NEUROPHARMACOLOGICAL STUDIES OF EPILEPSY USING CRAB AND MOUSE AS MODELS

A Thesis Submitted to the Goa University for the Award of the Degree of DOCTOR OF PHILOSOPHY

in

ZOOLOGY

By

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INDIA

FEB 2013

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Research Guide

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INDIA

FEB 2013

Special Dedication of these Grateful Feelings to My….

Beloved husband,

Cute three year old daughter 'Suzanne'

My guide and parents……

As required under the university ordinance, I state that the thesis entitled "NEUROPHARMACOLOGICAL STUDIES OF EPILEPSY USING CRAB AND MOUSE AS MODELS", is my original contribution and that the same has not been submitted on any previous occasion to the best of my knowledge. The present study is the first comprehensive study of its kind from the area mentioned. The literature conceiving the problem investigated has been cited. Due acknowledgements have been made wherever facilities have been availed of.

K.K.Therisa (Beena)

Date:

This is to certify that the thesis entitled "**Neuropharmacological studies of epilepsy using Crab and Mouse as models**", submitted by **Ms Karingada Kochu Therisa (Beena)** for the award of the Degree of **Doctor of Philosophy in Zoology** is based on the results of laboratory experiments carried out by her under my supervision. The thesis or any part thereof has not previously been submitted for any other degree or diploma.

Dr. P.V. Desai
Ex-Professor & Head,
Department of Zoology,
Goa University.

Date:

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K. K. Therisa (Beena)

PREFACE

There is significant evidence that the global load of mental and neurological disorders is enormous and increasing. At the same time, little is known about the resources available to meet this burden. Epilepsy is one such a chronic neurological disorder of the brain that affects people in every country of the world. Around 50 million people worldwide have epilepsy. Estimated rate of this disorder in the general population at a given time is between 6 to 10 per 1000 people. Among primary disorders of the brain, this burden ranks with depression and other affective disorders, Alzheimer's disease and other dementias, and substance abuse. Among all medical conditions, it ranks with breast cancer in women and lung cancer in men.

The nervous system has a limited repertoire of responses to insult; it can underact, causing negative signs and symptoms such as paralysis and blindness, or it can overact, causing positive signs and symptoms such as pain, hallucinations, and epileptic seizures. All epileptic seizures, however, are not epilepsy, which is defined as a condition associated with recurrent epileptic seizures. Epilepsy occurs when permanent changes in brain tissue cause the brain to be too excitable or jumpy. The brain sends out abnormal signals. This results in repeated, unpredictable seizures. Common causes of epilepsy include: Stroke or transient ischemic attack; Dementia; Traumatic brain injury; Infections such as meningitis, encephalitis; Congenital brain defect; Metabolism disorders present at birth; other damage that damage brain tissue; use of certain medications, including antidepressants, etc.

The treatment of epilepsy is done using allopathic drugs, but there are more chances of recurring of seizures, once the medications are stopped. Also, it is known that the anticonvulsant drugs on prolonged usage lead to other organ dysfunctions. Besides, the fear, misunderstanding and the resulting social stigma and discrimination surrounding epilepsy often force people with this disorder "into the shadows". The social effects may vary from country to country and culture to culture, but it is clear that all over the world the social consequences of epilepsy are often more difficult to overcome than the seizures themselves. Significant problems are often experienced by people with epilepsy in the areas of personal relationships and, sometimes, legislation. These problems may in turn undermine the treatment of epilepsy.

There is a necessity to gather more information, on treatment of epilepsy or in other words neuropharmacological studies of epilepsy using more animal models. In the present investigation, we have established a higher invertebrate animal such as Crab *Scylla serrata* as a model for neuropharmacological studies. Also, attempt is made to study the neuropharmacological effect of combined drug treatment (allopathic + homeopathic) for epilepsy in rodent such as Mice *Mus musculus*. Our intention was to understand the effect of this combined drug on the brain tissues of mice and its effectiveness to control seizure.

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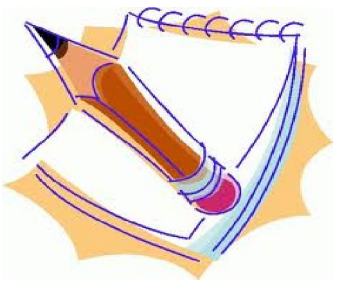
ABBREVIATIONS:

AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
GDH	Glutamate dehydrogenase
GS	Glutamine Synthetase
AChE	Acetylcholinesterase
LDH	Lactate dehydrogenase
PTZ	Pentylenetetrazole
Sod Val	Sodium Valproate
Cup	Cuprum metallicum
OL	Olfactory Lobe
CC	Cerebral Cortex
CoC	Corpus callosum
CG	Cingulate Gyrus
H	Hippocampus
CQ	Corpora Quadrigemina
C	Cerebellum
P	Pons
MO	Medulla Oblongata
GABA	Gamma-amino butyric acid
Glu	Glutamate

INTRODUCTION



Epilepsy is one of the most common disorders of the brain, and 0.1% people will have at least one seizure during their normal lifespan, and few among these will develop into epilepsy (Engel and Pedley, 2008). Worldwide, epilepsy affects 50 million people (Brodie and French, 2000). According to a World Health Organization (WHO) survey, epilepsy accounts for 1% of the global burden of disease, a figure equivalent to breast cancer in women and lung cancer in men (Engel and Pedley, 2008).

Epilepsy is a group of neurologic conditions, the fundamental characteristics of which are recurrent, usually unprovoked, epileptic seizures. Epilepsy is, of course, not a specific disease, or even a single syndrome, but rather a broad category of symptom complexes arising from a number of disordered brain functions that themselves may be secondary to a variety of pathologic processes or conditions. The terms convulsive disorder, seizure disorder, and cerebral seizures are used synonymously with epilepsy. They all refer to recurrent paroxysmal episodes of brain dysfunction manifested by stereotyped alterations in behavior (Engel and Pedley, 2008).

Epilepsy is a tendency for recurrent seizures- 'a chronic neurological condition where the individual is susceptible to several seizures'. People have seizures when the electrical signals in the brain misfire. These overactive electrical discharges disrupt the brain's normal electrical activity, causing a temporary communication problem between neurons. A seizure is a sudden, temporary interruption of normal electrical/ chemical activity in the brain, resulting in the change in sensation, awareness or behaviour. It can range from a brief disruption of senses, brief stares, muscle spasms or odd sensations to short periods of unconsciousness or convulsions, and can last

from a few seconds to a few minutes (Elaine, 2008). Some people have just one type of seizure, while others are susceptible to more.

About 80 percent of those diagnosed with epilepsy, seizures can be controlled with modern medicines and surgical techniques. However, about 25 to 30 percent of people with epilepsy will continue to experience seizures even with the best available treatment. Doctors call this situation intractable epilepsy. Having a seizure does not necessarily mean that a person has epilepsy. Only when a person has had two or more seizures, he or she is considered to have epilepsy.

Epilepsy is not contagious and is not caused by mental illness or mental retardation. Some people with mental retardation may experience seizures, but seizures do not necessarily mean the person has or will develop mental impairment. Many people with epilepsy have normal or above-average intelligence. Famous people who are known or rumored to have had epilepsy include the Russian writer Dostoyevsky, the philosopher Socrates, the military general Napoleon, and the inventor of dynamite, Alfred Nobel, who established the Nobel Prize (Elaine, 2008). Several Olympic medalists and other athletes also have had epilepsy. Seizures sometimes do cause brain damage, particularly if they are severe. However, most of the time recurrent seizures on the developing brain do not seem to have a detrimental effect (Camfield, 1997). Any changes that do occur are usually subtle, and it is often unclear whether these changes are caused by the seizures themselves or by the underlying problem that caused the seizures.

Epilepsy can develop in any person at any age. Approximately, 0.5% to 2% of people will develop epilepsy during their lifetime (Schachter, 2007). New cases of epilepsy are most common among children, especially during the first year of life. The rate of new cases gradually declines until about age 10, and then becomes stable.

After age 55 or 60, the rate starts to increase, as people develop strokes, brain tumors, or Alzheimer's disease (Kramer, 2001). Up to 5% of the world's population may have a single seizure at some time in their lives. It is likely that around 60 million people in the world have epilepsy at any one time (Elaine, 2008). Children and adolescents are more likely to have epilepsy of unknown or genetic origin than adults. Epilepsy can start at any age. Recent studies show that seizures in, up to 70% of children and adults with newly diagnosed epilepsy can be controlled with medications; however, many of these people experience treatment- related side effects, especially in a study carried out in Europe (Baker et.al, 1997). Seizures in up to 30% of people with epilepsy do not respond to available medications.

It is estimated that there are more than 10 million persons with epilepsy in India (Tripathi et.al, 2012). Its prevalence is about 1% of our population (Sridharan and Murthy, 1999), this being higher in the rural (1.9%) as compared with the urban population (0.6%) (Leonardi and Ustun, 2002; Pahl and de Boer, 2005). The treatment of epilepsy involves both direct and indirect economic costs, besides the health sufferings by the patients. Direct cost includes the cost of the hospitalization, treatment, medicines, homecare and ancillary services. The indirect costs include loss of time and productivity, the income lost by family members and the foregone leisure time. The cost attributed to pain, suffering and social stigma comes under intangible costs (Tripathi et.al, 2012). The direct and the indirect cost of treatment represented 27.1 and 72.9% of the total cost, respectively (Thomas et. al, 2001). Hence, it is mandatory to study more about the cause and treatment of epilepsy, using more animal models as well as by screening various types of antiepileptic drugs, especially those with less side-effects.

Epilepsy encompasses a diverse group of seizure disorders caused by a variety of structural, cellular and molecular alterations of the brain primarily affecting the cerebral cortex, leading to recurrent unprovoked epileptic seizures (Badawy et. al. 2009) as depicted in the following figure 1.

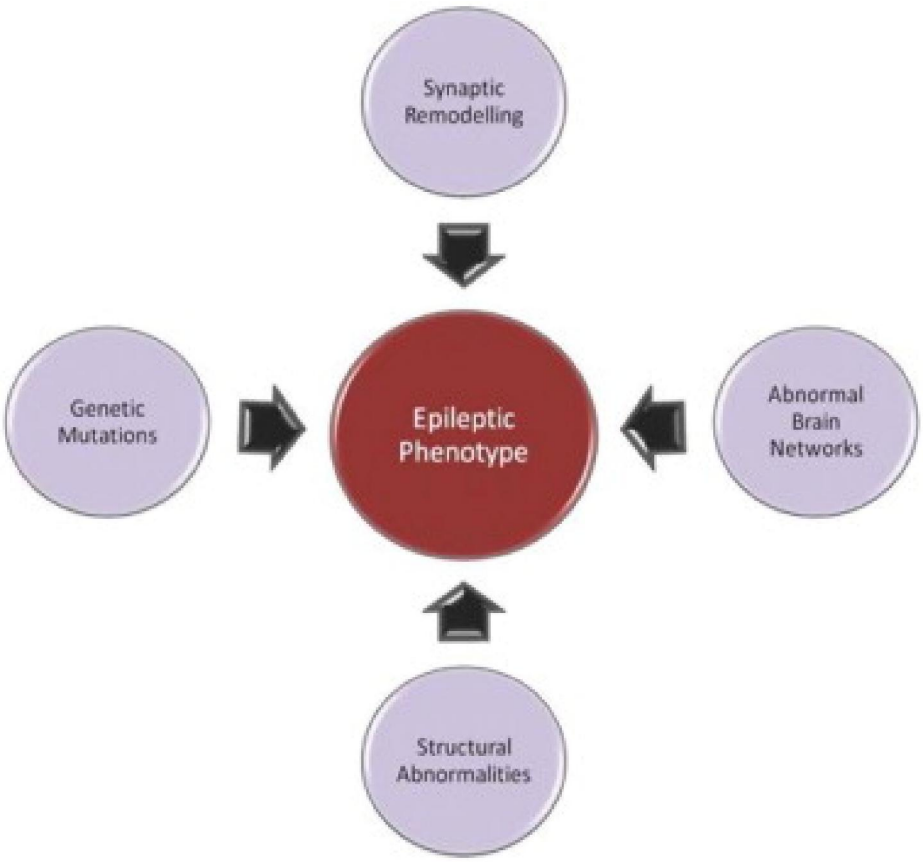


Figure 1: Different factors that interact to lead to the final presentation of the epileptic phenotype. (***Source***: Badawy et.al. 2009).

Besides, as reported by Rugg-Gunn and Holdright (2010), it is possible that patients with epilepsy are predisposed to developing cardiac arrhythmias during seizures due to a number of factors. These include the chronic exposure to potentially destabilising anti-epileptic medications, which may have additional, adverse cardiac and autonomic effects, the possibility of a shared genetic susceptibility for epilepsy and cardiac channelopathies and the long-term effects of recurrent seizures on the

heart leading to structural cardiac abnormalities or on the autonomic nervous system resulting in, for example, changes in heart rate variability. It has been represented in the following Figure 2.

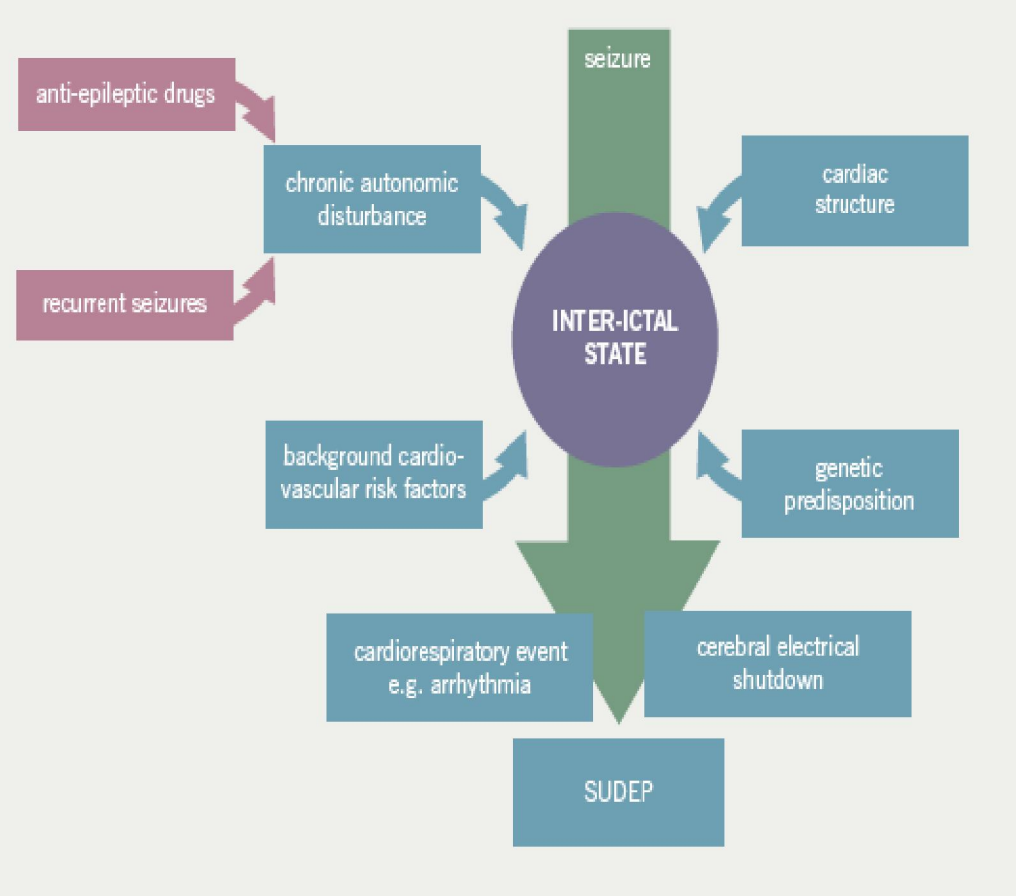


Figure 2: Schematic representation of the interaction between seizure activity and a primed inter-ictal state resulting in a cardio-respiratory event. (*Source*: Rugg-Gunn & Holdright, 2010).

Note the presence of post-ictal cerebral electrical shutdown which has been proposed as an alternative patho-physiological mechanism for Sudden unexplained death in epilepsy (SUDEP) (Rugg-Gunn and Holdright, 2010).

1.1. Definitions and Classifications of Epilepsy:

Jackson (1873) defined **epileptic seizures as the result of an occasional, sudden and excessive discharge of gray matter**. Epilepsy is present when seizures are recurrent and are not due to easily reversed, transient metabolic or toxic disorders. Seizures are fundamental elements of epilepsy (Gurnett and Dodson, 2009).

Classifications of seizure types:

I. Earlier Classification:

The classification of seizures was published in 1981 by Commission on Classification and Terminology of the International League against Epilepsy.

The primary dichotomy in classifying seizure types depends on whether seizure arises in one hemisphere or appears to involve both hemispheres from the onset. Accordingly, seizures are classified into Partial or Generalized seizure.

1. **Partial Seizures**:

i) **Simple Partial**: Consciousness is unimpaired during the episode.

ii) **Complex Partial**: Consciousness is impaired but may not be fully lost at any point during the ictus.

iii) **Partial Secondarily generalized**: Partial seizures (simple, complex or simple evolving to complex) may spread to become generalized.

2. **Generalised seizures:**

i) **Typical absence seizures (Petitmal seizures)**: Consciousness is lost and regained in an abrupt off-on pattern, usually quiet brief, often less than 5seconds.

ii) **Atypical absence seizures**: Like typical absence seizures begin and end abruptly, but associated with longer duration, decreased postural tone and

tonic activity. They form part of the Lennox-Gastaut Syndrome and they may occur at any age.

iii) **Myoclonic seizures**: A myoclonic seizure is a brief contraction of muscle, muscle group or several muscle groups due to cortical discharge. It can be single or repetitive, varying in severity from almost an imperceptible twitch to a severe jerking, resulting for instance, in a sudden fall or the propulsion of hand-held objects (the 'flying saucer' syndrome). Recovery is immediate, and the patient often maintains that consciousness was not lost.

iv) **Clonic seizures**: Clonic seizures consist of clonic jerking which is often asymmetric and irregular. Clonic seizures are most frequent in neonates, infants or young children, and are always symptomatic.

v) **Tonic seizures**: Tonic seizures take the form of a tonic muscle contraction with altered consciousness without a clonic phase. The tonic contraction causes extension of the neck; contraction of the facial muscles, with the eyes opening widely; upturning of the eyeballs; contraction of the muscles of respiration; and spasm of the proximal upper limb muscles causing the abduction and elevation of the semiflexed arms and the shoulders.

vi) **Tonic-Clonic seizures (Grand mal seizures):** This is the classic form of epileptic attack, the 'convulsion' or 'fit' that typifies epilepsy in the public imagination. The seizure is initiated by loss of consciousness, and sometimes an epileptic cry. The patient will fall if standing, there is a brief period of tonic flexion, and then a longer phase of rigidity and axial extension, with the eyes rolled up, the jaw clamped shut, the limbs stiff, abducted and extended. This tonic stage lasts on average 10-30 seconds and is followed by the clonic phase.

II. ILAE Report on Classification and Terminology of epileptic seizures and Epileptic syndrome in 2001 is as follows:

As reported by Engel (2001), The International League Against Epilepsy (ILAE) made a major contribution when it established standardized classifications and terminology for epileptic seizures and syndromes. This provided a universal vocabulary that not only facilitated communication among clinicians, but also established a taxonomic foundation for performing quantitative clinical and basic research on epilepsy. Much, however, has changed since the adoption of the currently used Classification of Epileptic Seizures in 1981 and the Classification of Epilepsies and Epileptic Syndromes in 1989. Consequently, the Executive Committee of the ILAE, which took office in July 1997, agreed that review and revision of the current classification system would be a priority for this Executive term.

Epileptic Seizure types:

1. **Self-limited seizure types**:-
    a) **Generalized seizures**: Such as Tonic-Clonic seizures; Clonic seizures; Typical absence seizures; Atypical absence seizures; Myoclonic absence seizures; Tonic seizures; Spasms; Myoclonic seizures; Eyelid Myoclonia; Myoclonic atonic seizures; Negative Myoclonus; Atonic Seizures; Reflex seizures in generalized epilepsy syndromes, etc.
    b) **Focal seizures**: Such as Focal Sensory seizures; Focal Motor seizures; Gelastic seizures; Hemiclonic seizures; Secondarily generalized seizures; Reflex seizures in Focal epilepsy Syndrome.

2. **Continuous Seizure types:-**

   a) **Generalised Status Epilepticus:** Such as Generalised tonic-Clonic status epilepticus; Clonic Status Epilepticus; Absence Status Epilepticus; Tonic status epilepticus; Myoclonic status epilepticus.

   b) **Focal Status Epilepticus:** Such as Epilepsia partialis continua of Kojevnikov; Aura continua; Limbic Status Epilepticus; Hemiconvulsive status with hemiparesis.

3. **Precipitating Stimuli for reflex seizures:-** Such as Visual Stimuli; Thinking; Music; Eating; Praxis; Somatosensory; Proprioceptive; Reading; Hot water; Startle.

Epilepsy syndromes and related conditions:

1. Benign familial neonatal seizures
2. Early Myoclonic encephalopathy
3. Ohtahara syndrome
4. Migrating Partial seizures of Infancy
5. West Syndrome
6. Benign Myoclonic epilepsy in infancy
7. Benign familial infantile seizures
8. Benign infantile seizures (nonfamilial)
9. Dravet's syndrome
10. HH syndrome
11. Myoclonic status in nonprogressive encephalopathies
12. Benign childhood epilepsy with centrotemporal spikes
13. Early-onset benign childhood occipital epilepsy (Panayiotopoulos type)
14. Late-onset childhood occipital epilepsy (Gastaut type)

15. Epilepsy with myoclonic absences
16. Epilepsy with myoclonic–astatic seizures
17. Lennox–Gastaut syndrome
18. Landau–Kleffner syndrome (LKS)
19. Epilepsy with continuous spike-and-waves during slow-wave sleep (other than LKS)
20. Childhood absence epilepsy
21. Progressive myoclonus epilepsies
22. Idiopathic generalized epilepsies with variable phenotypes
    a) Juvenile absence epilepsy
    b) Juvenile myoclonic epilepsy
    c) Epilepsy with generalized tonic–clonic seizures only
23. Reflex epilepsies
    a) Idiopathic photosensitive occipital lobe epilepsy
    b) Other visual sensitive epilepsies
    c) Primary reading epilepsy
    d) Startle epilepsy
24. Autosomal dominant nocturnal frontal lobe epilepsy
25. Familial temporal lobe epilepsies
26. Generalized epilepsies with febrile seizures plus
27. Familial focal epilepsy with variable foci
28. Symptomatic (or probably symptomatic) focal epilepsies
    a) Limbic epilepsies
        (i) Mesial temporal lobe epilepsy with hippocampal sclerosis
        (ii) Mesial temporal lobe epilepsy defined by specific etiologies

(iii) Other types defined by location and etiology

b) Neocortical epilepsies

(i) Rasmussen syndrome

(ii) Other types defined by location and etiology

29. Conditions with epileptic seizures that do not require a diagnosis of epilepsy

a) Benign neonatal seizures

b) Febrile seizures

c) Reflex seizures

d) Alcohol-withdrawal seizures

e) Drug or other chemically induced seizures

f) Immediate and early posttraumatic seizures

g) Single seizures or isolated clusters of seizures

h) Rarely repeated seizures (oligo-epilepsy)

III. Recently Recommended Classification of seizures and epilepsy:

The Commission on Classification and Terminology of the ILAE made the bold if not entirely popular step to break with the nearly century-old concepts and language of the ILAE classification systems and propose some new, alternative concepts and terminology (Berg et al., 2010).

1. **Focal seizures** are conceptualized as originating at some point within networks limited to one hemisphere.
2. **Generalized seizures** are conceptualized as originating at some point within and rapidly engaging bilaterally distributed networks.

Etiology:

1. **Genetic**: The epilepsy is, as best as understood, the direct result of a known or presumed genetic defect(s) in which seizures are the core symptom of the disorder. This attribution must be supported by specific forms of evidence.
2. **Structural/metabolic**: There is a distinct other structural or metabolic condition or disease that has been demonstrated to be associated with a substantially increased risk of developing epilepsy. These disorders may be of acquired or genetic origin. When of genetic origin, there is a separate disorder interposed between the gene defect and the epilepsy.
3. **Unknown**: The nature of the underlying cause is unknown; it may have a fundamental genetic basis (e.g., a previously unrecognized channelopathy) or it may be the consequence of an unrecognized structural or metabolic disorder not yet identified.

1.2. Mechanisms underlying epilepsy:

Epilepsy is a brain disorder in which clusters of nerve cells, or neurons, in the brain sometimes signal abnormally. Neurons normally generate electrochemical impulses that act on other neurons, glands, and muscles to produce human thoughts, feelings, and actions. In epilepsy, the normal pattern of neuronal activity becomes disturbed, causing strange sensations, emotions, and behaviour, or sometimes convulsions, muscle spasms, and loss of consciousness (Hosseini et.al. 2010). During a seizure, neurons may fire as many as 500 times a second, much faster than normal. The normal function of the central nervous system in any animal depends on adequate maintenance of the brain as well as neuronal microenvironment. This requires regulation of the extracellular along with intracellular ionic composition, osmolarity,

pH, also prevention of accumulation of neurotransmitters at the synaptic space. There also should be a continuous supply of fuel for oxidative neuronal metabolism and local increase in blood flow to meet the demands of the active neuronal populations. And this is been fulfilled within the brain tissue with the help of astrocytes.

It is a well known fact that glutamate is responsible for most of the fast excitatory synaptic transmission within the central nervous system. Sometimes, if the level of glutamate exceeds the normal level then it may cause havoc within the brain neuronal tissue causing excitotoxicity, but the astrocytes try to minimize glutamate effect by reuptaking it and metabolically converting it either into glutamine or promoting its oxidative metabolism. Astrocytes express several types of glutamate receptors, including AMPA and NMDA receptors and metabotropic glutamate receptors (Verkhratsky and Steinhauser, 2000; Bezzi et.al., 2001; Newman, 2003). Glu released from excitatory synapses elicits depolarization and increase in intracellular Ca^{+2} in the astrocytes by triggering IP3 production. Activation of IP3 receptors elicits mobilization of Ca^{+2} from endoplasmic reticulum, that in turn trigger a signaling process within the astrocytic network (Verkhratsky and Kettenmann, 1996).

Astrocytes are the main elements in the brain that regulate the synaptic levels of glutamate and the uptake of synaptic glutamate via the excitatory amino acid transporters (EAATs) present in the astrocyte, is the major mechanism preventing accumulation of glutamate in the synaptic space thereby, protecting neurons from excessive activation of their glutamate receptors and excitotoxic injury (Danbolt, 2001; Amara and Fontana, 2002; Sonnewald et.al., 2002; Hertz and Zielke, 2004). Glutamate uptake is driven by the electrochemical gradient of Na^{+}, which is maintained by the action of Na^{+}/K^{+}-ATPase and is therefore, critically dependent on

the energy metabolism. Impairement of glutamate uptake by the astrocyte in the setting of ATP depletion, as occurs with hypoxia, ischemia or hypoglycemia, is considered a primary mechanism of excessive accumulation of glutamate in the synaptic space, leading to neuronal injury. Besides, neuron and astrocyte interactions are important for maintaining a balance between excitatory and inhibitory synaptic transmission, disruption of which may lead to abnormal neuronal activity that occurs in seizures (Benarroch, 2005).

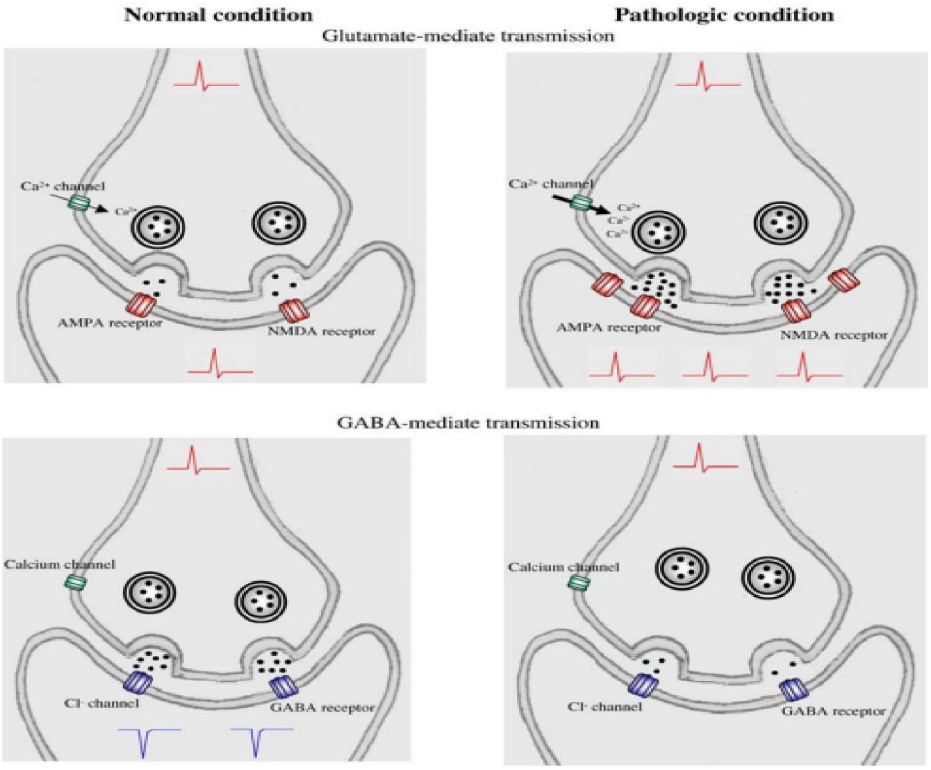


Figure:3 Molecular pathways involved in abnormal excitability of the brain tissue. (*Source*: Mortari et.al. 2007)

In the astrocyte, uptake of 1 molecule of glutamate is accompanied by cotransport of $3Na^+$, $1H^+$, and $1Cl^-$, antiport of $1K^+$ ion, and consumption of 1ATP molecule (Danbolt, 2001; Amara and Fontana, 2002) along with co transport of water

and cations (MacAulay et.al., 2004). The increase in intacellular Na^+ resulting from glutamate transport into the astrocytes has important metabolic consequences because it may serve as a signal that couples synaptic activity with glucose consumption (Magistretti and Pellerin, 1999). Intracellular Na^+ accumulation activates the Na^+/K^+-ATPase, and the resulting increase in the ADP/ATP ratio leads to activation of glycolysis.

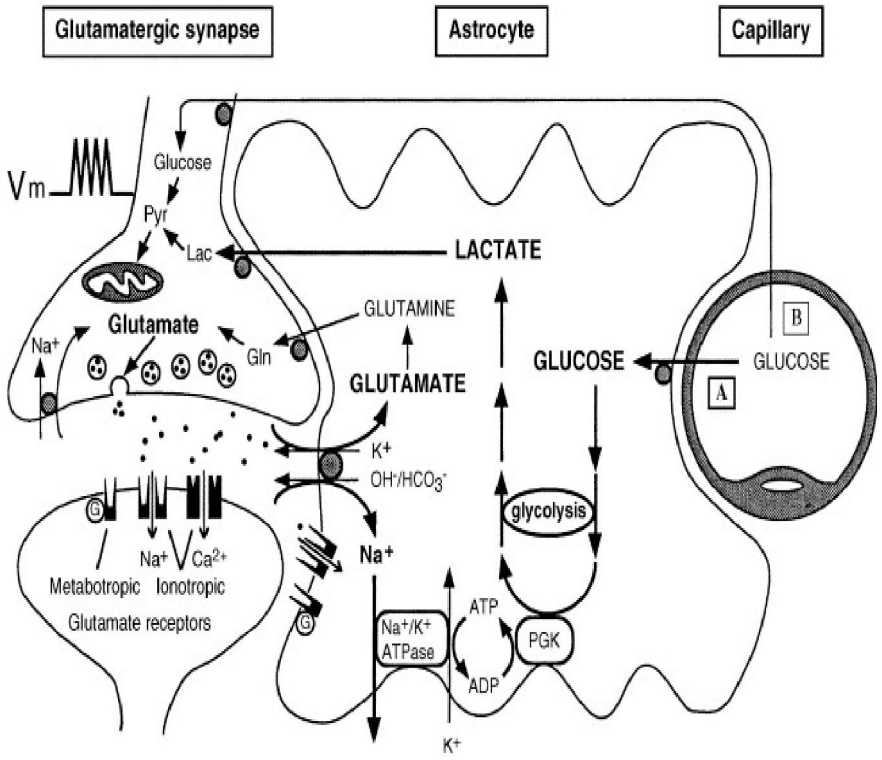


Figure 4: Schematic for the mechanism of glutamate-induced glycolysis in astrocytes during physiological activation, (*Source*: Nair, 2005)**.**

Besides, as Jefferys (2003) stated in his report that the excitatory neurons in an epileptic focus must be interconnected by excitatory synapses that (a) are relatively effective, (b) sufficiently densely connected, and (c) part of a large enough population. These three conditions provide for a chain reaction that can result in the whole neuronal population becoming synchronised relatively quickly (Traub and

Jefferys, 1997) causing the propagation of seizure in the brain. The cellular and network mechanisms of the transition of brief interictal discharges to prolonged seizures are a crucial issue in epilepsy (Jefferys, 2003).

1.3. Metabolic aspects in the brain during epilepsy:

Epilepsy involves a disruption of brain energy homeostasis and is potentially manageable through principles of metabolic control theory. Metabolism is an essential process underlying all phenotypes and an alteration in metabolic process can modify phenotype. The theory is based on the idea that compensatory genetic and biochemical networks, operating through flexible biological systems, are capable of modulating the bioenergetics of glycolysis, the TCA cycle, electron transport and oxidative phosphorylation (Greenspan 2001; Strohman 2002).

Metabolic enzyme such as aminotransferases like AST and ALT serve as a strategic link between carbohydrate and protein metabolism under pathophysiologic stress (Philip et al. 1994). Similarly, LDH is an enzyme that transforms lactate into pyruvate in brain tissue and thus, prevents acidosis and provides a substrate for TCA cycle. ATPases, also play an important role in the maintenance of ionic gradient by coupling ATP hydrolysis with energy processes (Kodama, 1985). ATP itself, as a neurotransmitter and neuromodulator, may influence the release of other neurotransmitters by acting through its own receptors or by altering the neurotransmitter receptors (Littleton and Bellen, 1995; Gendron et al. 2002). Na^+, K^+ -ATPase is a membrane bound enzyme and inactivation of this enzyme is an important factor in epileptization of neurons (Kryzhanovskii et al. 1987). Similarly it is demonstrated that inhibition of microsomal Mg^{2+}-ATPase, Ca^{2+}-ATPase may be associated with long-term plasticity changes associated with epileptogenesis (Parsons

et al. 2001). Idiopathic epilepsies involve the Na^+, K^+, or Ca^{2+} channels and the activities of Na^+, K^+ - or Ca^{2+} ATPases are responsible for maintaining ionic balance in the cell (Arundhati et al. 2003).

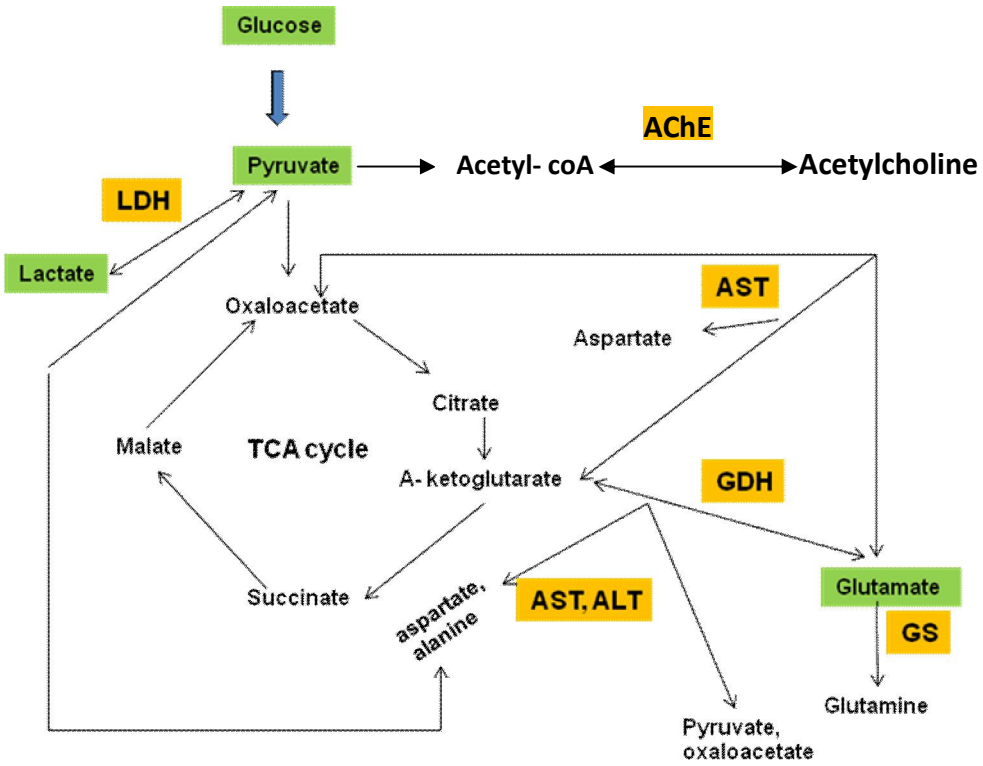


Figure 5: The correlation between a few metabolic enzymes and metabolites during metabolism in brain.

Glutamine synthetase (GS; EC 6.3.1.2) is a key enzyme in the regulation of glutamate neurotransmission in the central nervous system (CNS). It catalyzes the synthesis of glutamine from glutamate, and is responsible for the detoxification of ammonia in the brain (Meister, 1974). GS is crucial for nitrogen homeostasis, as the glutamine formed by this enzyme is a constituent of proteins and serves as a nitrogen source for a number of biosynthetic pathways (Meister, 1980). It has been demonstrated to be specially located in the glial fraction of the brain, primarily in astrocytes (Martinez-Hernandez et al., 1977; Norenberg, 1979; Norenberg and

Martinez-Hernandez, 1979). Glutamine which is synthesized in astrocytes via GS is then transported into neurons, where it serves as a precursor for the formation of the neurotransmitters glutamate and gamma aminobutyric acid (GABA) (Waniewski & Martin, 1986; Waniewski, 1992). GS is of particular importance in the brain, as although glutamate has a number of metabolic enzymes, the only known pathway for the synthesis of glutamine is via GS (Cooper et al., 1983).

Acetylcholinesterase (AChE) is an important enzyme which regulates the effects of acetylcholine at cholinergic synapses. AChE's main function is to terminate the effects of ACh after it is released, acting like an off switch. ACh is synthesized in neuron terminals in a reaction catalyzed by the enzyme choline acetyltransferase (ChAT). The choline is derived from membrane phospholipids while the acetyl CoA is a breakdown product of glucose. ACh is degraded into choline and acetate by acetylcholinesterase (AChE). ACh, one of the most important neurotransmitters of central nervous system, plays important roles in many physiologic events such as learning and memory, and pathologic events such as epilepsy (Decker and McGaugh, 1991; Rasmusson, 2000).

As far as energy metabolites in brain are concerned, glucose forms the obligatory energy substrate for brain and it is almost entirely oxidized to CO_2 and H_2O. Glucose can be incorporated into lipids, proteins, and glycogen, and it is also the precursor of certain neurotransmitters such as γ-aminobutyric acid (GABA), glutamate, and acetylcholine (Edvinsson et al. 1993; Sokoloff, 1989). Numerous studies have been performed to identify molecules that could substitute for glucose as an alternative substrate for brain energy metabolism. Among the vast array of molecules tested, mannose is the only one that can sustain normal brain function in the absence of glucose (Sloviter and Kamimoto, 1970). However, mannose is not

normally present in the blood and cannot therefore be considered a physiological substrate for brain energy metabolism. Whereas, lactate and pyruvate can sustain synaptic activity in vitro (Mata et al. 1980; Schurr et al. 1988), hence, act as an alternative energy substrate in the brain. Glutamate is an amino acid neurotransmitter that can behave as an endogenous convulsant as well as energy metabolite. Microdialysis measurements from humans with spontaneous seizures from the hippocampus show transient release of glutamate (During and Spencer, 1993).

In the brain tissue, the primary ions of electrolytes are Sodium (Na+), potassium (K+), calcium (Ca+2), magnesium (Mg+2), and Chloride (Cl-). Besides, other factors that cause seizures, ionic abnormalities also act as one cause for occurrence of seizures. Disturbance in ionic gradients across cellular membranes can have direct and indirect effects on neuronal discharge that may facilitate epileptiform activities (Scwartzkroin et.al. 1998). Disorders of sodium and osmolality exhibits neuronal depression, causing neuronal irritability (Luis et.al., 2006). Seizures are common in patients with Sodium abnormality, hypocalcemia, and hypomagnesemia especially causing generalized tonic-clonic and partial seizures (Victor and Ropper, 2001; Riggs, 2002, Luis et.al. 2006).

1.4. HISTORICAL PERSPECTIVE:

Among the diseases that have plagued humans over the centuries, few exhibit the brief, frightening manifestations of an epileptic attack and the relatively quick, seemingly miraculous recovery. Accounts of what may have been epileptic seizures can be found in several ancient scriptural accounts such as reference to the prophet Balaam falling down with the eyes open and to King Saul's fits of rage. Initially, these ancient accounts of falling attacks attributed such to an evil entity or punishment

inflicted by a god or, later, to some natural cause. Not until Hippocrates was the origin of theory of epilepsy placed it in the brain. Temkin (1945), in his book, “The Falling Sickness”, describes a battle between rational, scientific thinking and magical beliefs that started with Hippocrates' connection of epilepsy to the brain and continued at least until Jackson's time (Temkin, 1971). The three ancient Indian medical systems of Siddha, Ayurveda, and Unani all recognized epilepsy (Sieveking, 1858).

The most elaborate descriptions are found in the Ayurveda (science of life), the oldest known medical system that evolved continuously from 4500 to 1500 BCE. The views on epilepsy are attributed to the physician Atreya (about 900 BCE). The compendium of Ayurvedic medicine known as Charaka Samhita (6th century BCE) used the term apasmara (apa, loss of; smara, consciousness or memory) for epilepsy (Bharucha and Bharucha, 1989). Visual hallucinations; twitching of the tongue, eyes, and eyebrows; and jerking of the hands and feet accompanied by excessive salivation were some of the symptoms noted, as well as the observation of a patient awakening after the attack as if from sleep (Mansford, 1819). The term Apasmara poorva roopa was used for auras that included visual, auditory, and somatic symptoms, as well as behavioral disturbances.

The Hippocratic treatise On the Sacred Disease (Hippocrates, 1984) is not a medical text; it was apparently written for the layperson. It begins with an attack against common popular superstitions and all those who labelled epilepsy as to conceal their ignorance about its cause and justify their fraudulent practices. Contrary to the Babylonian text, the Hippocratic writings challenged the widespread beliefs of the time that epileptic seizures were caused by actions of demons or gods.

The Babylonian text described in detail various epileptic symptoms, as caused by a particular demon, while Hippocrates attempted to disconnect the physical

phenomena from supernatural forces. After a brief description of the generalized epileptic attack, the author recognized the hereditary nature of the disease and the greater frequency with which children were affected. Hippocrates importantly dissociated epilepsy from religion and magic, arguing forcibly and eloquently that epilepsy was properly a subject not for incantation but for medical investigation and study. His explanation that phlegm rushes into the cerebral vessels, preventing air from flowing into the brain, was the first attempt to explain the cause of epilepsy based on a physiologic process that affected the brain. Another important Hippocratic contribution to medicine was the introduction of prognosis. In the essay On the Sacred Disease, he commented on the grave outcome of status epilepticus and the spontaneous remission of epilepsy in children as they mature.

Attempts to define epilepsy started during Hellenistic times and continued during the time of the Roman Empire. By 1860 special hospitals for epileptics had been founded in Germany, France, and Britain. One of these was the Hospital for Epilepsy and Paralysis in Queen Square, London (later the National Hospital for Nervous Diseases). This meant that epileptics were increasingly seen and treated by physicians attached to such institutions, who became particularly experienced in the diagnosis and treatment of the disease. This led in particular to a more detailed differentiation and classification of epilepsy, including terms still in use today, such as grand mal, petit mal, absence seizures, and *status epilepticus*. By the latter part of the nineteenth century, the theories of the British neurologist, John Hughlings Jackson (1835-1911), and his French counterpart Jean Charcot (1825-93) began to define the neurological basis of the disease and its complex symptomatology. W. R. Gowers (1845-1915) authoritatively described the ‘aura’ that preceded a *grand mal* attack (although Hippocrates had not been ignorant of it).

Over the centuries attempts to understand and explain epilepsy have been plagued by religious, social, and even scientific explanations. Demonic possessions, the vital spirits, metabolic causes, psychoanalytic explanations, or even personal dislikes as well as political oppression were among the factors that had to be fought against to allow progress. Similarly, the progress in anatomy and physiology, and in particular the observations and experiments in cortical localization of specific functions, influenced Jackson's innovative concepts. The discovery by Galvani and others of nerve and muscle electricity led eventually to Berger's invention of EEG (Daras et al, 2008), which revolutionized modern epilepsy. The subsequent development of imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) as well as functional imaging such as single photon emission computed tomography (SPECT), positron emission tomography (PET), and functional MRI gave new insights into our understanding of epilepsy.

1.5. LITERATURE SURVEY:

Epilepsy is usually controlled, but not cured, with medication. However, over 30% of people with epilepsy do not have seizure control even with the best available medications. Surgery may be considered in difficult cases (Cascino 1994, Engel, 1996). Not all epilepsy syndromes are lifelong – some forms are confined to particular stages of childhood. Epilepsy should not be understood as a single disorder, but rather as syndromic with vastly divergent symptoms, all involving episodic abnormal electrical activity in the brain and numerous seizures.

1.5.1. Treatment of epilepsy:

Although epilepsy is characterized by episodic attacks, it exerts a significant psychological burden on the patients, their families, and on society in general. Furthermore, epilepsy is associated with high rates of psychiatric co-morbidity, which additionally affect the quality of life even in the absence of active seizures (Kanner, 2006; Pulsipher et al., 2006; Schachter, 2006). Among the potential neurobiological and psychosocial determinants, epilepsy-related variables (age at onset of seizures, temporal lobe epilepsy and frequency of seizures) and the antiepileptic drug treatment have been associated with depression (Marco, 2009). Approximately a third of patients undergoing epilepsy surgery have a history of major depression with prevalence figures ranging from 10% to 40% (Stub Naylor et al., 1994; Altshuler et al., 1999; Derry et al., 2000; Glosser et al., 2000; Ionue and Mihara, 2001; Malmgren et al., 2002; Quigg et al., 2003; Reuber et al., 2004; Wrench et al., 2004; Cankurtaran et al., 2005; Devinsky et al., 2005). Although, surgical excision of epileptogenic tissue via anterior temporal lobectomy (ATL) is a well-established method of controlling intractable complex partial seizures, still patients are at risk of psychiatric complications, including depression (Wrench et al., 2004; Devinsky et al., 2005) after surgery. Similarly, long term association between seizure outcome and depression after respective epilepsy surgery has been reported by Hamid et.al. (2011).

Besides, Corpus callosotomy was introduced as a surgical technique for the treatment for epilepsy in 1939 (Van Wagenen and Herren, 1940). It is often used to treat atonic, clonic, myoclonic and generalized tonic-clonic seizures (Gates and Courtney, 1993). Also, the ketogenic diet (KD) is a high fat, low protein, low carbohydrate diet, considered as anticonvulsant in many drug resistant epileptic children (Vining et al., 1998; Vining, 1999; Sinha and Kossoff, 2005; Freeman et al.,

2006, 2007; Hartman and Vining, 2007; Samala et.al., 2008). A meta-analysis of the second-generation AEDs used as adjunctive therapies shows that 12% to 29% of patients had a 50% or greater reduction in seizure frequency. Surgery and the vagus nerve stimulator provide important therapeutic options in patients whose seizures are not controlled by AEDs (Nadkarni et. al, 2005).

The mainstay of treatment of epilepsy is anticonvulsant medications. Often, anticonvulsant medication treatment will be lifelong and can have major effects on quality of life. Adverse effects are a major concern when starting antiepileptic drug (AED) treatment. The study reported by Perucca and Tomson (2011) quantified the extent to which adverse effects, reporting in people with new-onset seizures, started on AEDs was attributable to the medication per se, and investigated variables contributing to adverse effects reported. The choice among anticonvulsants and their effectiveness differs by epilepsy syndrome. Mechanisms, effectiveness for particular epilepsy syndromes, and side-effects differ among the individual anticonvulsant medications. Antiepileptic drugs provide satisfactory control of seizures for most patients with epilepsy.

1.5.2. Antiepileptic drugs for treating epilepsy:

In terms of treatment, *bromides* were the first successful medicine for epilepsy, from the 1850s onwards, abolishing attacks in some, and diminishing them in number or violence in most others; they started to be displaced only after the introduction of *phenobarbital*, first prescribed in epilepsy (Hauptmann, 1912). Barbiturates continue to be one of the effective treatments, although further understanding of the underlying mechanisms have led to the development of alternative modern anticonvulsant drugs: those that potentiate or imitate the inhibitory

neurotransmitter GABA, or stabilize the neuronal cell membrane, and thus prevent excessive firing. According to Perucca and Tomson (2011) Antiepileptic drug choice is primarily based on evidence of efficacy and effectiveness for the individual's seizure type, but other patient-specific factors need to be considered, including age, sex, childbearing potential, co morbidities, and concomitant medications.

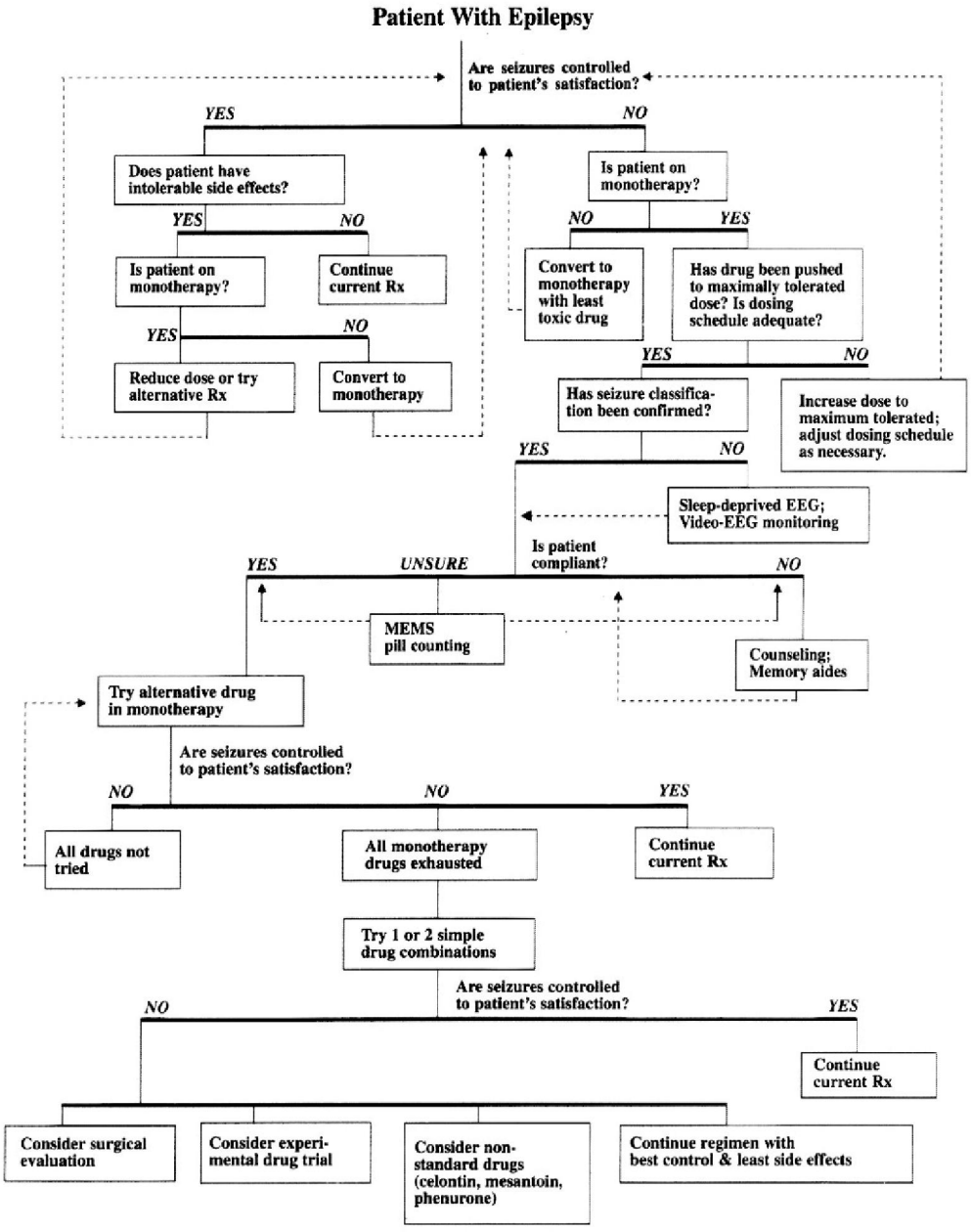


Figure 6: Long-term management paradigm for patients with epilepsy. (*Source*: Jacqueline, 1994)

Valproate is currently one of the major antiepileptic drugs (AEDs) with efficacy for the treatment of both generalized and partial epilepsies in adults and childrens (Brodie and Dichter, 1996; Loscher, 1999; Johannessen, 2000). As many as one-third of epilepsy patients continue to exhibit signs of seizure activity in spite of medical treatment with antiepileptic drugs (AEDs) (Deckers et al., 2003). Phenobarbital, Phenytoin, Primidone etc. are used as potent anticonvulsant drug, but they do cause teratogenic effect as reported in mice by Sullivan and McElhatton (1975). Even for conventional medications adverse effects can be difficult to assess, with many confounding variables leading to low levels of imputation agreements between decision algorithms (Macedo et al., 2003). Currently there are 20 medications approved by the Food and Drug Administration for the use of treatment of epileptic seizures in the US: carbamazepine (common US brand name Tegretol), clorazepate (Tranxene), clonazepam (Klonopin), ethosuximide (Zarontin), felbamate (Felbatol), fosphenytoin (Cerebyx), gabapentin (Neurontin), lacosamide (Vimpat), lamotrigine (Lamictal), levetiracetam (Keppra), oxcarbazepine (Trileptal), phenobarbital (Luminal), phenytoin (Dilantin), pregabalin (Lyrica), primidone (Mysoline), tiagabine (Gabitril), topiramate (Topamax), valproate semisodium (Depakote), valproic acid (Depakene), and zonisamide (Zonegran). Most of these appeared after 1990. As reported by Deckers et.al. (2003) Patients are usually aware of aggravation and may express a “dislike” for a particular AED as a warning sign for physicians to modify the medication. The availability of numerous AEDs, particularly with single mechanisms of action, has increased the risk of paradoxical effects that may go undetected in clinical trials and only surface during astute clinical observations (Deckers et. al. 2003). Also, as reviewed by Singh et al (2005), there is an added risk of causing cancer due to the prolonged usage of antiepileptic drugs. Yet,

complimentary medication such as homeopathy is strongly recommended as supportive line of treatment along with conventional treatment, in most cases. It may be stated that homeopathy alone may not help all the cases of epilepsy. At the same time, it is worth making a note that many cases of epilepsy which were resistant to the conventional medication, do respond significantly to homeopathy (Ricotii and Delanty, 2006).

1.5.3. Homeopathy treatment for epilepsy:

Homeopathy (also homœopathy or homoeopathy; from the Greek hómoios, "similar" +, páthos, "suffering" or "disease") is a form of alternative medicine first defined by Samuel Hahnemann in the 18th century (1833). A central thesis of homeopathy is that an ill person can be treated using a substance that can produce, in a healthy person, symptoms similar to those of the illness. Practitioners select treatments according to a patient consultation that explores the physical and psychological state (Hahnemann, 1833) of the patient, both of which are considered important to selecting the remedy. Homeopathy is a form of therapy that is based on two major principles: the “dynamization” principle and the “similia” principle. The first principle is that remedies that come from natural elements are prepared by a process of serial dilutions and succussions (vigorous shaking) (Ricotti and Delanty, 2006). The more times this process is performed, the greater the potency of the remedy. In high-potency remedies (ultra-high dilutions of C12 and above), substances have been diluted and succussed beyond Avogadro’s number, so they should not contain a single molecule of the original substance.

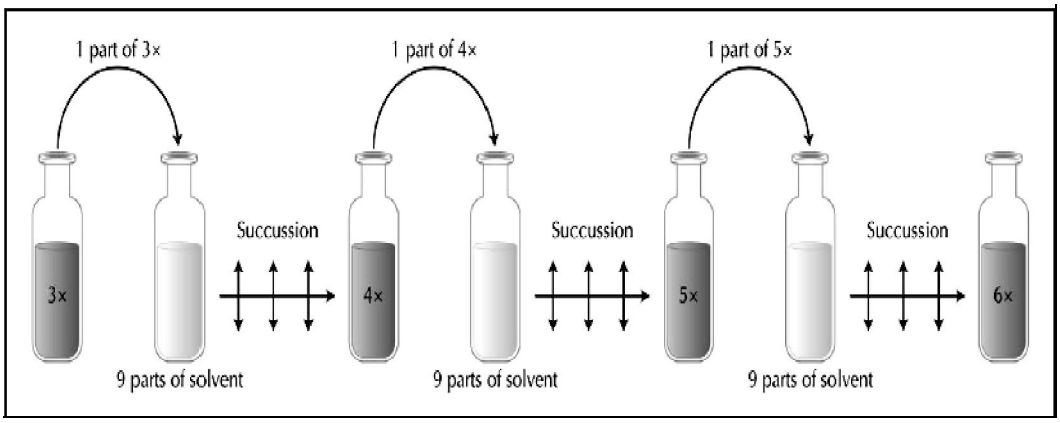


Figure 7: A Schematic representation of the method of preparation of homeopathic remedies (*Source*: Ricotti and Delanty, 2006).

It is believed that during the process of serial dilutions, bioenergy is transferred from the original substance to the solvent. The latter principle states that a minimal dose of a compound, which in a higher dose disturbed the biologic system, is used to cure it (homologous). A minimal dose of a different compound, which at a higher dose causes the same biologic disturbance, is used to cure it (heterogeneous). As a clinical example, homeopathic dilutions of bee venom appear to be capable of inhibiting cutaneous erythema induced both by bee venom itself (homologous) as well as by ultraviolet rays (heterogeneous) (Ricotti and Delanty, 2006). According to homeopaths, serial dilution, with shaking between each dilution, removes the toxic effects of the substance, while the essential qualities are retained by the diluent (water, sugar, or alcohol). The most frequently used homeopathic remedies in epilepsy are **silicea, cuprum, causticum, hyosciamus, Aethusa cynapium, Agaricus muscaricus, Artemesia absinthium, stramonium**, and **Cicuta virosa** (Ricotti and Delanty, 2006).

CUPRUM METALLICUM: Copper used as a homeopathic medicine gets the Latin name Cuprum metallicum. Copper is a metal with a characteristic red colour, very malleable, ductile and tenacious. At fresh air it's covered by green form hydrocarbonate (grayish green). It's found in the nature mainly in the state of pyrite of copper and iron sulphate, often associated to antimony, silver, lead and arsenic sulphates, and also in the state of oxide and hydrocarbonate. It is also present in the most of vegetal and animal food (Lathoud, 1980).

Copper acts on the spinal medulla and the sympathetic nervous system selectively, performing leading influence in the whole body. It also acts, in the motor and sensitive innervations and in the trophic enervation affecting the nutrition deep and directly (Lathoud, 1980). Cuprum is used in individuals who present with clonic spasm after mental and physical overexertion and patients who present after sleep deprivation. As children, these patients often suffered from febrile convulsions, especially during teething. If the physical and mental strain becomes prolonged, it could lead to a generalized seizure (Ricotti and Delanty, 2006).

1.5.4. Experimental models of epilepsy:

There are various experimental models of epilepsy according to the type of crisis that they can reproduce. A few chemically induced epilepsy models are Pentylenetetrazole- evoked seizures, Kainate evoked seizures, Picrotoxin-evoked seizures, Caffeine-evoked seizures, RDX-evoked seizures, (Desmond et.al., 2012).

Pentylenetetrazole -evoked seizures:

Pentylenetetrazole is a chemoconvulsant used to induce epilepsy in the laboratory. The use of the pentylenetetrazol for the discovery of new antiepileptic drugs began in 1944 when Everett and Richards demonstrated that trimethadione and

Phenobarbital, but not phenytoin, could block PTZ- induced seizures (White, 1998). PTZ is a selective blocker of the chloride ionophore complex to the GABAA receptor (Ramanjaneyulu and Ticku, 1984; Huang et al., 2001), it has convulsant effects after repeated or single dose administration and it affects several neurotransmitter systems, such as the GABAergic (Psarropoulou et al., 1994; Rocha et al., 1996a,b; Panagopoulos et al., 1998; Walsh et al., 1999), the adenosinergic (Pagonopoulou and Angelatou, 1998) and the glutamatergic system (Jensen et al., 1997; Ekonomou and Angelatou, 1999; Thomsen, 1999), in many brain areas including the cerebral cortex. Glutamine synthetase nitration in epilepsy was studied in PTZ model of epilepsy in rats as reported by Bidmon et.al. (2008). This model of epilepsy are used for the study of antiepileptic efficacy of different drugs for example: antiepileptic effects of quinine in the pentylenetetrazole model of epilepsy (Nassiri-Asl, 2009); anticonvulsant activities of various extracts of Erythrina sp. are studied using PTZ model of epilepsy (Nagaraja et.al., 2012).

1.5.5. Animal models of epilepsy:

As Nobert Weiner (1946) said, “The best model of a cat is a cat—preferably the same cat.” Unfortunately, using humans with epilepsy to identify basic mechanisms presents many problems, notably ethics, reproducibility, confounding effects of medication, and applying the experimental interventions needed to dissect the various cellular and synaptic processes that contribute to epileptic activity (Jefferys, 2003). An **animal model** is a living, non-human animal used during the research as well as investigation of human disease, for the purpose of better understanding of the disease, without any added risk of causing harm, to an actual human being during the process. Animal models are used to learn more about a

disease, its diagnosis and its treatment. Over the years numerous animal models have been developed to study the basic mechanisms of epilepsy (Holopainen, 2008) mainly in rodents, such as PTZ mediated changes in metabolomic profile in rat brain regions (Eloqayli et.al., 2003; Carmody and Brennan, 2010). Besides, there are many experiments carried out in mice as a model for studying various aspects during epileptic seizures (Li et.al., 2004). Mammalian neuronal cultures have been used for epileptic and antiepileptic studies (Macdonald and Barker, 1978). Invertebrate animal models such as *Helix aspera* have also been used to study the effects of epileptic drug at the neuronal level (Janahmadi et.al., 2006; Farajnia et. al., 2011). Mechanisms of epileptic activity in nervous systems were studied in the buccal ganglionic neurons of the snail *Helix pomatia* as a model system (Altrup, 2004). Williams et al (2004) reported that convulsions mimicking epilepsy can be induced by a mutation in a nematode *Ceanorhabditis elegans*, in combination with a chemical antagonist of gamma- aminobutyric acid (GABA) neurotransmitter signaling. Besides, Zebra fish (*Danio rerio*) has been used as an experimental animal model for epilepsy (Wong et.al., 2010).

With regard to discovery and testing of anticonvulsant substances the best results were achieved by implementation of experimental models. Animal models of epilepsy are useful in acquiring basic knowledge regarding pathogenesis, neurotransmitters (glutamate) receptors (NMDA/AIPA/kainate), propagation of epileptic seizures and preclinical assessment of antiepileptics (competitive and non-competitive NMDA antagonists).

Animal models of epileptogenesis provide a way to circumvent the practical issues associated with human studies by allowing the reproducible induction of epilepsy with short latent periods, reliable quantification of seizure burden and the

ability to correlate findings to histological measures. The investigator is in control of the epileptogenic insult making it possible to assess the pre-, developing and chronic epileptic states. The major work has been carried out in Rodents as animal models for epilepsy.

1.5.5.1. Epilepsy Research carried out in Rodent animal models:

The study of seizures in Mice was first reported by Dice (1935) and later studied by Watson (1939) for the deer mouse, *Peromyscus*, whereas in Rats it was reported earlier by Maier (1939). In the laboratory stocks of the house mouse, the occurrence of audiogenic seizures was first reported by Mirsky et.al. (1943). A detailed description of the behavior of mice subjected to relatively intense auditory stimulation and seizures were studied by Frings et.al. (1951). Kopeloff (1960) studied Chronic epilepsy in mouse by cerebral implantation of various powdered metals notably cobalt and nickel. In addition to spontaneous focal generalized seizures and epileptic status was evaluated by response, to challenge injections of Metrazol and semicarbazide, as well as to electroshock. The productions of Cobalt induced experimental epilepsy in rat were also reported by Dow et.al. (1962), exhibiting the ECoG changes consisted of slow waves, sharp waves and spikes and their climax, often accompanied by clonic movements of body musculature. Study of pathophysiological mechanisms of Cerebral haemorrhages provoked by reflex epileptic seizures in rats have been reported by Krushinsky (1962). Methoxypyridoxine convulsions have been studied in epileptic and non-epileptic Mice, showing the minimal effective dose of pyridoxine capable of preventing seizures (Kopeloff and Chusid, 1963).

Besides, L-Glutamic acid dose dependent seizures response were reported in rats by Hennecke and Weichert (1970) and they also reported that this model of seizures are useful to investigate metabolic events preceding seizures and also to test anticonvulsant drugs. In rodents, genetic manipulations relating to the expression or function of glutamate receptor proteins can be possible to induce epilepsy syndromes or raise seizure threshold as reported by (Meldrum et.al, 1999). The relationship of the PTZ-induced seizure sequence of myoclonus, clonus and hindlimb extension to brain PTZ levels has been reported in mice by Yonekawa et.al. (1980).

A study was carried out in mice, of the effects of certain drugs of the barbiturate and hydantoin groups, used in the treatment of grand-mal epilepsy, on the GABA content of normal cerebral hemispheres and cerebral hemisphere depleted of GABA (Saad et.al., 1972). The flash evoked after discharge (FEAD) has been proposed as a model of the petit mal seizure and the effects of d-amphetamine and apomorphine for controlling FEAD were reported (King and Burnham, 1980).

Behavioral, electroencephalographic and neuropathological responses to increasing doses of Pilocarpine administered to rats showed epileptiform activity in the limbic structures accompanied by motor limbic seizures, limbic status epilepticus and widespread brain damage (Turski et.al.1983). The genetically epilepsy prone (GEP) rat is susceptible to seizure induction by acoustic stimuli and the inferior colliculus has been implicated as being critically important in audiogenic seizure susceptibility (Faingold et al., 1986). Yet in another research carried out in rats, amygdala kindled female rats were used to compare the effects of seven antiepileptic drugs such as Phenobarbital, phenytoin, Carbamazepine, Valproic acid, Diazepam, Clonazepam, Primidone (Loscher et.al., 1986). Long term amygdala kindling in rats as a model for the study of interictal emotionality in temporal lobe epilepsy is been

reported. These long term kindled rats display profound changes in fearful and defensive behaviors which last for at least two months after the final stimulation (Kalynchuk, 2000). Edwards et.al. (2000) reported effects of seizures and kindling on reproductive hormones in rats.

The efficacy of ketogenic diet in controlling seizure and its role in amino acid metabolism has been demonstrated in rats (Thavendiranathan, 2000). The influence of Caloric restrictions on seizure susceptibility was investigated at both juvenile and adult in the EL mouse, a genetic model of multifactorial idiopathic epilepsy (Greene et al, 2002). Besides, as reported by Drage et.al. (2002), EL mouse can be a useful model for evaluating neuron-glia interactions related to idiopathic epilepsy. Also, genetic model of absence epilepsy is been exhibited by WAG/Rij strains of rats (Coenen and van Luijtelaar, 2003). Altered function of neuronal acetylcholine receptors in the brain has recently been associated with an idiopathic form of partial epilepsy and the antagonist for this receptor were evaluated in Mice and Rats, to study its involvement in generalized epilepsy and the information could be used for therapeutic approach (Loscher et.al., 2003).

Topiramate, a new generation antiepileptic drug was investigated for its anticonvulsant effects in various models of (genetically determined and chemically induced) epilepsy in rodents (Russo et al., 2004). Effects of novel antiepileptic drug Lacosamide were studied on the development of amygdala kindling induced epileptogenesis in rats (Brandt et.al., 2006). Behavioral alterations in the Pilocarpine model of temporal lobe epilepsy in mice has been reported by Groticke et.al., (2007). Voss et.al. (2009) reported the effects of gap junction blockade on seizure-like activity in rat and mouse cerebral cortex slices.

1.5.6. Need for additional animal models of epilepsy:

Control of epilepsy has primarily focused on suppressing seizure activity by antiepileptic drugs (AEDs) after epilepsy has developed. AEDs have greatly improved the lives of people with epilepsy. However, the belief that AEDs, in addition to suppressing seizures, alter the underlying epileptogenic process and, in doing so, the course of the disease and its prognosis, is not supported by the current clinical and experimental data. An intriguing possibility is to control acquired epilepsy by preventing epileptogenesis, the process by which the brain becomes epileptic. A number of AEDs have been evaluated in clinical trials to test whether they prevent epileptogenesis in humans, but to date no drug has been shown to be effective in such trials. Thus, there is a pressing need for drugs that are truly antiepileptogenic to either prevent epilepsy or alter its natural course. For this purpose, animal models of epilepsy are an important prerequisite (Loscher 2002). The need to develop better animal models of epileptogenesis and chronic epilepsy has been widely acknowledged. Pre-clinical evaluation for new antiepileptic drugs (AEDs) requires experimental models to assess the spectrum of antiepileptic activity (Meldrum, 1986). However, what a better model might look like largely depends on what aspect of the epileptic condition is intended for modeling. Merely reproducing a presumed etiological factor, such as status epilepticus, cortical malformation, stroke, fever or traumatic brain injury may not be that challenging. However, as the ultimate goal of an animal model is to understand the pathophysiology of the disease and to develop effective therapeutic interventions, a better model of epileptogenesis would be one that comes closest to reproducing the mechanisms and pharmacological profile.

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Currently, the kindling model and post-status models, such as the pilocarpine (Leite and Cavalheiro, 1994) or kainate models (Ben-Ari and Cossart, 2000) are the most widely used models for studies on epileptogenic processes and on drug targets by which epilepsy can be prevented or modified. Furthermore, the seizures in these models can be used for testing of antiepileptic drug effects (Loscher, 2002). A comparison of the pharmacology of chronic models with models of acute (reactive or provoked) seizures in previously healthy (non-epileptic) animals, such as the maximal electroshock seizure test, demonstrates that drug testing in chronic models of epilepsy

yields data which are more predictive of clinical efficacy and adverse effects, so that chronic models should be used relatively early in drug development to minimize false positives (Loscher, 2002). Interestingly, the pharmacology of elicited kindled seizures in fully kindled rats and spontaneous recurrent seizures in post-status models is remarkably similar. However, when these models are used for studying the anti-epileptogenic effects of drugs, marked differences between models exist, indicating that the processes underlying epileptogenesis differ among models, even among different post-status models of TLE. A problem for clinical validation of TLE models is the lack of an AED, which effectively prevents epilepsy in humans (Loscher 2002). Thus, at present, it is not possible to judge which chronic model is best suited for developing new strategies in the search for anti-epileptogenic and disease-modifying drugs, but rather a battery of models should be used to avoid false negative or positive predictions (Loscher, 2002).

1.5.7. Invertebrates in Biomedical research and neuroscience:

Invertebrate animals have been used as medicinals for 4,000 years and have served as models for research and teaching since the late 1800s. Interest in invertebrate models has increased over the past several decades as the research community has responded to public concerns about the use of vertebrate animals in research. As a result, invertebrates are being evaluated and recognized as models for many diseases and conditions. Their use has led to discoveries in almost every area of biology and medicine from embryonic development to aging processes. Species range from terrestrial invertebrates such as nematodes and insects to freshwater and marine life including planarians, crustaceans, molluscs, and many others (Wilson-Sanders, 2011). The most often used models are the fruit fly *Drosophila melanogaster* and the

minuscule nematode *Caenorhabditis elegans* (Nass et. al., 2008; Wilson-Sanders, 2011).

Invertebrates can serve as replacements for their vertebrate counterparts in many areas of research, testing, and education (Wilson-Sanders, 2011). Invertebrates may also play a pivotal role in toxicity and efficacy testing of new pharmaceuticals for both human and animal diseases, sparing vertebrate animals from preliminary testing (Wilson-Sanders, 2011). The fruit fly (*Drosophila melanogaster*) is one of the most studied organisms in the animal kingdom (Wilson-Sanders, 2011). Cytogenetic research has led to the complete mapping and sequencing of its chromosomes, enabling its use in an array of biological and biomedical investigations (Gilbert, 2008). *Caenorhabditis elegans* models have many advantages over vertebrate animals for use in biological and biomedical studies (Nass et al, 2008). These small worms are highly prolific reproducers with a short generation time, easily grown under laboratory conditions, and inexpensive to care for (Riddle 1997; Wood 1997; Nass et al. 2008). Additionally, *C. elegans* is anatomically simple and has a fully mapped nervous system (White et al. 1976). Humans and *C. elegans* have virtually the same number of genes, and there are many parallels in the ways that these divergent species operate on genetic and molecular levels (Nass et.al., 2008). As a result, *C. elegans* has become an instrumental model for understanding the molecular mechanisms involved in many human diseases (Nass et al. 2008).

The California sea slug (*Aplysia californica*), opalescent sea slug (*Hermissenda crassicornis*), and pond snail (*Lymnaea stagnalis*)—all gastropod molluscs—have served as models in studies of neuronal mechanisms of learning and memory (Alkon 1987; Lukowiak et al. 1996; Glanzman 2006, 2008, 2009). Drosophila models will also play a vital role in the identification and evaluation of

new therapies for neurological diseases, providing a preliminary animal model before the use of mammals (Marsh and Thompson 2004, 2006; Whitworth et al. 2006). *C. elegans* is an important model for understanding the pathophysiology and molecular mechanisms of neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's (Troulinaki and Tavernarakis, 2005; Johnson et al. 2010). Additionally, octopi and squids are excellent models for neural electrophysiology, neurochemistry, and neurosecretion (Young 1967, 1971; Packard 1972; Sanders et al. 1975). Research utilizing the long-finned squid (*Loligo pealei*) in nerve conduction studies garnered Andrew Huxley and Alan Hodgkin the 1963 Nobel Prize for Medicine. The brain of freshwater planarians (Platyhelminthes) may appear to be very simple in comparison to that of humans, but these organisms have many neural genes and transcription factors that are homologous to those that cause pathology in humans (Cebria, 2007). Furthermore, planarians are known for their ability to regenerate even their central nervous system (CNS) and researchers using gene silencing techniques hope to elucidate the molecular mechanisms and genes that enable the animal's regeneration (Cebria, 2007). Understanding this process may yield important information for treating human CNS injuries and disease. The highly sensitive auditory system of the cricket can be an effective model in studies of the development of dendrites and their response to injury (Horch et al. 2009). The cricket has also contributed to the understanding of adult neurogenesis (the production of new neurons throughout life), which occurs in most species including humans (Cayre et al. 2007). Invertebrate neuronal cells are also used as experimental models for studying the action of epileptogenic drugs in order to gain a better understanding of the neurophysiological basis of epilepsy (Sugaya et.al., 1973; Faugier-Grimaud, 1974); besides, they are also used to determine the mode of action of anticonvulsants on bioelectrical activity

(Faugier-Grimaud, 1974). Farajinia et.al, (2011) demonstrated that sub esophageal ganglion of *Helix aspera* shows paroxysmal depolarization shift (PDS), on treatment with PTZ an epileptogenic drug, that resembles closely with the epileptic activity in mammalian neurons.

1.6. Need for more epilepsy studies:

Epilepsy is a symptom of numerous disorders, but in the majority of sufferers the cause remains unclear despite a careful analysis of history, examination and investigation of the cases. Even a cursory glance at Fig:8 explains the need of more and more epilepsy studies. Since in 75% cases, the cause of epilepsy are not ascertained indicating the dearth of information on etimology of this disease.

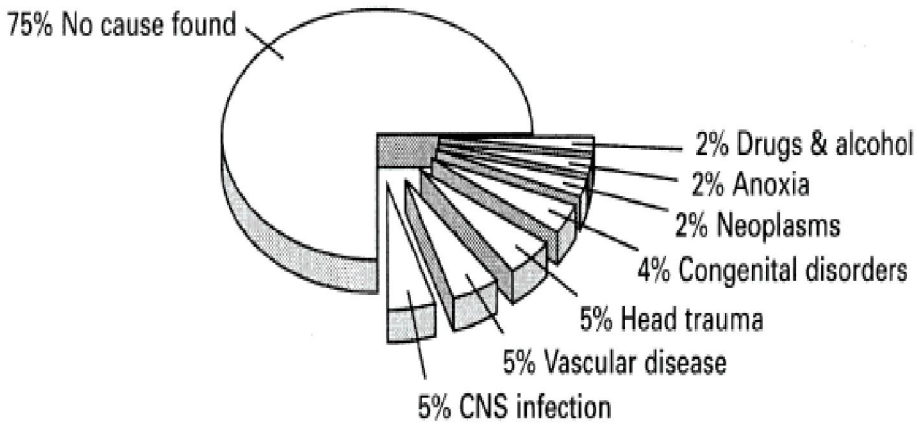


Figure: 8 Probable causes of epilepsy

(**Source :** http://www.med.muni.cz/~mbrazd/epilepsy.ppt)

Over the past few decades, intensive biological research on epilepsy has been carried out in the field of electrophysiology, while the biochemical approaches have been under examination since 1950s. The advantage of biochemical research is that it investigates biological phenomena in terms of the substances involved. Despite the folkloric importance of homeopathic medicine, there is a dearth of literature on the pharmacological effects of homeopathic preparations on epilepsy either alone or in combination with some potent drug like valproate. The present study is, therefore, designed to investigate the anticonvulsant potential of Cuprum metallicum (homeopathic preparation) alone and in combination with sodium valproate, a widely used antiepileptic drug (AED) against pentylenetetrazole (PTZ) mediated generalized epilepsy.

1.7. CHOICE OF PARAMETERS IN THE PRESENT STUDY:

Epilepsy is a brain disorder in which neurons, in the brain sometimes signal abnormally. Epileptic seizures develop as a result of both enhancement of excitatory mechanisms and impairment of inhibitory mechanisms between neurons. It is possible to induce such state in the brain by using convulsant drug on the animal models for epilepsy. Therefore, in the present study two experimental animals i.e. an invertebrate (Crab) and a vertebrate model (Mouse) have been opted.

Till today most of the work in epilepsy is carried out in rodents, but there are some invertebrates used for such study like *Helix pomatia*, yet the mechanisms involved during seizures are not known completely, leading a scope of more research. There is a need of more extensive research using various animal models, for both physiological as well as biochemical studies in order to understand the mechanisms involved in brain during expression of epilepsy as well as during treatment with

antiepileptic drugs. Therefore, in the present work, an attempt is made to develop a new model like Mud crab *Scylla serrata* by inducing PTZ mediated epilepsy as *Scylla serrata* is considered as a higher invertebrate having the structure required for the seizure to occur. Also, attempt is made to study epilepsy in Mice *Mus musculus* especially with reference to few metabolic parameters during PTZ mediated epileptic seizures, so as to throw more light on the ill effects of seizures on the brain metabolic pathways.

Pentylenetetrazole (PTZ) is a **chemoconvulsant drug** used experimentally to study expression of seizures and to identify pharmaceuticals that control them (White, 1998). PTZ treatment leads to the convulsions in experimental animals that resemble the one that occurs in Human epilepsy. Therefore, in the present study PTZ is used as the chemoconvulsant drug for inducing a generalized epilepsy.

According to Dr. Robert Fisher (2009), who is an epilepsy specialist, there is no formula to choose for seizure medicine to use for a particular patient. No one medicine dominates for effectiveness, and all have various side effects. Doctors and patients choose AEDs after considering which side effects should be avoided in particular cases, convenience of use, cost and physician's experience. Epilepsy Research Association UK was formed following the merger of the Epilepsy Research Foundation. The Funds for Epilepsy are made available to unravel some of the questions like what causes epilepsy, who gets it, what goes on in the brain during a seizure, and safer drugs and better surgical techniques to treat it. Considering this, an attempt is made to study the efficacy of Homeopathic preparations such as Cuprum Metallicum to protect the experimental animals from the convulsion seen during PTZ mediated epileptic seizures. Since homeopathic preparations are believed to be safe and are free from side-effects owing to extreme dilutions of the chemicals. Also, the

combined effect of this homeopathic drug with Sodium Valproate a known antiepileptic drug that is used to treat most of the type of epilepsy in humans is tested using the experimental models in the present study, since combinations of homeopathic and allopathic preparations are considered to be suitable and need of the hour.

In case of Crab, it was easy to expose Cerebral ganglion for surface electrical recordings during both epileptic as well as antiepileptic treatment, and hence, it was used for recording electrical activities. Similar recordings of the electrical events in the neurons or of the neurons of higher vertebrates including human are not possible. Besides the behavioral study in Crab, the biochemical assays of a few metabolic enzymes, related metabolites as well as the Total ATPases and electrolyte contents from both ganglia were carried out during various treatments as such type of work is not reported till this date. Similarly, the identical biochemical analysis in various regions of brain of Mice was carried out during epileptic and antiepileptic treatment. Due to complicated nervous organization and difficulty to record effectively the surface electrical recordings in brain of Mice, with reduced trauma in the experimental model. The electrical activities of the mice brain were avoided and emphasis was given on biochemical analysis of epilepsy and AEDs.

1.8. Aims and Objectives:

1. To induce epilepsy in Invertebrate and Vertebrate models i.e., in Crab ***Scylla serrata*** and Mouse ***Mus musculus***.

2. To study the effects of epilepsy on electrical activity in Crab ganglion and metabolism of neuronal tissues of Mice.

Invertebrate tissue (Crab): **Cerebral ganglion**, **Thoracic ganglion**

Vertebrate tissues (Mice): Discrete brain regions such as **Olfactory lobe**, **Cerebral cortex, Corpus callosum, Cingulate gyrus, Hippocampus, Corpora quadrigemina, Cerebellum, Pons, Medulla Oblongata.**

3. To evaluate the effects of **antiepileptic drugs** on Electrophysiology and Biochemistry vis-à-vis metabolism of neurons, i.e., of the brain regions of the chosen animal models.

Hypothesis 1:

1. It is possible to induce epilepsy in Crab.

2. Behavioral studies, surface electrical recordings, biochemical assays can establish crab as a model for epilepsy.

3. Screening of few antiepileptic drugs would help test the applicability of new model for epilepsy.

Hypothesis 2:

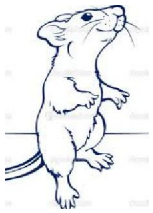
1. Inducing epilepsy in mouse (*Mus musculus*), a known model for epilepsy, would throw more light on the epileptic seizures.

2. Metabolic activities are believed to be altered in the discrete brain regions during epilepsy as well as during treatment. Therefore, it is essential to understand the

metabolic variables during the epilepsy vis-a-vis seizures particularly with reference to a few metabolites and metabolic enzymes in discrete brain regions.

3. Homeopathic preparations are believed to be effective in containing epileptic seizures; however, there is no scientific experimental proof for their efficacy. Hence, one could test one of the homeopathic preparations in Mice, after screening the preliminary effects in Crabs (*Scylla serrata*).

MATERIALS AND METHODS



2.1 Experimental Animals:

Two different experimental animals were used for neuropharmacological studies of epilepsy. An Invertebrate animal (Crab) and Vertebrate animal (Mice).

2.1.1 Mud Crab (*Scylla serrata)*

Scylla serrata **(Forkssal 1775) (**often called Mud Crab or Mangroove Crab as well as Black Crab) is an economically important Crab species found in the estuaries and mangroves of Africa, Australia and Asia. In their most common form, the shell colour varies from a deep, mottled green to very dark brown.

Scientific Classification:

Kingdom	**:** Animalia
Phylum	**:** Arthropoda
Subphylum	**:** Crustacea
Class	**:** Malacostraca
Order	**:** Decapoda
Infraorder	**:** Brachyura
Family	**:** Portunidae
Genus	**:** ***Scylla***
Species	**:** ***serrata***

S. serrata can be kept easily in home aquaria when smaller, but tends to outgrow very soon in small setups. They are very active and eat almost any conventional sinking pellets; they also appreciate some small fish pieces and vegetable matter. They are tolerant to most water conditions and are generally a very hardy and entertaining species. It is easy to study the behaviour of Crab in the laboratory because of its easy maintenance and acclimatization that it shows to the

environment. Therefore, it is opted as an experimental animal for the study of epilepsy, as it is known to have more developed nervous system compared to other invertebrates, yet simpler form of nervous organization as compared to the higher vertebrate rodent models.

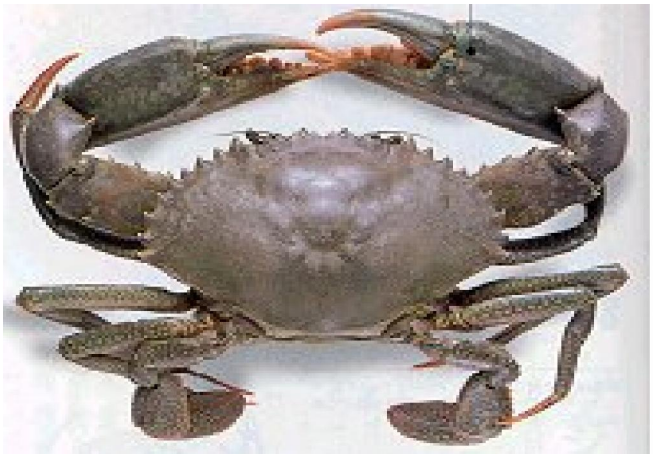


Figure 9: Mud Crab *Scylla serrata*

Adult male and female Mud crabs ranging in weight from 100 – 120 g with a carapace width 6.0 to 8.0 cm, were collected from the field and maintained in the laboratory on a 12:12 h light: dark cycle, in well aerated glass tanks (42cm x 90 cm x 42cm) (10 animals/ tank) filled to a depth of 4 cm with artificial sea water, pH 7.4-7.6.The water temperature was maintained between 22-24^{o}C. Experiments were carried out on crabs after a minimum of one week of acclimatization to the laboratory conditions. Experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, USA and Institutional Animal Ethical committee regulations.

Tissues such as Cerebral ganglion and Thoracic ganglion were considered for the present study in Crab. Cerebral ganglion was used for surface electrical recordings due to its easy access whereas; thoracic ganglion was used for the biochemical study

along with cerebral ganglion during various treatments as mentioned in the protocol below. Besides, it was noted that the electrical recordings of both the thoracic and cerebral ganglia were always similar during epilepsy in invitro state. Therefore, it was believed that both the types of ganglia would respond biochemically in the similar manner during epilepsy.

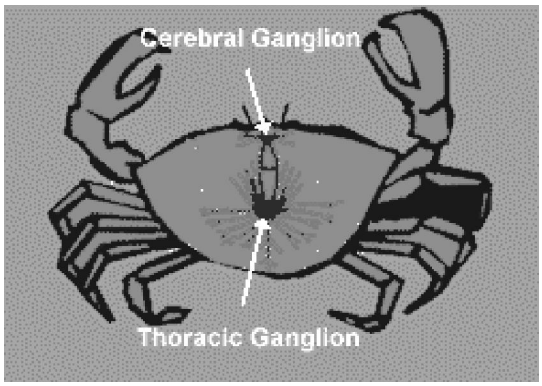


Figure 10: showing the Cerebral Ganglion and Thoracic ganglion

2.1.2 Mouse (*Mus musculus*)

A **mouse** (plural: **mice**) is a small mammal belonging to the order of rodents, characteristically having a pointed snout, small rounded ears, and a long naked or almost hairless tail. Mice are common experimentals animals in biology primarily because they are mammals, and also because they share a high degree of homology with humans.

Scientific Classification:

Kingdom : Animalia

Phylum : Chordata

Class : Mammalia

Order : Rodentia

Family : Muridae

Genus : *Mus*

Species : *musculus* (Linnaeus, 1758)

Figure 11: Mouse *Mus musculus*

Experiments were carried out on Mice (*Mus musculus*) weighing 25-30 g. The animals were housed in groups (n=5) to acclimatize to the laboratory conditions for seven days before the start of the experiment. The animals were kept on a 12 – h light/ dark cycle and at 22±1^{0}C with a free access to food and water. All experiments were performed between 10.00 am to 14.00 pm in a silent room at 22-24^{0}C. All animal use and procedures were approved by the Institutional ethical committee.

2.2. DRUGS USED:

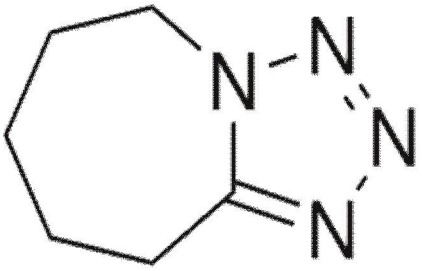
Drugs used for the present investigations were Pentylenetetrazole (Convulsive drug), Sodium Valproate (antiepileptic drug) and Cuprum metallicum (antiepileptic Homeopathic preparation).

2.2.1 PENTYLENETETRAZOL

Pentylenetetrazol is a drug used as a circulatory and respiratory stimulant, non-specific CNS stimulant and convulsant particularly high doses cause convulsions. It has been used in convulsive therapy, but was never considered to be effective, and side-effects such as seizures were difficult to avoid. Its approval by the FDA was

revoked in 1982. Pentylenetetrazole was obtained from Sigma-Aldrich, USA (Product code no: P6500).

Chemical structure:



Systematic (IUPAC) name: 6,7,8,9-Tetrahydro-5H-tetrazolo(1,5-a)azepine

Synonyms: 1,5- pentamethylenetetrazole; α,β-cyclopentamethylenetetrazole; Pentamethazol; Pentamethazolum; Pentamethylenetetrazal; Pentamethylenetetrazole; Pentamethylene-1,5-tetrazole; 6,7,8,9-tetrahydro-5-azepotetrazole; 6,7,8,9-tetrahydro-5H-tetrazoloazepine; 6,7,8,9-tetrahydro-5H-tetrazolo[1,5-a]azepine; Angiazol; Angioton; Angiotonin; Cardiazol; Cardiazole; Cardiol; Metrazole; Nauranzol; Naurazol; Pentrasol; Penetiazol; Pentacard; Pentazol; Pentazolum; Pentetrazol.

Physical properties: Colourless crystal or white crystalline powder, soluble in water.

Chemical data: Empirical Formula - $C_6H_{10}N_4$

Molecular mass - 138.17

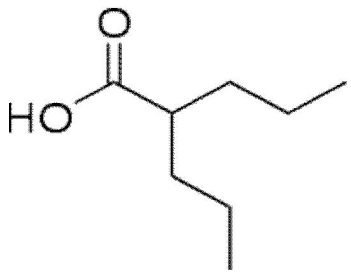
2.2.2. SODIUM VALPROATE:

Valproic acid (VPA) is a chemical compound and an acid that has been found suitable for clinical use as an anticonvulsant and mood-stabilizing drug, primarily in

the treatment of epilepsy, bipolar disorder, and less commonly in a major depression. It is also used to treat migraine headaches and schizophrenia. Valproic acid reacts with a base such as sodium hydroxide to form the salt **sodium valproate**, which is a solid.

Formulations: Branded products include: Convulex; Depakene; Depakine; Deprakine; Encorate; Epival; Epilim; Stavzor; Valcote, Valparin.

Chemical structure:



Systematic name: 2 - propylpentanoic acid

Valparin 200 was obtained from local commercial pharmacy (Company name: **Sanofi-synthelabo**; Batch number: 059013).

2.2.3. CUPRUM METALLICUM:

This was obtained from Kamaxi Homeopathic College, Shiroda, Goa, India and was manufactured as a dilution (6 CH potency) by Dr. Wilmar Schwabe India, Pvt. Ltd. with batch no: PA844. Potentised medicines are called Dilutions. Dilutions are the core of homeopathy treatment.

2.3. DOSAGE DETERMINATION OF DRUGS FOR THE EXPERIMENTAL MODELS:

Initial experiments were performed to screen drugs of interest for behavioral effects. These experiments were conducted for each drug on ten animals per batch. Groups exposed to various drugs at different concentrations were examined to catalogue the nature and type of movements and/or postures expressed, as a function of a concentration of the drug tested. These expressions were of the epileptic seizures and of prevention of seizures in case of anticonvulsant drugs. Drugs were screened in preliminary experiments to correlate them with the behaviours after their administration (Table 1). The test concentrations chosen for analysis were based on concentrations known to evoke behaviours in similar experiments on other rodents (White, 1998) and invertebrate models such as mollusca (Altrup, 2004) for epilepsy. The medium effective dose (ED_{50}) and 95% confidence interval were considered in the present study.

2.3.1. Determination of Dosage of Pentylenetetrazol (PTZ) in Crabs and Mice to induce epileptic seizure:

2.3.1.1 Induction of epileptic seizures in crabs using PTZ:

The preliminary experiments were designed to record behaviours associated/ correlated with the doses and duration of administration of PTZ. The test concentrations chosen for analysis were based on concentrations known to evoke epileptic behaviours in similar experiments on other invertebrate animals like mollusc snail, *Helix pomatia* (Altrup, 2004). In *Helix pomatia* buccal ganglion were bathed with solutions containing 1mM to 40 mM PTZ in order to induce epileptiform activity. Considering above reference, various concentrations of PTZ were used such

as 40, 50, 60, 70, 80, 90, 100, 110 mM/ litre PTZ (n=10 per concentrations) prepared in Crab Physiological saline, respectively, and the electrical potentials developed correlated with injection of PTZ drug, are plotted in the form of Dose-Response curve (Fig:12) and the behaviours exhibiting seizures in crabs are listed in Table 1. The screening of different doses of PTZ, revealed better epileptic responses in crabs treated with PTZ at concentration of 100 mM/litre (Therisa and Desai, 2011). The doses eliciting epileptic seizures in 97% of the test animals were considered as convulsive dose (CD97) as reported by White (1998) in rodents and the effects were observed for 30 mins from the time of injection of drug in Crabs. Therefore, 100 mM/litre PTZ was considered as CD97 in case of Crabs as they exhibited epileptiform activity within 30 mins of the administration of the drug without any mortality of the test animals, and regained normalcy after 30 mins period.

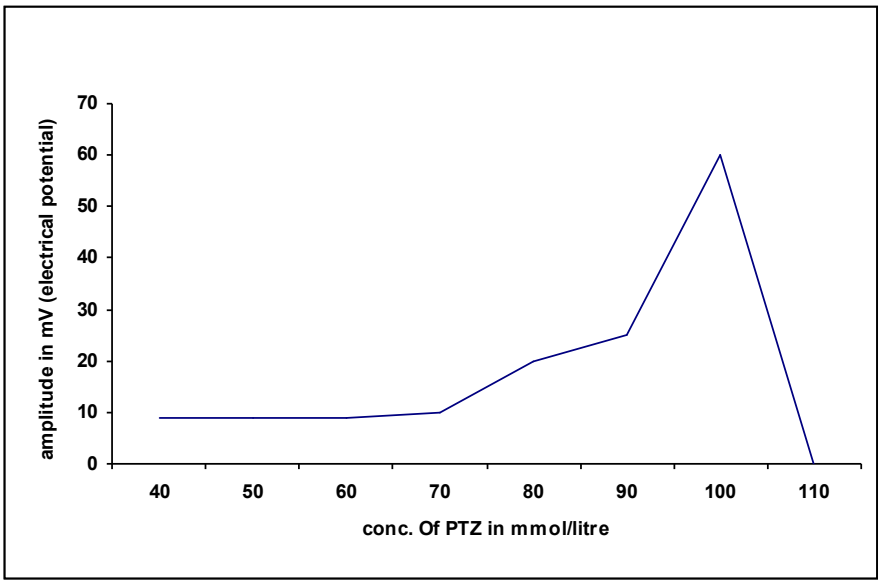


Figure: 12 Effects of different concentrations of PTZ (based on development of high electrical potentials at the surface of Crab Cerebral ganglia).

2.3.1.2. Induction of epileptic seizures in Mice using PTZ:

The dosage of PTZ for mice was determined on the basis of earlier report on rodents (White, 1998). The convulsive dose that elicits seizures in 97% of the mice was considered as CD97. The mice were subjected to different concentrations of PTZ and a dose- response curve was plotted to determine the effective dose. As per the dose-response curve (Fig: 13) a dose equivalent to 70 mg/kg body weight was selected for subsequent studies, since it produced seizures in majority of the animals and was not lethal to the test animals. The subcutaneous injection of PTZ test provides a quantal evaluation of the ability of a drug to promote seizure threshold. Since it is known that the seizure threshold can vary among various rodent strains, it becomes imperative that each individual laboratory establishes its own CD97 values in the particular mouse or rat strain that they intend to use (White, 1998). A set of ten animals was used for every desired behavioral study.

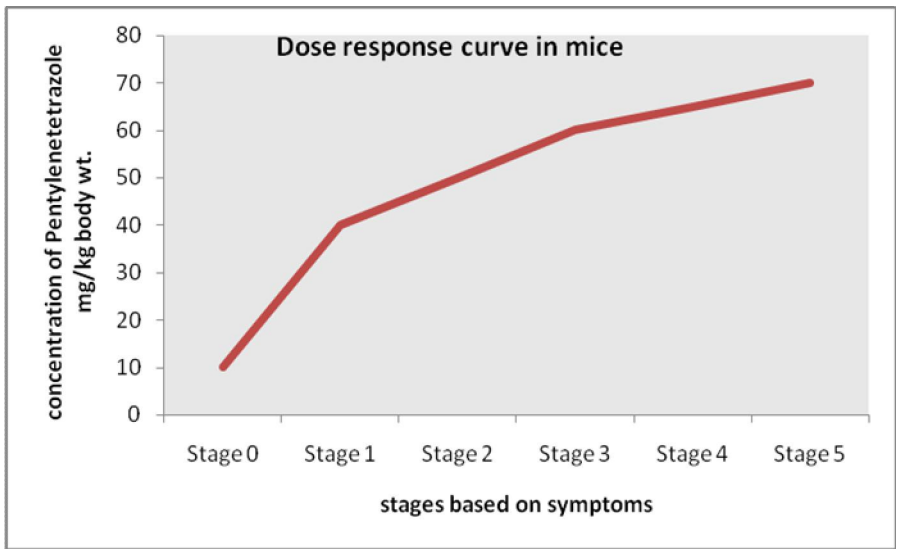


Figure: 13 Dose-response curve for Pentylenetetrazole treated mice, as per the stages described in section 2.5 of this chapter.

Table: 1 Few Convulsive responses considered as a representative for PTZ mediated epileptic seizures in Crab and Mice

Sr .no.	Epileptic behaviors of Crabs	Epileptic behaviors of Mice
1.	Body jerks.	Body jerks (myoclonic).
2.	Brief loss of balance.	Brief loss of balance.
3.	Oozing of froth from mouth.	Depressed locomotion.
4.	Rapid movements of antennules	Tonic extension of forelimbs and hindlimbs.
5.	Rapid retractions of the eyes.	Clonic forelimb and hindlimb seizures.
6.	Wide lateral extension of the chelae.	Loss of righting reflex.
7.	Defecation.	Defecation.

2.3.2. Determination of Dosage of Sodium Valproate for Crabs and Mice:

The concentrations normally used (400-600 mg/day, Jeavons and Clarke, 1974) to treat generalized epilepsy in humans were considered for converting them to doses equivalent to mg of drug per kilogram of an average body weight of human, and then equivalent doses were given to Crabs and Mice (n=10) across a wide variety of sizes. The doses thus determined were administered in order to study their protective efficacy against epileptic seizures induced by PTZ. Besides, these doses were not lethal to the test animals. Therefore, in Crab it was observed that 120 µmol/litre (200

mg/kg body weight) and in Mice 200 mg/kg body weight were effective doses that could protect the animals from PTZ mediated seizures.

2.4. EPILEPTIC AND ANTIEPILEPTIC TREATMENTS TO CRABS AND MICE:

2.4.1. Solutions :

Crab Physiological Saline (Cooke et al. 1989): Crab physiological saline was prepared by adding NaCl 440.0 mmol, KCl 11.3 mmol, $CaCl_2$ 13.3 mmol, $MgCl_2$ 26.0 mmol, Na_2SO_4 23.0 mmol, HEPES 10.0 mmol, in deionised water and pH adjusted to 7.4 with 0.1 N NaOH.

PTZ test solution: Pentylenetetrazole was obtained from Sigma Chemical Co, (US) (product code no:P6500). PTZ (100 mmol/liter) was prepared in Crab Physiological Saline (CPS) for inducing epileptic seizures in Crab. PTZ (70 mg/kg body weight) was prepared in mammalian saline (0.9% NaCl) for injecting subcutaneously in mice for inducing generalized tonic-clonic epilepsy.

Sodium Valproate test solution: Sodium Valproate (Valparin 200**)** tablets were obtained from a local pharmacy (Manufactured by: **Sanofi-synthelabo , Batch no: 059013**). Sodium Valproate (120 µmol/litre equivalent to approx. 200 mg/kg body weight) was prepared in CPS for treatment to Crabs. Sodium Valproate (200 mg/kg body wt.) was prepared in mammalian saline for Mice.

Homeopathic preparations: Homeopathic preparations such as Cuprum metallicum (6 CH potency) were procured for the present study. For treating Crabs, 1:1 dilution

i.e., 1 part of Cuprum metallicum mixed with 1 part of Crab saline were used. Whereas, for treating mice, one part of Cuprum metallicum (6 CH potency) was mixed with 99 parts of mammalian saline, thus, after dilution of Cuprum metallicum the final concentration of the preparation became 7 CH potency.

Usually, homeopathy medicines are prescribed for humans based on the type of symptoms and the history of the patients. In the present study, dosage was decided for the test animals by trial and error method after trying various potencies. Therefore, effective doses that reduce the seizure threshold induced by PTZ, without causing mortality to the test animals, were used.

2.4.2. Mode of Drugs delivery in Crab (*Scylla serrata*) and Mice (*Mus musculus*):

2.4.2.1. Drug delivery in Crab: (Wood et.al. 1995)

All drugs were injected into the infrabranchial sinus using a 22 gauge needle inserted through the membrane between the cephalothorax and the coxa of the fifth leg, in order to study the behavioral changes as well as for biochemical studies. But for electrical recording, drugs were applied directly onto the desheathed ganglia. The needles used for infrabranchial injection were of such a length that the release of the drug was within millimeters of the heart and thus, was distributed through the circulation (Wood et al. 1995). The volume injected into both the treated and control animals were 0.5 ml (V/V).

2.4.2.2. Drug delivery in Mice:

A subcutaneous dose of PTZ sufficient to induce seizures in 97% of the test animals was referred to as the convulsive dose 97 (CD97). At the time of the test, PTZ

(70 mg/kg) was injected sub-cutaneously into the loose fold of skin in the midline of the neck. The site of injection was massaged in order to distribute the PTZ solution prepared in saline throughout the subcutaneous tissue. If the PTZ solution is injected into adipose tissue and care is not taken to distribute it by massage, the likelihood of false positives is increased (White, 1998). PTZ was administered in a volume of 0.01 ml/g body weight. Mice were then placed in separate cages and observed over the course of next 30 minutes for the presence or absence of tonic- clonic seizures. Since a brief episode of seizures persists for only 3 to 5 seconds, it was imperative to pay extremely close attention to each animal. Animals not displaying a minimal clonic seizure are considered protected.

2.4.3. Drug treatments to Crabs (*Scylla serrata*) and Mice (*Mus musculus*):

The animals were randomly divided into groups:

(a) **Controls:** The animals (n=5) with no previous history of seizures were chosen and administered saline (0.9% NaCl in case of mice and Crab Physiological saline in case of Crab) injections.

(b) **Experimentals A (Group I):** The animals (n=5) received PTZ in the desired concentrations as described earlier through its respective vehicle i.e. Crab's received Crab Physiological Saline and Mice received Mammalian saline.

(c) **Experimentals B (Group II):** The animals (n=5) received only sodium valproate in doses described earlier prepared in respective Saline for respective animals (crabs and mice).

(d) **Experimentals C (Group III):** These animals (n=5) received cuprum metallicum in doses described earlier and mixed in respective saline (with final potency of 7 CH) for the specific group.

(e) **Experimentals D (Group IV):** These animals (n=5) (only mice) received equal quantity of both Sodium valproate and Cuprum metallicum in doses described earlier and mixed in saline (1:1 v/v) in order to study the combination of allopathic and homeopathic drug effects.

(f) **Experimentals E (Group V):** These animals received Sodium valproate followed by PTZ, after 1 hr of valproate injection in doses described earlier.

(g) **Experimentals F (Group VI):** These animals received Cuprum metallicum followed by PTZ after 1 hrs of injecting cuprum metallicum in Crabs and 2hrs in mice in their respective doses as described earlier .

(h) **Experimentals G (Group VII):** received by mice only, the mixture of Sodium valproate and Cuprum metallicum followed by PTZ after 2 hrs of injecting Sodium valproate +Cuprum metallicum in doses described earlier.

Similarly, Sodium valproate and Cuprum metallicum were given s.c. prior to PTZ and then observed for any behavioral changes. Animals not displaying a minimal clonic seizure were considered to be protected. Similarly, parallel experiments were carried out in controls by giving saline injections subcutaneously.

2.5. BEHAVIORAL STUDY IN CRABS AS WELL AS MICE:

In order to study the convulsive response after administration of the drugs, the physically isolated individual animals were maintained. If the animals are aggregated, they display a different response than when tested individually (George and Wolf,

1966). For example, when placed in a group, a seizing animal may lower the threshold of remaining animals. Thus, a relatively small drug-induced elevation of seizures thresholds could be masked by such an aggregate effect. Likewise, a drug-induced lowering of seizure threshold could be artificially enhanced by an aggregate effect. The behavioral changes (Table:1) of crab were recorded, when kept in aquarium tank having water and large stones. Besides, their behavioral changes during treatment were recorded when they were exclusively out of water. The behavioral changes under such conditions were minutely observed for any difference under the two set conditions.

Similarly, behavioral changes were studied in mice placed in individual cages for 30 minutes after the s.c. injection of PTZ as it was proved already that PTZ shows effect for 30 min (White, 1998). Yet, animals were observed for any other symptom for next few hours.

Racine's five point scale (Racine 1972), modified by Fathollahi et.al. (1997) was used for quantification of behavioral seizures in mice:

Stages 0 : no response;

Stage 1 : ear and facial twitching;

Stage 2 : myoclonic jerks, without upright position;

Stage 3 : myoclonic jerks, upright position with bilateral forelimb clonus;

Stage 4 : Clonic-tonic seizure;

Stage 5 : generalized clonic-tonic seizures, loss of postural control.

Seizure susceptibility was based on the latency, duration, total duration, number and severity of seizures during the various treatment regimens. The seizure severity was based on a maximal seizure score graded from 0 to 5, as described

previously (Lothman and Collins, 1981; and Sookyong and Frances, 2001; Hoffman et.al., 2003).

2.6. ELECTROPHYSIOLOGICAL TECHNIQUE USED FOR CRAB CEREBRAL GANGLION:

2.6.1 Experimental design:

Crabs were cold narcotized and immediately after dissection, cerebral ganglion was exposed and desheathed carefully for the invivo surface electrical recordings under various drug regimens in order to study the epileptic and antiepileptic effects i.e. neuropharmacological study with reference to epilepsy. Cerebral ganglion was kept perfused with crab physiological saline in order to avoid desiccation of the tissue. After exposure of ganglion, PTZ test solution (100 mM/ litre) prepared in Crab Physiological saline was added directly onto the desheathed ganglion and surface electrical activities were recorded for 10 to 50 seconds. In case of Sodium Valproate treatment groups, first Sodium Valproate (120 mM/ litre) prepared in Crab Physiological Saline was added onto the desheathed ganglion and after 15 minutes of pretreatment with Sodium Valproate, PTZ test solution (100 mM/litre) was added and surface electrical activities were recorded for 10 to 50 seconds.

Similarly, Using Cuprum metallicum, similar protocol was followed for the study of antiepileptic efficacy of the drug. Besides, controls receiving only Crab Physiological Saline were used for recordings surface electrical activities. The surface electrical recordings of the thoracic ganglion were not taken in vivo owing to the difficulty in reaching to the ganglion without disturbing and damaging other organs. However, invitro experiments were conducted.

For the purpose of recording electrical activities two identical platinum electrodes (0.35 mm diameter and resistance 0.1 Ω) were used. The electrodes were connected to the computerized recording unit, Unkeloscope (MIT, USA:version 1984) via a 20 port cord. The electrical recordings consisted of a horizontal scale, recording the duration and a vertical scale, measuring the voltage of the electrical activity. The horizontal trace was 10 to 50 seconds full scale and the recording frequency was set at an interval of 0.1 second at 5 Hz. The source of Vertical trace was analog 0, with a span of 50 mV full scale.

The surface electrical recordings from the desheathed cerebral ganglia were obtained from the treated and controls under normothermic conditions. The electrical properties of the cerebral ganglia were recorded by placing platinum electrodes (0.35 mm diam. and resistance 0.1 Ω) on the surface of the ganglia and connecting them to the computerized Unkelscope (MIT, USA; version 1984, Bhat & Desai 1998). For each experiment 5 animals were used. The readings were taken only when they steadied relatively and consistently showed the same pattern, i.e. after 10 min of drug administration. Recordings were made for 10 to 100 seconds.

2.7. BIOCHEMICAL ASSAYS:

2.7.1 Sample preparations for biochemical assays:

Crabs were cold narcotized after treatments with drugs and Cerebral ganglia were isolated and placed on ice. Tissue was homogenized in 0.25M sucrose. Glutamate and GABA level was measured under different treatment regimens using the similar protocol used for mice brain tissues as presented in section 2.7.2.4.

Similarly, mice were ether anesthetized and sacrificed by decapitation. The brains were quickly removed and placed on ice. The Olfactory Lobe, Cerebral cortex,

Cerebellum, Corpus callosum, Cingulate gyrus, Hippocampus, Pons, Medulla Oblongata and Corpora quadrigemina, were dissected from the mice brain and immediately stored at -20^{0}C.

Tissue sample were thawed and homogenized in:

a) **0.32 M sucrose** for **AST, ALT, LDH, Na^+/K^+-ATPase, Mg^{+2}-ATPase**, and **Ca^{2+} ATPase** assay (Venugopal et.al. 2005).

b) For **Glutamate dehydrogenase** assay tissues were homogenized in **100 mM Triethanolamine buffer** (Schimizu 1979).

c) For **Glutamine synthetase** assay, tissues were homogenized in **Tris buffer (**Kim and Rhee, 1987)

d) For **Acetylcholinesterase assay**, tissues were homogenized in **0.1 M phosphate Buffer (**Ellman et al. 1961).

e) Tissues homogenized in **Ringer bicarbonate buffer** were used for **Glucose estimation (Lutz et.al. 2003).**

f) **0.25 M sucrose** was used as homogenizing medium for estimations of **Glutamate, GABA (Nayak and Chatterjee, 2004) and Pyruvate (Lardy et.al., 1945)**

g) **10% TCA** for **lactate estimation (Jayaraman, 1981)**.

h) **Electrolyte estimations** were carried out from tissues homogenized in **milli-Q water**.

2.7.2. Enzymatic assays in Mice brain regions:

Enzymatic assays such as metabolic enzymes and Total ATPases were carried out in brain samples.

2.7.2.1 Aminotransferases:

Transamination is the process in which an amino group is transferred from amino acid to a keto acid. The enzymes responsible for transamination are called transaminases. The substrates in the reaction are α-ketoglutaric acid and L-aspartate for AST, and α-ketoglutaric acid and L-alanine for ALT. The products formed by enzyme action are glutamate and oxaloacetate for AST and glutamate and pyruvate due to ALT. Addition of 2,4,dinitrophenyl hydrazine results in the formation of hydrazone complex with the ketoacids. A brown colour is produced on the addition of sodium hydroxide. The intensity of the colour is related to enzymic activity.

Aminotransferases i.e. ALT (EC 2.6.1.2) and AST (EC 2.6.1.1) were measured according to 2,4-dinitrophenyl hydrazine (2,4-DNPH) method of Reitman and Frankel (1957) and also as described previously by (Shanti et al. 2004).

Reagents:

(a) Alanine-α-ketoglutarate

(b) 2,4-dinitrophenyl hydrazine

(c) 0.4 N NaOH

(d) Aspartate-α-ketoglutarate

Protocols:

The incubation mixture for ALT contained 500 µl of alanine and α-ketoglutarate, 100 µl of tissue extract (enzyme source), 500 µl of 2,4- DNPH and 5 ml of 0.4 N NaOH. The incubation mixture for AST contained 500 µl of aspartate and α-ketoglutarate, 100 µl of tissue extract (enzyme source), 500 µl of 2,4-DNPH and 5 ml of 0.4 N NaOH. Optical density of corresponding brown colored hydrazone formed in alkaline medium was read at 505 nm.

Calculations: were carried out the using the Standard curve

Units: mol pyruvate/min/mg protein

2.7.2.2 <u>Lactate dehydrogenase:</u> (Cabaud and Wroblewski, 1958)

LDH (EC 1.1.1.27) activity was assayed by 2, 4- DNPH method, using Span Diagnostic Reagent Kit (Product Code: 25903).

Lactate dehydrogenase catalyze the following reaction:

$$\text{Lactate} + \text{NAD} \xrightleftharpoons{\text{LDH}} \text{Pyruvate} + \text{NADH}$$

The enzyme lactate dehydrogenase catalyses the conversion of lactate in the buffered substrate into pyruvate in the presence of NAD. The increased concentration of the product (pyruvate) is measured colorimetrically using 2,4- dinitrophenyl hydrazine. Products so formed are coupled with 2,4- dinitrophenyl hydrazine (2,4-DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium and this is measured colorimetrically. Optical density was obtained at 440

nm using Nanodrop Spectrophotometer. Calculations were carried out using the standard curve.

Units: unit/min/mg proteins

2.7.2.3 Glutamate dehydrogenase:

Glutamic dehydrogenase

$$\alpha\text{-KG} + \text{ß-NADH} + NH4^{+} \longrightarrow \text{L-Glu} + \text{ß-NAD} + H_2O$$

Glutamate dehydrogenase (**EC 1.4.1.3)** assays were carried out using the method described by Schimizu (1979) by continuous spectrophotometric rate determination.

Reagents:

(a) 100 mM Triethanolamine Buffer with pH 7.3

(b) 200 mM α-ketoglutaric acid

(c) 3.2 M Ammonium acetate solution

(d) 10 mM β- Nicotinamide Adenine Dinucleotide

(e) 25 mM Ethylenediaminetetraacetic acid (EDTA)

(f) 1 M NaOH

(g) 1 M HCl

Protocols:

Brain tissues were homogenized in 100 mM Triethanolamine Buffer, pH 7.3. Centrifuged and the supernatant obtained was used for the assay. A reaction cocktail

was prepared by adding 26 ml of 100 mM Triethanolamine buffer, 200 mM α-ketoglutaric acid (2 ml), 0.5 ml of 3.2 M Ammonium acetate solution, 0.3 ml of β-Nicotinamide Adenine Dinucleotide (reduced form) and 0.3 ml 25 mM EDTA. The reaction mixture was equilibrated to 25°C after stirring. pH of the reaction cocktail was adjusted to 7.3 at 25°C with 1 M NaOH or 1 M HCl. To the 2.9 ml of reaction cocktail 0.1 ml of supernatant was added to the 'test'and 0.1 ml of buffer to the blank. Immediately after mixing the reaction mixture by inversion and the decrease in absorption was measured at 340nm for approximately 5 - 10 minutes.

Calculations:

Units/ml enzyme = $\frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340}\text{nm/min Blank})\ (3)\ (df)}{(6.22)(0.1)}$

3 = volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar Extinction Coefficient

0.1 = volume (in milliliter) enzyme used

Units/ mg protein= $\frac{\text{units/ml enzyme}}{\text{mg protein/ ml enzyme}}$

UNIT DEFINITION:

One unit will reduce 1.0 μmole of a-ketoglutarate to L-glutamate per minute at pH 7.3 at 25°C, in the presence of ammonium ions.

2.7.2.4 Glutamine synthetase assay (Kim and Rhee, 1987)**:**

Glutamine synthetase (EC 6.3.1.2) activity was determined by the formation of γ-glutamylhydroxamate from glutamine and hydroxylamine (Kim and Rhee, 1987). A coloured complex is then formed with ferric chloride, which was measured spectrophotometrically.

Reagents:

(a) Tris buffer

(b) 0.1 M imidazole-HCl

(c) 50 mM L-glutamine

(d) 0.4 mM Manganese Chloride

(e) 62.5 mM Hydroxylamine

(f) 10 mM Sodium Arsenate

(g) 1 mM Ferric Chloride

(h) 0.67 mM Hydrochloric acid

(i) 0.2 M Trichloroactetic acid

Protocols:

Supernantant (0.1ml) obtained after centrifuging the homogenate of the tissues prepared in Tris buffer were incubated at 37^0C with 0.1 M imidazole-HCl, 50 mM L-glutamine, 0.4 mM $MnCl_2$, 62.5 mM hydroxylamine and 10 mM sodium arsenate in a final volume of 0.5 ml (pH 7.2). The reaction was terminated by the addition of 1 mM $FeCl_3$, 0.67 mM HCl, 0.2 M TCA. Precipitated protein was removed by centrifugation at 10,000 g for 5 min and the absorbance of the supernatant was measured at 535 nm using Nanodrop Spectophotometer. γ-glutamylhydroxamate used as standard.

Calculations: Concentration of Sample = $\frac{\text{OD of sample}}{\text{OD of Std}}$ X Conc. Of Std.

Units: mmol of γ-glutamylhydroxamate / min/ mg protein

2.7.2.5 Acetylcholinesterase enzyme assay (Ellman et.al, 1961):

The activity of AChE was measured according to a method developed by Ellman et al. 1961. This method employs acetylthiocholine iodide (ATChI) as a synthetic substrate for AChE. ATChI is broken down to thiocholine and acetate by AChE and thiocholine reacts with dithiobisnitrobenzoate (DTNB) to produce a yellow color. The intensity of yellow color which develops over time is a measure of the activity of AChE and can be measured using a spectrophotometer. These coupled reactions are represented by the following equations:

AChE

acetylthiocholine iodide ==========> **thiocholine + acetate**

thiocholine + dithiobisnitrobenzoate ======> **yellow colored products***

* products of the reaction are 2-nitrobenzoate-5 mercaptothiocholine and 5-thio-2-nitrobenzoate (the latter is the yellow product).

The activity of an enzyme is generally expressed as a rate: the quantity of substrate (in moles) which is broken down by a known amount of enzyme per unit time. In this case, it will be the amount of ATChI which is broken down by AChE per minute.

Reagents:

(a) 0.1 M Phosphate buffer

(b) 0.01 M Dithiobisnitrobenzoate solution

(c) 0.1 M Acetylthiocholine iodide

Protocols:

Brain homogenate was prepared in 0.1 M phosphate buffer (30 mg/ml). In a test tube 0.2 ml sample was taken, and to this 0.1 ml 0.01M dithiobisnitrobenzoate (DTNB) solution was added, vortexed and kept at room temperature for 5 min. Using spectrophotometer, sample was set to zero absorbance at 412 nm. To this 0.02 ml of 0.1M acetylthiocholine iodide (ATChI) were added. Immediately, the reaction mixture was read for zero absorbance at 412 nm and the time was noted. Readings were taken at 30 sec, 60 sec, 2 min., and 3 min. Blank was prepared using the same method, instead of 0.02 ml of 0.1M acetylthiocholine iodide (ATChI), 0.02 ml of Phosphate buffer was added. Change in absorbance/min. against time was plotted into graph and calculation was done using the following formula.

Calculations:

$R = DA/(1.36*104) \times 1/(200/3320)Co = 1.22(10^{-3}) DA/Co$

R = rate, in moles substrate hydrolyzed/min/ g tissue

DA = change in absorbance/min.

Co = original concentration of tissue (mg/ml)

200/3320 is volume correction

1.36 (104) is the extinction coefficient of the yellow product

2.7.2.6 Total ATPases:

The activities of Total ATPases such as Na^{+}/K^{+}-ATPase, Mg^{+2}-ATPase, and Ca^{2+}-ATPase were determined from both control and experimental sets (treated with drugs), by following the method of Venugopal et.al. (2005) with a slight modification. The filtrate obtained after completion of the the reaction, and after precipitating protein i.e., enzyme by 10% TCA, was treated with an ammonium molybdate reagent, which reacted with inorganic phosphate to form phosphomolybdic acid. This was reduced by Metol to give a blue colour, which was measured spectrophotometrically.

Reagents:

(a) 0.32 M Sucrose

(b) 10% Trichloroacetic acid

(c) 30 mM Tris –HCl with pH 7.2

(d) 10 mM Sodium Chloride

(e) 0.2 mM Magnesium Chloride

(f) 0.1 mM Oubain

(g) 0.5 mM Adenosine triphosphate

(h) Copper Acetate buffer with pH 4

(i) 5 % Ammonium Molybdate

(j) 2 % Metol prepared in 5% Sodium Sulphite

(k) 0.3 mM Calcium Chloride

(l) 2 mM Potassium Cyanide

Protocols:

The brain tissues were homogenized in ice-cold 0.32 M sucrose and then were cold centrifuged for 5 min at 900 x g at 0^0C. The supernatant obtained was used for the enzyme assay after protein determination. To 0.1 ml of supernatant, 0.2 ml of 30 mM, Tris–HCl (pH 7.2), 0.2 ml of 10 mM NaCl, 0.2 mM KCl, 0.2 mM of $MgCl_2$ and 0.1 ml of 0.1 mM Oubain were added for determining the activity of Mg^{2+}-ATPase. In another set, Oubain was omitted and this set was used for the assay of Na^+,K^+-ATPase. The mixture was preincubated for 10 min at 37^0C in a water bath. The activities of Mg^{2+}-ATPase and total ATPases (Mg^{2+}-ATPase + Na^+,K^+-ATPase) were initiated by incubating the mixture with 0.2 ml of 0.5 mM ATP (Tris ATP salt, Sigma). The mixture was incubated at 37^0C for 10 min. The reagent blanks were made with 0.1 ml of 0.32 M sucrose. After 10 min of incubation, 0.2 ml of 10%TCA was added to the mixture and centrifuged at 1000 x g for 5 min. To 1 ml of the supernatant, 3 ml of reagent 'A' (copper acetate buffer, pH 4.0) was added and mixed well. This was followed by the addition of 0.5 ml of reagent 'B' (5% ammonium molybdate) and the mixture was shaken well and 0.5 ml of reagent 'C' (2% metol in 5% sodium sulphite) was added immediately and mixed thoroughly. After 7 min, the blue color developed in the mixture was read at 870 nm on a spectrophotometer. Phosphorous standard was used to calculate the concentration of inorganic phosphorous present in the sample. Na^+,K^+-ATPase activity was measured between 0.1 ml of 0.1 mM of oubain incubated and nonincubated with or without ATPase activities.

For Ca^{2+}-ATPase activity, 0.2 ml of 0.3 mM $CaCl_2$, 0.1 ml of 0.1 mM oubain, 0.1 ml of 2 mM KCN were added to the mixtures and all other ions were omitted. The rest of the processing was similar to that mentioned above. Seven minutes after the

addition of the reagents A, B and C, the color developed in the mixtures was recorded at 870 nm using Nanodrop spectrophotometer. Phosphorous standard was used to calculate the concentration of inorganic phosphorous present in the sample.

Calculations: Phosphate content = $\frac{\text{OD of unknown}}{\text{OD of Std}}$ X Conc. Of Std.

Units: Pi released in µg/mg protein/min

2.7.3. Biochemical assays of Energy metabolites in mice brain regions:

2.7.3.1 Glucose (Trinder, 1969):

Glucose was estimated from the supernatant obtained after centrifugation of the homogenated brain tissues, by Glucose oxidase- Peroxidase method using kits from Span Diagnostics (Product code: SKU # 93MB100-64).

Calculations: Glucose content = $\frac{\text{O.D of Sample}}{\text{O.D. of Std.}}$ X Known Conc. of Std.

Units: mM/mg wet wt of tissue

2.7.3.2. Pyruvate (Lardy et al. 1945):

To a 0.1 ml of supernatant, 1 ml of DNPH reagent was added and then incubated for 15 minutes at 37^0C. The reaction was terminated by adding 4N NaOH

and readings were taken at 440nm after 5 minutes. 1mM pyruvate was used as a standard.

Calculations: Pyruvate contents = $\frac{\text{O.D of Sample}}{\text{O.D. of Std.}}$ X Known Conc. of Std.

Units: Pyruvate in μM/ mg wet wt. of tissue

2.7.3.3. Lactate:

Lactate concentrations from the tissues were obtained by following the method of Jayaraman, (1981).

Reagents: (a) 10% Trichloroacetic acid

(b) 20% Copper Sulphate solution

(c) 1 gm Calcium hydroxide

(d) 0.4 % Copper Sulphate

(e) Concentrated Sulphuric acid

(f) p-hydroxy diphenyl reagent.

Protocols:

Brain tissues were homogenized in 10% TCA (about 10 ml per gram tissue weight) and then centrifuged at 3000 rpm for 15 min. The supernatant thus obtained was used for lactate estimation. To the 0.1 ml of supernatant, 20% $CuSO_4$ solution and 8 ml H_2O was added. To this 1 gm of powdered Calcium hydroxide was added and the mixture was kept at room temperature for 30 min after thorough mixing. This step helps in removing glucose and other interfering material. After centrifugation at 1000 rpm, 0.05 ml of 0.4% $CuSO_4$ was added into 1 ml of supernatant, followed by 6

ml of Conc. H_2SO_4, and the mixture was kept in a boiling water bath for 5 min. After cooling, 0.1 ml of p-hydroxy diphenyl reagent was added and the mixture was allowed to stand at room temperature for 30 min and then transferred to a boiling water bath for 90 seconds. After cooling, OD was obtained at 560 nm. Standard, lactate solutions (0.01 mg/ml) was treated similarly to obtain the standard graph.

Calculations: Lactate content was calculated using the following formula:

$$\text{Lactate contents} = \frac{\text{O.D of Sample}}{\text{O.D. of Std.}} \times \text{Known Conc. of Std.}$$

Units: μM/ mg wet wt. of tissue

2.7.3.4. Glutamate and Gamma amino butyric acid (GABA):

Following the method of Lowe et.al. (1958) and Nayak and Chatterjee (2001), the glutamic acid and γ-amino butyric acid (GABA) contents were measured in all the brain regions.

Reagents:

(a) 0.25 M Sucrose:-

(b) 10% TCA:- 10 gm Trichloroacetic acid dissolved in 100 ml milli-Q water.

(c) 14 mM Ninhydrin:- prepared in 0.5 M Carbonate buffer with pH 9.95.

(d) Copper tartarate reagent:- containing 160 mg% Na_2CO_3, 32.9 % tartaric acid, 30 mg% $CuSO_4.5H_2O$.

(e) Sodium tartarate reagent:- prepared by 160 mg% Na_2CO_3, 32.9 % tartaric acid in milli-Q water.

Protocol:

Brain regions were homogenized in 0.25M cold Sucrose. The 10% (w/v) brain homogenates in 0.2 ml portions were taken and protein-free supernatants were prepared by mixing the homogenates with same volume (0.2ml) of 10% TCA, followed by centrifugation at 3000 rpm for 15 min. For the development of fluorophores, 0.1 ml from each supernatant was taken and mixed with 0.2 ml ninhydrin (14 mM solution in 0.5 M carbonate buffer, pH 9.95). The mixed solutions were incubated at 60^0C water bath in capped tubes. After 30 min, the tubes were cooled and contents were mixed with 5 ml of copper tatarate reagent containing 160 mg % Na_2CO_3, 32.9 mg % tartaric acid and 30 mg % $CuSO_4.5H_2O$. Waiting for 25 min, the γ-amino butyric acid (GABA) concentrations were measured spectrofluorometrically using excitation wavelength at 377 nm and emission wavelength at 451 nm. A blank was prepared using 0.2 ml of 0.25 M sucrose instead of tissue homogenate, whereas the calibration curve was prepared using standard solutions of GABA containing 1 mg/ml, 2 mg/ml, 3 mg/ml GABA solutions and following the same fluorophore development procedure stated above.

On the other hand, the glutamic acid contents of brain regions were measured by running a parallel procedure with another 0.1 ml supernatant. They were incubated with 0.2 ml ninhydrin soultuion at 60^0C for 30 min., cooled and then mixed with Na-tartrate reagent (omitting the $CuSO_4.5H_2O$ from Cu-tratrate reagent). Measuring spectrofluorometrically (excitation wavelength 377 nm and emission wavelength 451 nm) summated absorbances of both glutamic acid and GABA was obtained. The absorbances of glutamic acids of brain regions were determined by subtracting the absorbances of GABA from the respective summated absorbances. The amounts of

glutamic acids and GABA of brain regions were determined from a standard curve using standard solutions of glutamic acids containing 1 mg/ml, 2 mg/ml, and 3 mg/ml.

Calculations: Amount of Glutamate and GABA was calculated using the same following formula.

$$\text{mM /mg wet wt. of tissue} = \frac{\text{O.D of Sample}}{\text{O.D. of Std.}} \times \text{Known Conc. Of Std.}$$

Units: Glutamte as well as GABA in mM /mg wet. wt. of tissue

2.7.4 Estimations of Electrolyte contents in mice brain regions:

Estimation of Sodium, Potassium, Magnesium, Calcium and Chloride were carried out using Kits from CREST BIOSYSTEMS for Na^+, K^+, Cl^- kit (ELY 3127A), Mg^{+2} kit (MAG 1102) and Ca^{+2} Kit (CAL-AS-01M).

2.7.4.1 Sodium: (Trinder, 1951 and Maruna, 1958)

Sodium was precipitated as a triple salt with magnesium and Uranyl acetate. The excess of uranyl ions were reacted with ferrocyanide in an acidic medium to develop a brownish colour. The intensity of the colour produced was inversely proportional to the concentration of sodium in sample. Colour produced was read at 530 nm using Nanodrop spectrophotometer.

Uranyl ions + Mg ions + Na^+ ⟶ UranylMgNa precipitates

Free Uranyl ions + $K_4Fe(CN)_6$ ⟶ Brown coloured complex

Calculations : $$\text{Sodium in mol} = \frac{\text{O.D of unknown}}{\text{O.D of Std.}} \times \text{Conc. Of Std}$$

Units of Sodium ion was finally calculated into mol/gm protein.

2.7.4.2 Potassium: (Terri and Sesin, 1958; Sunderman and Sunderman, 1959)

Potassium reacts with sodium tetraphenyl boron in a specially prepared buffer to form a colloidal suspension. The amount of the turbidity produced is directly proportional to the concentration of potassium in the sample. It was read using Nanodrop spectrophotometer at 630 nm.

Tetraphenyl boron + K^+ ⟶ White turbidity

Calculations: Potassium in mol = $\frac{\text{O.D of unknown}}{\text{O.D of Std.}}$ X Conc. Of Std

Units for Potassium ions was calculated as mol/gm protein.

2.7.4.3. Magnesium: (Gindler and Heth, 1971)

Magnesium was determined from the brain sample by Calmagite method. Magnesium combines with Calmagite in an alkaline medium to form a red coloured complex. Interference of calcium and proteins is eliminated by the addition of specific chelating agents and detergents. Intensity of the colour formed is directly proportional to the amount of magnesium present in the sample. O.D was taken at 510 nm.

Calculations: Magnesium in mmol = $\frac{\text{O.D of unknown}}{\text{O.D of Std.}}$ X Conc. Of Std

Units: Magnesium in mmol/gm proteins

2.7.4.4. Calcium: (Moorehead and Biggs, 1974)

Calcium was measured by following Modified Arsenazo method. Calcium reacts with the dye Arsenazo at specific pH to form bluish purple coloured complex. The intensity of the colour formed was directly proportional to the amount of calcium

present in the sample. The intensity of colour was determined using Nanodrop spectrophotometer at 620 nm.

Calculations: Calcium in mol = $\frac{\text{O.D of unknown}}{\text{O.D of Std.}}$ X Conc. Of Std

Units: calcium in mol / gm protein

2.7.4.5. Chloride:(Schales and Schales, 1941; Schoenfeld and Lewellen, 1964)

Chloride ions combine with free mercuric ions and release thiocyanate from mercuric thiocyanate. The thiocyanate released combines with the ferric ions to form a red brown ferric thiocyanate complex. Intensity of the colour is directly proportional to the amount of chloride present in the sample, and it is read at 505 nm using UV/Visible spectrophotometer.

$$2Cl^- + Hg(SCN)_2 \longrightarrow HgCl_2 + 2(SCN)$$

$$3(SCN) + Fe^{3+} \longrightarrow Fe(SCN)_3$$

Calculations: Chloride in mol = $\frac{\text{O.D of unknown}}{\text{O.D of Std.}}$ X Conc. Of Std

Units: Chloride ions: mol/ gm proteins.

2.8. Determination of Enzyme Protein:

Determination of the enzyme protein was made according to the method of Lowry et.al. (1952).

Protein reacts with the Folin – Ciocalteau reagent to give a coloured complex. The colour so formd is due to the reaction of the alkaline copper with the protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the colour depends on the amount of these aromatic amino acids present.

Reagents:

(a) Alkaline Sodium Carbonate solution (20gm/litre Na_2CO3 in 0.1 mol/litre NaOH).

(b) Copper sulphate- Sodium potassium tartarate solution (5 gm/litre CuSO4.5H2O in 10gm/litre Na,K tartarate), prepared fresh by mixing stock solutions.

(c) 'Alkaline solution', prepared by mixing 50 ml of solution (a) and 1 ml of solution (b).

(d) Folin-Ciocalteau reagent:- Commercially available reagent was diluted with an equal volume of milli-Q water on the day of use. This is a sodium tungstate and sodium molybdate in phosphoric and hydrochloric acid.

(e) Standard Protein:- Albumin solution 0.2 mg/ml was used.

Protocol:

To 5 ml of 'alkaline solution' 1 ml of supernatant obtained after centrifuging all the homogenates (the one used for enzyme assay), was added. Mixed thoroughly and allowed to stand at room temperature for 10 min. To this, 0.5 ml of diluted Folin-Ciocalteau reagent was added and after 30 min reading was taken using Nanodrop

Spectrophotometer at 750 nm. Protein concentration was obtained by using the standard curve. Bovine albumin was used as standard.

Calculations:

The Optical density was converted to gm protein/ gm wet wt. of tissue using the following formula.

Formula:

$$\text{mg protein/gm wet wt. of tissue} = \frac{\text{O.D of Sample}}{\text{O.D. of Std.}} \times \text{Known Conc. Of Std.}$$

2.9. Statistics:

All experimental data is presented as the mean ± SE. Student's t-test was used to analyze the results, using Graphpad Quickcalc software. $P < 0.05$ was considered significant.

RESULTS

In the present work, an attempt is made to study, epileptic seizures induced by Pentylenetetrazole. Two different experimental models i.e. Mud Crab (*Scylla serrata)* and Mouse (*Mus musculus)*, were used. Neuropharmacological aspects of epileptic seizures were carried out using Sodium valproate, an allopathic antiepileptic drug and Cuprum metallicum, a homeopathic preparations usually prescribed during epilepsy in humans. Besides, a combination of Sodium valproate and Cuprum metallicum were also used for controlling epileptic seizures using the experimental animals i.e. Mice. Also, a Crab (*Scylla serrata)*, an invertebrate is introduced as a new model for the first time, to study epilepsy.

3.1. NEUROPHARMACOLOGICAL STUDIES OF EPILEPSY IN CRAB:

The present work shows that Crabs (*Scylla serrata)* exhibit epileptiform activities mediated by Pentylenetetrazole (PTZ). Besides, they exhibited antiepileptic actions of anticonvulsant drugs such as Sodium valproate (Sod. Valp.) and Cuprum metallicum (cup). The experimental Crabs (*Scylla serrata)* were divided into six groups as mentioned below:

1. Controls -- These animals received plain crab physiological saline.
2. Pentylenetetrazole treatment—Theses groups received 100 mmol/litre PTZ.
3. Sodium valproate treatment--- These groups received 120 mmol/liter Sod.Valp.
4. Groups exposed to Cuprum metallicum---- received 7 CH potency of Cup.
5. Groups that were primed with Sodium valproate (120 mmol/litre) and then exposed to Pentylenetetrazole (100 mmol/litre) after 1 hour.
6. Groups primed with Cuprum metallicum (7CH) and then exposed after 1 hour, to Pentylenetetrazole (100mmol/litre).

Pentylenetetrazole was used to induce epileptiform activities and Sodium valproate and Cuprum metallicum were used for suppression of the same activities. Data obtained after experimentation has been distributed into three sub-sections. First section presents, behavioral responses related with different drug treatments, followed by the second section showing Surface electrical recordings of the treated animals and the third represents the biochemical assays.

3.1.1. Behavioral observations on crabs showing epileptic and non-epileptic activities during various treatments:

Behavioral responses of Crabs during various treatment regimens are compiled in table 2. There was no mortality observed during or after the treatment regimens in Crabs, except for those exposed to PTZ (10% mortality). The behavioral responses of crabs were nearly similar to those expressed by Rodent model. Also, the antiepileptic drug seemed to protect the animals from getting seizures. The data in the present work convincingly shows the utility of Crab, *Scylla serrata* for such Neuropharmacological studies due to its ability to express, behavioral patterns comparable to the rodents during epileptic activities.

It is clear from the table that PTZ treated crabs exhibited body jerks, loss of balance, froth oozing from mouth, rapid movements of antennules and eye stalks, fast running and frequent defecation. Whereas, only thirty percent of control crabs showed oozing of froth from the mouth after they received PTZ vehicle sans PTZ which 20% control crabs showed defecation and antennular as well as eyestalk movements after they were handled and received PTZ vehicle only i.e., Crab saline. Sodium Valproate and Cuprum metallicum significantly reduced PTZ effects. Besides, the Crabs primed

with Valproate or Cuprum showed lesser response in terms of behavioral changes to PTZ.

Table 2: Depicts treatment dependent behavioral responses in Crab (*Scylla serrata)* (data provided in percentage)

Behavioral responses ↓	← Treatments →					
	Control (saline) (n=10)	**PTZ (n=10)**	**Sod. Valp. (n=10)**	**Cup. (n=10)**	**Sod. + PTZ (n=10)**	**Cup. + PTZ (n=10)**
Body jerks	0	100 %	0	0	20%	30%
Brief loss of balance	0	100%	0	0	20%	20%
Oozing of froth from mouth	30%	100%	20%	0	30%	0
Rapid movements of antennules and eyestalks	20%	100%	20%	0	20%	20%
Sedation	0	0	80%	40%	60%	0
Fast running	30%	90%	20%	20%	10%	20%
Frequent defecation	20%	100%	20%	0	20%	10%
Mortality	0	10%	0	0	0	0

3.1.2. Surface electrical recordings of cerebral ganglion of Crab (*Scylla serrata)*:

Surface electrical recordings of the cerebral ganglion of Crab (*Scylla serrata)* (Control and experimentals) were recorded for 100 seconds, but a representative segments of 10 seconds are shown in the Figure 14a to 14f. The Figure 14a represents

surface electrical recordings (SECs) of controls while Figure 14b expresses the Surface electrical recordings (SECs) of the Crabs exposed to PTZ. Figure 14c represents SECs of the crabs exposed to 120 μmol/litre Valproate treatment, and Figure 14d shows SECs of crabs primed with Sodium Valproate and then exposed to PTZ. Figure 14e and 14f represents SECs of crabs treated with Cuprum treatment and crabs primed with Cuprum metallicum and then exposed to PTZ, respectively.

The Surface electrical recording corresponds to the pattern observed in humans Electroencephalogram. Control represented by Figure 14a, corresponds to Beta waves, whereas, rest all i.e. Fig 14c, 14d, 14e, 14f, corresponds to alpha waves. But Figure14b i.e. epileptic crabs exhibit a type of abnormal beta waves, representing hyper-excitability and seizure wave patterns.

Surface electrical recordings of cerebral ganglia showed abnormal synchronous electrical discharges. Control crabs' ganglia bathed with crab saline exhibited normal neuronal electrical discharges in the range of -20 to + 20 mV (Fig: 14a). PTZ (100 mmol/liter) treated crabs (Fig: 14b) showed ganglionic electrical discharges in the range of -50 to +50 mV.

Sodium Valproate (20 mg/kg body weight) promoted a decrease in electrical discharges of cerebral ganglia in comparison to those of controls, and the electrical discharges were in the range of -30 to +20 mV (Fig: 14c) but with reduced spike frequency as shown in Figure 14c, cerebral ganglia of crabs exposed, twenty minutes after pretreatment of Sodium Valproate to similar dose of PTZ, showed lower electrical discharges in the range of -40 mV to +10 mV (Fig: 14d) with reduced spike frequency, indicating a protective action of Sodium Valproate against PTZ induced seizures. Similar results were obtained in Crabs treated with cuprum metallicum exhibiting electrical discharge in the range of -30 to +10mV (Fig:14e) and also, crabs

cerebral ganglion exposed to Cuprum metallicum followed by PTZ revealed electrical discharge in the range of -30 to -10 mV (Fig:14f). Results indicate a protective action of Sodium Valproate and Cuprum metallicum against PTZ induced seizures.

Fig: 14a. Represents surface electrical recording in control. (duration of 10 secs)

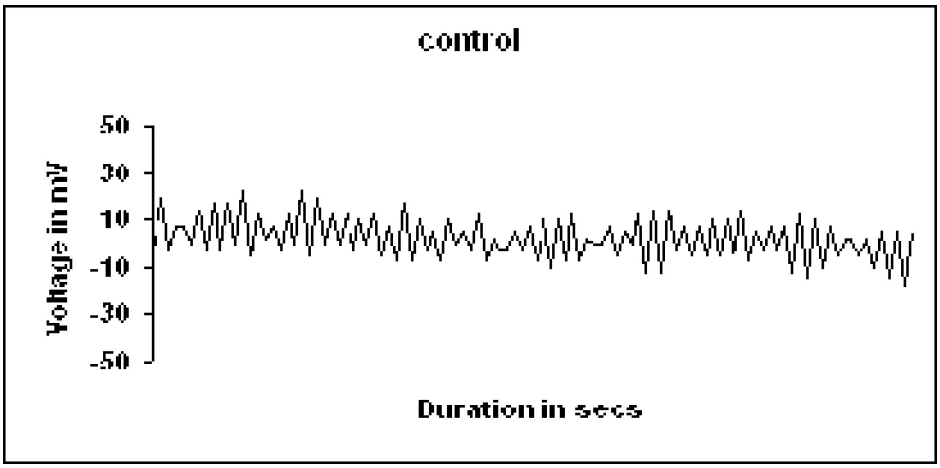


Fig: 14b. Represents surface electrical recording in Pentylenetetrazole treated. (duration of 10 secs)

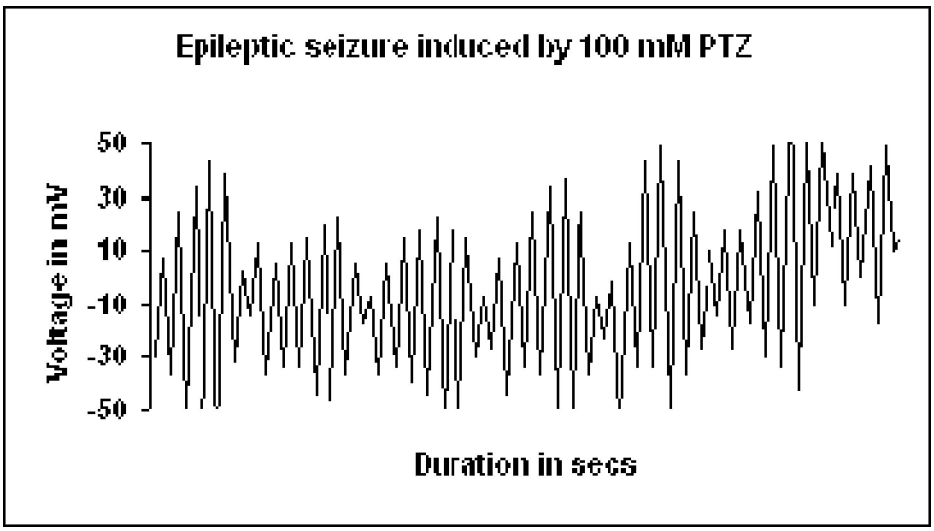


Fig: 14c. Represents Surface electrical recording in Sodium valproate treated. (duration of 10 secs)

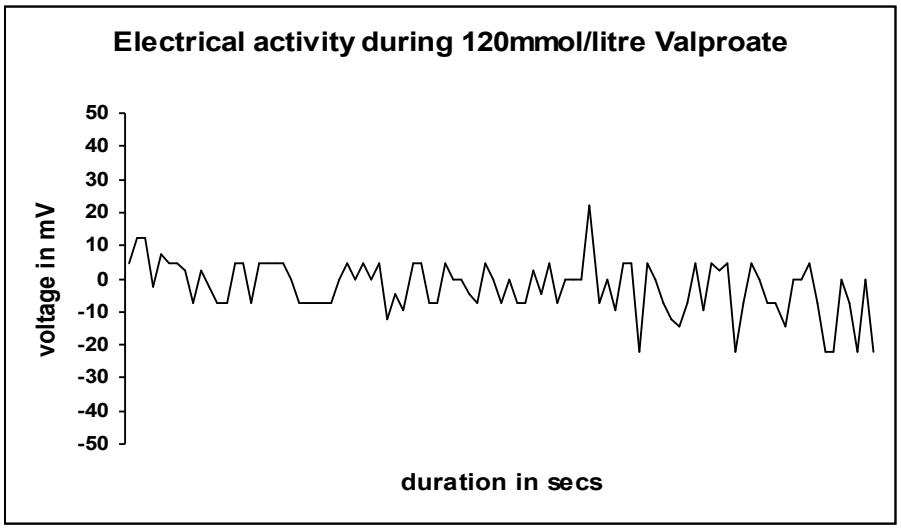


Fig: 14d. Represents Surface electrical recording in Crabs pretreated with Sodium Valproate followed by Pentylenetetrazole (duration of 10 secs)

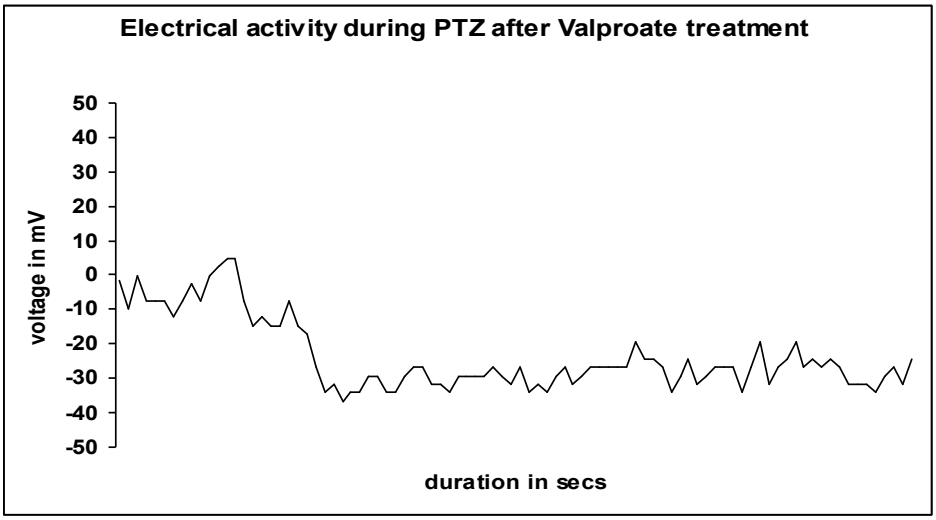


Fig: 14e Represents Surface electrical recording in Crabs treated with Cuprum metallicum (duration of 10 secs)

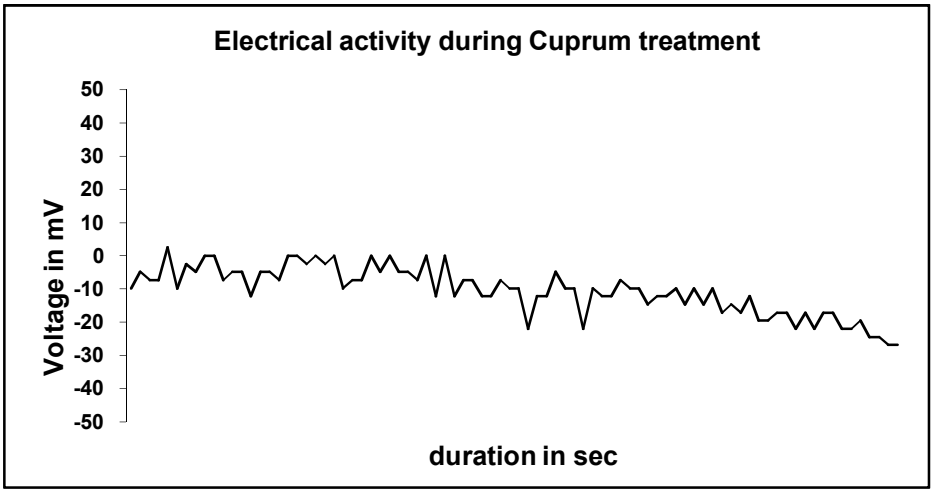
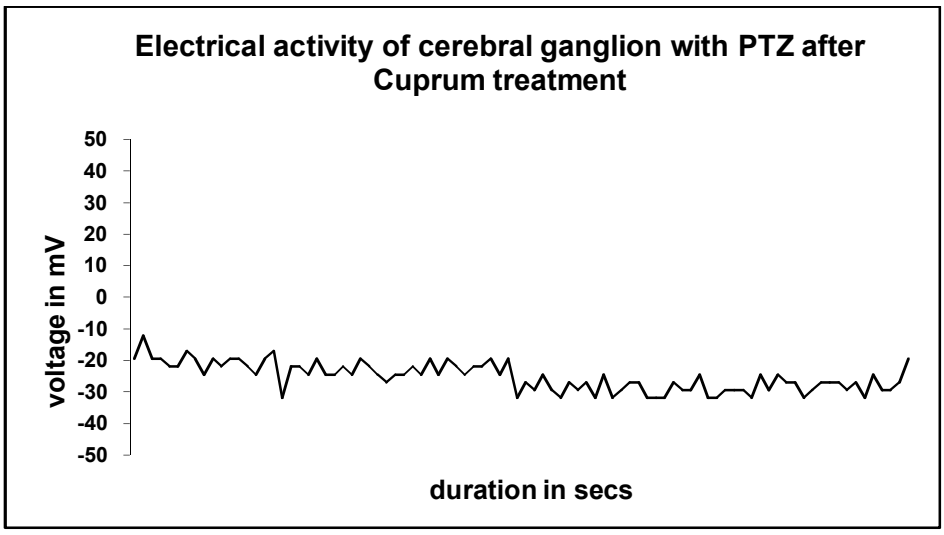


Fig: 14f. Represents Surface electrical recording in Crabs pretreated with Cuprum metallicum followed by Pentylenetetrazole (duration of 10 secs)



3.1.3 Glutamate and GABA assay in Cerebral and Thoracic ganglion of Crabs:

Glutamate and GABA concentrations of controls were equivalent to 4.81 ± 0.98 and 0.74 ± 0.01 mg/gm wet weight of tissue in Cerebral ganglion, respectively. Exposure to 100 mmol/liter dose of PTZ, elevated Glutamate concentrations of brain by 31.45% and decreased GABA concentration by 43.93% (Fig: 15.1). However, exposure of crabs to Sodium Valproate alone effected no significant change in Glutamate levels, however it induced a significant increase (24.75%) in GABA level. The Crabs pretreated with Sodium Valproate and then exposed to PTZ after one hours showed a significant increase (31.03%) in Glutamate level without any change in GABA level with respect to controls. The crabs treated with Cuprum metallicum alone exhibited a significant decrease in Glutamate level by 35.73% and GABA level by 37.16%, respectively whereas Crabs primed with Cuprum metallicum followed by PTZ after one hour revealed decrease in GABA level by 30.44% without altering Glutamate level (Fig:15.1).

Similar results were obtained in Thoracic ganglion of Crab during different treatment regimens (Fig: 15.2). In Pentylenetetrazole treated crab Thoracic ganglion showed significant increase by 14.56% glutamate and significant decrement in GABA by 48.10%, respectively. Sodium Valproate treatment exhibited no change in glutamate level whereas, increase in GABA level by 16.57% in thoracic ganglion. While Crab primed with Sodium Valproate followed by PTZ did not show any significant change. Cuprum metallicum treated crabs revealed a decrement in Glutamate and GABA level by 32.42% and 26.25%, while crabs primed with Cuprum metallicum followed by PTZ showed significant decrease in Glutamate level by 22.12%, in GABA level by 30.41%, respectively.

Fig: 15.1. Alterations in Glutamate and GABA level in cerebral ganglion of Crab during various drug treatment regimen (* represents probability p< 0.05, standard bar represents SE)

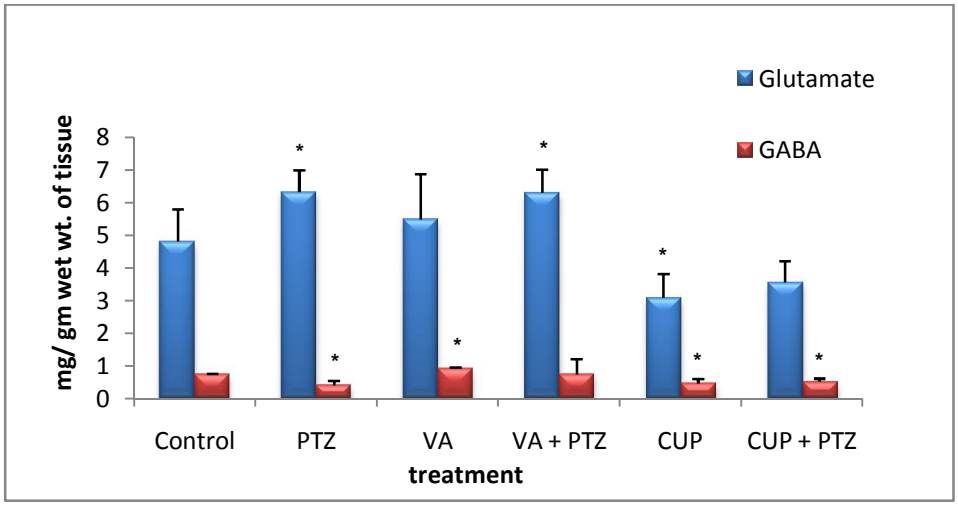
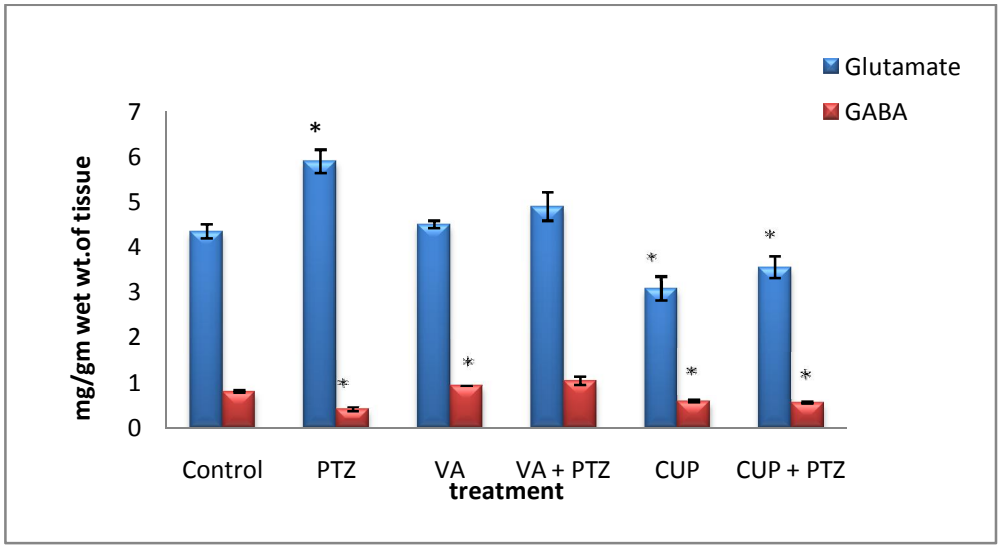


Fig: 15.2. Alterations in Glutamate and GABA level in Thoracic ganglion of Crab during various drug treatment regimen (* represents probability p□ 0.05, standard bar represents SE)



3.2. NEUROPHARMACOLOGICAL STUDIES OF EPILEPSY IN MICE:

3.2.1. Behavioral studies in Mice *Mus musculus*:

Behavioral studies data of Mice (n=10) is been provided in the following table, during various treatment regimen such as Pentylenetetrazole (PTZ) treatment, Sodium Valproate treated, Cuprum metallicum treated, Combined drug (Sodium Valproate + Cuprum metallicum) treated, Sodium Valproate followed by Pentylenetetrazole, Cuprum metallicum followed by Pentylenetetrazole treated, Combined drug (Sodium Valproate + Cuprum metallicum) followed by Pentylenetetrazole treated mice.

Table: 3 Depicts treatment dependent behavioral responses in Mice (*Mus musculus*) (data provided in percentage)

Behavioral response	control	PTZ	Sod. Val.	Cup. met	Sod. + Cup	Sod. + PTZ	Cup. + PTZ	(Sod.+Cup) + PTZ
Stage 0	100%	0	0	0	0	0	0	0
Stage 1	0	100%	0	20%	20%	30%	20%	30%
Stage 2	0	100%	0	0	0	20%	20%	10%
Stage 3	0	100%	0	0	0	10%	20%	0
Stage 4	0	100%	0	0	0	0	0	0
Stage 5	0	80%	0	0	0	0	0	0
Sedation	0	0	80%	0	50%	0	0	0
Mortality	0	0	0	0	0	0	0	0

Stages 0 : no response;

Stage 1 : ear and facial twitching;

Stage 2 : myoclonic jerks, without upright position;

Stage 3 : myoclonic jerks, upright position with bilateral forelimb clonus;

Stage 4 : Clonic-tonic seizure;

Stage 5 : generalized clonic-tonic seizures, loss of postural control.

No noticeable abnormal behavioral responses were observed in mice injected with plain mammalian saline as represented in Table 3. Pentylenetetrazole treated mice exhibited 100% responses from stage 1 to stage 4 i.e., ear and facial twitching; myoclonic jerks, without upright position, myoclonic jerks, upright position with bilateral forelimb clonus; Clonic-tonic seizure and 80% stage 5 response. Whereas, Sodium valproate treated mice revealed only sedation in 80% of animals. Cuprum metallicum treated mice exhibited Stage 1 response in 20% of test animals and none of the animals exhibited sedation. Combined drug (Sodium Valproate + Cuprum metallicum) treated groups showed 20% animals with stage 1 (ear and facial twitching) response and 50% showed sedation. Mice primed with Sodium Valproate followed by Pentylenetetrazole revealed responses such as Stage 1 (30%), Stage 2 (20%) and 10% of animals exhibited Stage 3 responses. Similarly, Mice primed with Cuprum metallicum followed by PTZ exhibited responses like Stage 1 (20%), Stage 2 (20%), Stage 3 (20%) respectively. Finally, Groups primed with Combined drug (Sodium Valproate + Cuprum metallicum) followed by PTZ exhibited responses like Stage 1(i.e. ear and facial twitching) by 30% of animals, Stage 2 (myoclonic jerks without upright position) by 10% of mice. None of the animals treated with various drug regimens exhibited Stage 0, except controls.

3.2.2. Biochemical assays of brain of mice exposed to Neuropharmacological preparations:

Biochemical studies such as Enzymes; Total –ATPases, Metabolites and Electrolytes were assayed in discrete brain regions of mice, after their exposure to various neuropharmacological preparations.

3.2.2.1. Enzyme assays of discrete brain regions of Mice:

Enzymes such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH), Glutamate dehydrogenase (GDH), Glutamine synthetase (GS) and Acetylcholinesterase (AChE) were assayed from the discrete brain regions of mice such as Olfactory Lobes, Cerebral Cortex, Corpus Callosum, Cingulate gyrus, Hippocampus, Corpora Quadrigemina, Cerebellum, Pons, Medulla Oblongata after exposing them to neuropharmacological preparations. The neuropharmacological preparations like Epileptic drug (PTZ), and antiepileptic drugs both allopathic (Sodium Valproate) as well as homeopathic (Cuprum metallicum), were used in various permutation and combinations. The results obtained after the treatments are presented graphically in below Fig: 16.1 to Fig: 16.9.

Note: Units for specific Enzyme activities are expressed as below:

a) **Aspartate Aminotransferase**: mol pyruvate/min/mg protein

b) **Alanine Aminotransferase**: mol pyruvate/min/mg protein

c) **Lactate dehydrogenase**: unit/min/mg protein

d) **Glutamate dehydrogenase**: pmol α-ketoglutarate reduced to glutamate/min/mg protein.

e) **Glutamine synthetase**: mmol/min/mg protein

f) **Acetylcholinesterase**: nmol Ach hydrolyzed/ min/mg protein

3.2.2.1.1. Alterations in Enzymes of Olfactory Lobes (OL):

AST, ALT, GS, GDH, LDH and AChE enzyme activities of the Olfactory lobes fluctuated during various drug treatment regimens (Fig: 16.1 and Fig: 16.2). There was a significant decrease in the activities of AST enzymes by 66.23%, 53.02%, 89.16%, 60.42%, 69.33%, 65.56%, 64.07%, in Pentylenetetrazole treated groups (epileptic mice), Sodium Valproate treated groups, Cuprum metallicum treated groups, Combined drug (Sodium val. + Cuprum met.) treated groups, Sodium valproate primed and then pentylenetetrazole treated groups, Cuprum metallicum primed and then Pentylenetetrazole treated groups and finally, Combined drugs (Sod val. +Cup. Met) followed by Pentylenetetrazole treated groups, respectively.

ALT activities significantly increased in Pentylenetetrazole treated mice by 21.21% , Whereas declined (19.22 %) in Sodium valproate treated groups while Combined drug (Sodium valproate + Cuprum metallicum) treated groups exhibited 37.11% decrement in the ALT activity, (Fig: 16.1).

Glutamine synthetase (GS) activities decreased in Pentylenetetrazole treated (epileptic) groups by 52% and elevated significantly in all other treatment groups such as Sodium valproate (134.99 %); Cuprum metallicum (232.06%) and Sodium valproate + Cuprum metallicum combination (7.79%). Groups pretreated with Sodium valproate followed by Pentylenetetrazole showed increase of 257.90%; Cuprum metallicum followed by Pentylenetetrazole treated groups had 8.24% elevation of GS activities and finally, while mice pretreated with Combined drug (Sodium valproate + Cuprum metallicum) followed by Pentylenetetrazole exhibited increased of 277.81% (Fig: 16.1).

Alterations in GDH, LDH and AChE of OL are expressed graphically in Fig: 16.2. Glutamate dehydrogenase (GDH) activities decreased significantly in all the

treated groups as compared to control groups. Pentylenetetrazole treated (epileptic) mice showed decrease of GDH activity by 36.50%; Sodium valproate and Cuprum metallicum treated groups showed decline in GDH activities by 12.46% and 84.77%, respectively. Mice exposed to Sodium valproate + Cuprum metallicum combination showed decrease in GDH activity by 86.92%; Sodium valproate followed by Pentylenetetrazole treated groups exhibited maximum decline in GDH activity of OL by 96.60%; Exposure to Cuprum metallicum followed by Pentylenetetrazole treatment promoted reduction of GDH by 27.17%; and lastly, combined drug treatment of Sodium valproate + Cuprum metallicum followed by Pentylenetetrazole reduced GDH activity of Olfactory lobes of mice by 87.16% (Fig: 16.2).

Lactate dehydrogenase (LDH) activity, of olfactory lobes did not show any significant alterations except in epileptic i.e., Pentylenetetrazole treated groups, the LDH activity diminished by 48.24%.

Acetylcholinesterase (AChE) enzyme assay showed decrement in its activity by 49.67% in Pentylenetetrazole treated mice, whereas, all other groups exhibited significant increase in the activity by 202.73% (Sodium valproate treated mice); 54.04% (Cuprum metallicum treated mice); 232.86% (combined drug treated mice i.e. Sodium valproate + Cuprum metallicum); 636.41% (Sodium valproate followed by Pentylenetetrazole treated); 636.41% (Cuprum metallicum followed by pentylenetetrazole treatment); 33.56% (pretreated with Sodium valproate + Cuprum metallicum, followed by Pentylenetetrazole treatment), respectively.

Fig 16.1: Alterations in AST, ALT, GS activities of Olfactory Lobes during various drug treatment. (* represents probability p< 0.05, standard bar represents SE)

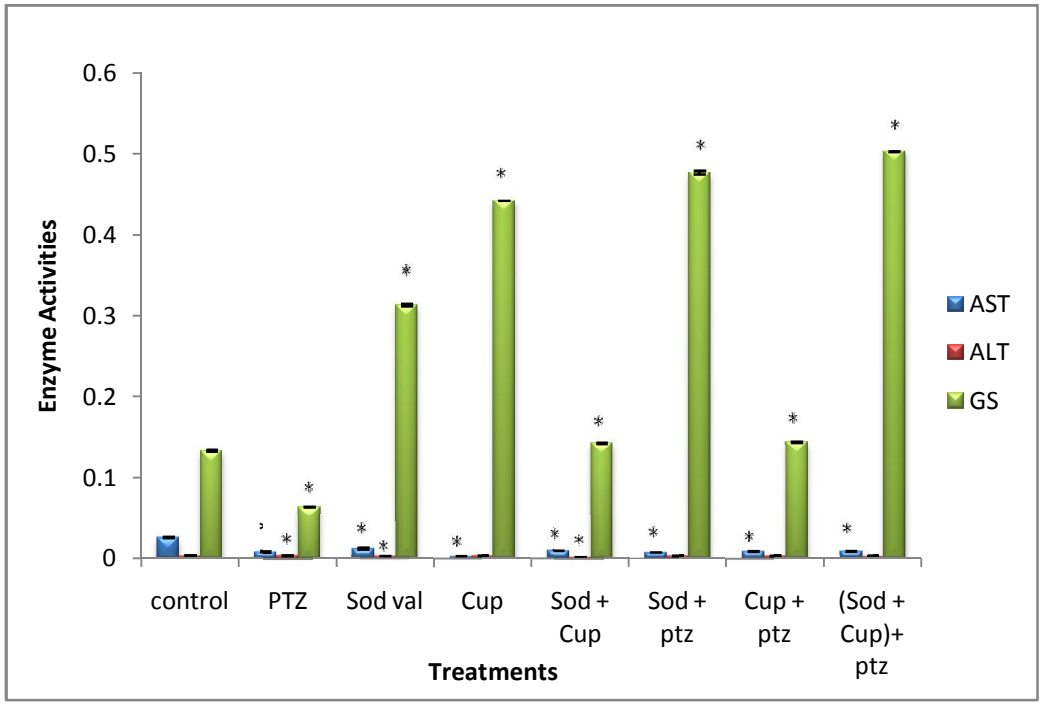
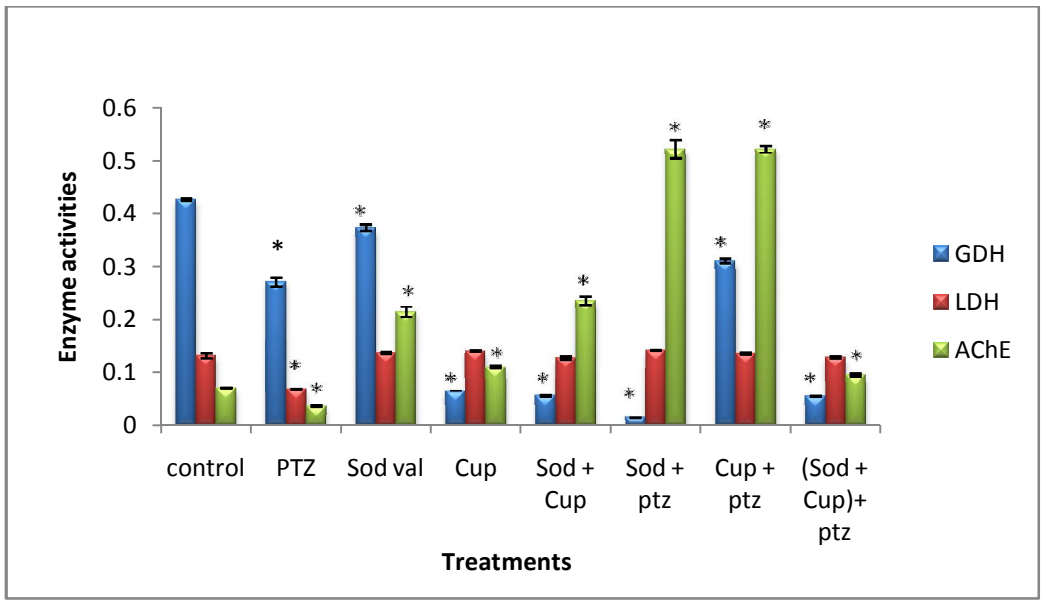


Fig 16.2: Alterations in GDH, LDH, AChE activities of Olfactory Lobes during various drug treatments. (* represents probability p< 0.05, standard bar represents SE)



3.2.2.1.2. Alterations in enzymes of Cerebral cortex:

Aspartate-aminotransferase (AST) activity is observed to be increased significantly by 131.51% in Cerebral cortex during Pentylenetetrazole treatment (Fig16.3).

Alanine-aminotransferase (ALT) activity showed significant increase in Pentylenetetrazole treated groups by 204.74%; Sodium valproate followed by Pentylenetetrazole treated mice by 107.68%; Groups pretreated with Cuprum metallicum followed by Pentylenetetrazole by 129.79%; whereas, Groups treated with combined (sodium valproate + Cuprum metallicum) drug followed by Pentylenetetrazole, showed no change in enzyme activity (Fig:16.3).

Glutamine synthetase (GS) activity decreased significantly in Pentylenetetrazole treated ones by 53.90%; whereas, it were observed to be elevated significantly in Sodium valproate treated groups by 290.70%; Cuprum metallicum treated groups by 506.72%; Sodium valproate + Cuprum metallicum treated groups by 34.018%; groups pretreated with Sodium valproate followed by Pentylenetetrazole by 43.86%; Cuprum metallicum with post-Pentylenetetrazole treated ones by 287.92% and combined drug (Sodium valproate + Cuprum metallicum) treated followed by Pentylenetetrazole exhibited 302.48% increase in enzyme activity, respectively (Fig: 16.3).

Figure16.4 represents enzymes such as Glutamate dehydrogenase, Lactate dehydrogenase and Acetylcholinesterases in Cerebral cortex of treated mice. Glutamate dehydrogenase (GDH) activity exhibited a significant elevation in Pentylenetetrazole treated mice by 71.4%; Sodium valproate treated groups by 54.61%; Cuprum metallicum treated groups by 135.87%; Groups pre-treated with Sodium valproate followed by Pentylenetetrazole injection exhibited increase in

activity by 40.58%; Cuprum metallicum treated followed by Pentylenetetrazole group by 135.87%, respectively. Besides, Groups such as combined drug (Sodium valproate+ Cuprum metallicum) treated showed decrement in GDH activity by 81.30% and similar group treated with combined drug following Pentylenetetrazole exhibited significant decrease by 37.14%.

Lactate dehydrogenase activity did not show any significant change in the Cerebral cortex, compared to its control counterparts.

Acetylcholinesterase (AChE) enzymes assay reveals, alterations in its activity in Cerebral cortex of mice during different drug treatment regimens. Pentylenetetrazole treated groups, Sodium Valproate with post Pentylenetetrazole treated groups, Cuprum metallicum with post Pentylenetetrazole treated groups of mice exhibits a significant decrease in enzyme activities by 94.98%, 94.14% and 52.20%, respectively. Whereas, groups that are treated with Sodium Valproate alone, Cuprum metallicum alone, Combine drug (Sodium Valproate + Cuprum metallicum) treated, Combine drug with post Pentylenetetrazole treated ones exhibited a significant elevations in AChE activities by 38.7%, 276.48%, 151.27% and 280.05%, respectively (Fig. 16.4).

3.2.2.1.3. **Alterations in enzymes of Corpus Callosum:**

Figure 16.5 represents the AST, ALT and GS enzyme activities in Corpus Callosum and Figure 16.6, depicts the enzyme activities in graphical form of GDH, LDH and AChE.

Aspartate amino-transferase (AST) activities were significantly elevated in five treatment regimens. Pentylenetetrazole treated groups showed elevation by

Fig 16.3: Alterations in AST, ALT, GS activities of Cerebral Cortex during various drug treatment. (* represents probability p< 0.05, standard bar represents SE**)**

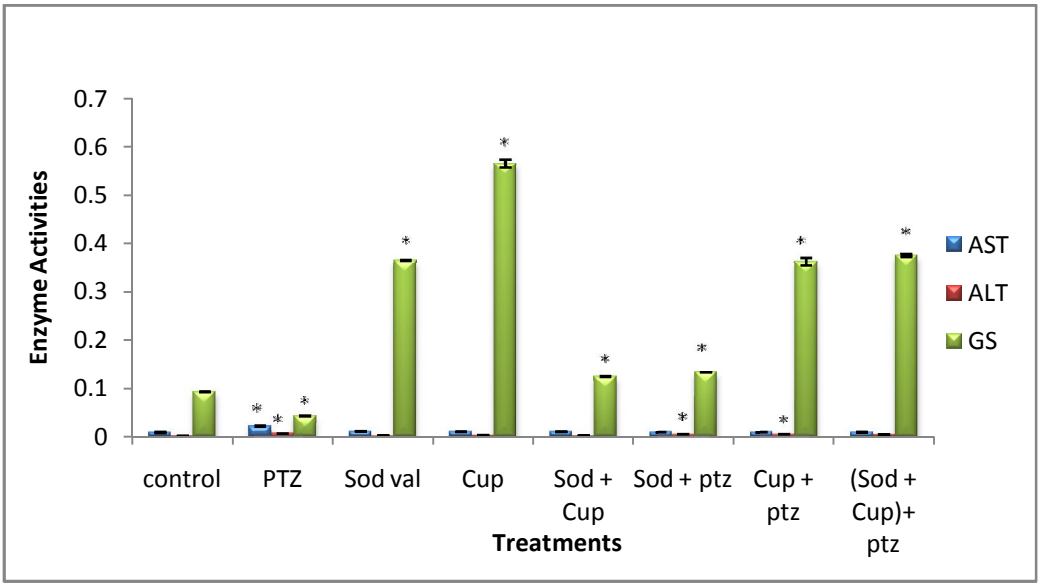
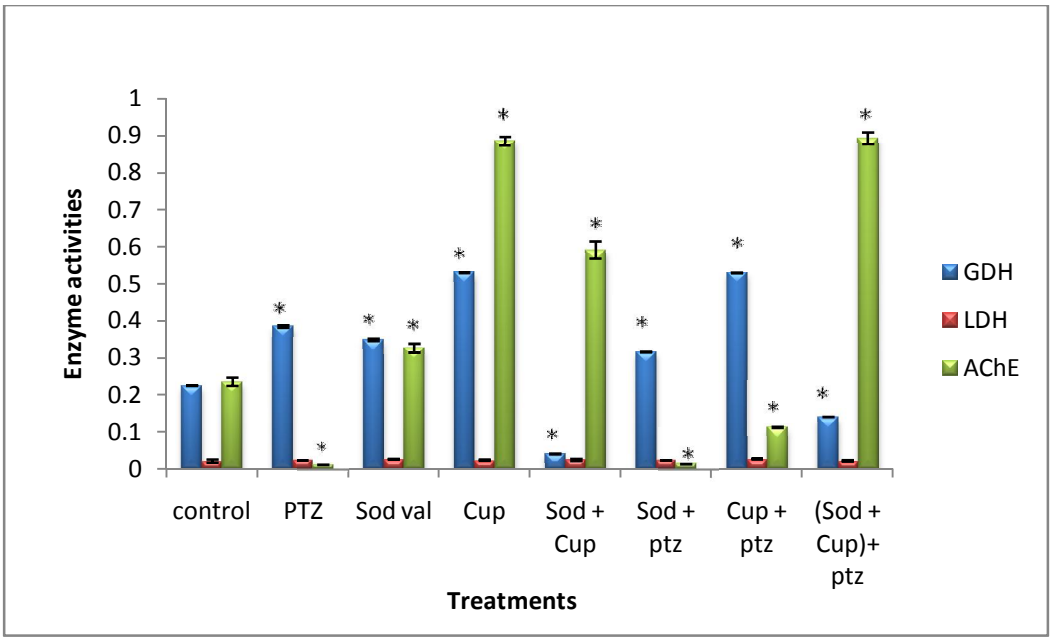


Fig 16.4: Alterations in GDH, LDH, AChE activities of Cerebral cortex during various drug treatment. (* represents probability p< 0.05, standard bar represents SE**)**



175.14%, Sodium valproate treated ones by 15.81%, Cuprum metallicum treated ones by 26.10% , Combined drugs (Sodium valproate+ Cuprum metallicum) treated groups by 24.06% and Combined drugs (Sodium valproate+ Cuprum metallicum) with post Pentylenetetrazole treated animals showed increase in enzyme activity by 102.93%, respectively.

Alanine amino-transferase (ALT) enzymes had elevated activities in groups treated with Pentylenetetrazole alone (by 181.55%), Sodium Valproate alone by (42.65%), Cuprum metallicum by (39.15%), Sodium valproate with post Pentylenetetrazole (by 106.06%), Cuprum metallicum followed by Pentylenetetrazole (by 145.32%) and Combined drug (Sodium valproate+ Cuprum metallicum) with post Pentylenetetrazole injected mice (by 146.96%), respectively.

Glutamine Synthetase (GS) activities fluctuated in all the different drug treatment regimens. Enzyme exhibited significant decrements in its activity upon treatment with Pentylenetetrazole (epileptic mice), Cuprum metallicum alone, Cuprum metallicum followed by Pentylenetetrazole, Combined drug (Sodium Vlaproate + Cuprum metallicum) with post treatment of Pentylenetetrazole by 92.94%, 43.58%, 66.57%, 66.14%, respectively. Besides, GS activities shown increments in Sodium Valproate treated, Combined drug (Sodium Valproate + Cuprum metallicum) treated and Sodium Valproate with post PTZ treated by 25.52%, 13.39% and 154.86%, respectively.

Glutamate dehydrogenase (GDH) exhibited a significant increase in enzyme activities in Pentylenetetrazole treated (epileptic mice) by 59.52%; Sodium valproate treated ones by 40.86%; Cuprum metallicum treated ones by 16.17%; Combined drugs (Sodium Valproate + Cuprum metallicum) treated ones by 42.56%; Cuprum

Fig 16.5: Alterations in AST, ALT, GS activities of Corpus Callosum during various drug treatment. (* represents probability p< 0.05, standard bar represents SE)

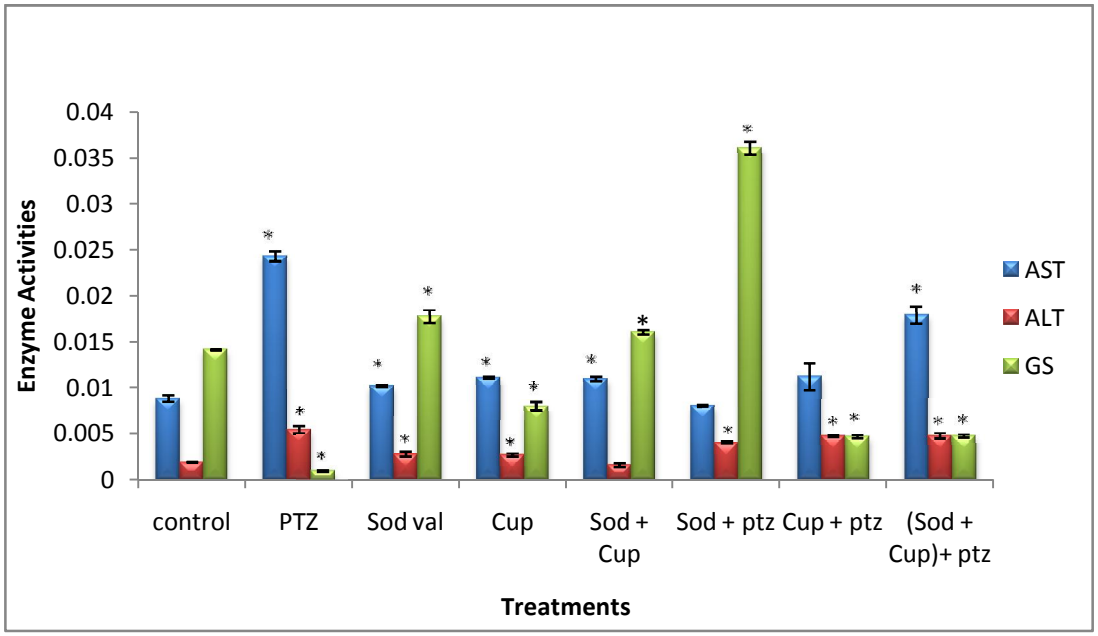
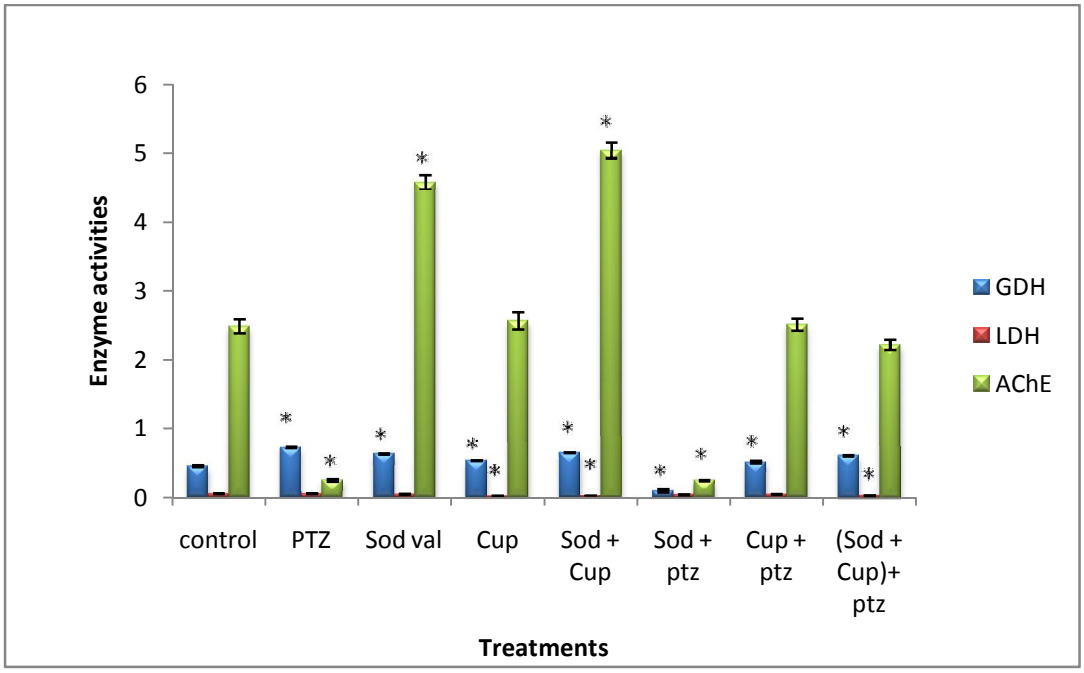


Fig 16.6: Alterations in GDH, LDH, AChE activities of Corpus callosum during various drug treatment. (* represents probability p< 0.05, standard bar represents SE)



metallicum followed by Pentylenetetrazole groups by 15.03%; Combined drugs (Sodium Valproate + Cuprum metallicum) with post Pentylenetetrazole injection, by 32.29%, respectively. Whereas, the group treated with Sodium valproate followed by Pentylenetetrazole showed reduced activity by 77.42% (Fig: 16.6).

Lactate dehydrogenase (LDH) activity was reduced significantly in Cuprum metallicum treated, Combined drugs (Sodium Valproate + Cuprum metallicum) treated, Combined drugs (Sodium Valproate + Cuprum metallicum) followed by Pentylenetetrazole by 54.38%, 30.27%, 27.33%, respectively (Fig: 16.6).

Acetylcholinesterase (AChE) activities in Corpus callosum, were found to be reduced significantly in Pentylenetetrazole treated (epileptic mice) by 89.55%; Sodium valproate followed by Pentylenetetrazole treated ones by 89.91%. Whereas, Sodium valproate alone treated and Combined drugs (Sodium Valproate + Cuprum metallicum) treated groups exhibited increased activities by 84.32% and 102.63%, respectively (Fig. 16.6).

3.2.2.1.4. Alterations in enzymes of Cingulate gyrus:

Alterations in the metabolic enzymes activities have been observed in the Cingulate gyrus during different treatment regimens, represented in Figure 16.7 and Figure 16.8.

Aspartate amino-transferase (AST) did not exhibit much changes compared to the control except in Epileptic mice i.e., Pentylenetetrazole treated groups, that showed significant increase by 61.22%.

Alanine amino-transferase (ALT) showed a significant increase in its activities during Pentylenetetrazole treatment by 31.08%; Cuprum metallicum treated mice by 21.67%; Sodium Valproate with post-Pentylenetetrazole treatment by 67.55%; and

Cuprum metallicum primed mice followed by PTZ treatment lead to increase in activity by 35.85%, respectively.

Glutamine synthetase (GS) activities were significantly decreased in Pentylenetetrazole treated mice by 60.70%; Cuprum metallicum treated mice by 29.31%; Sodium Valproate with post-Pentylenetetrazole treated by 61.11%; Combined drug (Sodium valproate + Cuprum metallicum) followed by Pentylenetetrazole treatment caused decrease in activity by 22.99%, respectively. Whereas, Sodium valproate treated mice, Combined drug (Sodium Valproate + Cuprum metallicum) treated mice and Cuprum metallicum with post pentylenetetrazole treatment lead to increase in enzyme activities significantly by 108.75%, 56.93% and 413.38%, respectively.

Glutamate dehydrogenase (GDH) enzyme study demonstrated a significant elevation in its activities in the entire treatment regimen considered in the present work. In Pentylenetetrazole treated group of mice showed increase by 215.91%; similarly, in Sodium Valproate treated groups by 105.84%; Cuprum metallicum treated group by 64.41%; Combined drug (Sodium valproate + Cuprum metallicum) treated ones by 225.28%; groups pre-treated with Sodium Valproate followed by Pentylenetetrazole shown increase in activity by 64.58%; Cuprum metallicum with post- Pentylenetetrazole injected groups by 17.04%; Combined drug (Sodium valproate + Cuprum metallicum) followed by Pentylenetetrazole showed 64.51% of increased activity.

Lactate dehydrogenase (LDH) activity was reduced in Pentyleneterazole (epileptic mice) treated groups by 39.83%; Sodium Valproate treated groups by

Fig 16.7: Alterations in AST, ALT, GS activities of Cingulate gyrus duing various drug treatment. (* represents probability p< 0.05, standard bar represents SE**)**

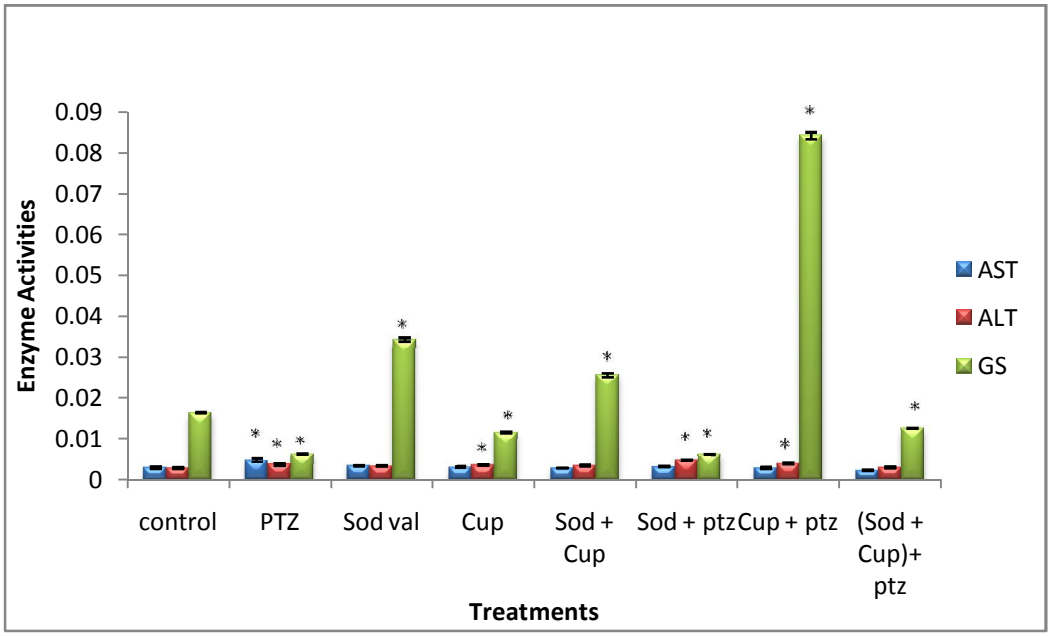
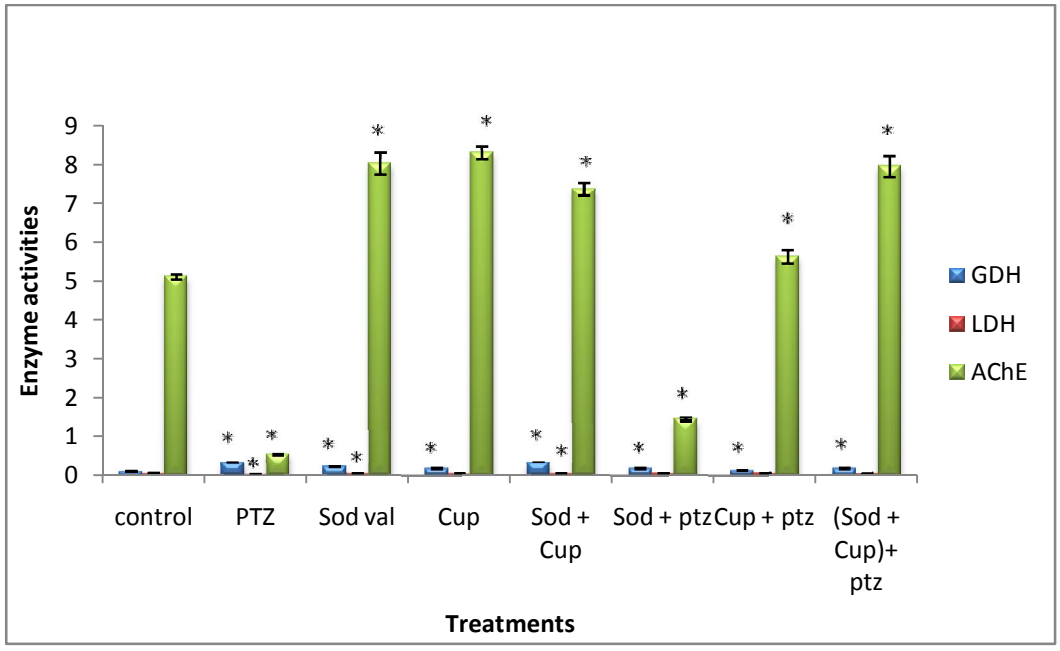


Fig 16.8: Alterations in GDH, LDH, AChE activities of Cingulate gyrus during various drug treatment. (* represents probability p< 0.05, standard bar represents SE**)**



14.20% and in Combined drug (Sodium valproate + Cuprum metallicum) treated groups by 12.78%, respectively (Fig: 16.8).

Acetylcholinesterase (AChE) activities were altered in Cingulate gyrus on treatment with various drug regimens. In Pentylenetetrazole treated ones 89.31 % decrease were observed. Similarly, in Sodium Valproate followed by Pentylenetetrazole, a decrement of 71.61% of activity was seen. Whereas, the one that is treated with Sodium Valproate, Cuprum metallicum, Combined drug (Sodium valproate + Cuprum metallicum), Cuprum metallicum followed by Pentylenetetrazole, Combined drug (Sodium valproate + Cuprum metallicum) with post –treatment with Pentylenetetrazole revealed a significant elevations in enzyme activities by 56.90%, 62.32%, 44.06%, 10.06%, 55.43%, respectively (Fig:16.8).

3.2.2.1.5. Alterations in enzymes of Hippocampus:

Metabolic enzymes such as AST, ALT, GS, GDH, LDH, AChE are altered during various drug treatment for both to induce epilepsy as well as anticonvulsant drugs and the data has been presented in the form of graphs, in the following Fig:16.9 and Fig:16.10.

Aspartate Amino-transferase (AST) activity were increased significantly in Pentylenetetrazole treated groups by 120.08% and in Sodium valproate treated mice by 93.11%, respectively. Whereas, remaining treated ones showed insignificant change when compared to its control.

Alanine Amino-transferase (ALT) activities elevated significantly in Sodium Valproate followed by PTZ treated mice by 44.12%; Cuprum metallicum primed mice followed by PTZ treatment by 87.24%; Combined drug (Sodium Valproate+

Cuprum metallicum) followed by Pentylenetetrazole exhibited 44.88% increase in enzyme activity (Fig:16.9).

Glutamine Synthetase (GS) enzyme activity reduced significantly during Pentylenetetrazole treatment, Cuprum metallicum treatment and in groups treated with Cuprum metallicum followed by Pentylenetetrazole by 79.90%, respectively. Whereas, Sodium Valproate treated groups by 1021.8%; Combined drug (Sodium Valproate + Cuprum metallicum) treated group by 840.31%; Sodium Valproate followed by Pentylenetetrazole treated ones by 44.94%; Combined drug (Sodium Valproate + Cuprum metallicum) followed by Pentylenetetrazole treated groups by 354.32%, respectively (Fig:16.9).

Glutamate dehydrogenase (GDH) enzyme assay during various treatments revealed a significant reduction in its activities in Hippocampus by 13.80% in Pentylenetetrazole treated groups; by 72.36% in Sodium Valproate treated groups; by 72.21% in Cuprum metallicum treated groups; by 72.32% in Combined drug (Sodium Valproate + Cuprum metallicum) treated; by 80.55% in Sodium Valproate followed by Pentylenetetrazole treated; by 35.08% in Cuprum metallicum followed by Pentylenetetrazole treated; by 73.95% in Combined drug (Sodium Valproate + Cuprum metallicum) followed by Pentylenetetrazole treated groups, respectively.

Lactate dehydrogenase (LDH) activities reduced significantly in Pentylenetetrazole treated (epileptic) mice by 79.20%; Sodium Valproate treated groups by 14.07%, respectively. LDH activity in Combined drug (Sodium Valproate + Cuprum metallicum) treated group showed significant elevation by 34.28%; similarly, in Sodium Valproate followed by Pentylenetetrazole treated ones by 41.59%; Cuprum metallicum followed by Pentylentetrazole by 37.77%; Combined

Fig 16.9: Alterations in AST, ALT, GS activities of Hippocampus during various drug treatment. (* represents probability p< 0.05, standard bar represents SE)

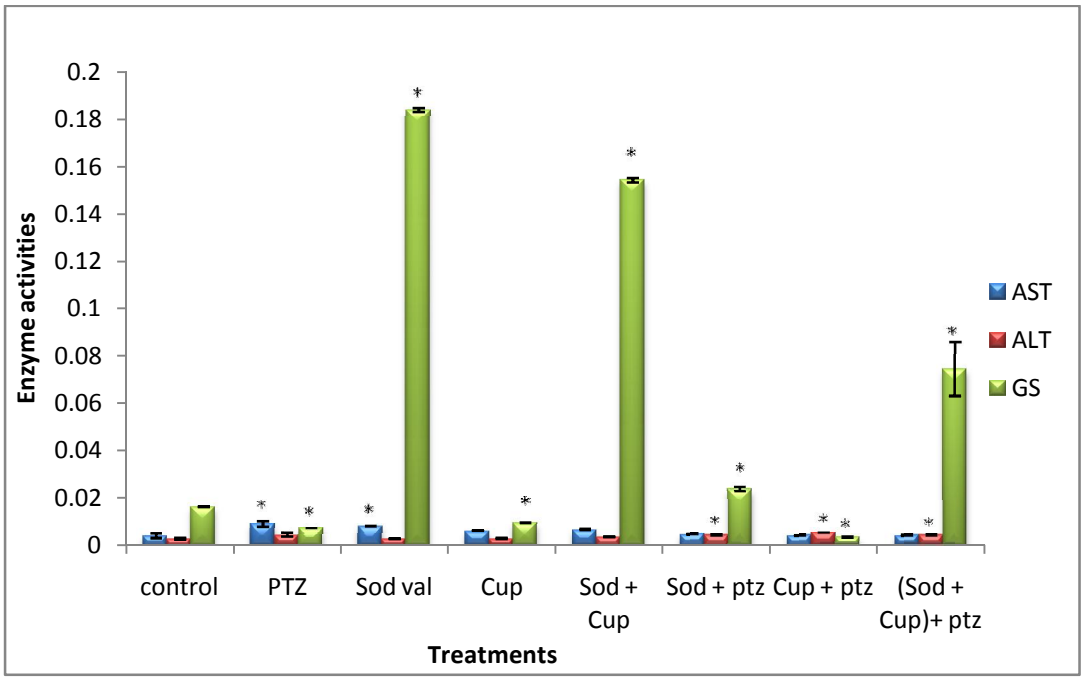
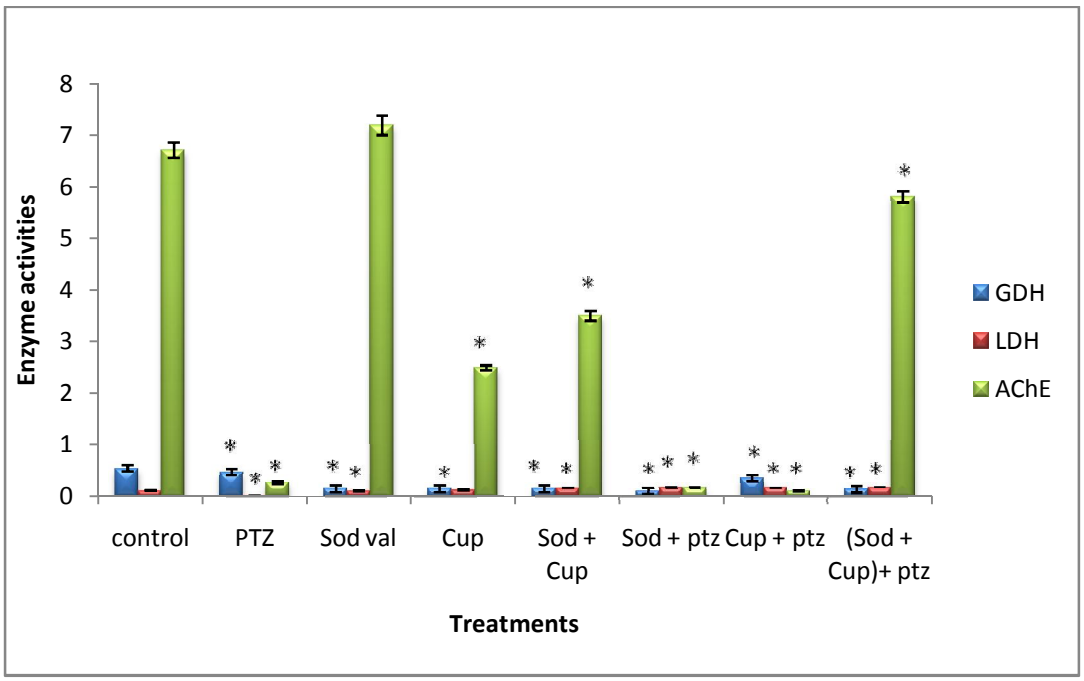


Fig 16.10: Alterations in GDH, LDH, AChE activities of Hippocampus during various drug treatment. (* represents probability p< 0.05, standard bar represents SE)



drug (Sodium Valproate + Cuprum metallicum) followed by Pentylenetetrazole by 44.42 %, respectively (Fig:16.10).

Acetylcholinesterase (AChE) activity exhibited a significant reduction in Pentylenetetrazole (epileptic) mice by 96.02%; Cuprum metallicum treated ones by 62.69%; Combined drug (Sodium Valproate + Cuprum metallicum) treated groups by 47.72%; Sodium Valproate followed by Pentylenetetrazole treated ones by 97.46%; Cuprum metallicum followed by Pentylenetetrazole treated ones by 98.42%; Combined drug (Sodium Valproate + Cuprum metallicum) followed by Pentylenetetrazole treated groups by 13.41%, respectively (Fig: 16.10).

3.2.2.1.6. Alterations in enzymes of Corpora Quadrigemina:

Metabolic enzyme activities during neuropharmacological study of epilepsy shows variation in its behavior and the data obtained from Corpora quadrigemina has been represented in Figure 16.11 and Fig: 16.12.

Aspartate Amino-transferase (AST) activity shows reduction in activity only in Pentylenetetrazole treated mice by 30.71%. Whereas, remaining groups showed insignificant change.

Alanine Amino-transferase (ALT) activities study revealed a significant reduction in Pentylenetetrazole treated mice by 99.48%; Sodium Valproate treated mice by 99.29%; Cuprum metallicum treated groups and Combined drug (Sodium Valproate + Cuprum metallicum) treated ones showed decrement of 99.25%, respectively.

Glutamine Synthetase (GS) activity showed fluctuations depending upon the type of treatment. Pentylenetetrazole (epileptic) mice; Cuprum metallicum treated mice; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice showed

reduced activity by 67.47%; 76.71%; 64.26%, respectively. Whereas, Sodium Valproate treated groups, Sodium Valproate followed by Pentylenetetrazole treated groups, Cuprum metallicum followed by Pentylenetetrazole treated groups, Combined drug (Sodium Valproate + Cuprum metallicum) followed by Pentylenetetrazole treated groups exhibited a significant increase in enzyme activity by 47.85%, 199.30%, 79.22% and 347.92%, respectively (Fig:16.11).

Glutamate dehydrogenase (GDH) activities altered throughout the treatment regimens. Mice treated with Pentylenetetrazole (epileptic) showed increase in activity by 648.19%; similarly, significant elevations in their activities seen in Cuprum metallicum treated mice by 343.312%; Combined drug (Sodium Valproate + Cuprum metallicum) treated ones by 246.07%; Sodium Valproate followed by Pentylenetetrazole treated groups by 142.03%; Cuprum metallicum followed by Pentylenetetrazole treated groups by 197.23%, respectively. Whereas, Sodium Valproate treated and Combined drug (Sodium Valproate + Cuprum metallicum) with post Pentylenetetrazole treated groups showed significant reduction in activity by 17.35% and 5.09%, respectively (Fig:16.12).

Lactate dehydrogenase (LDH) activities in Corpora Quadrigemina increased significantly in Sodium Valproate followed by Pentylenetetrazole treated groups by 38.33%; Cuprum metallicum followed by Pentylenetetrazole treated mice by 37.20% and Combined drug (Sodium Valproate + Cuprum metallicum) followed by Pentylenetetrazole treated groups by 48.48%, respectively (Fig:16.12).

Acetylcholinesterase (AChE) activities were exhibited significant decrements in groups such as Pentylenetetrazole treated (epileptic) mice by 99.49%; Cuprum

Fig 16.11: Alterations in AST, ALT, GS activities of Corpora Quadrigemina during various drug treatment. (* represents probability p< 0.05, standard bar represents SE**)**

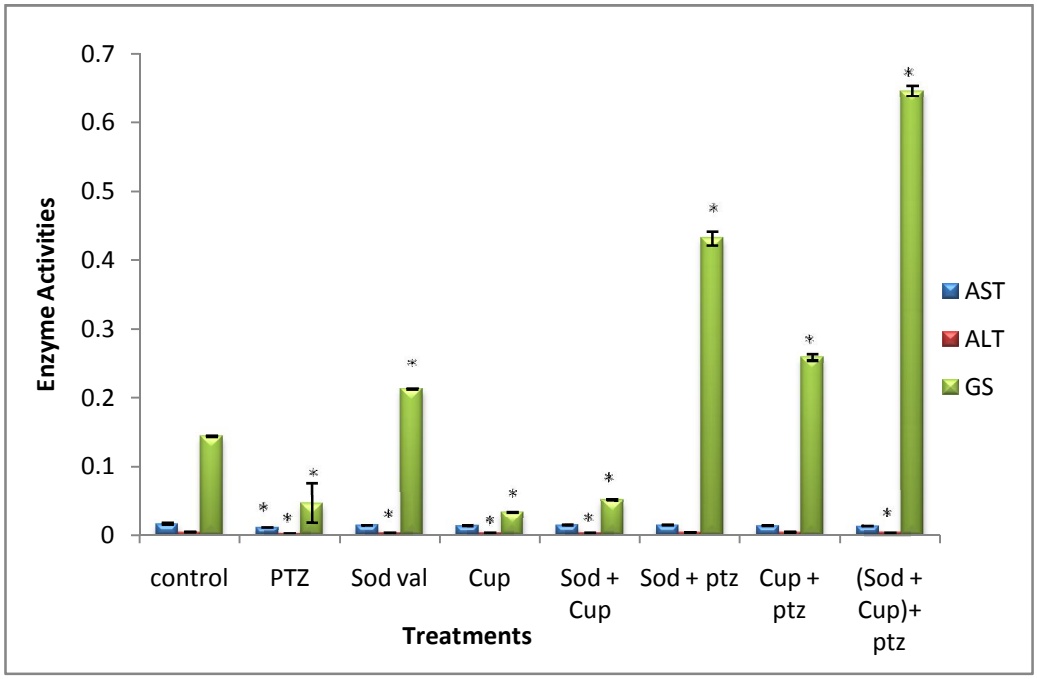
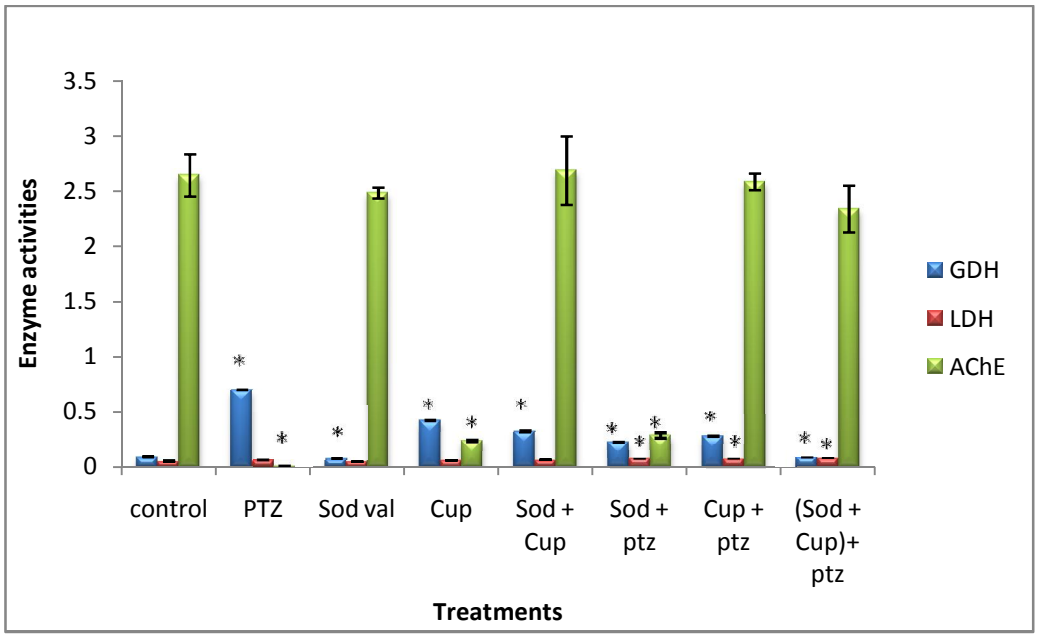


Fig 16.12: Alterations in GDH, LDH, AChE activities of Corpora Quadrigemina during drug treatment. (* represents probability p< 0.05, standard bar represents SE**)**



metallicum treated mice by 90.94% and Sodium Valproate followed by Pentylenetetrazole by 89.23%, respectively (Fig:16.12).

3.2.2.1.7. Alterations in enzymes of Cerebellum:

Aspartate amino-transferase (AST) activity in Cerebellum (Fig: 16.13) showed significant increase in Pentylenetetrazole treated (epileptic) mice by 17.75%; Cuprum metallicum treated mice by 10.17%; and Combined drug (Sodium Valproate + Cuprum metallicum) by 10.62%, respectively.

Alanine amino-transferase (ALT) activity in Cerebellum (Fig: 16.13) exhibited significant reduction by 97.61% in Pentylenetetrazole treated (epileptic) mice. Remaining groups were insignificant.

Glutamine sythetase (GS) in Cerebellum (Fig: 16.13) exhibited alterations in entire drug treatment regimens in the present study. Pentylenetetrazole treated (epileptic) group, mice treated with Sodium Valproate followed by Pentylenetetrazole, group treated with Cuprum metallicum followed by Pentylenetetrazole showed significant reduction in enzyme activities by 87.30%, 27.74%, 32.30%, respectively. Besides, groups such as Sodium Valproate treated, Cuprum metallicum treated, Combined drug (Sodium Valproate + Cuprum metallicum) treated, Combined drug (Sodium Valproate + Cuprum metallicum) with post treated Pentylenetetrazole revealed significant increase in activities by 138.74%, 20%, 16.96%, 114.63%, respectively.

Glutamate dehydrogenase (GDH) activity significantly increased in Pentylenetetrazole treated (epileptic) group by 187.34 %; Sodium Valproate treated mice by 7.32 %; Cuprum metallicum treated groups by 13.39 %; Sodium Valproate

Fig 16.13: Alterations in AST, ALT, GS activities of Cerebellum during various drug treatment. (* represents probability p< 0.05, standard bar represents SE)

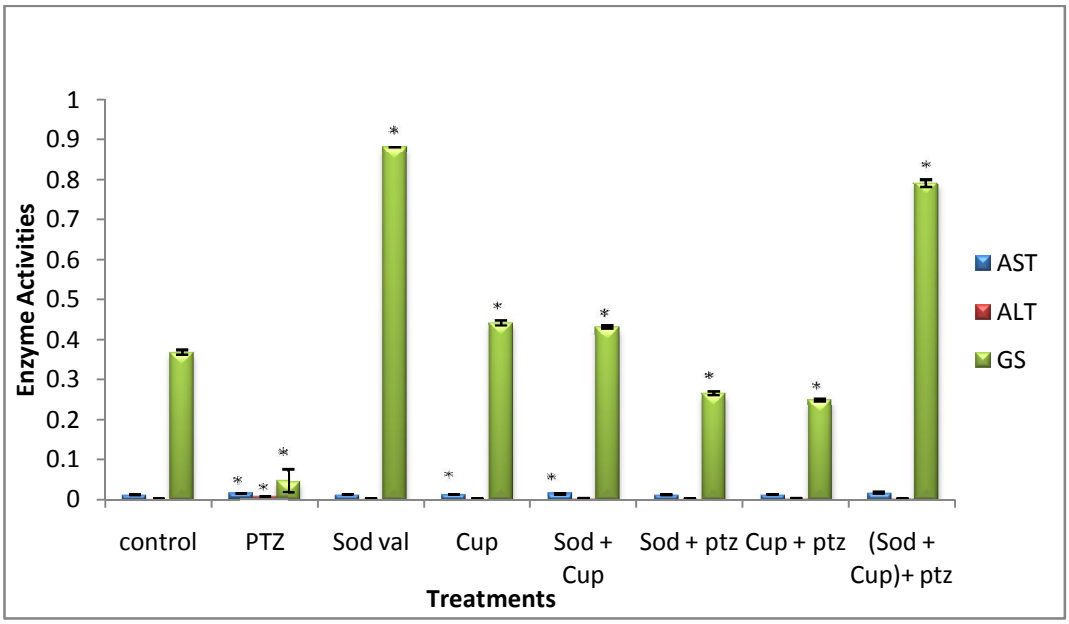
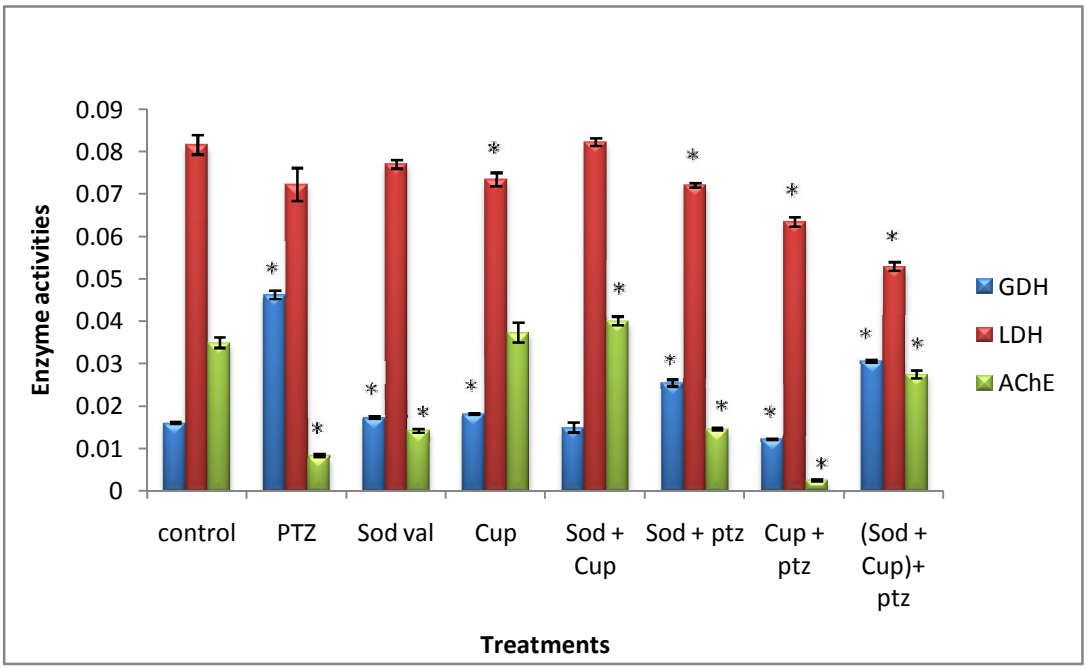


Fig 16.14: Alterations in GDH, LDH, AChE activities of Cerebellum during various drug treatment. (* represents probability p< 0.05, standard bar represents SE)



primed mice followed by Pentylenetetrazole treatment by 58.06 %; and Combined drug (Sodium Valproate + Cuprum metallicum) primed groups followed by Pentylenetetrazole by 90.44 %, respectively. Besides, the groups with Cuprum metallicum primed followed by Pentylenetetrazole treatment reduced activity by 23.82 % significantly (Fig: 16.14).

Lactate dehydrogenase (LDH) enzyme activity significantly reduced in the groups such as Cuprum metallicum treated by 9.98 %; Sodium Valproate followed by Pentylenetetrazole by 11.69 %; Cuprum metallicum with post treated Pentylenetetrazole by 22.21 %; Combined drug (Sodium Valproate + Cuprum metallicum) primed followed by Pentylenetetrazole treatment by 35.17 %, respectively (Fig: 16.14).

Acetylcholinesterase (AChE) activity decreased significantly during Pentylenetetrazole treatment (epileptic mice) by 76.10 %; Sodium Valproate treated by 59.37 %; Sodium Valproate followed by Pentylenetetrazole by 58.22 %; Cuprum metallicum primed mice followed by Pentylenetetrazole by 92.80 %; Combined drug (Sodium Valproate + Cuprum metallicum) primed groups followed by Pentylenetetrazole reduced by 21.24 %, respectively. Whereas, Combined drug (Sodium Valproate + Cuprum metallicum) primed mice significantly increased by 14.63 %, when compared to control (Fig: 16.14).

3.2.2.1.8. Alterations in enzymes of Pons:

Metabolic enzymes in Pons are represented in Figure 15.15 and Fig: 15.16, during different drug treatment regimen for study of epilepsy.

Aspartate amino-transferase (AST) activities decreased significantly in two groups i.e., Sodium Valproate treated ones and Cuprum metallicum treated ones by

39.78 % and 37.41 %, respectively. Whereas, remaining groups under the study did not show any significant changes (Fig: 16.15).

Alanine amino-transferase (ALT) showed insignificant change in entire treatment regimens (Fig: 16.15).

Glutamine Synthetase (GS) activities exhibited significant reduction in its activity especially in groups such as those mice treated with Pentylenetetrazole (epileptic) mice by 69.35 %; Sodium Valproate treated by 19.92 %; Cuprum metallicum treated groups by 59.5 %; Combined drug (Sodium Valproate + Cuprum metallicum) treated groups by 58.64 %; Cuprum metallicum primed followed by Pentylenetetrazole treated groups by 64.62 % and finally, Combined drug (Sodium Valproate + Cuprum metallicum) with post Pentylenetetrazole treated ones by 65.45 %, respectively. Whereas, Sodium Valproare primed mice followed by PTZ treatment exhibited an increment in GS activity (Fig:16.15).

Glutamate dehydrogenase (GDH) activities significantly increased by 147.63 % in Pentylenetetrazole (epileptic) mice; 66.09 % in Cuprum metallicum treated mice; 51.72 % in Combined drug (Sodium Valproate + Cuprum metallicum) treated groups; 42.90 % in Sodium Valproate primed followed by Pentylenetetrazole treated ones; 79.34 % in Cuprum metallicum primed followed by Pentylenetetrazole treated groups; 15.96 % in Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by Pentylenetetrazole treatment (Fig: 16.16).

Lactate dehydrogenase (LDH) activity exhibited significant deduction in Pentylenetetrazole treated groups by 78.24 %. Whereas, a significant elevation were found in Cuprum metallicum treated mice by 22.34 %; Combined drug (Sodium Valproate + Cuprum metallicum) treated groups by 57.95 %; Sodium Valproate

Fig 16.15: Alterations in AST, ALT, GS activities of Pons during various drug treatment. (* represents probability p< 0.05, standard bar represents SE)

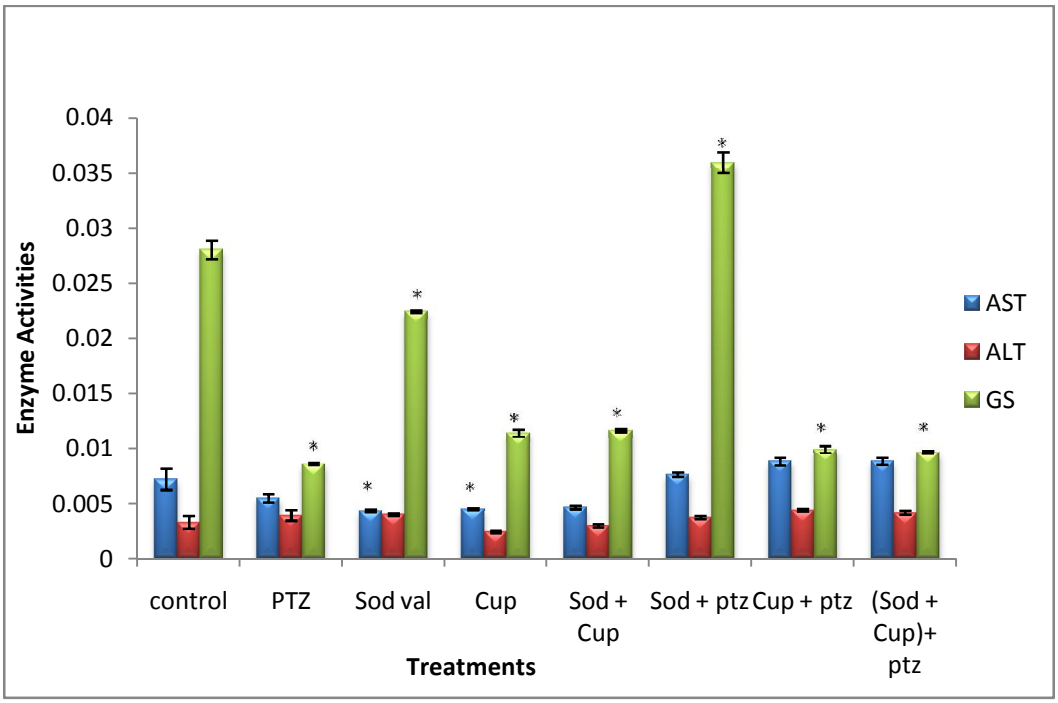
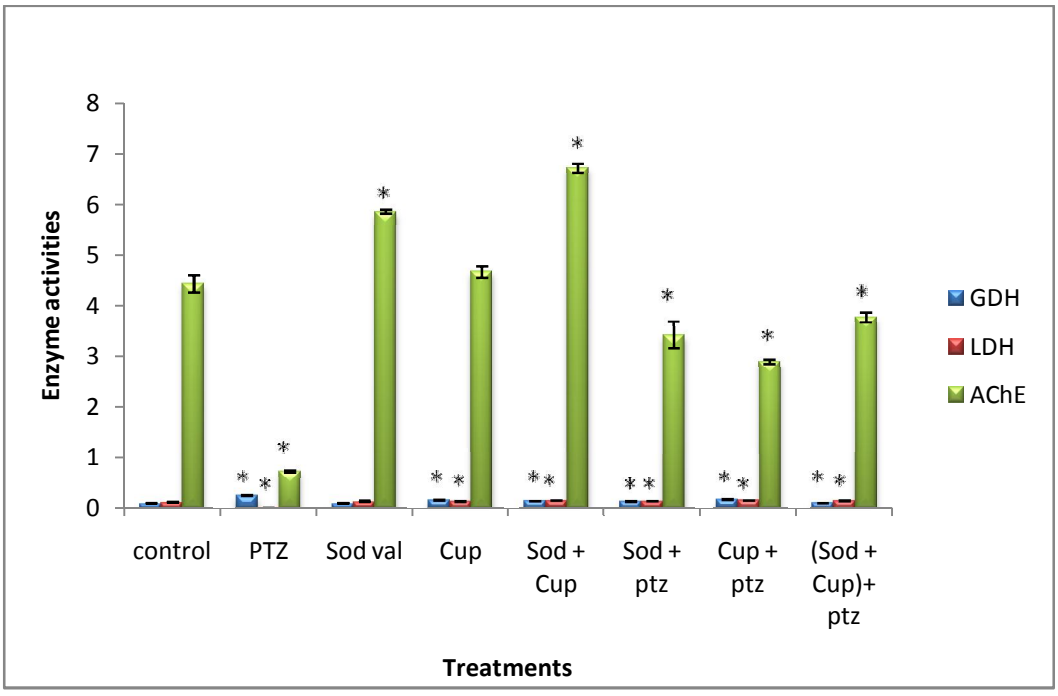


Fig 16.16: Alterations in GDH, LDH, AChE activities of Pons during various drug treatment. (* represents probability p< 0.05, standard bar represents SE)



primed followed by Pentylenetetrazole treated groups by 24.93 %; Cuprum metallicum with post Pentylenetetrazole treated groups by 37.75 %; Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by Pentylenetetrazole treatments by 31.91 %, respectively (Fig: 16.16).

Acetylcholinesterase (AChE) decreased significantly in Pentylenetetrazole treated mice by 83.51 %; Sodium Valproate followed by Pentylenetetrazole by 22.79 %; Cuprum metallicum followed by Pentylenetetrazole treated by 34.58 %; Combined drug (Sodium Valproate + Cuprum metallicum) primed groups followed by Pentylenetetrazole treated by 14.83 %, respectively. Besides, AChE activity elevated significantly by 32.18 % in Sodium Valproate treated mice and by 51.49 % in Combined drug (Sodium Valproate + Cuprum metallicum) treated groups (Fig: 16.16).

3.2.2.1.9 Alterations in enzymes of Medulla Oblongata:

Data obtained from enzymes assay in Medulla Oblongata, during different neuropharmacological treatments of epilepsy in mice has been presented in Figure 16.17 and Figure 16.18, respectively.

Aspartate amino-transferase (AST) reduced significantly in enzyme activity by 27.47 % in Pentylenetetrazole treated (epileptic) mice, and exhibited significant increase of 2.50 % in Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by Pentylenetetrazole treatment (Fig: 16.17).

Alanine amino-transferase (ALT) activity exhibited a significant reduction in the entire regimen of epilepsy treatments in Medulla Oblongata. In Pentylenetetrazole treated (epileptic) mice reduced significantly by 53.68 %; Sodium Valproate treated mice by 28.74 %; Cuprum metallicum treated mice by 45.08 %; Combined drug

(Sodium Valproate + Cuprum metallicum) treated group by 22.68 %; Sodium Valproate primed groups followed by Pentylenetetrazole treated by 29.86 %; Cuprum metallicum primed mice followed by Pentylenetetrazole treatment by 31 %; Combined drug (Sodium Valproate + Cuprum metallicum) primed mice with post Pentylenetetrazole treated group by 41.26 %, respectively (Fig: 16.17).

Glutamine Synthetase (GS) significant decrements in its activity by 74.71 % in Pentylenetetrazole treated (epileptic) mice; 49.70 % in Sodium Valproate treated mice; 67.24 % in Cuprum metallicum treated group; 60.19 % in Combined drug (Sodium Valproate + Cuprum metallicum) treated mice; 65.65 % in Sodium Valproate primed mice followed by Pentylenetetrazole treated; 92.76 % in Cuprum metallicum primed mice with post Pentylenetetrazole treatment; 79.29 % in Combined drug (Sodium Valproate + Cuprum metallicum) primed mice with post PTZ treated mice, respectively (Fig:16.17).

Glutamate dehydrogenase (GDH) assays revealed alterations in enzyme activities in Medulla oblongata (Fig: 15.18). Pentylenetetrazole treated (epileptic) mice exhibited increment of 142.74 %. Similarly, Cuprum metallicum treated and Sodium Valproate primed mice followed by Pentylenetetrazole treated exhibited increment by 118.56 % and 264.45 %, respectively. Besides, Sodium Valproate treated; Combined drug (Sodium Valproate + Cuprum metallicum) treated; Cuprum metallicum primed mice with post PTZ treated; Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ treated groups significantly reduced in enzyme activities by 86.72 %; 20.47 %; 30.17 %; 62.68%, respectively.

Lactate dehydrogenase (LDH) activity was significantly reduced in Sodium Valproate primed mice followed by PTZ treatment by 11.71 % and Cuprum metallicum primed mice followed by PTZ treatment by 20.57 %, respectively (Fig:16.18).

Fig 16.17: Alteration in AST, ALT, GS activities of Medulla Oblongata during various drug treatment. (*represents probability p< 0.05, standard bar represents SE)

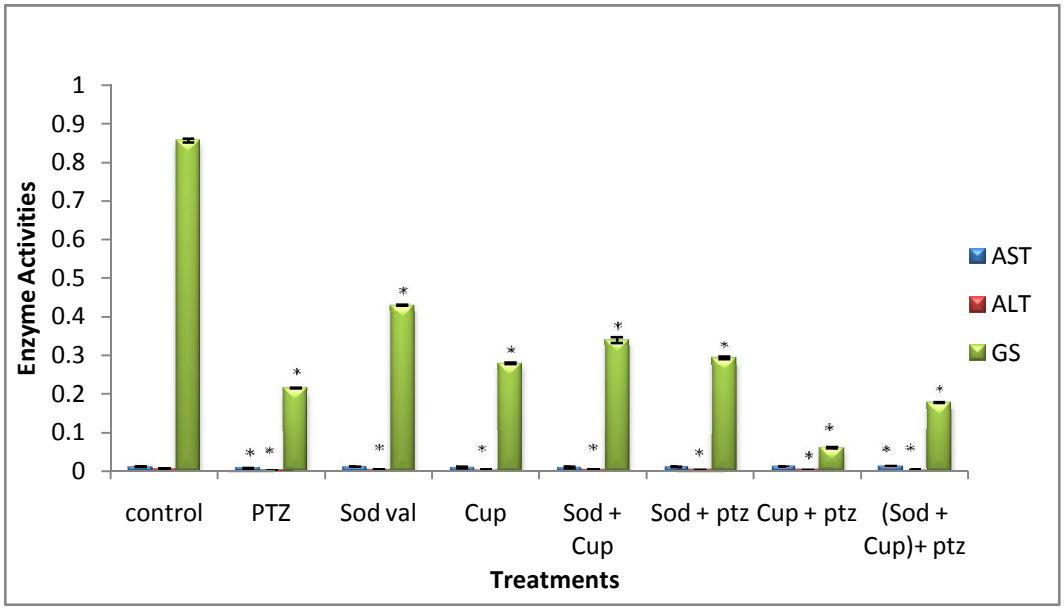
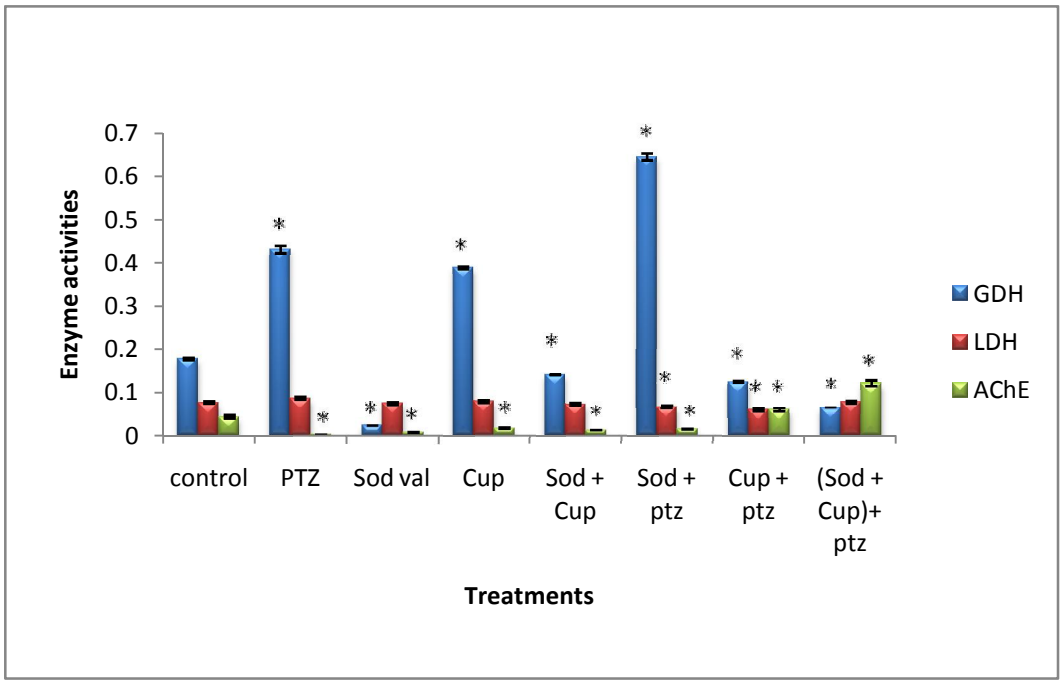


Fig 16.18: Alteration in GDH, LDH, AChE activities of Medulla Oblongata during various drug treatment. (* represents probability p< 0.05, standard bar represents SE)



Acetylcholinesterase (AChE) enzyme activity decreased significantly in Pentylenetetrazole treated (epileptic) mice by 93.60 %; Sodium Valproate treated groups by 83 %; Cuprum metallicum treated groups by 61.36 %; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 71.34 %; Sodium Valproate primed mice followed by PTZ treated groups by 64.73 %, respectively. Besides, AChE activities in Cuprum metallicum primed mice followed by PTZ treatment and Combined drug (Sodium Valproate + Cuprum metallicum) primed mice with post PTZ treatment exhibited significant increment by 37.70 % and 173.87 %, respectively (Fig:16.18).

3.2.2.2.Changes in Total ATPases in discrete brain regions of mice during various treatments:

Total ATPases such as Na^{+}-K^{+}-ATPase, Mg^{+2}-ATPase and Ca^{+2}-ATPase, were studied during various drug treatment regimens throughout epilepsy study in mice. Discrete mice brain regions such as Olfactory Lobe, Cerebral Cortex, Corpus Callosum, Cingulate gyrus, Hippocampus, Corpora Quadrigemina, Cerebellum, Pons, Medulla Oblongata, respectively were considered for studying changes in Total ATPases.

The course of drug treatments comprised of

1. Control (treated with saline);
2. PTZ (epileptic) treated mice;
3. Sodium Valproate treated mice;
4. Cuprum metallicum treated groups;
5. Combined drug (Sodium Valproate + Cuprum metallicum) treated groups;
6. Sodium Valproate primed followed by PTZ treated groups;

7. Cuprum metallicum primed followed by PTZ treated groups;
8. Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ treatment.

Note:

Units for Total ATPase enzyme activities is given as **Pi released in µg/mg protein/min**

3.2.2.2.1. Changes in Total ATPases of Olfactory Lobes:

Na^{+}-K^{+}-ATPase activities were increased in the entire set of treatment regimens in Olfactory Lobes of mice. It exhibited a significant increase by 272.76 % in PTZ treated (epileptic) mice; by 92.96 % in Sodium Valproate treated mice; 39.61 % in Cuprum metallicum treated groups; 59.84 % in Combined drug (Sodium Valproate + Cuprum metallicum) treated groups; 125.99 % in Sodium Valproate primed followed by PTZ treated groups. Similarly, an increase of 95.78 % in Cuprum metallicum primed followed by PTZ treated mice and 109.85 % increase in Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ treatment were exhibited (Fig:17.1).

Mg^{+2}-ATPase activities decreased considerably in almost entire set of experiments, by 52.66 % in PTZ treated (epileptic) mice; by 22.79 % in Sodium Valproate treated mice; 39.21 % in Cuprum metallicum treated groups; 24.11 % in Combined drug (Sodium Valproate + Cuprum metallicum) treated groups; 24.81 % decrease in Sodium Valproate primed followed by PTZ treated mice; 49.54 % in Cuprum metallicum primed mice followed by PTZ treatment (Fig:17.1).

Ca^{+2}-ATPase activities were altered appreciably, in different experimental groups under treatment such as PTZ treated (epileptic) mice with a significant

decrease by 32.26 %, Sodium Valproate primed mice followed by PTZ treatment by 25.01 %, Cuprum metallicum primed followed by PTZ treated by 36.89 % decrease were observed. Whereas, Combined drug (Sodium Valproate + Cuprum metallicum) treated mice showed considerable increment in its activity by 27.60 % (Fig: 17.1).

3.2.2.2.2. Changes in Total ATPases of Cerebral Cortex:

Na^{+}-K^{+}-ATPase activities altered in the entire set of treatment regimens in Cerebral Cortex of mice. It exhibited a significant decrement by 9.95 % in Pentylenetetrazole treated (epileptic) mice; 18.48 % in Cuprum metallicum primed mice followed by PTZ treated groups; 9.48 % in Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by pentylenetetrazole treatment, respectively. Besides, an appreciable increment of 15.87 % exhibited in Cuprum metallicum treated groups; similarly, in Combined drug (Sodium Valproate + Cuprum metallicum) treated group by 25.97 %; in Sodium Valproate followed by Pentylenetetrazole treated mice by 21.76 %, respectively (Fig:17.2).

Mg^{+2}-ATPase activities was decreased considerably in PTZ treated (epileptic) mice by 59.63 %; in Cuprum metallicum treated groups by 25.97 %; in Cuprum metallicum primed mice followed by PTZ treatment by 45.62 %, respectively. But a significant increase in enzyme activity was exhibited in Combined drug (Sodium Valproate + Cuprum metallicum) treated group by 43.50 % (Fig: 17.2).

Ca^{+2}-ATPase activities were appreciably decreased, in different groups such as PTZ treated (epileptic) mice by 40.26 %, Sodium Valproate treated mice by 35.22 %, Cuprum metallicum treated group by 48.23 %, Combined drug (Sodium Valproate + Cuprum metallicum) treated ones by 22.27 %, Sodium Valproate primed mice followed by PTZ treatment by 27.19 %, Cuprum metallicum primed groups followed

Fig: 17.1 Changes in Total ATPases during various drug treatment in Olfactory Lobe. (* represents probability p< 0.05, standard bar represents SE**)**

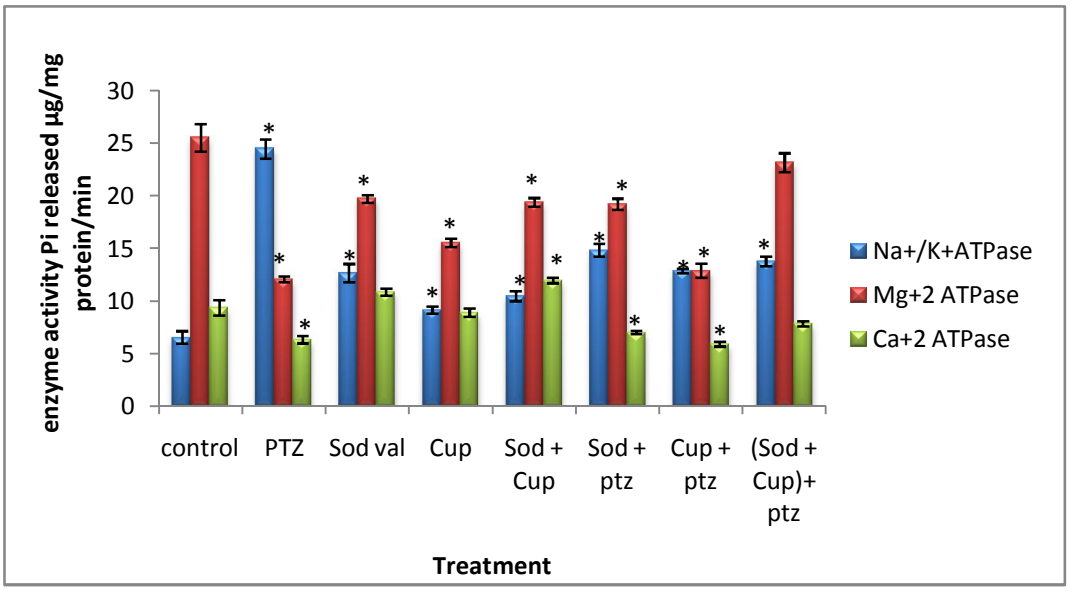
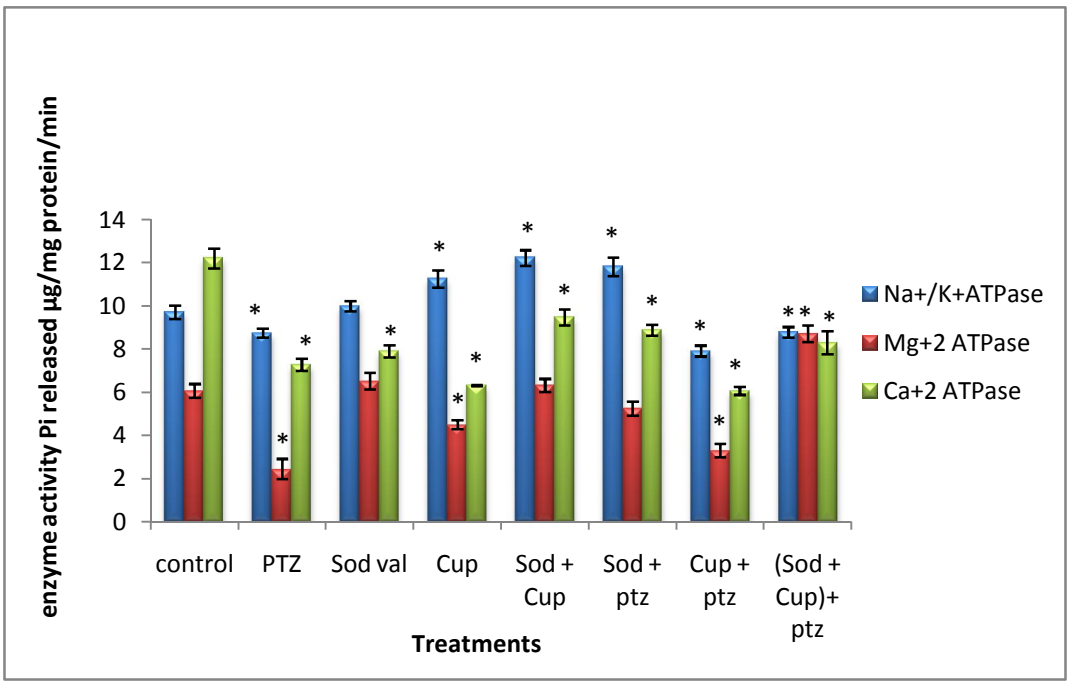


Fig: 17.2 Changes inTotal ATPases during various drug treatment in Cerebral cortex. (* represents probability p< 0.05, standard bar represents SE**)**



by PTZ treatment by 50.28 %, Combined drug (Sodium Valproate + Cuprum metallicum) primed followed by PTZ treated mice exhibited 32.01 % decrease (Fig:17.2).

3.2.2.2.3. Changes in Total ATPases of Corpus Callosum:

Na^{+}-K^{+-}ATPase activities exhibited a significant increase in activity by 33.63 % in PTZ treated (epileptic) mice; Sodium Valproate treated mice by 50.55 %, Cuprum metallicum treated groups by 56.86 %, Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 40.90 %, Cuprum metallicum followed by PTZ treated groups by 47.27 %, respectively (Fig:17.3).

Mg^{+2}-ATPase activities were decreased considerably in PTZ treated (epileptic) mice by 54.24 %; in Sodium Valproate treated mice by 34.81 %; in Cuprum metallicum treated groups by 22.72 %, respectively (Fig:17.3).

Ca^{+2}-ATPase activities were reduced, in all groups of mice under present study, such as PTZ treated (epileptic) mice 34.27 %, Sodium Valproate treated mice by 17.53 %, Cuprum metallicum treated groups by 9.01 %, Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 30.37 %, Sodium Valproate primed followed by PTZ treatment by 30.47 %, Cuprum metallicum primed mice followed by PTZ treated by 16.35 %, Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ treatment by 16.03 %, respectively (Fig:17.3)

3.2.2.2.4. Changes in Total ATPases of Cingulate gyrus:

Na^{+}-K^{+}-ATPase activities exhibited a considerable decrease in all the sets of treatment regimens in Cingulate gyrus of mice by 84.23 % in PTZ treated (epileptic) mice; by 41.61 % in Sodium Valproate treated groups; 67.26 % in Cuprum

metallicum treated groups; 53.79 % in Combined drug (Sodium Valproate + Cuprum metallicum) treated mice; by 69.02 % in Sodium Valproate primed followed by PTZ treated groups; by 69.49 % in Cuprum metallicum primed mice followed by PTZ treated groups; by 39.64 % in Combined drug (Sodium Valproate + Cuprum metallicum) primed followed by PTZ treated groups; respectively (Fig:17.4).

Mg^{+2}-ATPase activities significantly decreased in PTZ treated (epileptic) mice by 48.03 %; in Sodium Valproate treated mice by 40.91 %; in Cuprum metallicum treated groups by 34.30 %; Combined drug (Sodium Valproate + Cuprum metallicum) treated group by 27.73 %; in Sodium Valproate treated mice with post PTZ by 27.47 %; in Cuprum metallicum primed mice followed by post PTZ treated groups by 27.08 %, respectively (Fig:17.4).

Ca^{+2}-ATPase activities were appreciably decreased, in different groups such as PTZ treated (epileptic) mice (36.14 %), Sodium Valproate treated mice (33.08 %), Cuprum metallicum treated groups (18.64 %), Combined drug (Sodium Valproate + Cuprum metallicum) treated ones (22.27%), Sodium Valproate primed mice followed by PTZ treatment (30.80%), Cuprum metallicum primed mice followed by PTZ treated (15.87 %), Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ treated mice (25.63 %), respectively (Fig:17.4).

3.2.2.2.5. Changes in Total ATPases of Hippocampus:

Na^{+}-K^{+}-ATPase activities reduced significantly by19.26% in Pentylenetetrazole treated (epileptic) mice; 19.26 % in Sodium Valproate treated mice; 14.90 % in Cuprum metallicum treated groups; 41.11 % in Sodium Valproate primed mice followed by Pentylenetetrazole treatment, respectively. Besides,

Fig: 17.3 Changes in Total ATPases during various drug treatment in Corpus callosum. (* represents probability p< 0.05, standard bar represents SE**).**

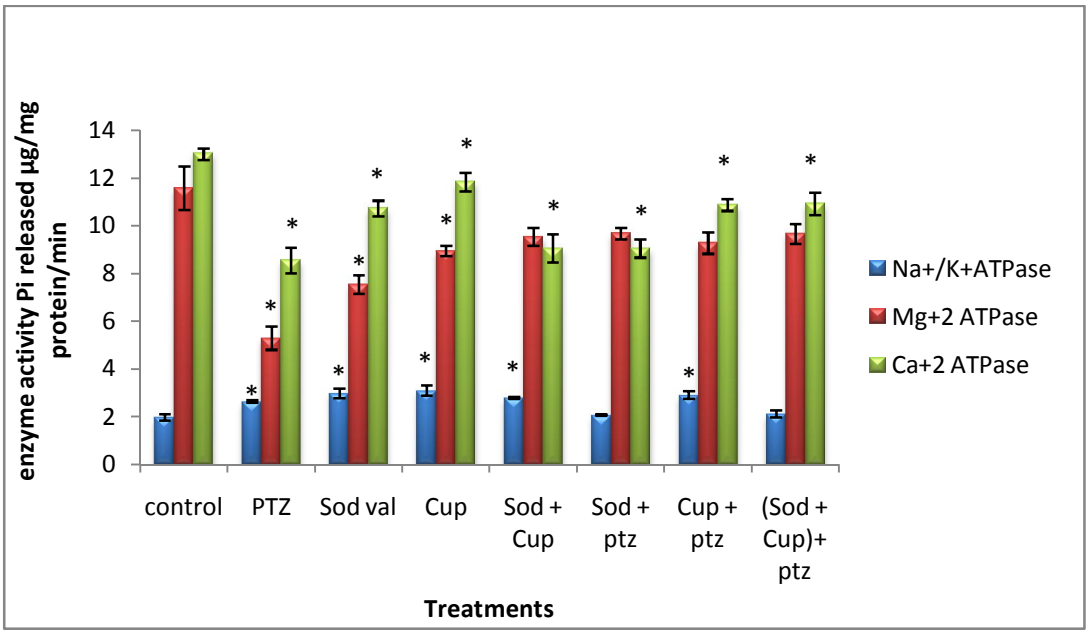
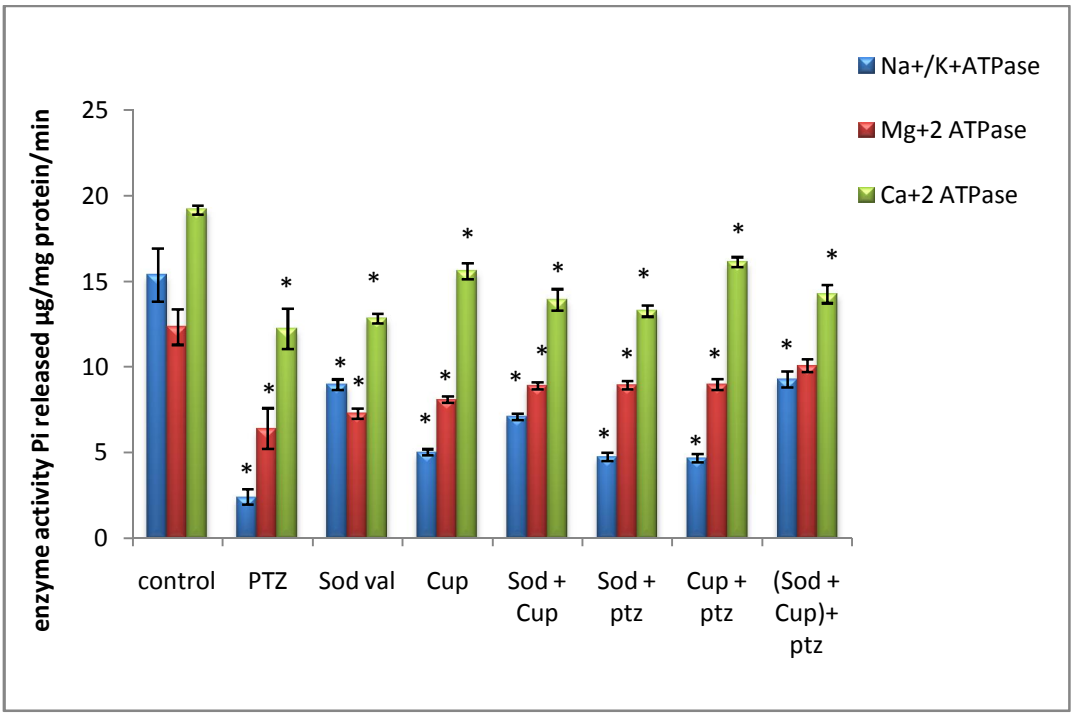


Fig: 17.4 Changes in Total ATPases during various drug treatment in Cingulate gyrus. (* represents probability p< 0.05, standard bar represents SE**)**



significant increment in enzyme activities by 40.30 % in Combined drug (Sodium Valproate + Cuprum metallicum) treated mice and 17.94 % in Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by Pentylenetetrazole treatment (Fig:17.5).

Mg^{+2}-ATPase activities decreased considerably under the influence of drugs such as Pentylenetetrazole (epileptic) by 39.74 %; Combined drug (Sodium Valproate + Cuprum metallicum) by 18.89 %; Cuprum metallicum primed followed by Pentylenetetrazole by 21.64 %; Combined drug (Sodium Valproate + Cuprum metallicum) primed with post Pentylenetetrazole by 19.41 %, respectively (Fig:17.5).

Ca^{+2}-ATPase activities reduced significantly in Hippocampus under the influence of various drug regimen such as Pentylenetetrazole (epileptic drug) by 29.68 %; Sodium Valproate by 22.02 %; Cuprum metallicum by 31.93 %, respectively. Besides, Sodium Valproate primed mice followed by Pentylenetetrazole increased by 14.65 %; Cuprum metallicum primed groups followed by Pentylenetetrazole significantly increased by 16.13 %, in Ca^{+2}-ATPase activities, Combined drug (Sodium Valproate + Cuprum metallicum) primed groups with post Pentylenetetrazole by 12.41 % increase, respectively (Fig:17.5).

3.2.2.2.6. Changes in Total ATPases of Corpora Quadrigemina:

The Total ATPases activities such as Na^{+}-K^{+}-ATPase, Mg^{+2}-ATPase, Ca^{+2}-ATPase in Corpora quadrigemina region of mice brain varied upon treatment with various epileptic and antiepileptic drugs, which is represented in Figure 17.6, respectively.

Na^{+}-K^{+}-ATPase, activities reduced appreciably by 87.62 % in Pentylenetetrazole (epileptic) treated mice; 52.02 % in Sodium Valproate treated

group; 74.84 % in Cuprum metallicum treated group; 57.61% in Combined drug (Sodium Valproate + Cuprum metallicum) treated group; 57.10% in Sodium Valproate primed mice followed by Pentylenetetrazole treatment; 78.31% in Cuprum metallicum primed groups followed by Pentylenetetrazole treatment; 27.47 % in Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by Pentylenetetrazole treated group, respectively.

Mg^{+2}-ATPase activities significantly increased only in Sodium Valproate primed followed by PTZ treated mice by 70.88 %, rest other treated groups exhibited insignificant changes when compared to its control.

Ca^{+2}-ATPase activities increased considerably in groups such as Pentylenetetrazole treated (epileptic) mice by 39.70 %; in Sodium Valproate primed groups followed by Pentylenetetrazole treated by 65.58 %; Cuprum metallicum primed mice followed by Pentylenetetrazole treated by 46.47 % and finally, in Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by Pentylenetetrazole treated by 64.13 %, respectively (Fig:17.6).

3.2.2.2.7. Changes in Total ATPases of Cerebellum:

Na^{+}-K^{+}-ATPase activities reduced significantly by 57.59% in Pentylenetetrazole treated (epileptic) mice; 37.69 % in Sodium Valproate treated mice; 64.77 % in Cuprum metallicum treated groups; 26.37 % in Combined drug (Sodium Valproate + Cuprum metallicum) treated mice; 58.98 % of Sodium Valproate primed mice followed by Pentylenetetrazole treated and 24.79 % in Cuprum metallicum primed mice followed by Pentylenetetrazole treatment, respectively (Fig:17.7)

Fig: 17.5 Changes in Total ATPases during various drug treatment in Hippocampus. (* represents probability p< 0.05, standard bar represents SE)

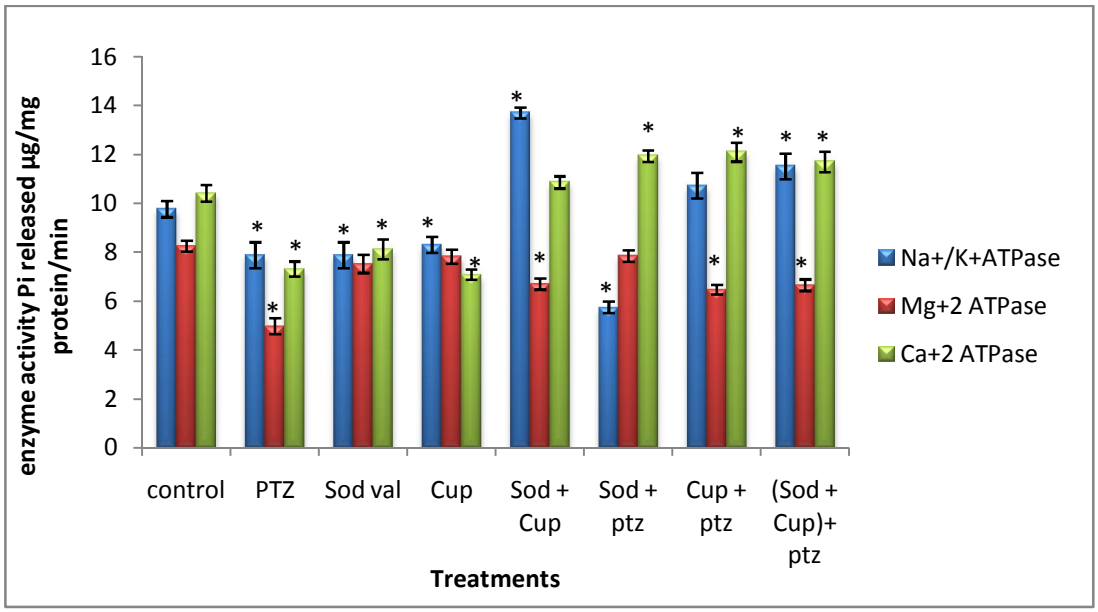
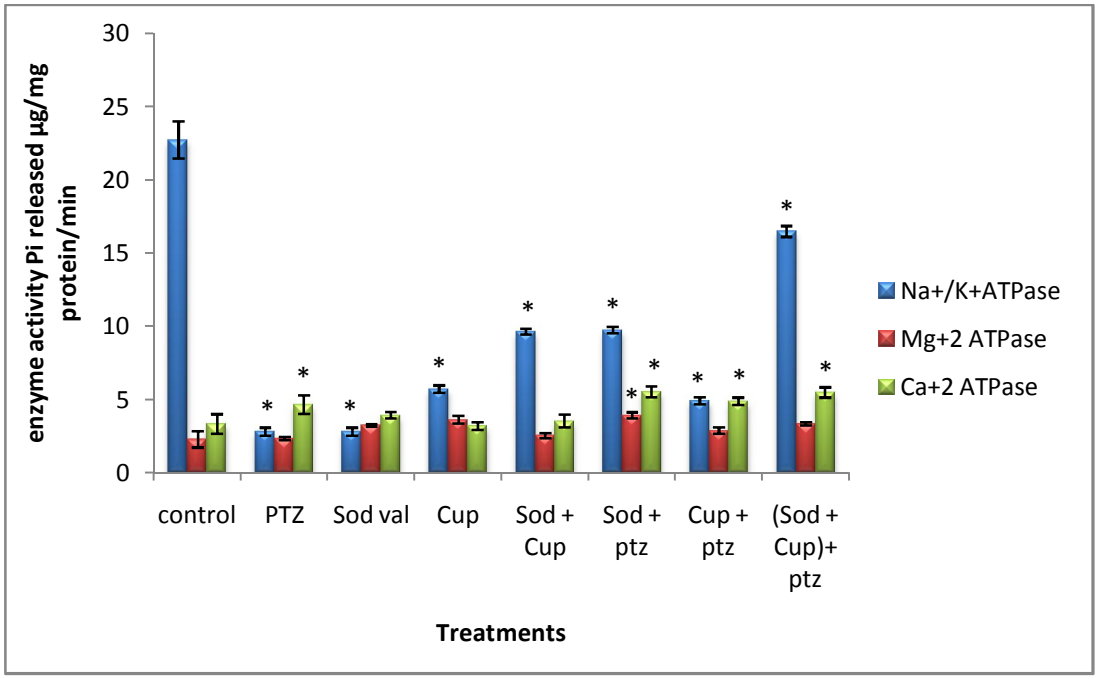


Fig: 17.6 Changes in Total ATPases during various drug treatment in Corpora quadrigemina. (* represents probability p< 0.05, standard bar represents SE)



Mg^{+2}-ATPase activities decreased considerably under the influence of drugs such as Pentylenetetrazole (epileptic) by 43.30 %; Sodium Valproate treated mice by 24.76 %; Sodium Valproate primed mice followed with Pentylenetetrazole by 25.56 %, respectively (Fig:17.7).

Ca^{+2}-ATPase activities reduced significantly in Cerebellum under the influence of various drug regimens such as Pentylenetetrazole (epileptic drug) by 73.03 %; Sodium Valproate by 46.19%; Cuprum metallicum by 50.13 %; Combined drug (Sodium Valproate + Cuprum metallicum) treated group by 44.02 %; Sodium Valproate primed mice followed by Pentylenetetrazole by 42.88 %; Cuprum metallicum primed mice followed by Pentylenetetrazole by 47.45 %; Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by Pentylenetetrazole by 27.06 %, respectively (Fig:17.7).

3.2.2.2.8. Changes in Total ATPases of Pons:

Na^{+}-K^{+}-ATPase, activities reduced appreciably by 75.27 % in Pentylenetetrazole (epileptic) treated mice; 31.50 % in Sodium Valproate treated group; whereas an increase by 23.60 % in Sodium Valproate primed mice followed by Pentylenetetrazole treated, respectively (Fig:17.8).

Mg^{+2}-ATPase activities significantly decreased in Pentylenetetrazole treated (epileptic) mice by 67.37 %; Sodium Valproate primed followed by PTZ treated mice by 23.72 %, Cuprum metallicum primed followed by PTZ treated group by 32.43 %; Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by Pentylenetetrazole treated groups by 22.25%, respectively (Fig:17.8).

Ca^{+2}-ATPase activities were reduced considerably in groups such as Pentylenetetrazole treated (epileptic) mice by 21.86 %; in Sodium Valproate by 33.36

%; in Cuprum metallicum treated group by 30.41 %; Combined drug (Sodium Valproate + Cuprum metallicum) treated groups by 21.12 %; in Sodium Valproate primed mice followed by Pentylenetetrazole treatment reduced by 44.10 %. Similarly, Cuprum metallicum primed mice followed by Pentylenetetrazole treatment by 50.66 % and finally, in Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by Pentylenetetrazole treated group by 37.79 % decrements, respectively (Fig:17.8).

3.2.2.2.9. Changes in Total ATPases of Medulla Oblongata:

Na^{+}-K^{+}-ATPase activities increased significantly by 184.81% in Pentylenetetrazole treated (epileptic) mice; 82.47 % in Sodium Valproate treated mice; 148.13 % in Cuprum metallicum treated groups; 73.59 % in Combined drug (Sodium Valproate + Cuprum metallicum) treated mice; 113.73 % Cuprum metallicum primed mice followed by Pentylenetetrazole treatment, respectively (Fig:17.9).

Mg^{+2}-ATPase activities was decreased considerably under the influence of drugs such as Pentylenetetrazole (epileptic) by 13.83 %; Sodium Valproate treated groups by 4.44 %; Combined drug (Sodium Valproate + Cuprum metallicum) by 20.44 %; Sodium Valproate with post Pentylenetetrazole by 25.77 %; Cuprum metallicum primed mice followed by Pentylenetetrazole by 3.59 %, respectively (Fig:17.9).

Ca^{+2}-ATPase activities were reduced significantly in Medulla Oblongata under the influence of various drug regimen such as Pentylenetetrazole (epileptic drug) by 16.48 %; Sodium Valproate by 23.66 %; Cuprum metallicum by 18.44 %, respectively (Fig:17.9).

Fig: 17.7 Changes in Total ATPases during various drug treatment in Cerebellum. (* represents probability p< 0.05, standard bar represents SE)

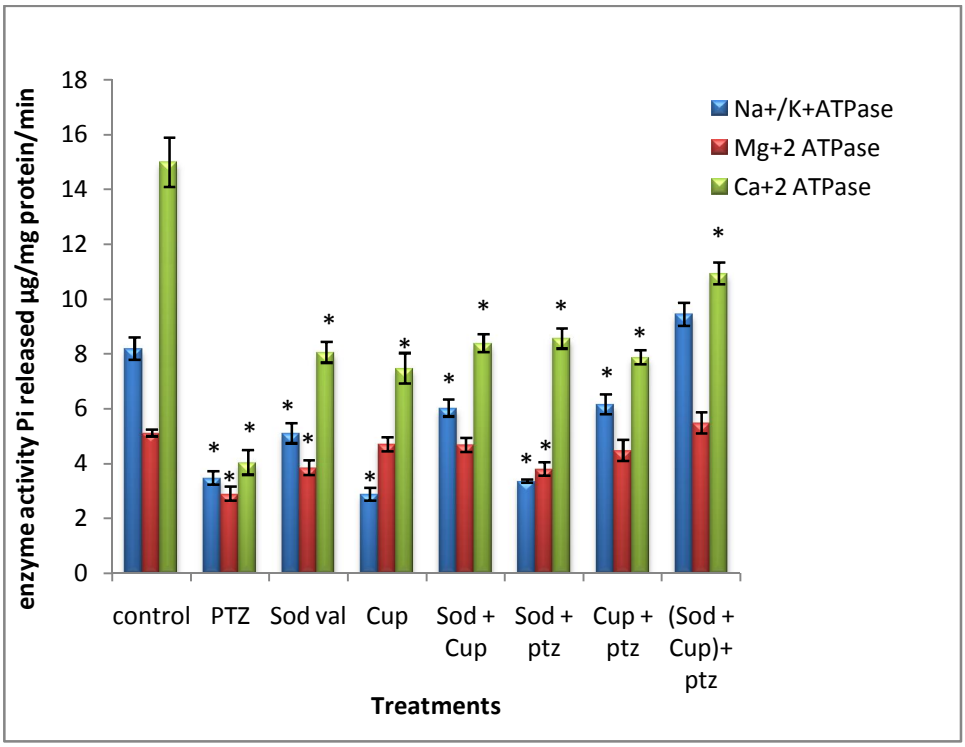


Fig 17.8: Changes in Total ATPases during various drug treatment in Pons. (*represents probability p<0.05, standard bar represents SE)

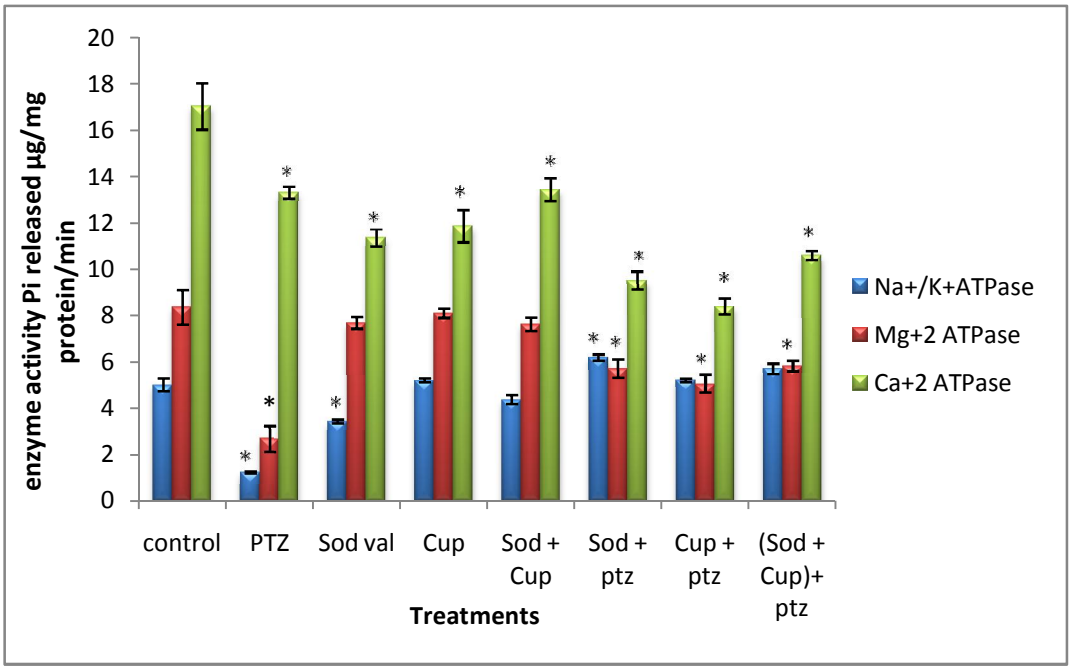
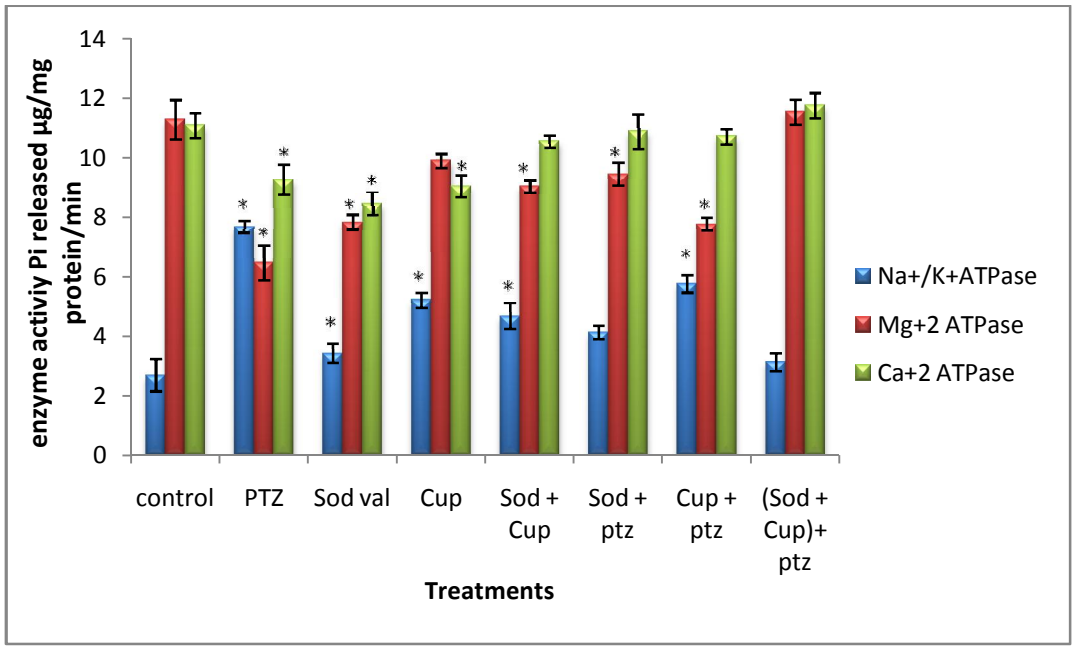


Fig 17.9: Changes in Total ATPases during various drug treatment in Medulla Oblongata. (* represents probability p< 0.05, standard bar represents SE)



3.2.2.3.Variations in Metabolite contents of discrete brain regions of mice during various Treatment:

Metabolites such as Pyruvate, Lactate, Glutamate, Glucose and Gama-amino butyric acid (GABA) has been assayed in the discrete mice brain regions such as Olfactory Lobe, Cerebral Cortex, Corpus Callosum, Cingulate gyrus, Hippocampus, Corpora Quadrigemina, Cerebellum, Pons, Medulla Oblongata during various drug treatments. The neuropharmacological treatments during epilepsy in Mice comprised of different drugs such as Epileptic drug (PTZ), and antiepileptic drugs both allopathic (Sodium Valproate) as well as homeopathic preparations (Cuprum metallicum) and combination of the two antiepileptic drugs. The results obtained have been represented in the form of graphs below (Fig: 18.1-18.18). The X-axis depicts the different treatments and Y-axis shows the metabolite level.

Note: Units for metabolite content are as follows:

a) Pyruvate level -- µM/ mg wet weight of tissue
b) Lactate -- µM/ mg wet wt. of tissue
c) Glutamate -- mM/mg wet wt of tissue
d) Glucose -- mM/mg wet wt of tissue
e) GABA -- mM/mg wet wt of tissue

3.2.2.3.1. Variations in Metabolite contents of Olfactory Lobe: (Fig:18.1 & 18.2)

Pyruvate contents did not change significantly in Olfactory Lobe, during various drug treatment regimens.

Lactate contents increased significantly in entire set of treatments, such as in Pentylenetetrazole treated (epileptic) mice by 197.40 %; Sodium Valproate treated mice by 244.97 %; Cuprum metallicum treated mice by 43.89 %; Combined drug

(Sodium Valproate + Cuprum metallicum) treated ones by 161.07 %; Sodium Valproate primed mice followed by Pentylenetetrazole by 141.26 %; Cuprum metallicum primed followed by Pentylenetetrazole treated by 63.34 %; Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ treatment by 32.12 %, respectively.

Glutamate content exhibited increment in Pentylenetetrazole treated (epileptic) mice by 89.97 %. Whereas, it decreased significantly in Sodium Valproate treated group by 36.94 %; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 30.04 %; Sodium Valproate primed mice followed by Pentylenetetrazole by 34.10 %; Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by Pentylenetetrazole treatment by 30.04%, respectively.

Gamma-amino butyric acid content decreased precipitously in Pentylenetetrazole treated (epileptic) mice by 43.10 %. Besides, it increased significantly in Sodium Valproate treated mice by 43.66 %, Combined drug (Sodium Valproate + Cuprum metallicum) treated groups by 28.44 %, Sodium Valproate primed mice followed by Pentylenetetrazole by 41.82 %, Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by Pentylenetetrazole treatment by 38.79%, respectively.

Glucose content decreased considerably by 27.53 % in Pentylenetetrazole treated mice; 56.52 % in Sodium Valproate treated mice; 46.26 % in Combined drug (Sodium Valproate + Cuprum metallicum) treated groups; 30.11 % in Sodium Valproate primed mice followed by Pentylenetetrazole treatment; 38.79 % in Cuprum metallicum primed mice followed by Pentyleneterazole treatment respectively.

Fig: 18.1 Alterations in Pyruvate, Lactate, Glutamate contents of Olfactory Lobe during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**

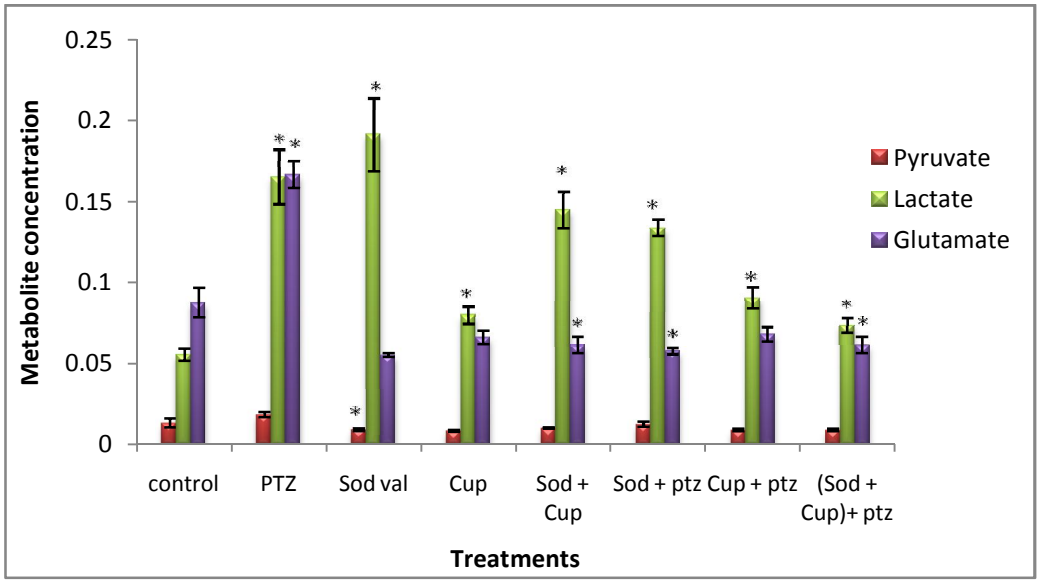
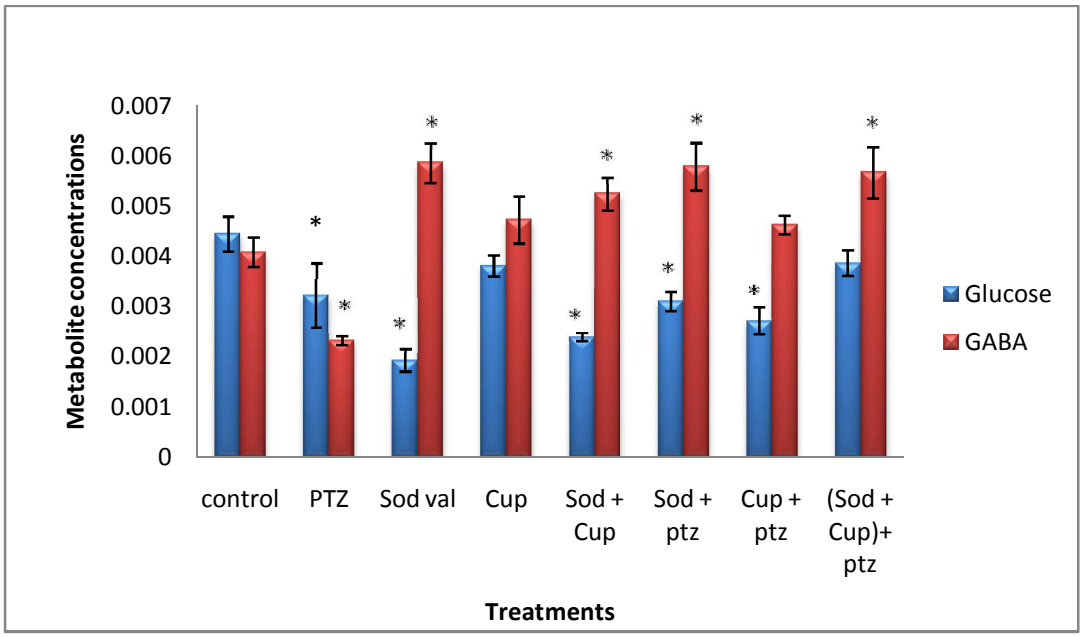


Fig: 18.2 Alterations in Glucose, GABA contents of Olfactory Lobe during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**



3.2.2.3.2. Variations in Metabolite contents of Cerebral Cortex:(Fig: 18.3 & 18.4)

Pyruvate content showed to be decreased significantly by 44.76 % in Pentylenetetrazole treated mice; 18.81% in Sodium Valproate treated mice; 14.43 % in Cuprum metallicum treated mice; 22.01% in Combined drug (Sodium Valproate + Cuprum metallicum) treated ones; 12.98 % in Sodium Valproate followed by Pentylenetetrazole treated mice; 7.87% in Cuprum metallicum followed by Pentylenetetrazole treated mice; 5.99% in Combined drug (Sodium Valproate + Cuprum metallicum) followed by Pentylenetetrazole treated groups, respectively.

Lactate level were found to be elevated appreciably by 26.32% in Pentylenetetrazole treated (epileptic) mice; 22.97% in Sodium Valproate treated mice; 20.39% in Cuprum metallicum treated mice; 25.03% in Combined drug (Sodium Valproate + Cuprum metallicum) treated ones, respectively.

Glutamate was found to be increased during Pentylenetetrazole treatment in mice by 87.74%.

Gamma-amino butyric acid content was decreased in Pentylenetetrazole treated mice by 51.57 % and increase significantly in Cuprum metalicum treated group by 14.92%.

Glucose content were significantly reduced by 9.89 % in Pentylenetetrazole treated (epileptic) mice; by 50.81 % Sodium Valproate treated mice; by 45.36 % in Combined drug (Sodium Valproate + Cuprum metallicum) treated groups; by 27.69 % in Sodium Valproate primed mice followed by Pentylenetetrazole treatment, respectively.

Fig: 18.3 Alterations in Pyruvate, Lactate, Glutamate contents of Cerebral Cortex during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**

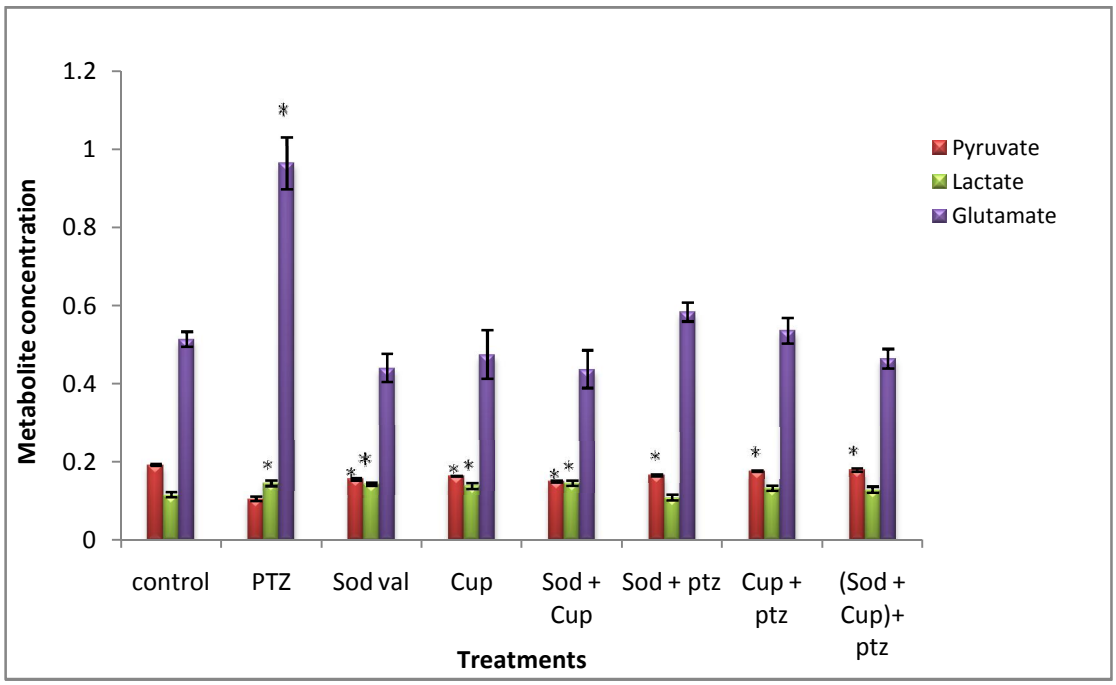
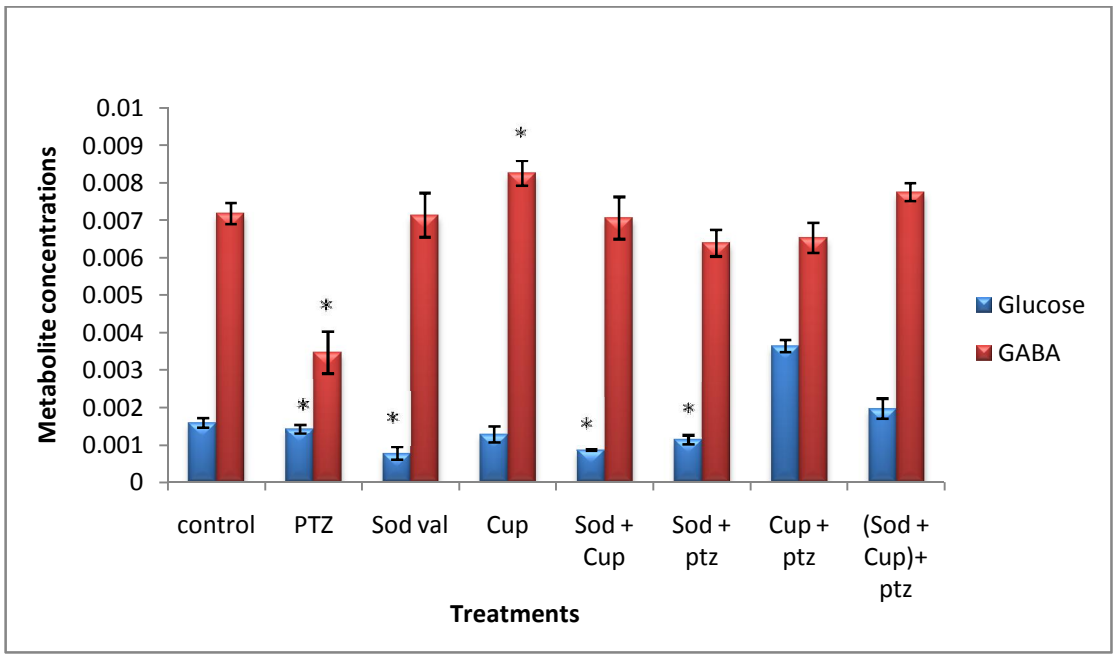


Fig: 18.4 Alteration in Glucose, GABA contents of Cerebral Cortex during various drug treatment (* represents probability p< 0.05, standard bar represents SE)



3.2.2.3.3. Variations in Metabolite contents of Corpus callosum: (Fig:18.5 & 18.6)

Pyruvate level significantly elevated by 37.54% in Pentylenetetrazole treated (epileptic) mice; 43.12 % in Combined drug (Sodium Valproate + Cuprum metallicum) treated groups; by 54.62 % in Sodium Valproate primed mice followed by PTZ treament, respectively.

Lactate concentration reduced in Pentylenetetrazole treated mice by 52.31 % and increased significantly in Sodium Valproate treated mice by 33.56 %, respectively.

Glutamate content increased considerably only in Pentylenetetrazole treated mice by 26.91%.

Gamma-amino butyric acid (GABA) was decreased appreciably in Pentylenetetrazole treated mice by 44.11%; Whereas, it was increased significantly in Sodium Valproate treated mice by 149.26%; Cuprum metallicum treated mice by 75.74%; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 111.71%; Sodium Valproate primed mice followed by Pentylenetetrazole treatment by 99.06%; Combined drug (Sodium Valproate + Cuprum metallicum) with post Pentylenetetrazole treated mice by 63.90%, respectively.

Glucose content increased significantly in Pentylenetetrazole treated mice by 44.30%.

3.2.2.3.4. Variations in Metabolite contents of Cingulate gyrus: (Fig: 18.7 &18.8)

Pyruvate content were increased significantly in the entire set of treatment such as Pentylenetetrazole by 115.44 %; Sodium Valproate treated mice by 25.25 %; Cuprum metallicum treated mice by 67.94%; Combined drug (Sodium Valproate +

Fig: 18.5 Alterations in Pyruvate, Lactate, Glutamate contents of Corpus callosum during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**

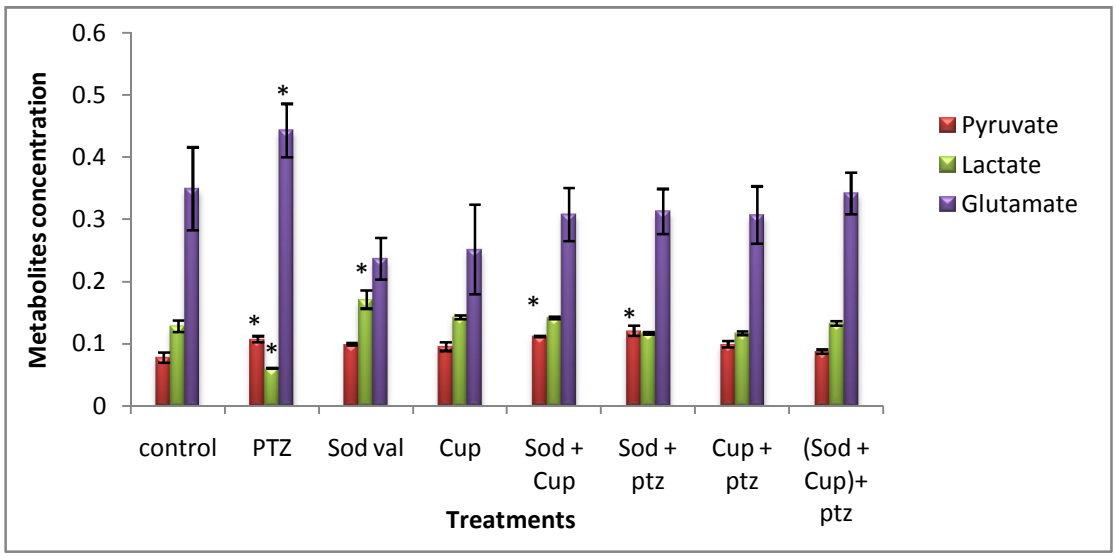
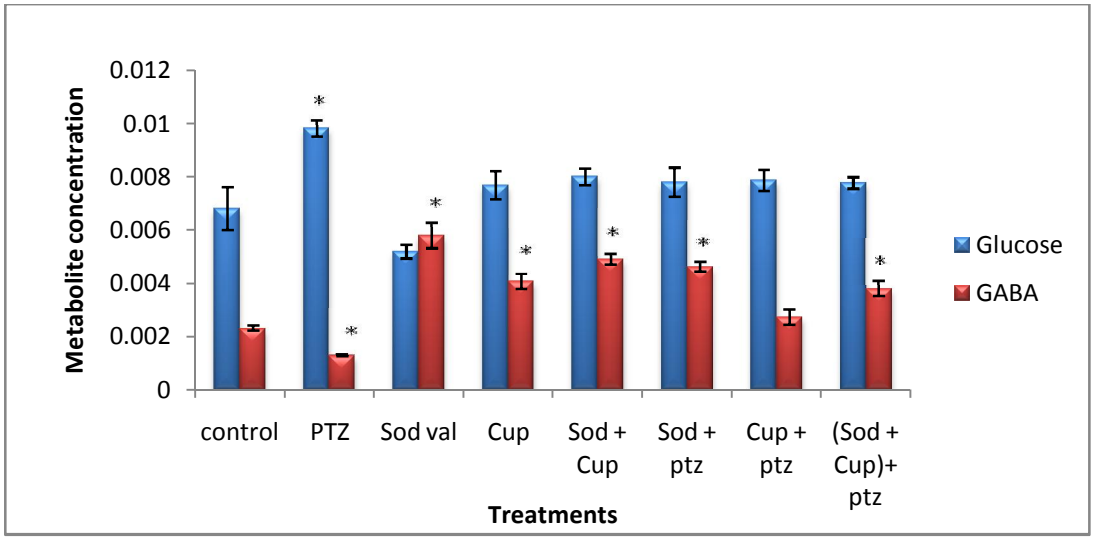


Fig: 18.6 Alterations in Glucose, GABA contents in Corpus Callosum during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**



Cuprum metallicum) treated mice by 49.34%; Sodium Valproate primed mice followed by PTZ treatment mice by 37.47%; Cuprum metallicum followed by Pentylenetetrazole treated mice by 34.91%; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice followed by Pentylenetetrazole by 26.73%, respectively.

Lactate contents elevated considerably in Pentylenetetrazole by 19.77 %; Sodium Valproate treated mice by 39.52 %; Cuprum metallicum treated mice by 34.48%; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 10.37%; Sodium Valproate followed by Pentylenetetrazole treated mice by 27.03%; Cuprum metallicum followed by Pentylenetetrazole treated mice by 18.49%; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice followed by Pentylenetetrazole by 12.17%, respectively.

Glutamate levels exhibited an significant increment by 59.02 % in Pentylenetetrazole treated mice; Whereas, it decreased significantly in Sodium Valproate treated by 11.52%, Cuprum metallicum treated by 10.37%, Cuprum metallicum followed by Pentylenetetrazole treated mice by 12.56%, respectively.

Gamma-amino butyric acid (GABA) shown significant decrement in its concentration in Pentylenetetrazole treated mice by 33.04% and in Cuprum metallicum treated mice by 20.77%.

Glucose level were decreased in all set of treatments by 16.64% in Pentylenetetrazole treated mice; 26.54% in Sodium Valproate treated mice; 52.15% in Cuprum metallicum treated mice; by 32.47% in Sodium Valproate followed by Pentylenetetrazole treated mice; by 39.23% in Cuprum metallicum followed by Pentylenetetrazole treated mice; by 20.56% in Combined drug (Sodium Valproate + Cuprum metallicum) treated mice followed by Pentylenetetrazole, respectively.

Fig: 18.7 Alterations in Pyruvate, Lactate, Glutamate contents of Cingulate gyrus during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**

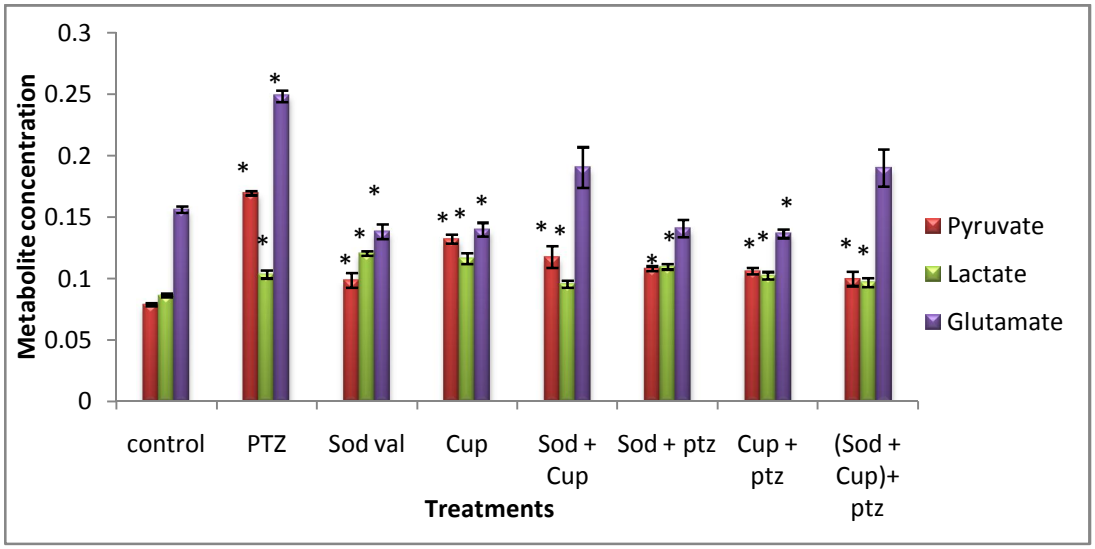
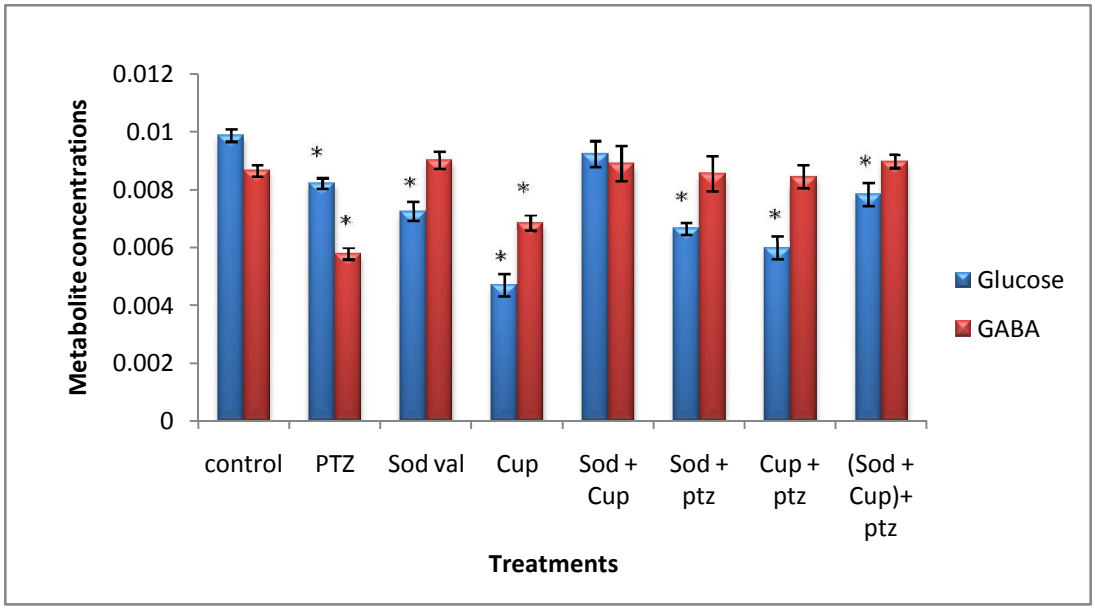


Fig: 18.8 Alterations in Glucose, GABA contents of Cingulate gyrus during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**



3.2.2.3.5. Variations in Metabolite contents of Hippocampus: (Fig:18.9 & 18.10)

Pyruvate contents were increased significantly in the entire set of treatment such as Pentylenetetrazole by 133.10 %; Sodium Valproate treated mice by 37.36 %; Cuprum metallicum treated mice by 43.22%; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 35.21%; Sodium Valproate followed by Pentylenetetrazole treated mice by 72.77%; Cuprum metallicum followed by Pentylenetetrazole treated mice by 47.78%; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice followed by Pentylenetetrazole by 29.35%, respectively.

Lactate contents elevated considerably in Pentylenetetrazole by 166.77 %; Sodium Valproate treated mice by 55.22 %; Cuprum metallicum treated mice by 21.81%; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 50.40%; Sodium Valproate followed by Pentylenetetrazole treated mice by 100.45%; Cuprum metallicum followed by Pentylenetetrazole treated mice by 92.64%; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice followed by Pentylenetetrazole by 106.72%, respectively.

Glutamate levels increased significantly by 27.52 % in Pentylenetetrazole treated mice; Whereas, it decreased significantly in all treated groups such as Sodium Valproate treated by 25.95%, Cuprum metallicum treated by 38.38%, Combined drug treated by 31.88% , Sodium Valproate primed followed by PTZ by 31.63%, Cuprum metallicum followed by Pentylenetetrazole treated mice by 19.44%, Combined drug (Sodium Valproate + Cuprum metallicum) by 16.41% respectively.

Gamma-amino butyric acid (GABA) exhibited significant decrement in its concentration in Pentylenetetrazole treated mice by 47.44% and in Cuprum

Fig: 18.9 Alterations in Pyruvate, Lactate, Glutamate contents of Hippocampus during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**

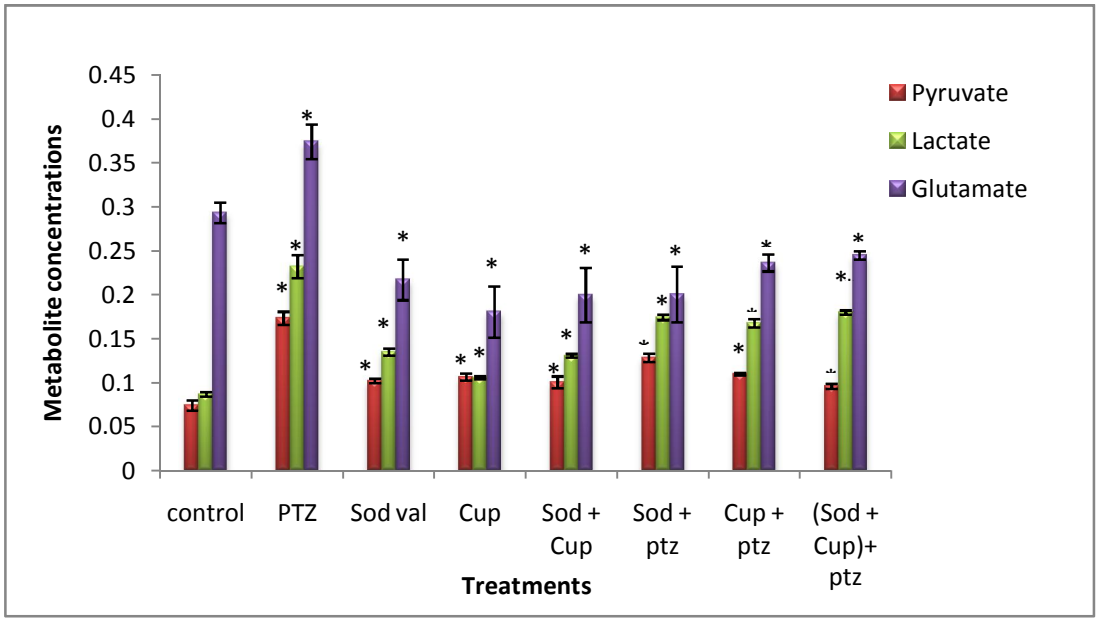
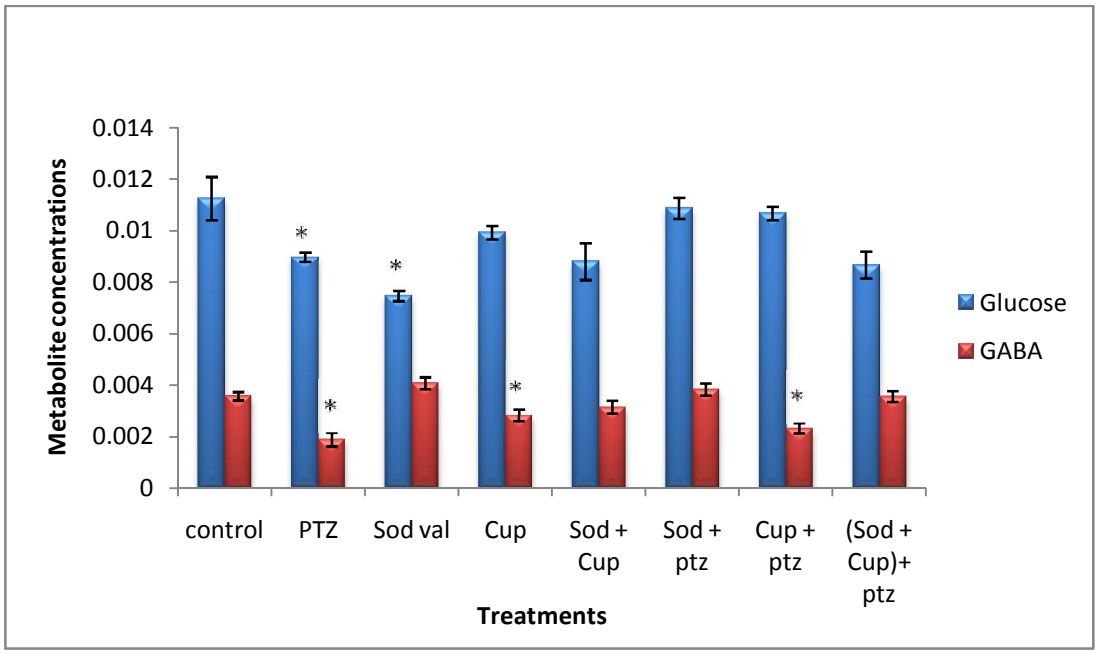


Fig: 18.10 Alterations in Glucose, GABA contents of Hippocampus during various drug treatment (*represents probability p< 0.05, standard bar represent SE**)**



metallicum treated mice by 20.75%, Cuprum metallicum followed by Pentylenetetrazole treated mice by 34.94%, respectively.

Glucose concentrations were decreased in Pentylenetetrazole treated (epileptic) mice by 20.21% and Sodium Valproate treated mice by 33.61%.

3.2.2.3.6. Variations in Metabolite contents of Corpora Quadrigemina: (Fig: 18.11 & 18.12)

Pyruvate content were found to be elevated considerably in Sodium Valproate treated mice by 35.45 %; Cuprum metallicum treated mice by 62.15 %; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 87.85%; Cuprum metallicum followed by Pentylenetetrazole treated mice by 84.89 %; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice followed by Pentylenetetrazole by 51.27%, respectively.

Lactate content showed to be decreased considerably in Pentylenetetrazole by 61.28 %; Sodium Valproate treated mice by 28.19 %; Cuprum metallicum treated mice by 31.93%; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 36.16%; Sodium Valproate followed by Pentylenetetrazole treated mice by 39.70%, respectively.

Glutamate content found to be increased appreciably in Pentylenetetrazole treated mice by 89.13%, whereas, it was found to be decreased in Sodium Valproate treated mice by 29.91% and Combined drug (Sodium Valproate + Cuprum metallicum) treated ones by 18.47%, respectively.

Gamma-amino butyric acid (GABA) level were showed to be increased significantly by 173.02% in Sodium Valproate treated mice; in Cuprum metallicum treated ones by 76.08%; Combined drug (Sodium Valproate + Cuprum metallicum)

treated group by 112.04%; Combined drug (Sodium Valproate + Cuprum metallicum) followed by Pentylenetetrazole by 151.98%, respectively.

Glucose level remained unaltered in all the treated groups.

3.2.2.3.7. Variations in Metabolite contents of Cerebellum: (Fig: 18.13 & 18.14)

Pyruvate content were reduced appreciably in Sodium Valproate treated mice (53.91%); Cuprum metallicum treated mice (65.94%); Combined drug (Sodium valproate + Cuprum metallicum) treated ones (63.66%) respectively.

Lactate level were reduced significantly in the entire sets of treatment in the present study except in Combined drug (Sodium valproate + Cuprum metallicum) treated ones by 25.99 % of increment was seen. Other group such as Pentylenetetrazole treated showed 52.07% decrease, similarly, Sodium Valproate treated ones by 33.17%, Cuprum metallicum treated ones by 36.19%; Sodium Valproate followed by Pentylenetetrazole treated ones by 36.14% and finally, 32.41% of reduction were observed in Cuprum metallicum followed by Pentylenetetrazole, respectively.

Glutamate increased only in Pentylenetetrazole treated mice by 10.93%.

Gamma- amino butyric acid level was dropped significantly in Pentylenetetrazole treated mice by 54.11%.

Glucose content exhibited significant decrement in entire sets of treatments such as Pentylenetetrazole treated mice by 23.81%; Cuprum metallicum treated mice by 36.32%; Sodium Valproate + Cuprum metallicum together shown 51.92 % decrement; Sodium Valproate followed by Pentylenetetrazole treated ones by 29.60%; Cuprum metallicum followed by Pentylenetetrazole treated group by 32.13%;

Fig: 18.11 Alterations in Pyruvate, Lactate, Glutamate contents of Corpora quadrigemina during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**

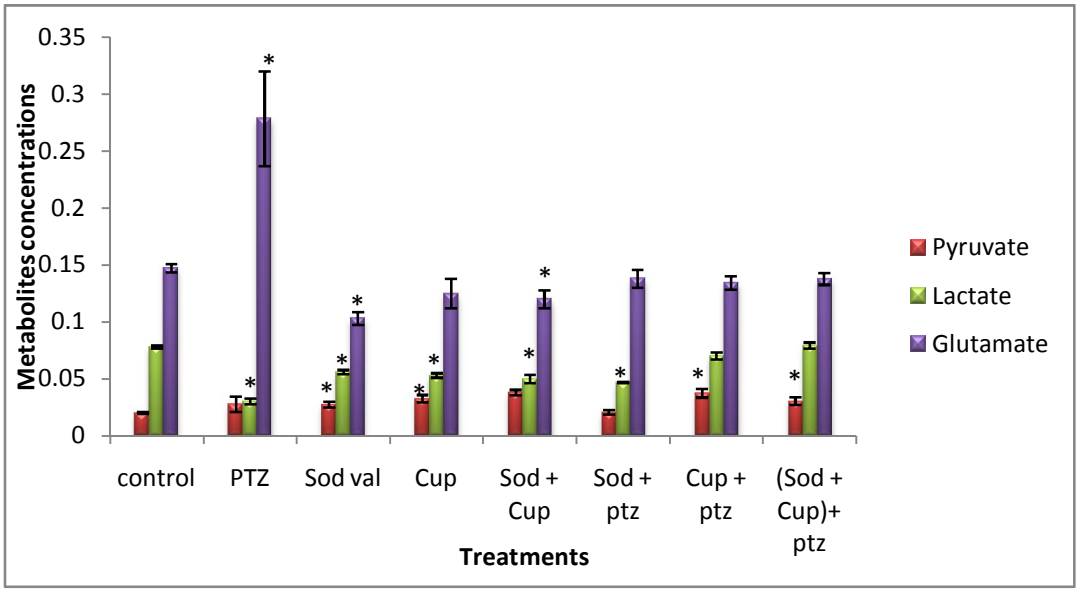
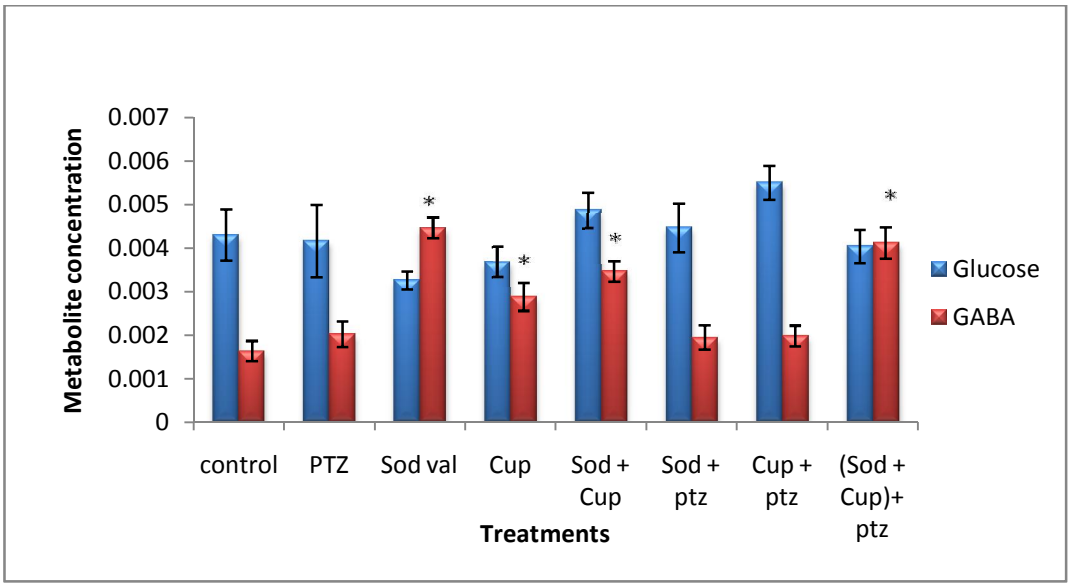


Fig: 18.12 Alterations in Glucose, GABA contents of Corpora quadrigemina during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**



Combined drug (Sodium valproate + Cuprum metallicum) treated followed by Pentylenetetrazole treated mice by 40.73%, respectively.

3.2.2.3.8. Variations in Metabolite contents of Pons: (Fig: 18.15 & 18.16)

Pyruvate level was reduced significantly in Combined drug (Sodium valproate + Cuprum metallicum) treated ones by 80.64%.

Lactate contents revealed significant decrease in Pentylenetetrazole treated mice by 52.31%; Sodium Valproate treated followed by Pentylenetetrazole by 12.43%. Whereas, in Sodium Valproate treated mice showed significant increase by 39.17%, in Cuprum metallicum treated mice by 12.64% increment and finally in Combined drug (Sodium valproate + Cuprum metallicum) treated ones showed 10.77% of significant increment, respectively.

Glutamate contents altered in the entire sets of treatments. In Pentylenetetrazole treated group it was increase considerably by 32.44%. Whereas, in Sodium Valproate treated groups, Cuprum metallicum treated groups, Combined drug (Sodium valproate + Cuprum metallicum) treated ones, Cuprum metallicum followed by Pentylenetetrazole treated, Combined drug (Sodium valproate + Cuprum metallicum Treated ones with post Pentylenetetrazole treated mice showed 21.07, 43.89, 39.39, 44.47 and 16.04% of significant increments, respectively.

Gamma- amino butyric acid were decreased considerably in Pentylenetetrazole treated mice by 93.45%; Whereas in Sodium Valproate, Cuprum metallicum, Combined drug (Sodium valproate + Cuprum metallicum) treated ones showed significant increase by 94.98%, 71.25% and 113.69%, respectively.

Glucose was decreased significantly in Pentylenetetrazole treated group by 27.40%.

Fig: 18.13 Alterations in Pyruvate, Lactate, Glutamate contents of Cerebellum during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**

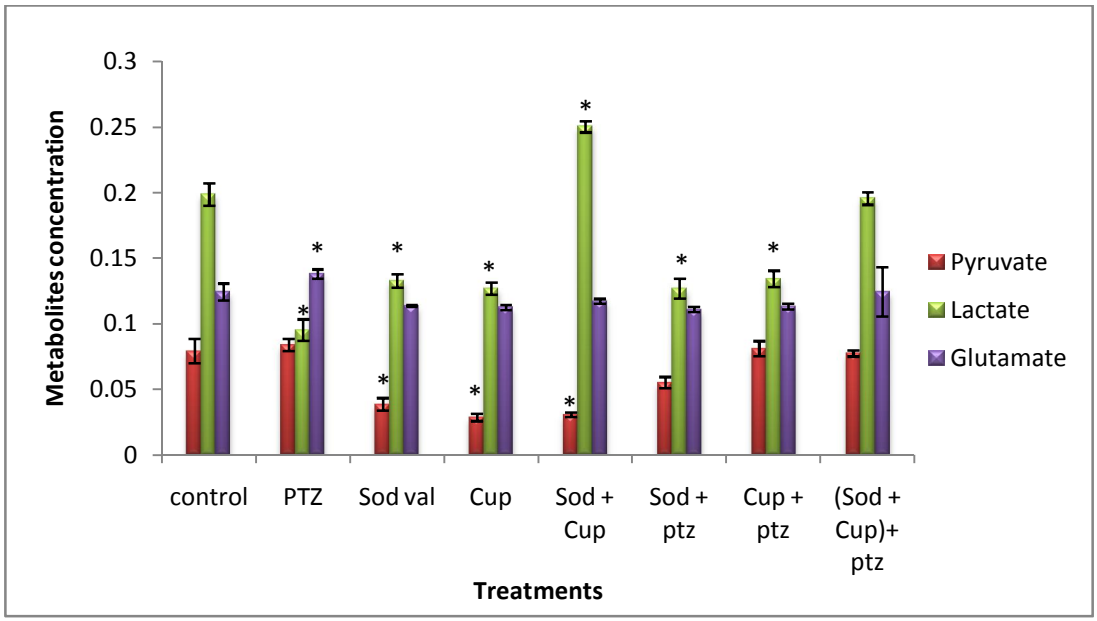
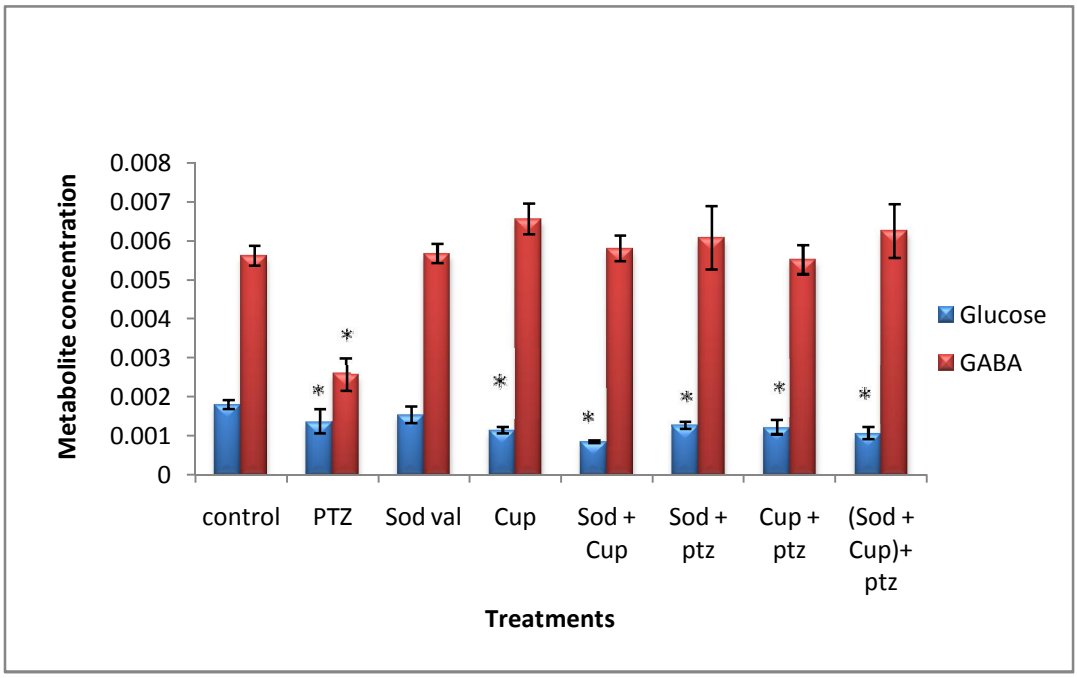


Fig: 18.14 Alterations in Glucose, GABA contents of Cerebellum during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**



3.2.2.3.9. Variations in Metabolite contents in Medulla Oblongata: (Fig: 18.17 & 18.18)

Pyruvate showed insignificant change in entire set of treatments. Lactate dropped in all group by 85.24%, 63.48%, 65.68%, 37.87%, 67.01%, 38.25%, respectively in Pentylenetetrazole, Sodium Valproate, Cuprum metallicum, Combined drug (Sodium valproate + Cuprum metallicum) treated ones, Cuprum metallicum followed by Pentylenetetrazole, Combined drug (Sodium valproate + Cuprum metallicum) treated ones followed by Pentylenetetrazole , respectively.

Glutamate level was increased in Pentylenetetrazole treated mice by 17.42%, significantly.

Gamma amino butyric acid dropped in Pentylenetetrazole group by 41.16%, Whereas 43.20%, 56.26%, 44.45%, 26.25%, 30.54% of significant increase were observed in Sodium Valproate, Cuprum metallicum, Combined drug (Sodium valproate + Cuprum metallicum) treated ones, Sodium Valproate followed by Pentylenetetrazole treated mice, Cuprum metallicum followed by Pentylenetetrazole, respectively.

Glucose was found to be increased in Pentylenetetrazole treated mice by 74.28%.

Fig: 18.15 Alterations in Pyruvate, Lactate, Glutamate contents of Pons during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**

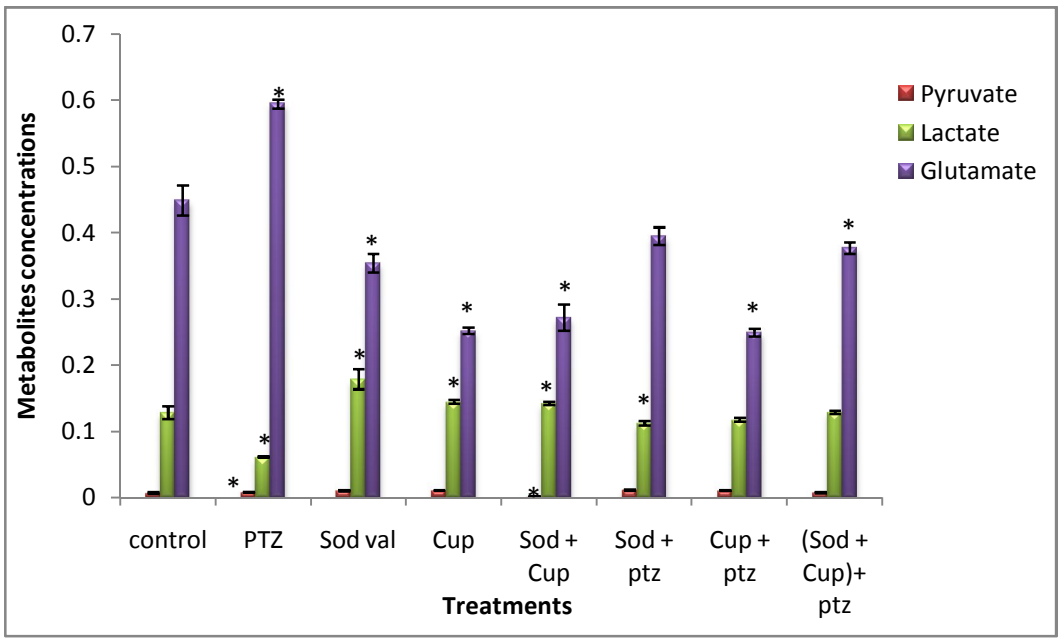


Fig: 18.16 Alterations in Glucose, GABA contents of Pons during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**

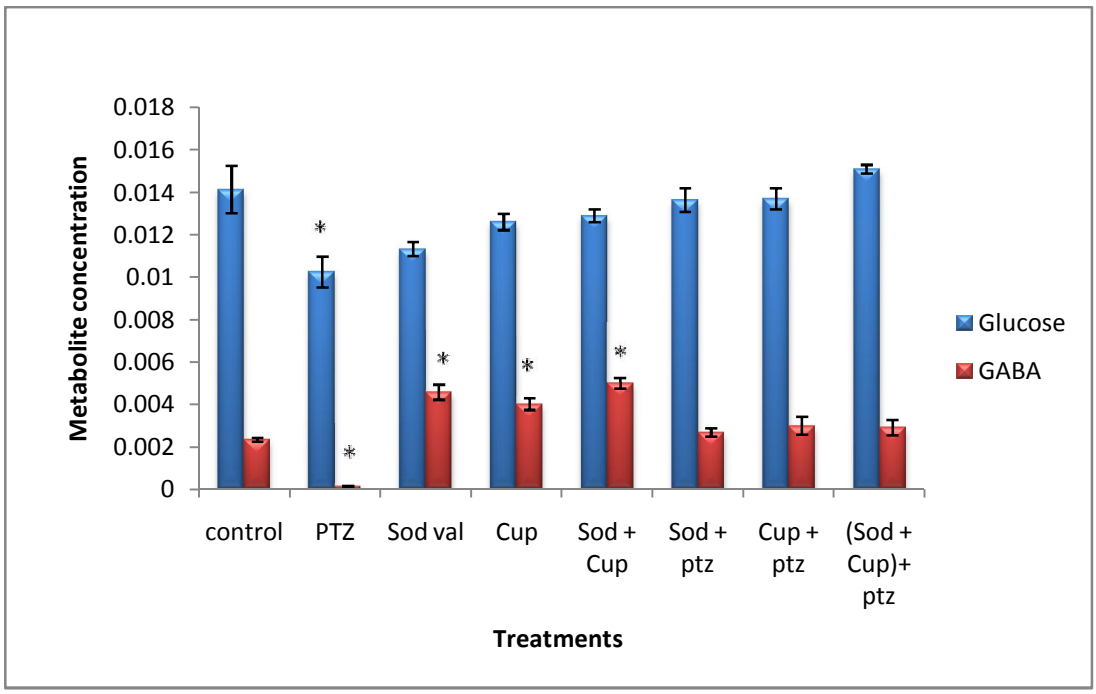


Fig: 18.17 Alterations in Pyruvate, Lactate, Glutamate contents of Medulla Oblongata during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**

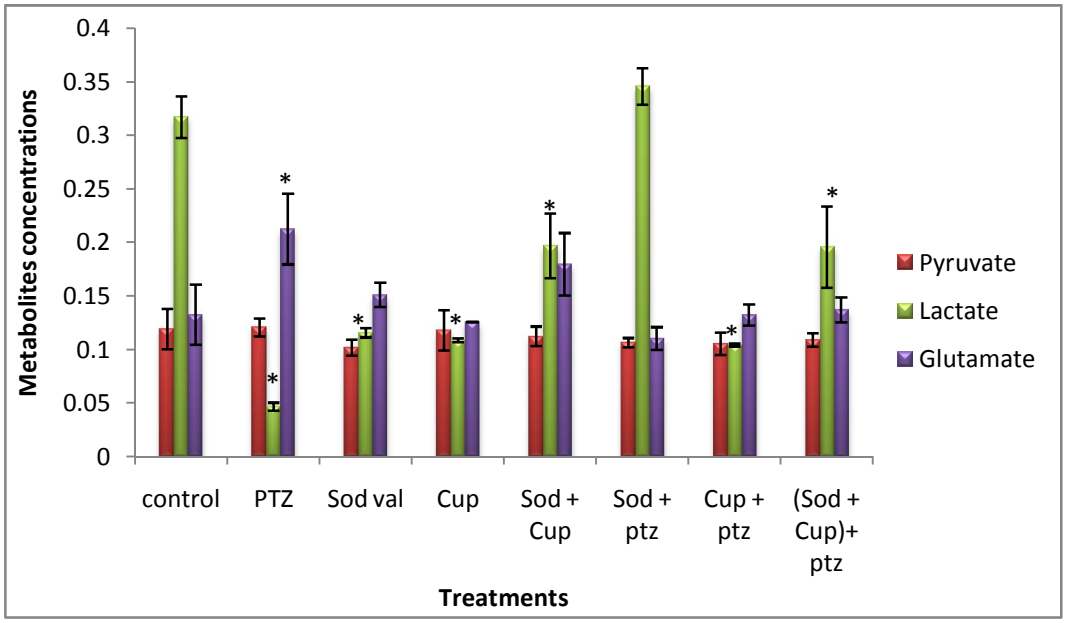
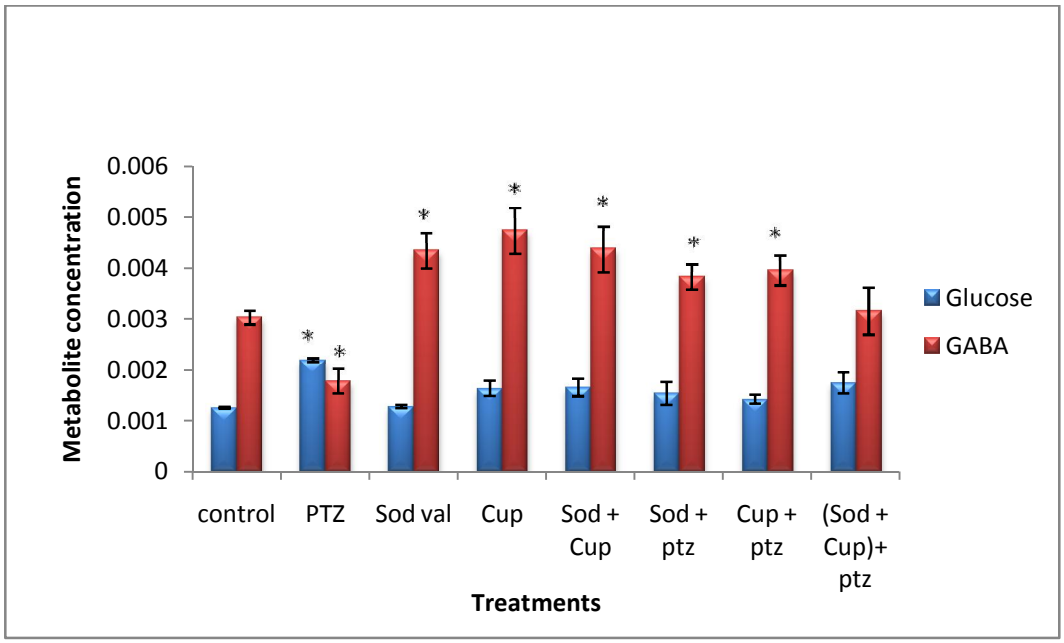


Fig: 18.18 Alterations in Glucose, GABA contents of Medulla Oblongata during various drug treatment (* represents probability p< 0.05, standard bar represents SE**)**



3.2.2.4. Electrolytes in discrete mice brain regions:

Electrolyte contents in discrete brain regions of mice such as Olfactory lobe, Cerebral cortex, Corpus callosum, Cingulate gyrus, Hippocampus, Corpora quadrigemina, Cerebellum, Pons and Medulla Oblongata during treatments with epileptic and antiepileptic drugs. Electrolyte such as Na^{+2}, K^{+}, Mg^{+2}, Ca^{+2} and Cl^{-} showed alterations during the various drug treatment regimens such as Pentylenetetrazole treatment, Sodium Valproate, Cuprum metallicum, Combined drug (Sodium Valproate + Cuprum metallicum), Sodium valproate with post-PTZ, Cuprum metallicum followed by PTZ, Combined drugs (Sodium Valproate + Cuprum metallicum) followed by PTZ respectively, represented in Figures 18.1 to 18.18.

Note:

Units for electrolyte contents are as follows:

1. **Sodium ion**: mol/ gm proteins
2. **Potassium ion**: mol/ gm proteins
3. **Magnesium ion**: mmol/ gm proteins
4. **Calcium ions**: mol/ gm proteins
5. **Chloride ions**: mol/ gm proteins

3.2.2.4.1. Alterations in Electrolyte contents of Olfactory Lobes: (Fig: 19.1 &19.2)

Electrolyte levels exhibits regional variations in the mice brain Olfactory Lobes, upon treatments, with epileptic and antiepileptic drugs.

Sodium contents decreased significantly in Pentelenetetrazole (PTZ) treated (epileptic) mice by 18.66% and in Cuprum metallicum primed mice followed by PTZ showed decrease by 8.92% (Fig:19.1).

Potassium ions revealed considerable increments in most of the treatment regimens i.e., Pentylenetetrazole treated (epileptic) mice by 135.91%, Cuprum metallicum treated group by 61.28%, Sodium valproate primed mice followed by PTZ treatment by 64.80%, Cuprum metallicum primed groups with post-PTZ treatment by 65.77% and finally, Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ treatment exhibited an increment of Potassium ion by 69.91% (Fig: 19.1).

Magnesium ion was decreased precipitously in PTZ treated (epileptic) mice by 49.26%. Whereas, 33.74% significant increase revealed in Sodium valproate treated mice and 48.61% increase in Combined drug (Sodium Valproate + Cuprum metallicum) treated mice (Fig: 19.1).

Calcium content exhibited decrement in PTZ treated group by 61.05% and Sodium Valproate primed mice followed by PTZ by 89.70%. Whereas, an increment of 56.80% in Combined drug (Sodium Valproate + Cuprum metallicum) treated mice was present (Fig: 19.2).

Chloride level were decreased significantly in Olfactory Lobes of mice during Pentylenetetrazole treated (epileptic) mice by 47.97%, Sodium Valproate treated mice by 39.19% and in Sodium Valproate primed mice followed with PTZ by 49.44%. Whereas, in Combined drug (Sodium Valproate) treated mice exhibited 25.65% of increment (Fig:19.2).

Fig 19.1: Electrolyte (Na^{+}, K^{+}, Mg^{+2}) contents of Olfactory lobes during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)

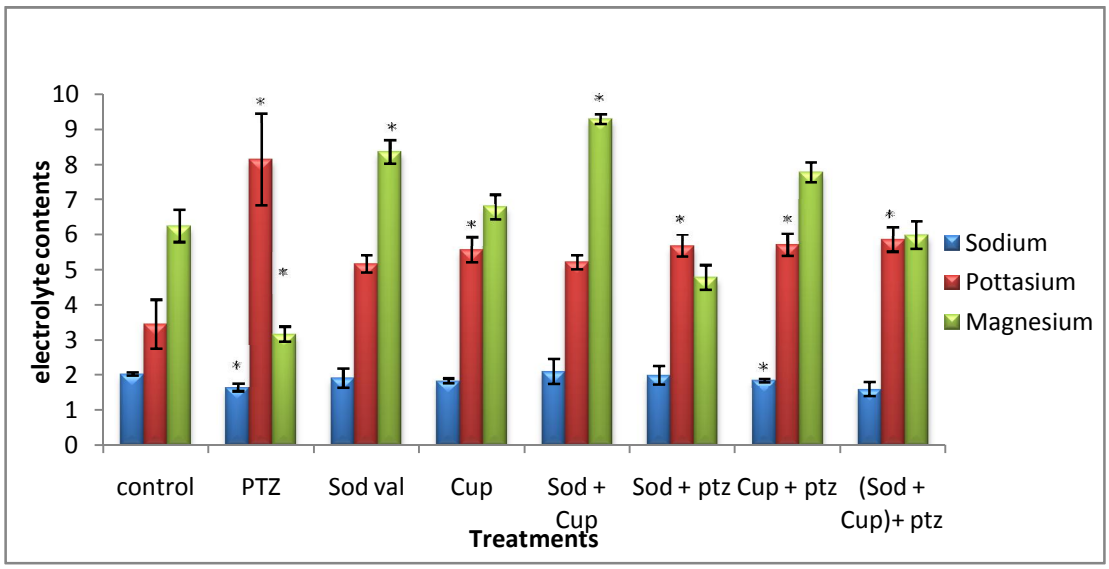
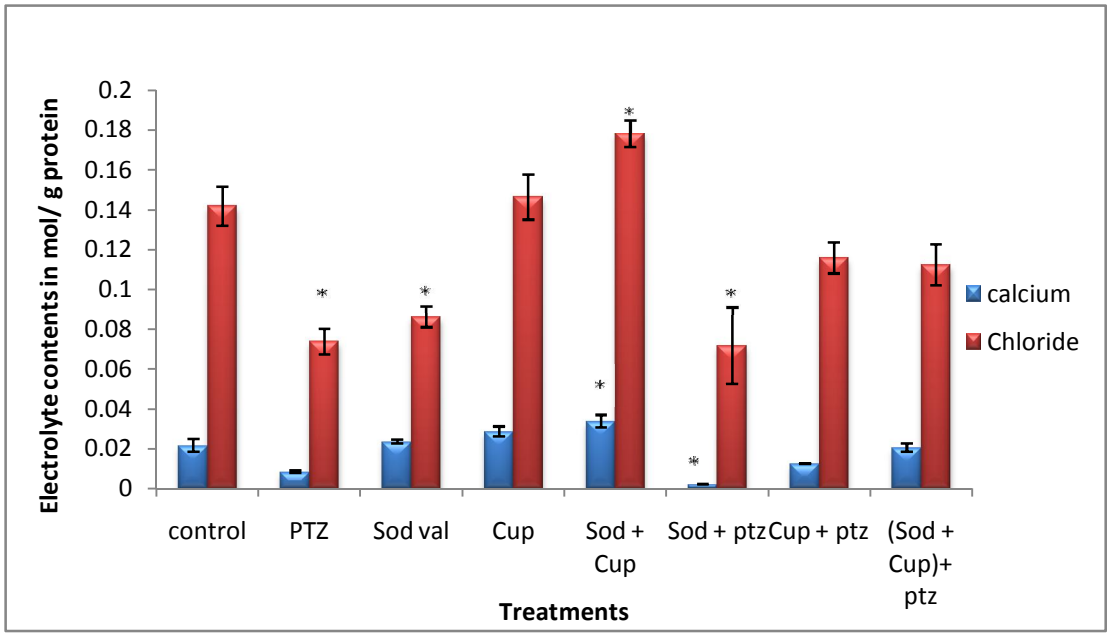


Fig 19.2: Electrolyte (Ca^{+2}, Cl^{-}) contents of Olfactory lobes during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)



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3.2.2.4.2. Alterations in Electrolyte contents of Cerebral Cortex:(Fig: 19.3 &19.4)

An electrolyte of cerebral cortex exhibits treatment dependent changes. Sodium decreased significantly in Pentylenetetrazole treated mice by 48.30% and Cuprum metallicum primed mice followed by Pentylenetetrazole by 42.70%, respectively (Fig:19.3).

Potassium level in cerebral cortex increased significantly by 51.73% in Sodium Valproate treated mice; by 113.68% in Sodium Valproate primed followed by Pentylenetetrazole treatment; by 151.50% in Cuprum metallicum primed followed by Pentylenetetrazole treatment and by 119.56% increase in Combined drug (Sodium Valproate+ Cuprum metallicum) primed mice followed by PTZ treatment, respectively (Fig:19.3).

Magnesium content in the cerebral cortex revealed a decrease in Pentylenetetrazole treated (epileptic) mice by 84.30%. Whereas, in mice treated with Sodium Valproate exhibited 80.08% of increase; similarly in Cuprum metallicum treated mice showed increase of 63.37%, Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 149.63%, Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ by 138.51% increase were observed, (Fig:19.3).

Calcium content did not show any significant alterations in cerebral cortex under various drug treatment regimens (Fig:19.4).

Chloride content in cerebral cortex exhibited an increase in its level by 230.20% in Combined drug (Sodium Valproate + Cuprum metallicum) treated mice, (Fig: 19.4). Rest of the groups revealed insignificant change compared to its control.

Fig 19.3: Electrolyte (Na^+, K^+, Mg^{+2}) contents of Cerebral cortex during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)

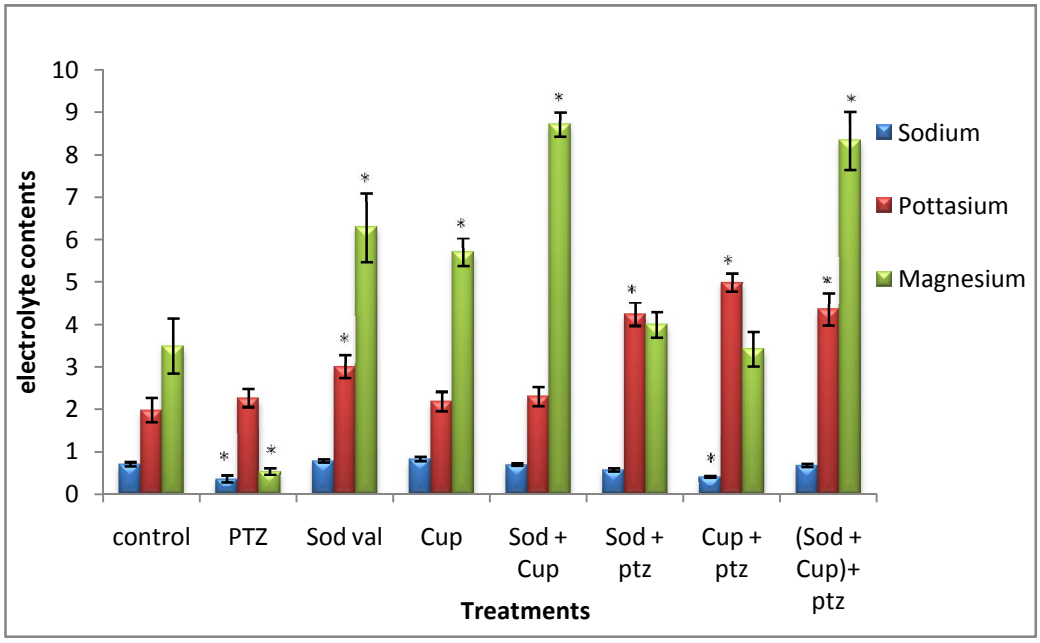
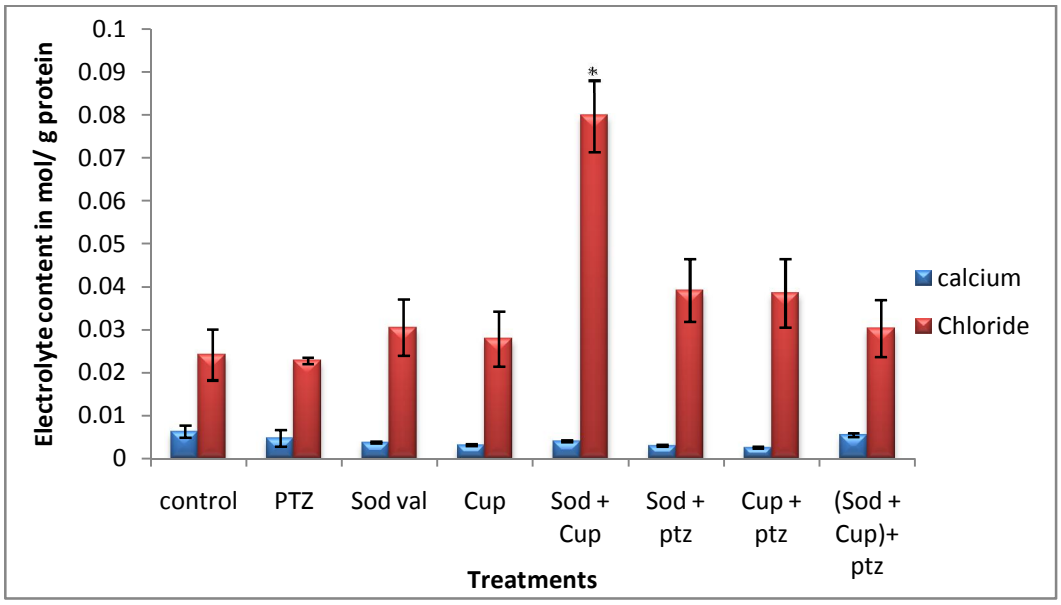


Fig 19.4: Electrolyte (Ca^{+2}, Cl^-) contents of Cerebral cortex during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)



3.2.2.4.3. Alterations in Electrolyte contents of Corpus Callosum: (Fig: 19.5 &19.6)

Alterations in the electrolyte contents of Corpus callosum under various treatment regimens were represented in Figures 19.5 and 19.6.. Sodium level did not exhibit any significant changes in Corpus callosum under various drug treatment regimens (Fig:19.5).

Potassium level in Pentylenetetrazole treated (epileptic) mice revealed a significant decrease of 39.89%, rest of the treated groups exhibited insignificant changes (Fig:19.5).

Magnesium contents in Corpus callosum exhibited a significant reduction in level by 86.99% in Pentylenetetrazole treated mice; by 34.31% in Sodium valproate primed mice followed by PTZ treatment and finally in Cuprum metallicum primed mice followed by PTZ revealed 48.76% decrement. Whereas, in Sodium valproate treated mice exhibited an increment of 23.79% in magnesium level (Fig: 19.5).

Calcium levels exhibited a considerable increment of 42.59% in Sodium Valproate treated mice, similarly an increment of 26.66% revealed in Combined drug (Sodium Valproate + Cuprum metallicum) treated mice. Whereas, a decrease of 34.74% exhibited in Sodium Valproate primed mice followed by PTZ treatment, respectively (Fig:19.6).

Chloride content in Corpus callosum revealed significant reduction of 48.34% in Pentylenetetrazole treated (epileptic) mice. Whereas, a considerable increment was exhibited in Cuprum metallicum primed mice followed by PTZ treatment by 40.93% and Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ treatment by 52.15%, respectively (Fig:19.6).

Fig 19.5: Electrolyte (Na^+, K^+, Mg^{+2}) contents of Corpus callosum during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)

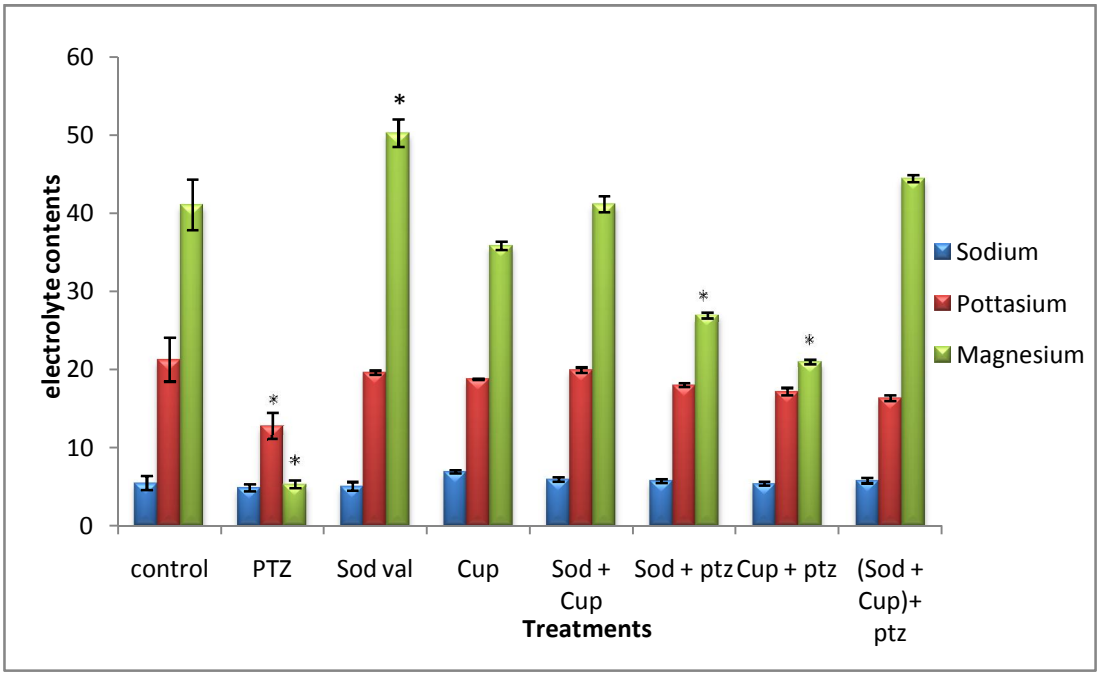
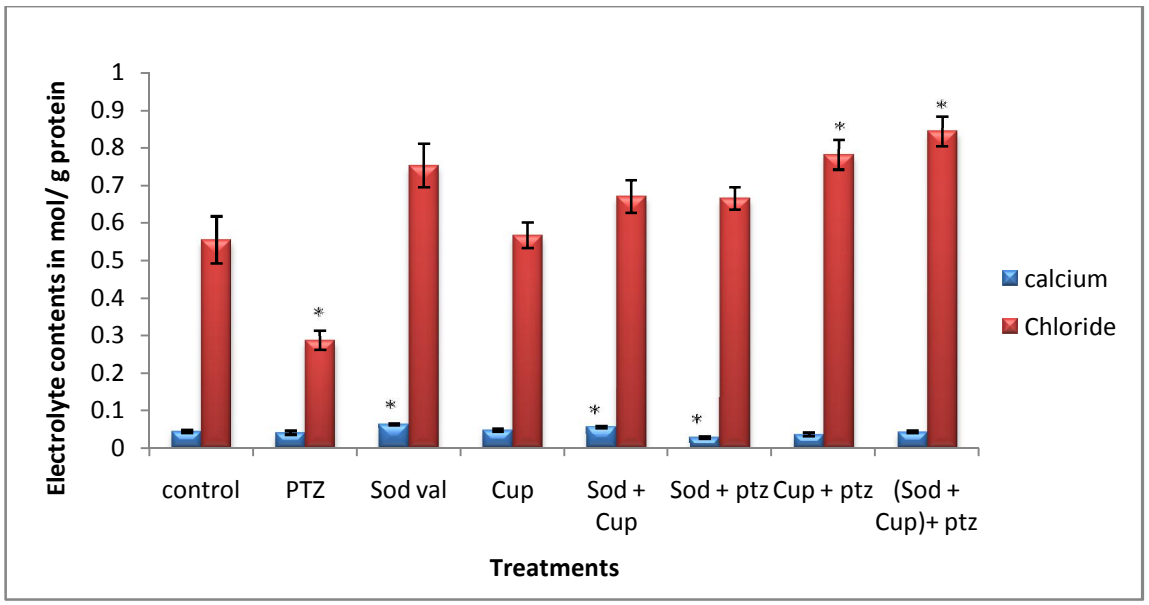


Fig 19.6: Electrolyte (Ca^{+2}, Cl^-) contents of Corpus callosum during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)



3.2.2.4.4. Alterations in Electrolyte contents of Cingulate gyrus: (Fig: 19.7 &19.8)

Variations in the electrolyte content under various treatment regimens has been exhibited in Cingulate gyrus of mice brain represented in Figures 19.7 and 19.8.

Sodium level increased in Sodium Valproate treated mice by 29.37%; Cuprum metallicum treated mice by 38.50%; and Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ treatment by 34.75%, respectively (Fig:19.7).

Potassium contents decreased significantly in the entire treated groups such Pentylenetetrazole treated mice by 47.12%; Sodium Valproate treated mice by 16.36%; Cuprum metallicum treated mice by 36.78%; Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 56.58%, respectively. Similarly, in Sodium Valproate primed mice followed by Pentylenetetrazole exhibited decrement by 45.51%; also, Cuprum metallicum primed groups followed by Pentylenetetrazole by 37.96% and Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ revealed decreased potassium level by 17.38%, respectively (Fig: 19.7).

Magnesium levels in Cingulate gyrus decreased considerably in Pentylenetetrazole treated (epileptic) mice by 73.79%, whereas, Combined drug (Sodium Valproate + Cuprum metallicum) treated groups exhibited an increment by 53.41% and Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ by 19.39%, (Fig:19.7).

Calcium levels decreased significantly by 31.35% in PTZ treated mice; by 46.91% in Sodium Valproate primed mice followed by PTZ and similarly, in Cuprum metallicum primed groups followed by PTZ exhibited 42.49% decrease, respectively (Fig: 19.8).

Fig 19.7: Electrolyte (Na^{+}, K^{+}, Mg^{+2}) contents of Cingulate gyrus during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)

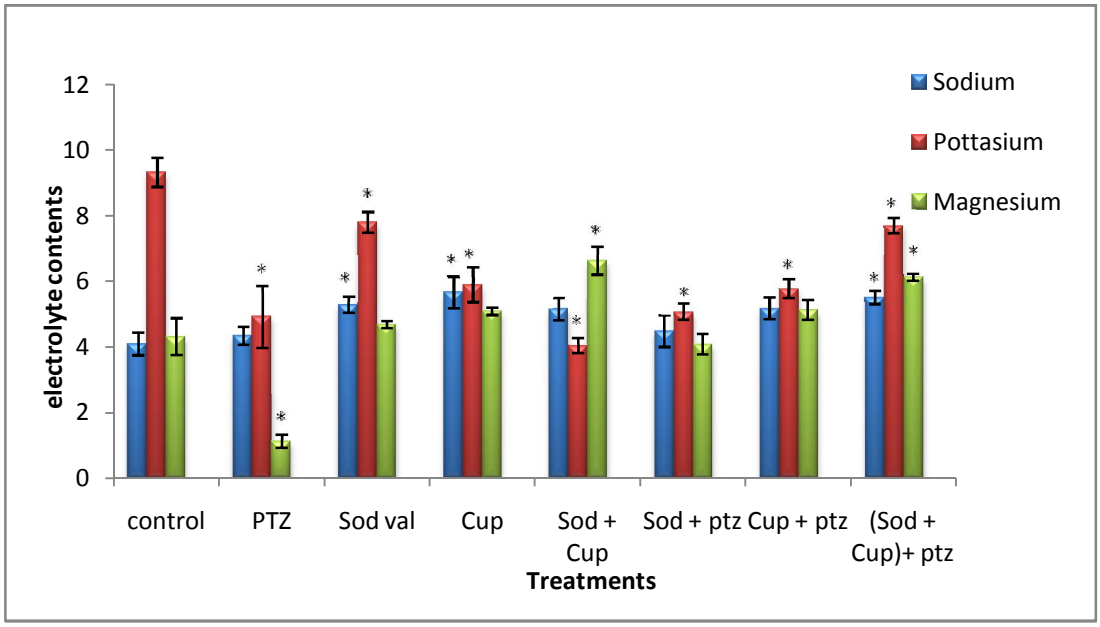
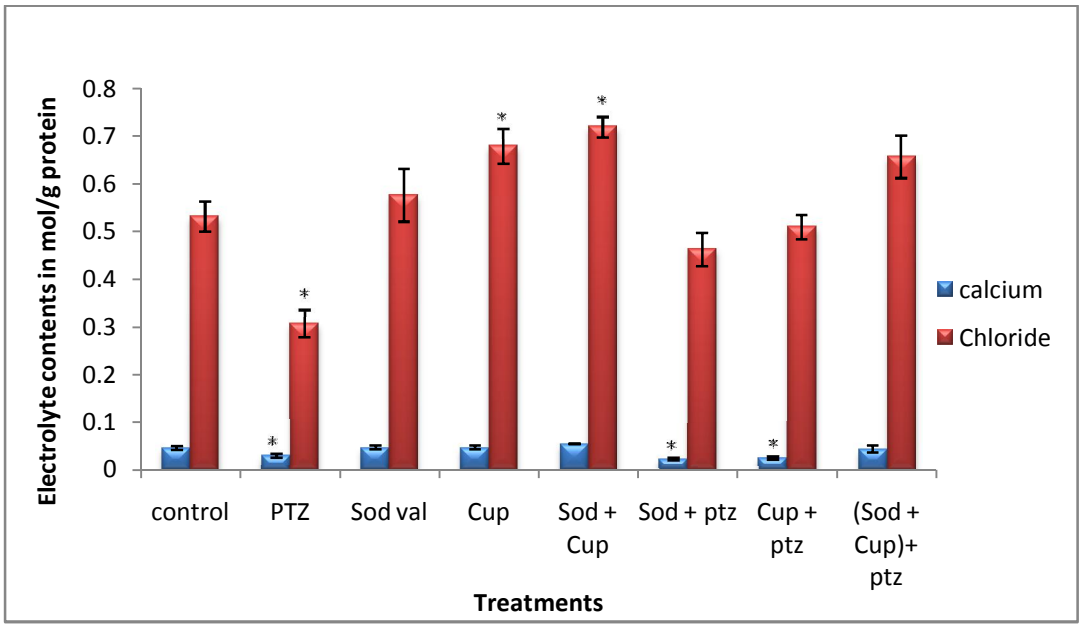


Fig 19.8: Electrolyte (Ca^{+2}, Cl^{-}) contents of Cingulate gyrus during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)



Chloride contents decreased in Pentylenetetrazole treated mice by 42.20%, whereas, in Cuprum metallicum treated mice and Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ treatment exhibited an increment of 27.64% and 35.23%, respectively (Fig: 19.8).

3.2.2.4.5. Alterations in Electrolyte contents of Hippocampus: (Fig: 19.9 &19.10)

Sodium ion levels decreased significantly in Hippocampus during Pentylenetetrazole treated mice by 42.44% (Fig: 19.9).

Potassium level were decreased precipitously in PTZ treated mice by 43.66%; in Combined drug (Sodium valproate + Cuprum metallicum) treated mice by 34.20% and 34.72% decrease in Sodium Valproate primed mice followed by PTZ treatment.

Magnesium contents reduce significantly in PTZ treated mice by 80.45%, similarly, in Cuprum metallicum treated mice, significant drop in magnesium in Hippocampus by 17.84%. Whereas, in Sodium Valproate treated mice exhibited an increment of 21.71%, (Fig: 19.9).

Calcium levels in hippocampus during PTZ treatment dropped precipitously by 51.45%, whereas, in Cuprum metallicum treated mice, calcium content increased by 111.59%. Similarly, in Combined drug (Sodium Valproate + Cuprum metallicum) treated groups exhibited 69.41% and Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ treatment revealed an increment of 131.30%, (Fig: 19.10).

Fig 19.9: Electrolyte (Na^+, K^+, Mg^{+2}) contents of Hippocampus during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)

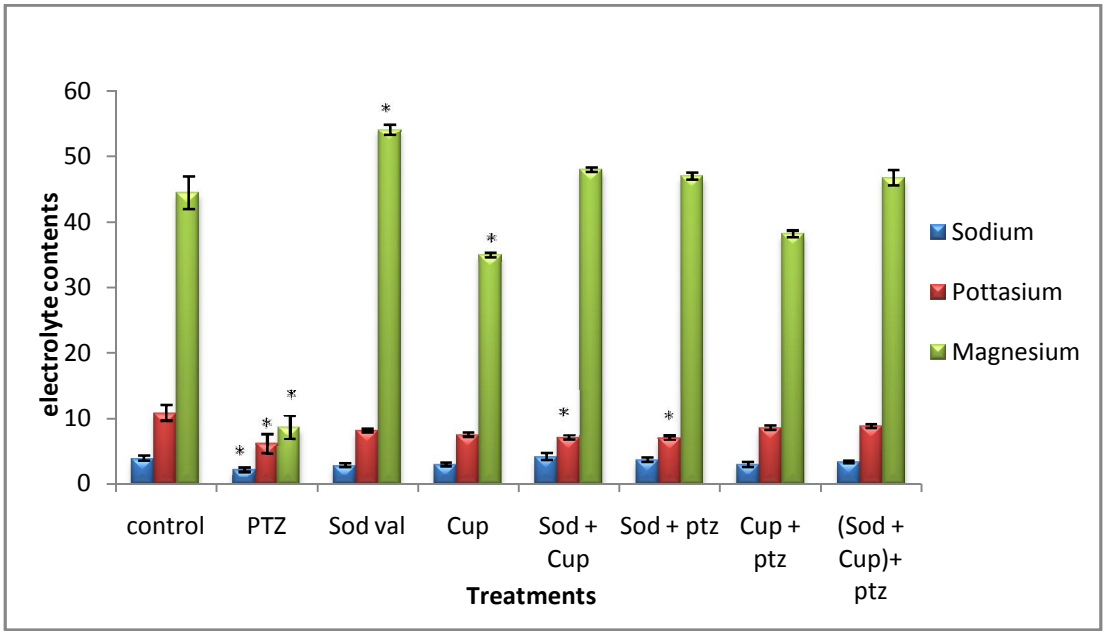
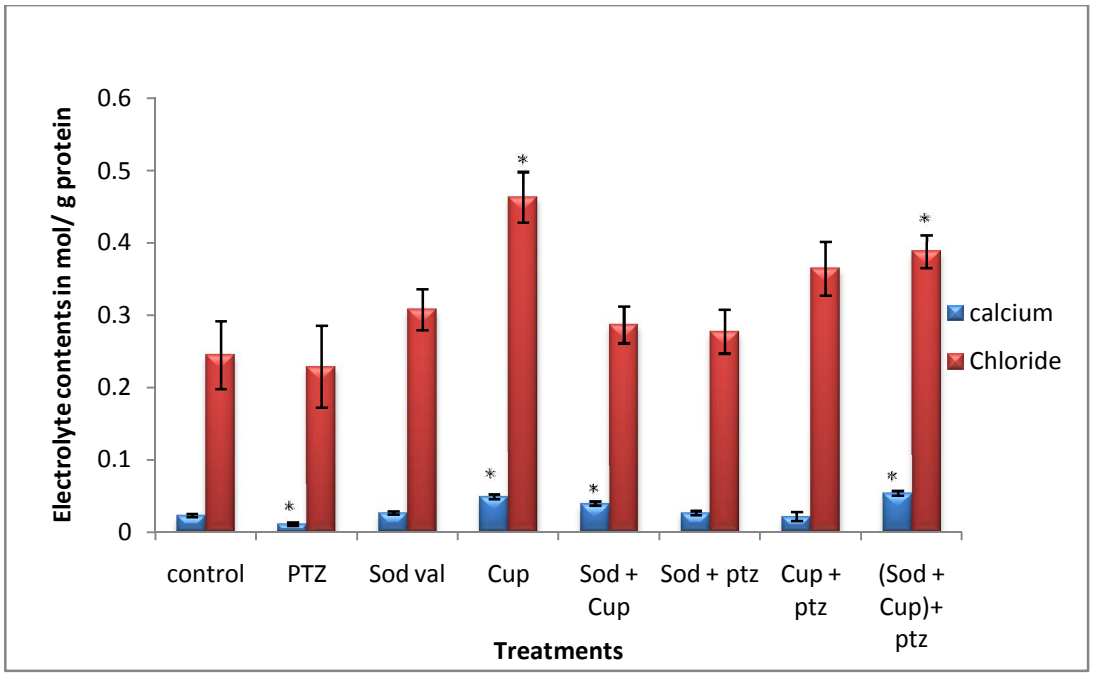


Fig 19.10: Electrolyte (Ca^{+2}, Cl^-) contents of Hippocampus during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)



Chloride levels were increased in Cuprum metallicum treated groups by 89.20% and in Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ treatment by 58.56%, (Fig: 19.10).

3.2.2.4.6. Alterations in Electrolyte contents of Corpora Quadrigemina: (Fig: 19.11 & 19.12)

Sodium, Magnesium and Chloride contents in Corpora quadrigemina of Mice treated with different drug regimens exhibited alterations, whereas, Potassium and Calcium levels were unaltered and the data is represented in Figures 19.11 and 19.12.

Sodium content decreased appreciably in Pentylenetetrazole treated mice by 37.20%. Whereas, groups treated with Cuprum metallicum showed increment of 91.68%, mice treated with Combined drug (Sodium Valproate + Cuprum metallicum) increased by 82.66% and groups primed with Sodium Valproate followed by Pentylenetetrazole exhibited increment of 62.21%, respectively (Fig:19.11).

Magnesium levels exhibited decrement of 97.43% in Pentylenetetrazole treated mice; of 51.79% in Sodium Valproate treated groups; deduction of 37.45% in Cuprum metallicum treated; reduction of 25.48% in Combined drug (Sodium Valproate + Cuprum metallicum) treated mice. Similarly, reduction of 40.30% in Sodium Valproate primed mice followed by PTZ and of 31.24% in Cuprum metallicum primed followed with Pentylenetetrazole treatment were exhibited (Fig:19.11).

Chloride contents was increased significantly in Sodium Valproate treated mice by 54.91% and in Combined drug (Sodium Valproate + Cuprum metallicum) treated mice by 64.89%, respectively (19.12).

Fig 19.11: Electrolyte (Na^{+}, K^{+}, Mg^{+2}) contents of Corpora quadrigemina during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)

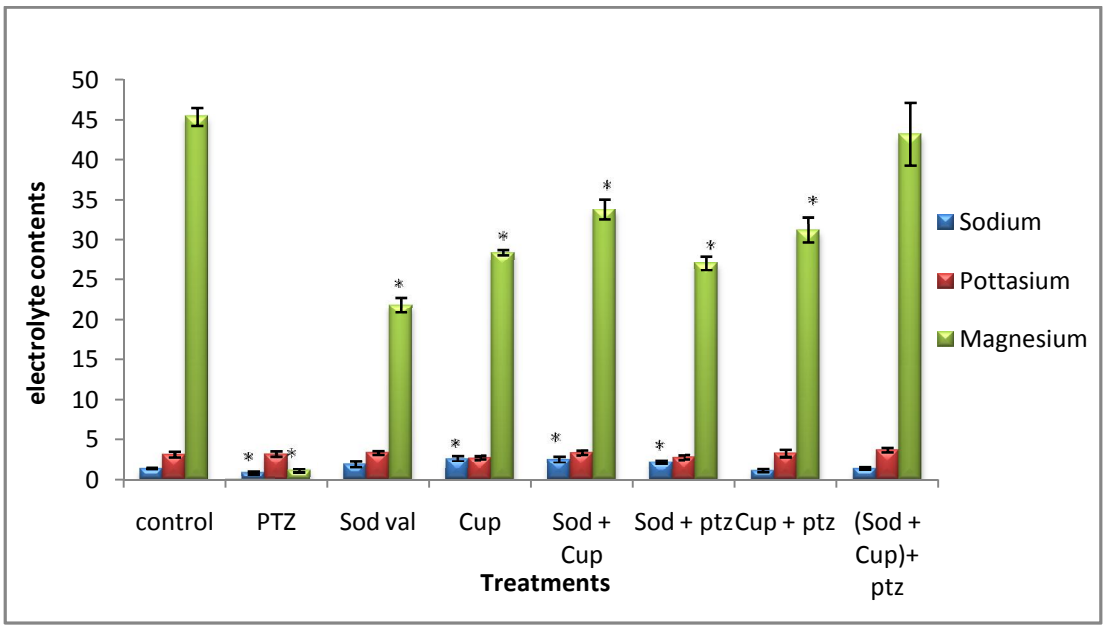
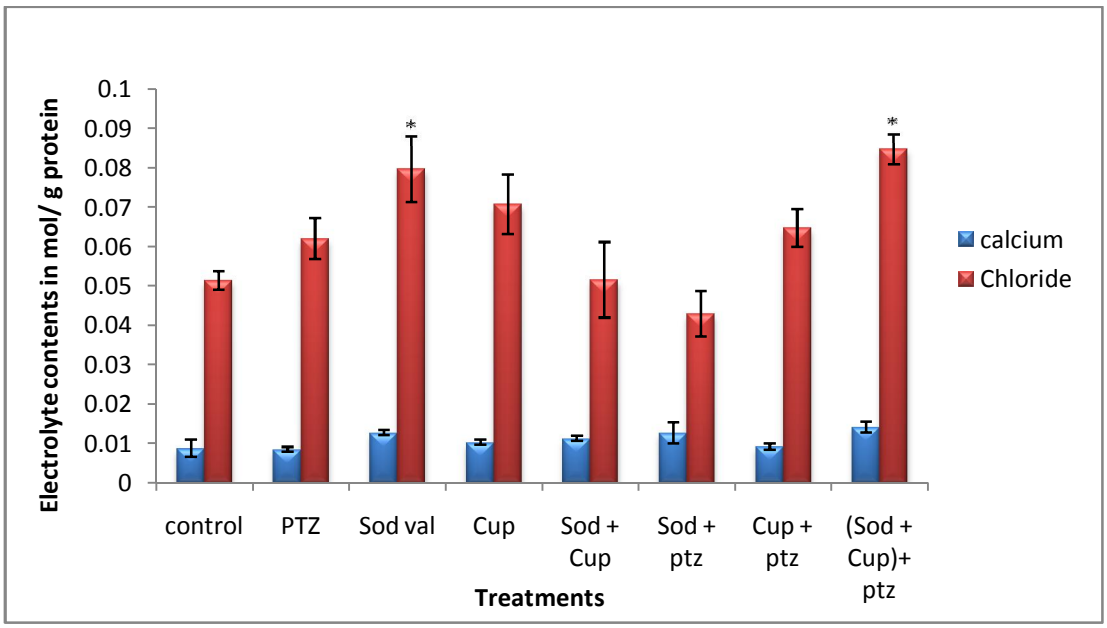


Fig 19.12: Electrolyte (Ca^{+2}, Cl^{-}) contents of Corpora quadrigemina during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)



3.2.2.4.7. Alterations in Electrolyte contents of Cerebellum: (Fig: 19.13 &19.14)

Alterations in the magnesium content were revealed in Cerebellum under various drug treatment regimens. Sodium, Potassium, Calcium and Chloride levels were unaltered when compared to the control and represented in Figures 19.13 and 19.14.

Magnesium contents decreased in Pentylenetetrazole treated mice by 48.43%, Cuprum metallicum treated mice by 17.80% and Sodium Valproate primed mice followed by Pentylenetetrazole treatment by 17.80% respectively (Fig:19.13).

3.2.2.4.8. Alterations in Electrolyte contents of Pons: (Fig: 19.15 & 19.16)

Electrolyte contents varied depending upon different drug treatment regimens in Pons of mice brain region, except Sodium level remained unaltered (Fig: 19.15).

Potassium levels in Pons exhibited reduction by 28.45% in Pentylenetetrazole treated (epileptic) mice, 19.25% reduction of potassium in Sodium Valproate treated mice. Similarly, in Cuprum metallicum treated mice reduced by 17.87%, Combined drug (Sodium Valproate + Cuprum metallicum) treated groups by 19.58%, Sodium Valproate primed mice followed by PTZ by 15.53%, Cuprum metallicum primed mice followed by PTZ by 19.41% and finally in Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ exhibited decrement of 19.87%, respectively (Fig: 19.15).

Magnesium contents reduced in Pons by 93.58% in Pentylenetetrazole treated mice, in Sodium Valproate primed mice followed by Pentylenetetrazole reduced by 22.67% and in Cuprum metallicum primed mice followed by PTZ by 48.94%, respectively (Fig: 19.15).

Fig 19.13: Electrolyte (Na^{+}, K^{+}, Mg^{+2}) contents of Cerebellum during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)

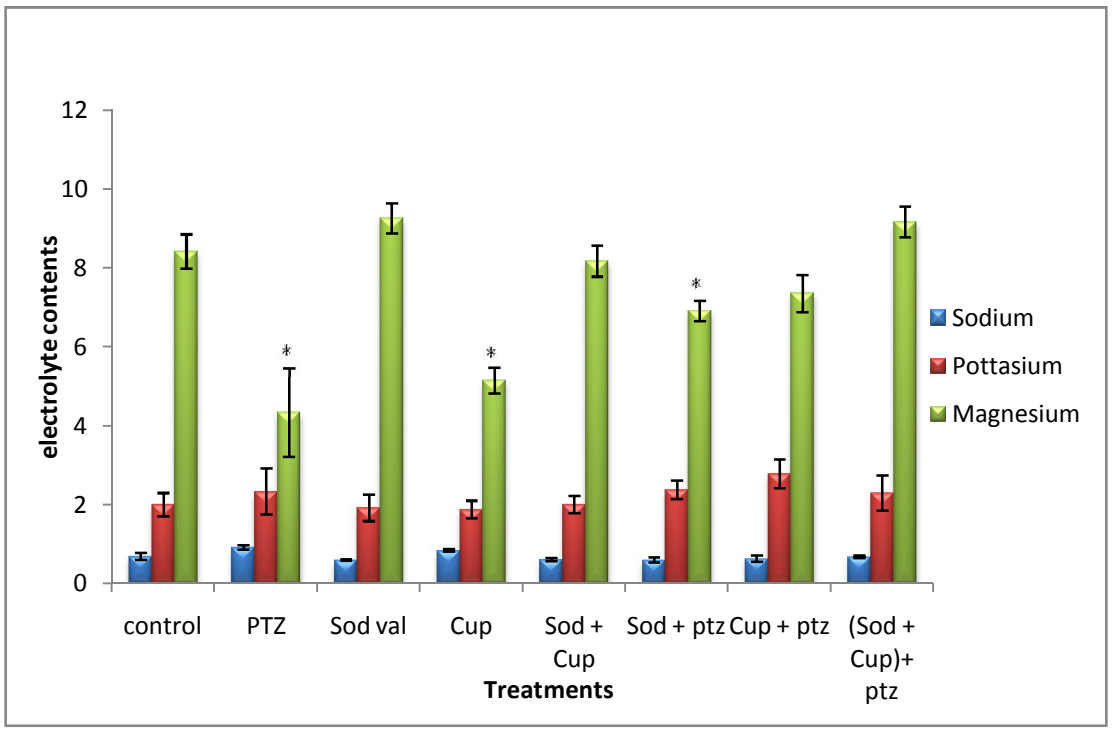


Fig 19.14: Electrolyte (Ca^{+2}, Cl^{-}) contents of Cerebellum during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)

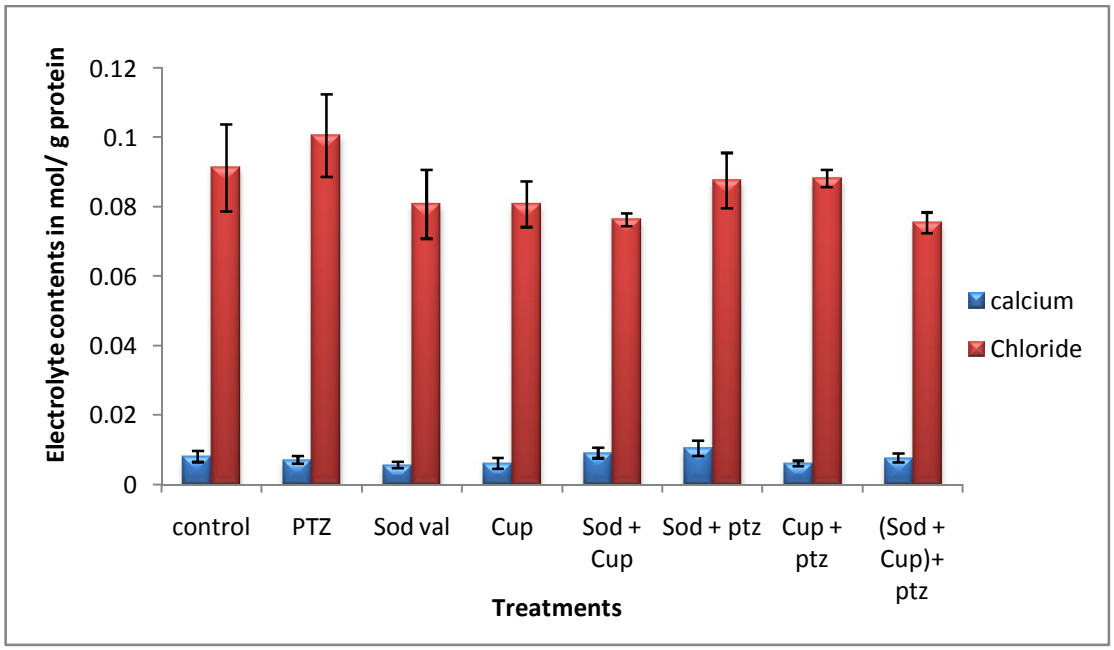


Fig 19.15: Electrolyte (Na^{+}, K^{+}, Mg^{+2}) contents of Pons during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)

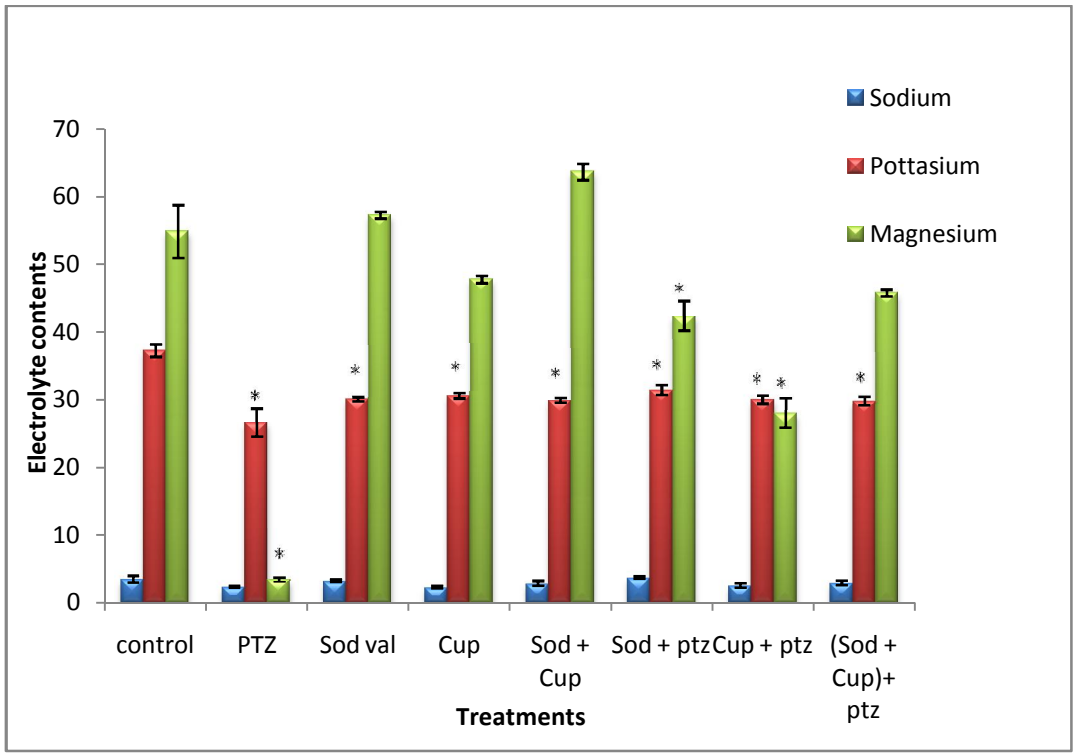
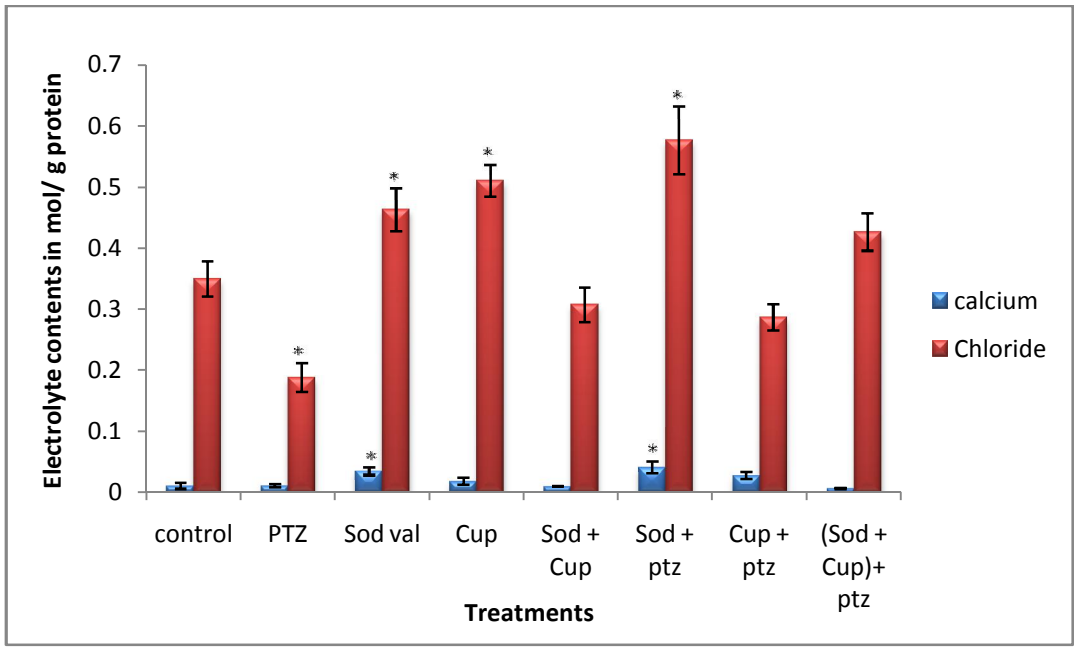


Fig 19.16: Electrolyte (Ca^{+2}, Cl^{-}) contents of Pons during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)



Calcium levels in Sodium Valproate treated mice exhibited increment of 225.22%, similarly, in Sodium Valproate primed mice followed by PTZ increased significantly by 285.86%, (Fig: 19.16). Rest other groups were insignificant compared to control.

Chloride contents in pons reduced appreciably in Pentylenetetrazole treated mice by 46.15%. Whereas, in Sodium Valproate treated mice revealed 32.29% increase, in Cuprum metallicum treated mice by 45.72% increment and in Sodium Valproate primed mice followed by Pentylenetetrazole exhibited increment of 64.74%, respectively (Fig: 19.16).

3.2.2.4.9. Alterations in Electrolyte contents of Medulla Oblongata: (Fig: 19.17 & 19.18)

Electrolyte such as Sodium ion exhibited significant increase of 53.96% in only Cuprum metallicum treated mice, rest of the groups were unaltered (Fig: 19.17).

Potassium showed decrease by 44.97% in Pentylenetetrazole treated mice only.

Magnesium level varied in the entire group upon treatments with drugs. Pentylenetetrazole treated mice revealed a reduction in magnesium content in Medulla Oblongata by 50.92%. Whereas, a significant increment revealed in Sodium Valproate treated mice by 172.94%, Cuprum metallicum treated mice by 103.84% increase, Combined drug (Sodium Valproate + Curpum metallicum) treated mice by 233.35% increase, Sodium Valproate primed mice followed by PTZ exhibited increment of 43.84% and in Combined drug (Sodium Valproate + Cuprum metallicum) primed mice followed by PTZ increased by 29.65% (Fig: 19.17).

Fig 19.17: Electrolyte (Na^+, K^+, Mg^{+2}) contents of Medulla Oblongata during various drug treatment regimens. (* represents probability p< 0.05, standard bar representsSE)

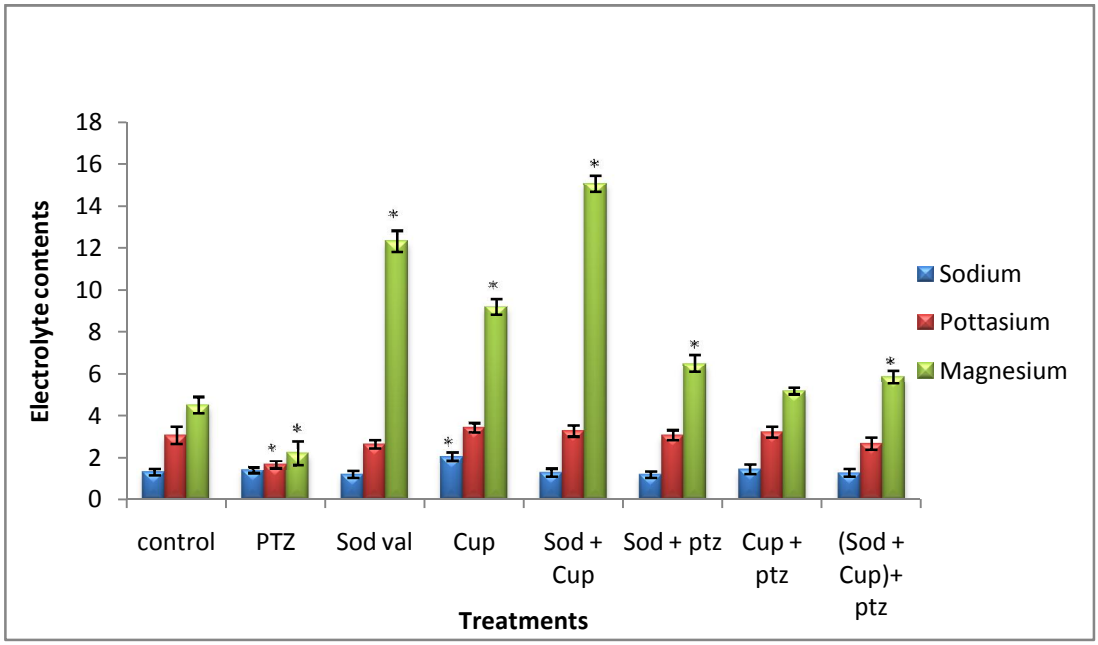
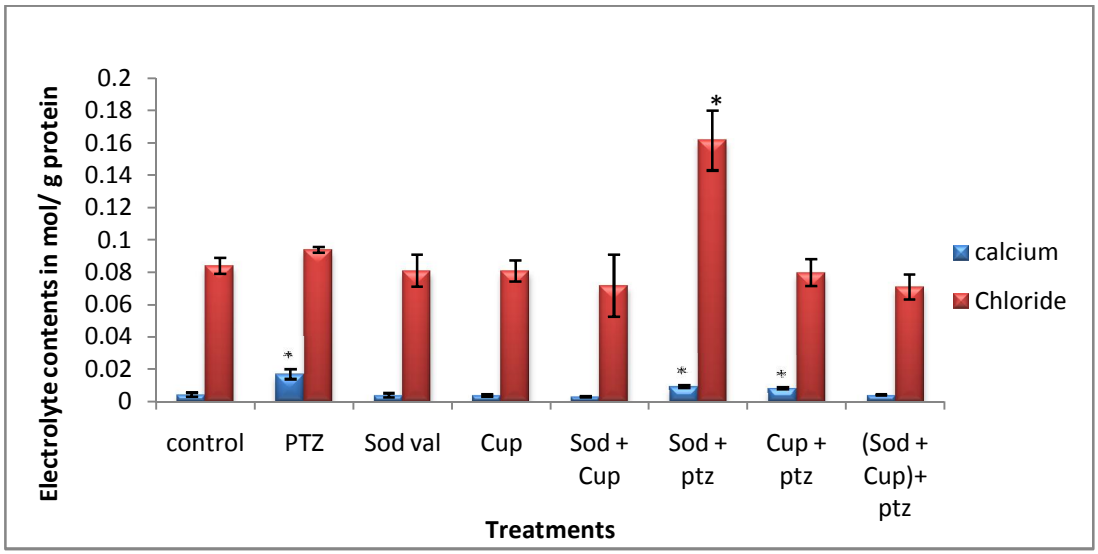
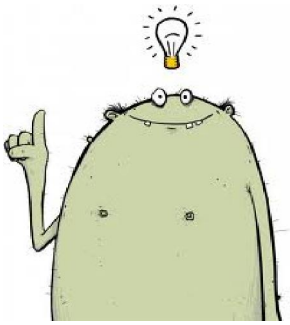


Fig 19.18: Electrolyte (Ca^{+2}, Cl^-) contents of Medulla Oblongata during various drug treatment regimens. (* represents probability p< 0.05, standard bar represents SE)



Calcium content increased significantly in PTZ treated mice by 296.79%, Sodium Valproate primed followed by PTZ by 116.72%, Cuprum metallicum primed followed by PTZ treated groups exhibited increment by 89.25%, (Fig: 18.18). Chloride content revealed increase only in Sodium Valproate primed followed by PTZ treated group by 92.51% (Fig:19.18).

DISCUSSIONS



The World Health Organization (WHO) is responsible for providing technical information and advice to its Member States to help them to improve the health of their citizens. This task is facilitated by collaboration with various scientific and professional groups that have similar goals. To bring epilepsy "out of the shadows", a Global Campaign Against Epilepsy was launched in 1997 "to improve acceptability, treatment, services and prevention of epilepsy worldwide". The Campaign was conducted in the year 2005 by WHO in partnership with the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). The aim of the Campaign was principally to reduce the treatment gap by providing better information about epilepsy and its consequences and to assist governments and those concerned with epilepsy to reduce the burden of the disorder. Epilepsy is a common medical and social disorder or group of disorders with unique characteristics (Reynolds, 2005). Hence, it becomes a necessity to study more about this neurological disorder, either on humans directly, or by mimicking the symptoms in the experimental animals, and learning more about the cause and its etiologies. Thus, such knowledge may lead for the better treatment for epilepsy with reduced side-effects on the body and leading the patients to live a healthy life. In the present study, an attempt is made to unravel some aspects related to the treatment for epilepsy.

Neuropharmacological studies of epilepsy directly in human are not possible due to the ethical factors and heterogeneity present among the patient populations such as age, type of epilepsy, course of the epilepsy, AED regime and genetic background. Also, it is difficult to study therapy related effects in a systematic way as reported by Dedeurwaerdere et.al. (2006). Animal models mimicking human epilepsies are peculiar, and each animal model most likely attributes a specific aspect, or a mixture of some aspects of the variety of human epilepsies. Nonetheless, these

models have high utility and are important prerequisites for research of epilepsy, as they allow us to approach specific point(s) of the human pathology experimentally. They are equally important in making it possible to pinpoint a specific aspect of epilepsy and to analyze it in a "simpler" context than in human pathologic conditions (Cloix and Hevor, 2009). Therefore, it becomes mandatory to work by using animal models and then through clinical trials on humans. Hence, in the present investigations, a neuropharmacological study of epilepsy is carried out in two different types of animal models of epilepsy. It is for the first time, an attempt is made to induce epilepsy in Crab (*Scylla serrata*), a higher invertebrate in order to establish it as a model for studies of epilepsy. Also, we have attempted more detailed analysis on the brain metabolic aspects in Rodent model such as Mouse (*Mus musculus*) during the epileptic seizures as well as after treating with two antiepileptic drugs. This work also highlights the impact of allopathic antiepileptic drugs, homeopathic drugs and combined drugs on a few components of metabolic pathways in the brain tissue of mice.

4.1. Neuropharmacological studies of epilepsy in Crab as a model:

A basic phenomenon of epilepsy consists of typical neuronal discharges induced in the nervous systems of many organisms. It becomes easier to study such phenomena in higher invertebrates due to their simpler nervous organization compared to that of higher vertebrate models. The present work shows convincingly that Crab cerebral ganglia are able to produce epileptiform electrical discharges upon exposure to Pentylenetetrazole (PTZ). The effect of antiepileptic drug such as Sodium Valproate normally used to treat Human epilepsy was used in Crab for studying its efficacy in controlling seizures in Crab. Besides, a homeopathic preparation (Cuprum

metallicum), a known antiepileptic drug is tested for Mice and Crabs to assess its efficacy. Epilepsy has already been studied in various invertebrate systems in the past, indicating attempts by scientists to establish them as models for such studies. Epileptic electrical discharges of invertebrate ganglia are easy to investigate at the level of a single neuron. The buccal ganglia of *Helix pomatia* were studied to understand the mechanisms underlying epilepsy (Altrup et al. 1992). Speckmann and Caspers (1973) used Pentylenetetrazole (PTZ) to promote epileptic electrical discharges (EED) in isolated neurons of *Helix pomatia,* while Altrup et al. (1991) used hypnotic drug Etomidate for producing EED in mollusks. In the present work, the results obtained during the study of epilepsy in Crabs (*Scylla serrata)* are discussed.

4.1.1. Behavioral changes in Crabs under treatment:

Decapod crustaceans such as crayfish, lobsters, are used extensively in neuroethology (Kravitz 2000; Edwards et al., 2003; Panksepp and Huber, 2004) for studying neurobehavioral aspects using various neurological drugs. The present work shows that with induction of epileptiform activities mediated through Pentylenetetrazole, normal electrical rhythms are altered and new epileptic rhythms appear. The behavioral changes of crabs like fast running and sudden loss of postures, vigorous movements of eyestalks and antennules, frequent body jerks, oozing of froth from mouth followed by brief loss of posture after PTZ treatment, are quite comparable to tonic-clonic epileptic behaviors in rats (Maciejak et al. 2009). Besides, human epileptic patients exhibit oozing of froth from mouth and brief loss of postures along with frequent body jerks. Therefore, the epileptiform behavior of crab is partly comparable to the human generalized seizure pattern. Besides, the results revealed

that Sodium Valproate, a antiepileptic drug used to treat human epilepsy is effective in blocking PTZ mediated seizures in Crabs which suggests, that Crabs too respond to human antiepileptic drug. Also, a homeopathic preparation Cuprum metallicum, similarly, prescribed for treating epilepsy in humans is also as effective as Sodium Valproate, proving its suitability for such studies. These observations clearly suggest that the Crabs do have nearly identical neural architectural set up responsible for induction and treatment of epilepsy.

4.1.2. Electrical activities in Cerebral ganglion of Crabs:

The altered rhythms of neuronal discharges are nothing but paroxysmal depolarization shifts (Goldenshohn & Purpura 1963; Matsumoto & Ajmone-Marson 1964). It is known that 40 mmol/liter dose of PTZ can induce epileptic discharges in mollusks (Altrup et al. 1992). PTZ promotes volleys of electrical discharges of high frequency and amplitudes. The control crabs exhibited normal neuronal firing approximately in between -20 mV to +20 mV. The rate and pattern of volleys of electrical discharges induced by PTZ in crabs indicate the onset of epileptiform activities. PTZ produces large swings from +ve to –ve electrical potentials wherein, more positive effect indicates hyper-excitability while negative recordings indicate hyperpolarization suggesting membrane potentials have gone below the value of resting membrane potential. Further, they indicate involvement of excitatory circuits for progression of epilepsy (Khalilov et al. 2003).

Surface electrical recordings of cerebral ganglion of Crabs exhibit a pattern of brain waves observed in humans electroencephalography. In humans, a normal brain wave patterns are beta, alpha, theta and gamma waves (Guyton, 1971). In the present study, similar pattern in control crabs especially beta wave pattern was revealed

whereas, epileptic (PTZ treated) crabs showed abnormal beta waves corresponding to hyperexcitability. Besides, the crabs treated with Sodium Valproate and Cuprum metallicum alone showed theta wave pattern similar to that of humans, indicating relaxed state. Also, similar results were obtained in Crabs primed with Sodium Valproate followed by PTZ and Crabs primed with Cuprum metallicum followed by PTZ. These results indicate, the usefulness of crabs for such studies, as it represents similar brain wave patterns seen in humans.

4.1.3. Glutamate and GABA contents in Crab ganglions:

Millimolar concentrations of PTZ, the most extensively used epileptogenic drug, have been shown to affect a variety of ion-selective channels and neurotransmitter receptors (Hartung & Hermann 1987; Madeja et al. 1991; Bloms et al. 1992; Bloms-Funke et al. 1994; Madeja et al. 1996). These effects can lead to enhanced excitability of nerve cells by membrane depolarization, or to a hyper polarization that suppresses small depolarization and allows re-initiation of seizure activity (Madeja et al. 1996). The present work demonstrated that 100 millimolar concentration of PTZ increases the Glutamate level above that of control. Glutamate is a source of energy in oxidative phosphorylation and acts as a building unit for brain protein synthesis as well as an excitatory neurotransmitter. In addition to that, Glutamate has several specific functions. It is a detoxifying agent that binds NH_3 to form glutamine, a precursor for GABA, an inhibitory neurotransmitter and proline synthesis (Cooper et al. 1996). Therefore, it has been extremely difficult to dissociate the role Glutamate plays in neuronal metabolism and as a precursor for GABA from its possible role as a neurotransmitter. The present investigation indicates that the elevations in electrical activities could be due to elevations in the levels of Glutamate

and a reduced level of GABA suggesting the role of altered Glutamate and GABA levels in induction of epilepsy (Freitas et al. 2007). Controls show Glutamate level equal to 4.81 ± 0.98 mg/gm wet weight of tissue indicating as a requisite level for a normal functioning of cerebral ganglion, but larger increase in Glutamate indicate release of Glutamate on administration of PTZ which leads to hyperexcitation of neurons leading to epileptiform activity. Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system, but it has been established that an excessive neurotransmission at Glutamatergic synapses, mediated mainly by the *N*-methyl-D-aspartate (NMDA) receptor type, is greatly involved in epilepsy and neurodegeneration (Choi 1988; Meldrum 1991; Tapia et al. 1999). Sodium valproate reverses the effect of PTZ. The Sodium Valproate mediated declines in electrical discharges of neurons of both the control and PTZ administered crabs indicate involvement of GABA. Sodium Valproate is known to increase GABA in the brain tissue (Arroyo 2004). Sodium Valproate increases synaptosomal GABA concentrations through the activation of the GABA-synthesizing enzyme Glutamic acid decarboxylase (Arroyo 2004). In addition, Sodium Valproate inhibits GABA catabolism through inhibition of GABA transaminase and succinic semialdehyde dehydrogenase (Arroyo 2004). Sodium Valproate also inhibits the excitatory neurotransmission mediated by aspartic acid, Glutamic acid (Arroyo 2004). Furthermore, Valproate reduces cellular excitability through modulation of voltage – dependent sodium currents (Vreugdenhil & Wadman 1999). Valproate has shown efficacy in animal models of absence, partial and generalized seizures induced by chemical (bicuculline, PTZ, Picrotoxin, Strychnine, quinolinic acid), electrical (maximal electroshock) or sensory stimuli (photic). Valproate prevents the development of kindled seizures, thus, has an antiepileptogenic effect (Silver et al.

1991). The prevention of epileptic activities in the form of synchronized electrical discharges as well as change in the behavioral pattern and suppression of Glutamate rise by Valproate, (although it does not decrease Glutamate significantly), suggest a condition similar to that observed in experimental rodent models and human epilepsy. The present research indicates that Valproate alone promotes rise in GABA but PTZ probably suppresses Valproate ability to promote rise in GABA. But the –ve trace indicates that the factors other than GABA are suppressing the action of PTZ. Hence, it is necessary to further investigate the levels of other inhibitory neurotransmitters in the cerebral ganglion.

The present work also shows the presence of Glutamate and GABA in crab brain with possibility of presence of their receptors on the neurons. Khalilov et al. (2003) reported that for the propagation of seizures, excitatory neural circuits are essential and the PTZ mediated induction of seizures suggests the existence of such circuit in crab brain. The expression of behavioral pattern, synchronous abnormal electrical discharges by the brain of crab and abolishment of these responses by the antiepileptic drugs indicate the suitability of crab for studying epileptiform activities. Since the CNS of crab is a simple ganglionic tissue comprised of neurons, it would be possible to study the effects of epilepsy and antiepileptic drugs at cellular level quite easily as compared to the complex rodent brain.

Therefore, it is concluded that the cerebral ganglion of Mud crab, *Scylla serrata* can be used for studying epileptiform activities and physiological as well as biochemical changes associated with it. The present study indicates that the crab neurons have the requisite paraphernalia for producing volleys of electrical discharges associated with epilepsy. With caution, the information obtained from invertebrate studies could be applicable to higher systems.

4.2. Neuropharmacological studies of epilepsy using mice as model:

The second main objective of the present study was to understand the effect of epileptic seizures on some components involved in metabolic pathways in discrete mice brain regions such as Olfactory lobes (OL), Cerebral cortex (CC), Corpus callosum (CoC), Cingulate gyrus (CG), Hippocampus (H), Corpora Quadrigemina (CQ), Cerebellum (C), Pons (P), and Medulla Oblongata (MO). Besides, the effects of antiepileptic drugs such as Sodium Valproate and Cuprum metallicum, as well as the combined treatment of both drugs have been investigated. The results obtained show convincingly, the efficacy of this combined drug (Sodium valproate + Cuprum metallicum) therapy for controlling seizures and its impact on brain tissue metabolism.

4.2.1. Behavioral changes in Mice during epileptic and antiepileptic treatments:

Subcutaneous dose (CD 97) of Pentylenetetrazole induced generalized tonic-clonic seizures and loss of postural balance along with brief loss of consciousness in mice in the present investigations which also are known to be observed in Human epileptic conditions. The same has been reported in rats by White et.al. (1998) Besides, Sodium Valproate a known antiepileptic drug treatment showed a sedative effect on mice. Cuprum metallicum a homeopathic preparation did not show any noticeable behavioral change when given alone to the non-epileptic mice. Similarly, Combined drug (Sodium Valproate + Cuprum metallicum) treatment did not exhibit any noticeable behavioral change, and also it was not lethal to the animals that were not exposed to PTZ. It was noticed in the present investigation that combined drug

primed mice were more resistant to the PTZ effects, and did not show tonic-clonic seizures.

4.2.2. Biochemical changes in mice brain regions during treatments:

Among different regions of brain, the hippocampus is often the focus of epileptic seizures: hippocampal sclerosis is the most commonly visible type of tissue damage in temporal lobe epilepsy (Chang and Lowenstein, 2003). It is not yet clear, though, whether the epilepsy is usually caused by hippocampal abnormalities, or the hippocampus is damaged by cumulative effects of seizures (Sloviter, 2005). In experimental settings where repetitive seizures are artificially induced in animals, hippocampal damage is a frequent result: this may be a consequence of the hippocampus being one of the most electrically excitable parts of the brain that is responsible for developing long term potentiations (LTPs). It may also have something to do with the fact that the hippocampus is one of very few brain regions where new neurons continue to be created throughout life (Kuruba et al. 2009). In the present study PTZ induced epileptic seizures are observed in Mice with alterations in the metabolic activities in all the discrete brain regions such as OL, CC, CoC, CG, H, CQ, C, P, MO, under present study. Injection of convulsive doses of PTZ is thought to modulate patterns of generalised tonic- clonic seizures in humans (Fisher 1989; Psarropoulou et al. 1994; Loscher 2002). Based on the behavioral study in the present work the CD97 dose of PTZ is enough to induce generalized tonic-clonic seizures in mice which is akin to a type of epilepsy seen in humans. Therefore, it is suggested here, that the disturbances which are observed in the metabolic pathways involving different enzymes, metabolites, electrolytes, etc in discrete mice brain regions may be occuring in humans as well during generalized tonic-clonic seizures.

4.2.2.1. Alterations in Enzymes in Mice brain regions:

Enzymes such as AST, ALT, GS, GDH, LDH, AChE and Total ATPases show significant changes in their activities as compared to controls. All the enzyme activities varied depending upon the treatment regimen. Besides, AST, ALT, GS, and GDH are involved in the glutamate metabolism. Glutamate is always been known as an excitatory neurotransmitter.

The present results indicate that there are significant regional alterations in AST and ALT activities in mice brain regions. Alanine is an important nitrogen donor for the formation of Glutamate by ALT. Similarly, AST also contributes to the biosynthesis of Glu, but mostly in relation to the malate shuttle (Erakovic et.al., 2001). AST and ALT facilitates conversion of aspartate and alanine to glutamate, and serve as a link between carbohydrate and protein metabolism under stress (Philip et.al., 1994). Increased AST in CC, CoC, CG, H, C and ALT enzyme activities in brain regions such as OL, CC, CoC, CG during PTZ treatment, indicate pronounced metabolism of amino acids associated with seizure (Erakovic et al. 2001). OL exhibited either reduction or no significant change in AST, ALT activities in all the antiepileptic treated groups. Whereas, CC did not show any change in enzyme activities during Combined drug priming followed by PTZ treatment. However, ALT activities decreased in Sodium Valproate and Cuprum metallicum groups when primed separately and then exposed to PTZ, thus indicating the normalcy of these two enzymes in OL and CC during Combined drug treatment even after exposure to PTZ. CoC showed increase in AST and ALT activities in Cuprum metallicum treated mice and Combined drug primed mice which were then exposed to PTZ. This indicates increase in amino acid metabolism. ALT activities slightly increased in CoC, CG, H, MO regions of brain of mice receiving antiepileptic drugs. However, in CoC ALT

activities did not increase in mice receiving Combined drug treatment. The changes in ALT were insignificant in CG of mice exposed to Sodium Valproate/ Combined drugs before exposure to PTZ. Mice receiving only PTZ exhibit increased ALT activities. These results indicate that antiepileptic drugs slightly elevate alanine metabolism, but this effect is quite insignificant when compared to that observed in epileptic animals. However, it is clear that all the brain regions under study do not respond to antiepileptic drugs in the identical and predictable manner. Mostly, no significant changes in AST were observed in CG, H and CQ in antiepileptic treated mice except in H and CQ during Sodium Valproate treatment and in MO during Combined drug primed followed by PTZ treatment. Similarly, in regions like C and P exhibited no significant change in ALT activities, in all antiepileptic treatment indicating involvement of antiepileptic drugs to reduce the impact of seizure. Although, there are report (Erkovick et.al. 2001) on alterations of AST, ALT activities in mice brain regions during epilepsy, there is more need for investigation in these aspects during antiepileptic treatment. As it is been shown in the present work, Combined drug treatment exhibits a protective effect from seizure in most of the regions, still, there is treatment dependent regional alterations in AST, ALT enzyme activities indicating metabolic stress particularly in protein metabolism even during the treatment with antiepileptic drugs. This indicates that though epilepsy is controlled, prolonged exposure to antiepileptic drugs would have side effects on different brain regions to a different degree. Further, it appears that Cuprum metallicum is relatively safe.

Glutamine synthetase (GS) is a key enzyme in the regulation of glutamate neurotransmission in the CNS (Fraser et.al., 1999). It catalyzes the synthesis of glutamine from glutamate, and is responsible for the detoxification of ammonia in the brain (Meister, 1974). In the present investigation, GS activities exhibited decrease in

almost all brain regions during PTZ mediated epilepsy, indicating an increase in glutamate levels causing hyper-excitability of neurons leading to seizures similar observations are reported by Meister (1980); Laming et.al., (1989) and Carl et.al. (1993). Whereas, Sodium Valproate treatment as well as Combined drug (Sodium Valproate + Cuprum metallicum) treatment to non epileptic animals exhibited an increase in GS activities in almost all brain regions except CQ, P and MO. It has been reported that the AEDs such as Sodium Valproate enhances the activity of GS in rat cortical and cerebellar homogenates (Nolan et.al. 1985; Phelan et.al., 1985). Whereas, Cuprum metallicum treatment revealed decrease in this enzyme activity in most of the brain regions. But proved to be extremely protective against PTZ as far as GS activity is concerned in most of the regions except CoC, CG, P and MO. These results indicate involvement of Combined drug treatment in increasing the level of glutamine as GS is the only known enzyme involved in synthesis of glutamine in CNS (Cooper et al, 1983) and without getting affected much with PTZ induced seizure. The rise in GS activities by the antiepileptic drugs in nonepileptic groups indicate their sedative action, since loss of glutamate and rise in glutamine would reduce the excitation of a normal order.

Glutamate dehydrogenase (GDH) is responsible for catalyzing a reversible reaction mediating the interconversion of glutamate from alpha ketoglutarate and vice-versa as reported by Dennis et.al. (1977). It is observed in the present investigations, that Glutamate dehydrogenase activities increased in epileptic mice especially in regions such as CC, CoC, CG, CQ, C, P and only found reduced in OL and H. This result indicates an involvement of GDH for glutamate synthesis during seizures and glutamate is known to be associated with epilepsy. Further, the results indicate the involvement of CC, CoC, CG, CQ, C and P in genesis of seizure through

elevated GDH vis-a-viz glutamate in these regions as glutamates' association with epilepsy is well established (Rowley et.al., 1995; Takazawa et al. 1995; Ueda and Tsuru, 1995 and Li et. al., 2004). Whereas, reductions in GDH activities were observed in antiepileptic drug treated mice in comparison to control mice, indicating role of GDH in reducing in glutamate mediated seizures. Alterations such as increase in enzyme activities in CC, CoC except during Sodium Valproate priming followed by PTZ treatment, increase CG, CQ except in Sodium Valproate and Combined drug priming followed by PTZ, elevation in C except in Cuprum metallicum primed mice followed by PTZ, and finally in P were noticed. Therefore any increase in GDH activity could be considered as a step towards induction/ onset of epilepsy through synthesis of glutamate whereas, significant decrease in its activity in OL was observed during entire treatment regimen. But Combined drug primed animals exhibited decrease in GDH activity in regions such as OL, CC, H, MO indicating synthesis of less glutamate through alpha-ketoglutarate breakdown in the tissues, even after treatment with PTZ, resulting into containment of epilepsy through reduced levels of glutamate. Besides, there are reports suggesting prolonged intake of monosodium glutamate reduced invivo GDH levels (Yoon et.al., 2002). However, since seizures and epilepsy expression doesn't continue for long, there are less chances of inhibition of GDH by Glutamate itself. But one need to find out what concentration of glutamate inhibit GDH.

Lactate dehydrogenase catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+ (Markert, 1984). It converts pyruvate, the final product of glycolysis to lactic acid when oxygen is absent or in short supply, and it performs the reverse reaction during the Cori cycle in the liver. The results indicate reduction of LDH in the OL, CC, CG, H, and P during PTZ

induced epileptic seizures. There could be some factors that inhibit its activity in these regions of brain during seizures or it may be just because of increase in lactate as well as pyruvate levels together, thereby causing energy crisis, which cause morbidity of epilepsy. However, it needs further investigation. High LDH activities in H, CQ and P during entire antiepileptic treatment regimen with small exceptions suggest its involvement in interconversion of pyruvate and lactate for the energy demand, in these brain tissues. Besides, OL and CC did not show any significant change in LDH activity upon treatments with antiepileptic drugs.

It was reported earlier by Giovagnoli and Avanzini (2006) that the impairments in learning, memory and behavior in patients with epilepsy are caused due to cholinergic system dysfunction. Also there are reports which give consistent evidence that high levels of acetylcholine in the brain are associated with cognitive dysfunction (Giacobini, 2000; Freitas et.al., 2006). Cholinergic transmission is mainly terminated by Acetylcholine hydrolysis by enzyme acetylcholinesterase (Sales et.al. 2010). The increased activities of Acetylcholinestetrase in the regions such as OL, CC, CG, C and MO, etc, upon treatment with Combined drug followed by PTZ indicate that brain tissues in these regions get protected from cholinergic dysfunction to be developed due to epilepsy by elevating AChE activity as earlier reported by Sales et.al., (2010). Besides, high levels of ACh due to decreased AChE activities could be leading to cognitive dysfunctions in the state of epilepsy as reported by Giacobini, (2000) and Freitas et. al. (2006). Whereas decrease in its activities in PTZ treatment, in all regions indicates impairement in Acetylcholinesterase activities, inturn increasing the acetylcholine level, which may be adding to the seizure threshold in those brain regions. Besides, constant reduction in acetylcholinesterase enzyme during seizures may cause memory deficit as reported by Sales et.al. (2010).

Cuprum metallicum treatment alone exhibits decrease in enzyme activity in H, CQ and MO which may elevate Ach levels while increased in activity in OL, CC would reduce Ach levels in these tissues. Sodium Valproate alone shows increase in AChE activity in OL, CC, CoC, CG and P, with decrease in C alone. Increased AChE could reduce Ach level in these regions. Similarly, Combined drug treatment exhibits increase in AChE in OL, CC, CoC, CG, P and a slight decrease in activity in regions such as H, and MO, indicating that the Cuprum metallicum drug behaves similar to that of Sodium Valproate which is already known as conventional antiepileptic drug. Increase in AChE activity could be contributing to sedation through reduced Ach.

The Na^+,K^+-ATPase harness energy stored in the phosphate bond to exchange Na^+ ions inside to K^+ ions outside of the axonal membrane (Vatta et al. 1999). This transmembrane gradient of Na^+ and K^+ ions facilitates the neurons to propagate nerve impulses from one point to another. Other enzymes such as Mg^{2+}- ATPase and Ca^{2+}-ATPase also take part in energy production and transmission of nerve impulses. In the present investigation these three ATPases show a significant decrease in activity in most of the brain regions as compared to controls during epileptic seizures which is in agreement with the observations of Visweswari et al. (2010), except OL, CoC, MO wherein Na^+,K^+-ATPase activity was high and in CQ, both Mg^{2+}- ATPase and Ca^{2+}-ATPase was increased. Inhibition in the activity reveals induction of energy crisis through disruption in oxidative phosphorylating process during epileptic seizure. The decrement in total ATPases, especially Na^+,K^+-ATPases indicate impairement of ion/electrolyte homeostasis during epilepsy. The decline in Na^+,K^+-ATPase enzyme activity has been reported to be associated with excitotoxicity by Golden et al. (2001). Besides, Xiao et al. (2002) demonstrated that neuronal death associated with inhibition of Na^+,K^+-ATPase activity and is mediated through intracellular depletion

of K+ and accumulation of Ca^{2+} and Na^{+}, indicating its role in seizures induced by PTZ, in the present study. Also, While rise of ATPase in CoC indicate significantly increased role in impulse transmissions as well as stress on ionic balance in epilepsy. As CoC is the principal anatomical and neurophysiological track linking the cerebral cortices, the increased ATPases clearly indicate the increased neural transmission load in it. Besides, most of the regions shows alteration in Total ATPases activity, especially decrease except a few during all the treatment regimens. But, the noticeable aspect is that Combined drug (Sodium Valproate + Cuprum metallicum) primed mice show less impact on all three enzyme activities even after PTZ treatment indicating the protective nature of this combined drug against seizures and excitotoxicity.

Thus, it can be concluded from the above enzyme studies that, due the impact of seizures, metabolic pathways get affected in every brain region. Therefore, the treatment for epilepsy should be such that the drug should be able to keep a normal or at least near normalcy in metabolic pathways to control the occurrence of seizures.

4.2.2.2. Alterations in Metabolite contents in Mice brain regions:

The brain is extremely metabolically active, with its local energy demands fluctuating rapidly between high and low activity states. However, the brain's endogenous stores of metabolic energy substrates are small. Consequently, normal brain function requires a continuous supply of exogenous substrates obtained through blood flow. Under normal physiological conditions, glucose is the dominant exogenous energy substrate in the adult brain (Clarke and Sokoloff, 1999). However, other exogenous or endogenous energy substrates can serve as alternatives to glucose under certain physiological and pathological conditions. In vitro studies have shown that cultured brain cells or isolated brain tissues can oxidize a variety of substrates.

These substrates include glycolytic intermediates such as pyruvate (Peng et al. 1994) and lactate (McKenna, 1993; Itoh, 2003), amino acids such as glutamate and glutamine (Hertz and Hertz, 2003). Seizures usually lead to uncoupling between glucose uptake and oxygen consumption and increases lactate accumulation while energy homeostasis is maintained (Folbergova, 1997). In the present study, Energy metabolites such as Pyruvate, Lactate, Glutamate, Gamma amino butyric acid, glucose have been studied during various epileptic and antiepileptic drug treatment regimens, particularly in Mice brain regions such as OL, CC, CoC, CG, H, CQ, C, P and MO.

Glucose level is decreased in OL, CC, CG, H, C, P and MO during epileptic seizures indicating energy crisis developed by the over excitation and stepped up glucose metabolism. The high glucose metabolism could rise in levels of lactate in CC, CG, H, and pyruvate level in CoC, CG, H, as a result of stepped up metabolism and could be used as alternative substrate for glycolysis besides glucose. Decreased glucose consumption was reported by Eloqayli et al. (2002) in cerebellar granular cells exposed to PTZ. Elevated lactic acid concentration is observed in the brain of piglets with experimentally induced convulsions and in the extracellular fluid of the hippocampus of humans during spontaneous seizures (During et al. 1994 and Thoresen et al. 1998). CQ did not show much change in glucose and pyruvate and a decrease in lactate could be due to the presence of sufficient substrate for activity as well as due to breakdown of lactate. Less change in glucose and pyruvate in CQ suggest lesser metabolite crisis. Besides, during antiepileptic treatment such as Sodium Valproate alone shows decrease in glucose in regions such as OL, CC, CG, H, CQ; at the same time Pyruvate were decreased in CC, C and increased in CQ, CG, C, H; similarly, Lactate level increased in CC, CoC, CG, H, P and decreased in CQ,

indicating that either of the substrate is present for energy formation but reduction of glucose, a prime source of energy to the brain is an attempt to curtail epilepsy vis-a-viz over-excitation by depriving the system of energy coinage. Whereas, Cuprum metallicum treatment alone lead to increase in pyruvate, lactate content in CG with less glucose, etc. Hence, there is a regional difference in level of metabolic substrates both during treatment with antiepileptic as well as during antiepileptic priming before exposure to PTZ. But a noticeable fact in these results is that the animals primed with Combined drug (Sodium Valproate + Cuprum metallicum) and then exposed to PTZ did not show much alterations in the substrate such as glucose, pyruvate and lactate contents in discrete brain regions, suggesting that the combination of drug therapy doesn't contain epileptic seizures by reducing substrates for energy but by controlling the other metabolic pathways.

Also an increase in glutamate level in all the brain regions of mice is observed in the present study during PTZ induced epileptic seizures, which is in agreement with the observations of Li et al. 2004; Rowley et al. 1995; Takazawa et al. 1995; Ueda and Tsuru, 1995. Thus, suggesting that glutamate, an excitatory neurotransmitter is involved in seizures in almost all parts of brain. A decline in GABA is observed during epilepsy indicating its role during seizures GABA being an inhibitory neurotransmitter, a decline in its level could pave way for development of seizures and uncontrolled excitability. In epilepsy, GABA is thought to be a key component underlying the abnormal hyperexcitability (Pan et.al., 2008). The results show that, upon treatment with Sodium Valproate, Cuprum Metallicum, or Combined drug (Sodium Valproate + Cuprum metallicum), some of the brain regions exhibit either decrease in Glutamate and increase in GABA level or no significant change, indicating the efficacy of these drugs for controlling the level of these two substrates,

which act as a key for unbalanced excitability. Besides, the results also represent the fact that Combined drug is far better in controlling seizures induced by PTZ.

4.2.2.3. Alterations in Electrolyte contents of Mice brain regions:

Electrolyte homeostasis is essential in the central nervous system for a normal brain function. Regulation of electrolyte balance is a critical process involving a complex array of molecules for moving ions into and out of the brain and involving blood–brain barrier function as well as mechanisms in the membranes of both neurons and glia (Guerra et.al. 2006). Altered ionic gradients across the membranes can have direct and indirect effects on neuronal electrical discharges that may lead to epileptiform activities (Scwartzkroin et al. 1998).

There are several reports that relate the change in electrolyte contents in the blood to brain stroke or brain edema. Kiviranta et. al. (1996) observed that the febrile children, with and without seizures, have altered osmolarity and electrolyte concentration in the cerebrospinal fluid and serum, especially altered serum calcium levels leading to convulsions. Neonatal serum hypocalcaemia and hypomagnesaemia are known to promote convulsions (Lynch and Rust, 1994; Sheth, 1997). A change in the serum electrolytes in childhood epilepsy has been studied by Shah et. al. (2001) who reported a reduced K^{+} along with unaltered Na^{+2} levels in serum. Hameed and Abdellah (2004) conducted research on trace elements, electrolyte homeostasis and their relation to antioxidant enzyme activity in brain and hyper excitability of epileptic patients. However, there is hardly any report on the actual electrolyte contents of the different brain regions in the state of epilepsy. In the present study, the electrolyte levels in the brain regions of Mice *Mus musculus,* during the state of epilepsy and during antiepileptic treatment are investigated.

Hyponatremia was observed in four regions of the brain such as CC, OL, H, CQ during PTZ induced seizures. Hyponatremia indicates an impairement of electrolyte homeostasis in the brain. Altered osmolality and sodium levels lead to CNS neuronal depression, with encephalopathy as the major clinical manifestation, thus affecting neuronal irritability (Victor and Ropper, 2001). Hypernatremia was seen in CG, CQ, MO during treatment with Cuprum metallicum, while CG and CQ in Sodium Valproate treatment. Thus, it may be considered that these drugs may help in stabilizing Sodium level in brain tissues, as Combination of these drugs show normalcy even after PTZ treatment.

Except CC, C, and CQ, rest all other regions, showed hypokalemia. But hyperkalemia was observed in OL only. Hypokalemia observed in the state of epilepsy draws parallel to the reported serum hypokalemia (Shah et.al, 2001; Natelson, 1979). The onset of hypokalemia in the state of epilepsy is attributed to the impairment of neuronal membranes. Serum hypokalaemia and normal sodium levels are reported during seizures (Shah et.al, 2001; Natelson, 1979). In the present work similar relationships of sodium and potassium in MO, P, CoC and CG is observed indicating their involvement in seizures. Similar results as that of Sodium were observed for Potassium contents during antiepileptic treatments.

Magnesium is known for the anesthetic effect on the brain and particularly for its ability to counter excessive electrical discharges by the neurons. Therefore, lowering of magnesium (Hypomagnesaemia) level in the brain would result in induction of epilepsy for want of counter measures by the neurons. Shah et.al. (2001) reported serum hypomagnesaemia in epileptic patients. In the present work all the mice brain regions showed hypomagnesemia during PTZ induced epileptic seizures. Sodium Valproate treatment exhibited hypermagnesemia in OL, CC, CoC, H, MO;

Cuprum metallicum treatment revealed hypermagnesemia in CC, MO; Combined drug treatment exhibited hypermagnesemia in OL, CC, CG, MO. But individual antiepileptic drug primed mice exposed to PTZ exhibited hypomagnesemia in a few brain regions but the mice primed with Combined drug did not show hypomagnesemia.

Hypocalcaemia was observed only in OL and CG during seizures. Hypocalcaemia and hypomagnesaemia are known to promote neuronal irritability with seizures (Riggs, 2002). Besides, reduced Ca^{+2} are treated as a marker of excessive seizures (Hamed and Abdellah, 2004). Also reduction in Ca^{+2} in 3 brain regions indicate blockade of synaptic transmission and onset of ephatic/ electrotonic transmission during seizures. Under the antiepileptic treatment hypercalcemia was found in few a brain regions while the rest remained unaltered (e.g. CC, CQ, C).

Chloride is an important anion responsible for the maintenance of osmolarity within the tissue. In the present study it remained unaltered in CC, C, H, CQ indicating no osmotic stress in these regions while hypochloremia in OL, P, CoC, CG indicates osmotic stress in these regions during the state of epilepsy. Few mice brain regions such as OL, CoC, CG and H showed hyperchloremia during Combined drug primed mice with post priming PTZ treatment.

The hyponatremia, hypokalemia, hypocalcaemia and hypomagnesaemia observed in discrete brain regions in the present work clearly indicate that not only the serum hyponatremia, hypokalemia, hypocalcaemia and hypomagnesaemia (Kiviranta, 1996; Lynch and Rust, 1994; Sheth, 1997; Shah et al, 2001; Hamed and Abdellah, 2004; Natelson, 1979) are responsible for epileptic tonic-clonic seizures vis-à-vis epilepsy, but such events in the discrete brain regions could be considered as causative factors for epilepsy. Since it is difficult to assay such hyponatremia,

hypokalemia, hypocalcaemia, hypomagnesaemia and hypochloremia in the human brain, occurrence of such events in the serum have always been correlated with the epileptic events. However, correlation of such events of lowered sodium, potassium, magnesium, calcium and chloride i.e., electrolytes in general could be directly responsible for the induction of epilepsy. Further, the predominant hypokalemia and hypomagnesaemia found in brain regions such as MO, H, P, CoC, CG and similarly, hypocalcaemia and hypochloremia in MO, OL, H, P, CoC and hyponatremia in CC, OL, H, CQ suggests their involvement in epilepsy, indicating etiology of epilepsy in the brain.

Therefore, it is concluded that during the state of epilepsy there is not only serum hyponatremia, hypokalemia, hypocalcaemia, hypomagnesaemia but also similar situation occurs in the discrete brain regions involved in epilepsy. This is altered when treated with antiepileptic drug such as Sodium Valproate and Cuprum metallicum, especially Combined drug primed mice exhibited better control on PTZ induced epileptic seizures in mice.

4.2.3. Utility of homeopathic drugs combined with allopathic for controlling seizures:

Sodium Valproate is a conventional AEDs used commonly for treatment of epilepsy (Sanders, 2004). It is known to be effective in spike-wave EEG discharges in humans with idiopathic generalized epilepsy (Sato et.al. 1982). Yet its mechanism of action has not fully been elucidated. However, most animal studies have demonstrated its effect on GABA system (Arroyo, 2004). Sodium Valproate increases synaptosomal GABA concentrations through the activation of the GABA- synthesizing enzyme glutamic acid decarboxylase. Also, it inhibits GABA catabolism through inhibition of

GABA transaminase and succinic semialdehyde dehydrogenase. Besides, Valproate inhibits the excitatory neurotransmission mediated by aspartic acid, glutamic acid, and gamma-hydroxybutyric acid. Furthermore, it reduces cellular excitability through modulation of voltage dependent sodium currents (Vreugdenhil and Wadman, 1999).

Yet, it has a unique place in epilepsy therapy because of its efficacy in generalized seizures, including generalized tonic-clonic seizures (Perucca, 2002). However, Valproate has substantial disadvantages in that it is a teratogen, causes weight gain and reproductive dysfunction, occasionally associated with hepatic and pancreatic toxicity (Rogawski, 2006). After prolonged usage it leads to cancer. Valproate administration caused uterine adenocarcinomas in Wistar rats (Watkins et. al., 1992). In contrast, an anti-tumour effect of valproate was discovered serendipitously, when clinical experience with the drug raised concerns about its teratogenicity, specifically the development of human neural tube defects (Blaheta et al., 2004). Its teratogenicity was then studied in vitro using neuroectodermal cell lines, such as neuroblastoma cells (Regan, 1985; Blaheta and Cinatl, 2002). An antiproliferative and differentiating effect was revealed in these experiments and was later extended to other cell lines, such as glioma, breast cancer, prostate cancer and teratocarcinoma cell lines and leukaemia progenitors (Cinatl et al., 1997; Knupfer et al., 1998; Blaheta and Cinatl, 2002). The effect was subsequently confirmed in vivo (Michaelis et al., 2004).

The present work shows that during epileptic seizures, the metabolism is altered in the brain tissue, which is actually the impact of the seizures that is seen in human with generalised epilepsy. Besides, it should be noted that even with antiepileptic drugs a slight variation in the metabolic pathways occur although, the seizure threshold would be under control. Cuprum metallicum a homeopathic

preparations exhibited similar results as that of Sodium Valproate with a slight variation. However, Combination of both drugs revealed better results in containment of seizures induced by PTZ in mice. A detailed study in this field is required which will help to treat the post epileptic effect, infact the antiepileptic drug that could be given as a precautionary medicine should have the ability of not altering the basic metabolic processes, which, may in turn lead to control the seizure impact.

In the present investigation, if one compares the results of the metabolic studies on Mice brain, many a time it is noticeable that Combined drug (Sodium Valproate + Cuprum metallicum) can reduce the impact on the metabolism as compared to Sodium Valproate alone, or Cuprum metallicum alone. Also, it should be noted that, as Cuprum metallicum is a homeopathic preparations, it does not have any side-effects due to its infinitesimal quantities. Therefore, if it is combined with an allopathic drug, thus reducing the quantity of allopathic chemical that enters the body as a part of chronic treatment during epilepsy. Hence, one can reduce the other organ dysfunction, besides brain as reported in case of Sodium Valproate in humans.

There should be more detailed study conducted wherein, alternative and complementary medicines should be used in combination with allopathic drugs, as it will have less deleterious effects on the health of a person for long term, since the neurological disorders like epilepsy requires long term treatment. The present work is just a preliminary study in this regards, but still more detailed investigation is required before going for clinical trials.

1. As we proposed in our first hypothesis, it is possible to induce epileptic seizure in Crab *Scylla serrata* by using 100mM PTZ concentration, which is in agreement with **Altrup et.al., 1992**, who showed the same effect in Mollusc *Helix pomatia*. Therefore, it is clear that the cerebral ganglion of Crab has all potentials for the development of seizure.
2. The behavioral changes of crabs like fast running, and sudden loss of postures, vigorous movements of eyestalks and antennules, frequent body jerks, oozing of froth from mouth followed by brief loss of posture after PTZ treatment, are quite comparable to tonic-clonic epileptic behaviors in rats (**Maciejak et al. 2009**).
3. The surface electrical recordings of cerebral ganglion of control Crabs exhibits normal neuronal firing in the range of approximately between **-20 mV to +20 mV**. PTZ produces large swings from +ve to –ve wherein, more positive effect indicates hyperexcitability while negative recording indicate hyperpolarization indicating membrane potential have gone below the value of resting membrane potential which is in agreement with work done in mice (**Khalilov et al. 2003**).
4. The rate and pattern of volleys of electrical discharges induced by PTZ in crabs indicate the onset of epileptiform activities.
5. Hence, this enhanced excitability of nerve cells by membrane depolarization, or to a hyper polarization within the cerebral ganglion suppresses small depolarization and allows re-initiation of seizure activity (**Madeja et al. 1996**).

6. Sodium valproate reverses the effect of PTZ in crab cerebral ganglion, indicating the involvement of GABA, as valproate is known to increase GABA in the brain tissue (**Arroyo, 2004**).
7. Studies with the homeopathic preparations, shows convincingly that Crab could be considered for the preliminary screening of antiepileptic drug, as it shows the effect rapidly as compared to the higher vertebrate model due to its simpler nervous system.
8. The present work also shows the presence of glutamate and GABA in crab brain with possibility of presence of their receptors on the neurons.
9. The expression of behavioral pattern, synchronous abnormal electrical discharges by the brain of crab and abolishment of these responses by the antiepileptic drugs indicate the suitability of crab for studying epileptiform activities.
10. The behavioral study in the present work the CD97 dose of PTZ is enough to induce generalized tonic-clonic seizure in mice, which akin to a type of epilepsy seen in humans (**Loscher, 2002**).
11. Increased AST and ALT level in most of the brain regions indicate pronounced metabolism of amino acids associated with seizure (**Erkovic, 2001**).
12. Rise in GDH activity during PTZ treatment in most of the regions except H and MO, indicates high glutamate metabolism as, it catalyzes a reversible reaction, mediating the interconversion of glutamate from α- ketoglutarate and vice versa (**Dennis, 1977).**
13. Also, GDH activity is seen to be reduced in most of the brain regions except MO, C, CoC during combined pre-treatment of sodium valproate and cuprum

metallicum, suggesting the possibility of reduced glutamate metabolism in the brain.

14. Increased GS activity in Cuprum treated Mice, shows that the glutamate metabolism is increased by increasing glutamine synthesis from glutamate and detoxification of ammonia, rather, than other antiepileptic drug that usually causes decreased GS activity as reported by **Fraser et al. (1999)**.
15. Besides, Glutamate content in all regions is seen to be increase during PTZ treatment, and this could be the main reason for development of seizure.
16. Similarly, GABA is reduced in PTZ treated mice, it is considered as the inhibitory neurotransmitter. But, it rises in Sodium valproate and Cuprum metallicum pretreated mice.
17. Electrolytes are ionized molecules found throughout the blood, tissues, and cells of the body. These molecules, which are either positive (cations) or negative (anions), conduct an electric current and help to balance pH and acid-base levels in the body. Most of the time, disturbances of serum sodium, magnesium, potassium, and calcium is reviewed with regard to their propensity to provoke seizures. But there are no reports of the electrolyte content in the brain tissues found during various disorders in the brain. In the present study, electrolyte content in the different brain regions are measured underlying epileptic seizure, induced by PTZ in Mice *Mus musculus* as well as during antiepileptic treatment. We found alterations in the various electrolytes content such as Sodium, Potassium, Calcium, Magnesium, and Chloride in brain tissues during neuropharmacological studies of generalized epilepsy.

18. Metabolic pathways studied in the present work during various treatment, reveals convincingly, that combination of Sodium valproate and Cuprum metallicum, protects from PTZ mediated epileptic seizures.

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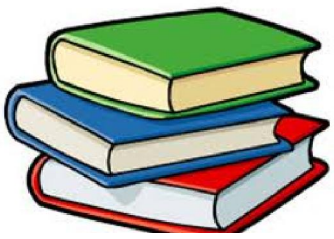

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ORIGINAL PAPER

Study of epileptiform activity in cerebral ganglion of mud crab *Scylla serrata*

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Abstract An attempt is made to induce in mud crab (*Scylla serrata)* epileptiform activities that resemble the generalized epileptic seizures. Cerebral ganglion of crab was exposed in situ, to a convulsant drug pentylenetetrazole (PTZ) 100 mM, for induction of seizures. Also, crabs were pretreated with antiepileptic drug viz sodium valproate (120 μmol/l) to inhibit epileptiform activities. The surface electrical discharges of cerebral ganglion were recorded using Unkelscope (MIT, USA) in control as well as experimental animals. The cerebral ganglion of crab showed a pattern of high cerebral electrical discharges after PTZ treatment compared to control. The sodium valproate promoted sedative action in control and prevented PTZ-mediated epileptiform discharges. Glutamate and GABA contents in cerebral ganglion were assayed. Glutamate level increased (31.45%) during PTZ treatment with concomitant decrease (43.93%) in GABA. Sodium valproate had no effect on glutamate concentration, but it decreased GABA by 24.75%. The present study shows that epileptiform activities can be induced in crabs.

Keywords Crab · Pentylenetetrazole · Sodium valproate · Glutamate · GABA

Introduction

Invertebrate systems are useful for understanding processes in the central nervous system (CNS). To understand the basic mechanisms of epilepsy, it is essential to know how populations of neurons interact and how the physiological processes in individual neurons are altered. Owing to an easy access to invertebrate neurons, it is possible to examine the basic mechanisms controlling neuronal excitability and to predict the electrophysiological mechanisms that may be present in central nervous system (Lewis et al. 1986).

Epilepsy is a common neurological disorder evidenced by the occurrence of spontaneous seizures with diverse etiologies (Liu et al. 2009). Although the causes of epilepsy are many, the fundamental disorder is secondary to abnormal synchronous discharges of a network of neurons and can be caused by either abnormal ionic conductance or alterations of neuronal membranes, or an imbalance between excitatory and inhibitory neurotransmitters. Epilepsy is characterized by intense electrical discharges in localized brain areas, associated with a change in glutamate (Meldrum 1994; Urbanska et al. 1998) as well as ϒ-aminobutyric acid (GABA) levels (Heinemann and Hamon 1986).

Invertebrates have often been used as an experimental model for studying the effect of convulsant and anticonvulsant agents at the cellular level for understanding the cellular and neurophysiological mechanisms of epilepsy (Janahmadi et al. 2006). It is known that pentylenetetrazole (PTZ) induces a pattern of electrical discharges in molluscan neurons, resembling the paroxysmal depolarization shift (PDS) of epilepsy observed in mammalian neurons (Goldenshohn and Purpura 1963; Matsumoto and Ajmone-Marson 1964; Sugaya et al. 1973). PTZ is used experimentally to study expression of seizures and to identify pharmaceuticals that control them (Janahmadi et al. 2006).

Earlier, attempts were made to study mechanism of epilepsy using invertebrate animal models such as gastropod *Helix pomatia* (Altrup et al. 1992) at a single neuron

The authors declare that the experiments comply with the current Institutional ethical laws.

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Metabolic responses in discrete, Mice brain regions like CC, CG, H and CQ during PTZ induced epileptic seizures.

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Abstract

Epilepsy, a neurological disorder with recurrent seizures, involves disruption of different metabolic enzymes and its related metabolites altering the normal processes of metabolism, either during the onset or post epilepsy in the brain. In the present work, it is convincingly, observed that the Mice brain regions such as Corpus callosum (CC), Cingulate gyrus (CG), Hippocampus (H) and Corpora quadrigemina (CQ) shows significant changes in the activities of metabolic enzymes such as AST, ALT, LDH; ATPases like Na^+, K^+-ATPase, Mg^{2+}-ATPase, Ca^{2+}-ATPase along with their metabolites such as Glucose, Pyruvate, Lactate and Glutamate, altering the metabolic integrity during Pentylenetetrazole (PTZ) induced epileptic seizure. We report from the present work that, H and CG are affected completely during epileptic seizure as compared to its control but CC and CQ shows partly altered as far as metabolism in brain is concerned.

Keywords: Corpus callosum, Hippocampus, Cingulate gyrus, Corpora Quadrigemina, Metabolic enzymes and metabolites.

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Introduction

Epilepsy, involves a disruption of brain energy homeostasis and is potentially manageable through principles of metabolic control theory. Metabolism is an essential process underlying all phenotypes and an alteration in metabolic process can modify phenotype. The theory is based on the idea that compensatory genetic and biochemical networks, operating through flexible biological systems, are capable of modulating the bioenergetics of glycolysis, the TCA cycle, electron transport and oxidative phosphorylation [1,2].

Although, the work in this aspect has been done in Cerebral cortex, Cerebellum, Medulla Oblongata, Hippocampus (H) etc, yet the regions such as Corpus Callosum (CC), Cingulate gyrus (CG), Corpora Quadrigemina (CQ) are not explored in this regards to a large extent.

The corpus callosum is the principal anatomical and neurophysiological track linking the cerebral cortices [3]. Corpus callosotomy was first introduced as a surgical technique for the treatment for epilepsy in 1939 [4]. It is often used to treat atonic, clonic, myoclonic and generalized tonic-clonic seizures [5]. The cingulate gyrus is an arched convolution that lies next to the corpus callosum and is separated from it by the sulcus of the corpus callosum. Cingulate gyrus epilepsy is controversial because it may overlap with other frontal lobe epilepsy syndromes. But, an aberrant behaviors observed in epileptic patients completely resolved after lesionectomy of Cingulate gyrus [6]. Hippocampus and Cingulate gyrus forms the part of limbic system. Corpora quadrigemina are reflex centers involving vision and hearing. In the brain, the corpora quadrigemina are the four colliculi, two inferior, two superior located on the tectum of the dorsal aspect of the midbrain [7].

Metabolic enzyme such as aminotransferases like AST and ALT serve as a strategic link between carbohydrate and protein metabolism under pathophysiologic stress [8]. Similarly, LDH is an enzyme that transforms lactate into pyruvate in brain tissue and thus, prevents acidosis and provides a substrate for TCA cycle. ATPases, also play an important role in the maintenance of ionic gradient by coupling ATP hydrolysis with energy processes [9]. ATP itself, as a neurotransmitter and neuromodulator, may influence the release of other neurotransmitters by acting through its own receptors or by altering the neurotransmitter receptors [10,11]. Na^+, K^+ -ATPase is a membrane bound enzyme and inactivation of this enzyme is an important factor in epileptization of neurons [12]. Similarly it is demonstrated that inhibition of microsomal Mg^{2+}-ATPase, Ca^{2+}-ATPase may be associated with long-term plasticity changes associated with epileptogenesis [13]. Idiopathic epilepsies involve the Na^+, K^+, or Ca^{2+} chan-

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Epilepsy mediated alterations in the electrolyte contents of the discrete mice brain regions

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Key words: *Sodium, Potassium, Calcium, Magnesium, Chloride, Epilepsy, Pentylenetetrazole, Brain.*

Electrolyte homeostasis is essential in the central nervous system for a normal brain function. Regulation of electrolyte balance is a critical process involving a complex array of molecules for moving ions into and out of the brain and involving blood–brain barrier function as well as mechanisms in the membranes of both neurons and glia [1]. Altered ionic gradients across the membranes can have direct and indirect effects on neuronal electrical discharges that may lead to epileptiform activities [2].

There are several reports that relate the change in electrolyte contents in the blood to brain stroke or brain edema. Kiviranta et. al. [3] observed that the febrile children, with and without seizures, have altered osmolarity and electrolyte concentration in the cerebrospinal fluid and serum, especially altered serum calcium levels leading to convulsions. Neonatal serum hypocalcaemia and hypomagnesaemia are known to promote convulsions [4, 5]. A change in the serum electrolytes in childhood epilepsy has been studied by Shah *et al.* [6] who reported a reduced K^+ along with unaltered Na^{+2} levels in serum. Hameed and Abdellah [7] conducted research on trace elements, electrolyte homeostasis and their relation to antioxidant enzyme activity in brain and hyper excitability of epileptic patients. However, there is hardly any report on the actual electrolyte contents of the different brain regions in the state of epilepsy. In the present study, we investigated the electrolyte levels in the brain regions of Mice *Mus musculus*, during the state of epilepsy.