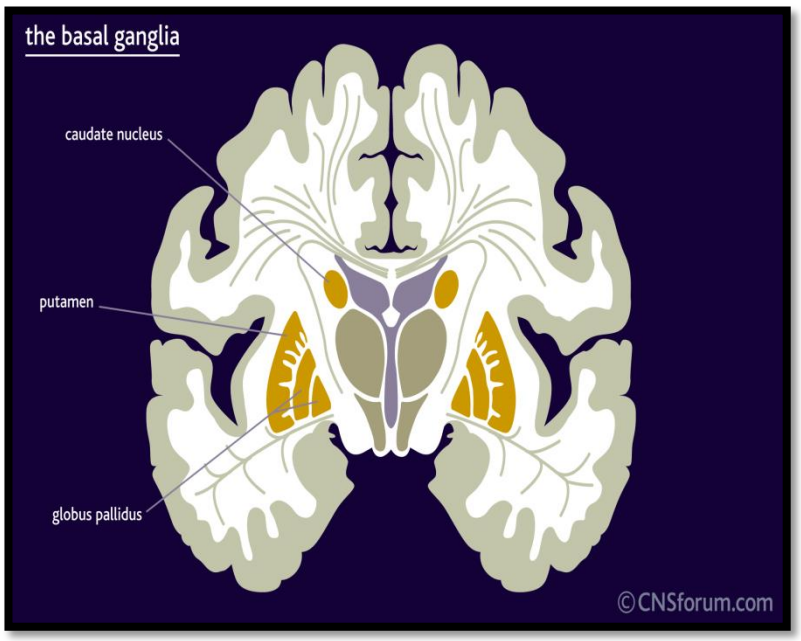
(http://thebrain.mcgill.ca/)

The motor cortex is situated in the rear portion of the frontal lobe, just before the central sulcus (furrow) which separates the frontal lobe from the parietal lobe. The motor cortex is divided into two main areas, Area 4 and 6. Area 4, also known as the primary motor cortex, forms a thin band along the central sulcus. Area 6 lies immediately forward of Area 4. Area 6 is wider and is further subdivided into two distinct sub-areas – Premotor area (PMA) and Supplementary motor area (SMA). In order to carry out goal-directed movements, motor cortex must first receive various kinds of information from the various lobes of the brain: information about the body's position will be provided by the parietal lobe; the goal to

be attained and an appropriate strategy for attaining it will be provided by the anterior portion of the frontal lobe; memories of past strategies will be provided by the temporal lobe.

Basal Ganglia

Figure 6: Basal Ganglia



(Crossman and Neary, 1995)

The basal ganglia are made up of distributed set of brain structures in the telencephalon, diencephalon, and mesencephalon. The forebrain structures is made up of caudate nucleus, the putamen, the nucleus accumbens (or ventral striatum) and the globus pallidus. Together, these structures are named the corpus striatum. The caudate nucleus is a C-shaped structure that is closely associated with the lateral wall of the lateral ventricle. It is largest at its anterior pole (the head), and its size diminishes posteriorly as it follows the course of the lateral ventricle (the body) all the way to the temporal lobe (the tail), where it terminates at the amygdaloid nuclei. The putamen is also a large structure that is separated from the caudate nucleus by the anterior limb of the internal capsule. The putamen is connected to the caudate head by bridges of cells that cut across the internal capsule. Because of the striated appearance of these cell bridges, the caudate and putamen are collectively referred to as the striatum or neostriatum, and the nucleus accumbens is often called the ventral striatum. Functionally, the caudate nucleus and the putamen are considered equivalent to each other. There are two different pathways that process signals through the basal ganglia:

1. The Direct Pathway

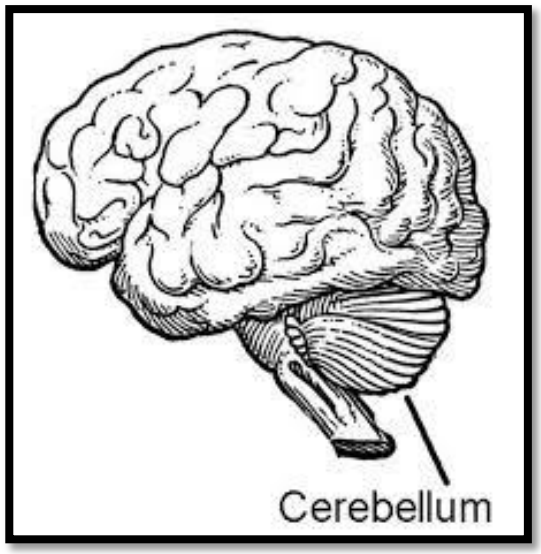
2. The Indirect Pathway.

These two pathways have opposite net effects on thalamic target structures. Excitation of the direct pathway will result into the net effect of exciting thalamic neurons (which in turn make excitatory connections with the cortical neurons). Excitation of the indirect pathway has the net effect of inhibiting thalamic neurons (rendering them unable to excite motor cortex neurons). The normal functioning of the basal ganglia apparently involves a proper balance between the activities of these two pathways. Basal ganglia is involved in the enabling of practiced motor acts and in gating the initiation of voluntary movements by modulating motor programs stored in the motor cortex and elsewhere in the motor hierarchy. Thus, voluntary movements are not initiated in the basal ganglia (they are initiated in the cortex); however, proper functioning of the basal ganglia appears to be necessary in order for the motor cortex to relay the appropriate motor commands to the lower levels of the hierarchy (Knierim, 1997).

The Cerebellum

The cerebellum also known as the little brain is a structure that is located at the back of the brain, underlying the occipital and temporal lobes of the cerebral cortex. Cerebellum accounts for about 10% of the brain's volume, but it contains over 50% of the total number of neurons in the brain. The cerebellum is considered as the motor structure, because cerebellar damage leads to impairments in motor control and posture and because the majority of the cerebellum's outputs are to parts of the motor system. Motor commands are not initiated in the cerebellum; rather, the cerebellum modifies the motor commands of the descending pathways to make movements more adaptive and accurate. The cerebellum is important for motor learning. The cerebellum plays a major role in adapting and fine-tuning motor programs to make accurate movements through a trial-and-error process (Knierim, 1997).

Figure 7: Cerebellum



(http://www.intropsych.com)

Thus the motor activity of the rats was assessed by performing two behavioural studies namely the wheel running test and the staircase test.

2.8 Epilepsy and Memory - Cognition impairment

Cognition is the ability to think, learn, and remember. It is the basis for how we reason, judge, concentrate, plan, and organize. The cognitive system is responsible for the temporary maintenance and processing of information during complex cognitive activities, is important for many everyday functions including reading comprehension, mental arithmetic, following instructions, and reasoning (Dunning and Holmes, 2014).

Memory and recognition involves making judgments about whether a stimulus has been encountered before. However, it can be argued that recognition memory is not a unitary process, as distinct types of information are used to form judgments of prior occurrence, including the relative familiarity of an object or location or when or where an object was previously encountered (Mumby and Pinel, 1994; Ennaceur *et al.*, 1996; Bussey *et al.*, 1999; Norman and Eacott, 2004).

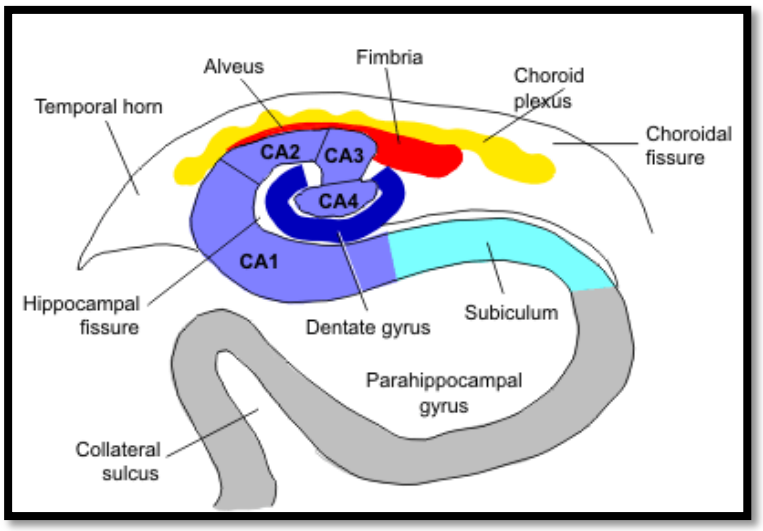
Investigations on the neural basis of recognition and memory have implicated several brain regions. Lesions in the perirhinal cortex severely disrupt object recognition and object-in-place memory and temporal order recognition memory but not object location memory (Hannesson *et al.*, 2004; Barker *et al.*, 2007).

Lesions of the medial prefrontal cortex disrupt temporal order and object-in-place memory yet have no effect on object recognition. Further disconnection of the perirhinal and medial prefrontal cortices significantly impairs both object-in-place and temporal order memory, showing that, depending on the nature of the recognition memory judgment, these cortical regions may function within a neural circuit (Barker *et al.*, 2007).

Hippocampus has been shown to be involved in memory and cognitive functions. It is important for a special kind of memory called the declarative memory. It is also involved temporarily to bind together the distributed sites in frontal cortex that together represents a whole memory (Squire, 1992). It also plays an important role in conversion of temporary memory to long term memory by a process called Long Term Potentiation (LTP) (Siegel, 2006).

Normally, in the hippocampus, CA3 pyramidal cells fire bursts of action potentials and via their Schaffer collaterals, drive CA1 cells. However extensive inhibition from GABAergic interneurons prevents burst firing of CA1 cells. In epileptic hippocampal slices however, CA3 cells do trigger bursting of CA1 cells, and burst firing of CA3 cells is itself exaggerated by paroxysmal depolarizing shifts (PDS) due to Ca^{2+} influx. PDSs cause abnormal interictal spikes in EEG during seizures (Longstaff, 2002).

Figure 8: Hippocampal Anatomy



(Hesselink, FACR, 2014)

Hippocampus, being a part of Temporal Lobe, is the key site to undergo damage during TLE and hence loss of memory is one of the major consequences of TLE.

2.9 HIF-1-α

HIF-1α and Nerve Growth Factor (NGF) are involved in ischemic and hypoxic cerebral injury. The HIF signalling pathway plays an important role in intrinsic neuro-protection. Up regulation and maintenance of HIF-1α and NGF expression may attenuate Vascular Cognitive Impairment (VCI) (Ke and Zhang, 2013). HIF-1 is a key mediator in spontaneous recovery and after Traumatic Brain Injury (TBI). HIF-1 inhibition resulted in deterioration of motor function, increased lesion volume, hypothermia, and reduced oedema formation (Umschweif *et al.,* 2013).

It has been well elucidated that HIF-1 plays a central role in regulating glucose metabolism under hypoxia (Shen *et al.,* 2012). Chronic hypoxia of high altitude is associated with decreased serum glucose and insulin concentrations. Other investigators reported that HIF-1 promotes cellular glucose uptake by increased expression of GLUT1 and increased glycolysis (McClain *et al.,* 2013). In ischemia/reperfusion injured brains, increase of IGF-1 secretion and GLUT3 up-regulation, that is essential for glucose passage across cell membrane, are regarded as protective processes. Works have shown that various growth factors and cytokines including IGF-1 can stimulate HIF-1α expression and also the expression of GLUT3 in response to IGF-1 was dependent on PI-3-kinase and mTOR activity, and required the transcription factor HIF-1α (Yu *et al.,* 2012).

2.10 Lactate Dehydrogenase

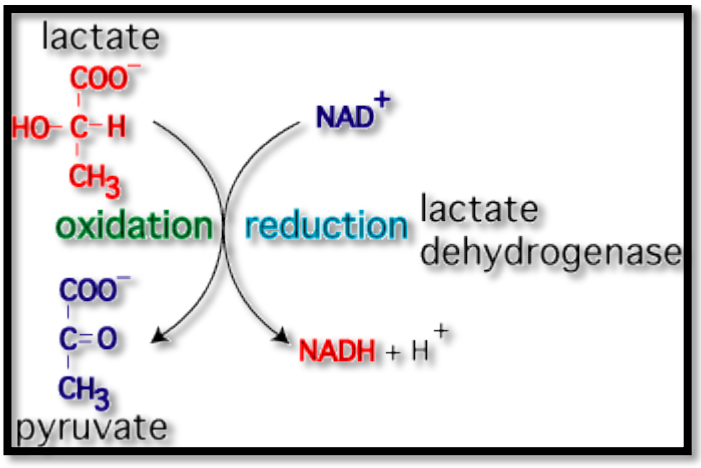
LDH (EC no. – 1.1.1.27) catalyses the reversible reduction of pyruvate to lactate. Under the influence of enzyme LDH, accumulation of lactate in brain will continue and the reversible conversion of NAD^{+}/NADH coupled redox reaction will occur. The extracellular lactate is transported to the cell and is oxidized to pyruvate and lactate. Lactate synthesized within astrocyte and released into the interstitial space in the brain may serve as energy fuel for neurons. Astrocytes release lactate at the greater rate than neurons and the lactate is preferentially metabolized in neural cells to produce energy (Beltran *et al.,* 2012). Hypoxia and anoxia leads the muscle to produce lactate from pyruvate (Bakker *et al.,* 2013). The pathways using the NADP system are relatively protected from the rapid fluctuations that seizure and anoxia can produce (Merrill and Guynn, 1981).

LDH overexpression shows decrease in the glucose oxidation rate at glucose concentrations little more than the control cells, whereas this condition remains unchanged at lower glucose concentrations. Lactate release gets increased in parallel with a decrease in the glucose oxidation rate (Alcazar *et al.*, 2000).

The level of LDH expression in insulin-secreting cells is an important determinant of the physiological insulin-secretory capacity, and also determines how pyruvate and lactate affect, insulin secretion. Gluco-kinase-mediated glycolytic flux decreases after overexpression of LDH. Thus low level of LDH helps glucose to undergo aerobic metabolism (Alcazar *et al.*, 2000). Epileptic seizures and other neurological disorders shows increase in the level of LDH in their CSF (Nelson *et al.*, 1975.)

LDH permits organisms to generate temporary oxygen debt energy in the form of accumulated lactate which later gets discharged by re-oxidation of lactate to pyruvate when oxygen becomes available (Markert, 1984).

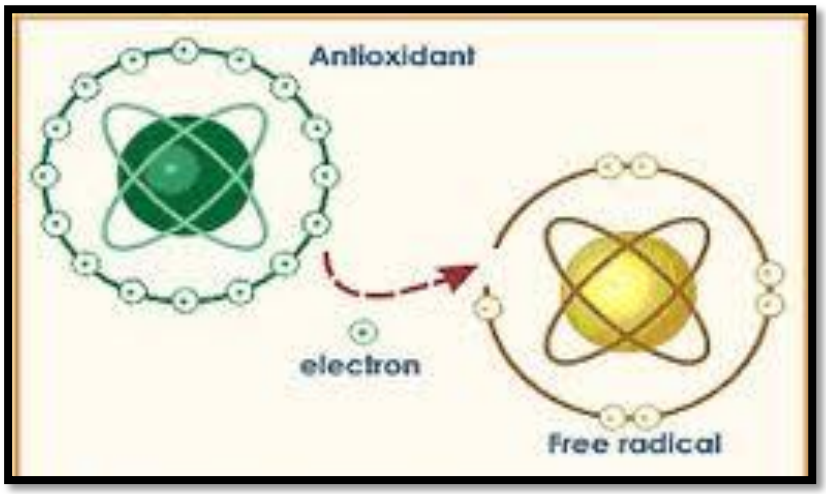
Figure 9: LDH enzymatic reaction



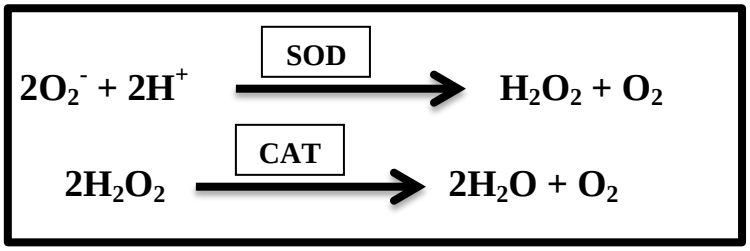
(http://academic.brooklyn.cuny.edu)

2.11 Antioxidant Enzymes

Figure 10: Antioxidant neutralizing a free radical



(http://acaiberryeducation.com)

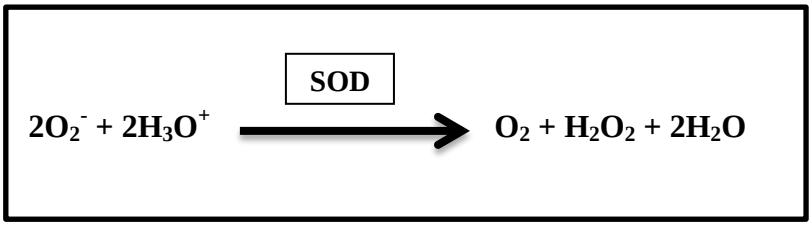


2.11.1 Superoxide Dismutase

One of the most effective intracellular enzymatic antioxidants is SOD (EC no. – 1.15.1.1). One family of antioxidant enzymes, SOD are metalloenzymes that function to remove damaging ROS from the cellular environment by catalysing the dismutation of superoxide radical into hydrogen peroxide + molecular oxygen and consequently provide an important defence mechanism against superoxide radical toxicity.

SOD exists in several isoforms, differing in the nature of active metal centre and amino acid constituency, as well as their number of subunits, cofactors and other features. Under physiological conditions, a balance exists between the levels of ROS produced during normal cellular metabolism and the level of endogenous antioxidants, which serve to protect tissues from oxidative damage.

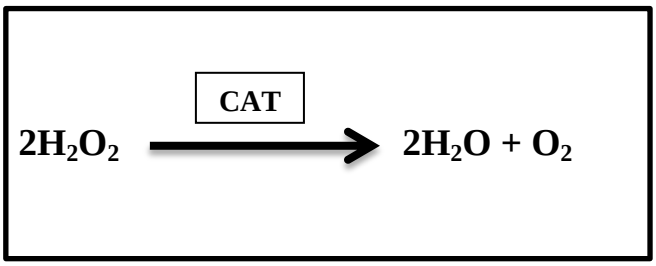
SOD exists in several isoforms, differing in the nature of active metal centre and amino acid constituency, as well as their number of subunits, cofactors and other features. Under physiological conditions, a balance exists between the levels of ROS produced during normal cellular metabolism and the level of endogenous antioxidants, which serve to protect tissues from oxidative damage.

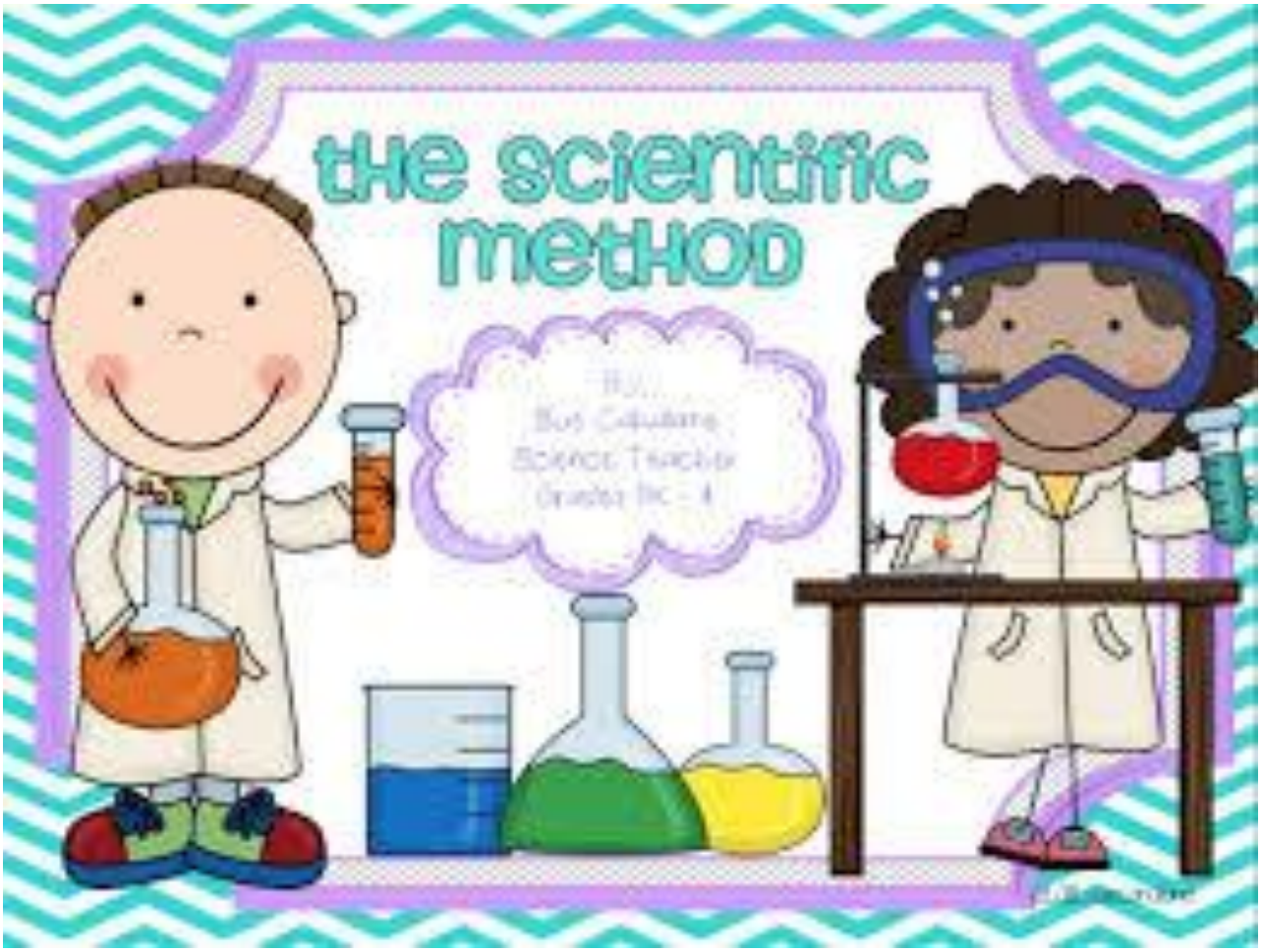


The SOD activity was estimated according to Kono, (1978) by monitoring its ability to inhibit the photochemical reduction of Nitroblue Tetrazolium (NBT) dye by superoxide radicals, which are generated by the auto oxidation of hydroxylamine hydrochloride.

2.11.2 Catalase

Oxidative metabolism and energy production in the body generate free radicals and non-radical derivatives of oxygen and of nitrogen. CAT (EC no. 1.11.1.6) catalyses the decomposition of hydrogen peroxide to oxygen and water. It is an important antioxidant which protects the cell from oxidative damage rendered by reactive oxygen species (Armogida M *et al.*, 2011). ROS are generated due to the oxidative stress. The enzyme CAT acts as a natural antioxidant to supress these ROS. In brain it acts as a powerful reactive oxygen species scavenger and protects the brain from any severe damage caused by them. The hydrogen peroxide produced by the SOD will be converted into oxygen and water molecule by the enzyme catalase in a normal condition (Waldbaum and Patel, 2010).





MATERIALS

&

METHODS

3. Materials and Methods

3.1 Chemicals

Pilocarpine (Sigma Aldrich Ltd.,), Atropine, Sodium Chloride (Merck Specialities Pvt. Ltd., Mumbai), Glucose (Glucon-D, Heinz India Pvt. Ltd.), Actrapid Human Insulin (Novo Nordisk India Pvt. Ltd., Bangalore), Glucose Estimation Kit – GOD-POD method (Lab Care Diagnostics Pvt. Ltd., Gujarat), Sucrose (Merck Specialities Pvt. Ltd., Mumbai), EGTA (Sisco Research Laboratories Pvt. Ltd., Mumbai), HEPES (Spectrochem Pvt. Ltd., Bombay), Sodium Phosphate Monobasic Anhydrous (Sisco Research Laboratories Pvt. Ltd., Mumbai), Di - Sodium Hydrogen Phosphate Anhydrous (Merck Specialities Pvt. Ltd., Mumbai), NADH (Sisco Research Laboratories Pvt. Ltd., Mumbai), Sodium Pyruvate (S. D. Fine – Chem Ltd., Mumbai), Hydrogen Peroxide (Merck Specialities Pvt. Ltd., Mumbai), BSA (Central Drug House Pvt. Ltd., New Delhi), Sodium Carbonate (Merck Specialities Pvt. Ltd., Mumbai), Copper Sulphate (S. D. Fine – Chem Ltd., Mumbai), Sodium Potassium Tartarate (Finar Chemicals India Pvt. Ltd., Ahmedabad), Hydrochloric acid, Hydroxylamine HCl, Triton-X 100, NBT (Nitro blue tetrazolium) (Hi Media , Mumbai) ,Sodium Hydroxide Pellets (Merck Specialities Pvt. Ltd., Mumbai), Folin Ciocalteu Reagent (Merck Specialities Pvt. Ltd., Mumbai), Tri Reagent (Sigma Aldrich Ltd.), cDNA synthesis kit (Thermo Scientific), Primers (Eurofins), PCR kit (Thermo scientific), Agar Agar (Hi-Media, Mumbai), Sodium Hypochlorite (Merck Specialities Pvt. Ltd., Mumbai), Chloroform, Isopropanol, Ethanol.

All Biochemicals used in the present study were of analytical grade.

3.2 Animals

Adult male Wistar rats weighing 200 – 250 g and age between 6 to 8 weeks were purchased from Haffkines Biopharmaeuticals Ltd., Mumbai and used for the experiments. They were housed in separate cages for 12 hr. light and 12 hr. dark periods and were maintained on standard food pellets and water freshly before administration of pilocarpine. Animal care and procedures were carried out following the Institutional and National Institute of Health Guidelines.

3.3 Model Induction

3.3.1 Chemical

The chemical used for the induction of TLE was pilocarpine hydrochloride. Pilocarpine is a cholinergic para sympathomimetic agent exerting a broad spectrum of pharmacologic effects with predominant muscarinic action.

3.3.2 Induction of Epilepsy

The experiment was done by dividing sets of animals in the following groups:

Table 2: Model Induction

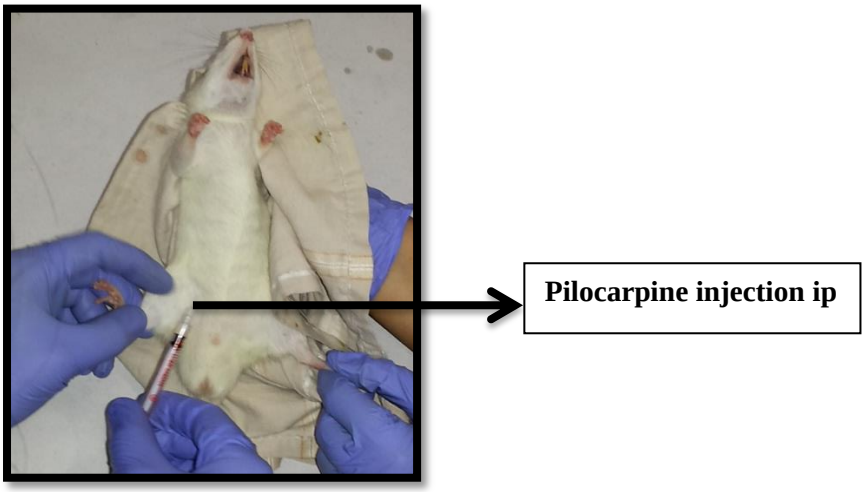
Group No.	Experimental Groups	Treatment
1	Control	-
2	Epilepsy	Pilocarpine 350mg/kg body weight by ip
On 16th Day of model induction		
3	Control + Glucose + Insulin	Glucose 500mg/ kg body weight s.c. Insulin 0.01 IU/kg body weight s.c.
4	Epilepsy + Glucose + Insulin	Pilocarpine-350mg/kg body weight by ip Glucose 500mg/ kg body weight s.c. Insulin 0.01 IU/kg body weight s.c.

(n=3 rats/group)

(Krishnakumar A. *et al.*, 2009)

Experimental rats were induced epilepsy by injecting rats with a single intra peritoneal dose of pilocarpine hydrochloride (350 mg/kg body weight ip). Control rats were not injected with any drugs. Within 20 – 40 min. of the pilocarpine injection, the rats showed the development of primary seizures. Glucose + insulin treatment was given by injecting 500 mg/kg body weight of glucose and 0.01 IU/kg body weight of insulin sc.

Figure 11: Intraperitoneal injection of Pilocarpine



3.4 Food – Water Intake

Food and Water intake of all the animals were monitored from a week prior to the model induction until dissection, and appropriate changes were noted.

3.5 Glucose Estimation

Blood glucose was estimated by glucose estimation kit (Lab Care Diagnostics Pvt. Ltd., Gujarat) using the Glucose Oxidase – Peroxidase (GOD-POD) method. Glucose is oxidized to gluconic acid and hydrogen peroxide in presence of GOD. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of POD to form a red coloured quinone-imine dye complex. Intensity of the colour formed is directly proportional to the amount of glucose concentration measured at 505 nm.

1. Glucose $\xrightarrow{\text{GOD}}$ Gluconic acid + H_2O_2

2. H_2O_2 + Phenol + 4-AAP $\xrightarrow{\text{POD}}$ Red Complex + Water + O_2

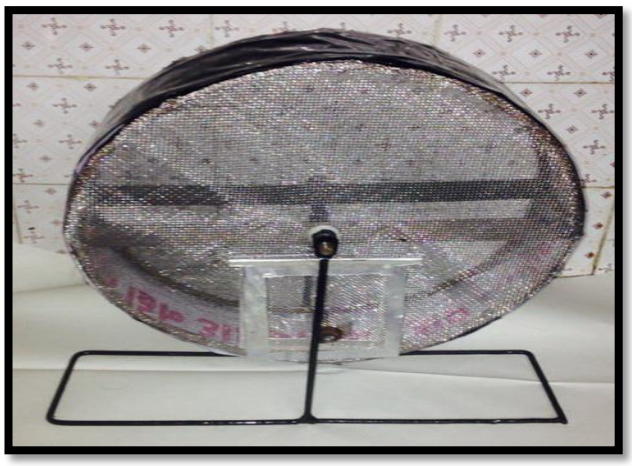
3.6. Behavioural Study

Behavioural studies were carried out for all the four experimental groups of rats.

3.6.1Wheel Running Test

Wheel running test were performed to assess the motor activity. First all the rats were trained for 2 days so that they get familiar with wheel. After training they were kept 3 minutes in the wheel for test. Rotations of wheel, raising attempts, and immobile period were recorded, and speed was calculated.

Figure 12: Model for Wheel Running Test



3.6.2 Staircase Test

3.6.2.1 Instrumentation

A staircase of wooden ply was built with the dimension as follows

Height -5.5cm

Width -6.5cm

Length –8.5cm

Number of stairs – 12

Figure 13: Model for Staircase Test

The Staircase test assesses forelimb extension, grasping skills, side bias and independent use of forelimbs. The staircase test was developed to assess the independent use of forelimbs of rats and was later adopted to assess skilled reaching in rats. This test allows for the bilateral measurement of an animal's forelimb extension, its grasping skills and side bias by observing its behaviour in the given task. Advantage of this task is the ability to evaluate skilled use of the limbs independently following the impairment caused by hypoxia and ischemia. Training of a few weeks is generally required to teach the animals (Schaar *et al.*, 2010)

3.6.2.2 Procedure

Rats were trained over the span of three days and then from the other day actual readings were taken where the motor ability of the individual rats were accessed based on the parameter given as follows:

Immobile period

Time taken to reach the last staircase

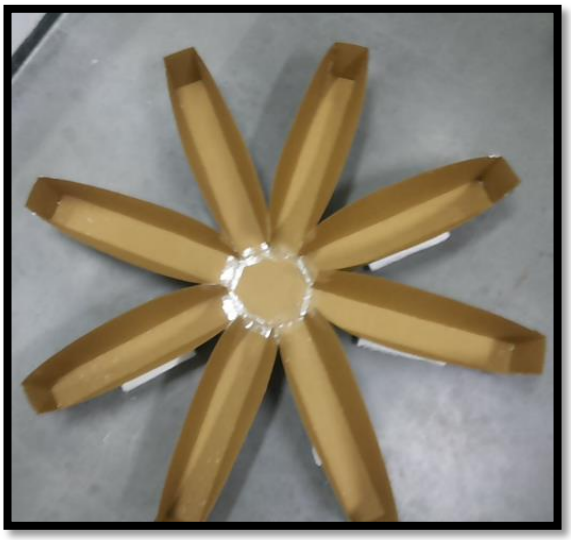
Grooming attempts

Raising attempts

3.6.3 Radial Arm Maze Test:

The Radial Arm Maze (RAM) Test exercise can facilitate acquisition of hippocampal – related spatial learning and short-term memory (Anderson *et al.*, 2000). In RAM, 8 arms were arranged radially around a central platform. The rat, that is to be subjected to test, was placed on the central platform and was allowed to move freely in the arms. 2 of the 8 arms are to be supplemented with some food that can act as a trigger. And the frequency of visiting any arm in given 5 min. of time was noted. And lastly a record of the rearing attempts of the experimental group of animals was noted.

Figure 14: Model for Radial Arm Maze Test



3.6.3.1 Dimensions

Base Platform diameter: 76 cm
Width of Base of each Arm: 9 cm
Length of each Arm: 58 cm
Height of each Arm: 20 cm

3.6.3.2 Fabrication

The apparatus of the RAM was designed from cardboard and thermocol sheets. The arms of the maze and the central platform were designed from the cardboard sheet, and these arms were provided support with a thermocol base. 8 arms of the above mentioned dimensions were attached to the central platform in a circular manner without leaving any space in between two arms, and were assembled. The dimensions of the maze were decided according to the protocol mentioned by Anderson *et al.*, 2000).

3.6.3.3 Procedure

This test was carried out in two phases with all the experimental group of rats:

i. Training Phase
ii. Test Phase

For the training phase, the rats were trained in the maze for a period of a week and during this training period all the armz except the stimulus arm were blocked.

During the test phase, all the blocked arms were opened, that means all the 8 arms are accessible to the rat, but the food stimulus was placed in only 2 arms, and it was tested whether the rat recognizes the arm with the food stimulus and the frequency of the rat visiting that arm was noted. Also, the rearing attempts made by the rats were noted.

3.6.4 Novel Object Recognition Test

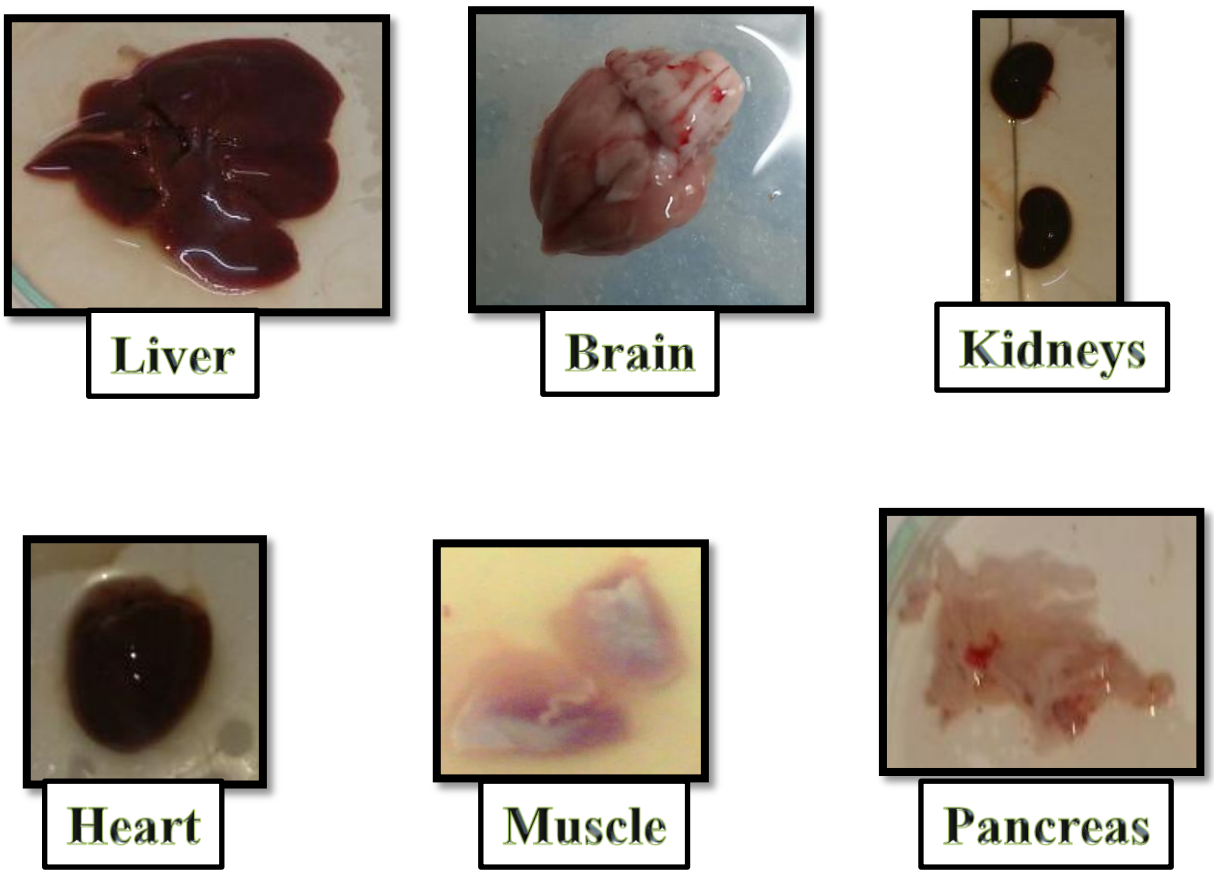
NOR test was performed to assess the cognition. No training was provided prior to the study. All the experimental groups of rats were kept 3 minutes in novelty environment. Raising attempts, climbing attempts, and immobile period, sniffing of object were recorded. The number of novel objects recognized and interactions with them were also recorded.

. **Figure 15: Model for Novel Object Recognition Test**

3.7 Dissection and Tissue Preparation

Rats were sacrificed by cervical dislocation on the 65th day of the experiment. The hypothalamus, cerebellum, brainstem, cerebral cortex and corpus striatum, spinal cord from brain and liver, kidney, pancreas, heart and muscle from body were dissected out quickly over ice according to the procedure of Glowinski and Iversen, 1966.The tissues were stored at -80ºC until assay.

Figure 16: Tissues



3.8 Enzyme Assays

3.8.1 Lactate Dehydrogenase

LDH enzyme assay was done from skeletal muscle, heart using a spectrophotometric method.

3.8.1.1 Preparation of Homogenate

5% homogenate of the required tissues (skeletal muscle, heart) was prepared using a homogenizer with an isolation medium containing 250 mM Sucrose, 10mM HEPES Buffer and 1 mM EGTA (pH – 7.2), according to the protocol of Ling *et al.*, 2012.

3.8.1.2 Mitochondrial Isolation

The prepared homogenate was the centrifuged at 1000 rpm for 8 min at 4ºC. The supernatant obtained, was collected and centrifuged at 10,000 rpm for 10 min at 4ºC. The pellet obtained here is the mitochondrial pellet, which is collected and washed twice with the washing medium containing 250 mM Sucrose, 10 mM HEPES and 0.1 mM EGTA. After this, the final pellets of mitochondria were suspended in 2 ml of isolation medium without EGTA i.e. the suspension medium (Ling *et al.*, 2012).

3.8.1.3 Assay Mixture

The assay was performed according to the protocol of Plummer, 2005. The assay was performed in a 1 ml reaction mixture containing varying concentrations of substrate sodium pyruvate, ranging from 150µM to 300µM, 0.1 mM NADH, 100µl of enzyme and 0.1 M sodium phosphate buffer, pH 7.4 to make up the volume to 1.0 ml. Here NADH acts as the reducing agent, which reduces the substrate pyruvate to produce lactate. The utilization of enzyme in making up the enzyme – substrate complex, and thus the product is noted by taking the change in absorbance at 340 nm, by using Agilent Technologies Ltd. UV – Visible spectrophotometer.

3.8.2 Superoxide Dismutase

SOD enzyme assay was done from liver, skeletal muscle using a spectrophotometric method.

3.8.2.1 Preparation of Homogenate

10% tissue homogenate was prepared following in 0.1M Na-PO_4 pH 7.4 by the method of Kono *et al.*, (1997).

3.8.2.2 Cytosolic Isolation

The homogenenate was centrifuged at 1000 rpm for 10 min. at 4ºC. Supernatant was collected. Collected supernatant was centrifuged at 10,000 rpm for 20 min. at 4ºC. The obtained supernatant was used to determine enzyme activity.

3.8.2.3 Assay Mixture

The reaction mixture contains Na-carbonate buffer (50 mM, pH 10) NBT (200 µmole) and Triton X 100 (0.6%). The reaction was initiated by addition of hydroxylamine-HCl (20 mM, pH 6.0) and different concentration of enzymes (10-50µl). Change in absorbance was measured at 560nm. Optical density was measured at 10 seconds intervals for 1 minute at room temperature.

3.8.3 Catalase

CAT enzyme assay was done from liver, skeletal muscle using a spectrophotometric method.

3.8.3.1 Preparation of Homogenate

10% homogenate of the required tissues (liver, skeletal muscle) was prepared using a homogenizer preceding a wash with 250 mM Sucrose followed by homogenization with an Isolation medium containing 0.1 M Sodium Phosphate Buffer, pH 7.0, according to the protocol of Wielosz *et al.,* 2003.

3.8.3.2 Cytosolic Isolation

The prepared homogenate was the centrifuged at 1000 rpm for 10 min at 4ºC. The supernatant obtained, was collected and centrifuged at 10,000 rpm for 20 min at 4ºC. The supernatant obtained is used for determining enzyme activity.

3.8.3.3 Assay Mixture

The assay was performed in a 1 ml reaction mixture containing varying concentrations of enzyme, ranging from 5µl to 50µl, 0.5% substrate H_2O_2, 0.1 M sodium phosphate buffer, pH 7.4 to make up the volume to 1.0 ml. The utilization of enzyme in breaking the substrate to its by-product H_2O and O_2 was noted and by taking the change in

absorbance of H_2O_2 at 240 nm, by using Agilent Technologies Ltd. UV – Visible spectrophotometer.

3.8.4 Determination of Enzyme Activity:

Activity of all the three enzymes – LDH, SOD and CAT, was expressed in terms of Specific Activity, represented as Enzyme Units (EU)/mg protein. Kinetic parameters - Km and Vmax were calculated from the data.

3.8.5 Determination of Protein Concentrations:

Protein concentration, in the sample tissue homogenate was measured by using BSA as standard, by Folin Lowry's method for protein estimation (Lowry et.al., 1951).

3.9 Gene Expression

3.9.1 Total RNA Isolation

Total RNA was isolated using Tri reagent from the cerebral cortex of the control and experimental group of rats' brain. RNA purity was checked at 260/280 nm in Agilent Technologies Ltd. UV – Visible spectrophotometer.

Isolated RNA was observed on an agarose gel using agarose gel electrophoresis.

3.9.2 cDNA synthesis

cDNA synthesis was performed from the total isolated RNA by using Thermo Scientific's cDNA synthesis kit.

3.9.3 PCR Amplification of HIF-1-α

PCR amplification was performed with gene specific primers. Gene specific primers were designed using IDT (Integrated DNA Technologies) and NCBI's BLAST softwares.

Amplified PCR product was observed on agarose gel using agarose gel electrophoresis.

Table 3: HIF-1-α gene specific primers

Primers	Forward	Reverse	Product Length
HIF-1-α	3' GACCCAGCTGTTCACTAAAG 5'	3' GAGTTTCAGAGGCAGGTAATG 5'	260 bp

Table 4: PCR Thermo Profile

Steps	Temperature	Time
Initial Denaturation	**94º**	**3 min**
Denaturation	**94º**	**30 sec.**
Annealing	**52.9 º**	**45 sec.**
Amplification	**72 º**	**45 sec.**
Final Extension	**72 º**	**10 min.**

3.10 Statistical Analysis

Statistical Evaluations were done by ANOVA by using Ms Excel (2010) software.

RESULTS & DISCUSSION

4. Results and Discussion

4.1 Changes in behaviour post Model Induction

Following model induction with pilocarpine, immediate behavioural changes were observed in rats. Initially, upon injecting pilocarpine, chewing, behaviour was observed. Immobility followed and was accompanied with hyper-salivation, excessive urination and excessive defecation, which lasted for about 50 to 60 min. After about 60 to 65 min, the effect of pilocarpine started decreasing. Creep walking began followed by head nodding. After about 70 to 75 min of drug administration, the abnormal behaviour decreased, and gradually exploration behaviour initiated. After about 1.5 to 2 hrs, the behaviour started to become normal and increased exploration behaviour, sniffing and grooming behaviour prevailed. Behavioural changes that were observed immediately following Pilocarpine induction were scored based on the Racine scale of seizure classification and the following postural changes were also observed post-induction.

4.1.1 Racine Scale for Seizure Classification

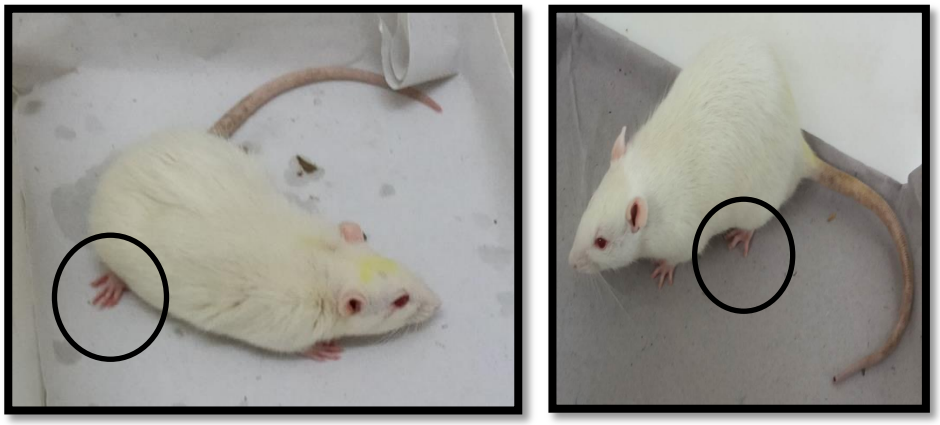
Table 5: Racine and Pinel – Rovner Beahavioural Scale

Racine Behavioural Scale	Stage	Behavioural Alterations
	1	Oro-Alimentary Movement or Mouth and Facial Movement
	2	Head nodding
	3	Fore limb clonus
	4	Dorsal extension or Rearing with Forelimb Clonus
	5	Repeated falling or Rearing and Falling with Fore Limb Clonus
Pinel and Rovner Behavioural Scale	6	Repeated falling
	7	Violent Jumping and Running
	8	Stage 7 with Tonic period

(Cordiero *et al.*, 2009)

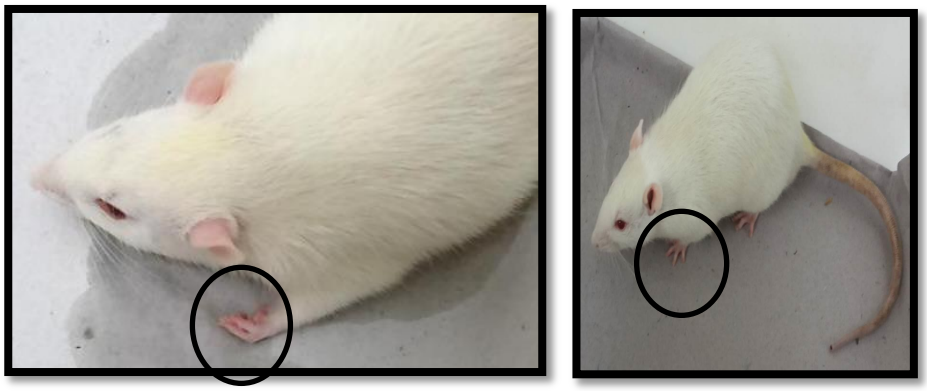
4.1.2 Postural changes observed Post Model Induction

Figure 17: Hind Limb Clonus



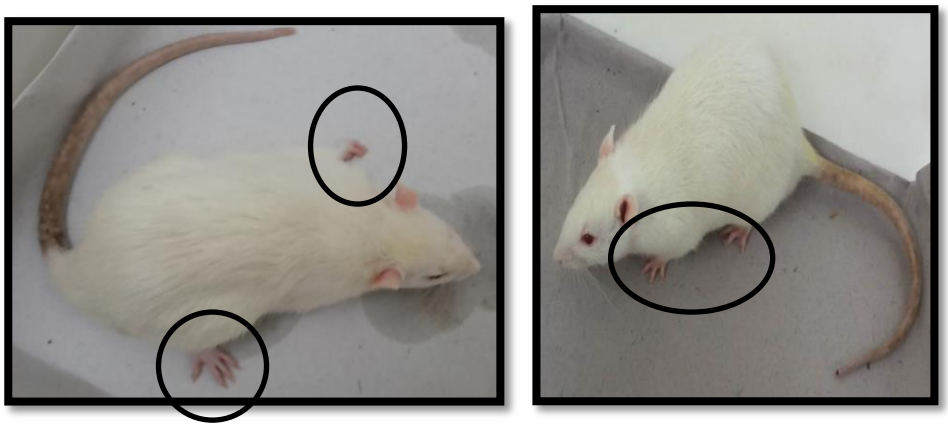
Epileptic Rat **Control Rat**

Figure 18: Fore Limb Clonus



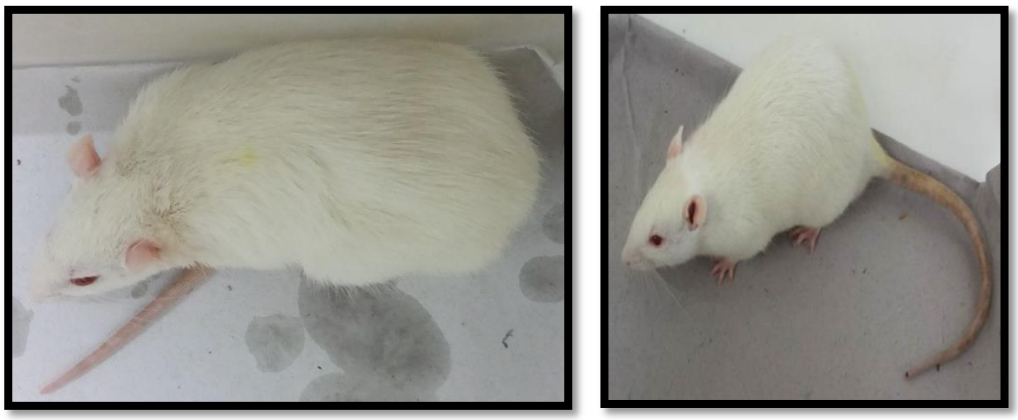
Epileptic Rat **Control Rat**

Figure 19: Complete Clonus



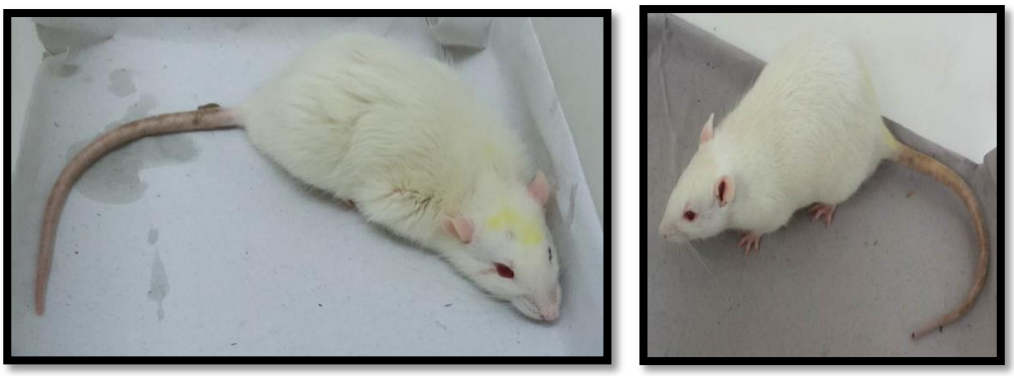
Epileptic Rat **Control Rat**

Figure 20: Hunch Back



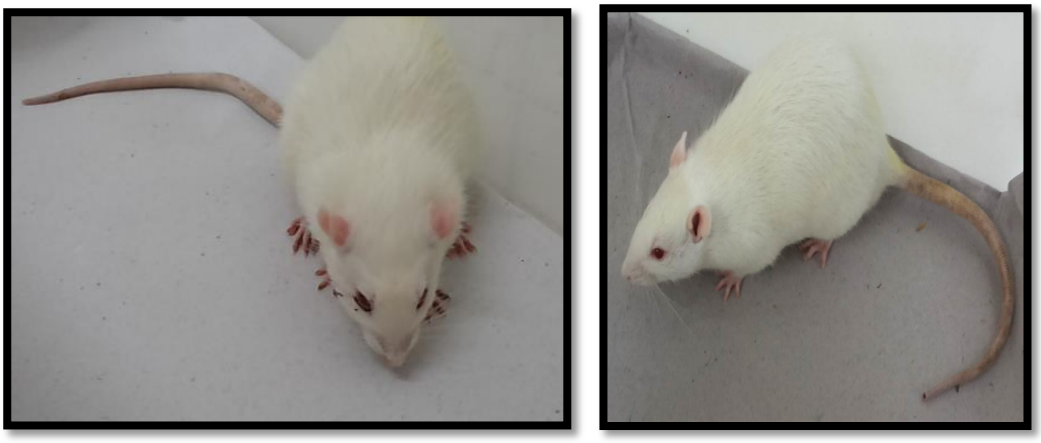
Epileptic Rat **Control Rat**

Figure 21: Immobility



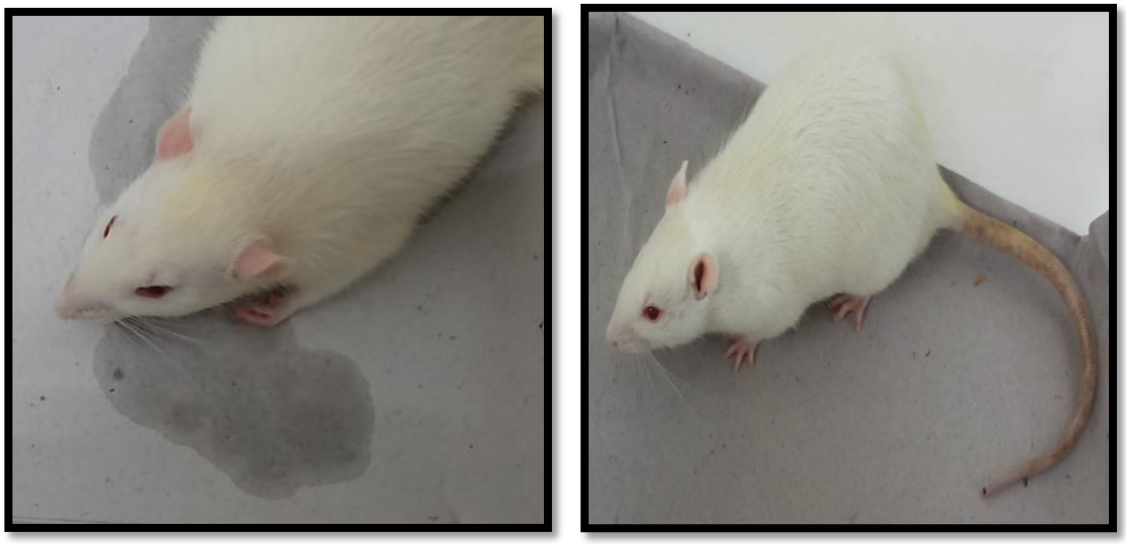
Epileptic Rat **Control Rat**

Figure 22: Sniffing



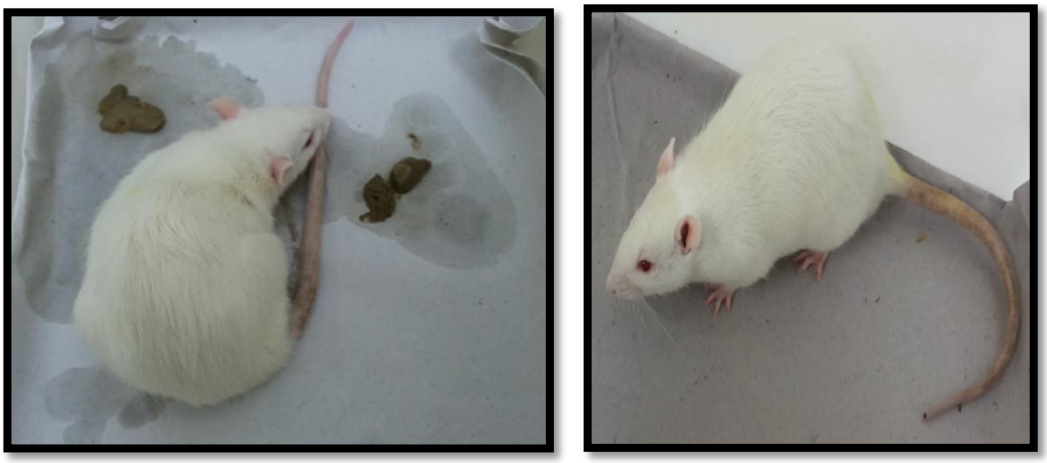
Epileptic Rat **Control Rat**

Figure 23: Excessive Salivation



Epileptic Rat **Control Rat**

Figure 24: Excessive Urination and Defecation

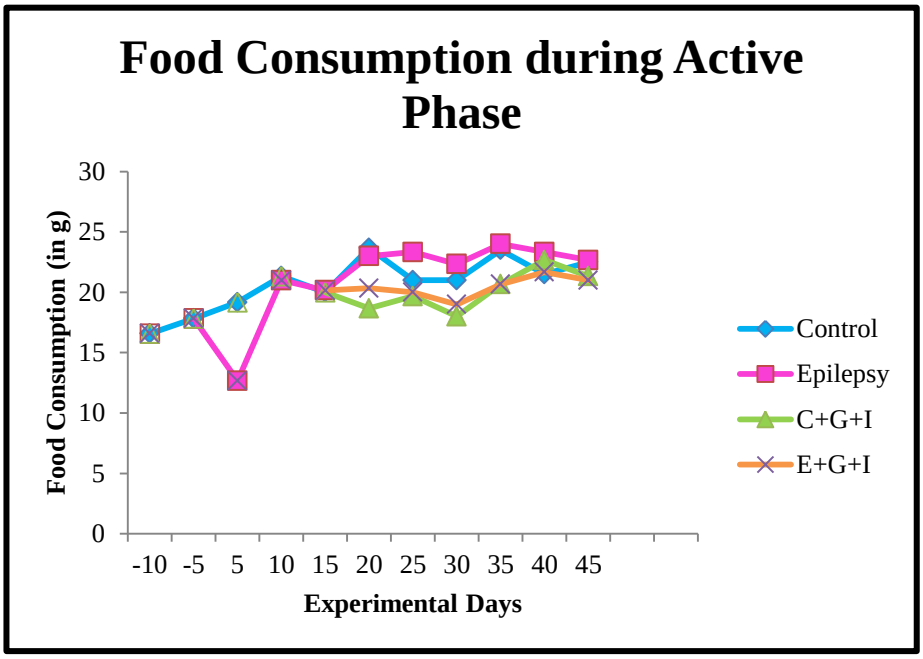


Epileptic Rat **Control Rat**

4.2 Food Consumption

4.2.1 Food Consumption during Active Phase

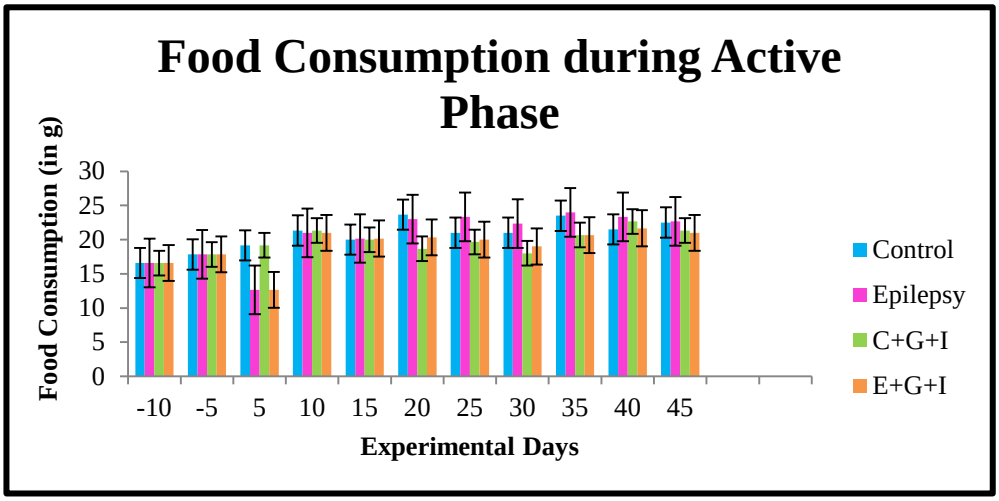
Figure 25: Food Consumption during Active phase



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Figure 26: Food Consumption during Active phase



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Table 6: Food Consumption during active phase

Experimental groups	Food Consumption during active phase (in g)
C	20.73 ± 2.22
E	20.63 ± 3.56
C+G+I	19.63 ± 1.80
E+G+I	19.17 ± 2.63

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

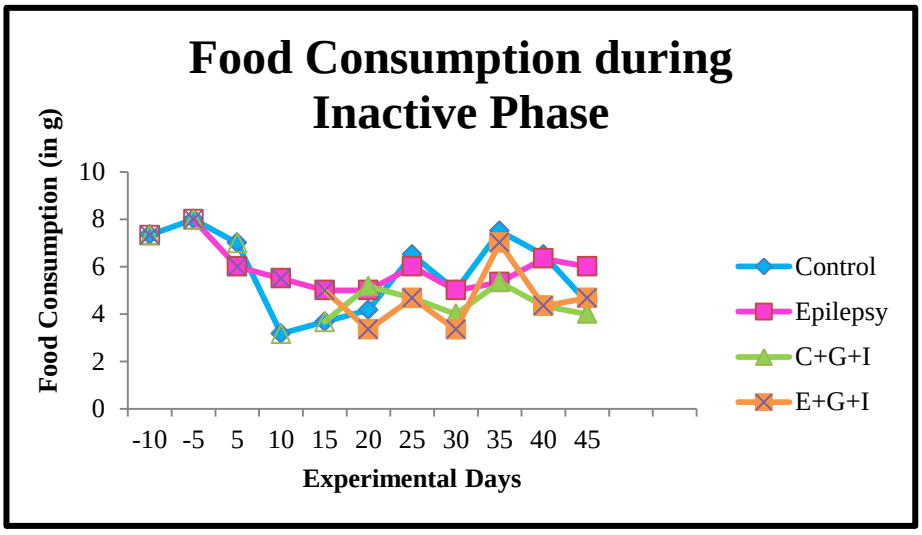
Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Discussion

Pre-induction the food consumption of all rats was almost the same. Post induction the epileptic rats showed decrease in food consumption for couple of days due to the primary seizures. After that food consumption of epileptic rats increases in comparison with control rats .After 15th day when treatment of glucose and insulin was given to rats, water consumption of E+G+I group of rats was decreased in comparison with control rats .Whereas there was no difference observed in C+G+I group of rats in comparison with control rats.

4.2.2 Food Consumption during Inactive Phase

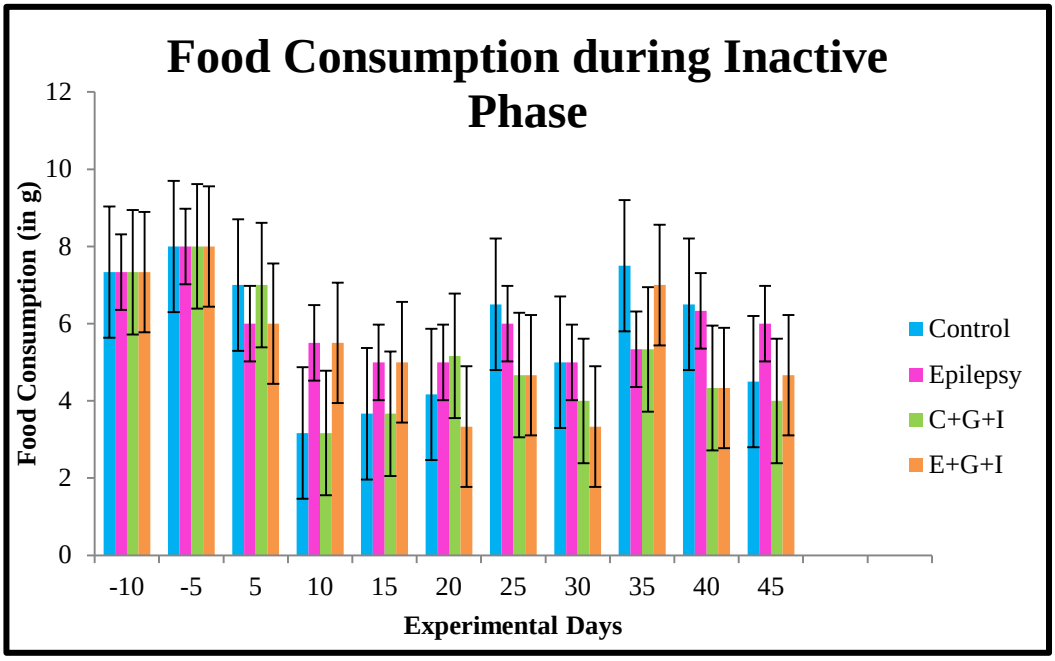
Figure 27: Food Consumption during inactive phase



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Figure 28: Food Consumption during inactive phase



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Table 7: Food Consumption during inactive phase

Experimental Groups	Food Consumption during inactive phase (in g)
C	5.76 ± 1.70
E	5.95 ± 0.98
C+G+I	5.15 ± 1.61
E+G+I	5.38 ± 1.56

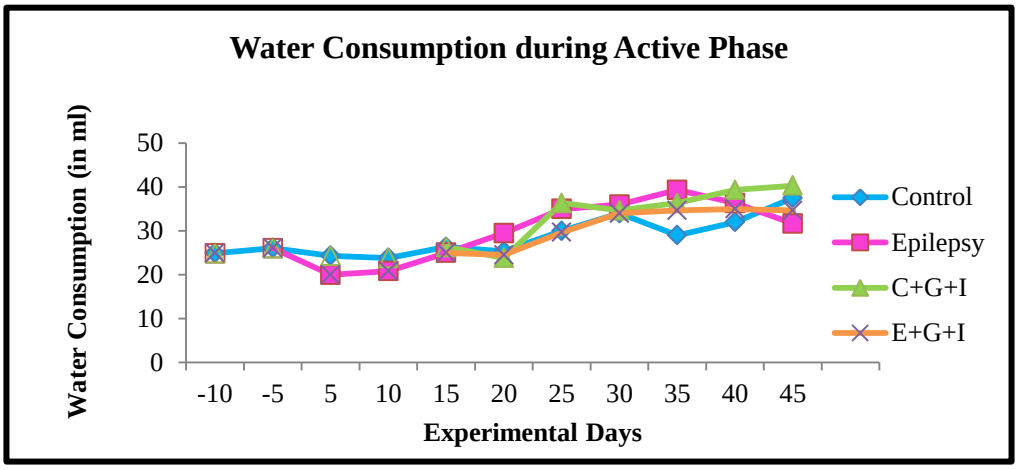
C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

4.3 Water Consumption

4.3.1 Water Consumption during Active Phase

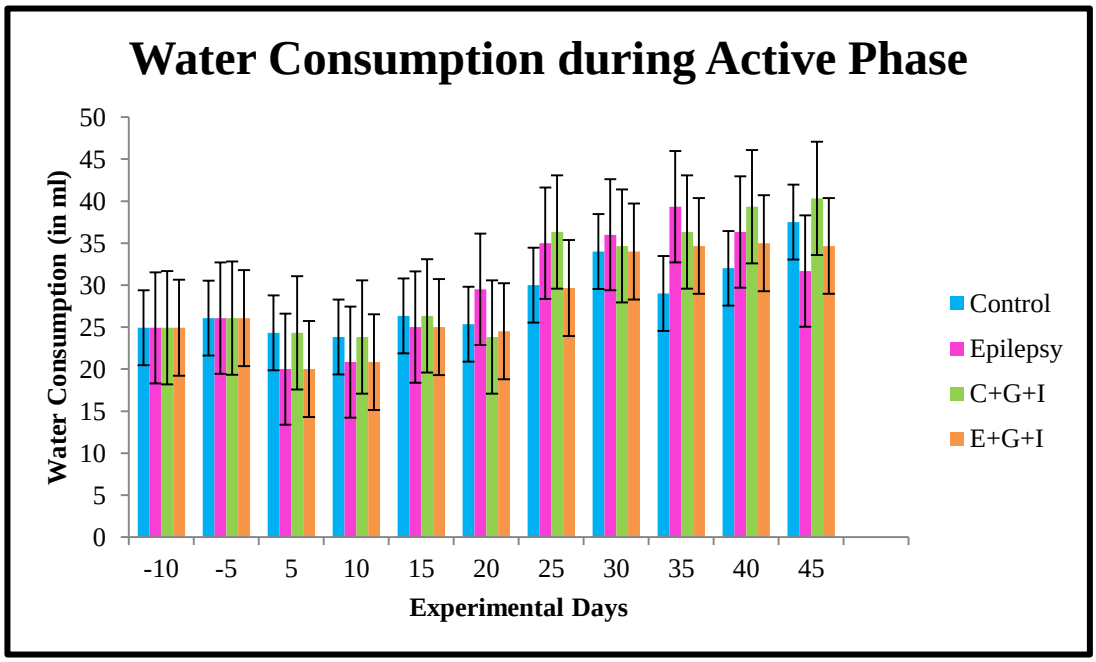
Figure 29: Water Consumption during active phase



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Figure 30: Water Consumption during active phase



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Table 8: Water Consumption during active phase

Experimental Groups	Water Consumption during active phase (in ml)
C	28.49 ± 4.46
E	29.52 ± 6.62
C+G+I	30.58 ± 6.75
E+G+I	28.12 ± 5.71

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin

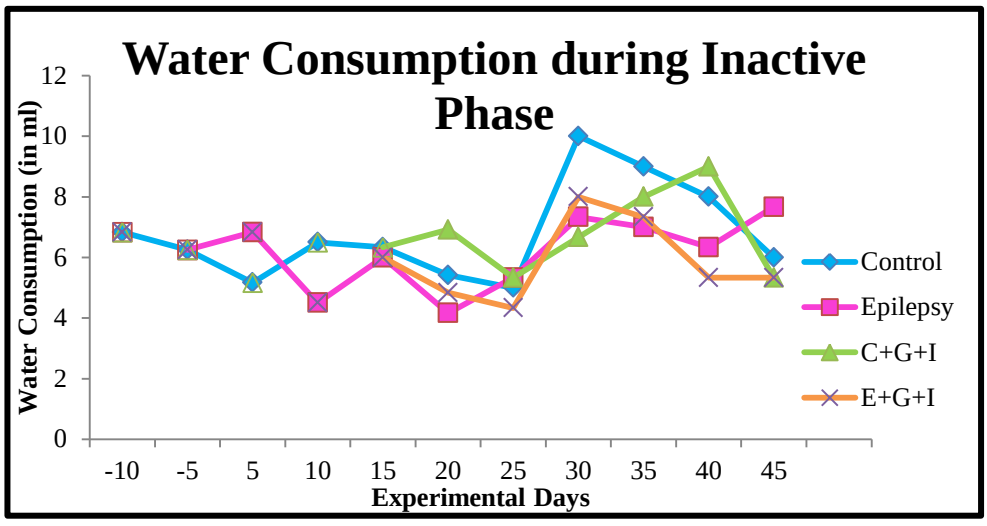
Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Discussion

In the Pre-induction phase, the water consumption of all rats was almost the same. Post induction of the epileptic rats showed decrease in water consumption for couple of days due to the primary seizures. After that water consumption of epileptic rats increases in comparison with control rats .After 15th day when treatment of glucose and insulin was given to rats, water consumption of E+G+I group of rats was decreased in comparison with C+G+I rats and epileptic group of rats showed decrease in comparison with control rats.

4.3.2 Water Consumption during Inactive Phase

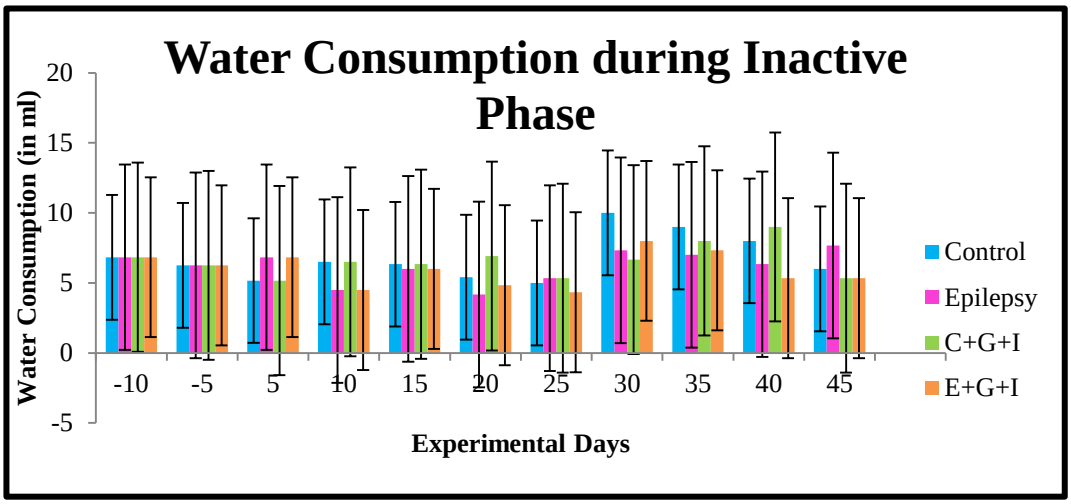
Figure 31: Water Consumption during inactive phase



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Figure 32: Water Consumption during inactive phase



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Table 9: Water Consumption during inactive phase

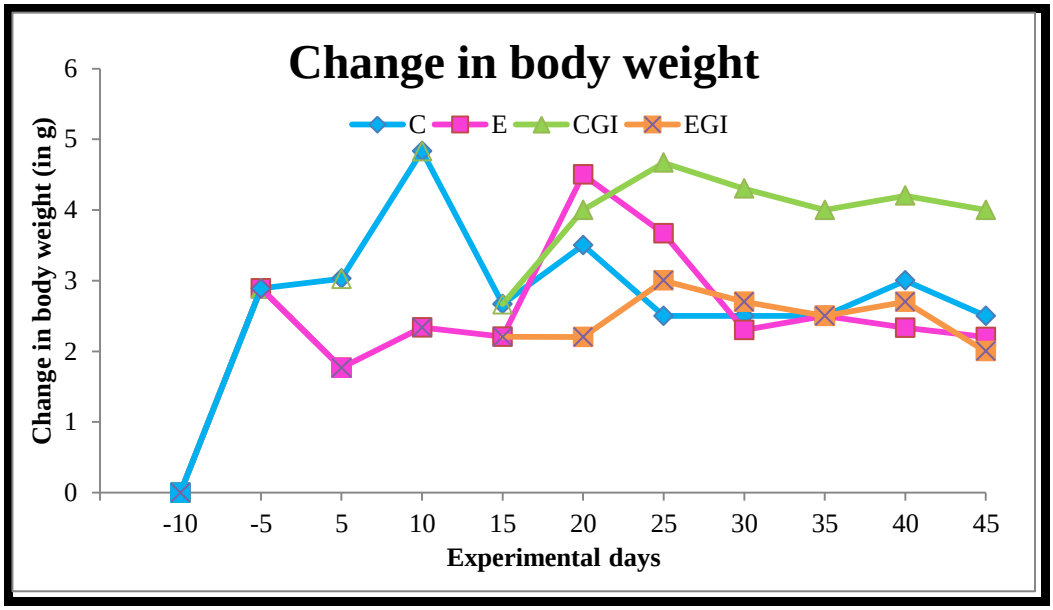
Experimental Groups	Water Consumption during inactive phase (in ml)
C	6.77 ± 1.60
E	6.21 + 1.13
C+G+I	6.58 ± 1.15
E+G+I	5.96 ± 1.203

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

4.4 Change in Body Weight

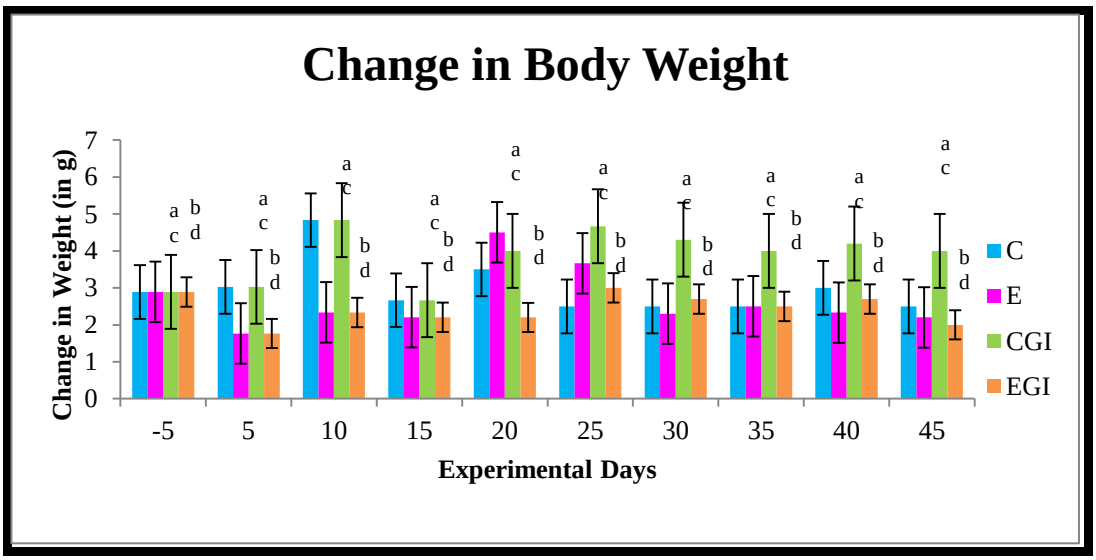
Figure 33: Total change in the body weight of the Experimental Rats



C-Control rats; **E**-Epileptic rats; **CGI -** Control rats treated with Glucose and Insulin, **EGI** - Epileptic rats treated with Glucose and Insulin

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Figure 34: Total change in the body weight of the Experimental Rats



C-Control rats; **E**-Epileptic rats; **CGI -** Control rats treated with Glucose and Insulin, **EGI** - Epileptic rats treated with Glucose and Insulin

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Table 10: Total change in the body weight of the Experimental Rats

Experimental Groups	Total change in the body weight of the Experimental Rats (in g)
C	2.71 ± 1.13
E	2.42 ± 1.11
C+G+I	3.50 ± 1.36** ##
E+G+I	2.20 ± 0.82*π π π

C-Control rats; **E-**Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

a ** P <0.01 when compare to C

b * P < 0.05 when compare to C

c ## P <0.01 when compare to E

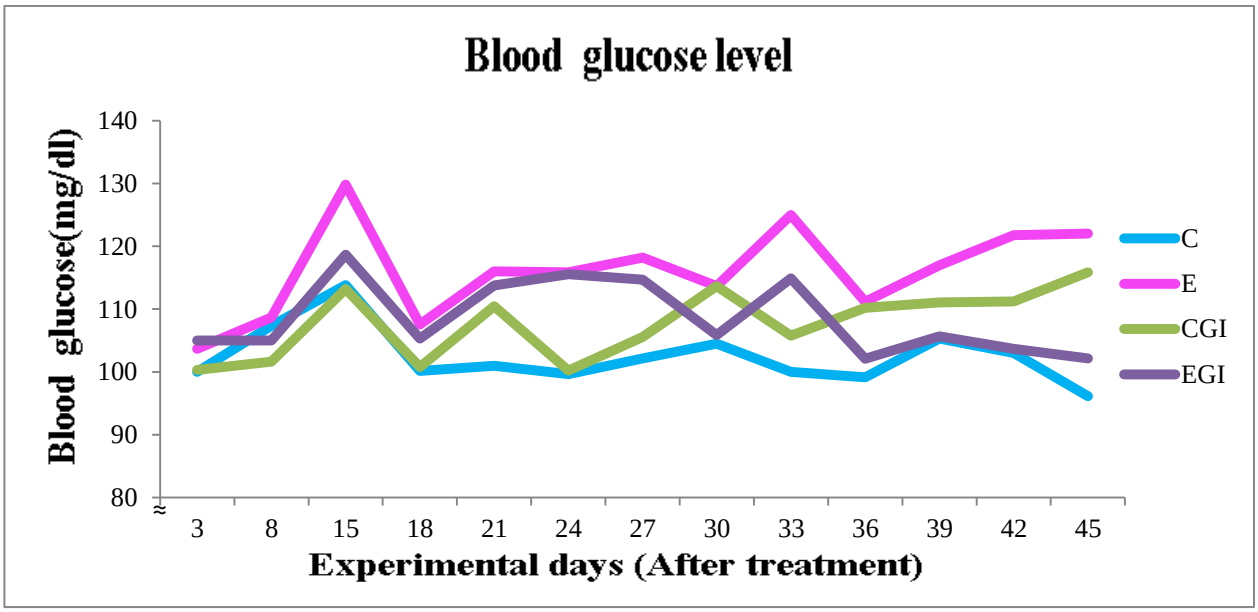
d π π π P< 0.001 when compare to C + G + I

Discussion

In pre induction phase there is a steep increase in the body weight of all the control group of rats. Post induction the rats were divided into two groups namely Epileptic and Control group of rats. Change in body weight of epileptic rats decreased for couple of days after the model induction in comparison with control rats. This is due to the primary seizure attained by them due to the pilocarpine induction. After that change in body weight of epileptic rats increases in comparison with control rats. After 15th day when treatment of glucose and insulin was given to the rats, change in body weight of E+G+I and Epileptic group of rats decreased in comparison with C+G+I group of rats. Control Group of rat does not show any difference or much more variation.

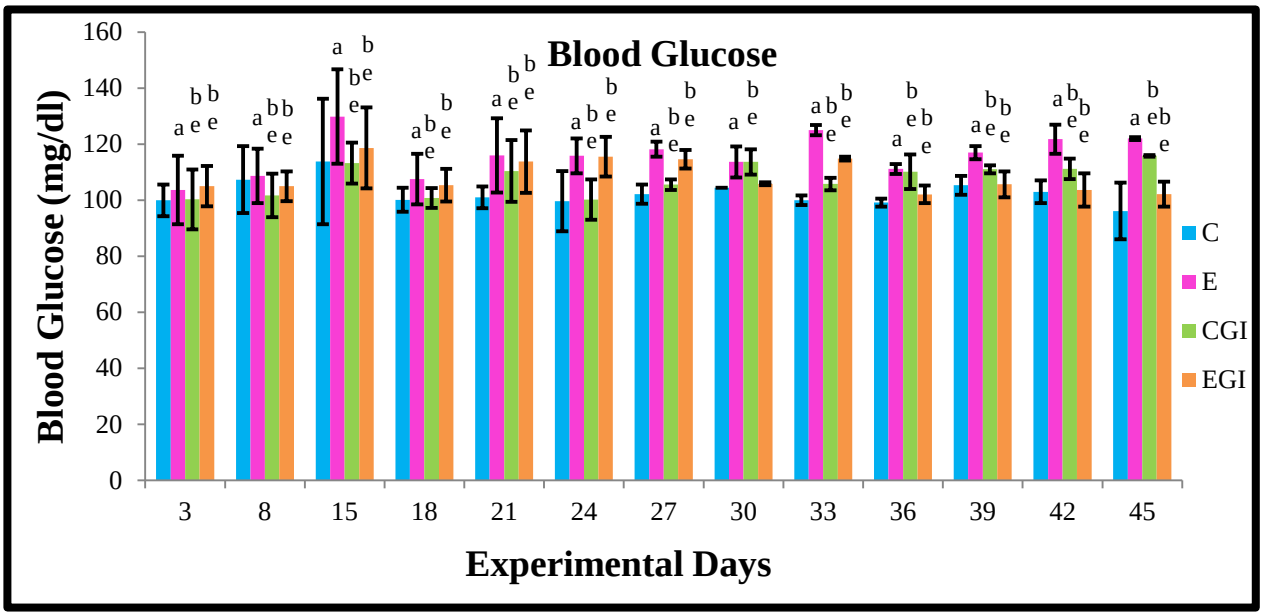
4.5 Blood Glucose Levels

Figure 35: Blood glucose level in Experimental Group of rats



C-Control rats; E-Epileptic rats; CGI - Control rats treated with Glucose and Insulin (0.01IU/kg body weight), EGI - Epileptic rats treated with Glucose and Insulin (0.01 IU/ kg body weight)

Figure 36: Blood glucose level in Experimental Group of rats



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

Table 11: Blood glucose level in Experimental Group of Rats

Experimental Days	Blood Glucose Levels (in mg/dl)			
	C	**E**	**C+G+I**	**E+G+I**
3	100 ± 5.65	103.6667 ±12.22 a	103.333±10.69 b e	105 ± 7.21 b e
8	107.3333 ± 11.93	108.6667 ±9.71 a	101.6667 7.76 b e	105 ± 5.29 be
15	113.83 ± 22.37	129.84 ± 16.85 a	113.22 ± 7.31 b e	118.67 ± 14.42 b e
18	100.17 ± 4.25	107.56 ± 9.00 a	100.78 ± 3.56 b e	105.33 ± 5.84 b e
21	101 ± 3.90	116 ± 13.29 a	110.44 ± 11.02 b e	113.78 ± 11.18 b e
24	99.67 ± 10.75	115.83 ± 6.21 a	100.22 ± 7.16 b e	115.55 ± 7.06 b e
27	102.17 ± 3.40	118.22 ± 2.67 a	105.55 ± 1.89 b e	114.67 ± 3.32 b e
30	104.5 ± 0	113.67 ± 5.50 a	113.67 ± 4.51 b e	105.89 ± 0.50 b e
33	100 ± 1.73	125 ± 1.85 a	105.78 ± 2.26 b e	114.89 ± 0.69 b e
36	99.17 ± 1.44	111.17 ± 1.75 a	110.22 ± 6.17 b e	102.11 ± 3.17 b e
39	105.33 ± 3.34	117 ± 2.33 a	111.06 ± 1.42 b e	105.67 ± 4.63 b e
42	103 ± 4.04	121.78 ± 5.18 a	111.22 ± 3.67 b e	103.67 ± 5.96 b e
45	96.17 ± 10.13	122 ± 0.47 a	115.83 ± 0.23 b e	102.17 ± 4.48 b e

C-Control rats; **E-**Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 to 4 separate experiment (n=3 rats/ group)

*** P < 0.001 When Compare to C = a

** P < 0.01 When Compare to C = b

* P < 0.05 When Compare to C = c

π π π P < 0.001 When Compare to C + G + I = h

P < 0.001 When Compare to E = e

P < 0.01 When Compare to E = f

# P < 0.05 When Compare to E = g

π π P< 0.01 When Compare to C + G + I = i

π P < 0.05 When Compare to C + G + I = j

Discussion

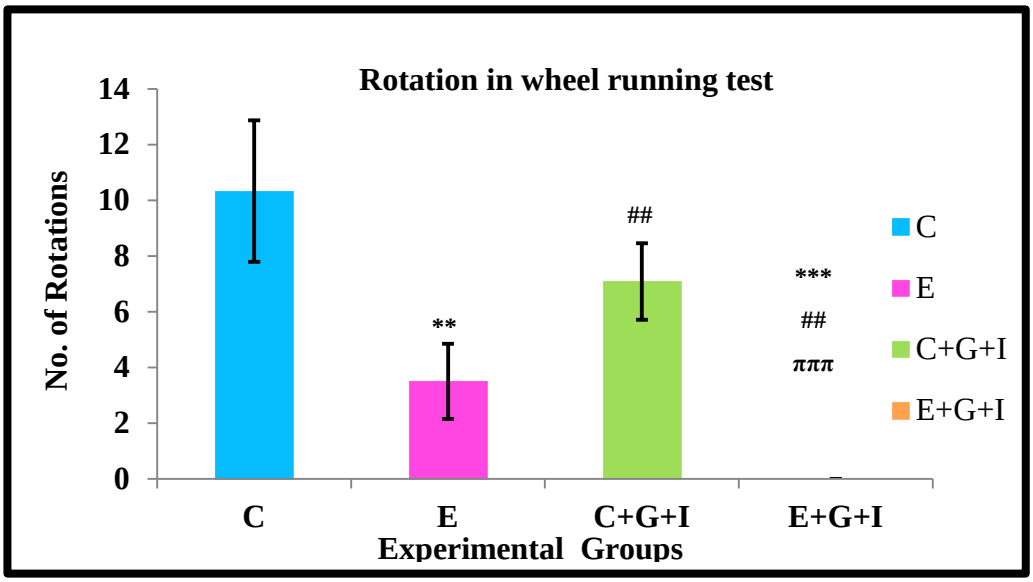
Blood glucose level of epileptic rats is significantly higher in comparison with control rats. After treatment with glucose and insulin blood glucose level of E+G+I group of rats significantly decrease in comparison with epileptic rats where as there is no significant difference between C+G+I and Control group of rats.

4.6 Behavioural Studies

4.6.1 Wheel Running Test

4.6.1.1 No. of Rotations

Figure 37: No. of Rotations in Wheel Running Test



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 12 No. of Rotations in Wheel Running Test

Experimental Groups	No. of Rotations
C	10.3333 ± 2.5
E	3.5 ± 1.3 **
C+G+I	7.0833 ± 1.4 ##
E+G+I	0 ± 0 *** ## $^{\pi\pi\pi}$

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

*** P < 0.001 When Compare to C

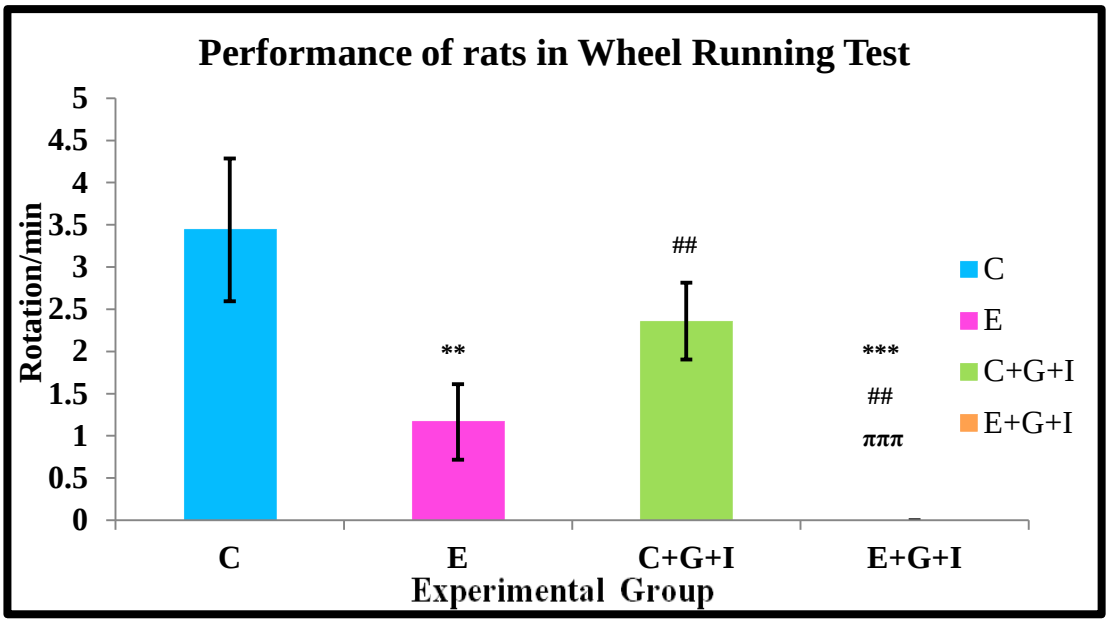
** P < 0.01 When Compare to C

P < 0.01 When Compare to E

$^{\pi\pi\pi}$ P < 0.001 When Compare to C + G + I

4.6.1.2 Performance of rats in Wheel Running Test

Figure 38: Performance of rats in Wheel Running Test



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 13: Performance of rats in Wheel running test

Experimental Groups	Rotations/min.
C	3.4408 ± 0.85
E	1.165 ± 0.45 **
C+G+I	2.3591 ± 0.46 ##
E+G+I	0 ± 0 *** ## $^{\pi\pi\pi}$

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

*** P < 0.001 When Compare to C

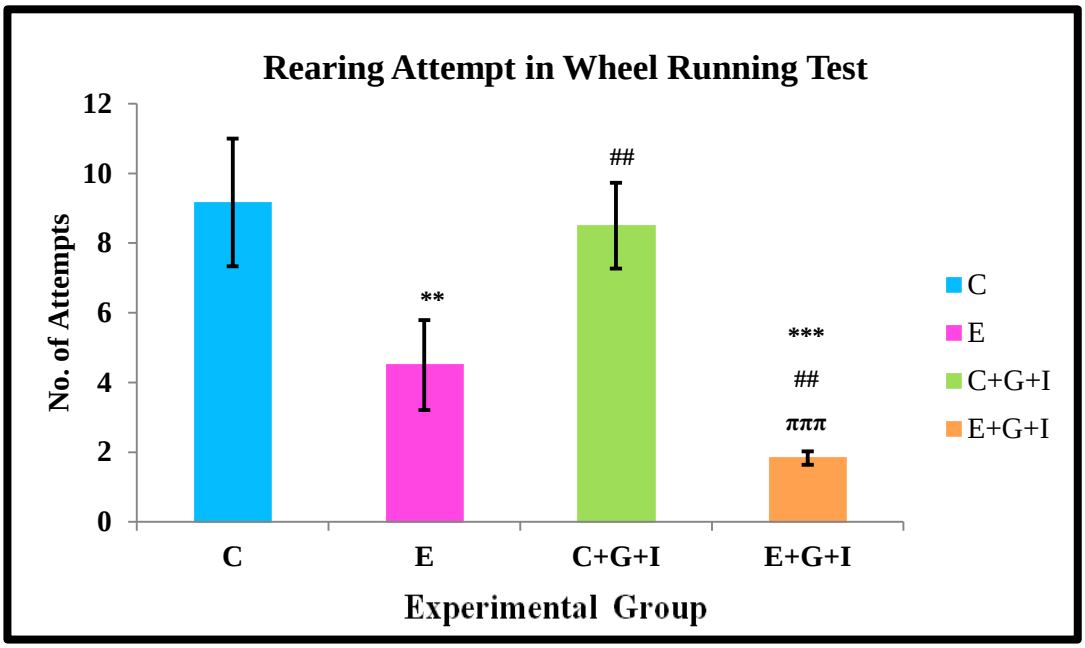
** P < 0.01 When Compare to C

P < 0.01 When Compare to E

$^{\pi\pi\pi}$ P < 0.001 When Compare to C + G + I

4.6.1.3 Rearing Attempts in Wheel Running Test

Figure 39: Rearing Attempts Wheel Running Test



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 14: Rearing Attempts of Rats

Experimental Groups	Rearing Attempts
C	3.4408 ± 1.8
E	1.165 ± 1.3 **
C+G+I	2.3591 ± 1.2 ##
E+G+I	0 ± 0 *** ## $^{\pi\pi\pi}$

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

*** P < 0.001 When Compare to C

** P < 0.01 When Compare to C

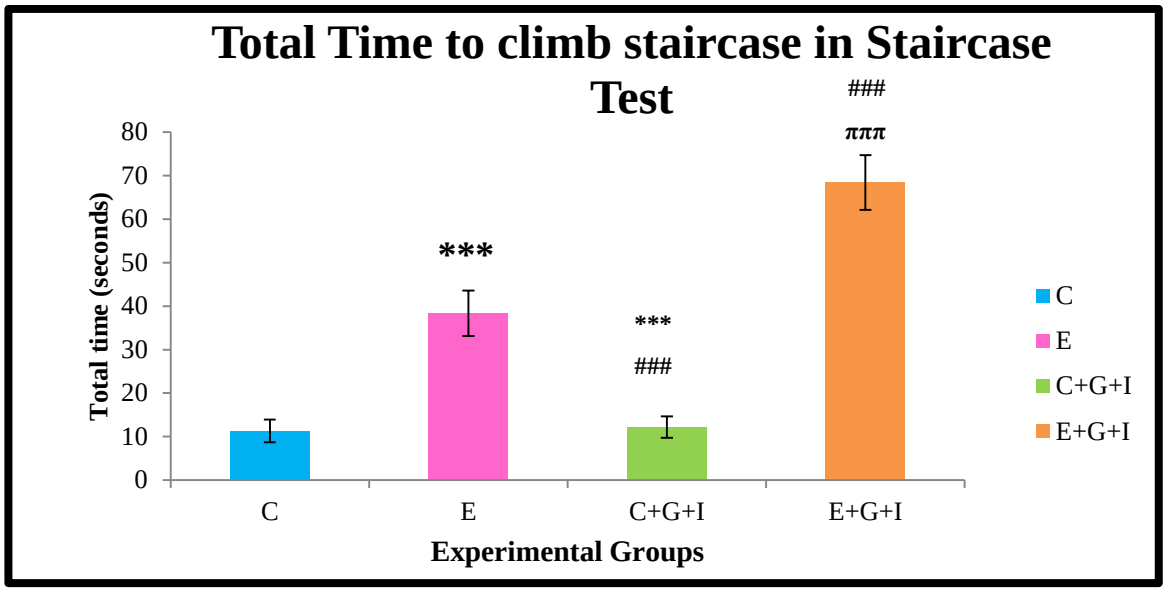
P < 0.01 When Compare to E

$^{\pi\pi\pi}$ P < 0.001 When Compare to C + G + I

4.6.2 Staircase Test

4.6.2.1 Total Time in Staircase Test

Figure 40: Total Time in Staircase Test



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 15: Total Time in Staircase Test

Experimental Groups	Total time taken (in sec.)
C	11.3± 2.61
E	38.3±5.21***
C+G+I	12.2±2.47 *** ###
E+G+I	68.4±6.28 ### $^{\pi\pi\pi}$

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

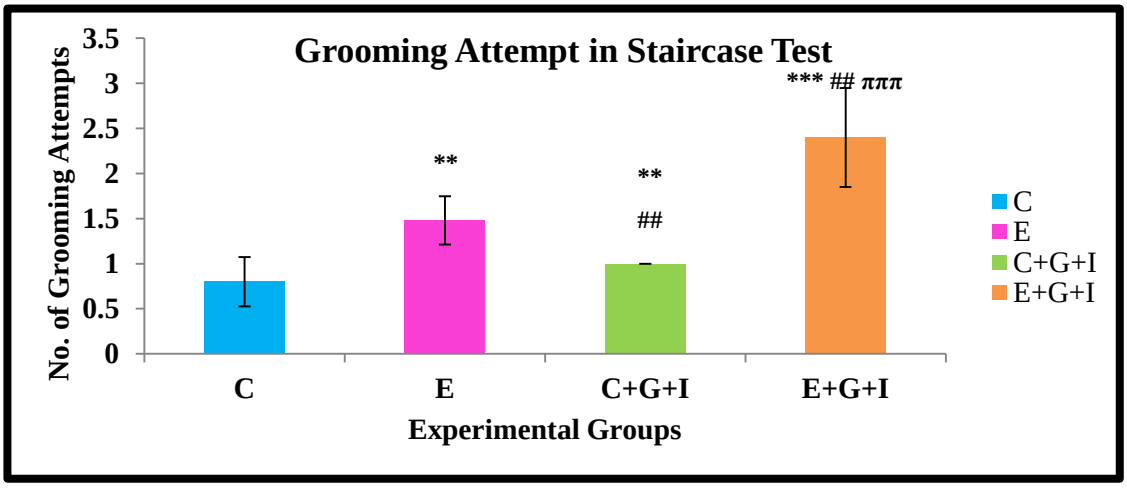
***P <0.001 when compared to C

###P <0.001 when compared to E

$^{\pi\pi\pi}$P <0.001 when compared to C + G + I

4.6.2.2 Grooming Attempts in Staircase Test

Figure 41: Grooming Attempts in Staircase Test



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 16: Grooming Attempts in Staircase Test

Experimental groups	Grooming Attempts
C	0.8±0.2
E	1.48 ± 0.26**
C+G+I	1 ± 0 ** ##
E+G+I	2.4 ± 0.54*** ## $^{\pi\pi\pi}$

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

**P<0.01 when compared to C

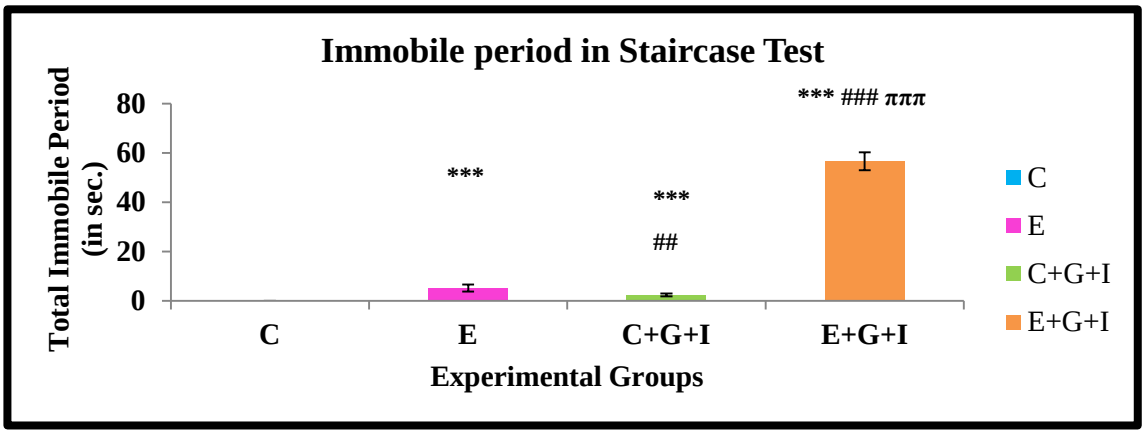
***P < 0.001 when compared to C

##P <0.01 when compared to E

$^{\pi\pi\pi}$ P<0.001 when compared to C + G + I

4.6.2.3 Immobile Period in Staircase Test

Figure 42: Immobile Period in Staircase Test



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 17: Immobile Period in Staircase Test

Experimental Groups	Immobile period (in sec.)
C	0±0
E	5.13 ± 1.48***
C+G+I	2.33 ± 0.57*** ##
E+G+I	56.6 ± 3.61*** ### $^{\pi\pi\pi}$

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

***P<0.001 when compared to C

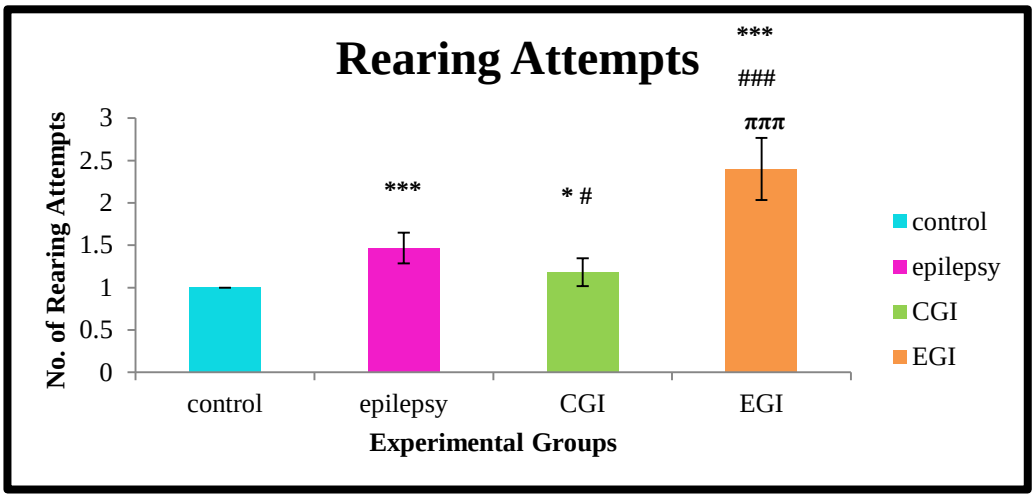
P<0.01 when compared to E

###P<0.001 when compared to E

$^{\pi\pi\pi}$ P<0.001 when compared to C + G + I

4.6.2.4 Rearing Attempts in Staircase Test

Figure 43: Rearing attempts in Staircase Test



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 18: Rearing attempts in Staircase Test

Experimental Groups	Rearing attempts
C	1 ± 0
E	1.46 ± 0.18***
C+G+I	1.18 ± 0.16* #
E+G+I	2.4 ± 0.36*** ### $^{\pi\pi\pi}$

C-Control rats; **E-**Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

***P<0.001 when compared to C

*P< 0.05 when compared to C

# P<0.05 when compared to E

P<0.001 when compared to E

$^{\pi\pi\pi}$ P<0.001 when compared to C + G + I

Discussion

The total time taken by the control group of rats to climb the stairs was significantly three fold lesser as compared to the epileptic group of rats. There is no difference in total time taken to climb the stair by the control group of rats as compared to the C + G + I. The time taken by control and C + G + I group of rats is almost the same. The total time taken by the control group of rats was significantly six folds lesser as compared to that of the E + G + I group of rat. The epileptic group of rats takes significantly more time to climb the stairs as compared to the C + G + I group of rats. The epileptic group of rats takes significantly more time to reach the top of the stair as compared to the E + G + I. When C + G + I and E + G + I were compared the C + G + I group of rats were significantly more active as compared to the E + G + I group of rats.

The grooming attempts by the control group of are significantly half fold lesser as compared to the epileptic group of rats. There is no difference in grooming attempts by the control group of rats as compared to the C + G + I .The grooming attempts by control and C + G + I group of rats is almost the same. The total grooming attempts by the E + G + I group of rats was significantly two folds higher as compared to that of the control group of

rat. The epileptic group of rats show significantly higher tendency to perform the grooming attempts as compared to the C + G + I group of rats. The E + G + I group of rats shows significantly two folds more grooming attempts as compared to the epileptic group of rats. When C + G + I and E + G + I were compared the C + G + I group of rats were significantly more active as compared to the E + G + I group of rats because they have significantly low grooming attempts.

The immobile period by the control group of rats was significantly less as compared to the epileptic, C + G + I and E + G + I by five, two and fifty six folds. Thus the control group of rats are most active in comparision with the other group of rats. The epileptic group of rats have significantly two folds less immobile period as compared to the C + G + I. The epileptic group of rats have significantly eleven folds less immobile period as compared to the E + G + I group of rats. The C + G + I group of rats have significantly twenty eight folds less immobile period as compared to the E + G + I group of rats.

The raising attempts by the control group of rats were significantly less as compared to the epileptic, C + G + I and E + G + I by 1.4, 1.18 and 2.4 folds. The epileptic group of rats have significantly 0.8 folds more immobile period as compared to the C + G + I. The epileptic group of rats have significantly 1.7 folds less immobile period as compared to the E + G + I group of rats. The C + G + I group of rats have significantly two folds less immobile period as compared to the E + G + I group of rats.

When seizures occur, every muscle will contract in the body. This is referred to as a grand mal seizure. However, involuntary muscle contractions can also occur in isolated areas of the body. Weakness can occur during seizures. The cerebral cortex controls the motor , cognition and physical movement of an individual.

The cerebral cortex is the only area from which epileptic form activity arises with any frequency. Even still, not all areas of the cerebral cortex have the same tendency to epileptic activity: most of the neocortex is relatively resistant, while the temporal lobes and frontal lobes (particularly the limbic areas) are highly susceptible. (Reeves and Swenson, 2008)

The cerebellum plays a major role in the amalgamation of sensory perception, memory consolidation, coordination, and motor control. To coordinate motor control, there are many neural pathways linking the cerebellum with the cerebral motor cortex and the spinocerebellar tract (Witter and Canto, 2013). In epilepsy there is cerebellar dysfunction due to the misfiring of the neurons. Cerebellar dysfunction is the complication

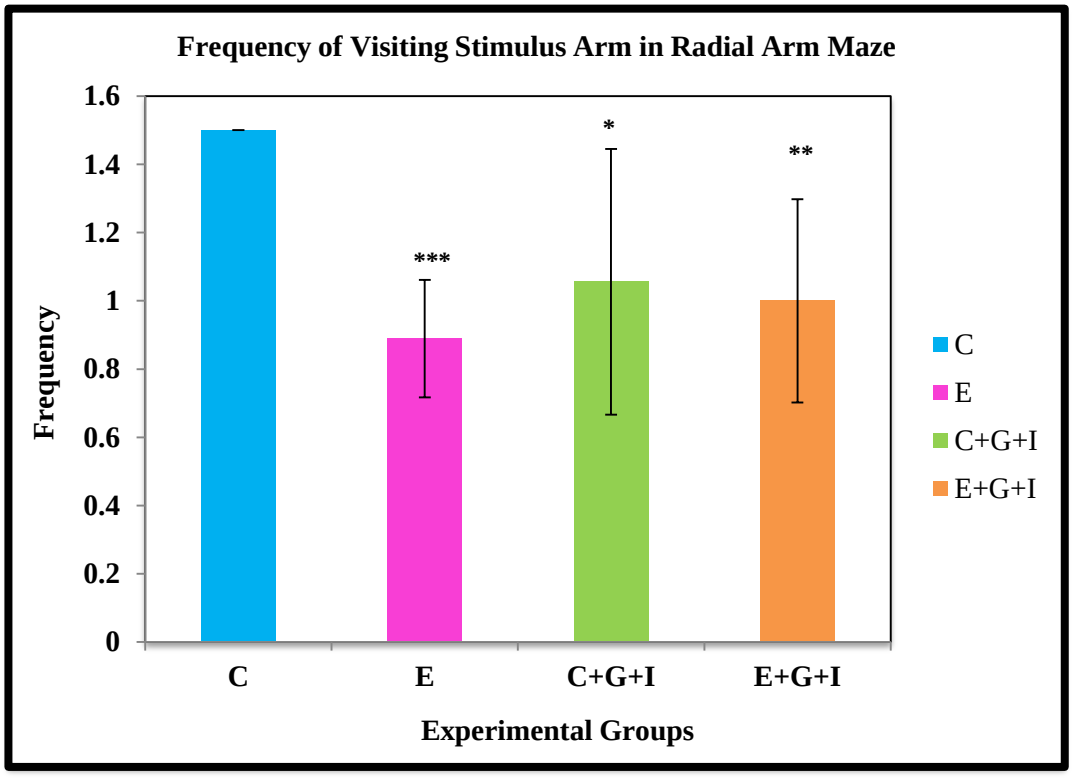
associated with the TLE and it is associated with seizure generation, motor deficits, and memory impairment.

Thus the control group of rats, free from the diseased condition are active as they climb the staircase very easily and with low grooming attempt , rearing attempt and immobile period. The epileptic rats shows high immobile period, grooming and rearing attempts . The time to climb stair was very high as compared to the control group of rats. The C + G + I group of rats actively performed the task as compared to E + G + I rats. The E + G + I and epileptic group of rats show poor performance due to the seizures and other physical stress acquired due to hypoxia.

4.6.3 Radial Arm Maze

4.6.3.1 Frequency of visiting stimulus arm

Figure 44: Frequency of visiting stimulus arm



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 19: Frequency of visiting the stimulus arm

Experimental Groups	Frequency of Visiting Stimulus Arm
C	1.5 ± 0
E	0.89 ± 0.17***
C + G + I	1.056 ± 0.39*
E + G + I	1.0 ± 0.30**

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

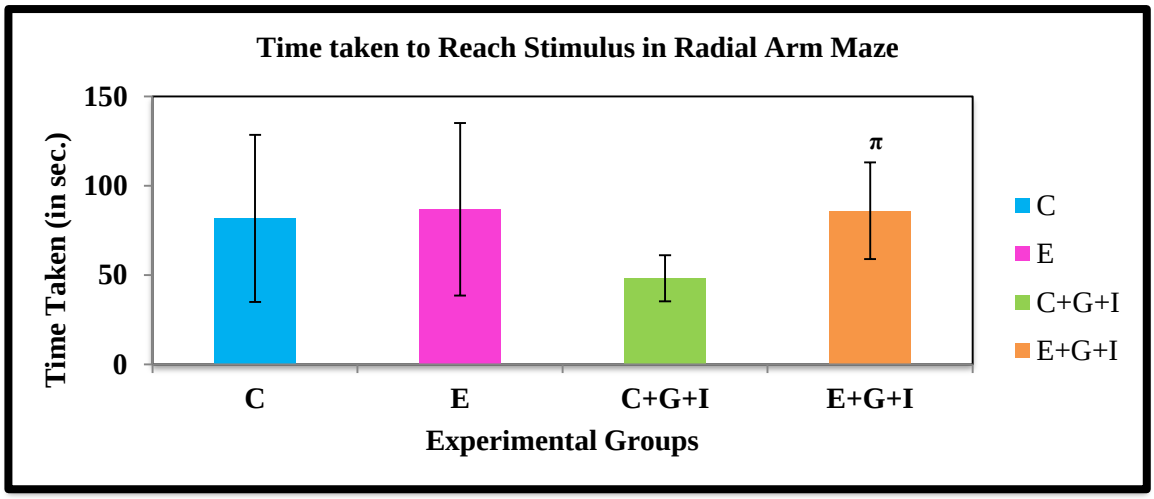
*** P<0.001 when compared to C

* P<0.05 when compared to C

** P<0.01 when compared to C

4.6.3.2 Time taken to reach stimulus arm for the 1st time

Figure 45: Time taken to reach Stimulus arm for the 1st Time



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 20: Time taken to reach Stimulus arm for the 1st Time

Experimental Groups	Time taken to reach Stimulus (in sec.)
C	81.67 ± 46.74
E	86.78 ± 48.30
C + G + I	48.17 ± 12.95
E + G + I	85.89 ± 27.06 $^{\pi}$

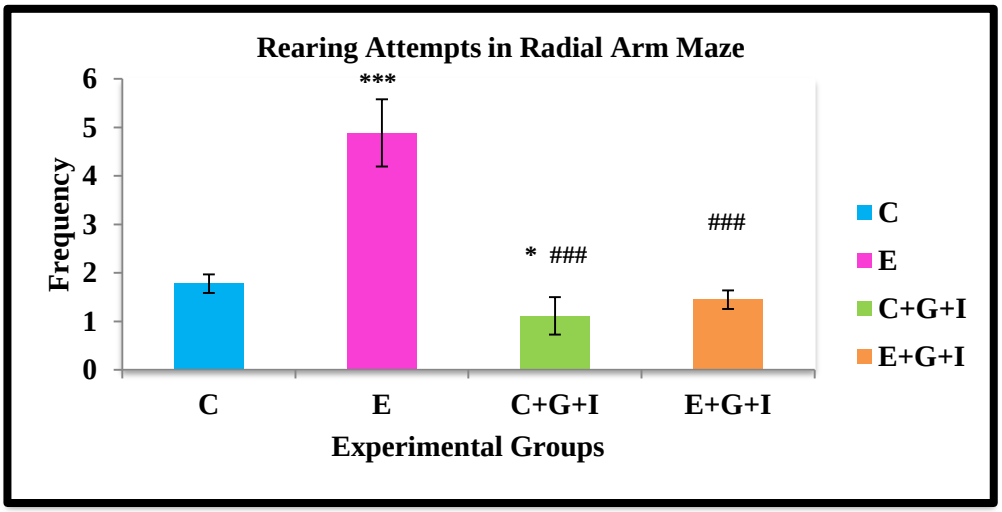
C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

$^{\pi}$ P<0.05 when compared to C + G + I

4.6.3.3 Rearing Attempts in Radial Arm Maze

Figure 46: Rearing Attempts in Radial Arm Maze



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 21: Rearing Attempts in Radial Arm Maze

Experimental Groups	Rearing Attempts
C	1.77±0.19
E	4.88±0.69 ***
C + G + I	1.11±0.38 *###
E + G + I	1.44±0.19 ###

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

***P<0.001 when compared to C

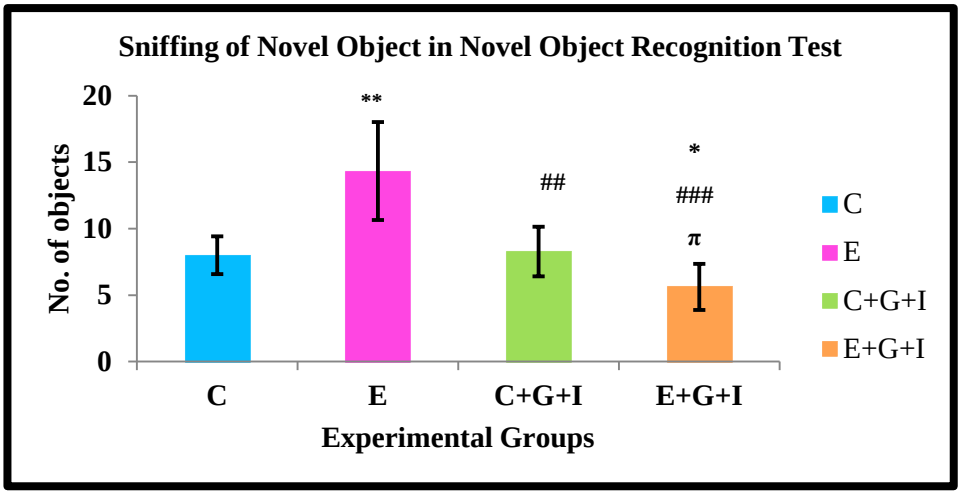
*P< 0.05 when compared to C

P<0.001 when compared to C + G + I

4.6.4 Novel Object Recognition Test

4.6.4.1 Sniffing of Novel Object

Fig 47: Sniffing of Novel Object



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 22: Sniffing Of Novel Object by Rats

Experimental Group	No. of Object
C	8 ± 1.4
E	14.33 ± 3.7 **
C+G+I	8.2777 ± 1.9 ##
E+G+I	5.6111 ± 1.7 * ### $^{\pi}$

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

* P < 0.05 When Compare to C

** P < 0.01 When Compare to C

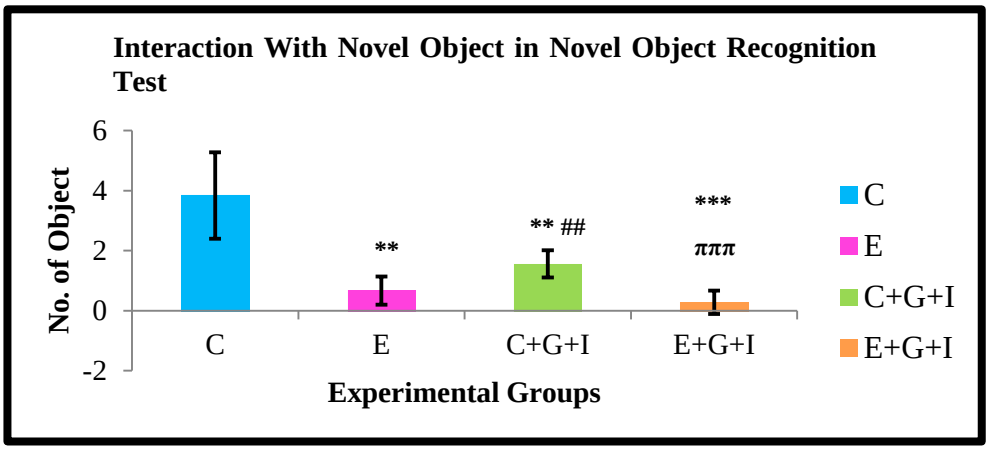
P < 0.001 When Compare to E

P < 0.01 When Compare to E

$^{\pi}$ P < 0.05 When Compare to C + G + I.

4.6.4.2 Interaction with Novel object

Fig 48:- Interaction with Novel Object by Rat



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 23:- Interaction with Novel Object

Experimental Groups	No. of Object
C	3.8333 ± 1.4
E	0.6666 ± 0.48 ***
C+G+I	1.5555 ± 0.46 ** ##
E+G+I	0.2777 ± 0.39 *** πππ

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

*** P < 0.001 When Compare to C

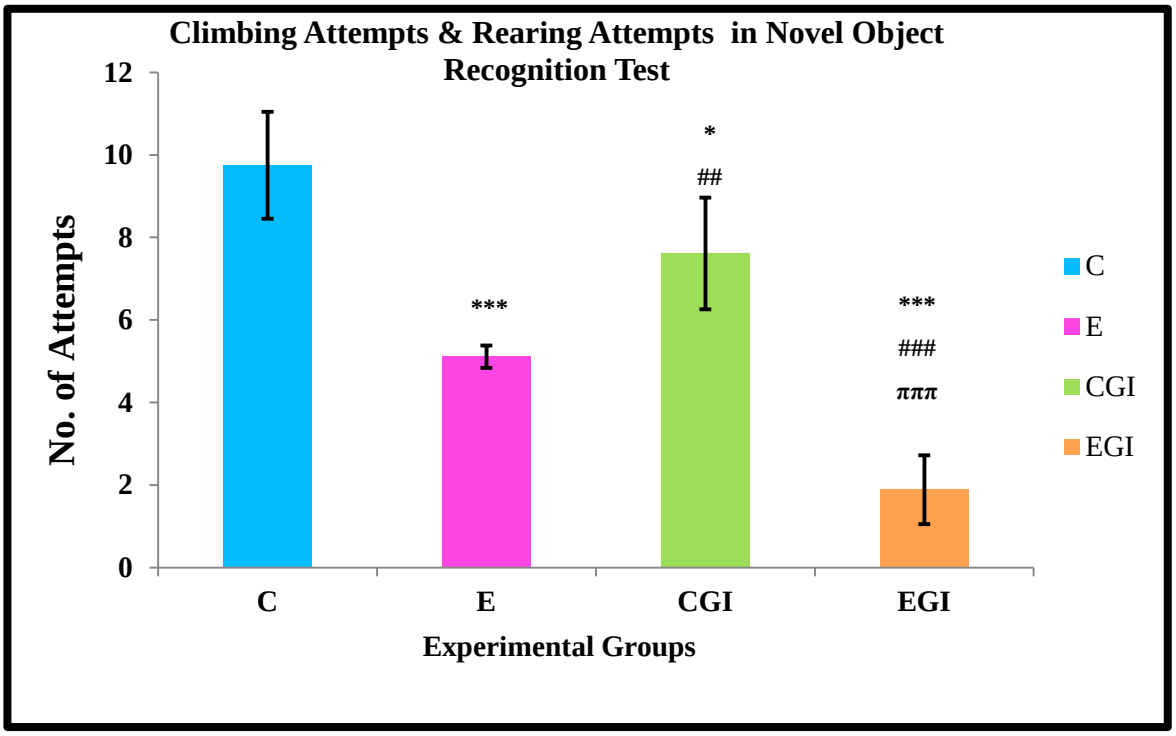
** P < 0.01 When Compare to C

P < 0.01 When Compare to E

πππ P < 0.001 When Compare to C + G + I

4.6.4.3 Climbing and Rearing Attempts in Novel Object Recognition Test

Figure 49: Climbing and Rearing Attempts by Rats



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 24: Climbing and Rearing Attempts by Rats

Experimental Groups	No. of Attempts
C	9.75 ± 1.3
E	5.1111 ± 0.28 ***
C+G+I	7.6111 ± 1.4 * ##
E+G+I	1.8888 ± 0.84 *** ### $^{\pi\pi\pi}$

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

*** P < 0.001 When Compare to C

* P < 0.05 When Compare to C

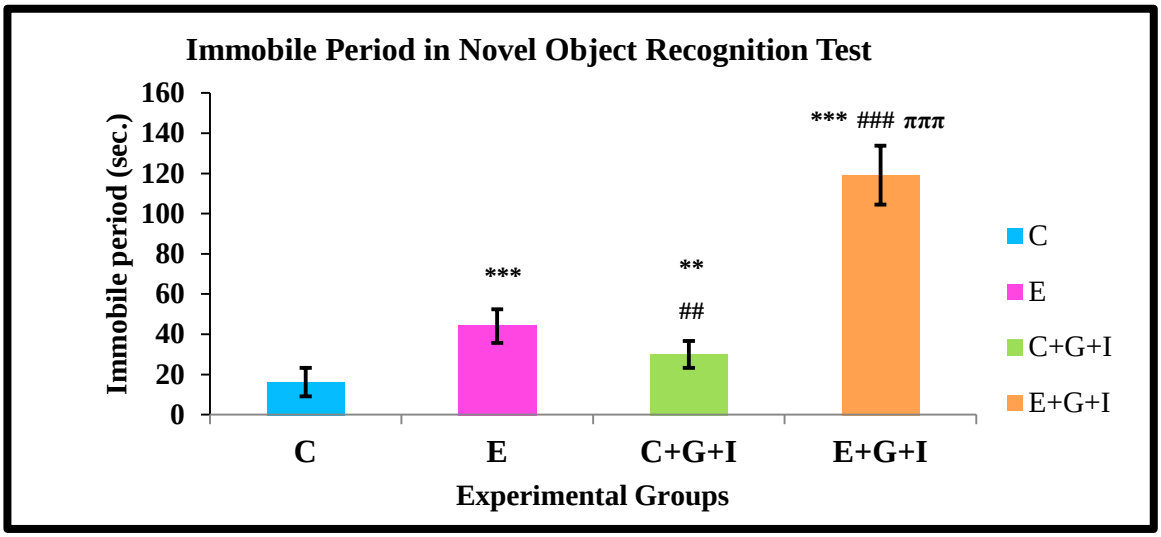
P < 0.001 When Compare to E

P < 0.01 When Compare to E

$^{\pi\pi\pi}$ P < 0.001 When Compare to C + G + I

4.6.4.4 Immobile Period in Novel Object recognition Test

Figure 50: Immobile Period in Novel Object recognition Test



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 25: Immobile Period in Novel Object recognition Test

Experimental Groups	Time (in sec.)
C	16.1666 ± 7.1
E	44.1111 ± 8.4 ***
C+G+I	30 ± 6.7 ** ##
E+G+I	119.1667 ± 14.6 *** ### $^{\pi\pi\pi}$

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

*** $P < 0.001$ When Compare to C

** $P < 0.01$ When Compare to C

$P < 0.001$ When Compare to E

$P < 0.01$ When Compare to E

$^{\pi\pi\pi}$ $P < 0.001$ When Compare to C + G + I

Discussion

Memory formation and retrieval are based on complex cellular and molecular processes. Neuronal glucose metabolism and its control by the Insulin signal transduction cascade are mainly involved in such processes. The brain glucose/energy metabolism and its regulatory Insulin signal transduction cascade is mainly involved in diverse memory processes (Hoyer, 2003). Cognition can be defined as the capacity of the brain to process information precisely. The most reported cognitive complaints in epilepsy are mental slowness, memory impairment and attention deficits (Van Rijckevorsel, 2006). Radial Arm Maze (RAM) is a test to assess memory and cognitive impairments.

In the present study, when the Epileptic rats were subjected to the test, all the three parameters that were assessed for the study demonstrated that there is impairment for memory in epileptic rats as compared to the control rats. In the first parameter, which includes the frequency of visiting the arm by the experimental group of rats, it is observed that the epileptic group of rats show a significant decrease in the frequency then the control rats. Also, this frequency is less than the frequency of all other experimental animals visiting the stimulus arm. In the second parameter where it was checked for the time taken by the rats to remember the stimuli and reach the stimuli arm for the 1st time it visits that arm, it can be

observed that Epileptic group of rats take comparatively more time to reach the stimulus arm as compared to that of the control group. In the third parameter, that is the rearing attempts, epileptic group of rats show significant increase in the rearing attempts compared to the control group, whereas C + G + I shows a significant decrease as compared to control and C + G + I and E + G + I group shows a significant decrease in rearing attempts compared to the epileptic group of rats.

These increased rearing attempts in case of Epileptic rats indicate a condition of fear and an attempt to look for an exit from the maze. C + G + I and E + G + I group of rats shows decrease in rearing attempts possibly because of the effect of Glucose Insulin treatment.

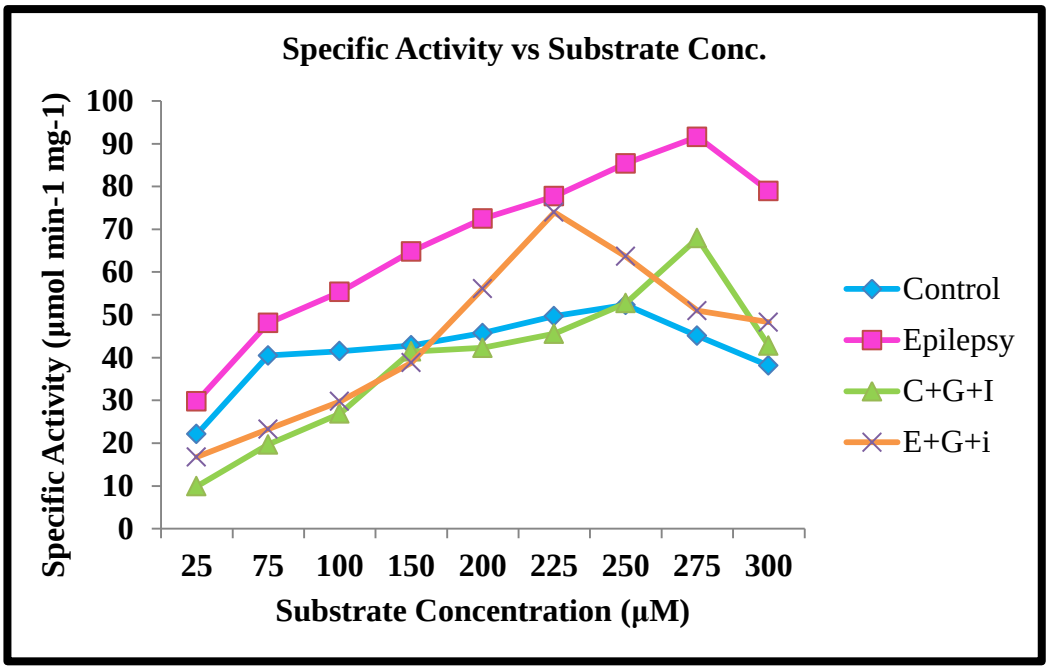
A probable explanation for this may be the damage to Temporal Lobe. In particular, principal cells in CA3, CA1 and the dentate gyrus are lost. Loss of entorhinal neurons, in particular those in layer III, and of amygdala neurons have also been documented to be lost previously. Axonal sprouting may accompany neuronal cell death. More notably, mossy fibre sprouting has been found after both human and animal seizures. It seems apparent that, neuronal cell death would contribute to the behavioral disruptions after seizures. However, the brain may undergo functional reorganization after neuronal cell loss, which may compensate for possible behavioral disruptions.

4.7 Enzyme Assays

4.7.1 Lactate dehydrogenase

4.7.1.1 LDH Assay in Heart

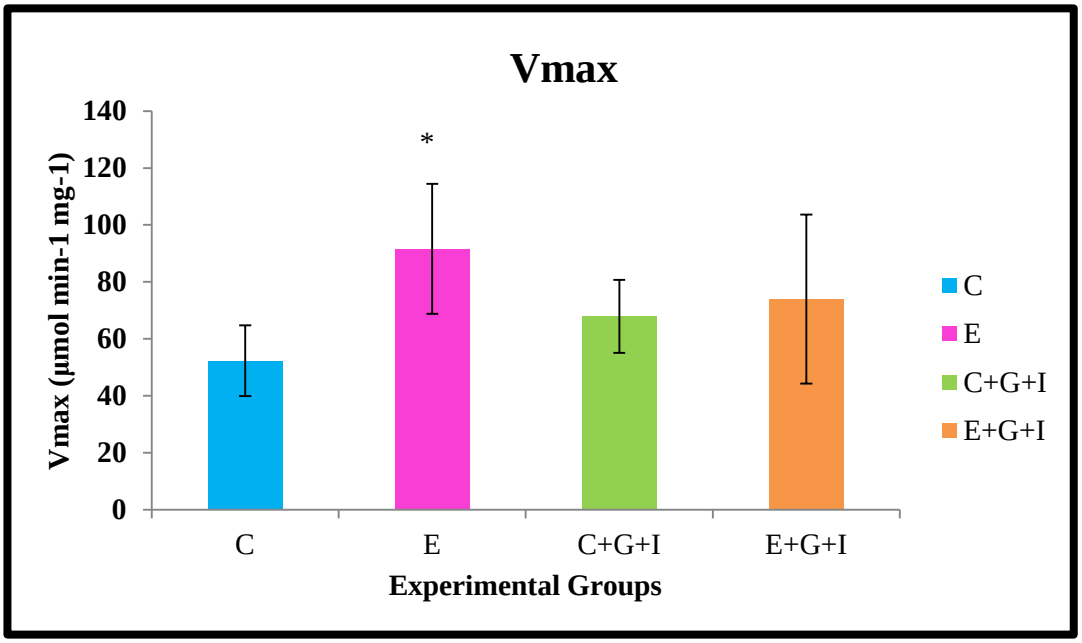
Figure 51: Specific Activity vs Substrate Conc. for LDH in Heart



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Figure 52: Vmax for LDH in Heart



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin

Values are Mean ± SD for 3 separate experiment (n=3 rats/experimental group)

Table 26: Vmax and Km for LDH in Heart

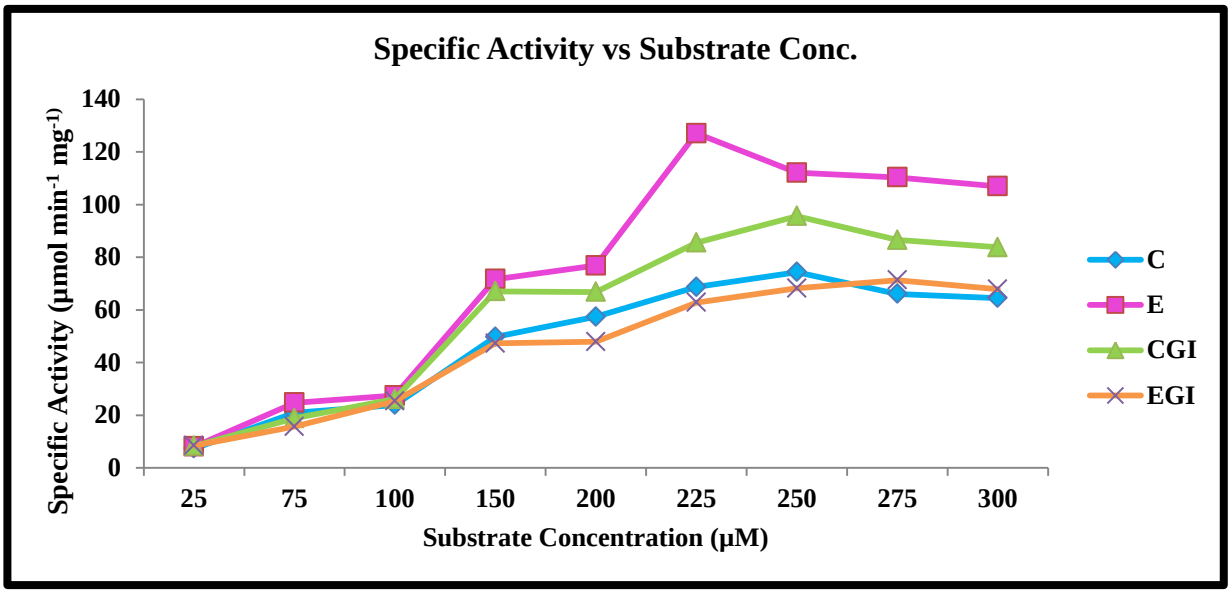
Experimental Groups	Vmax	Km
C	52.30 ± 12.43	33.50 ± 7.35
E	91.59 ± 22.81 *	73.50 ± 72.09
C+G+I	67.91 ± 12.82	219.04 ± 185.70
E+G+I	73.99 ± 29.68	101.58 ± 77.30

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin

*P <0.05 when compared to C

4.7.1.2 LDH Assay in Muscle

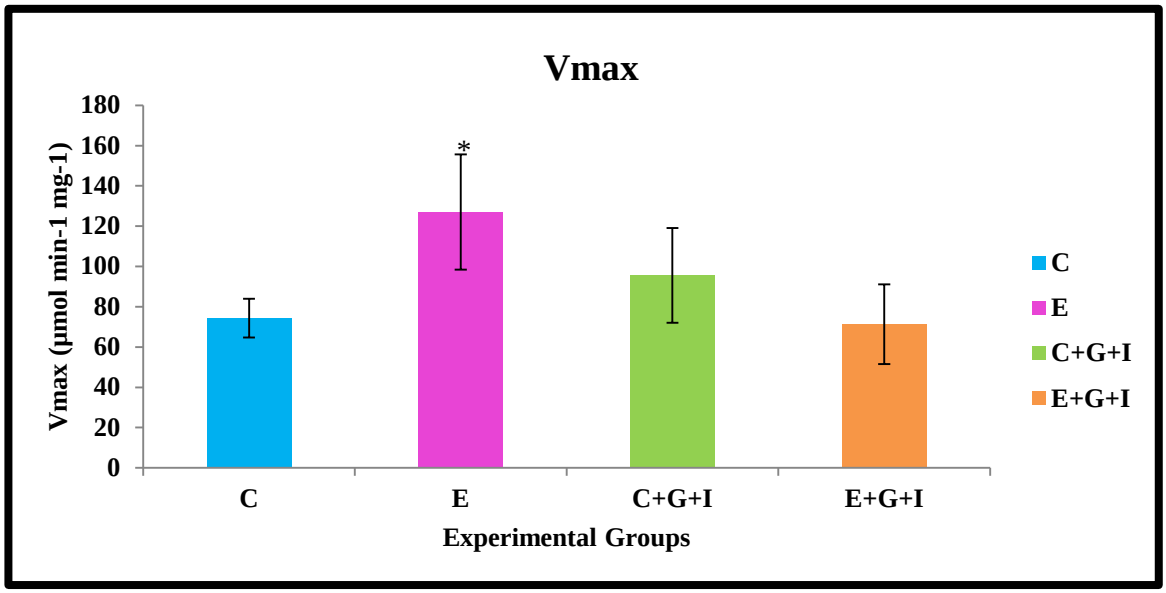
Figure 53: Specific Activity vs Substrate Conc. for LDH in muscle



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Figure 54: Vmax for LDH in muscle



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 27: Vmax and Km for LDH in Muscle

Experimental Groups	Vmax	Km
C	74.34 ± 9.56	413.88 ± 312.25
E	127.02 ± 28.56*	527.16 ± 353.40
C+G+I	95.61 ± 23.52	400.08 ± 296.48
E+G+I	71.31 ± 19.76	349.93 ± 365.68

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

*P <0.05 when compared to C

Discussion

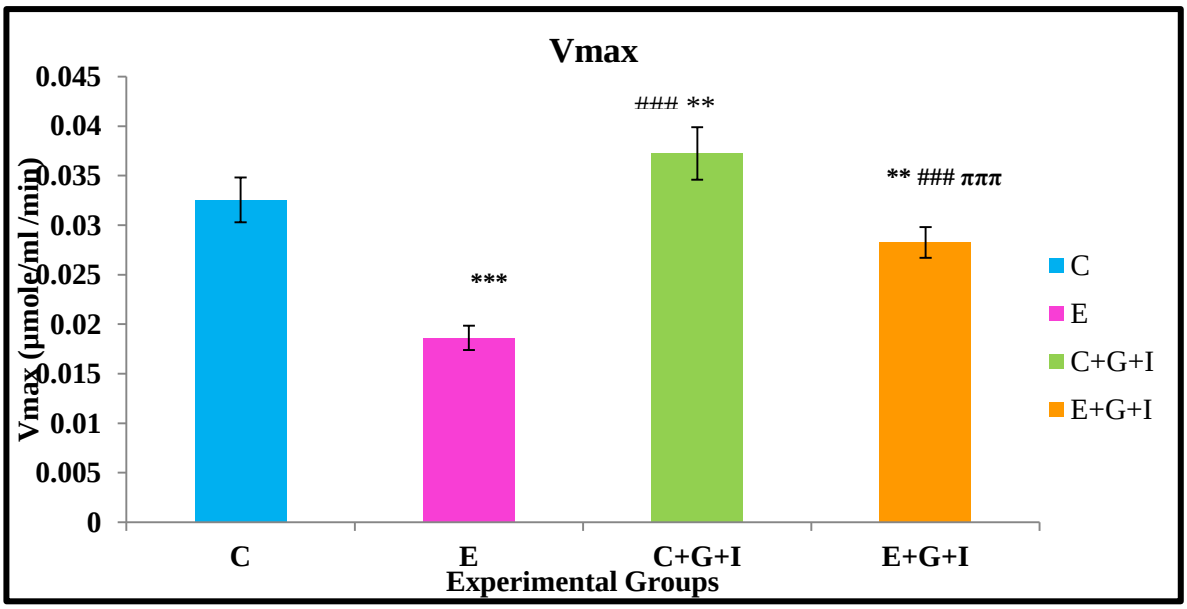
LDH is an enzyme that marks the conversion of pyruvate to lactate and vice versa by inter-conversion of NADH to NAD+. During the epileptic seizures, there is a continuous need of energy, because of high oxidative stress and hyper-excitability (Beltran *et al.*, 2012. In such condition, neurons metabolize lactate that has been suggested to originate from astrocytic glycolysis (Pellerin and Magistretti, 2003). Heart and Skeletal muscles are the

main tissues, which undergo anaerobic respiration during stress. In this study, LDH activity was assessed from heart and muscle, and in both the tissues, the activity of LDH in Epileptic group of rats was significantly (P<0.05) high than the Control group of rats, indicating a prevalent hypoxic condition, and an increased damage to the tissue caused by high oxidative stress, which becomes irreversible because of extensive metabolism and leads to Lactate accumulation. This can cause lactic acidosis, which might get reversed again after oxygen becomes available, but the damage caused is irreversible (Magistretti et al., 1999).

4.7.2 Super Oxide Dismutase

4.7.2.1 Superoxide Dismutase assay in liver

Figure 55: Vmax for SOD in liver



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 28: Vmax for SOD in liver

Experimental Groups	Vmax
C	0.032544±0.002254
E	0.018632±0.001229***
C+G+I	0.037223±0.00265**###
E+G+I	0.028247± 0.001554**### πππ

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

***P <0.001 when compared to C

**P < 0.01 when compared to C

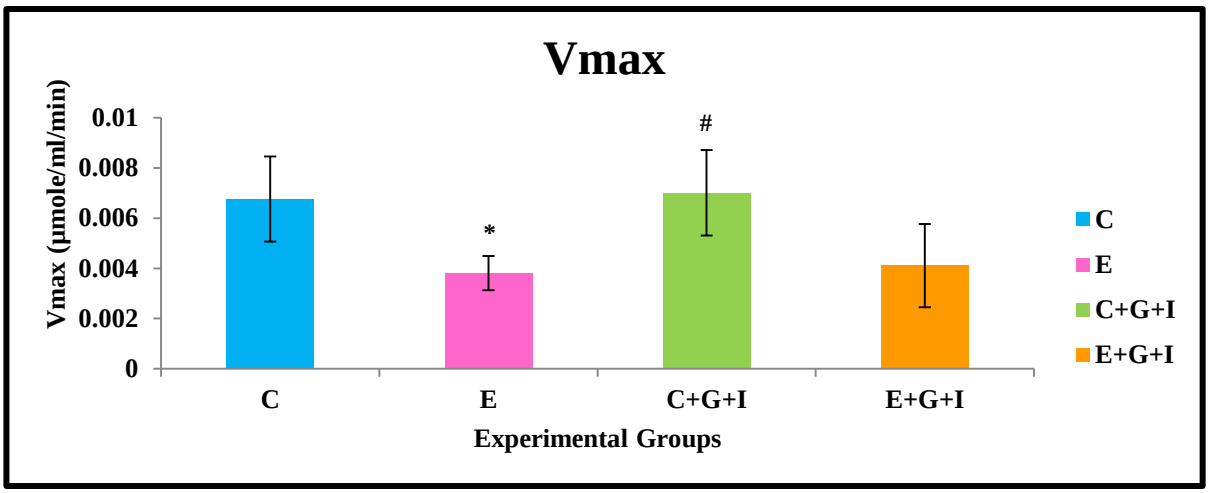
###P <0.001 when compared to E

##P <0.01 when compared to E

$^{\pi\pi\pi}$P<0.001 when compared to C+G+I

4.7.2.2 Superoxide Dismutase assay in muscle

Figure 56: Vmax for SOD in muscle



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 29: Vmax for SOD in Muscle

Experimental Groups	Vmax
C	0.006± 0.001
E	0.003± 0.0006*
C+G+I	0.007± 0.001#
E+G+I	0.004± 0.001

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

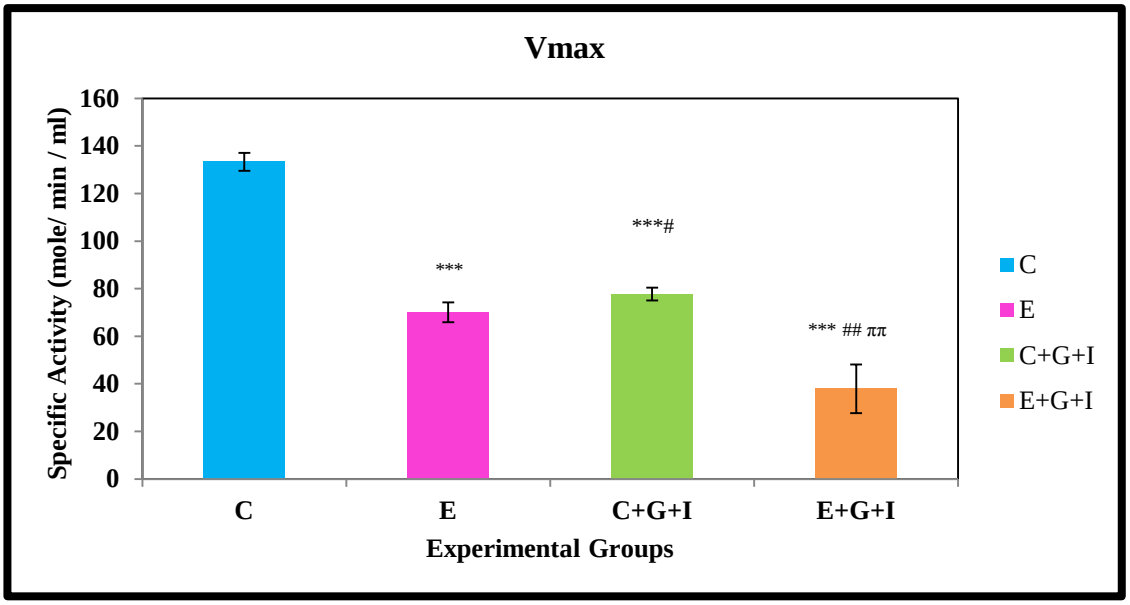
*P <0.05 when compared to C

#P < 0.05 when compared to E

4.7.3 Catalase

4.7.3.1 Catalase Assay in Liver

Figure 57: Vmax for CAT in liver



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 30: Vmax for CAT in liver

Experimental Groups	Vmax
C	133.31 ± 3.71
E	70.06 ± 4.18***
C+G+I	77.74 ± 2.71***#
E+G+I	37.92 ± 10.21***## $^{\pi\pi}$

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

***P <0.001 when compared to C

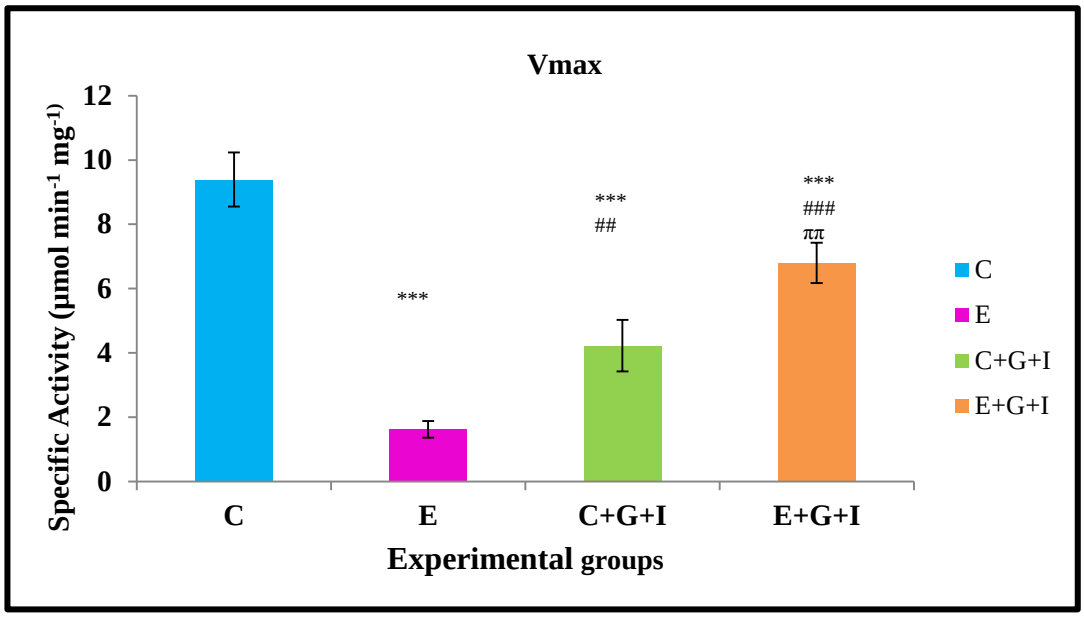
##P < 0.01 when compared to E

< 0.05 when compared to E

$^{\pi\pi}$ < 0.01 when compared to C+G+I

4.7.3.2 Catalase Assay in Muscle

Figure 58: Vmax for CAT in muscle



C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

Table 31: Vmax for CAT in muscle

Experimental Groups	Vmax
C	9.39±0.84
E	1.62±0.26***
C+G+I	4.22±0.79***##
E+G+I	6.79±0.62**###ππ

C-Control rats; **E**-Epileptic rats; **C+G+I** Control rats treated with Glucose and Insulin , **E+G+I** Epileptic rats treated with Glucose and Insulin.

Values are Mean ± SD for 3 separate experiment (n=3 rats/ group)

***P <0.001 when compared to C

##P < 0.01 when compared to E

P <0.001 when compared to E

ππ p< 0.05 when compared to C + G + I

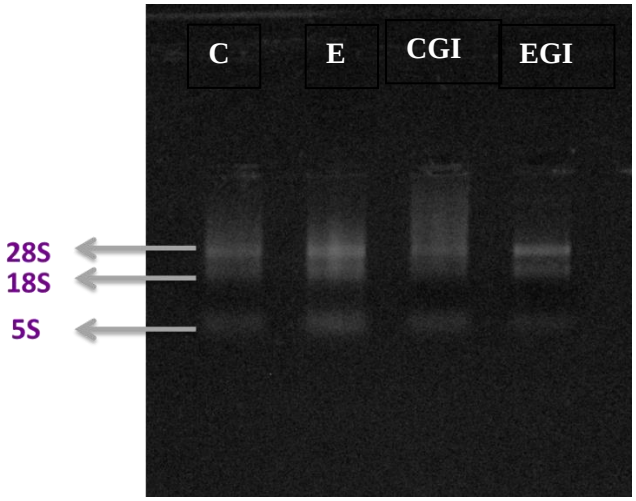
Discussion

The specific activity of the antioxidant enzymes in control group of rats, in both liver and muscle is significantly high when compared to the epileptic rats. When antioxidants cannot breakdown ROS efficiently, oxidative damage occurs (Sies, 1993). Decrease in regional antioxidant levels have also been reported in pilocarpine induced epileptic model. In the pilocarpine model of epilepsy it is reported that there is lower amount of antioxidants, suggesting impaired and not increased antioxidant defence (Erakovic et al., 2000). In liver C + G + I group of rats showed significantly high specific activity when compared to the control, epilepsy and E + G + I group of rats. In muscle the C + G + I group of rats showed significantly high specific activity when compared to the epileptic rats. In liver E + G + I group of rats showed significantly high specific activity as compared to the control and C + G + I group of rats but significantly low specific activity as compared to the epileptic rats. But there was no such significant results obtained when the assay was performed from the muscle. This indicates that in hypoxic condition ROS generation is less due to the lack of oxygen but the free radicles production is high. The free radicals which are produced during the seizures are very reactive and they might produce oxidative damage in DNA, proteins and lipids leading to the neuro degenerative disease progression (Peterson et al., 2002).

4.8 Gene Expression of HIF-1-α

4.8.1 Agarose Gel Electrophoresis of total RNA

Figure 59: Total RNA from cerebral cortex



C – Control E – Epilepsy

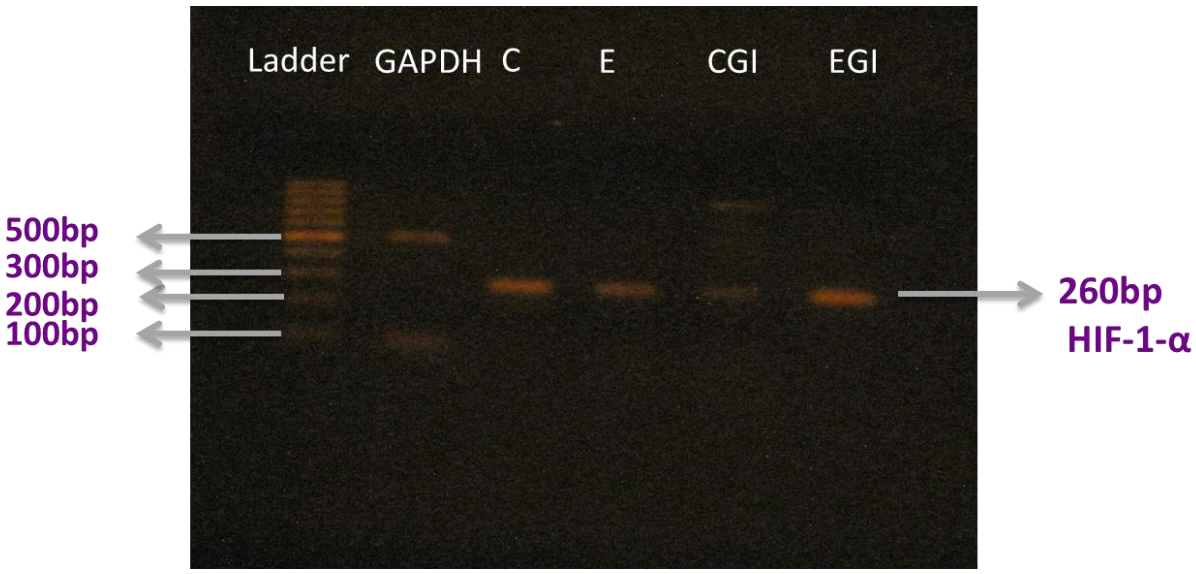
CGI – Glucose and Insulin treated Control

EGI – Glucose and Insulin treated Epilepsy

Three bands of RNA were clearly observed in the gel, indicating a proper isolation of RNA. Three different components of rRNA – 28S, 18S and 5S could be resolved in the gel and could be distinguished. 28S and 18S bands were close to each other while the 5S band has migrated more in the gel and could be seen separated. This shows a good quality of isolated RNA without DNA contamination.

4.8.2 PCR Results

Figure 60: PCR Result



Lane 1 – Ladder 1500 bp Lane 2 – GAPDH positive control

Lane 3 – Control Lane 4 – Epilepsy

Lane 5 – C + G + I – Glucose and Insulin treated Control group

Lane 6 - E + G + I – Glucose and Insulin treated Epileptic group

The bands in the GAPDH lane show GAPDH expression. GAPDH is a conserved gene sequence. Hence its expression indicates proper cDNA preparation.

HIF-1-α gene expression in the Cerebral cortex of the Control and experimental groups was observed. In the PCR amplification, an upregulation of gene expression was observed in EGI and Control group, whereas, it was down-regulated in Epileptic group and a very fade band was observed in CGI group. However, since PCR was performed only once, no significant concluding results were obtained.

SUMMARY

5. SUMMARY

In the present study, significant role of glucose (500mg/kg body weight) and insulin (0.01 IU/ kg body weight) treatment was observed in antioxidant assays in muscle, but no significant results were obtained in other studies. Behavioural study of Wheel Running test and Stair case test were done to check motor activity. Significant results were observed in Epileptic rats compared to Control group of rats. Radial Arm Maze and NOR test were carried out to check memory and cognition. Significant results were observed in Epileptic rats compared to Control group of rats. whereas no significant results were observed in C+G+I and E+G+I group of rats. From the enzymes assays SOD and Catalase it can be concluded that glucose and insulin treatment has its role in correcting abnormal glucose and oxidative stress during epilepsy, but in activity of LDH no significant results were obtained. HIF-1α gene expression studies were performed but since it was performed only once no significant results were obtaines for the same. From this study it can be concluded that during Epileptic seizures, hypoxic condition prevails, motor, memory and cognitive impairments occurs along with increased ROS and Lactate production. Also, therapeutic effect of Glucose and Insulin can be observed in antioxidant assays, but cannot be observed in LDH assay and Behavioural studies assessing motor activity, memory and cognition.

REFERENCES

Alcazar O, Tiedge M and Lenzen S. Importance of lactate dehydrogenase for the regulation of glycolytic flux and insulin secretion in insulin-producing cells, Biochem. J., 352: 373 – 380 (2000).

Armogida M, Spalloni A, Amantea D, Nutini M, Petrelli F, Longone P, Bagetta G, Nistico R and Mercuri NB. The protective role of Catalase against cerebral ischemia in vitro and in vivo, 24 (3): 735 – 747 (2011).

Banks WA, Jaspan JB and Kastin AJ. Selective, physiological transport of insulin across the blood brain barrier: Novel demonstration by species-specific radioimmunoassays, Peptides, 28:1257 – 1262 (1997).

Bao Z, Lei Yang, Luyang Yu, Bo Lin, Yanan Hou, Jun Wu, Qin Huang, Yifan Han, Lihe Guo, Qi Ouyang, Bo Zhang, Lu Lu, and Xuejun Zhang Acetylcholinesterase is associated with apoptosis in b cells and contributes to insulin-dependent diabetes mellitus pathogenesis Acta Biochim Biophys, 44: 207–216(2012).

Barker GR, Bird F, Alexander V, Warburton EC. Recognition Memory for objects, place and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex, J Neuroscience , 27:2948 –2957 (2007).

Bartolomei F, Chauvel P and Wendling F. Epileptogenicity of brain structures in human temporal lobe epilepsy: A quantified study from intracerebral EEG, Brain, 131: 1818 – 1830 (2008).

Beltran FA, Acuna AI, Miro MP and Castro MA, Brain energy metabolism in health and disease, Neuroscience – dealing with frontiers, Intech (2012).

Beltrán FA, Acuña AI, Miró MP and Castro MA. Neuroscience – Dealing with Frontiers, InTech, (2012).

Benton D. Nutritional Neuroscience, Taylor and Francis Group, LLC (2005).

Beresford HR, Posner JB and Plum F. Changes in Brain Lactate during induced Cerebral seizures, Arch Neurol., 20(3): 243 – 248 (1969).

Bingham EM, Hopkins D, Smith D, Pernet A, Hallett W, Reed L, Marsden PK and Amiel SA. The role of Insulin in Human Brain Glucose Metabolism: An Fluoro-Deoxyglucose Positron Emission Tomography Study, Diabetes, 51 (2002).

Blair RDG. Temporal Lobe Epilepsy Semiology, Epilepsy Research and Treatment, 2012 (2011).

Briellmann RS, Hopwood MJ and Jackson GD. Major depression in temporal lobe epilepsy with hippocampal sclerosis: Clinical and imaging correlates, J Neurol Neurosurg Psychiatry, 78: 1226 – 1230 (2007).

Brooks GA. Cell-Cell and intracellular lactate shuttles, J. Physiol., 587 (Pt 23): 5591 – 5600 (2005).

Buckmaster PS. Laboratory animal models of Temporal Lobe Epilepsy, Comparitive Medicine, 54 (5): 473 – 485 (2004).

Buettner GR. Superoxide dismutase in redox Biology: The roles of superoxide and hydrogen peroxide, Anticancer Agents Med Chem., 11 (4): 341 – 346 (2011).

Bussey TJ, Muir JL, Aggleton JP. Functionally dissociating aspects of event memory: the effects of combined perirhinal and postrhinal cortex lesions on object and place memory in the rat, J Neurosci , 19:495–502 (1999).

Bussey TJ, Muir JL, Aggleton JP. Functionally dissociating aspects of event memory: The effects of combined perirhinal and postrhinal cortex lesions on object and place memory in the rat, J Neurosci 19: 495–502 (1999).

Chang BS and Lowenstein DH, Epilepsy, The New England Journal of Medicine, 349: 1257 – 1266 (2003).

Chowers I, Lavy S and Halpern L. Effect of insulin administered intra-cisternally in dogs on the glucose level of the blood and the cerebrospinal fluid, Exp Neurol. 3(2): 197–205 (1961).

Cigdem IA, Ichise M, Olsavsky A, Tikofsky RS, Van Heertum RL. Gilliam F. Epilepsy duration impacts on brain glucose metabolism in temporal lobe epilepsy: Results of voxel-based mapping, Epilepsy and Behaviour, 17 (3): 373 – 380 (2010).

Clanton TL. Hypoxia-induced reactive oxygen species formation in skeletal muscle, J Appl Physiol. 102: 2379 – 2388 (2007).

Clifford D. B., Olney J. W., Maniotis A., Collins R. C. and Zorumski C. F. The functional anatomy and pathology of lithium-pilocarpine and high-dose pilocarpine seizures, J. Neuroscience, 23:953–968 (1987).

Dienel GA. Brain lactate metabolism: The discoveries and the controversies, Journal of Cerebral Blood Flow and Metabolism, 32: 1107 – 1138 (2012).

Dienel GA. Fueling and imaging brain activation , Asn neuro , 4(5): (2012).

Engel J Jr., Williamson PD and Wieser HG. Mesiel Temporal Lobe Epilepsy – Epilepsy: A comprehensive textbook, Lippincott-Raven, Philadelphia, 2417 – 2726 (1997).

Ennaceur A, Neave N, Aggleton JP. Neurotoxic lesions of the perirhinal cortex do not mimic the behavioural effects of fornix transection in the rat, Behav Brain Res, 80:9 –25 (1996).

Epilepsy Foundation of America, (www.epilepsyfoundation.org) (2009).

Erakovic V, Zupan G, Varljen J, Laginja J, Simonic A. Lithium plus pilocarpine induced status epilepticus-biochemical changes, Neurosci Res., 36(2):157-66 (2000).

Fisher R and Saul M. Overview of Epilepsy, (2010).

Gareth R. I. Barker and Elizabeth C. Warburton. When Is the Hippocampus Involved in Recognition Memory?, The Journal of Neuroscience, 31(29):10721–10731 (2011).

Ghasemi R, Haeri A, Dargahi L, Mohamed Z, and Ahmadiani A. Insulin in the Brain: Sources, Localization and Functions, Molecular Neurobiology, 47: 145 – 171 (2013).

Goldstein T, Mazet J.A.K., Zabka T. S., Langlois G., Colegrove K. M., Silver M., Bargu S., Van D.F., Leighfield T., Conrad P. A., Barakos J., Williams D. C., Dennison S., Haulena M. and Gulland F.M.D. Novel Symptomatology and Changing Epidemiology of Domoic Acid Toxicosis in California Sea Lions (Zalophus californianus): An Increasing Risk to Marine Mammal, Health Proc Biol Sci., 275: 267–276 (2008).

Hamilton SE, Loose MD, Qi M, Levey AI, Hille B. and McKnight GS. Disruption of the M1 receptor gene ablates muscarinic receptor-dependent M current regulation and seizure activity in mice, Proc Natl Acad Sci USA, 94: 13311–13316 (1997).

Hannesson DK, Vacca G, Howland JG, Phillips AG. Medial prefrontal cortex is involved in spatial temporal order memory but not spatial recognition memory in tests relying on spontaneous exploration in rats, Behav Brain Res, 153:273–285 (2004).

Harik SI, Hritz MA and LaManna JC. Hypoxia-induced braain angiogenesis in the adult rat, Journal of Physiology, 485.2: 525 – 530 (1995).

Helmstaedter C and Elger CE. Chronic temporal lobe epilepsy: A neurodevelopmental or progressively dementing disease?, Brain: A journal of Neurology, 132: 2822 – 2830 (2009).

Helmut Sies. Strategies of antioxidant defense, Eur. J. Biochem., 215: 213-219 (1993).

Hesselink JR. The temporal lobe and the limbic system, FACR, (http://spinwarp.ucsd.edu/neuroweb/Text/br-800epi/br-800epi1.gif)

Higashida RT. (University of California-Centre for Cerebrovascular Research/American Stroke Association) (https://avm.ucsf.edu/Figures/Figure015.gif) (2012).

Hirvonen J, Virtanen KA, Nummenmaa L, Hannukainen JC, Honka MJ, Bucci M, Nesterov SV, Parkkola R, Rinne J, Iozzo P and Nuutila P. Effects of Insulin on Brain Glucose Metabolism in imapaired Glucose Tolerance, Diabetes, 60: 443 – 446 (2011).

Hopkins DF, Williams G. Insulin receptors are widely distributed in human brain and bind human and porcine insulin with equal affinity, Diabet Med., 14: 1044 – 1050 (1997).

http://academic.brooklyn.cuny.edu/biology/bio4fv/page/couple.gif

http://acaiberryeducation.com/wp-content/uploads/2011/04/antioxidant_diag.gif

http://thebrain.cgill.ca/flash/d/d_06/d_06_cr/d_06_cr_mou/d_06_cr_mou.html

http://thebrain.mcgill.ca/flash/d/d_06/d_06_cr/d_06_cr_mou/d_06_cr_mou.html

http://www.cnsforum.com/educationalresources/Figurebank/pd_affectedareas/brain_struc_bagang_alz

http://www.ebmedicine.net/topics.php?paction=showTopicSegandtopic_id=112&seg_id=2167

http://www.intropsych.com/ch02_human_nervous_system/02cerebellum.jpg

http://www.rcsb.org/pdb/101/motm.do?momID=102&evtc=Suggest&evta=Moleculeof%20the%20Month&evtl=OtherOptions

http://www.rcsb.org/pdb/101/motm.do?momID=57&evtc=Suggest&evta=Moleculeof%20the%20Month&evtl=OtherOptions

James Knierim. Overview Functions of the Cerebellum, Neuroscience online an electronic textbook for the neuroscience , (1997).

Jeffrey Lopeza, Maria E. Gonzaleza, Lourdes Lorigadosa, Lilia Moralesa, Gretel Riveronb, Jose Y. Bauzaa. Oxidative stress markers in surgically treated patients with refractory epilepsy, Clinical biochemistry , 40(5-6): 292-298 (2007).

Kawasaki K, Traynelis SF and Dingledine R. Different responses of CA1 and CA3 regions to hypoxia in rat hippocampal slice , Journal of Neurophysiology, 63: 385 – 394 (1990).

Ke X and Zhang J. Changes in HIF-1α, VEGF, NGF and BDNF levels in cerebrospinal fluid and their relationship with cognitive impairment in patients with cerebral infarction, Journal of Huazhong University of Sciences and Technology, Medical Science, 33(3):433-7(2013).

Kneussel M. Dynamic regulation of GABAA receptors at synaptic sites, Brain Res. Rev, 39:74–83 (2002).

Krishnakumar A, Abraham PM, Paul J and Paulose CS. Down-regulatin of cerebellar 5-HT_{2C} receptors in pilocarpine-induced epilepsy in rats: Therapeutic role of Bacopa monnieri extract, Journal of the Neurological Sciences, 284: 124 – 128 (2009).

Krystal L Schaar, Miranda M Brenneman, Sean I Savitz. Functional assessments in the rodent stroke model, Experimental & Translational Stroke Medicine, 2:13 2010.

Kuo LW, Lee CY, Chen JH, Wedeen VJ, Chen CC, Liou HH and Tseng WYI. Mossy fibre sprouting in Pilocarpine-induced status epilepticus rat hippocampus: A correlative study of diffusion spectrum imaging and histology, NeuroFigure, 41: 789 – 800 (2008).

Lanteaume L, Guedj E, Bastien-Toniazzo M, Magalahaes A, Mundler O, Bartolomei F. Cognitive and metabolic correlates of emotional vulnerability in patients with temporal lobe epilepsy, J Neurol Neurosurg Psychiatry , 83(5):522-8.(2012).

Laurens Witter, Cathrin B. Canto, Tycho M. Hoogland, Jornt R. de Gruijl, and Chris I. De Zeeuw.Strength and timing of motor responses mediated by rebound firing in the cerebellar nuclei after Purkinje cell activation, Front Neural Circuits, 7: 133 (2013).

Leite JP1, Bortolotto ZA, Cavalheiro EA. Spontaneous recurrent seizures in rats: an experimental model of partial epilepsy", Neurosci Biobehav Rev.,14(4):511-7 (1990).

Leona Plum, Markus Schubert, Jens C. Bruning. The role of insulin receptor signaling in the brain, Trends in Endocrinology & Metabolism , 25(5): 225-282 (2005).

Ling B, Peng F, Alcorn J, Lohmann K, Bandy B and Zello G. D-Lactate altered mitochondrial energy production in rat brain and heart but not liver, Nutrition and Metabolism, 9:6 (2012).

Longstaff A. Instant notes on Neuroscience, Viva Books Pvt. Ltd. (2002).

Magistretti PJ, Pellerin L, Rothman DL and Shulman RG. Energy on demand, Science, 283 (5401): 496 – 497 (1999).

Mantis JG, Centeno NA, Todorova MT, McGowan R and Seyfried TN. Management of multifactorial idiopathic epilepsy in EL mice with caloric restriction and the ketogenic diet: role of glucose and ketone bodies, Nutrition and Metabolism, 1:11 (2004).

Markert CL. Lactate Dehydrogenase: Biochemistry and function of Lactate Dehydrogenase, Cell biochem. Funct., 2: 131 – 134 (1984).

Mathern GW, Adelson PD, Cahan LD, Leite JP. Hippocampal neuron damage in human epilepsy: Meyer's hypothesis revisited , Prog Brain Res, 135:237-51 (2002).

McClain D, Abuelgasim K, Nouraie M, Salomon-Andonie J, Niu X, Miasnikova G, Polyakova L, Sargueeva A, Okhotin O, Cherqaoui R, Okhotin D, Cox J, Swierczek S, Song J, Simon M, Huang J, Simcox J, Yoon D, Prchal J and Gordeuk V. Decreased serum glucose and glycosylated hemoglobin levels in patients with Chuvash polycythemia: a role for HIF in glucose metabolism, Journal of Molecular Medicine (Berlin), 91(1):59-67 (2013).

McCord J and Fridovich I. Superoxide Dismutase: An enzymic Function for Erythrocuprein (Hemocuprein), The Journal of Biological Chemistry, 244(22):49-55. (1969).

Muller CJ, Bankstahl M, Groticke I, Losher W. Pilocarpine vs. lithium-pilocarpine for induction of status epilepticus in mice: development of spontaneous seizures, behavioral alterations and neuronal damage, Eur J Pharmacol , 619(1-3):15-24 (2009) .

Mumby DG, Pinel JP. Rhinal cortex lesions and object recognition in Rats, Behav Neuroscience , 108:11–18 (1994).

Nadler JV. The recurrent mossy fibre pathway of the epileptic brain. Neurochem Res; 28: 1649 – 1659 (2003).

Naik P, Prasad S and Cucullo L. Role and function of Dehydrogenases in CNS and Blood-Brain Barrier Pathophysiology, Intech.

Natelson S, Miletich DJ, Seals CF, Visintine DJ and Albrecht RF. Clinical Biochemistry of Epilepsy – I – Nature of diseases and a Review of the Chemical Findings in Epilepsy, Clinical Chemistry, 25(6): 889 – 897 (1979).

Neuroscience Core Concepts, The essential principles of neuroscience. http://emotion.psychdept.arizona.edu/SfnLocal/misc/core_concepts.pdf (2008).

Norman G, Eacott MJ. Impaired object recognition with increasing levels of feature ambiguity in rats with perirhinal cortex lesions, Behav Brain Res, 148:79 –91 (2004).

Owen L and Sunram-Lea SI. Metabolic agents that enhance ATP can improve Cognitive Functioning: A review of the evidence for Glucose, Oxygen, Pyruvate, Creatine and L-Carnitine. Nutrients, 3: 735 – 755 (2011).

Pellerin L and Magistretti PJ. Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. Proc. Natl. Acad. Sci. 91: 10625 – 10629 (1994).

Pellerin L and Magistretti PJ. How to balance the brain energy budget while spending glucose differently, Journal of Physiology, 546.2: 325 (2003).

Pellerin L, Magistretti PJ. Food for thought: challenging the dogmas. J. Cereb, Blood Flow Metab , 23: 1282–6 (2003).

Percy ME. Catalase: an old enzyme with a new role?, Can J Biochem Cell Biol., 62(10):1006-14 (1984)

Peterson SL,Morrow D, Liu S & Liu KJ. Hydroethidine detection of superoxide during lithium pilocarpine model of status epilepticus. Epilepsy Research, 49:226- 238 (2002).

Plummer DT. An Introduction to Practical Biochemistry, 3rd Edition; 24th reprint, Tata McGraw Hill (2005).

Priel MR. and Albuquerque Ex.Short-term effect of pilocarpine on rat hippocampal neurons in culture, Epilepsia , 43:40–46 (2002).

Purves D, Augustine G, Fitzpatrick D, Hall W, Lamitia AS, McNarmra J and While LE. Neuroscience, Sinauer Associates, Inc. Publishers (2008).

Rayner DV. Glucose transporters in the brain. Proceedings of the Nutrition Society. 55 (1b): 209 – 219 (1996).

Reeves and Swenson . Chapter 22 Epilepsy, Disorders of the Nervous System, (2008).

Scharfman HE. The Neurobiology of Epilepsy, Curr NeuNeurosci Rep., 7(4): 348 – 354 (2007).

Schauwecker PE. The effects of glycemic control on seizures and seizure-induced excitotoxic cell death, BMC Neuroscience, 13:94 (2012).

Schmidt RP and Wilder BJ, Epilepsy, Philadelphia, FA Davis Co., 55 – 59 (1968).

Scholey A, Harper S and Kennedy D. Cognitive demand and blood glucose, Physiol. Behav. 73: 585–592 (2001).

Schulingkamp RJ, Pagano TC, Hung D and Raffa RB. Insulin receptors and insulin action in the brain: review and clinical implications, Neuroscience and Behavioural Reviews, 24: 855 – 872 (2000).

Schwartz MW, Sipols AJ, Kahn SE, Lattemann DP, Taborsky Jr GJ, Bergman RN, Woods SC and Porte Jr D. Kinetics and specificity of insulin uptake from plasma into cerebrospinal fluid, Am J Physiol, 259:E378 – 383 (1990).

Scorza FA, Arida RM, Priel MR, Calderazzo L and Cavalheiro EA. Glucose Utilisation during staus epilepticus in an epilepsy model induced by Pilocarpine – A qualitative study, Arq Neuropsiquiatr, 60(2-A): 198 – 203 (2002).

Sfar S, Boussoffara R, Sfar MT and Kerkeni A, Antioxidant enzymes activities in obese Tunisian children, Nutrition Journal, 12:18 (2013).

Shen G, Zhao Y, Chen M, Zhang F, Liu X, Wang Y, Liu C, Yu J and Zhang J. Hypoxia-inducible factor-1 (HIF-1) promotes LDL and VLDL uptake through inducing VLDLR under hypoxia, The Biochemical Journal, 441(2):675-83 (2012).

Siegel GJ. Basic Neurochemistry: Molecular, Cellular and Medical Aspects, 7th Edition, Elsevier Academic Press, UK (2006).

Smolders I., Khan G. M., Manil J., Ebinger G. and Michotte Y. NMDA receptor-mediated pilocarpine-induced seizures characterization in freely moving rats by microdialysis, Br J Pharmacol , 121: 1171–1179 (1997).

Stadelmann I and Sawyers W. Quantitative analysis of Pilocarpine in human plasma using LC-MS/MS (http://www.basinc.com/library/presentations/biochem/sawyers03/).

Sucholeiki, Roy. Temporal Lobe Epilepsy, Gale Encyclopedia of Neurological Disorders, (2005).

Sutula TP and Dudek FE. Unmasking recurrent excitation generated by mossy fibre sprouting in the epileptic dentate gyrus: An emergent property of the complex system In: Scharfman, HE., editor. The Dentate Gyrus: A Comprehensive Guide to Structure, Function, and Clinical Implications. Amsterdam: (2007).

Szymonik-Lesiuk A, Czechowska GY, Stryjecka-Zimmer M, Slomka M, Madro M, Celinski K and Wielosz M, Catalase, Superoxide Dismutase, and Glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication, J. Hepatobil. Pancreatic. Surg., 10: 309 - 315 (2003).

Umschweif G, Alexandrovich AG, Trembovler V, Horowitz M and Shohami E. Hypoxia-inducible factor 1 is essential for spontaneous recovery from traumatic brain injury and is a key mediator of heat acclimation induced neuroprotection. Journal of Cerebral Blood Flow and Metabolism, 33(4):524-31 (2013).

Waldbaum S and Patel M. Mitochondrial Oxidative Stress in Temporal Lobe Epilepsy, Epilepsy Research, 88(1):23-45 (2010).

Wan Q, Xiong ZG, Man HY, Ackerley CA, Braunton J, Lu WY. Recruitment of functional GABAA receptors to postsynaptic domains by insulin , Nature , 388**:**686–690 (1997).

Wieser HG and ILAE commission on neurosurgery of epilepsy. Mesial Temporal Lobe Epilepsy with hippocampal sclerosis. Epilepsia, 45: 695 – 714 (2004).

Williams PA, Wuarin JP, Duo P, Ferraro DJ and Dudek FE. Reassessment of the effects of cycloheximide on mossy fibre sprouting and epileptogenesis in the pilocarpine model of temporal lobe epilepsy, Journal of Neurophysiology, 88 (4): 2075 – 2087 (2002).

Willie CK and Smith KJ. Fueling the exercising brain: A regulatory quagmire for lactate metabolism, The Journal of Physiology, 589.4: 779 – 780 (2011).

www.drugs.com/pro/pilocarpine-eye-drops.html

Xiao H, Zhenyang G, Wang G, Zha T The Possible Mechanisms Underlying the Impairment of HIF-1α Pathway Signaling in Hyperglycemia and the Beneficial Effects of Certain Therapies, International Journal of Medical Sciences,10(10) :1412-1421(2013).

Yasuhisa kono. Generation of Superoxide Radical during Autoxidation of Hydroxylamine and an Assay for Superoxide Dismutase, Archives of biochemistry and biophysics, 186(1): 189-195 (1978).

Yu J, Li J, Zhang S, Xu X, Zheng M, Jiang G and Li F. IGF-1 induces hypoxia-inducible factor 1α-mediated GLUT3 expression through PI3K/Akt/mTOR dependent pathways in PC12 cells, Brain Research, 1430:18-24 (2012).

Zhang Z, Yan J, Chang Y, Yan S S, and Shi H Hypoxia Inducible Factor-1 as a Target for Neurodegenerative Diseases, Curr Med Chem, 18(28):4335–4343 (2011).