EFFECTS OF ADMINISTRATION OF CARBAMAZEPINE, PHENYTOIN AND THEIR COMBINATION ON NEUROBEHAVIOURAL, COGNITIVE HAEMATOLOGICAL AND SERUM BIOCHEMICAL CHANGES IN ALBINO RATS

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SEPTEMBER, 2012

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN VETERINARY PHARMACOLOGY

DEPARTMENT OF VETERINARY PHARMACOLOGYAND TOXICOLOGY
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DECLARATION

I declare that the work in the thesis entitled "Effects of Administration of Carbamazepine, Phenytoin and their combination on Neurobehavioural, Cognitive, Haematological and Serum biochemical Changes in Albino Rats" was performed by me in the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University Zaria under the supervision of Professor J. O. Ayo, Doctors S. F. Ambali and A. U. Zezi. The information derived from the literature has been duly acknowledged in the text and the list of references provided. No part of this thesis was previously presented for another degree or diploma at any other university.

_____ _____

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CERTIFICATION

This thesis, titled "Effects of Administration of Carbamazepine, Phenytoin and their combination on Neurobehavioural, Cognitive, Haematological and Serum biochemical Changes in Albino Rats" by Hadiza Aliyu meets the regulations governing the award of Master of Science of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This project is dedicated to all animal owners, who, due to their affection for animals, are always concerned with their well-being; they are the true animal care-givers.

ACKNOWLEDGEMENTS

All thanks are to almighty Allah for giving me the opportunity and for sparing my life to witness this period of my life's endeavour that is, the completion of my research work.

I give thanks to my parents; Prof. M. M. Aliyu, Alhajas Maryam Jiya and Muslimah Aliyu and my aunt, Alhaja Nurat Olaniyan for their moral support. I appreciate the words of encouragement given to me by all my siblings.

I am highly grateful to my supervisory committee; Professor J. O. Ayo, Drs. S. F. Ambali and A. U. Zezi for their patience, tolerance and encouragement with my research work and at vetting all my manuscripts.

I appreciate the continuous words of encouragement from my lecturers: Drs. M. U. Kawu, T. Aluwong, M. M. Suleiman, T. Dzenda; and from friends and colleagues, Drs. C. Uchendu, M. Shittu, V. O. Sinkalu, L. S. Yaqub, P.I. Kobo, M. T. Salawudeen and H. K. Yusuf. I thank Drs. C. Orieje and Richard for helping with my laboratory work.

I am also grateful to the technical staff of the Department of Veterinary Physiology, Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria; and Mr. F. O. Ayegbusi of the Department of Chemical Pathology and Haematology, Ahmadu Bello University Teaching Hospital, Shika, Zaria for helping with the analysis of the samples collected.

My appreciation cannot be complete without acknowledging the support, love, encouragement and advice I received from my darling husband, Luqman, I am most grateful.

ABSTRACT

The aim of the study was to evaluate the neurobehavioural patterns, cognitive functions and haemato-biochemical changes following the administration of carbamazepine (CBZ), phenytoin (PHE) and their combination. Forty, apparently healthy adult male Albino rats weighing between 144 and 300 g were divided into four groups of 10 animals each and used for the experiment. Group I (control) were administered distilled water per os at the dose rate of 10 mls/kg and they served as untreated control. CBZ at 20 mg/kg, PHE at 100 mg/kg and a combination of CBZ and PHE at 20 mg/kg and 100 mg/kg were administered per os to groups II, III and IV respectively. The regimens were given once daily for a period of eight weeks and the rats were monitored for neurobehavioural changes and cognitive functions using the method as described by Zhu et al., 2001. Blood and serum samples were collected for evaluation of full blood count, total protein, albumin, globulin, urea and some electrolytes. Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase activities were also determined. Results show that the administration of CBZ, PHE and their combination induced cognitive impairments. Combination of the drugs caused significant ($P < 0.05$) memory impairment. Locomotion of the rats administered CBZ decreased significantly at weeks 5, 7 ($P < 0.01$) and 2 ($P < 0.05$) when compared to week 1 of therapy, but increased at weeks 6 and 8 compared with week 1. The group administered CBZ+PHE had decreased locomotion at weeks 2, 5 ($P < 0.05$) and 8 ($P < 0.01$) when compared with week 1 and increased at weeks 4, 7 ($P < 0.05$) compared with week 1 also, at week 4 ($P < 0.01$) compared with week 3. Rearing activity reduced in the CBZ-treated group at weeks 4, 7 ($P < 0.05$) and week 5 ($P < 0.01$) compared to week 1. A significant ($P < 0.05$)

increase was observed at week 5 and a decrease ($P < 0.05$) at week 7 when the two weeks were compared to week 1 with the administration of the drug combination. There was no significant ($P < 0.05$) change in the value of the PCV and Hb but the RBC and platelets decreased in all the drug-treated groups. Leucocytosis, lymhpocytosis and neutrophilia were recorded in all the rats treated with either or combination of CBZ and PHE. Increased Na^+ and decreased K^+ were observed in the drug-treated groups, whereas Cl^- remained unaltered. Generally, there were increases in serum proteins, albumin and urea as globulin decreased. There was a significant increase in ALT activities in the CBZ and CBZ+PHE groups and an increase in AST activity in the PHE group. In conclusion, the administration of CBZ, PHE and their combination affect cognitive, neurobehaviour, serum biochemical and haematologic parameters. It is recommended that serum biochemical and haematological parameters must be observed and evaluated for all individuals undergoing therapy with CBZ, PHE or their combination as their effects are deleterious with use of over 8 weeks.

TABLE OF CONTENTS

TITLE PAGE - - - - - - - - - -

DECLARATION - - - - - - - - -i

CERTIFICATION - - - - - - - - -ii

DEDICATION - - - - - - - - -iii

ACKNOWLEDGEMENT - - - - - - - -iv

ABSTRACT - - - - - - - - - -v

TABLE OF CONTENTS - - - - - - - -vii

LIST OF FIGURES - - - - - - - - -x

CHAPTER 1 - - - -- - - - - -1

1.0 INTRODUCTION - - - - - - - -1

1.1 Statement of Research Problem - -- - - - -5

1.2 Justification - - - - - - - - -5

1.3 General Aim of Study - - - - - - - -6

1.4 Specific Objectives of Study - - - - - - -6

1.5 Statement of Research Hypothesis - - - - - -7

CHAPTER 2 - - - - - - - - - -8

2.0 LITERATURE REVIEW - - - - - - -8

2.1 Historical Perspective - - - - - - - -8

2.2 Types of Epilepsy - - - - - - - -8

2.2.1 Primary Epilepsy - - - - - - - -9

2.2.2 Secondary Epilepsy - - - - - - - -9

2.3 Types of Seizures - - - - - - - -10

2.3.1 Generalized Seizure - - - - - - - -10

2.3.2 Petit Mal Seizure - - - - - - - -11

2.3.3 Simple Partial Seizure - - - - - - - -11

2.3.4 Complex Partial Seizure - - - - - - -12

2.3.5 Status Epilepticus - - - - - - - -13

2.4 Pathophysiology of Epilepsy - - - - - -13

2.5 Molecular Basis of Epilepsy - - - - - - -14

2.6 Causes of Epilepsy - - - - - - - -15
2.7 Symptoms of Epilepsy - - - - - - -16
2.8 Seizure Threshold in Epilepsy - - - - - -16
2.9 Stages of a Seizure - - - - - - - -18
2.10 Diagnosis - - - - - - - - -19
2.11 Therapy - - - - - -- - - -19
2.12 Therapeutic Agents Used in Epilepsy- - -- - - -20
2.12.1 Antiepileptic Drugs - - - - -- - - -22
2.12.2 Phenobarbital - - - - - - - - -22
2.12.3 Phenytoin - - - - - - - - -24
2.12.4 Carbamazepine - - - - - - - -26
2.12.5 Levetiracetam - - - - - - - - -30
2.12.6 Valproic Acid - - - - - -- - - -31
2.13 Rational Polypharmacy of Antiepileptic Drugs - - - -32
2.13.1 Role of Rational Polypharmacy in the Treatment of Refractory Epilepsy -32
2.13.2 Antiepileptic Drugs used in Rational Polypharmacy - - - -33
2.14 Processes in the Treatment of Epilepsy - - - - -33
CHAPTER 3 - - - - - - - - - -35
3.0 MATERIALS AND METHODS - - - -- - -35
3.1 Animals - - - - - -- - - -35
3.1.1 Anticonvulsant Drugs - - - - -- - - -35
3.2 Experimental Protocol - - - -- - - -35
3.2.1 Evaluation of Motor Activity - - - - - - -36
3.2.2 Evaluation of Rearing Activity - - -- - - -36
3.2.3 Assessment of Learning - - -- - - -37
3.2.4 Assessment of Memory - - - - - -37
3.3 Evaluation of Weight - - - - -- - - -38
3.4 Evaluation of the Effect on Haematological Parameters - - -38
3.4.1 Determination of Packed Cell Volume - - -- - -38
3.4.2 Determination of Total Red Blood Cell Count - -- - -39

3.4.3 Determination of Total and Differential Leucocyte Count -- - -39
3.4.4 Determination of Haemoglobin Concentration - -- - -39
3.5 Serum Biochemical Examination - - - -- - -40
3.6 Statistical Analysis - - - - - -- - -41
CHAPTER 4 - - - - - - - -- - -42
4.0 RESULTS - - - - - - -- - -42
4.1 Open Field Test - - - - - - - -42
4.1.1 Effect of Treatments on Locomotion in Wistar Rats - -- - -42
4.1.2 Effect of Treatments on Rearing in Wistar Rats - - - -44
4.2 Cognition: Learning and Memory - - - -- - -46
4.2.1 Effect of Treatments on Learning Ability in Wistar Rats -- - -46
4.2.2 Effects of Treatments on Short-term Memory in Wistar Rats -- -48
4.3 Effects of Treatments on Body Weights in Wistar Rats - - -50
4.4 Effects of Treatments on Haematological Parameters - - -52
4.4.1 Effects of Treatments on Packed Cell Volume in Wistar Rats -- -52
4.4.2 Effects of Treatments on Red Blood Cell Counts in Wistar Rats -- -54
4.4.3 Effects of Treatments on Haemoglobin Concentration in Wistar Rats -56
4.4.4 Effects of Treatments on Platelet Counts in Wistar Rats - - -58
4.4.5 Effects of Treatments on Total Leucocyte Counts in Wistar Rats -- -60
4.4.6 Effects of Treatments on Neutrophil Counts in Wistar Rats - -- -62
4.4.7 Effects of Treatments on Lymphocyte Counts in Wistar Rats -- -62
4.5 Effects of Treatments on Serum Electrolytes - - - - -64
4.5.1 Effects of Treatments on Sodium ion Concentration - - -- -64
4.5.2 Effects of Treatments on Potassium ion Concentration -- -64
4.5.3 Effects of Treatments on Chloride ion Concentration - -- -64
4.6 Effects of Treatments on Serum Proteins - - - - -66
4.6.1 Effects of Treatments on Serum Total Proteins - - -- -66
4.6.2 Effects of Treatments on Serum Albumin Concentration - -- -66
4.6.3 Effects of Treatments on Serum Globulin Concentration - -- -66
4.7 Effects of Treatments on Urea Concentration - -- - -68

4.8 Effects of Treatments on Liver Enzyme Activities - -- - -70
4.8.1 Effects of Treatments on Alanine aminotransferase - -- - -70
4.8.2 Effects of Treatments on Aspartate aminotransferase -- - -70
4.8.3 Effects of Treatments on Alkaline Phosphatase - -- - -70
4.8.4 Effects of Treatments on Lactate Dehydrogenase - -- - -71
CHAPTER 5 - - - - - - - -- - -75
5.0 DISCUSSION - - - - - -- - -75
CHAPTER 6 - - - - - - - -- - -86
6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATION -- -86
6.1 Summary - - - - - - -- - -86
6.2 Conclusion - - - - - - - - -88
6.3 Recommendation - - - - - - - -88
7.0 REFERENCES - - - - - -- - -89
7.1 APPENDICES - - - - - - -- - 101

LIST OF FIGURES

Figure	Title	Page
4.1	Effects of Administration of Carbamazepine and/or Phenytoin on Locomotion in Wistar Rats	-43
4.2	Effects of Administration of Carbamazepine and/or Phenytoin on Rearing in Wistar Rats	-45
4.3	Effects of Administration of Carbamazepine and/or Phenytoin on Learning in Wistar Rats	-47
4.4	Effects of Administration of Carbamazepine and/or Phenytoin on Memory in Wistar Rats	-49
4.5	Effects of Administration of Carbamazepine and/or Phenytoin on Body Weights	-51
4.6	Effects of Administration of Carbamazepine and/or Phenytoin on Packed Cell Volume	-53
4.7	Effects of Administration of Carbamazepine and/or Phenytoin on Red Blood Cell Counts in Wistar Rats	-55
4.8	Effects of Administration of Carbamazepine and/or Phenytoin on Haemoglobin Concentration in Wistar Rats	-57
4.9	Effects of Administration of Carbamazepine and/or Phenytoin on Platelet Counts in Wistar Rats	-59
4.10	Effects of Administration of Carbamazepine and/or Phenytoin on Total Leucocyte Counts on Wistar Rats	-61
4.11	Effects of Administration of Carbamazepine and/or Phenytoin on Neutrophil Counts	-63
4.11	Effects of Administration of Carbamazepine and/or Phenytoin on Lymphocyte Counts in Wistar Rats	-63
4.12	Effects of Administration of Carbamazepine and/or Phenytoin on Sodium ion Concentrations in Wistar Rats	-65

4.12 Effects of Administration of Carbamazepine and/or Phenytoin on Potassium ion Concentration in Wistar Rats -- - - -65

4.12 Effects of Administration of Carbamazepine and/or Phenytoin on Chloride ion Concentration in Wistar Rats -- - - -65

Figure **Title**

Page

4.13 Effects of Administration of Carbamazepine and/or Phenytoin on Serum Proteins in Wistar Rats - - -- - - -67

4.13 Effects of Administration of Carbamazepine and/or Phenytoin on Albumin Concentration in Wistar Rats - -- - - -67

4.13 Effects of Administration of Carbamazepine and/or Phenytoin on Globulin Concentration in Wistar Rats - -- - - -66

4.14 Effects of Administration of Carbamazepine and/or Phenytoin on Urea Concentration in Wistar Rats -- - - - -69

4.15 Effects of Administration of Carbamazepine and/or Phenytoin on Alanine aminotransferase in Wistar Rats - -- - - -72

4.15 Effects of Administration of Carbamazepine and/or Phenytoin on Aspartate aminotransferase in Wistar Rats - - - -72

4.15 Effects of Administration of Carbamazepine and/or Phenytoin on Alkaline Phosphatase in Wistar Rats - - - - -72

4.15 Effects of Administration of Carbamazepine and/or Phenytoin on Lactate Dehydrogenase in Wistar Rats - -- - - -72

CHAPTER 1

1.0 INTODUCTION

Subtle impairment of cognitive function and behaviour occur with modest or therapeutic levels of phenytoin, valproate, phenobarbitone and carbamazepine (Balakrishnan *et al*., 1998). More recently, it was reported that rather than being overtly manifest, subtle changes in cognitive and psychomotor function do occur commonly with long-term antiepileptic drug therapy, especially phenytoin (Meador *et al.,* 1991). The complex relationship between fits, cognitive impairment, psychosocial difficulties and underlying cerebral pathology has been the subject of several recent investigations. There has been growing body of evidence that a fifth factor, the presence of antiepileptic drugs in the brain, contributes independently to disruption of intellectual functioning (Gillham *et al*., 1988).

The International League against Epilepsy (ILEA) and the International Bureau for Epilepsy (IBE) have defined epilepsy as a disorder of the brain, characterized by an enduring predisposition to generate seizures. It also includes neurobiologic, cognitive, psychological and social consequences of the condition. The definition of epilepsy requires the occurrence of at least one epileptic seizure (Fisher *et al.,* 2005). Seizures result from paroxysmal and excessive electroneuronal discharges in the brain that cause a variety of clinical manifestations (Anon, 2008a).

The term ‘epilepsy’ is usually restricted to those cases with a tendency for recurrent seizures (Nair, 2003). Seizures occur in many pure bred dogs, baboons and other species

(Anon, 2004a). Epilepsy is found in all breeds of dogs. Susceptibility is high in certain breeds, where there is a genetic predisposition. Tervueren is a breed of dog with a genetic predisposition to epilepsy (Oliver and Michael, 1993). Other breeds of dogs with high incidence of seizure are Beagle, Dachshund, German Shepherd, Keeshond, Boxers, Cockerspaniels, Collies, Golden Retrievers, Irish Setters, Labrador Retrievers, Miniature Schnauzers, Poodles, Saint Bernard, Siberian Huskies and Wire-Haired Terriers (Oliver and Michael, 1993). The prevalence of epilepsy in dog population in the United State has been estimated at 0.5-5.7% (Cunningham and Farnbach, 1988; Koestner, 1989).

Seizures can be severe, frequent and may occur in clusters (several times in a day) or progress to the life-threatening state of *status epilepticus* and in extreme cases where seizures cannot be controlled, a veterinarian may advise that the animal be euthanised (Marcinczyk, 1995). Although epilepsy presents itself at any age, the incidence and prevalence is highest in the very young and the elderly and causes differ widely depending on the age of manifestation (Nair, 2003). Zhou *et al*. (2007) reported that patients with epilepsy are at substantial risk of memory impairment, and results obtained from animal studies have demonstrated impaired hippocampal function as measured by spatial memory in rodents subjected to seizures. Chronic persistent dysfunction of limbic circuits, characteristic of epilepsy may also impair memory even in the absence of neuronal injury and seizures (Zhou *et al*., 2007); therefore, memory impairment is one of the neurobehavioural complications associated with epilepsy (Ali *et al*., 2003).

Epilepsy itself and its therapy are often accompanied by severe neurological symptoms such as drowsiness, ataxia, impairment of learning and memory (Balakrishnan *et al*.,

1998). Kšerk *et al*. (1998) reported that phenytoin affects the direct activation of the motor system by stimulation of the sensorimotor cortex in adult, but not in immature rats. Antiepileptic drugs are designed to modify the structures involved in the development of a seizure, including neurones, ion channels, receptors, glia and inhibitory or excitatory synapses. The processes are modified to favour inhibition over excitation in order to arrest or prevent seizure activity (Anon, 2004b). An ideal antiepileptic drug suppresses all seizures without causing any side-effects. Unfortunately, the drugs currently used not only fail to control seizure activity in some patients, but frequently cause side-effects that range from aplastic anaemia to hepatic failure (Anon, 2008b). However, recent findings indicate that rats with focal onset of spontaneous seizures respond to treatment following antiepileptic drug administration. Like in humans, the response to antiepileptic drugs can vary substantially between animals (e.g. dogs) (Nissinen and Pitkanen, 2007).

It has been established that polytherapy gives an improved control of epilepsy (Pearse, 1990; Sisson *et al*., 1990; LeCouteur and Child, 1998). Rational polypharmacy of antiepileptic drugs is one of the treatment strategies for refractory epilepsy (Sun *et al*., 2002). For example, a combination of carbamazepine and valproate has been tested in mice in different ratios and was more beneficial at a ratio of 1:20 in mice than when each of the drugs was administered (Sun *et al*., 2002). The observation that combination of antiepileptic drugs induces more pronounced neurotoxic effects offers one possible explanation for the increased risk of cognitive impairment associated with antiepileptic polytherapy (Zhan, 1998). However, the effects of some of the drug combinations on

neurobehavioural, haematological and biochemical changes have not been elucidated. Phenytoin, carbamazepine and phenobarbitone are the first line antiepileptic drugs. Despite the availability of newer antiepileptic drugs e.g. gabapentin, the first line drugs are commonly used because of their efficacy and low cost (Abbondanzo *et al*., 1995; De Vriese *et al.*, 1995). Carbamazepine is an anticonvulsant used to treat epilepsy and mood disorders (Almgren *et al*., 2008). It is administered alone or in combination with other medications to treat certain types of seizures in patients with epilepsy (Parks-Veal, 2008). Its main function is reduction of sustained repetitive firing in neurones by blocking voltage-gated sodium channels (Macdonald and Meldrum, 1995). It also potentiates gamma- aminobutyric acid (GABA) receptors (Granger *et al.,* 1995). Thus, carbamazepine exerts therapeutic effects via inhibition of brain neuronal activities. The drug is widely used in Nigeria for the treatment of seizure disorders and trigeminal and other neuralgias (Salawu and Danburam, 2007).

Phenytoin sodium is one of the classical antiepileptic drugs (Kšerk *et al.,* 1998). It acts by blocking sodium channels and inhibits persistent sodium currents in neurones, thus inhibiting neuronal firing in the brain (McCleane, 1999; Bryan and Waxman, 2005). It has also been shown to protect axons within white matter, subjected to anoxia (Fern *et al.,* 1993). None of the new antiepileptic drugs is superior in efficacy to the older drugs in terms of seizure remission (Schmidt, 2011).

1.1 STATEMENT OF RESEARCH PROBLEM

Epilepsy is a disorder that affects the nervous system of both humans and animals and its treatment or management is of great importance. Antiepileptic drugs have been found to exert some neurological and cognitive effects as well as some haematological and serum biochemical changes. Epilepsy was not induced in the rats used for this study so as to prevent an overlap between the adverse effects caused by epilepsy and those caused by the AEDs.

1.2 JUSTIFICATION

Dogs proof to be best friends for centuries now, and have served in man companionship and security. They have assumed economic importance in the provision of these functions and from breeding in Nigeria and many countries. Any disease or disorder that affects the optimal performance of dogs may create emotional and economic discomfort to the owner. Thus, many clients will still prefer to suppress canine epilepsy with drugs or even surgical procedures rather than euthanasia. The current management strategy of canine and even human epilepsy, apparently, involves a combination of two or more drugs to increase efficacy and reduce toxicity (Zhou *et al.,* 2008). However, the neurobehavioural and cognitive changes associated with prolonged exposure to these drugs have not been evaluated. Similarly, the effects of the drugs combination on haematological and biochemical parameters have not been elucidated. The role of spontaneous seizures and hippocampal injury in the mechanism underlying memory

deterioration may be investigated by examining how the elimination of spontaneous seizures by anticonvulsant treatment affects memory performance (Zhou *et al.,* 2008).

1.3 GENERAL AIM OF THE STUDY

The general aim of the study evaluated the effects of administration per os of CBZ, PHE and of a combination of CBZ and PHE on neurobehavioural pattern, cognitive function and haematological and biochemical changes in apparently healthy adult male Albino rats.

1.4 SPECIFIC OBJECTIVES

The objectives of the present study were to:

i) Evaluate the neurobehavioural patterns such as locomotion and rearing activities that may arise as a result prolonged exposure to CBZ, PHE and combination on male adult Albino rats

ii) Determine the haematological alterations that may accompany the use of these drugs in male adult Albino rats

iii) Evaluate the effects of administration of CBZ and PHE and CBZ+PHE combination on some serum biochemical parameters in male adult Albino rats.

1.5 STATEMENT OF RESEARCH HYPOTHESIS

The administration of phenytoin, carbamazepine and a combination of CBZ and PHE have no effect on neurobehavioural, cognitive, heamatological and serum biochemical parameters in adult male Albino rats.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 HISTORICAL PERSPECTIVES

In imperial Rome, epileptics were encouraged to enter the coliseum and drink the blood of wounded gladiators. This medical "cure" was thought to be effective in banishing the seizures that caused epileptics to be feared and shunned by other citizens (Anon, 2004a). Throughout the middle ages, epilepsy was believed to be an infectious disease, and epileptics were routinely confined to insane asylums during the 18th and 19th centuries. Even as late as 1933, epileptic inmates of U.S mental health institutions were forcibly sterilized in an erroneous attempt to prevent them from passing their genes to their children (Anon, 2004a).

2.2 TYPES OF EPILEPSY

Epilepsy is characterized by unprovoked seizures, canine epilepsy is often genetic but epilepsy in other pets is rare because there is no hereditary component to epilepsy in these animals (Anon, 2004b). The characteristic event in epilepsy is the seizure, which is associated with the episodic high frequency discharge of impulses by a group of neurones (Rang *et al.,* 2005). More than 40 distinct forms of epilepsy have been identified; epileptic seizures often cause transient impairment of consciousness leaving the individual at risk of bodily harm (McNamara, 2006).

*Anon = anonymous

2.2.1 Primary (Idiopathic, Genetic, Inherited or True) Epilepsy

There are no diagnostic findings that will substantiate this type of epilepsy. It is a case of ruling out every other possibility. The first seizure in dogs with primary epilepsy usually occurs between the ages of 6 months and 5 years (Oliver and Michael, 1993). However, the diagnosis of primary epilepsy is not a proof of a genetic defect; only careful breeding studies could prove that; the breed, age and history may suggest a genetic basis for primary epilepsy, if there is a familial history of seizures (Marcinczyk, 1995). According to Michelle (2010), the following tests are advised before a diagnosis of idiopathic/inherited epilepsy is made: glucose tolerance test; to check for hypoglycaemia, thyroid panel 6 tests, to determine low thyroid function/hypothyroidism. Electroencephalograph (EEG) is used to evaluate findings that may be suggestive of a lesion. Cerebrospinal fluid (CSF) analysis is used to evaluate encephalitis, distemper and other infections. Blood test is also conducted to determine lead (Pb) poisoning and cerebral tomography scan or magnetic resonance imaging (MRI) is used to detect brain lesion.

2.2.2 Secondary Epilepsy

This refers to seizures for which a cause can be determined. In dogs less than one year of age, the common causes of seizures are classified as: degenerative (storage diseases); developmental (hydrocephalus); toxic (lead, arsenic, organophosphates, chlorinated hydrocarbons, strychnine, and tetanus); infectious (distemper, encephalitis); metabolic (transient hypoglycaemia, enzyme deficiency, liver or kidney failure); nutritional

(thiamine deficiency, parasitism); and traumatic (acute injury). In dogs 1-3 years of age, a genetic factor is most highly suspected. In dogs 4 years of age and older, seizures are most commonly found in the metabolic (hypoglycaemia, cardiovascular arrhythmia, hypocalcaemia, cirrhosis) and neoplastic (brain tumour) classes (Oliver and Michael, 1993). Seizures are also associated with hypothyroidism with a familial (inherited) autoimmune disease of purebred dogs (Marcinczyk, 1995).

2.3 TYPES OF SEIZURES

Seizures occur when there is a sudden change in the normal way the brain cells communicate through electrical signals (Anon, 2008a). Seizures can be non-epileptic, when evoked in a normal brain by treatments such as electroshock, chemical convulsants or epileptic, when occurring without evident provocation (Rang *et al.,* 2005).

The diversity of symptoms that can result from an epileptic seizure arises from the differing brain regions. The regions, when deprived of their function, give rise to the particular features of an individual seizure, which range from a brief lapse of attention to a full convulsive fit, lasting several minutes, with odd sensations or behaviours (Nair, 2003; Rang *et al*., 2005).

2.3.1 Generalized Seizures: Tonic-clonic.

These involve the whole brain, including the reticular system, thus producing abnormal electrical activity throughout both hemispheres (Rang *et al.,* 2005). There are two important phases in generalized seizures; the tonic-clonic phase (*grand mal*) and the

absence seizure phase (*petit mal*). In *grand mal* seizure, the tonic phase occurs as the animal falls, loses consciousness and extends its limbs rigidly. Respiration also stops (apnoea). This phase usually lasts for 10-30 seconds before the clonic phase begins (Marcinczyk, 1995). Clonic movements include paddling of the limbs and/or chewing. Other signs that appear during the tonic or clonic phase are dilation of the pupils, salivation, urination and defaecation. The electroencephalograph tracings in *grand mal* seizure exhibit high amplitude and erratic pattern lasting several minutes. The mild seizure involves little or no paddling or extension of the limbs and usually no loss of consciousness. Generalized seizures are usually associated with primary epilepsy (Marcinczyk, 1995).

2.3.2 *Petit Mal* Seizure (Absence Seizure)

Petit mals are described as either very rare or usually unrecognized in animals. Signs are brief (seconds) duration of unconsciousness, loss of muscle tone, blank stare and possibly upward rotation of the eyes (Marcinczyk, 1995). According to Kay (1989), the term *petit mal* is misused by veterinarians and should only be accorded to cases manifesting very specific clinical signs and electroencephalographic abnormalities.

2.3.3 Simple Partial Seizures

Signs and symptoms of simple partial seizures depend on the location of the seizure focus (Anon, 2009d). Symptoms include involuntary muscle contractions, abnormal sensory experiences or autonomic discharge or affects mood and behaviour (psychomotor

epilepsy) (Rang *et al*., 2005). Movements are restricted to one area of the body, such as muscle jerking, movement of one limb, turning the head or bending the trunk to one side or facial twitches. A partial seizure can progress to (and be mistaken for) a generalized tonic-clonic seizure, but the difference can be established by noting whether or not a seizure starts with one specific area of the body. Partial seizures are usually associated with secondary epilepsy (Marcinczyk, 1995).

2.3.4 Complex Partial Seizures (Psychomotor or Behavioural)

Psychomotor or behavioural seizures are associated with bizarre or complex behaviours that are repeated during each seizure (Marcinczyk, 1995). All patients with complex partial seizures have impaired consciousness; the patient either does not respond to commands or respond in an abnormally slow manner (Anon, 2009d). People with complex partial seizure experience distortions in thoughts, perception or emotion (usually fear); sometimes with unusual visual, olfactory, auditory and gustatory sensations. In dogs the same experience may explain the lip-smacking, chewing, fly biting, aggression, vocalisation, hysterical running, cowering or hiding in otherwise normal animals. These abnormal behaviours may last for minutes or hours and can be followed by a generalized seizure. Complex partial seizures are usually associated with secondary epilepsy (Marcinczyk, 1995).

2.3.5 *Status Epilepticus*

This can occur as one continuous seizure lasting 10 minutes or more, or a series of multiple seizures in a short time with no period of normal consciousness, which may be life-threatening (Mitchelle, 2010). It can be difficult to differentiate *status epilepticus* from frequent cluster seizures, but both are considered life-threatening emergencies (Marcinczyk, 1995). Most *status epilepticus* patients usually suffer from generalized tonic-clonic seizures. Though *status epilepticus* can occur with either primary or secondary epilepsy, it may also suddenly arise in dogs with no previous history of seizures, especially if it is related to traumatic brain injury, toxins or diseases (Dyer and Shell, 1993).

2.4 PATHOPHYSIOLOGY OF EPILEPSY

Epilepsy encompasses a group of syndromes that vary in its associated pathology and seizure types (Nair, 2003). It may be associated with enhanced excitatory amino acid transmission, impaired inhibitory transmission or abnormal electrical properties of the affected cells (Rang *et al*., 2005). 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 (Anon, 2008b). Two sets of changes determine the epileptogenic properties of neuronal tissues; abnormal neuronal excitability is thought to occur as a result of disruption of the depolarisation and repolarisation mechanisms of the cell (Nair, 2003). Hyperexcitability of the neurones which results in random firing of cells may not by itself lead to propagation of an

epileptic seizure. Indeed, both normal and abnormal patterns of behaviour require a certain degree of synchronisation of firing in a population of neurones (Nair, 2003). Seizures can be triggered in any individual under certain conditions such as life-threatening dehydration or high temperature (Anon, 2008a).

Other types of seizures not classified as epilepsy include those caused by an imbalance of body fluids or chemicals or by alcohol or drug withdrawal. A single seizure does not mean the individual has epilepsy (Anon, 2008a). Thus, epileptic seizures originate in a setting of both altered excitability and altered synchronisation of neurones (Nair, 2003).

2.5 MOLECULAR BASIS OF EPILEPSY

Enormous progress has been made in understanding the genetics of mammalian epilepsy. Mutant genes have been identified for a number of symptomatic epilepsies, in which there seems to be a manifestation of some profound neurodegenerative disease because most patients with epilepsy are neurologically normal (Schaffer and Berkovic, 2003). Elucidating the mutant genes underlying familial epilepsy in otherwise normal individuals is of particular interest. This has led to a successful identification of eleven distinct genes implicated in distinct, though rare, idiopathic epileptic syndromes that account for less than 1% of all human epilepsies (Schaffer and Berkovic, 2003). Genetic causes contribute to a wide diversity of human epilepsies. They are solely responsible for some rare forms inherited in an autosomal dorminant or recessive manner. Genetic causes are also mainly responsible for some more common forms such as juvenile myoclonic

epilepsy or childhood absence epilepsy. Genetic determinants may also contribute some degree of risk to epilepsies caused by injury to the cerebral cortex (McNamara, 2006). Interestingly, almost the entire mutant gene encodes ion channels that are gated by voltage or ligands (Schaffer and Berkovic, 2003).

Rapid development in molecular biologic techniques for the study of the neurophysiology of epilepsy and the neurotransmitters, which are the target for the antiepileptic drugs, may also provide better insight into the interactions of antiepileptics with either ion channels or brain receptors (Emilien and Maloteaux, 1998). Mutation has been identified in voltage-gated sodium and potassium channels and in channels gated by γ-aminobutyric acid (GABA) and acetylcholine (Ptacek, 1997). The cellular electrophysiological consequences of some of the mutant genes exhibit an intriguing relationship to mechanisms of seizures and antiseizure drugs. For instance, generalized epilepsy with febrile seizures is caused by a point mutation in the β-subunit of a voltage-gated sodium channel (Wallace *et al*., 1998).

2.6 CAUSES OF EPILEPSY

Disruptions of GABAergic neurotransmission have been implicated in numerous central nervous system disorders, including epilepsy, depression, bipolar disorder and neuropathic pain (Smith-Yockman *et al.,* 2005). The aetiology commonly consists of a lesion in some parts of the cortex such as a tumour, developmental malformation, and damage due to trauma or stroke. Such lesions are often evident on brain magnetic resonance imaging. Alternatively, the aetiology may be genetic (McNamara, 2006). The

common causes of epilepsy include: injury to baby during delivery, infections of the brain; meningitis, encephalitis, hydrocephalus, delay in delivery with decreased oxygen supply to the brain; and drugs such as penicillin, chloroquine, and drugs used in the treatment of depression and angina (Anon, 2008a).

2.7 SYMPTOMS OF EPILEPSY

The particular symptoms produced depend on the function of the region of the brain that is affected. Thus, involvement of motor cortex causes convulsions; involvement of the hypothalamus evokes peripheral autonomic discharge and involvement of the reticular formation in the upper brain stem leads to a loss of consciousness (Rang *et al*., 2005). Some symptoms of epilepsy include fainting, memory loss, changes in mood or energy level, headache and confusion (Anon, 2008a). Disorders that can be confused with epilepsy include: migraine, syncope, transient ischaemic attacks, non-epileptic events (pseudoseizures), movement disorders, Maniere's disease, and rage attacks (Browne and Holmes, 2001).

2.8 SEIZURE THRESHOLD IN EPILEPSY

It is suggested that each animal inherits a genetically determined predisposition to seizure and that seizure occurs when this threshold is exceeded, that is a physical condition which may cause seizure in a low-threshold animal may not cause seizure in a normal animal (Cunningham and Farnbach, 1988).

Seizure threshold is, apparently, exceptionally low in animals that suffer from idiopathic (primary) epilepsy (Marcinczyk, 1995). An animal's threshold can also be altered by other means. For example, certain types of tranquillizers (for example, acepromazine) may induce seizures in an animal with low threshold. Medically, condition of alkalosis is reported to decrease the threshold to seizure (Shell, 1993a).

The mirror focus phenomenon is also important in seizure activity. Each hemisphere of the brain is a mirror image of the other. A seizure focus on one side of the brain will show itself as abnormal wave forms on the state in full EEG recordings. Within a period of weeks, the "normal" side of the brain will start to show similar EEG abnormalities. In time, the mirror focus becomes capable of causing seizure activity on its own. Therefore, repetitive uncontrolled seizures also lower the seizure threshold in any given animal. That is why early intervention is very important in the control of seizure (Marcinczyk, 1995).

Dyer and Shell (1993) reported that repetitive seizures can irreversibly lower threshold in a process called kindling. Kindling is described as a mechanism in which epileptic neurones in the brain 'recruit' normal neurones into the original seizure focus, enlarging the area of the brain that can produce seizure (Fenner and Haas, 1989). Quite often, an epileptic patient suffers from neurobehavioural problems such as impaired memory, depression, which may have pathological and/or iatrogenic basis. Patients with epilepsy are at substantial risk of memory impairment. Animal studies have paralleled these clinical observations, demonstrating impaired hippocampal function (Zhou *et al.,* 2007). A number of mechanisms may contribute to the disruption of memory function in

epilepsy patients. One commonly cited reason for memory function impairment is hippocampal neuronal cell loss that is due both to the precipitating insult; for example, *status epilepticus* or brain trauma, and recurrent seizures (Rang *et al.,* 2005). The understanding of the nature of memory deficits associated with epilepsy is important because it would define the therapeutic approaches to their management (Porter and Meldrum, 2007).

2.9 STAGES OF SEIZURE

There are four basic stages of a seizure which include: 1) prodrome; 2) the aura or preictus, 3) the ictus or seizure stages; and 4) postictus.

1) Prodrome - It is characterised by a change in mood or behaviour (Mitchelle, 2010). Human epileptics experience mood changes, headaches, insomnia or feelings about an impending seizure. It is not known if animals experience a prodrome, except for any behavioural changes observed by the owners (Shell, 1993b). This may precede the actual seizure by hours or days.

2) The aura - This signals the start of the seizure. Signs include restlessness, nervousness, whining, trembling, salivation, affection, wandering, hiding, hysterical running and apprehension (Mitchelle, 2010).

3) The ictus – This is the actual seizure, characterized by increased tone of all muscle groups, it is either tonic or tonic-clonic (Kay, 1989; Oliver and Michael, 1993). It is a period of intense physical activity, lasting 45 seconds to 3 minutes. A dog may lose consciousness and fall to the ground. There may be teeth gnashing, frantic thrashing of

limbs, excessive drooling salivation, vocalisation, paddling of feet, uncontrollable urination and defaecation (Mitchelle, 2010).

4) The postictus – This may be the only sign of epilepsy seen by the owner, particularly since many seizures occur at night or early in the morning (Shell 1993b; Oliver and Michael, 1993). After the seizure, the dog may pace endlessly, appear blind and deaf, and eat and drink excessively (Mitchelle, 2010).

2.10 DIAGNOSIS

The term epilepsy encompasses a group of syndromes that vary in its associated pathology and seizure types. The diagnosis of the epileptic syndrome is one of the primary objectives undertaken when managing a patient with seizures (Nair, 2003). The initial evaluation in patients who present with spells or seizures is to determine whether the episodes are epileptic in nature (Browne and Holmes, 2001). A false diagnosis can have severe repercussions for the patient, including the expense of medications and their potential adverse effects. The EEG helps to confirm the diagnosis of epilepsy and provides information regarding the epileptic syndrome and, in focal epilepsies, the location of the seizure focus (Browne and Holmes, 2001).

2.11 THERAPY

Once epileptic seizures have been diagnosed, the determination of the epileptic syndrome follows and then the seizure type. This is important in selecting appropriate medication and evaluating patients for surgical treatment (Nair, 2003). The first issue that arises is

whether and when to initiate treatment, for instance, it may not be necessary to initiate antiseizure therapy after an isolated tonic-clonic seizure in a healthy young adult, who lacks a familial history of epilepsy and who has a normal neurological examination, a normal EEG and a normal magnetic resonance imaging scan. That is the odds of seizure recurrence in the next year (15%) approximate the risk of a drug reaction sufficiently severe to warrant discontinuation of medication (Bazil and Pedley, 1998). The choice of an antiepileptic drug for any individual should take into cognizance information about seizure control, adverse effects and cost (Gamble *et al*., 2009).

2.12 THERAPEUTIC AGENTS USED IN EPILEPSY

Therapy is symptomatic because the available drugs only inhibit seizure, and neither effective prophylaxis nor cure is available (McNamara, 2006). Once a drug has been shown to be effective in preventing seizures in an animal model, the side-effects of the drug is considered and extent of the drug interference with motor coordination or memory is determined. The dose that will prevent seizures with the lowest incidence of side-effects is administered (Anon, 2004a).

2.12.1 Antiepileptic Drugs

The term antiepileptic is used synonymously with anticonvulsant to describe drugs that are used to treat “epilepsy” (which does not necessarily cause convulsions) as well as “non-epileptic” convulsive disorders (Rang *et al*., 2005). For the first time, it was assumed that a single drug could be developed for the treatment of all forms of epilepsy,

but the causes of epilepsy are extremely diverse, encompassing genetic and developmental defects, traumatic, neoplastic and degenerative disease processes (Porter and Meldrum, 2007). In 1857, Sir Charles Lolock evaluated the use of potassium bromide to control aberrant behaviours in the mentally ill; it later became the first antiepileptic drug used for the treatment of seizures due to its unexpected anticonvulsant properties (Parks-Veal, 2000). The introduction of bromides for the treatment of epilepsy was followed first by phenobarbital, the first synthetic organic agent having antiseizure activity, which was accidentally discovered in 1921, and then by phenytoin as therapeutic options (Parks-Veal, 2000; Jallon, 2007). Until 1990, approximately nineteen (19) antiseizure drugs are available and 13 of them can be classified into five very similar chemical groups (McNamara, 2006).

The antiepileptic drugs can be grouped according to their mechanism of action, although many have several different actions while others work through unknown mechanisms (Anon, 2008b). The chemical structures of most of the drugs introduced before 1965 were closely related to phenobarbital (McNamara, 2006). They included the hydantoins and succinimides; between 1965 and 1990, the chemically distinct structures of the benzodiazepines, an iminostilbene (carbamazepine) and a branched-chain acid (valproic acid) were introduced. This was followed in the 1990s by a phenyltriazine (lamotrigine), a cyclic analogue of GABA (gabapentine), a sulphamate-substituted monosaccharide (topiramate), a nipecotic acid derivative (tiagabine) and a pyrrolidine derivative (levetiracetam) (McNamara, 2006). These groups have in common a similar heterocyclic ring structure with a variety of substituents (Porter and Meldrum, 2007). Long established

antiepileptic drugs include phenytoin, carbamazepine and valproate, and ethusuximide, phenobarbital, together with various benzodiazepines such as diazepam, clonazepam and clozapam and the newer drugs include gabapentine, lamotrigine, felbamate, tiagabine, levetiracetam and zonisamide (Anon, 2008b).

Existing antiseizure drugs provide adequate seizure control in about two-thirds of patients, they exhibit similar pharmacokinetic properties including those with diverse structural and chemical properties because most have been selected for oral activity and all must enter the central nervous system (Porter and Meldrum, 2007). Antiepileptic drugs are known to cause a variety of adverse effects; such as idiosyncratic bone marrow suppression or dose-related bone marrow suppression or aplastic anaemia except gabapentine (Kaufman *et al.*, 1996 and Scheuer, 1996). Ashrafi *et al*., (2010) reported a decrease in immunoglobulins A and G following CBZ and PHE administration, this may cause reduced serum globulin. Antiseizure drugs are eliminated chiefly by hepatic mechanisms, although they have low extraction ratios. Many are converted to active metabolites that are also eliminated by the liver (Porter and Meldrum, 2007).

2.12.2 Phenobarbital

It was one of the first barbiturates to be developed and its antiepileptic properties were discovered in 1912 (Rang *et al.*, 2005). Although it has long been considered one of the safest of the antiseizure agents, the use of other medications with less sedative effects has been advocated (Porter and Meldrum, 2007). The mechanism by which phenobarbital

inhibit seizure may involve potentiation of synaptic inhibition of the GABA receptor. Intracellular recordings of mouse cortical or spinal cord neurones demonstrated that phenobarbital enhances responses to iontophoretically applied GABA. These effects have been observed at therapeutically relevant concentrations of phenobarbital. (Twyman *et al*., 1989). At levels exceeding therapeutic concentrations, phenobarbital also limits sustained repetitive firing; this may underlie some of the antiseizure effects of higher concentrations of phenobarbital that is achieved during therapy of *status epilepticus* (McNamara, 2006). It is assumed to act by enhancing the inhibitory processes and diminution of excitatory transmission. Recent data indicate that it may selectively suppress abnormal neurones, inhibiting the spread and suppressing firing from the epileptic foci (Porter and Meldrum, 2007). It is useful in the treatment of partial seizures and generalized tonic-clonic seizures, although it is often tried for virtually every type of seizure, especially when attacks are difficult to control (Porter and Meldrum, 2007).

Phenobarbital is well absorbed and about 50 % of the drug in the blood is bound to plasma albumin. It is eliminated slowly from the plasma (half-life, 50-140 hours) and about 25 % is excreted unchanged in the urine (Rang *et al*., 2005). The main unwanted effect of this drug is sedation, which often occurs at plasma concentrations within the therapeutic range for seizure control. Other unwanted effects that may occur with clinical dosage include osteomalacia (McNamara, 2006). Phenobarbital is contraindicated in patients with porphyria. Overdosage produces coma, respiratory and circulatory failure as do all barbiturates (Rang *et al*., 2005).

2.12.3 Phenytoin

It was first synthesized in 1908 by Biltz, but its anticonvulsant activity was not discovered until 1938 (McNamara, 2006). Phenytoin, known for decades as diphenylhydantoin is the oldest non-sedative antiseizure drug, introduced in 1938 after a systematic evaluation of compounds such as phenobarbital that altered electrically-induced seizures in laboratory animals (Porter and Meldrum, 2007). Phenytoin sodium is an anticonvulsant used to control *grand mal* and psychomotor seizures. It can cause gingival hyperplasia, agranulocytosis, aplastic anaemia and various neurological deficits when given for a long time. It produces chromosomal anomalies and increased incidence of malignant melanoma (Vijay *et al*., 2009). Systemic administration induces anticonvulsant effect in humans and experimental animals (Rykaczewska-Czerwińska, 2007). The most significant effect of phenytoin is its ability to modify the pattern of maximal electroshock seizures (McNamara, 2006).

Phenytoin blocks voltage-sensitive sodium ion channels and in this way inhibits neuronal firing in the brain (Rykaczewska-Czerwińska, 2007) and alters potassium and calcium ions conductance, membrane potentials, the concentrations of amino acids and the neurotransmitters - noradrenaline, acetylcholine and GABA (Porter and Meldrum, 2007). It limits the repetitive action potentials evoked by a sustained depolarisation of mouse spinal cord neurones maintained *in vitro*. This effect is mediated by a slowing of the rate of recovery of voltage-activated sodium ion channels from inactivation, and this action is both voltage- and use-dependent (McLean and Macdonald, 1986). Phenytoin

paradoxically causes excitation in some cerebral neurones; a reduction of calcium permeability with inhibition of calcium influx across the membrane. This may explain the ability of phenytoin to inhibit a variety of calcium-induced secretory processes, including the release of hormones and neurotransmitters (Porter and Meldrum, 2007 and Thakur *et al*., 2011a).

Absorption of phenytoin is highly dependent on the formulation of the dosage form and particle size, and pharmaceutical additives affect both the rate and the extent of absorption (Porter and Meldrum, 2007). It is well absorbed when given orally with about 80-90 % of the plasma content bound to albumin while drugs such as salicylates, phenylbutazone and valproate competitively inhibit this binding. Therefore, increasing free phenytoin concentration also increases hepatic clearance of phenytoin, thus enhancing or reducing the effect of phenytoin (Rang *et al.,* 2005). Absorption after intramuscular injection is unpredictable with some drug precipitation occurring in the muscle. This route of administration is not recommended for phenytoin, but for fosphenytoin (a phosphate pro-drug of phenytoin), which is well absorbed after intramuscular injection (Porter and Meldrum, 2007). Phenytoin is widely used, being effective against various forms of partial and generalized seizures; although not against absence seizures, which may even get worse (Rang *et al*., 2005).

Side-effects of phenytoin begin to appear at plasma concentration exceeding 100 μmol/L and may be severe above about 150 μmol/L, the milder side-effects include vertigo, ataxia, headache and nystagmus (Rang *et al*., 2005; Porter and Meldrum, 2007).

Phenytoin causes gingival hyperplasia which develops gradually, agranulocytosis, aplastic anaemia, leukemia, hirsutism and coarsening of features which may be due to increased androgen secretion, hypersensitivity reactions and various neurological deficits if given for a long period (Vijay *et al*., 2009). Thakur *et al.,* 2011b reported decrease in RBC counts by PHE and this may be due to the fact that the drug undergoes oxidative metabolism with the production of toxic arene oxide intermediate, which covalently binds with cell macromolecules, causing cytotoxic damage, bone marrow toxicity and aplastic anaemia. PHE has been shown to exert a transient inhibitory effect on antidiuretic hormone (ADH) therefore may increase sodium ion concentration (Liamis *et al*., 2009). Diphenylhydantoin has been reported to cause a more frequent and higher increase in ALT, AST and ALP than CBZ (Aldenhövel, 1988). The elimination of phenytoin is dose-dependent; at very low blood levels, phenytoin metabolism follows first-order kinetics, but as blood levels rise within the therapeutic range, the maximum capacity of the liver to metabolize phenytoin is approached. Further increases in dosage, however small, may produce very large changes in phenytoin concentrations (Porter and Meldrum, 2007).

2.12.4 Carbamazepine

Carbamazepine was discovered by a chemist Walter Schindler at J.R. Geigy AG (now part of Novartis) in Basel, Switzerland in 1953. Schindler then synthesized the drug in 1960, before its antiepileptic properties had been discovered (Schindler and Häfilger, 1954). Carbamazepine is an iminostilbene, a dibenzepine derivative that is chemically and pharmacologically related to tricyclic antidepressant agents (Bazil and Pedley, 2003).

It was approved for management of seizures in 1974, although, it had been introduced a decade earlier for the management of trigeminal neuralgia (Mattson *et al*, 1985). Carbamazepine is a highly conventionally used antiepileptic drug, which has efficacy in attenuating picrotoxin-induced convulsion (Ali *et al*., 2003). This may be attributed to its mechanism of action, that is, use dependent sodium channel blockade, weak GABAergic and antiglutamatergic effects (Motohashi, 1992).

Carbamazepine is marketed under different trade names which include; tegretol®, biston®, calepsil®, carbatrol®, epitol®, equetro®, finlepsin®, sirtal®, stazepine®, telesmin®, teril®, timonil®, trimonil®, epimaze®, carbama/carbamaze® (New Zealand), amizepine® (Poland), hermolepsin® (Sweden), and degranol® (South Africa) (Anon, 2009c). Carbamazepine has three main uses which are; prevention of mood swings in patients suffering from bipolar illness (manic depression); help in controlling fits or blackouts and to relieve the symptoms of trigeminal neuralgia (Anon, 2009c). Carbamazepine is the usual drug of choice for patients with newly diagnosed partial onset seizure (Gamble *et al*., 2009).

It acts by stabilizing inactivated sodium channels. Voltage-gated sodium channels which are the molecular pores that allow brain cells (neurones) to generate action potentials - the electrical events that allow neurones to communicate over long distances. After the sodium channels open to start the action potential, they are then inactivated, essentially closing the channels. Carbamazepine will then sustain this closure so that fewer of these channels are available to subsequently open, making brain cells less excitable (Granger *et al.,* 1995). Carbamazepine has also been shown to potentiate GABA receptors, made up

of α_1, β_2, γ_2 subunits (Granger *et al.,* 1995). Carbamazepine shows activity against maximal electroshock seizures. The rate of absorption after oral administration varies widely among patients, although almost complete absorption apparently occurs in all (Porter and Meldrum, 2007). Carbamazepine exhibits autoinduction, it induces the expression of the hepatic microsomal enzyme system, CYP3A4 which metabolizes carbamazepine itself. Upon initiation of therapy, concentrations are predictable and follow their respective base-line clearance/half life values that have been established for the specific patient (Bauer, 2008). However after enough carbamazepine has been presented to the liver, the CYP3A4 activity increases, speeding up its clearance and shortening the half life. Auto-induction will continue with subsequent increase in dose, but will reach a plateau within 5-7 days of a maintenance dose. Increase in dose of 200 mg every 1-2 weeks may be required to achieve a stable threshold. Stable carbamazepine concentrations occur within 2-3 weeks after initiation of therapy (Bauer, 2008).

Carbamazepine has a very high potential for drug interactions. Caution should be taken in combining other medicines with it, including other antiepileptics and mood stabilizers (Anon, 2009a). Lower levels of carbamazepine are used when co-administered with phenobarbital, phenytoin or primidone. Carbamazepine as a CYP_{450} inducer may increase clearance of many drugs and decreasing their blood levels (Anon, 2009b). Drugs that are more rapidly metabolized when administered with carbamazepine include warfarin, phenytoin, theophylline and valproic acid (Anon, 2009a). Drugs that decrease the metabolism of carbamazepine or otherwise increase its serum levels include

erythromycin (macrolide antibiotic), (Stafstrom *et al*., 1995), cimetidine (H_2-receptor blocker), propoxyphene (analgesic) and calcium channel blockers (such as verapamil) (Anon, 2009a). Carbamazepine also increases the metabolism of the hormones in birth control pills and can reduce their effectiveness leading to unexpected pregnancies (Anon, 2009a).

The most common dose-related adverse effects of carbamazepine are diplopia and ataxia in humans. Other side effects include mild digestive upsets and, at much higher doses, drowsiness, hyponatraemia and water intoxication have occasionally occurred (Porter and Meldrum, 2007). Carbamazepine has been reported to cause sedation which may be related to the induction of cytochrome P_{450} (Nowakowska, 2011). Luszcki (2004) observed a decrease in locomotor and rearing activities in mice administered CBZ. Carbamazepine has also been linked to serious cognitive anomalies and EEG slowing (Salinsky *et al*., 2002), Shannon and Love (2005) recorded higher errors of omission following CBZ administration relative to PHE administration, also more deterioration in memory was observed following CBZ administration than repeated dosing with PHE (Shannon and Love, 2004). Carbamazepine however, appears to have a more favourable profile than phenytoin and phenobarbital when a study was performed on cognitive effects of AEDs in humans (Ogunrin *et al.*, 2005). Haematological toxicity of CBZ is well documented; CBZ has been reported in an earlier study to cause decrease RBC counts, apparently due to isolated cessation of RBC production, as a result of pure RBC aplasia. However, McNamara (2006) reported that the prevalence of aplastic anaemia appears to be 1 in 200,000 patients treated with CBZ monotherapy. CBZ has been

reported to cause increased platelet counts which was suggested to be of the reactive type since the drug can cause inflammatory reaction, resulting in increased level of interleukin-6 and it has been proposed to induce direct stimulation of the platelet, this may have a compensatory effect in CBZ-induced thrombocytopenia (Tutor-Crespo *et al*. (2007). CBZ administration may cause increased lymphocytes; this may arise due to stimulation of formation of epoxides by the activity of cytochrome P_{450}. The epoxides bind covalently with macromolecules and act as hapten to stimulate immunologic actions, hence lymphocytosis (Gerson *et al*., 1983; Spielberg *et al*., 1986). Ashrafi *et al*. (2010) reported a decrease in immunoglobulins A and G following CBZ administration and this may cause decreased globulin concentration. Increased ALP activity following CBZ therapy was thought to be associated with an effect on bone formation possibly related to increased bone turnover (Merete *et al*., 2005). Hogan *et al*. (2000) associated adjunctive CBZ therapy with weight gain. Therefore, a patient undergoing carbamazepine therapy should be carefully monitored, especially for serious adverse reactions, including pure red cell aplasia (Tagawa *et al*., 1997).

2.12.5 Levetiracetam

Levetiracetam was used to improve cognitive function, but was accidentally found to have antiepileptic activity in animal models (Rang *et al*., 2005). A piracetam analogue that is ineffective against seizures induced by maximum electroshock or pentylenetetrazol, but has prominent activity in the kindling model (Porter and Meldrum, 2007). Levetiracetam monotherapy is a frequently chosen option in the intensive care unit

setting, particularly in neurosurgical patients (Alore *et al.*, 2005). It acts by binding selectively to a synaptic vesicular protein SV2A, and modifies the synaptic release of glutamate and GABA through an action on vesicular function (Porter and Meldrum, 2007). The drug is used for the treatment of partial seizures, its oral absorption is nearly complete and drug interaction is minimal. Plasma half-life of levetiracetam is 6-8 hours and may be longer in the elderly. Two-thirds of the drug is excreted unchanged in the urine and its adverse effects include somnolence, asthenia and dizziness. Idiosyncratic reactions are rare (Porter and Meldrum, 2007). The lack of interaction with other medications through binding or enzyme induction makes levetiracetam a particularly attractive alternative in a population of patients where the average number of medications taken on discharge is greater than nine (Alore *et al.*, 2005). Levetiracetam is effective not only as adjunctive therapy in refractory localisation-related, but also idiopathic generalized epilepsies (Pillay *et al*., 2005). Older patients and patients with previous complications related to either antiepileptic drugs or underlying disease were preferentially treated with Levetiracetam, and levetiracetam appears to be a safe alternative to other antiepileptic drugs (Meckler *et al.*, 2005).

2.12.6 Valproic Acid

It is a simple monocarboxylic acid (Rang *et al.,* 2005) which was discovered accidentally in 1963 to have anticonvulsant activity in mice, when it was used as a solvent in the search for other drugs that are effective against seizures. It was marketed in France in 1969, but was not licensed in the United States of America until 1978 (Porter and

Meldrum, 2007). It inhibits most kinds of experimentally-induced convulsions and also effective in many kinds of epilepsy, especially in absence seizures and patients with concomitant generalized tonic-clonic attacks (Rang *et al.,* 2005; Porter and Meldrum, 2007). At therapeutically relevant concentration, it inhibits sustained repetitive firing induced by depolarisation of mouse cortical or spinal cord neurones (McLean and Macdonald, 1986).

Valproate is well absorbed orally with plasma half-life of about 15 hours; and two-third of it is excreted as the glucuronide in the urine (Rang *et al.,* 2005) which may cause fulminant hepatitis that is frequently fatal (Dreifuss *et al*., 1989).

2.13 RATIONAL POLYPHARMACY OF ANTIEPILEPTIC DRUGS

Epilepsy treatment has evolved from institutionalised polytherapy to dogmatic monotherapy and most recently to rational polypharmacy (Jallon, 2007). The practice of rational polytherapy is the use of two effective antiepileptic drugs with differing modes of action, and which do not lead to worsening of adverse effects for the patients (Nair, 2003). It was recommended that after the use of a first and second antiepileptic drug without adequate improvement, a combination of two drugs is resorted to (Kwan and Brodie, 2000).

2.13.1 Role of Rational Polypharmacy in the Treatment of Refractory Epilepsy

Refractory epilepsy or intractable seizures refer to a situation where epileptic symptoms are not responding to treatment. Seizures are well controlled with a single anticonvulsant

in most patients with epilepsy. However, about 20% of patients with primary generalized epilepsy and 35% of patients with focal epilepsy have medically intractable seizures (Cascino, 1990; Reuten and Berkovic, 1995). If seizures are not controlled with initial dose of a first-line antiepileptic drug and there is no evidence of toxicity, the dose should be systematically increased, if the seizures are still not under control, a second, first-line drug should be tried or added to the first drug (Nair, 2005). Novel medical approaches now under exploration include the use of drugs with complementary mechanisms of action, stimulation of various components of the nervous system, biochemical manipulations, focal intracerebral drug perfusion and gene therapy (Jallon, 2007).

2.13.2 Antiepileptic Drugs used in Rational Polypharmacy

Combinations of levetiracetam and carbamazepine (Jinsook *et al*., 2007); combination of carbamazepine and valproate (Sun *et al.,* 2002); lamotrigine and valproate, lamotrigine and carbamazepine, lamotrigine and diphenylhydantoin (Luszczki, 2004); carbamazepine and phenytoin (Lai *et al*., 1992); carbamazepine and phenytoin (Perucca and Richens, 1980) are some of the drug combinations employed in rational antiepileptic polypharmacy.

2.14 PROCESSES IN THE TREATMENT OF EPILEPSY

The selection of an appropriate antiepileptic drug is based on proper diagnosis of the epileptic syndrome of the patient (Spanaki *et al*., 2005). The first-line therapy for patients with focal seizures includes the administration of phenytoin, carbamazepine and

valproate (Nair, 2003). Antiepileptic drugs are usually given in an initial dose, and then gradually increased over time until maximum seizure control is achieved with minimal side-effects (Anon, 2008b). Successful treatment consists of finding the balance between obtaining adequate seizure control and avoiding adverse effects (Chung *et al.,* 2005).

Monotherapy, using newly developed drugs such as levetiracetam, avoided side effects due to drug interactions but was ineffective in 20-30% of patients (Jallon, 2007). Despite the increased availability of new antiepileptic drugs with improved tolerability and reduced interaction, old generation antiepileptic drugs are extensively used outside specialized epilepsy centres. Phenytoin is still the most commonly prescribed antiepileptic drug for the elderly (Spanaki *et al.,* 2005). Selective inhibition of neuronal and glial GABA transporter subtypes may offer unique therapeutic options for regaining balance between inhibitory and excitatory systems (Smith-Yockman, 2005). Antiepileptic drugs prevent or interrupt seizures by blocking of voltage-dependent sodium channels, enhancement of GABA-mediated inhibition and blocking of glutamatergic excitatory neurotransmission (Taylor and Meldrum, 1995; Brodie and Dichter, 1996; Gidal *et al.,* 1999).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 ANIMALS

Forty adult male Albino rats weighing between 144 and 300 g were used for the experiment. The animals were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, and were housed in rat cages in the laboratory. The animals were fed pellets made from grower's mash (Grand Cereals, Jos), maize bran and groundnut cake in the ratio 4:2:1, with wheat flour serving as binder, and water was provided *ad libitum.* The animals were allowed to acclimatize for a period of two weeks before the experiment commenced.

3.1.1 Anticonvulsant Drugs

The anticonvulsant drugs used in this study were carbamazepine tablets (Hovid Bhd, Malaysia) at 20 mg/kg (Rajesh *et al.,* 1991) and phenytoin capsules (Biomedicine Belgium) at 100 mg/kg (Vijay *et al.,* 2009).

3.2 EXPERIMENTAL PROTOCOLS

The animals were divided at random into four groups of 10 animals each. Animals in groups 2, 3 and 4 were given carbamazepine (20 mg/kg) (CBZ), phenytoin (100 mg/kg) (PHE) and carbamazepine+phenytoin (20 and 100 mg/kg) (CBZ+PHE), respectively. Rats in group 1 were given distilled water at 10 mls/kg and served as untreated control.

All treatments were administered orally by gavage once daily for a period of eight weeks. During this period, the rats were monitored for clinical and neurobehavioural signs.

3.2. NEUROBEHAVIOURAL PATTERN

3.2.1 Evaluation of Locomotor Activity

The effect of the regimens on locomotor activity was evaluated weekly till the end of the experiment using the open-field apparatus as described by Zhu *et al*. (2001). The open-field apparatus was constructed using cardboard box (50 × 50 × 46 cm high) with clear Plexiglas on the floor. The floor of the box was divided into 25 equal squares. The locomotor activity was assessed by placing an animal in the box and allowing it to roam freely for 3 minutes to familiarize itself with the environment. The number of squares crossed with all the paws during the next 2 minutes was recorded. Soapy water followed by 90% alcohol solution was used to clean the arena between trials to eliminate odours from preceding animal.

3.2.2 Evaluation of Rearing Activity

Rearing activity was also evaluated weekly till the end of the experiment. The open-field apparatus as described by Zhu *et al.* (2001) was also used. Rearing was assessed by placing an animal in the box and allowing it to roam freely for 3 minutes to familiarize itself with environment. The number of times an animal stood on its hind limb trying to peep out of the box in the next 2 minutes was recorded. Soapy water followed by 90% alcohol solution was used to clean the arena.

3.2.3 Cognition

3.2.3 Assessment of Learning

This experiment was performed 48 hours prior to the termination of the study. It was done using the step-down inhibitory avoidance learning task as described by Zhu *et al*. (2001). The apparatus used was made of 40 × 25 × 25 cm acrylic chamber, consisting of a floor made of parallel 2 mm caliber stainless steel bars spaced 1cm apart. An electric shock was administered through the floor bars. A 25 cm high, 8cm by 25 cm wooden platform was placed at the extreme end of the chamber. Each animal was placed gently on the platform; upon stepping down, the rat received a single 80 volts foot shock. If the animal does not return to the platform, the foot-shock was repeated every 5 seconds. A rat is considered to have learned the avoidance task if it remains on the platform for more than 2 minutes. The number of foot-shocks applied before the animal learns the avoidance task was recorded as an index of learning acquisition.

3.2.4 Assessment of Memory

Memory was also assessed using the step-down inhibitory avoidance task as described by Zhu *et al.* (2001). The apparatus used was the same as that described for learning. Individual rats were again placed gently on the platform 24 hours after performing the learning task. The time during which the animal remains on the platform was recorded as an index of memory retention. Staying on the platform for 2 minutes (120 seconds) was counted as maximum memory retention (ceiling response).

3.3 EVALUATION OF WEIGHT

Rats were weighed weekly throughout the period of the experiment. Each rat in a group was weighed using a digital weighing balance and the weights were recorded.

3.4 HAEMATOLOGY

3.4 EVALUATION OF THE EFFECT OF CARBAMAZEPINE AND/OR PHENYTOIN ON HAEMATOLOGICAL PARAMETERS

At the end of the experimental protocols, rats from each group were sacrificed by decapitation after light ether anaesthesia. Blood was collected into sample bottles containing ethylenediaminetetreacetic acid (EDTA) for haematological analysis. The haematological parameters analysed were packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) counts, and total and differential leucocyte counts.

3.4.1 Determination of Packed Cell Volume

The PCV values were measured using the method described by Dacie and Lewis (1991). Heparinized capillary tubes were inserted into the sample bottles containing the collected blood, with about 15 mm of the tubes unfilled. The open end of each tube was then carefully sealed in a fine flame. The tubes were then loaded onto a microhaematocrit centrifuge and were centrifuged for 10 minutes at 1000 *g*. The PCV value for each of the tube was read using the haematocrit reader.

3.4.2 Determination of Total Red Blood Cell Counts

Blood (0.5 ml) was aspirated into an RBC pipette and then diluted with 100 ml of RBC diluting fluid (1:200). The counting was done using an improved Neubaeur counting chamber under a light microscope at × 40 magnification (Dacie and Lewis, 1991).

3.4.3 Determination of Total and Differential Leucocyte Counts

Leucocyte counts were measured using the method described by Dacie and Lewis (1991). A drop of blood was placed at one end of a glass slide. A spreader was used to make a thin blood film, which was allowed to dry by air. Leishman stain was poured to cover the film for 2 minutes. The film was then rinsed in buffered-distilled water and allowed to stand for 10 minutes. This was later examined under the microscope in an oil immersion at × 1000 magnification.

3.4.5 Determination of Haemoglobin (Hb) Concentration

The Hb concentration was determined using the cyanmethaemoglobin method as described by Baker and Silverton (1985). About 20 µl of blood was pipetted into a tube and diluted with 5 ml of modified Darkin's fluid and was allowed to stand for at least 3 minutes. The absorbance of the mixture was read using a spectrophotometer at a wavelength of 540 nm against reagent blank. The Hb, concentration was determined using the following formula:

$$Hb = \frac{T \times C \times D\ (g/dL)}{A \times 100}$$

Where T is the test absorbance, A is the standard absorbance, C concentration of cyanmethaemoglobin standard (mg/dL), while D is the dilution factor (1:250); 1000 converted the haemoglobin concentration from mg/dL to g/dL.

3.5 SERUM BIOCHEMICAL ANALYSIS

Blood was collected into test tubes and incubated for 60 minutes. The tubes were then centrifuged at 1,000 *g* for 10 minutes. Thereafter, the serum was collected from each test tube into clean sample tubes, which were subsequently used for the evaluation of serum biochemical parameters. Alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) were analysed using autoanalyzer (Bayer Clinical Chemistry Analyzer, Germany), lactate dehydrogenase (LDH), electrolytes (Na^+, K^+ and Cl^-), urea, total protein, albumin and globulin concentrations were also evaluated. Total serum proteins were analysed using the method of Lowry (1952), while serum albumin concentration was measured using the method described by Henry (1974). Serum globulin concentration was obtained by subtracting serum albumin concentration from total serum protein concentration. The LDH activity was estimated by optimised standard kit method (Roche/Hitachi), based on the principle that LDH catalysed the conversion of pyruvate to lactate; nicotinamide adenine dehydrogenase (NADH) was oxidized to nicotinamide adenine dinucleotide in the process. The rate of decrease in NADH was directly proportional to the LDH activity. The LDH activity was estimated using a kit

(Optimized Standard Kit; Roche/Hitachi), and the absorbance was read using a spectrophotometer [Shimadzu Double-beam Digital Atomic Absorption/Flame Spectrophotometer Model AA-650 (202-37200), Shimadzu Corporation, Tokyo, Japan].

3.6 STATISTICAL ANALYSIS

Values obtained were expressed as mean ± SEM and were subjected to statistical analysis using one way analysis of variance (ANOVA), the variant means were separated using Tukey's post hoc test. Using GraphPad Prism Version 4.0 for Windows from GraphPad Software, San Diego, Carlifornia, USA (www.graphpad.com). Values of $P < 0.05$ were considered significant.

CHAPTER 4

4.0 RESULTS

4.1 OPEN TEST FIELD

4.1.1 Effect of Treatments on Locomotor Activity in Albino Rats

The changes in the number of squares crossed by rats in the control group were relatively stable in all the experimental weeks. Similarly, changes observed in the number of squares crossed by rats in the PHE group at week 1 compared to the remaining experimental weeks did not differ significantly ($P > 0.05$). A significant ($P < 0.01$) decrease in the number of squares crossed by rats following CBZ administration was observed at weeks 5, 7, 4 and 2 ($P < 0.05$) when compared to week 1. An increase (P0.05) in the number of squares crossed by rats at weeks 6 and 8 compared to week when compared to week 1. There was a significant ($P < 0.05$) decrease in the number squares crossed by rats in the CBZ+PHE group at weeks 2, 5 and 8 ($P < 0.01$) compared to week 1 but the numbers of squares crossed increased ($P < 0.05$) at weeks 4 and 7 when compared to week 1 and week 4 when compared to week 3 (Figure 4.1).

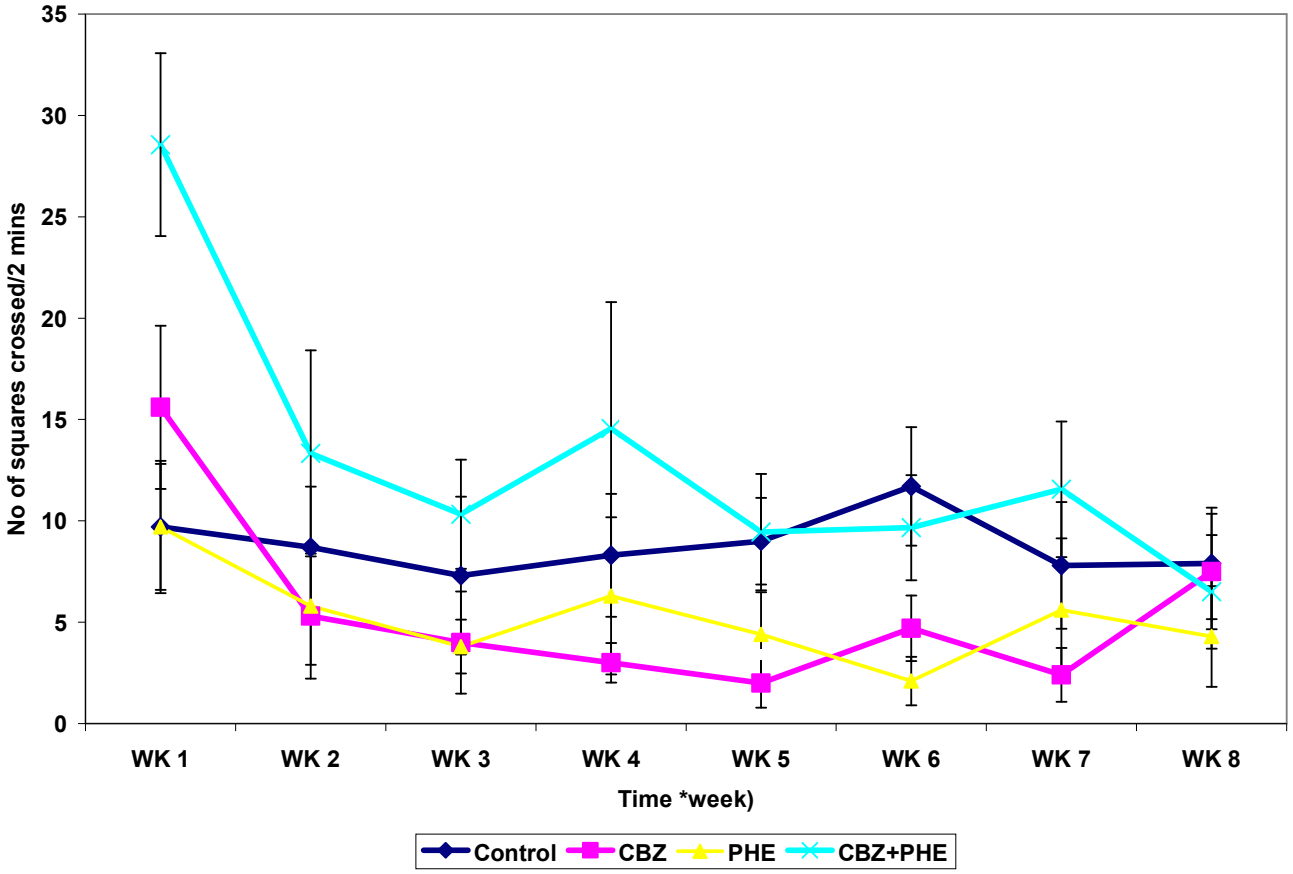


Figure 4.1: Locomotion activity decreased in all the drug-treated groups upon the administration of the drugs but undulated with subsequent administration after every week, at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks.

4.1.2 Effect of Treatments on Rearing Activity in Albino Rats

There was no significant ($P > 0.05$) change in rearing activity in the PHE-treated group when the treatment weeks were compared. Rearing activity decreased ($P < 0.05$) at weeks 4, 7 and 5 ($P < 0.01$) when compared to week 1 in the CBZ-treated group. A significant ($P < 0.05$) increase in rearing activity was observed in the CBZ+PHE-treated group at week 5 and a decrease ($P < 0.05$) at weeks 7 and 8 compared to week 1 (Figure 4.2).

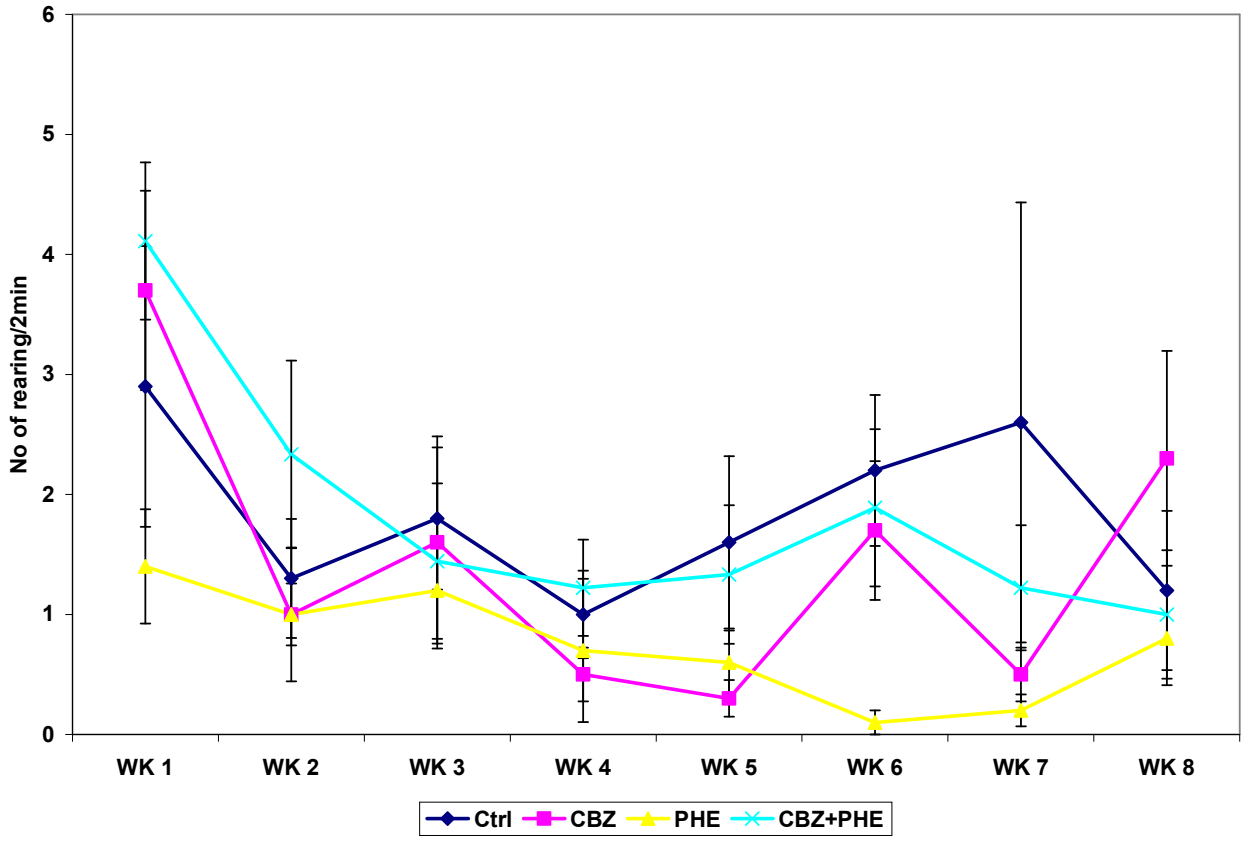


Figure 4.2: Rearing activity decreased in all the treated groups upon the administration of the drugs but undulated with subsequent administration after every week, at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks.

4.2 COGNITION: LEARNING AND MEMORY

4.2.1 Effect of Treatments on Learning Ability in Albino Rats

There was no significant ($P > 0.05$) change in the number of foot-shocks when the drug-treated groups were compared to the control group. Similarly, there was no significant ($P > 0.05$) change in the number of foot-shocks applied in between the treatment groups (Figure 4.3).

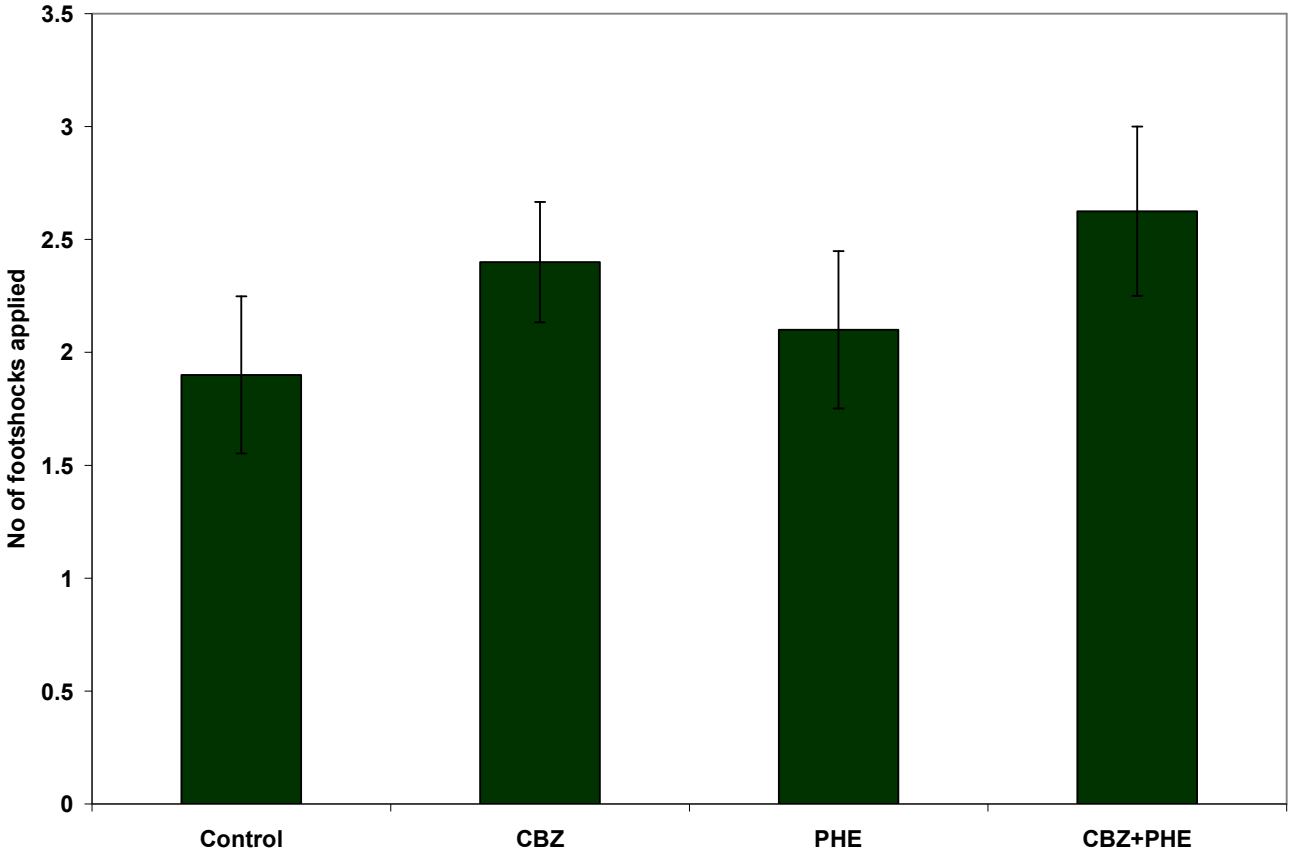


Figure 4.3: Learning ability decreased in all the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks when compared with the control group.

4.2.2 Effect of Treatments on Short-Term Memory in Albino Rats

There was no significant ($P > 0.05$) change in the duration of stay on the platform in the CBZ and PHE groups when respectively compared to the control group. The duration of stay on the platform in the CBZ+PHE group when compared to the control group decreased ($P < 0.05$). Insignificant ($P > 0.05$) change was observed between the treatment groups (Figure 4.4).

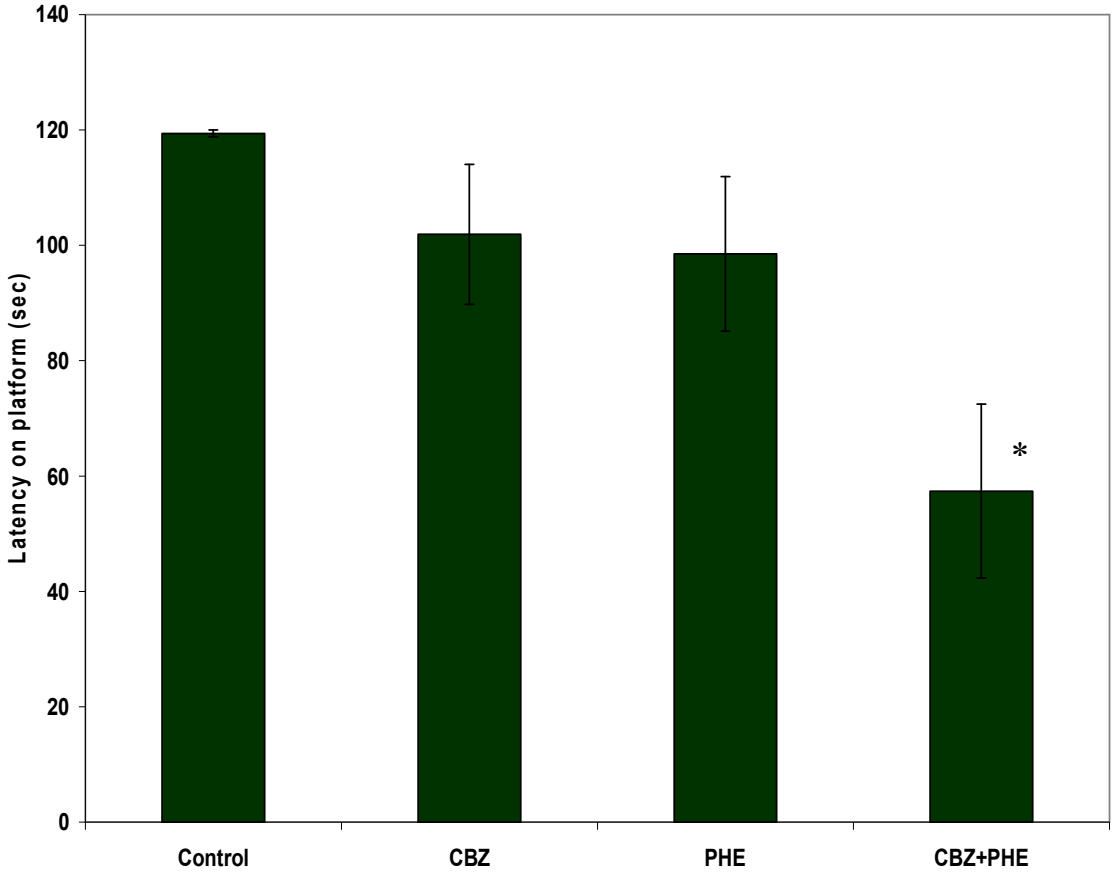


Figure 4.4: Short-term memory decreased in all the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks when compared with the control group.

*= $P < 0.05$ (CBZ+PHE Vs Control)

4.3 EFFECT OF TREATMENT ON BODY WEIGHT

Body weight rose significantly ($P < 0.001$) in the control, CBZ and PHE groups at week 1 compared to week 8. A non-significant ($P > 0.05$) increase in body weights recorded in the CBZ+PHE group at weeks 1 and week 8 did not differ (Figure 4.5).

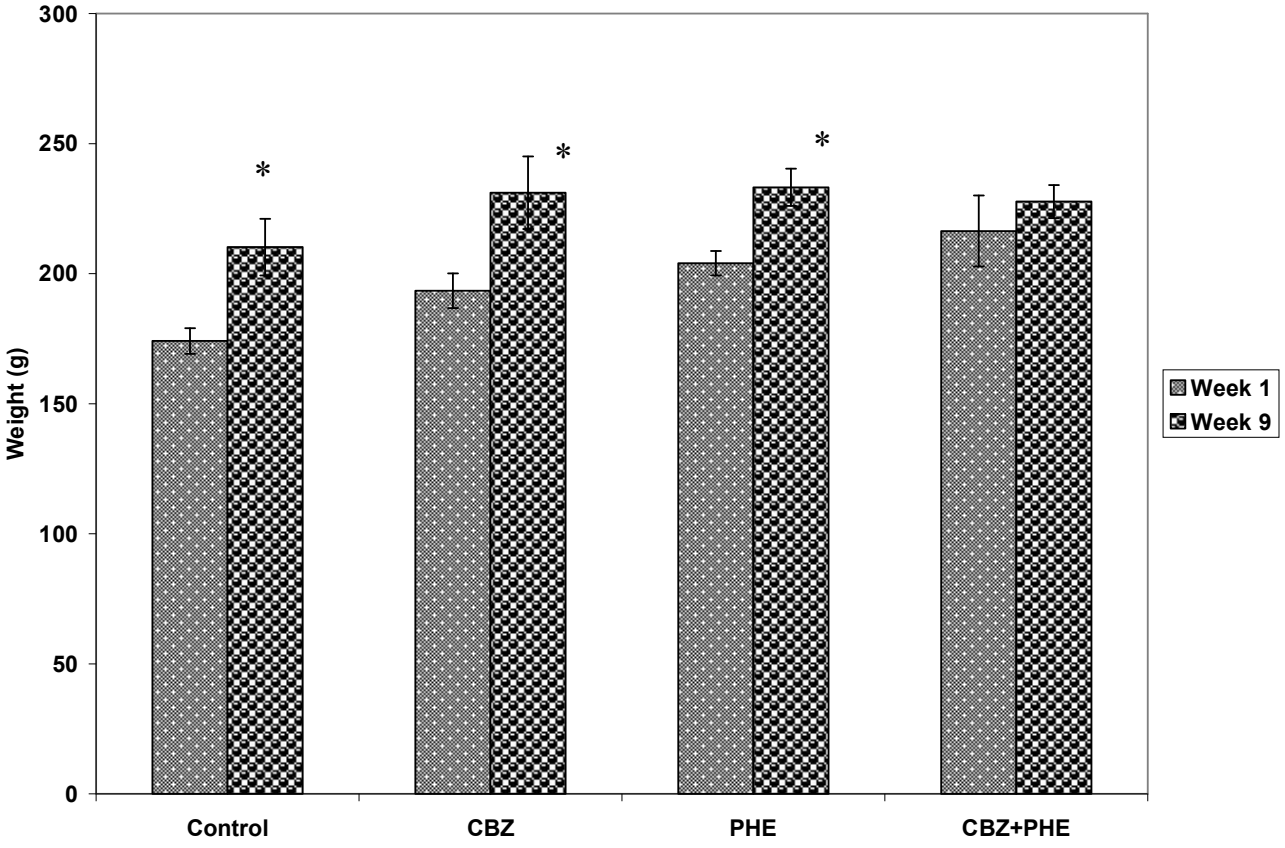


Figure 4.5: Body weights increased in all the treatment groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks when week 1 was compared with week 8.

*= $P < 0.001$ (Week 1 Vs Week 8)

4.4 EFFECT OF TREATMENT ON HAEMATOLOGICAL PARAMETERS

Effect of Treatments on Packed Cell Volume

There was no significant ($P > 0.05$) difference in the value of the PCV obtained in the CBZ, PHE and CBZ+PHE groups, compared to the control group. Also, there was no significant ($P > 0.05$) difference in PCV value between the drug-treated groups (Figure 4.6).

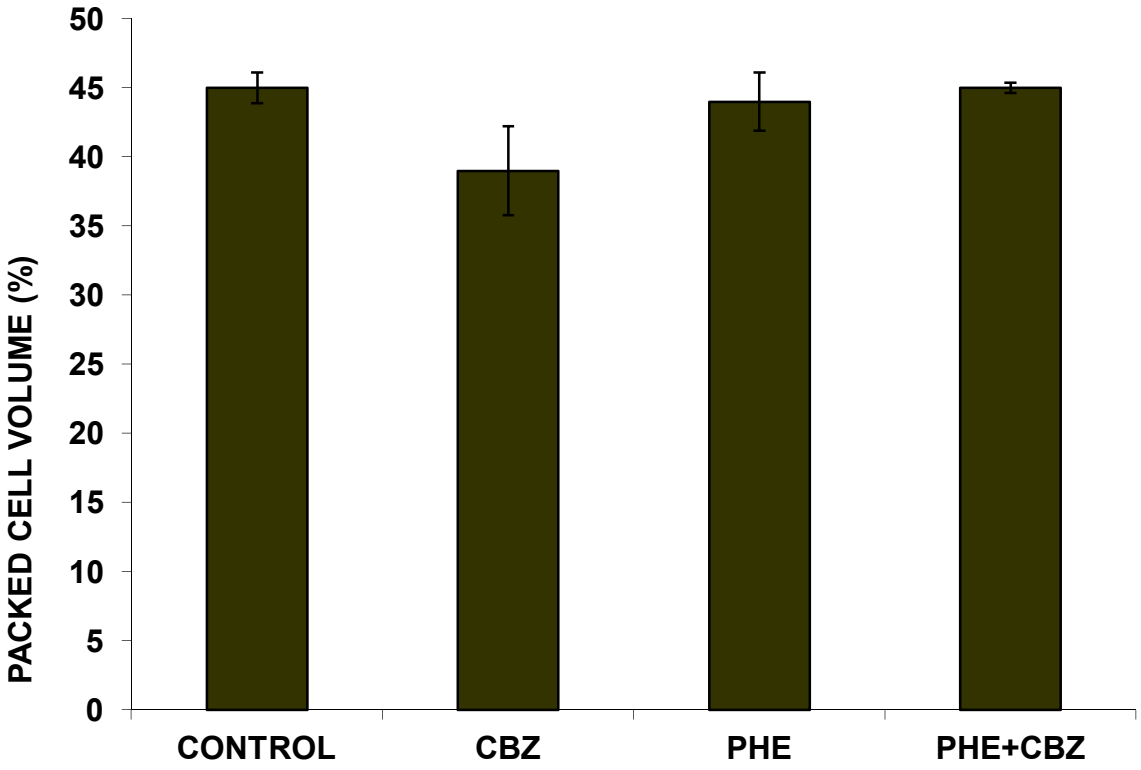


Figure 4.6: Changes in the value of the PCV in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks was not statistically significant when compared with the control group.

4.4.3 Effect of Treatments on Red Blood Cell Counts

The change in RBC counts obtained in PHE and CBZ+PHE groups when respectively compared to that of the control group were not significant ($P > 0.05$). The RBC counts in the CBZ group was lower ($P < 0.01$) than the value recorded in the control group. There was no significant ($P > 0.05$) change in RBC counts in between the drug-treated groups (Figure 4.6).

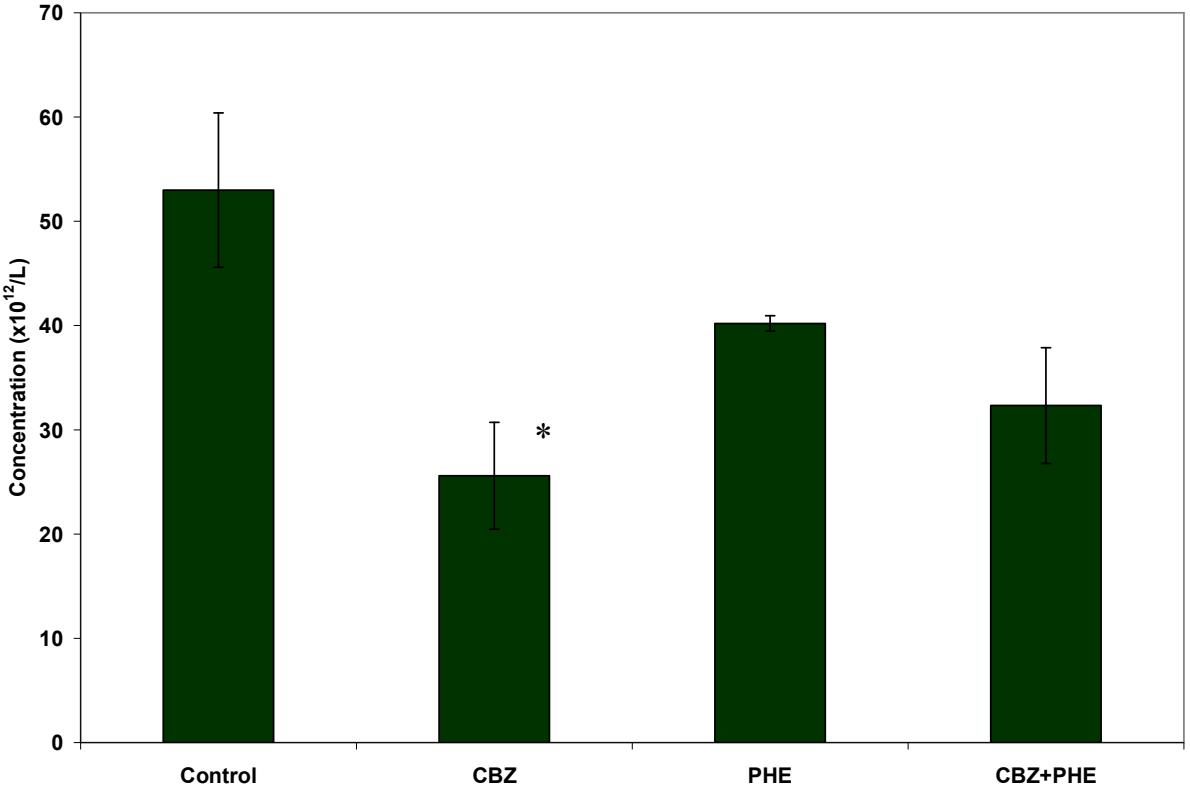


Figure 4.7: The value of RBC in the drug-treated groups decreased upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks when compared with control group.

*= $P < 0.01$ (CBZ Vs Control)

4.4.2 Effect of Treatments on Haemoglobin Concentration

There was no significant ($P > 0.05$) change in Hb concentrations between the experimental groups (Figure 4.7).

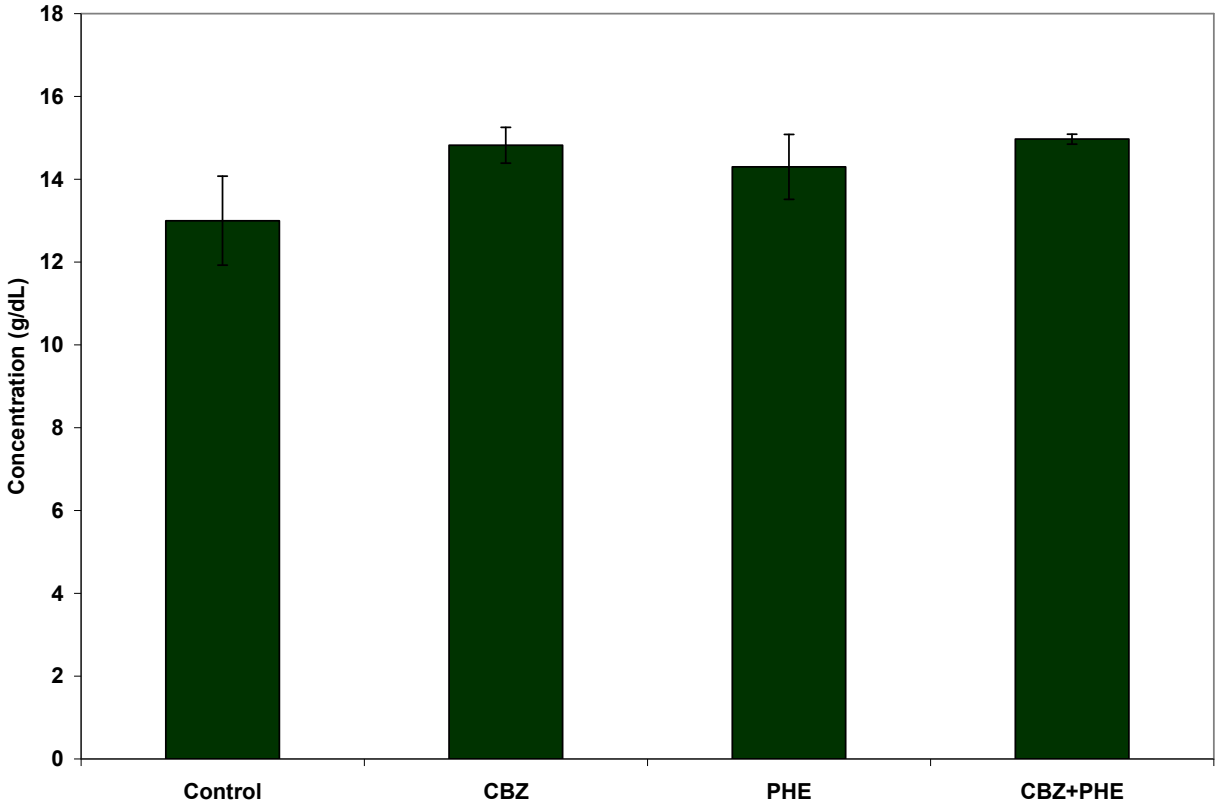


Figure 4.8: Changes in Hb concentration in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks were not statistically significant when compared with control group.

4.4.3 Effect of Treatments on Platelet Counts in Albino Rats

There was no significant ($P > 0.05$) change in the platelet counts when the values obtained in the drug-treated groups were respectively compared to that of the control group. Also, the changes recorded in the counts between the drug-treated groups were not different ($P > 0.05$) (Figure 4.8).

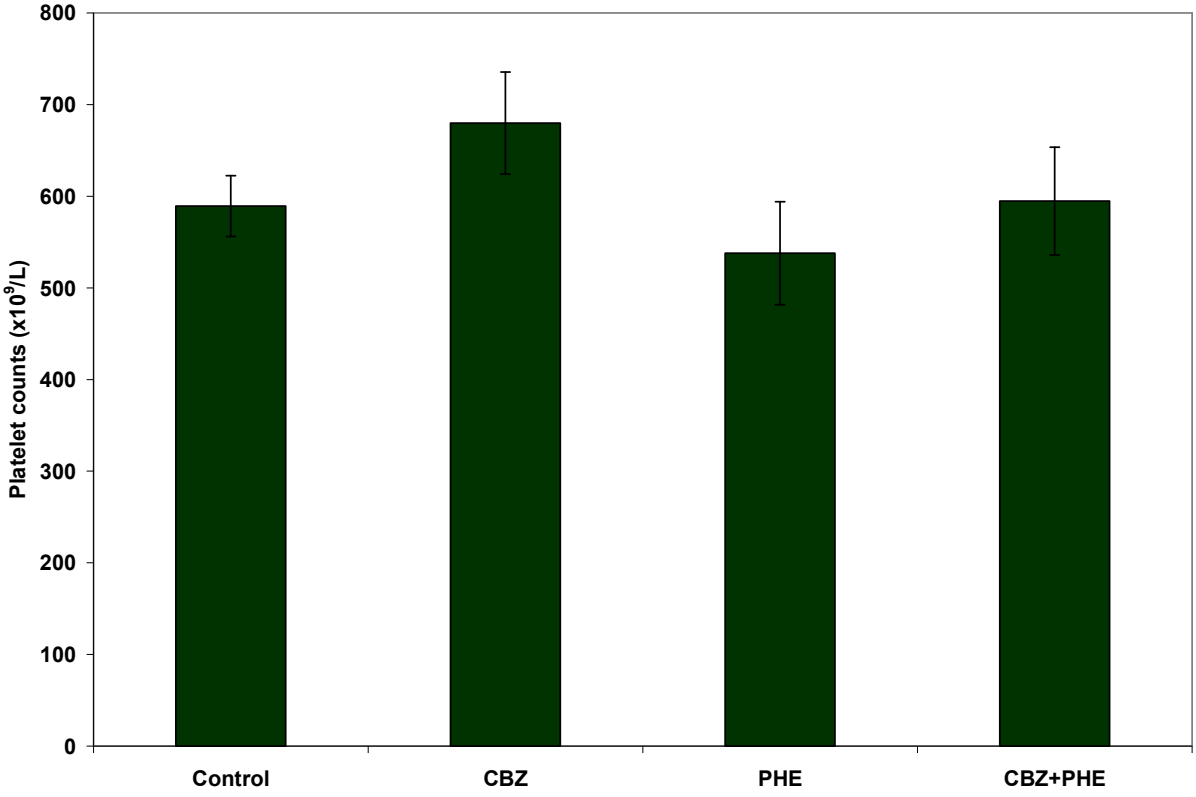


Figure 4.9: Changes in the platelet counts in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks were not statistically significant when compared with the control group.

4.4.4 Effect of Treatments on Total Leucocyte Counts in Albino Rats

An insignificant ($P > 0.05$) increase observed when the total leucocyte counts in each of the drug-treated group was compared to that of the control group. There was no significant ($P > 0.05$) increase in the total leucocyte counts obtained in the drug-treated groups (Figure 4.10).

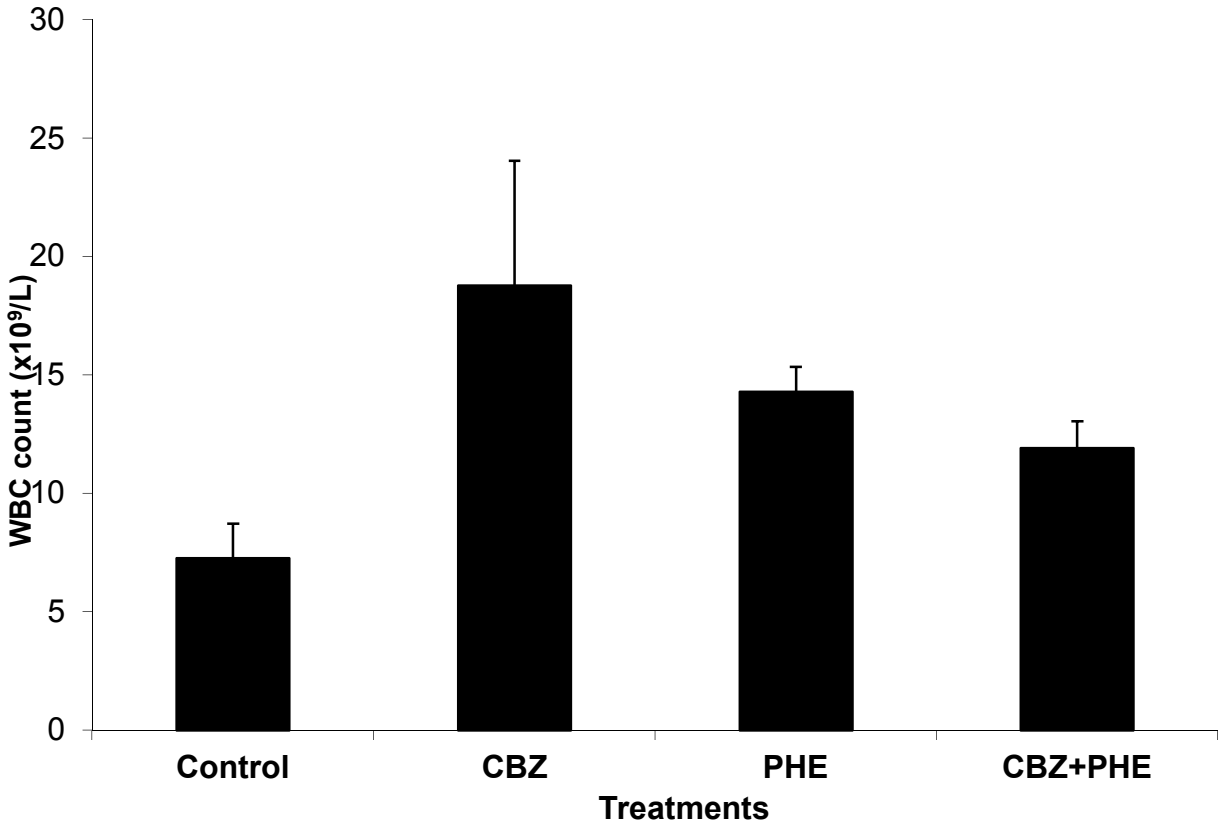


Figure 4.10: Changes in the WBC counts in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks were not statistically significant when compared with the control group.

4.4.5 Effect of Treatments on Neutrophil Counts in Albino Rats

Neutrophil counts in the CBZ group rose significantly ($P < 0.01$) when compared to that of the control group, but the increase in the PHE and CBZ+PHE groups was not different from that of the control group. There was no significant change in neutrophil counts obtained in between the treatment groups (Figure 4.11).

4.4.6 Effect of Treatments on Lymphocyte Counts in Albino Rats

The lymphocyte counts increased ($P < 0.05$) in the CBZ group when compared to that of the control group. There was no significant ($P > 0.05$) change in lymphocyte counts when the PHE and CBZ+PHE groups were respectively compared to the control group. Similarly, no significant ($P > 0.05$) changes in lymphocyte counts were recorded in between the drug-treated groups (Figure 4.11).

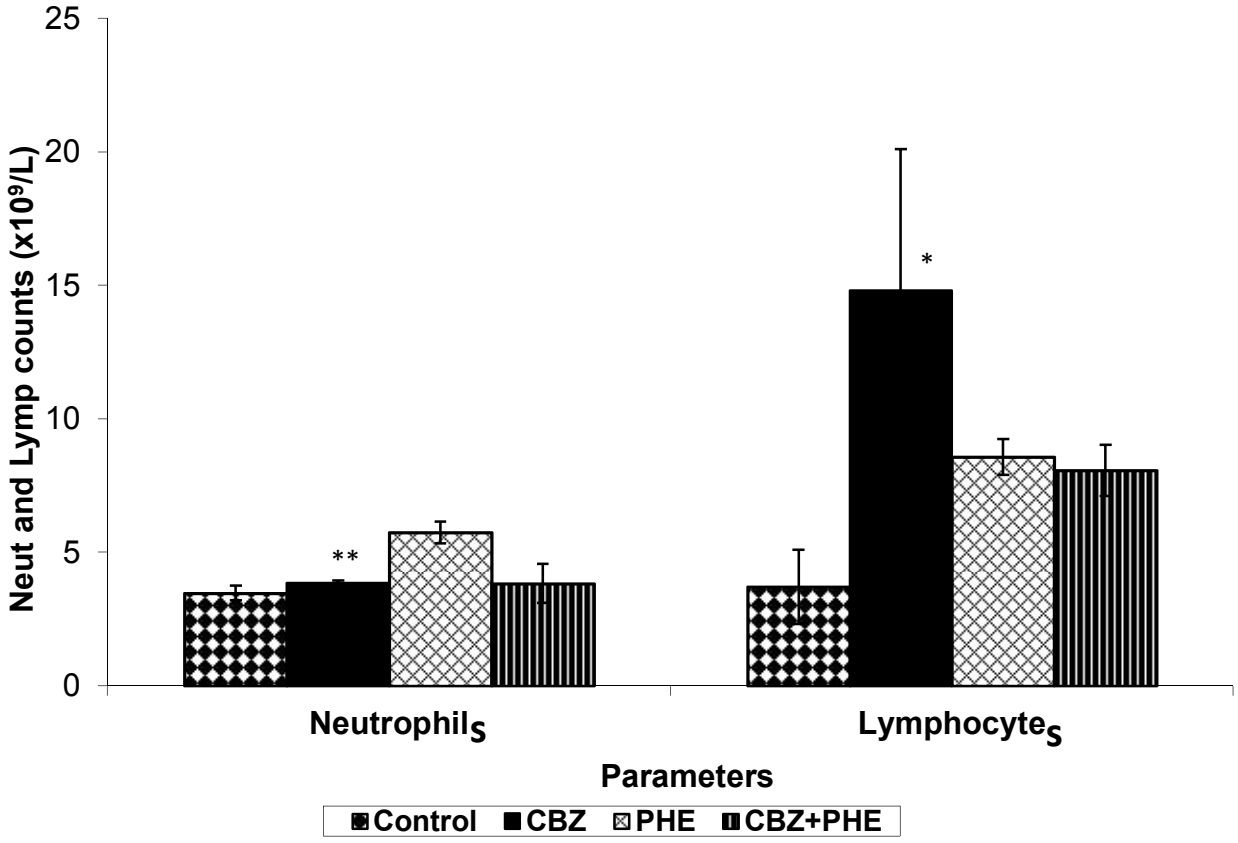


Figure 4.11: The neutrophil and lymphocyte counts increased in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks when compared with the control group.

*= $P < 0.05$ (CBZ Vs Control), **= $P < 0.01$ (CBZ Vs Control)

4.6 EFFECT OF TREATMENT ON SERUM ELECTROLYTE CONCENTRATIONS

4.6.1 Effect of Treatments on Sodium ion Concentration

There was a significant ($P < 0.001$) increase in Na^+ concentrations in the CBZ, PHE and CBZ+PHE-treated groups when compared to that of the control group. There was no significant ($P > 0.05$) change in Na^+ concentration in the CBZ group, when compared to that of the PHE or CBZ+PHE group. The difference in Na^+ concentration in the PHE group compared to that of the CBZ+PHE group was not significant ($P > 0.05$) (Figure 4.12).

4.6.2 Effect of Treatments on Potassium ion Concentration

The changes in K^+ concentration in the PHE group when compared to the control and the CBZ+PHE groups were not significantly ($P > 0.05$) different. There was a significant ($P < 0.05$) decrease in K^+ concentration in the CBZ and CBZ+PHE groups when respectively compared to the concentration recorded in the control group. Potassium ion concentrations obtained in between the treatment groups did not differ ($P > 0.05$) (Figure 4.12).

4.6.3 Effect of Treatments on Chloride ion Concentration

There was no significant ($P > 0.05$) change in Cl^- concentration in between the groups. The changes in the mean Cl^- concentration in the PHE and CBZ+PHE groups were not marginally different, when compared to that of the CBZ group (Figure 4.12).

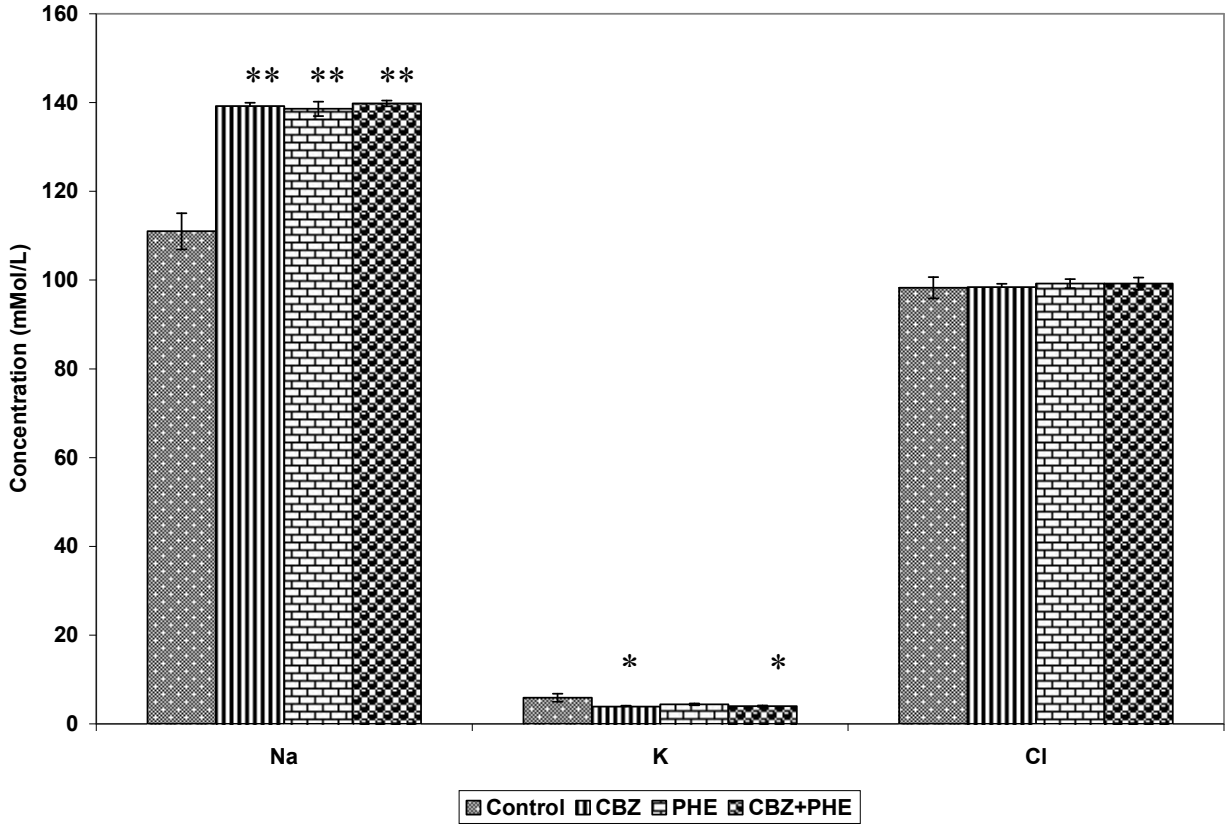


Figure 4.12: Na^+ concentration increased, K^+ decreased and Cl^- unaltered in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks when compared with the control group.

Na = Sodium ion, K = Potassium ion, Cl = Chloride ion

*= $P < 0.05$ (CBZ, CBZ+PHE Vs Control), **= $P < 0.001$ (CBZ, PHE, CBZ+PHE Vs Control)

4.7 EFFECT OF TREATMENT ON SERUM PROTEINS

4.7.1 Effect of Treatments on Serum Total Proteins

There were no significant ($P > 0.05$) changes in the concentrations of obtained total proteins in between the drug-treated groups and when the concentrations in each of the treatment groups was compared to that of the control group (Figure 4.13).

4.7.2 Effect of Treatments on Serum Albumin Concentration

Albumin concentrations in the CBZ, PHE and CBZ+PHE groups were higher ($P < 0.01$), when respectively compared to that of the control group. There were no significant ($P > 0.05$) changes in albumin concentrations recorded between the drug-treated groups (Figure 4.13).

4.7.3 Effect of Treatments on Globulin Concentration

There were significant ($P < 0.05$) decreases in globulin concentration in the CBZ and PHE groups, when respectively compared to that of the control group. However, there was no significant ($P > 0.05$) change in globulin concentration in the CBZ+PHE group when compared to that of the control group. Globulin concentrations in between the drug treatment groups were not different ($P > 0.05$) (Figure 4.13).

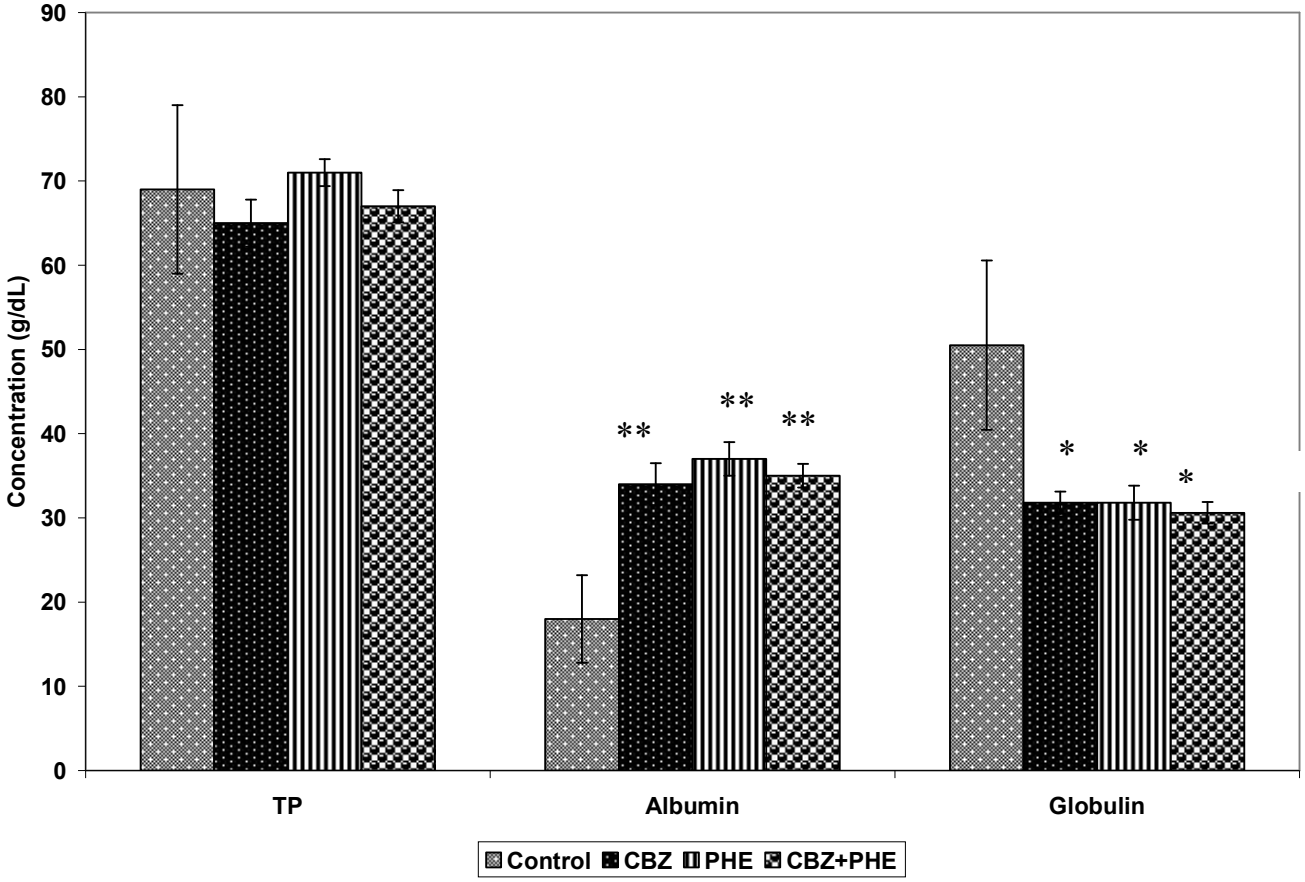


Figure 4.13: TP concentration changed, albumin increased and globulin decreased in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks when compared with the control group.

TP = Total proteins

*= P < 0.05 (CBZ, PHE, CBZ+PHE Vs Control), **= P < 0.01 (CBZ, PHE, CBZ+PHE Vs Control)

4.8 EFFECT OF TREATMENT ON SERUM UREA CONCENTRATION

Urea concentrations in the CBZ and PHE groups were significantly lower ($P < 0.05$) when each was compared to the value recorded in the control group. There was no significant ($P > 0.05$) change in urea concentrations obtained between the antiepileptic drug-treatment groups (Figure 4.14).

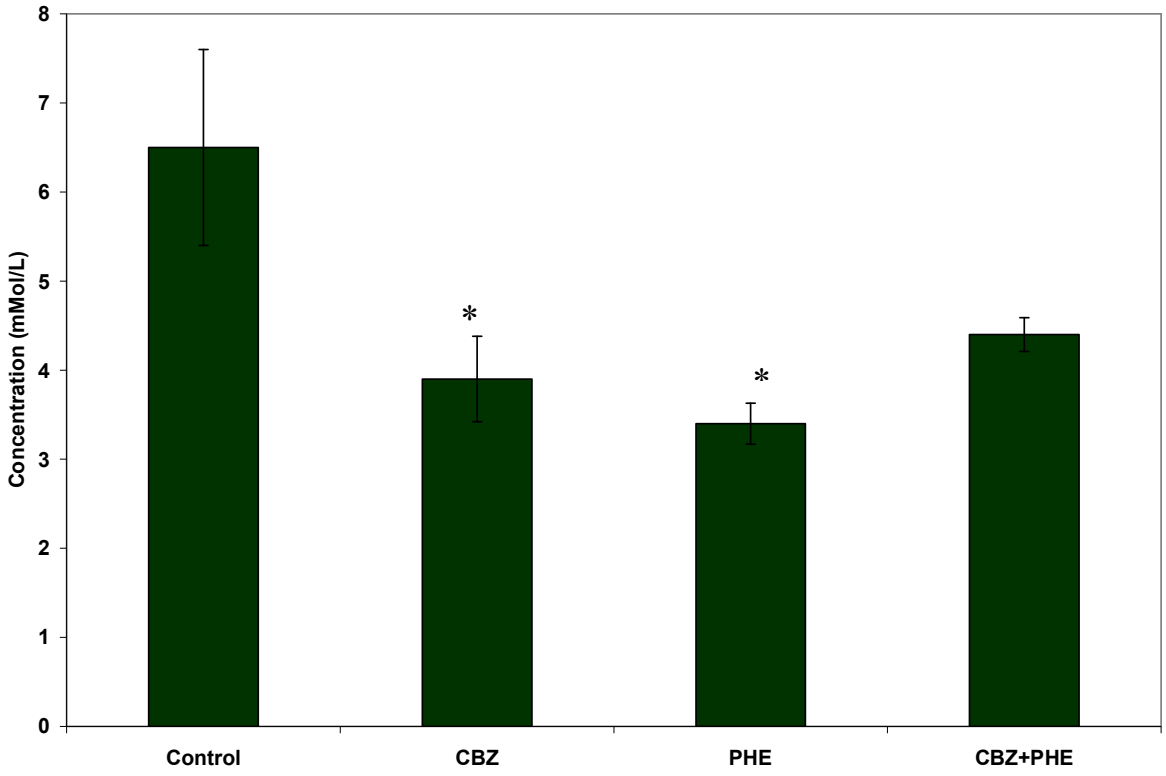


Figure 4.14: Urea concentration decreased in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks when compared with the control group.

*= $P < 0.05$ (CBZ, PHE Vs Control)

4.9 EFFECT OF TREATMENT ON LIVER ENZYME ACTIVITIES

4.9.1 Effect of Treatments on Alanine aminotransferase

The ALT activities in the CBZ and CBZ+PHE groups rose significantly ($P < 0.05$), when compared to that of the control group. The ALT activities in the PHE group and that of the control were not significant ($P > 0.05$). There were also no significant ($P > 0.05$) changes in ALT activities between the drug-treated groups (Figure 4.15).

4.9.2 Effects of Treatments on Aspartate aminotransferase

A significant ($P < 0.05$) increase in AST activities was recorded in the PHE group, when compared to that of the control group. Increases in AST activity in the CBZ and CBZ+PHE groups when respectively compared to that of the control group were not significant ($P > 0.05$). There was no significant ($P > 0.05$) change in AST activity when the treatment groups were compared (Figure 4.15).

4.9.3 Effect of Treatments on Alkaline phosphatase Activity

Changes in ALP activity in the CBZ, PHE and CBZ+PHE groups when respectively compared to that of the control group were insignificant ($P > 0.05$). The increase in ALP activity in the PHE group when compared to that of the CBZ or CBZ+PHE group was insignificant ($P > 0.05$). Similarly an insignificant ($P > 0.05$) increase was recorded in the CBZ+PHE group when compared to that of the CBZ group (Figure 4.15).

4.9.4 Effect of Treatments on Lactate dehydrogenase Activity

There were no significant ($P > 0.05$) changes in LDH activity in the CBZ, PHE and CBZ+PHE groups, when respectively compared to that of the control group. There was a relative but, insignificant ($P > 0.05$), decrease in LDH activity in the CBZ group compared to either that of the PHE or CBZ+PHE group. Although the LDH activity in the PHE group increased over that recorded in the CBZ+PHE group, the difference in the activities was not significant ($P > 0.05$) (Figure 4.15).

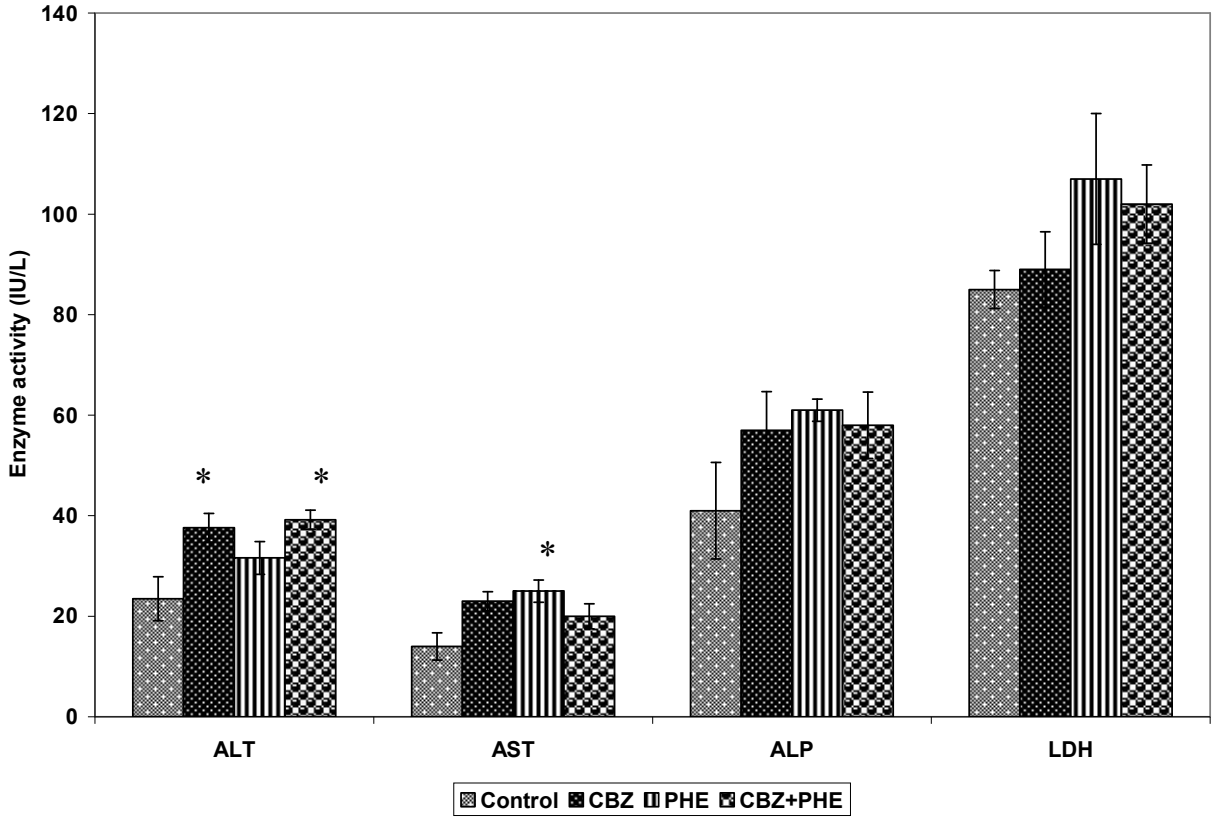


Figure 4.15: Enzyme activities increased in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks when compared with the control group.

ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase, LDH = Lactate dehydrogenase.

*= $P < 0.05$ (ALT = CBZ, CBZ+PHE Vs Control; AST = PHE Vs Control)

CHAPTER 5

5.0 DISCUSSION

The progressive decrease in locomotor activity in all the drug treatment groups demonstrated impairment of motor activity by the anticonvulsants. The decreased locomotion recorded in the CBZ group for the first few weeks of therapy agreed with the findings of Luszcki (2004), who reported a decrease in ambulatory activity and total distance covered in mice administered CBZ, and this may be due to the sedative effect of the drug. Nowakowska *et al*. (2011) also attributed the decrease in locomotor activity in the CBZ monotherapy in rats to the sedative effects of the drug, and suggested that this may be related to the induction of microsomal enzymes of the P_{450} cytochrome. Increased locomotion at week 8 of therapy may be due to the induction of tolerance to the drug. Development of tolerance is an adaptive response of the body to prolonged exposure to the drug and tolerance to AEDs is no exception (Löscher and Schmidt, 2006). Tolerance in the CBZ group may be due to the ability of the drug to induce hepatic microsomal enzymes, CYP34A which also metabolizes it, thereby enhancing its clearance from the system (Bauer, 2008).

The initial decrease in locomotor activity in the PHE-treated group agreed with the findings of Balakrishnan *et al.* (1998) and Thakur *et al.* (2011), who attributed the decrease to the central nervous system (CNS) depressant effect of the drug. Decreased locomotor activity may also be due to the inhibition of calcium-induced secretory processes, including hormones and neurotransmitter released as a result of decrease in

calcium permeability, with inhibition of calcium influx across the membrane (Porter and Meldrum, 2007). Kšerk *et al*. (1998) reported that phenytoin affects the direct activation of the motor system by stimulation of the sensorimotor cortex in adult, but not in immature rats. Increased locomotor activity observed towards the terminal part of the study in the cause of PHE therapy was due to the development of tolerance (Löscher and Schmidt, 2006). The decrease in locomotion observed with the polytherapy group was not as pronounced as that obtained with monotherapy groups. This indicates that the combination of CBZ and PHE did not affect locomotor activity in rats, apparently due to the fact that CBZ reduces the bioavailability of PHE thereby reducing the effects of the drugs (Lai *et al*., 1992). This disagrees with the findings of Luszcki (2004), who reported that combining two sodium channel blockers may result in a considerable reduction in locomotor activity of the animals tested, which, apparently, induced the potentiation rather than the summation of hypolocomotor effects produced by the combination of the AEDs.

The decrease in rearing recorded in the CBZ group agreed with the result obtained by Luszcki (2004), which showed a decrease in rearing activity in mice administered CBZ. Rearing along with locomotion is used to measure motor activity. The decrease in rearing in the CBZ group demonstrated that the drug impaired motor activity. This may be due partly to the mechanism of action of the drug, relating to its ability to decrease calcium permeability (Porter and Meldrum, 2007), stabilize sodium inactivated channels and potentiate GABA receptors (Granger *et al.,* 1995). The combined effect of these actions

is apparently manifested in the decrease in motor activity. The impairment of rearing is more pronounced in the CBZ+PHE group, reflecting the additive or synergistic effect of the drugs (Brodie, 1992) or as a result of potentiation rather than the summation effects of the drugs (Luszcki, 2004).

Decrease in learning ability was observed in the CBZ- and/or PHE-treated groups. For the CBZ-treated group, impairment in learning agreed with the work of Rajesh *et al*. (1991), who observed that CBZ at 10 mg/kg and 20 mg/kg significantly decreased learning. In the same vein, Gillham *et al*. (1988) recorded a negligible cognitive impairment following CBZ administration. The relative increase in learning ability engendered by CBZ compared with that provoked by PHE was in agreement with previous study (Ogunrin *et al*., 2005). Furthermore, Shannon and Love (2005) recorded higher errors of omission following CBZ administration relative to PHE administration. Similarly, Pulliainen and Jokolainen (2005) stated that the long-term effects of PHE as compared to those of CBZ on cognition are few and restricted to some visually-guided motor functions. The relatively higher learning impairment recorded in the CBZ+PHE group than when either of the antiepileptic drugs was administered is an indication that antiepileptic polytherapy caused more cognitive deficit. Zhan (1998) showed that antiepileptic drug polytherapy caused more pronounced cognitive impairment, affirming the finding of the present study. Jinsook *et al*. (2007) showed that CBZ exacerbated PHE-induced cell death. This may be responsible for the relatively higher cognitive impairment obtained in the present study following co-administration of CBZ and PHE.

The fact that AED also suppressed epilepsy through its effect on the hippocampus may be responsible for the cognitive impairment, since the same part of the brain plays an important role in memory. This, therefore, makes the hippocampus which plays an important role in epileptogenesis, memory and learning (Mathew *et al.,* 2011) to be central to the beneficial and unwanted (adverse) effects of the AED. Apart from its effect on epileptic patients, antiepileptics also induce cognitive impairments in healthy subjects (Shannon and Love, 2005).

The impairment of memory in the CBZ, PHE and CBZ+PHE groups was in agreement with the results obtained by Shannon and Love (2004) that AEDs disrupt working memory, but at different magnitude that does not correlate with the mechanism of action. The authors found more deterioration in memory following CBZ administration than those repeatedly dosed with PHE. However in the present study, PHE was found to disrupt short-term memory performance than that of the CBZ group. Furthermore, Wamil and McLean (1993) showed that phenytoin significantly decreased the retention latency in the passive avoidance test similar to what was recorded in the present study. In addition, Thakur *et al.* (2011a) reported that chronic phenytoin treatment caused memory impairment and that neuronal damage in the hippocampus, cortex, cerebellum and midbrain by phenytoin may be responsible for memory impairment.

Since memory is maintained by various groups of neurones present in the hippocampus, cerebral cortex, cerebellum and midbrain, studies have shown that phenytoin increased

lipid peroxidation in these regions causing peroxidative injury to the neuronal membranes and macromolecules, alteration of the neurotransmitters, disruption of key neuronal functions and perturbation of motor function (Markesbery, 1997; McIntosh *et al*., 1997). Phenytoin may induce memory deficit by blocking N-methyl-D-aspartate (NMDA) responses (Balakrishnan *et al*., 1998). NMDA receptors have been linked directly to such phenomenon as long-term potentiation, and indirectly to learning and memory (Bliss and Collingridge, 1993). Phenytoin has been shown to block NMDA responses in mouse central neurones (Wamil and McLean, 1993).

Given the complexity of memory and cognition mechanisms as well as the diversity underlying neuronal processes, it is unlikely that impaired memory and cognition in epilepsy can be explained by a single mechanism. Indeed, a variety of factors such as neuronal cell loss, recurrent seizures, intetrical perturbation and sustained tonic dysfunction of the limbic circuits are likely to contribute to the impairments of learning and memory (Mazarati, 2008). Lui *et al*. (2003) reported that the extent of both memory deterioration and hippocampal activity positively correlates with the severity of hippocampal neuronal injury and mossy fibre sprouting. The fact that greater depreciation of short-term memory was observed following co-administration of CBZ and PHE than when either of the drugs was used shows that the combination of the two drugs caused significant cognitive impairment, apparently due to the synergistic effects of the drugs.

The relative increase in body weight in all the groups at week 8 compared with week 1 showed that the anticonvulsant drugs when administered alone or in combination did not adversely affect body weight. The relative increase in weight with CBZ therapy agrees with the work of Hogan *et al.* (2000), who observed that adjunctive CBZ therapy was associated with weight gain. However, the result of the present study disagrees with the finding of Vanina *et al.* (2002), who reported that CBZ is less commonly associated with weight gain. The fact that co-administration of CBZ and PHE did not result in an increase in body weight shows that AED polytherapy did not induce body weight gain. The reason for this is not known and requires further investigation.

The decrease in RBC counts in the CBZ, PHE and CBZ+PHE groups agreed with the finding of Misra *et al*. (2003), who observed that PHE, phenobarbital and CBZ are highly toxic to the haemopoietic system. Thakur *et al.* (2011b) showed that decrease in RBC counts in the PHE group may be due to the fact that the drug undergoes oxidative metabolism. The metabolism resulted in the formation of a toxic arene oxide intermediate, which covalently binds with cell macromolecules, causing cytotoxic damage, bone marrow toxicity and aplastic anaemia. The severity of the significant decrease in RBC counts in the CBZ group was similar to that reported in an earlier study, apparently due to isolated cessation of RBC production, resulting from pure RBC aplasia. However, McNamara (2006) reported that the prevalence of aplastic anaemia appears to be 1 in 200,000 patients treated with CBZ monotherapy. Therefore, the concern that aplastic anaemia may be a frequent complication of long-term CBZ therapy may remain

controversial, despite the result obtained in the present study. The non-significant decrease in RBC counts in the polytherapy group indicated a stabilization effect of co-administration of the drugs on the RBCs.

Drugs have been shown to cause idiosyncratic bone marrow suppression or dose-related suppression (Kaufman *et al*., 1996). Idiosyncratic bone marrow suppression is a life-threatening event that is not related to dose or to the duration of administration and cannot be predicted by repeated blood draws (Young, 1994; Sepkuty and Kaplan, 2004). Indeed idiosyncratic aplastic anaemia is one of the adverse drug reactions associated with all major AEDs, except gabapentine (Scheuer, 1996). The AEDs that are primarily known to be associated with bone marrow suppression (although rare) are felbamate, CBZ, PHE, and valproate (Suchitra and Bussel, 2000).

There was an increase in platelet counts (thrombocytosis) in the CBZ and CBZ+PHE groups, with the highest increase in the CBZ-treated group. The thrombocytosis observed in the CBZ-treated group agrees with the report of Tutor-Crespo *et al*. (2007), who suggested it to be of the reactive type. CBZ can cause an inflammatory reaction within hours, with increased levels of serum interleukin-6. Reactive thrombocytosis may result from increase in interleukin-6, and it has been proposed that direct stimulation of platelet by this interleukin may have a compensatory effect in CBZ-induced thrombocytopenia. The increased platelet counts obtained with CBZ therapy in the present study disagreed with the finding of McNamara (2006), who reported a transient thrombocytopenia, but

agrees with the decrease observed in the PHE group. Thrombocytopenia may be due to hyper-destruction of peripheral platelets (Tutor-Crespo *et al.,* 2007). The increase recorded in the CBZ+PHE group was not as pronounced as that observed with the CBZ group; this means the combination therapy had less effect on the platelet counts coupled with the fact that PHE and CBZ had differing effects in this study.

Leucocytosis was observed in the CBZ, PHE and CBZ+PHE groups. This finding disagrees with the reports by McNamara (2006), where 10% of patients were observed to have transient, mild leucopenia during initiation of therapy with CBZ, which resolved within the first four months of continued treatment. Leucocytosis observed in this study agrees with the report of Flanagan and Dunk (2008), attributed to primary bone marrow disorder. Also, according to Ekaidem *et al*. (2006), leucocytosis may be as a result of cellular inflammation in the absence of an infection. Leucocytosis observed in this study could also be due to the lymphocytosis recorded. Neutrophilia was observed in all the AED-treated groups, with a significant increase in the CBZ- and PHE-treated groups, and the increase in neutrophil count may also be due to cellular inflammation. Neutrophilia may predorminantly be attributed to the leucocytosis observed.

Lymphocytosis was observed in the AED-treated groups. Therefore, the leucocytosis recorded in the AED-treated groups was due to lymphocytosis. Lymphocytosis was very significant in the CBZ-treated group, and may arise due to stimulation of formation of epoxides by the activity of cytochrome P_{450}. The epoxides bind covalently with

macromolecules and act as hapten to stimulate immunologic actions, hence lymphocytosis (Gerson *et al*., 1983; Spielberg *et al*., 1986). Lymphocytosis recorded in the PHE-treated group disagrees with the report of Kumar *et al.* (2007) who observed that PHE may suppress mitogen-induced activation of lymphocytes, and resulting in lymphopenia. CBZ+PHE group had the least increase in leucocyte, neutrophil and lymphocyte counts, indicating reduced effects of the combination of the drugs on these parameters. Minimal effects observed with co-administration of CBZ and PHE on haematological parameters compared to the monotherapy groups may be due to the fact that CBZ can reduce the bioavailability of serum PHE (Lai *et al*., 1992)

Sodium ion is involved in fluid balance, nerve functioning, heart activity and other metabolic activities (Myshkin, 2011). The increase in Na^+ concentration in the AED-treated groups agrees with the findings of Porter and Meldrum (2007) who demonstrated an increase in $Na,^+$ but disagrees with that of Kolb and Litt (2002), who reported hyponatraemia following the administration of CBZ and oxcarbamazepine. The result of the present study also disagrees with that of Salawu and Danburam (2007), who obtained hyponatraemia following CBZ administration. According to Liamis *et al.* (2009), PHE has been shown to exert a transient inhibitory effect on antidiuretic hormone (ADH). ADH is released by the posterior pituitary gland and it increases water reabsorption in the collecting duct of the kidneys. PHE has also been shown to reverse the hyponatraemia induced by CBZ therapy. The reason for the decrease in K^+ concentration in the AED-treated groups is not known. However, hypokalaemia may be observed with increased

activity of the adrenal cortex; this will cause the body to reclaim Na^+ from the urine in exchange for K^+ excretion. Thus, high Na^+ concentration was obtained in the present study with relative decrease in serum K^+. The very marginal increase in Cl^- concentration in the anticonvulsant drugs-treated groups relative to the control showed that either of the AEDs or their combination did not alter the concentration of the electrolyte.

The decrease in serum protein concentration in the CBZ and CBZ+PHE groups agrees with the work of Ekaidem *et al.* (2006), and it was attributed to impairment of hepatocellular integrity. This is because hepatocytes constitute the major source of serum proteins. The increase in albumin concentration in the AED-treated groups disagrees with the report of McNamara (2006), who reported extensive binding of both PHE (95%) and CBZ (75%) to serum proteins, particularly albumin. The increase in albumin concentration observed in the present study may be as a result of over-production of cortisol by the adrenal glands (Kaslow, 2011). According to Thakur *et al*. (2011b), the arene oxide metabolites of PHE may cause oxidative stress. CBZ+PHE group had a greater increase in serum albumin, indicating pronounced increase in cortisol production as a result of the synergistic effects of the drugs.

The decrease in globulin concentration in the AED-treated groups agrees with the results of Ashrafi *et al*. (2010) that CBZ therapy caused decrease in immunoglobulin A (IgA) and immunoglobulin G (IgG) levels. Also, PHE was reported to cause an induction of

transient selective IgA deficiency. The decrease globulin may also be due to nephrosis, a condition in which the kidneys do not filter the protein from the blood and it leaks into the urine (Kaslow, 2011). The group co-administered with CBZ and PHE had the greatest decrease in globulin concentration, indicating higher decrease in immunoglobulins A and G. This may be due to the synergistic effects of the drugs.

Urea is an organic chemical compound produced in the liver as a result of the breakdown of proteins or amino acids and ammonia (Bostwick, 2011). The decrease in urea concentration in the AED-treated groups may be due to impaired protein metabolism, apparently due to hepatic dysfunction. This is because the liver converts proteins into urea for excretion. This impairment may be due to persistent assault on the liver by the drugs or their metabolites, since the liver is the site of metabolism and detoxification of the drugs. Co-administration of CBZ and PHE gave the least increase, indicating reduced hepatocellular damage compared to the monotherapy groups. The metabolism of these AEDs results in the formation of toxic epoxides from the action of cytochrome P_{450s}, resulting in hepatic dysfunction (Spielberg, 1986).

There was an increase in ALT activity in all the AED-treated groups. This finding agrees with what was reported by McNamara (2006), who observed moderate elevation of ALT activity with PHE therapy. These changes were transient and may be due in part to induced synthesis of the enzymes. Transient elevation of ALT activity with CBZ therapy may be due to hepatocellular damage. The group co-administered with CBZ and PHE had

a very high ALT activity, demonstrating greater hepatocellular injury. AST activity was found to be the highest in the PHE-treated group, relative to the other AED-treated groups. This indicates that PHE caused more damage to any or all the organs (liver, cardiac and skeletal muscles, kidneys, brain and blood cells), where the enzyme is found. ALP activity was found to be highest in the PHE-treated group; this is an indication that PHE caused damage to either the liver and/or the bone. Increased ALP activity has been attributed to both altered metabolism of vitamin D and the attendant inhibition of intestinal absorption of calcium ion (McNamara, 2006). The increase in ALP in the CBZ group may partly be due to an effect on bone formation, possibly related to an increased bone turnover (Merete *et al.,* 2005). However, the contribution of liver damage to the high ALP activity may be more important. This requires further investigation.

Studies have shown that increases in AST, ALT and ALP activities are more frequent and higher with diphenylhydantoin than with CBZ (Aldenh□vel, 1988); except in the case of AST activity which was higher in the CBZ group, but the ALT and ALP activities were highest in the PHE group in the present study. Ekaidem *et al.* (2006) also reported increased activities of ALT, AST and ALP with long-term PHE therapy in rats and attributed this to hepatocellular damage. Phenytoin, phenobarbital and carbamazepine have been shown to be highly toxic to the skin, liver, brain, kidneys and gastro-intestinal tract (Misra *et al*., 2003).

The LDH enzyme is widely distributed throughout the body; cellular damage causes an elevation of the total serum LDH (Vijay *et al*., 2009). Thus, the estimation of LDH activity provides a quantitative basis for the loss of cell viability and its application in assessing the cytotoxicity of the cell (Decker *et al*., 1988; Adiga *et al*., 1999). The increase in LDH activity in all the AED-treated groups, with PHE having the highest LDH activity, may be as a result of cellular injury to the tissues containing the enzyme; the cells release LDH into the blood stream where it is identified in higher than normal levels (Vijay *et al*., 2009).

Severe adverse reactions occasionally encountered during PHE and CBZ therapy, such as hepatic necrosis (Spielberg, 1986) and aplastic anaemia (Gerson *et al*., 1983) are, apparently, mediated by chemically reactive epoxides, formed by cytochrome P_{450}. Such epoxides may in theory, covalently bind to cell macromolecules and cause genetic and cytotoxic damage and, by acting as haptens, lead to secondary immune reactions. The predisposition to the toxic effects of PHE and CBZ is presumed to be a consequence of an inherited deficiency in the detoxifying enzyme(s) epoxides hydrolase (Riley *et al*., 1989).

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

The study has demonstrated:

A decrease in locomotion and rearing activities in the AED-treated groups, indicating impairment of motor activity. The combination of CBZ and PHE showed a decrease in locomotion which, was mild relative to the monotherapy groups.

The AEDs caused cognitive impairment; apparently due to impairment in neuronal firing and function, involving, especially, the hippocampus. The impairment of cognition was higher in the CBZ+PHE group than that recorded when either of the drugs was administered.

An increase in the body weights of rats was obtained in the AED-treated groups, but the increase was least in the group co-administered with CBZ and PHE.

A decreased RBC concentration in the CBZ group was obtained, apparently due to aplastic anaemia, although the decrease was least in the group co-administered with CBZ and PHE.

Thrombocytosis occurred in rats administered with CBZ, which may be as a result of a compensatory effect on the CBZ-induced thrombocytopenia. Thrombocytosis observed with the polytherapy group was not as pronounced as that obtained in the CBZ group. Leucocytosis recorded may be due to primary bone marrow disorder or cellular inflammation. Also lymhpocytosis recorded in this study may be a contributory factor.

Neutrophilia recorded may be due to cellular inflammation and leucocytosis. CBZ+PHE group had the least increase in leucocyte, neutrophil and lymphocyte counts.

An increase in Na^+ concentration may be due to the inhibitory effect of PHE on ADH and the reversal of the CBZ-induced hyponatraemia. Cl^- concentration was not altered, indicating the AEDs administered did not significantly affect the electrolyte, although the decrease in K^+ concentration obtained may be due to increased activity of the adrenal cortex.

Increase in serum proteins in the PHE group and that recorded in albumin in the AED-treated groups could be ascribed to the hepatocellular damage, and increased cortisol production, respectively but globulin concentration decreased in the AED-treated groups, probably due to decreased immunoglobulins or renal damage. CBZ+PHE group had greater increase in total proteins, when compared to those of the control and CBZ groups and greatest decrease in globulins.

An increase in urea concentration, apparently due to hepatic dysfunction, and the polytherapy group had the least increase.

Increased activities of hepatic transaminases (ALT, AST) and ALP, was recorded which may in part be due to induced synthesis of the hepatic microsomal enzymes. Increased LDH activity was, apparently caused by cellular damage to tissues containing the enzyme. The increase in activities of hepatic transaminases in rats co-administered with CBZ and PHE was relative.

6.2 CONCLUSION

The administration of carbamazepine and/or phenytoin caused cognitive impairment, alterations in neurobehaviour and haemato-biochemical parameters.

6.3 RECOMMENDATION

There should be adequate monitoring of cognitive, neurobehavioural and haemato-biochemical parameters during therapy with either CBZ and/or PHE so as to prevent or reduce the adverse effects of the drugs.

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APPENDICES

Appendix 1.1 Locomotion of rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks

Control group

	Week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	0	0	1	2	0	1	3	0
	1	21	22	22	18	2	11	5
	22	22	0	1	18	12	0	2
	2	0	16	9	9	28	8	8
	7	16	34	0	2	25	0	26
	20	1	0	25	8	16	33	19
	13	16	0	16	4	4	12	12
	1	11	0	0	12	13	6	2
	5	0	0	8	16	10	5	0
	26	0	0	0	3	6	0	5
Mean	9.700	8.700	7.300	8.300	9.000	11.70	7.800	7.900
Std. error	±3.106	±2.985	±3.893	±3.030	±2.140	±2.918	±3.126	±2.747

CBZ group

	Week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	9	1	0	8	1	0	0	0
	15	1	0	3	0	13	0	26
	9	28	4	3	12	0	0	0
	47	18	0	8	0	8	0	5
	4	5	5	0	5	2	10	16
	17	0	0	0	0	0	0	0
	21	0	3	1	2	11	10	16
	17	0	2	0	0	0	0	0
	16	0	0	5	0	9	4	8
	1	0	26	2	0	4	10	4
Mean	15.60	5.300	4.000	3.000	2.000	4.700	2.400	7.500
Std. error	±4.020	±3.081	±2.517	±0.9775	±1.220	±1.613	±1.327	±2.849

PHE group

	Week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	26	0	0	1	10	0	20	0
	2	8	0	0	4	0	0	4
	7	11	9	0	0	3	4	1
	27	1	4	4	20	12	32	24
	14	0	0	0	0	2	0	0
	7	8	11	0	1	0	0	1
	0	0	2	34	1	0	0	1
	0	0	0	0	0	0	0	0
	0	1	4	0	0	0	0	0
	14	29	8	24	8	0	0	12
Mean	9.700	5.800	3.800	6.300	4.400	2.100	5.600	4.300
Std. error	±3.263	±2.898	±1.323	±3.876	±2.077	±1.197	±3.583	±2.481

CBZ+PHE group

	Week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	37	0	0	4	1	8	4	0
	16	32	5	2	1	0	12	0
	21	0	8	21	2	2	22	4
	33	0	26	1	13	12	14	8
	56	30	16	56	19	10	24	3
	38	0	15	1	0	0	0	17
	15	3	8	0	22	17	4	20
	22	30	13	18	16	18	24	0
	19	25	2	28	11	20	0	
Mean	28.56	13.33	10.33	14.56	9.444	9.667	11.56	6.500
Std. error	±4.513	±5.080	±2.693	±6.236	±2.873	±2.593	±3.346	±2.803

Appendix 1.2 Rearing activity of rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

Control group

	Week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	0	1	0	1	1	2	0	0
	0	0	3	0	1	1	1	1
	0	2	2	1	0	1	0	1
	0	0	3	1	2	2	1	0
	5	3	6	1	0	4	0	4
	8	4	1	4	7	7	19	6
	6	3	2	1	4	2	2	0
	0	0	1	0	1	0	1	0
	1	0	0	0	0	1	1	0
	9	0	0	1	0	2	0	0
Mean	2.900	1.300	1.800	1.000	1.600	2.200	2.600	1.200
Std. error	±1.169	±0.4955	±0.5925	±0.3651	±0.7180	±0.6289	±1.833	±0.6633

CBZ group

	Week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	3	0	0	1	0	0	0	0
	5	0	0	1	1	2	0	6
	3	5	3	0	0	0	1	1
	9	3	0	2	1	4	1	2
	0	2	1	0	0	5	0	0
	5	0	0	0	0	0	0	0
	4	0	2	0	0	0	2	4
	5	0	1	0	0	1	0	0
	3	0	0	1	1	3	0	2
	0	0	9	0	0	2	1	8
Mean	3.700	1.000	1.600	0.5000	0.3000	1.700	0.5000	2.300
Std. error	±0.8307	±0.5578	±0.8844	±0.2236	±0.1528	±0.5783	±0.2236	±0.8950

PHE group

	Week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	3	0	0	0	0	0	0	1
	3	1	0	0	0	1	0	3
	4	3	4	0	2	0	0	0
	1	1	1	1	0	0	1	3
	2	1	0	0	0	0	0	0
	1	1	3	0	0	0	1	3
	0	0	1	6	1	0	0	1
	0	1	0	0	0	0	0	0
	0	1	2	0	2	0	0	0
	0	1	1	0	1	0	0	0
Mean	1.400	1.000	1.2000	0.7000	0.6000	0.1000	0.2000	0.8000
Std. error	±0.4761	±0.2582	±0.4422	±0.5972	±0.2667	±0.1000	±0.1333	±0.3887

CBZ+PHE

	Week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	0	0	0	0	0	0	0	0
	2	2	0	1	0	0	2	0
	6	2	0	0	0	1	3	2
	6	1	6	1	4	5	4	0
	3	3	2	2	2	1	0	0
	1	0	2	1	0	0	0	2
	4	1	0	0	4	4	2	4
	3	7	2	3	3	0	2	0
		5	1	3	2	4	0	
Mean	4.111	2.333	1.444	1.222	1.333	1.889	1.222	1.000
Std. error	±0.6550	±0.7817	±0.6479	±0.4006	±0.5774	±0.6550	±0.5212	±0.5345

Appendix 1.3 Body weight changes of rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

Control

	Week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	165	162	160	170	167	177	157	176
	189	189	200	209	211	222	200	232
	156	157	156	166	166	178	168	186
	144	151	161	178	179	190	175	196
	176	188	198	228	235	258	264	284
	178	166	174	184	186	185	185	200
	181	185	183	190	185	183	185	196
	176	175	170	175	179	174	181	184
	178	176	180	199	191	184	184	197
	198	204	203	216	226	223	232	251
Mean	174.1	175.3	178.5	191.5	192.5	197.4	193.1	210.2
Std. error	±4.938	±5.224	±5.490	±6.600	±7.519	±8.736	±10.12	±10.91

CBZ

	Week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	211	217	219	243	201	244	258	280
	186	187	185	194	210	187	189	203
	195	201	198	211	199	198	213	222
	179	183	178	195	190	184	189	197
	164	168	166	172	210	172	167	174
	189	193	190	185	201	209	215	223
	240	247	257	266	292	294	303	329
	177	185	187	195	209	209	201	216
	203	209	209	205	212	219	225	234
	190	191	188	202	216	220	222	233
Mean	193.4	198.1	197.7	206.8	214.0	213.6	218.2	231.1
Std. error	±6.669	±6.977	±8.110	±8.811	±8.999	±11.08	±12.25	±13.97

PHE

	Week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	204	201	197	212	204	219	223	227
	198	199	201	206	210	213	225	233
	197	199	198	204	199	204	212	218
	191	200	200	196	190	204	213	218
	207	216	211	213	210	224	231	244
	239	253	245	260	255	271	271	284
	183	194	190	195	182	204	190	203
	203	202	183	204	202	218	212	224
	214	222	226	229	228	249	232	253
	204	199	208	215	207	219	213	228
Mean	204.0	208.5	205.9	213.4	208.7	222.5	222.2	233.2
Std. error	±4.749	±5.648	±5.716	±6.048	±6.452	±6.846	±6.648	±7.160

CBZ+PHE

	Week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
	248	255	254	217	203	199	175	226
	254	220	244	257	263	270	239	186
	218	220	210	223	222	223	202	207
	226	233	226	241	248	256	235	253
	301	292	286	296	296	304	272	245
	198	203	195	204	229	237	241	228
	211	217	217	229	204	216	225	220
	179	178	173	189	183	194	209	228
	164	178	169	174	193	204	219	236
	165	195	188	199	201	219	238	248
Mean	216.4	222.5	216.2	222.9	224.2	232.2	225.5	227.7
Std. error	±13.67	±11.61	±11.81	±11.24	±11.22	±11.08	±8.332	±6.368

Appendix 1.4 Changes in Learning ability in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	4	2	1	3
	1	3	2	2
	2	3	2	3
	2	1	1	4
	3	3	2	2
	3	2	1	2
	1	2	4	4
	1	2	2	1
	1	2	4	
	1	4	2	
Mean	1.900	2.400	2.100	2.625
Std. error	±0.3480	±0.2667	±0.3480	±0.3750

Appendix 1.5 Changes in Short-memory in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	120	120	120	75
	120	120	120	23
	114	120	108	21
	120	120	120	26
	120	37	23	26
	120	120	120	48
	120	120	14	120
	120	120	120	120
	120	120	120	
	120	22	120	
Mean	119.4	101.9	98.50	57.38
Std. error	±0.6000	±12.12	±13.40	±15.07

Appendix 1.6 Changes in values of Packed Cell Volume in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	45.3	45.3	44.1	44.4
	31	48.3	34.2	45.6
	37	41.4	45.6	44.7
	43	41.7	42.6	
		45.6	48	
Mean	39.08	44.46	42.90	44.90
Std. error	±3.210	±1.299	±2.351	±0.3606

Appendix 1.7 Changes in Red Blood Cell Counts in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	31	40	42	34
	58	10	41	41
	60	29	38	22
	63	30	41	
		19	39	
Mean	53.00	25.60	40.20	32.33
Std. error	±7.405	±5.124	±0.7348	±5.548

Appendix 1.8 Changes in Haemoglobin concentration in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	15.1	15.1	14.7	14.8
	10.3	16.1	11.4	15.2
	12.3	13.8	15.2	14.9
	14.3	13.9	14.2	
		15.2	16	
Mean	13.00	14.82	14.30	14.97
Std. error	±1.075	±0.4329	±0.7836	±0.1202

Appendix 1.9 Changes in Platelet Counts in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	652	788	334	489
	510	763	529	603
	560	512	631	692
	635	581	650	
		755	545	
Mean	589.3	679.8	537.8	594.7
Std. error	±33.12	±55.77	±56.10	±58.75

Appendix 1.10 Changes in Total Leucocyte Counts (WBC) in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	11.37	10.6	11.04	14.2
	7	39.02	15.01	10.49
	6.25	13.05	13.2	11.01
	4.4	12.21	14.93	
		19.06	17.32	
Mean	7.2	19	14	12
Std. error	±1.5	±5.3	±1.0	±1.2

Appendix 1.11 Changes in Lymphocyte Counts in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	7.8	6.4	6.4	9.4
	2.9	35.1	8.9	6.2
	2.5	9.3	8.2	8.6
	1.6	7.8	8.8	
		15.4	10.6	
Mean	47.00	73.20	59.80	67.67
Std. error	±7.450	±5.508	±0.7348	±5.548

Appendix 1.12 Changes in Neutrophil Counts in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	3.5	4.2	4.6	4.8
	4	3.9	6.2	4.3
	3.7	3.8	5	2.4
	2.7	3.7	6.1	
		3.6	6.8	
Mean	53.00	25.60	40.20	32.33
Std. error	±7.405	±5.124	±0.7348	±5.548

Appendix 1.13 Changes in Sodium ion Concentration in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	136	141	140	138
	119	137	133	140
	101	140	142	140
	198	138	137	
		140	141	
Mean	111.0	139.2	138.6	139.8
Std. error	±4.062	±0.7348	±1.631	±0.6633

Appendix 1.14 Changes in Potassium ion Concentration in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	4.1	3.7	4.7	3.6
	7.9	3.3	4	4.1
	4.6	4.2	5	4.4
	6.8	3.9	4.3	
		4.5	3.8	
Mean	5.9	3.9	4.4	4.0
Std. error	±0.90	±0.21	±0.22	±0.14

Appendix 1.16 Changes in Chloride ion Concentration in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	96	98	100	98
	104	100	96	104
	100	100	98	96
	93	96	100	
		98	102	
Mean	98.25	98.40	99.20	99.20
Std. error	±2.394	±0.7483	±1.020	±1.356

Appendix 1.17 Changes in Urea Concentration in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	4.1	3.3	2.8	4.3
	10	5.5	4.1	5
	7	3.2	3.6	4.7
	7	4.5	3	
		3	3.6	
Mean	6.5	3.9	3.4	4.4
Std. error	±1.1	±0.48	±0.23	±0.19

Appendix 1.18 Changes in Total Proteins in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	69	72	69	68
	52	66	77	71
	97	71	68	66
	56	58	72	60
		60	69	69
Mean	69	65	71	67
Std. error	±10	±2.8	±1.6	±1.9

Appendix 1.19 Changes in Albumin Concentration in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	33	41	36	33
	12	30	44	40
	17	38	33	35
	10	30	40	32
Mean	18	34	37	35
Std. error	±5.2	±2.5	±2.0	±1.4

Appendix 1.120 Changes in Globulin Concentration in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	40	31	24	35
	80	36	33	31
	46	33	35	31
	36	28	32	28
		31	35	28
Mean	50.50	31.80	31.80	30.60
Std. error	±10.05	±1.319	±2.035	±1.288

Appendix 1.21 Changes in Alanine aminotransferase activities in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	36	38	42	33
	18	27	36	41
	23	41	24	44
	17	44	29	37
		38	27	41
Mean	23.50	37.60	31.60	39.20
Std. error	±4.368	±2.874	±3.265	±1.908

Appendix 1.22 Changes in Aspartate aminotransferase activities in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	16	22	22	18
	95	20	26	12
	96	29	33	26
	205	24	24	24
		18	20	22
Mean	14	23	25	20
Std. error	±2.7	±1.9	±2.2	±2.5

Appendix 1.23 Changes in Alkaline phosphatase activities in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	51	47	58	69
	26	44	68	48
	247	80	60	40
	636	71	63	58
		43	55	76
Mean	41	57	61	58
Std. error	±9.6	±7.7	±2.2	±6.6

Appendix 1.24 Changes in Lactate dehydrogenase activities in rats administered CBZ, PHE and a combination of CBZ and PHE (Mean ±SEM) for a period of 8 weeks.

	Control group	CBZ group	PHE group	CBZ+PHE group
	85	105	117	89
	75	100	105	105
	93	96	98	131
	88	80	67	89
		64	150	97
Mean	85	89	107	102
Std. error	±3.8	±7.5	±1.3	±7.8