MODULATORY EFFECT OF PIRACETAM IN ISCHAEMIC BRAIN INJURY ON THE CEREBRUM, CEREBELLUM AND HIPPOCAMPUS OF WISTAR RAT

BY

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DECLARATION

I hereby declare that the work in the dissertation entitled "Modulatory Effect of Piracetam in Ischaemic Brain Injury on the Cerebrum, Cerebellum and Hippocampus of Wistar Rat" has been carried out by me in the Department of Human Anatomy under the supervision of Professor S.A. Ojo and Professor M.A. Mabrouk

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

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CERTIFICATION

I certify that this work titled "Modulatory Effect of Piracetam in ISCHAEMIC Brain Injury on the Cerebrum, Cerebellum and Hippocampus of Wistar Rat" was carried out by LAWAL Ahmed AbdulMumuni of the Department of Human Anatomy, Ahmadu Bello University Zaria, under my supervision.

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DEDICATION

This dissertation is dedicated to all those with impairment from ISCHAEMIC brain injury and to those whose lives and careers are dedicated to solving this menace.

Indeed, no riddle can outlast persistent human research…

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ABSTRACT

Ischaemic brain injuries, that results from stroke, are common and often lead to permanent residual disabilities. This work investigates the role Piracetam, a nootrophic drug, played in modulation of induced brain injury to key areas of the brain; cerebrum, cerebellum and hippocampus of adult Wistar rats. Drugs like Vitamin C, Vitamin E and corticosteroids have been known to improve damage resulting from ischaemic damage to the brain. Piracetam, like wise has been known to improve higher brain functions such as memory and mental alertness. The effect of paracetam was investigated on twenty wistar rats that were divided into five groups of four animals each. Ischaemic brain injury was then induced by transient bilateral occlusion of the carotid arteries. Motor function and memory was assessed and comparison made between those animals that did not receive any drugs and those that received Piracetam and or vitamin E. Serum and brain malonylaldehyde (MDA) levels, histological assessment of cerebrum, cerebellum and hippocampal brain were studied in animals in the various groups. Results show that the percentage and grade of excitability score, forepaw grip time and transfer latency period in an elevated plus maze was higher for those animals that received vitamin E or a combination of vitamin E and Piracetam when compared with those that received Piracetam only. The differences were however not statistically significant. Serum malonylaldehyde was found to be significantly higher in those animals that did not receive any drugs following induction of ischaemia when compared with those that received either Piracetam, vitamin E or a combination of the two drugs. Furthermore serum malonylaldehyde levels were significantly higher in those groups that received vitamin E when compared to those that received Piracetam only. Brain tissue malonylaldehyde levels were found to be higher in those animals that received Piracetam only when compared with the group that received a combination of the two drugs. When vitamin E alone was administered, malonylaldehyde levels in the brain were higher when compared to the group that received Piracetam only. Photomicrographs of the cerebral cortex, cerebellum and hippocampus showed marked evidence of neurodegeneration in the untreated group compared to those groups that received drug treatment. Among the groups treated, neuronal cell ischaemic changes were more evident in the group treated with Piracetam alone when compared with the groups treated with Piracetam and or vitamin E.

TABLE OF CONTENTS

Title page - - - - - - - - - i

Declaration - - - - - - - - - ii

Certification - - - - - - - - - iii

Dedication - - - - - - - - - iv

Acknowledgement - - - - - - - - - v

Abstract - - - - - - - - - vi

Table of contents - - - - - - - - - vii

List of tables - - - - - - - - - ix

List of figures - - - - - - - - - x

List of plates - - - - - - - - - xi

List of acronyms - - - - - - - - - xiv

Chapter One – Introduction - - - - - - - - - 1

1.1 Justification- - - - - - - - - - 3

1.2 Aim and Objective - - - - - - - - 5

1.3 Hypothesis - - - - - - - - 5

Chapter Two – Literature Review - - - - - - - - 6

2.1 Piracetam - - - - - - - - - - 6

2.1.1 Uses of Piracetam - - - 7

2.1.2 Mechanism of action of Piracetam - 9

2.1.3 Toxicity of Piracetam - - - 11

2.2 Ischaemia - - - - - - - - - 12

2.3 Brain - - - - - - - - - 19

2.4 Vertebrate Brain - - - - - - - 19

2.5 Human Brain - - - - - - - - 20

2.6 Memory - - - - - - - 24
2.7 Stroke - - - - - - - 25
2.7.1 Pathophysiology of Stroke - - 26
2.8 Wistar Rat Brain - - - - - - 29
Chapter Three – Materials and Methods - - - - - 36
3.1 Materials - - - - - - - 36
3.1.1 Instruments - - - - 36
3.1.2 Chemicals and Drugs - - - 36
3.1.3 Animals - - - - 36
3.2 Methods - - - - - - - 36
3.2.1 Design of the Study - - - 36
3.2.2 Experimental Induction of Ischaemia - 37
3.2.3 Apparatus for Physiological Test - 38
3.2.4 Assessment of Lipid Peroxidation - 38
Chapter Four – Results - - - - - - - 40
Chapter Five – Discussion - - - - - - - 96
5.1 Discussion - - - - - - - 96
Chapter Six – Conclusion and Recommendation - - - - - 100
6.1 Conclusion - - - - - - - 100
6.2 Recommendation - - - - - - 101
References - - - - - - - - - 102
Appendices - - - - - - - - - 105

LIST OF TABLES

1. **Table 4.1** Mean serum and brain tissue malonylaldehyde levels for wistar rats treated with Piracetam and vitamin E following induction of global ischaemia - - - - - - 40

LIST OF FIGURES

1. **Figure 4.1** Percentage excitability and grade of reaction in wistar rats following induction of ischaemia and treatment with Piracetam and vitamin E - - - - - - 42
2. **Figure 4.2** Forepaw grip time of wistar rats treated with Piracetam and vitamin E following induction of global ischaemia - 44
3. **Figure 4.3** Transfer latency time of wistar rats in an elevated plus maze after treatment with Piracetam and vitamin E following induction of global ischaemia - - - - - - 46

LIST OF PLATES

1. **Plate 1** Photomicrograph of hippocampus of wistar rats in the control group H&E stain - - - - - 49
2. **Plate 2** Photomicrograph of hippocampus of wistar rats in the control group cfv stain - - - - - - 51
3. **Plate 3** Photomicrograph of hippocampus of wistar rats in group II H&E stain - - - - - - - - - 52
4. **Plate 4** Photomicrograph of hippocampus of wistar rats in group II H&E stain - - - - - - - - - 54
5. **Plate 5** Photomicrograph of hippocampus of wistar rats in group II cfv stain - - - - - - - - - 55
6. **Plate 6** Photomicrograph of hippocampus of wistar rats in group III H&E stain - - - - - - - - - 56
7. **Plate 7** Photomicrograph of hippocampus of wistar rats in group III cfv stain - - - - - - - - - 58
8. **Plate 8** Photomicrograph of hippocampus of wistar rats in group IV H&E stain - - - - - - - - - 59
9. **Plate 9** Photomicrograph of hippocampus of wistar rats in group IV cfv stain - - - - - - - - - 61
10. **Plate 10** Photomicrograph of hippocampus of wistar rats in group V H&E stain - - - - - - - - - 62
11. **Plate 11** Photomicrograph of hippocampus of wistar rats in group II cfv stain - - - - - - - - - 64

12. Plate 12 Photomicrograph of cerebrum of wistar rats in control group H&E stain - - - - - - - 65

13. Plate 13 Photomicrograph of cerebrum of wistar rats in control group cfv stain - - - - - - - 67

14. Plate 14 Photomicrograph of cerebrum of wistar rats in group II H&E stain - - - - - - - - - 68

15. Plate 15 Photomicrograph of cerebrum of wistar rats in group II cfv stain - - - - - - - - - 70

16. Plate 16 Photomicrograph of cerebrum of wistar rats in group III H&E stain - - - - - - - - - 71

17. Plate 17 Photomicrograph of cerebrum of wistar rats in group III cfv stain - - - - - - - - - 73

18. Plate 18 Photomicrograph of cerebrum of wistar rats in group IV H&E stain - - - - - - - - - 74

19. Plate 19 Photomicrograph of cerebrum of wistar rats in group IV cfv stain - - - - - - - - - 76

20. Plate 20 Photomicrograph of cerebrum of wistar rats in group V H&E stain - - - - - - - - - 77

21. Plate 21 Photomicrograph of cerebrum of wistar rats in group V cfv stain - - - - - - - - - 79

22. Plate 22 Photomicrograph of cerebellum of wistar rats in group I H&E stain - - - - - - - - - 80

23. Plate 23 Photomicrograph of cerebellum of wistar rats in group I H&E stain - - - - - - - - - 82

24. Plate 24 Photomicrograph of cerebellum of wistar rats in group I cfv stain - - - - - - - - - 83

25. Plate 25 Photomicrograph of cerebellum of wistar rats in group II H&E stain - - - - - - - - - 84

26. Plate 26 Photomicrograph of cerebellum of wistar rats in group II H&E stain - - - - - - - - - 86

27. Plate 27 Photomicrograph of cerebellum of wistar rats in group II cfv stain - - - - - - - - - 87

28. Plate 28 Photomicrograph of cerebellum of wistar rats in group II cfv stain - - - - - - - - - 88

29. Plate 29 Photomicrograph of cerebellum of wistar rats in group III H&E stain - - - - - - - - - 89

30. Plate 30 Photomicrograph of cerebellum of wistar rats in group III H&E stain - - - - - - - - - 91

31. Plate 31 Photomicrograph of cerebellum of wistar rats in group IV H&E stain - - - - - - - - - 92

32. Plate 32 Photomicrograph of cerebellum of wistar rats in group V H&E stain - - - - - - - - - 94

LIST OF ABBREVIATIONS

AMPA	α – amino – 3 – hydroxy – 5 – methyl – 4 – isoxazole – propionic acid
ATP	Adenine triphosphate
CVA	Cerebro vascular accident
DVM	Doctor of veterinary Medicine
FCVSN	Fellow college of veterinary surgeons of Nigeria
FDA	U.S Food and drug administration
GABA	γ-Aminobutyric acid
MBA	Master in business administration
MBBch	Bachelor of Medicine, Bachelor of Surgery
MD	Doctor of Medicine
MDA	Malonylaldehyde
M.Sc	Master of science
NRC	National research council (U.S)
PhD	Doctor of Philosophy

CHAPTER ONE

INTRODUCTION

ischaemia is literally translated from Greek meaning a restriction in blood supply, with resultant damage or dysfunction of tissue (Lionel, 1990). This is different from hypoxia, a general term denoting oxygen shortage which usually results from lack of oxygen in the air.

ischaemia can be defined as an absolute or relative shortage of the blood supply to an organ (Klabunde, 2004). ischaemia does not occur without hypoxia, while the reverse is not the case. This implies that in ischaemia, there is a shortage of oxygen as well as glucose and other blood-borne nutrients and other materials. This mismatch in availability of oxygen and nutrients, in comparison to their need in the organisms metabolism results in more severe damage than that caused by hypoxia, because of the potential for toxic metabolic wastes to build up in the tissues. ischaemia commonly results from inadequate flow of blood to a part of the body, caused by constriction or blockage of the blood vessels supplying it (Hossmann, 1998). It is through such mechanism that stroke commonly occurs.

Neuronal cell damage is generally a potentially serious problem for two reasons: First, nerve cells do not regenerate: therefore neuronal cells with the exception of neural stem cells and a few other types of neurons, do not undergo mitotic cell division(Ruggiero *et al*., 2012). In most cases, neurons are generated by special types of stem cells. Astrocytes, a type of glial cell, have also been observed to turn into neurons by virtue of the stem cell characteristic pluripotency (Addis *et al*., 2011). In humans, neurogenesis largely ceases during adulthood. Only for two brain areas, the hippocampus and olfactory

bulb, is there strong evidence for generation of substantial numbers of new neurons (Gould *et al*., 1999). Secondly, brain function directly controls the activity in other parts of the body, thus, neuronal cell damage in a small region of the brain can significantly hamper activity and may even threaten life (Schmued *et al*., 2000).

Severe or prolonged brain ischaemia will result in unconsciousness, brain damage or death, mediated by the ISCHAEMIC cascade. Multiple cerebral ISCHAEMIC events may lead to subcortical ISCHAEMIC depression, also known as vascular depression. Late onset depression is increasingly seen as a distinct sub-type of depression, and can be detected with an MRI (Baldwin, 2004). ischaemia leads to alterations in brain metabolism, reduction in metabolic rates, and energy crisis.

There are two types of ischaemia: focal ischaemia, which is confined to a specific region of the brain; and global ischaemia, which encompasses wide areas of brain tissue. Their main symptoms involve impairments in vision, body movement, and speaking. The causes of brain ischaemia vary from sickle cell anemia to congenital heart defects.

Restoration of blood flow after a period of ischaemia can actually be more damaging than the ischaemia. Moreover, reintroduction of oxygen causes a greater production of damaging free radicals, resulting in reperfusion injury. With reperfusion injury, necrosis can be greatly accelerated (Jie *et al*., 2007).

Piracetam (2-oxo-1-pyrrolidine acetamide), is the first of the nootrophic drugs. This class of drugs affects mental function.

Piracetam was first synthesized in 1964 by the Belgian pharmaceutical company UCB (Shorvon, 2004). It has been shown to improve higher functions of the brain like learning

and memory, but, does not cause sedation, nor stimulate the central nervous system. Relatively few side effects are known. In the words of C.E. Giurgea, UCB's principal Piracetam researcher, nootropic drugs like Piracetam should be able to:

- Enhance learning and memory.
- Enhance the resistance of learned behaviors/memories to conditions which tend to disrupt them (e.g. hypoxia).
- Protect the brain against various physical or chemical injuries (e.g. barbiturates).
- Increase the efficacy of the tonic cortical/subcortical control mechanisms.
- Lack the usual pharmacology of other psychotropic drugs, Possess very few side effects and have extremely low toxicity (Gamzu *et al*., 1989).

However its mechanism of action is still generally unclear (Winblad, 2005). Complications of chronic diseases such as hypertension and diabetes, cardiovascular diseases and traumatic head injuries can commonly result to stroke which can be easily fatal. This study is designed to investigate outcome of ISCHAEMIC damage to the brain when Piracetam alone and in combination with a known anti oxidant, vitamin E is administered immediately following the induction of brain ischaemia.

1.1 JUSTIFICATION

According to the World Health Organization, 15 million people suffer stroke worldwide each year. Of these, 5 million die and another 5 million are permanently disabled.

Stroke is the third most common cause of death in the United States (US). More than 140,000 people die each year from stroke in the United States. Stroke also results in substantial health-care expenditures; the mean lifetime cost resulting from an

ISCHAEMIC stroke is estimated at $140,000 per patient in the US (Rosamond *et al*., 2007).

Europe averages approximately 650,000 stroke deaths each year (Redon *et al*., 2011). Comprehensive stroke surveillance data for Africa are lacking, the available data show that age-standardized mortality, case fatality, and prevalence of disabling stroke in Africa are similar to or higher than those measures in most high-income regions (Mensah, 2007).

The current prevalence of stroke in Nigeria is 1.14 per 1000 while the 30-day case fatality rate is as high as 40%. Management of the disease is largely conservative (Wahab, 2008).

Despite this heavy burden stroke has placed on society, the search for a ‘‘magic bullet’’ for the treatment of stroke or traumatic brain injury (TBI) has failed thus far. This is partly because the pathophysiology of ISCHAEMIC brain injury and TBI involves a number of mechanisms leading to neuronal injury, including excitotoxicity, free radical damage, inflammation, necrosis, and apoptosis (Sayeed & Stein, 2009).

The common pathway for all these factors in causing brain ischaemia is loss of cell membrane integrity.

A popular hypodissertation concerning Piracetam is it restores cell membrane fluidity following an insult (Muller, 1999; Winblad, 2005). This is the basis for which it is used in a wide range of diseases affecting many different organ systems.

This investigation is therefore pertinent to define the role Piracetam can play in modulating brain injury in stroke especially in developing countries like Nigeria.

1.2 AIM AND OBJECTIVES

Aim

The aim of this study is to determine the effect of Piracetam on ISCHAEMIC brain injury in Wistar rats when used alone and compare with its effect when used in conjunction with vitamin E which is a known anti oxidant.

Objectives

1. To assess the effect of Piracetam and or vitamin E on malonylaldehyde in blood and brain tissue of ISCHAEMIC Wistar rats
2. To study the effect of transient ischaemia on the cytoachitecture of cerebrum, cerebellum and hippocampus of Wistar rats
3. To determine the effect of Piracetam and or vitamin E on the cytoachitecture of cerebrum, cerebellum and hippocampus of ISCHAEMIC Wistar rats
4. To observe the effect of Piracetam and or vitamin E on memory and motor activity of ISCHAEMIC Wistar rats

1.3 HYPOTHESIS

ISCHAEMIC brain injury can be modulated by Piracetam and or Vitamin E.

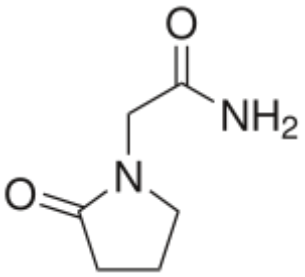
Null Hypotheses

ISCHAEMIC brain injury is not modulated by Piracetam and or vitamin E.

CHAPTER TWO

LITERATURE REVIEW

2.1 Piracetam



Molecular formula **$C_6H_{10}N_2O_2$**

Piracetam is a Nootrophic drug. It has the chemical structure represented above and has the chemical name, 2-oxo-1-pyrrolidine acetamide. It also has the same 2-oxo-pyrrolidine base structure as 2-oxo–pyrrolidine carboxylic acid (pyroglutamate).

Piracetam is a derivative of γ-Aminobutyric acid (GABA). GABA is chemically an amino acid that is not incorporated into a protein but serves as an inhibitory neurotransmitter in mammals. In humans GABA is responsible for the control of muscle tone (Watanabe *et al*., 2002).

Piracetam is also one of the groups of drugs that are termed Racetams. These are a class of nootrophic drugs that share the presence of a pyrrolidine ring as a common feature. Other examples of Racetams include: Aniracetam, Brivaracetam, Coluracetam, Dimiracetam, Etiracetam, Fasoracetam, Imuracetam, Levetiracetam, Nebracetam, Nefiracetam, Nicoracetam, Oxiracetam, Phenylpiracetam, Pramiracetam, Rolipram, Rolziracetam and Seletracetam.

Racetams are used in a wide variety of conditions including, epilepsy, psychosis, depression, ISCHAEMIC retinopathy, retinal and optic nerve injuries and neuropathic pain. Some are employed as anti inflammatory agents. However the mechanism of action of racetams in general is unclear (Gualtieri *et al*., 2002).

Piracetam is not a prescription drug. It is sold over the counter under a wide variety of trade names. Some of these names are listed in table 1 below. The extent of the variety suggests that it is common in use.

2.1.1 Uses of Piracetam

Piracetam has been held to improve memory in normal individuals for a long time (Dimond and Brouwers, 1976). Indeed, it is this ability to improve memory and other cognitive functions of the brain, combined with little known side effects that caused Dr Corneliu E. Giurgea to use the term 'nootrophic' in describing Piracetam and other substances. Until recently, for this reason, Piracetam was advertised as a food supplement in the United State of America (FDA, 2010) and many people, especially students still use it off-label.

The drug however is used in a wide variety of clinical settings with well documented efficacy.

It is used for example in cognitive disorders especially as affects the elderly in normal aging. Chouinard *et al*. in 1983 demonstrated that Piracetam improved mental alertness, cooperation and socialization in elderly people with known psychiatric illnesses including Schizophrenia without any effect on the psychiatric illness itself and its drug treatment.

Several other authors have subsequently reported similar beneficial uses of Piracetam in cognitive disorders of the elderly (Flicker and Grimley, 1997).

Piracetam is also used in vertigo. Vertigo is derived from the Latin word *vertō* which means "a whirling or spinning movement". It is a type of dizziness in which the individual feels a false spinning motion of self or environment. It results from disturbances in the vestibular system which may be peripheral (e.g., abnormalities in the vestibular apparatus) or central (e.g., cerebrovascular disease). Lack of sensory input from proprioceptive or visual sources can also contribute to symptoms. The clinical benefit of Piracetam in vertigo may result from its effects on neurotransmission and microcirculation. In a double-blind study of 143 elderly patients with chronic vertigo of central, peripheral or unspecified origin, Piracetam 2.4g /day was compared with placebo (Rosenhall *et al*., 1996). Several other studies indicated efficacy of Piracetam on vertigo of central, peripheral, or mixed origin (Oosterveld, 1996). Piracetam is also effective in the treatment of postconcussional vertigo (Hakkarainen and Hakamies, 1978).

Piracetam is used in the treatment of cortical myoclonus. This is a type of epilepsy that originates in the cerebral cortex. Abnormal electrical activity in the sensorimotor cortex causes uncontrollable jerking which usually involve only a few muscles in one part of the body. Jerks may also involve many muscle groups. Cortical myoclonus can be intensified when patients attempt to move in a certain way or perceive a particular sensation.

Piracetam has been reported to cause more marked improvements when used in the treatment of cortical myoclonus compared to when used in the treatment of other forms of myoclonus (Kojovic *et al*., 2011).

The use of Piracetam in the treatment of dyslexia is controversial. Dyslexia is a learning disability characterized by a specific difficulty in interpreting written language despite adequate intelligence and normal vision. It is often accompanied by problems with writing or spelling. Several double-blind studies have investigated Piracetam in the treatment of dyslexia, and, while the findings are not entirely consistent, most report a significant effect of Piracetam relative to placebo on reading speed and or accuracy. in a multicenter, 36-week, double-blind, placebo-controlled study among two hundred and twenty-five dyslexic children, it was shown that reading skills significantly improved following 12 weeks of treatment and this was sustained for the rest of the period of the experiment (Wilsher *et al*., 1987).

In sickle cell anemia, pain crises usually arise from occlusion of blood vessels following sickling of blood cells. Studies have suggested that Piracetam is useful as a remedy during sickle cell disease painful crises (Al Hajeri *et al*., 2007).

2.1.2 Mechanism of action of Piracetam

Hypotheses abound about the possible mechanism of action of Piracetam, but up till now, there is no universally acceptable explanation to how Piracetam has an effect on such a wide range of conditions with completely differing pathophysiology.

First and foremost, Piracetam has been shown unequivocally to affect higher mental function without acting either as a sedative or as a stimulant (Winblad, 2005). This ability quite differs from most other known drugs that influence the cognitive function of the brain. Secondly, it has been demonstrated that Piracetam can act at the vascular, cellular or at the molecular level to achieve results. For example Winblad in 2005, reported that at

the vascular level, Piracetam can prevent vasospasm while at a cellular level, it influences membrane stability. This is why it is thought that its mechanism of action must at least in part involve maintenance or restoration of cell membrane fluidity. Since such action can neither be cell nor organ specific it can explain its use in such a wide variety of clinical situations.

Workers demonstrated that the calcium channel blockers Nifedipine and Cadmium ion cause a weakening or reversal of the effects of Piracetam in an in vitro study using snail brain cells (Verbnyi *et al*., 1996). This suggests that actions of Piracetam were as a direct effect of influence on calcium ion channels.

Glutamate receptors are the most abundant excitatory receptors in the vertebrate brain. They mediate important higher functions of the brain such as learning and memory. They are also involved in disease processess like Alzheimer's disease, Parkinson's disease, epilepsy and ischaemic brain damage. α – amino – 3 – hydroxy – 5 – methyl – 4 – isoxazole – propionic acid (AMPA) is a synthetic glutamate receptor agonist. Researchers have shown that Piracetam has a positive allosteric modulatory influence on AMPA receptors (Ahmed and Robert, 2010). AMPA receptors are transmembrane receptors. This gives further credence to the theory that the influence of Piracetam is on cell membranes.

In a review of over 400 publications published over a period of more than 25 years, Gouliaev and Senning concluded that racetams including Piracetam must act by influencing ion exchange across cell membranes (Gouliaev and Senning, 1994). All these evidences suggest strongly that the mechanism of action of Piracetam has to do with its

ability to influence the cell membrane of cells. This, no doubt will mean that whatever effect is eventually observed will partly be due to the fact that it somehow gives stability and increases the integrity and viability of the cell membrane in the first place.

In the brain, Piracetam has been shown to improve the overall blood flow and also oxygen utilization of the brain. Such activity cannot be attributed to a single molecular activity such as action on cell membranes. Some researchers believe that such ability is a fall out from its effect on general increase in brain activity rather than on a specific mechanism of action on neuronal cells or vascular endothelium (Jordaan *et al*., 1996). This increase in blood flow to the brain and increased utilization of oxygen, whatever its mechanism of action must increase the ability to cope with healing in the face of injurious stimuli.

2.1.3 Toxicity of Piracetam

Piracetam is probably one of the safest drugs ever made. In acute toxicological studies, rats given 8gm/Kg intravenously and mice, rats and dogs treated with 10gm/Kg orally, failed to achieve an LD_{50} (Gouliaev and Senning, 1994).

This calculated is equivalent to giving a 70kg man 700gm per dose. Asides from this, Piracetam has actually been demonstrated to reverse the damaging effects on the brain of a wide range of chemicals and toxic agents e.g. Alcohol (Paula- Barbosa *et al*., 1991), oxydipentonilim, Hexachlorophene (Andreas, 1993), secobarbital (Grau *et al*., 1987), severe hypoxia, electroconvulsive shock and so on.

At present, doses used in Piracetam vary from 40-100mg/kg with the lower end of the range (40-50mg/kg) usually being used. Similar doses are used for youth with Dyslexia. For adults, doses range from 1200-4800mg per day; the largest effective dose appears to be 1600mg of Piracetam taken thrice a day in divided doses to total 4800mg overall.

2.2 ISCHAEMIA

Since oxygen is mainly bound to hemoglobin in red blood cells, insufficient blood supply causes tissue to become hypoxic, or, if no oxygen is supplied at all, anoxic.

In very aerobic tissues such as heart and brain, at body temperature necrosis due to ischaemia usually takes about 3-4 hours before becoming irreversible.

This and typically some collateral circulation to the ISCHAEMIC area accounts for the efficacy of "clot-buster" drugs such as Alteplase, given for stroke and heart attack within this time period.

However, complete cessation of oxygenation of such organs for more than 20 minutes typically results in irreversible damage.

ischaemia features in heart diseases, transient ischaemic attacks, cerebrovascular accidents, ruptured arteriovenous malformations, and peripheral artery occlusive disease.

The heart, the kidneys, and the brain are among the organs that are the most sensitive to inadequate blood supply.

ischaemia in brain tissue, for example due to stroke or head injury, causes a process called the ischaemic cascade to be unleashed, in which proteolytic enzymes, reactive

oxygen species, and other harmful chemicals damage and may ultimately kill brain tissue (Li *et al.,* 1996).

Restoration of blood flow after a period of ischaemia can actually be more damaging than the ischaemia.

Reintroduction of oxygen causes a greater production of damaging free radicals, resulting in reperfusion injury. With reperfusion injury, necrosis can be greatly accelerated.

Brain ischaemia, also known as cerebral ischaemia, is a condition in which there is insufficient blood flow to the brain to meet metabolic demand. This leads to poor oxygen supply or cerebral hypoxia and thus to the death of brain tissue or cerebral infarction / ischaemic stroke. It is a sub-type of stroke along with subarachnoid hemorrhage and intracerebral hemorrhage.

ischaemia leads to alterations in brain metabolism, reduction in metabolic rates, and energy crisis. Its main symptoms include impairments in vision, body movement, and speaking. The causes of brain ischaemia vary from sickle cell anemia to congenital heart defects.

Symptoms of brain ischaemia can include unconsciousness, blindness, problems with coordination, and weakness in the body. Other effects that may result from brain ischaemia are stroke, cardio respiratory arrest, and irreversible brain damage.

An interruption of blood flow to the brain for more than ten seconds results in loss of consciousness and leads to brain ischaemia, consequently resulting in irreversible brain damage.

The brain is one of the most important organs in the body, regulating all bodily functions and activities. The five essential parts of the brain are the cerebrum, cerebellum, brain stem, pituitary gland, and hypothalamus.

Generally, the proper functioning of all regions of the brain is imperative to normal activity.

It was believed for many years that multiple incidents of brain ischaemia would eventually lead to irreversible damage. In 1974, Hossmann and Zimmerman challenged this assumption using animal studies and demonstrated that ischaemia induced in mammalian brains for up to an hour can be at least partially recovered. Accordingly, this discovery raised the possibility of intervening after brain ischaemia before the damage becomes irreversible.

The broad term, "stroke" can be divided into three categories: brain ischaemia, subarachnoid hemorrhage and intracerebral hemorrhage.

There are two types of ischaemia: focal ischaemia, which is confined to a specific region of the brain; and global ischaemia, which encompasses wide areas of brain tissue.

Brain ischaemia can be further subdivided, by cause, into thrombotic, embolic, and hypoperfusion. Thrombotic and embolic are generally focal or multifocal in nature while hypoperfusion affects the brain globally.

Focal brain ischaemia occurs when a blood clot has occluded a cerebral vessel. Focal brain ischaemia reduces blood flow to a specific brain region, increasing the risk of cell death to that particular area. It can be either caused by thrombosis or embolism.

Global brain ischaemia occurs when blood flow to the brain is halted or drastically reduced. This is commonly caused by cardiac arrest. If sufficient circulation is restored within a short period of time, symptoms may be transient.

However, if a significant amount of time passes before restoration, brain damage may be permanent. While reperfusion may be essential to protecting as much brain tissue as possible, it may also lead to reperfusion injury. Reperfusion injury is classified as the damage that ensues after restoration of blood supply to ischaemic tissue.

The symptoms of brain ischaemia reflect the anatomical region undergoing blood and oxygen deprivation.

ischaemia within the arteries branching from the internal carotid artery may result in symptoms such as blindness in one eye, weakness in one arm or leg, or weakness in one entire side of the body.

ischaemia within the arteries branching from the vertebral arteries in the back of the brain may result in symptoms such as dizziness, vertigo, double vision, or weakness on both sides of the body. Other symptoms include difficulty in speech, slurred speech, and loss of coordination.

The symptoms of brain ischaemia range from mild to severe. Further, symptoms can last from a few seconds to a few minutes or extended periods of time. If the brain becomes damaged irreversibly and infarction occurs, the symptoms may be permanent.

Similar to cerebral hypoxia, severe or prolonged brain ischaemia will result in unconsciousness, brain damage or death, mediated by the ischaemic cascade.

Multiple cerebral ischaemic events may lead to subcortical ischaemic depression, also known as vascular depression. This condition is most commonly seen in elderly depressed patients. Late onset depression is increasingly seen as a distinct sub-type of depression, and can be detected with Magnetic Resonance Imaging (MRI).

Brain ischaemia has been linked to a variety of diseases or abnormalities. Individuals with sickle cell anemia, compressed blood vessels, ventricular tachycardia, plaque buildup in the arteries, blood clots, extremely low blood pressure as a result of heart attack, and congenital heart defects have a higher predisposition to brain ischaemia in comparison with their healthy counterparts.

Sickle cell anemia may cause brain ischaemia associated with the irregularly shaped blood cells. Sickle shaped blood cells clot more easily than normal blood cells, impeding blood flow to the brain.

Compression of blood vessels may also lead to brain ischaemia, by blocking the arteries that carry oxygen to the brain. Tumors are one cause of blood vessel compression.

Ventricular tachycardia represents a series of irregular heartbeats that may cause the heart to completely shut down resulting in cessation of oxygen flow. Further, irregular heartbeats may result in formation of blood clots, thus leading to oxygen deprivation to all organs.

Blockage of arteries due to plaque buildup may also result in ischaemia. Even a small amount of plaque build up can result in the narrowing of passageways, causing that area to become more prone to blood clots. Large blood clots can also cause ischaemia by blocking blood flow.

A heart attack can also cause brain ischaemia due to the correlation that exists between heart attack and low blood pressure. Extremely low blood pressure usually represents the inadequate oxygenation of tissues.

Untreated heart attacks may slow blood flow enough that blood may start to clot and prevent the flow of blood to the brain or other major organs. Extremely low blood pressure can also result from drug overdose and reactions to drugs. Therefore, brain ischaemia can result from events other than heart attacks.

Congenital heart defects may also cause brain ischaemia due to lack of appropriate artery formation and connection. People with congenital heart defects may also be prone to blood clots (Morian *et al*., 1993).

Other pathological events that may result in brain ischaemia include cardio respiratory arrest, stroke, and severe irreversible brain damage.

Recently, Moyamoya disease has also been identified as a potential cause for brain ischaemia. Moyamoya disease is an extremely rare cerebrovascular condition that limits blood circulation to the brain, consequently leading to oxygen deprivation.

During brain ischaemia, the brain cannot perform aerobic metabolism due to the loss of oxygen and substrate. The brain is not able to switch to anaerobic metabolism because it does not have any long term energy stored. Thus, the levels of adenosine triphosphate (ATP) drop rapidly.

In the absence of biochemical energy, cells begin to lose the ability to maintain electrochemical gradients. Consequently, there is a massive influx of calcium into the

cytosol, a massive release of glutamate from synaptic vesicles, lipolysis, calpain activation, and the arrest of protein syndissertation (Ferchmin, 1986).

Additionally, removal of metabolic wastes is slowed. The interruption of blood flow to the brain for ten seconds results in the immediate loss of consciousness. The interruption of blood flow for twenty seconds results in the stopping of electrical activity.

The outcome of brain ischaemia is influenced by the quality of subsequent supportive care. Systemic blood pressure (or slightly above) should be maintained so that cerebral blood flow is restored. Also, hypoxemia and hypercapnia should be avoided.

Seizures can induce more damage; accordingly, anticonvulsants should be prescribed and should a seizure occur, aggressive treatment should be undertaken. Hyperglycemia should also be avoided during brain ischaemia.

When someone presents with an ischaemic event, treatment of the underlying cause is critical for prevention of further episodes.

Anticoagulation with warfarin or heparin may be used if the patient has atrial fibrillation.

Operative procedures such as carotid endarterectomy may be performed if the patient has a significant amount of plaque in the carotid arteries associated with the local ischaemic events.

A study published in the British Medical Journal established, from the results of animal studies, a correlation between the effects of brain ischaemia and the type of ischaemia involved.

Therapeutic hypothermia has been attempted to improve results post brain ischaemia. This procedure was suggested to be beneficial based on its effects post cardiac arrest. Evidence supporting the use of therapeutic hypothermia after brain ischaemia, however, is limited.

2.3 BRAIN

The brain is a mass of nerve tissue located in an animal's head that controls the body's functions. In simple animals, the brain functions like a switchboard, picking up signals from sense organs and passing the information to muscles. In more advanced animals, particularly vertebrates, the brain is much more complex and is capable of far more advanced behaviors.

The brain is part of an animal's central nervous system, which receives and transmits impulses. It works with the peripheral nervous system, which carries impulses to and from the brain and spinal cord via nerves that run throughout the body.

2.4 VERTEBRATE BRAIN

The central nervous system of vertebrates consists of a single spinal cord, which runs along the animal's back, and a highly developed brain. The brain controls all body functions, analyzes and interprets complex information and behavior patterns. The peripheral nervous system, composed of nerves which run to all parts of the body, transmits information to and from the central nervous system.

The vertebrate brain is divided into three main divisions: the fore-brain, the midbrain, and the hindbrain.

The forebrain consists of the cerebrum, thalamus, and hypothalamus. In mammals the cerebrum is highly developed, operating as a complex processing center for information.

The midbrain is the location of the optic lobes, responsible for receiving and interpreting visual signals. The midbrain is also the source of an organism's motor responses.

The hindbrain connects the brain to the spinal cord, and a portion of it, called the medulla oblongata, controls important body functions such as the breathing rate and the heart rate. Also located in the hindbrain is the cerebellum, which controls balance.

2.5 HUMAN BRAIN

The living human brain is a soft, shiny, grayish white, mushroom-shaped structure. Encased within the skull, it is a 1.4-kilogram (3-pound) mass of nerve tissue. Averagely, the brain weighs 390 grams (13.7 ounces) at birth, and at fifteen years of age, grows to approximately 1,300 grams (46 ounces). The human brain is composed of up to one trillion nerve cells. One hundred billion of these are neurons, and the remainder are supporting (glial) cells. Neurons receive, process, and transmit impulses, while glial cells (neuroglia) protect, support, and assist neurons.

The brain is protected by the skull and by three membranes called the meninges. The outermost membrane is known as the Dura mater; the middle as the Arachnoid; and the innermost as the Pia mater. Also protecting the brain is cerebrospinal fluid, a liquid that circulates between the Arachnoid and Pia mater. Many bright red arteries and bluish veins on the surface of the brain penetrate inward. Glucose, oxygen, and certain ions pass easily from the blood into the brain, whereas other substances, such as antibiotics, may

not. Capillary walls are believed to create a blood-brain barrier that protects the brain from a number of biochemical substances circulating in the blood.

The parts of the brain can be studied in terms of structure and function. Four principal sections of the human brain are the brain stem (the hindbrain and midbrain), the diencephalon, the cerebrum, and the cerebellum.

The brain stem is the stalk of the brain, and is a continuation of the spinal cord. It consists of the medulla oblongata, pons, and midbrain.

The medulla oblongata is actually a portion of the spinal cord that extends into the brain. All messages that are transmitted between the brain and spinal cord pass through the medulla. Nerves on the right side of the medulla cross to the left side of the brain, and those on the left cross to the right. The result of this arrangement is that each side of the brain controls the opposite side of the body. Three vital centers in the medulla control heartbeat, rate of breathing, and diameter of the blood vessels. Centers that help coordinate swallowing, vomiting, hiccuping, coughing, and sneezing are also located in the medulla. A region within the medulla helps to maintain the conscious state.

The pons (meaning "bridge") conducts messages between the spinal cord and the rest of the brain, and between the different parts of the brain.

The midbrain conveys impulses from the cerebral cortex to the pons and spinal cord. It also contains visual and audio reflex centers involving the movement of eyeballs and head.

Twelve pairs of cranial nerves originate in the underside of the brain, mostly from the brain stem. They leave the skull through openings and extend as peripheral nerves to their destinations.

The diencephalon lies above the brain stem, and includes the thalamus and hypothalamus. The thalamus is an important relay station for sensory information coming to the cerebral cortex from other parts of the brain. The thalamus also interprets sensations of pain, pressure, temperature, and touch, and is concerned with some of our emotions and memory. It receives information from the environment in the form of sound, smell, and taste.

The hypothalamus performs numerous important functions. These include the control of the autonomic nervous system. It helps regulate the endocrine system and controls normal body temperature. It tells us when we are hungry, full, and thirsty. It helps regulate sleep and wakefulness, and is involved when we feel angry and aggressive.

The cerebrum makes up about 80 percent of the brain's weight. It lies above the diencephalon. The cerebral cortex is the outer layer of the brain and is made up of gray matter. The tissue of the cerebral cortex if spread out would have a surface area of about 1.5 square meters. White matter, composed of nerve fibers covered with myelin sheaths, lies beneath the gray matter.

A deep fissure separates the cerebrum into a left and right hemisphere. Each cerebral hemisphere is divided into regions known as frontal, temporal, parietal, and occipital lobes. The corpus callosum, a large bundle of fibers, connects the two cerebral hemispheres.

The cerebral cortex is the portion of the brain that provides the most important distinctions between humans and other animals. It is responsible for the vast majority of functions that define what we mean by "being human." It enables us not only to receive and interpret all kinds of sensory information, such as color, odor, taste, and sound, but also to remember, analyze, interpret, make decisions, and perform a host of other "higher" brain functions.

By studying animals and humans who have suffered damage to the cerebral cortex, scientists have found that various parts of this region have specific functions. For example, spoken and written language, are transmitted to a part of the cerebrum called Wernicke's area, where meaning is extracted. Instructions are then sent to Broca's area, which controls the movement of muscles throughout the body. Within Broca's area, thoughts are translated into speech and muscles are coordinated for speaking. Impulses from other motor areas direct our hand muscles when we write and our eye muscles when we scan the page for information.

Association areas of the cerebrum are concerned with emotions and intellectual processes, by connecting sensory and motor functions. In our association areas, innumerable impulses are processed that result in memory, emotions, judgment, personality, and intelligence.

The cerebellum is located below the cerebrum and behind the brain stem, and is shaped like a butterfly. The "wings" are the cerebellar hemispheres, and each consists of lobes that have distinct grooves or fissures. The cerebellum controls the movements of our muscular system needed for balance, posture, and maintaining posture.

2.6 MEMORY

One of the most fascinating of all brain functions is memory. Memory refers to the brain's ability to recall events that have taken place at some time in the past. Two kinds of memory exist, short-term and long-term memory. It is still uncertain how the brain remembers things. Memory trace is used to describe changes in the brain that correspond to the creation of memory, but no one knows exactly what a memory trace corresponds to in terms of brain structure, chemistry, or function.

According to the most popular current theory of memory, exposure to stimuli can cause changes in the connections that neurons make with each other. These changes may be the "memory traces" that scientists talk about. Those neural connections appear to be able to survive for very long periods of time and can be recalled when a person decides to recall them or when some stimulus causes them to reappear.

Some exciting research on memory has suggested that nerve cells may actually grow and change as they are exposed to light, sounds, chemicals, and other stimuli. The new patterns they form may in some way be connected to the development of a memory trace in the brain.

Two surprisingly small areas at the front of the cerebrum, located on each hemisphere roughly above the outer edge of the eyebrow, are the brain's centers for high-level thinking. In 2000, scientists announced that this paired region, called the lateral prefrontal cortex, was activated in people who were given tests involving verbal and spatial problems. This is the same region of the brain that previous research studies had shown to be important for solving novel tasks, keeping many things in mind at once, and screening

out irrelevant or unimportant information. Scientists also believe the lateral prefrontal cortex acts as a global workspace for organizing and coordinating information and carrying it back to other parts of the brain as needed.

Certain structures in the cerebrum and diencephalon make up the limbic system. These regions are responsible for memory and emotions, and are associated with pain and pleasure.

By studying patients whose corpus callosum had been destroyed, scientists have learned that differences exist between the left and right sides of the cerebral cortex. The left side of the brain functions mainly in speech, logic, writing, and arithmetic. The right side of the brain, on the other hand, is more concerned with imagination, art, symbols, and spatial relations.

2.7 STROKE

Stroke is a medical condition characterized by the sudden loss of consciousness, sensation, and voluntary movement caused by the loss of blood flow to the brain. Stroke is also called a cerebral vascular accident or CVA. It is caused by one of two events, a ruptured artery or an artery that has become closed off because a blood clot has lodged in it. Stroke resulting from a burst blood vessel is called a hemorrhagic stroke, while one caused by a clot is called a thrombotic stroke. Blood circulation to the area of the brain served by that artery stops at the point of disturbance, and brain tissue beyond that point is damaged by the lack of oxygen and begins to die.

Stroke is the third leading cause of death in the United States after heart attack and all forms of cancer. Approximately 500,000 strokes, new and recurrent, are reported each

year. Of these, about 150,000 will be fatal. Today approximately 3,000,000 Americans who have had a stroke are alive.

2.7.1 Pathophysiology of Stroke

The brain requires a constant and steady flow of blood in order to carry out its functions. Blood delivers the oxygen and nutrients needed by the brain cells. If this blood flow is interrupted for any period of time and for any reason, brain cells begin to die quickly.

A stroke occurs in one of two ways; a burst blood vessel may occur in a weak area in the artery, or a blood vessel becomes plugged by a floating blood clot. In either case, blood is no longer supplied to brain tissue beyond the point of the occurrence. Brain tissue ceases to function if deprived of oxygen for more than 60 to 90 seconds and after approximately three hours, will suffer irreversible injury possibly leading to death of the tissue, i.e., infarction. This infarction results from damage incurred from products of the ischaemic cascade.

The ischaemic cascade is a series of biochemical reactions that are initiated in the brain and other aerobic tissues after seconds to minutes of ischaemia ("eMedicine - Stroke, ischaemic: Article by Joseph U Becker") typically secondary to stroke injury, or cardiac arrest. Most ischaemic neurons that die do so due to the activation of chemicals produced during and after ischaemia (Stroke Center of the Washington University School of Medicine). The ischaemic cascade usually goes on for two to three hours but can last for days, even after normal blood flow returns ("Stroke: Hope Through Research: National Institute of Neurological Disorders and Stroke (NINDS)".

The ischaemic cascade can be generally characterized as follows:

1. Lack of oxygen causes the neuron's normal process for making Adenine Triphphosphate (ATP) for energy to fail.
2. The cell switches to anaerobic metabolism producing lactic acid.
3. ATP-reliant ion transport pumps fail, causing the cell to become depolarized, allowing ions, including calcium (Ca^{++}), to flow into the cell.
4. The ion pumps can no longer transport calcium out of the cell, and intracellular calcium levels get too high.
5. The presence of calcium triggers the release of the excitatory amino acid neurotransmitter glutamate.
6. Glutamate stimulates AMPA receptors and Ca^{++}- permeable Nmalonylaldehyde receptors, which open to allow more calcium into cells.
7. Excess calcium entry over excites cells and causes the generation of harmful chemicals like free radicals, reactive oxygen species and calcium-dependent enzymes such as calpain, endonucleases, ATPases and phospholipases in a process called excitotoxicity (Jill Conway, 2000. Diseases at the cellular level lecture handout) (e-Medicine Edward C. Jauch) Calcium can also cause the release of more glutamate.
8. As the cell's membrane is broken down by phospholipases, it becomes more permeable, and more ions and harmful chemicals flow into the cell.
9. Mitochondria break down, releasing toxins and apoptotic factors into the cell.
10. The caspase-dependent apoptosis cascade is initiated, causing cells to "commit suicide."

11. If the cell dies through necrosis, it releases glutamate and toxic chemicals into the environment around it. Toxins poison nearby neurons, and glutamate can overexcite them.
12. If and when the brain is reperfused, a number of factors lead to reperfusion injury.
13. An inflammatory response is mounted, and phagocytic cells engulf damaged but still viable tissue.
14. Harmful chemicals damage the blood brain barrier.
15. Cerebral edema (swelling of the brain) occurs due to leakage of large molecules like albumins from blood vessels through the damaged blood brain barrier. These large molecules pull water into the brain tissue after them by osmosis. This 'vasogenic edema' causes compression of and damage to brain tissue.

The effect of the damage to the brain depends upon the area of the brain that is involved. Interruption of a small blood vessel may result in a speech impediment or difficulty in hearing or an unsteady gait. If a larger blood vessel is involved, the result may be total paralysis of one side of the body.

Recovery from a stroke varies from one person to the next. Swift treatment followed by effective physical therapy may restore nearly full function of an affected area of the body. Some individuals have experienced severe enough damage that their recovery is minimal.

Current therapy includes medications that can be given to dissolve clots, thereby removing the barrier to blood flow. If blood flow can be reinstated quickly enough, brain tissue may suffer less damage.

2.8 WISTAR RAT BRAIN

Adult Wistar Rats rarely weigh more than 500 grams (1.1 lb) and has a life span of about one year. Since 1895, rats have increasingly been used for scientific laboratory experiments. Wistar rats have been easy to use because they are calm, reproduce early, produce many offspring and tolerate crowding. Further, their viscera such as the brain, liver and kidney are small (Barnett, 2002). When it comes to conducting tests related to intelligence, learning, and drug abuse, rats are a popular choice due to their high intelligence, ingenuity, aggressiveness, and adaptability. Their psychology, in many ways, seems to be similar to humans. A 2007 study found rats to possess metacognition, a mental ability previously only documented in humans and some primates (Foote and Jonathon, 2007).



Figure 2.1: Rat brain

A rat weighing about 400g has a brain weighing a mere 1.4g. Like the brains of all vertebrates, including man, it can be divided into three anatomical categories: the hindbrain, midbrain and forebrain.

The Hindbrain

The hindbrain, or rhombencephalon, connects the brain to the spinal cord and consists of the medulla, pons and cerebellum. It is responsible for balance and muscle control as well as autonomic functions such as heart rate and respiration.

The Midbrain

As its name suggests, the midbrain, or mesencephalon, sits between the hindbrain and the forebrain. All information passed between the higher brain and the brain stem has to traverse the midbrain, which has structures involved in the receiving and interpreting of auditory and visual signals.

The Forebrain

Also known as the prosencephalon, the forebrain consists of the cerebrum (telencephalon) and the thalamus and hypothalamus (diencephalon). The cerebrum is instrumental in conscious behavior, learning and sensory perception. The hypothalamus plays an essential role in endocrine functions and governs various responses such as fight, flight and sexual behavior. The thalamus conveys sensory information to higher brain centers in the cortex.

Cerebral Cortex

The tissue that makes up the outer layer of the mouse brain is referred to as the cerebral cortex. The right and left sides of the cerebral cortex are connected by the corpus callosum, a thick band of nerve fibers. Bumps and grooves on the cortex are known as gyri and sulci and while they are plentiful in higher mammals, in mice there are very few.

The genes responsible for building mouse and human brains are 90 percent identical, which is why mouse brains are being used to study mental disorders of the human kind.

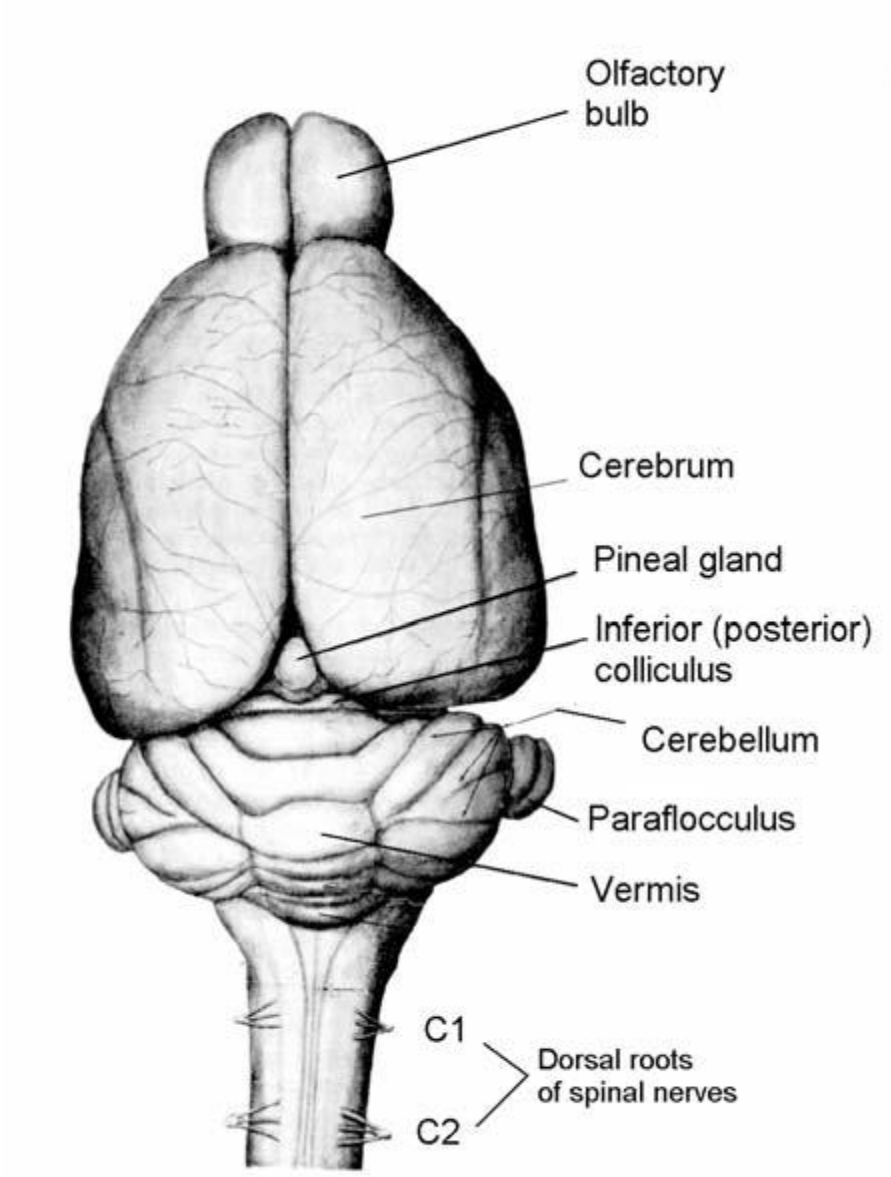


Figure 2.2: Anatomy of Wistar rat brain, Dorsal view

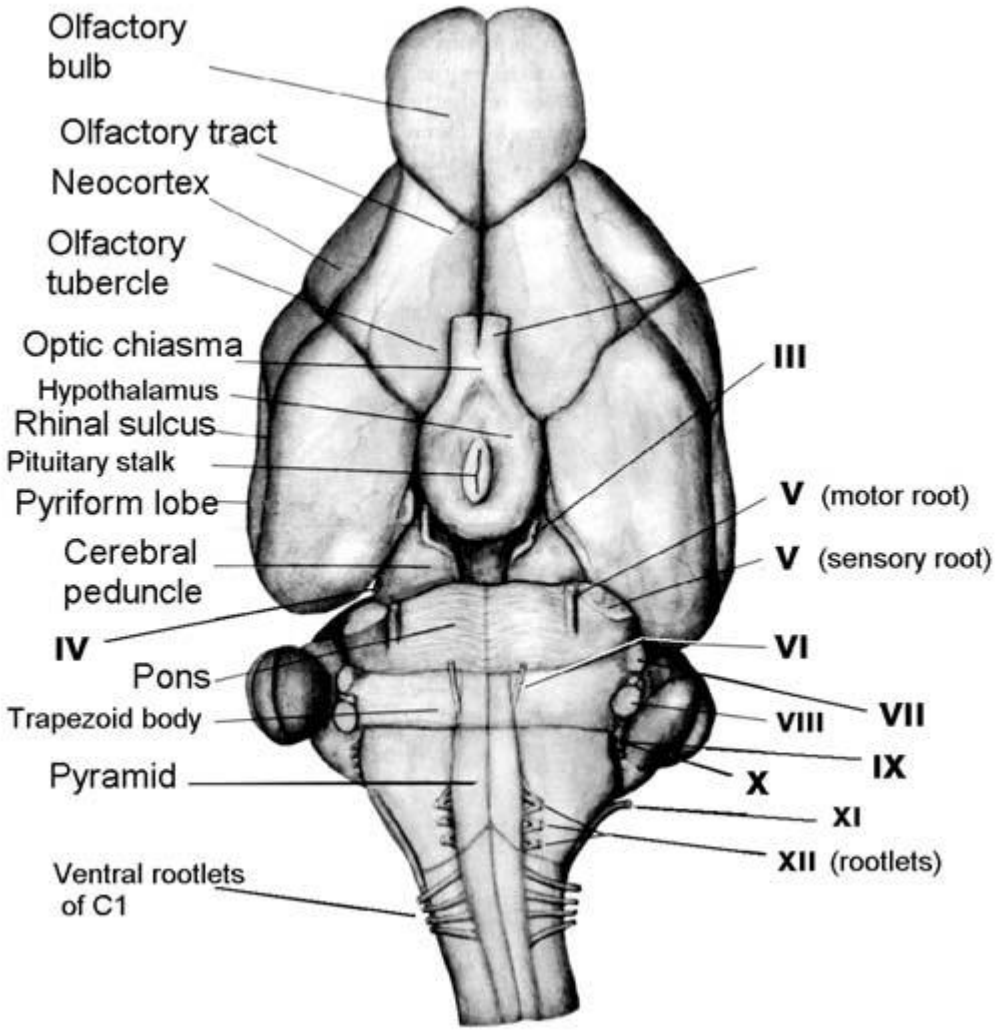


Figure 2.3: Anatomy of Wistar rat brain, Ventral view

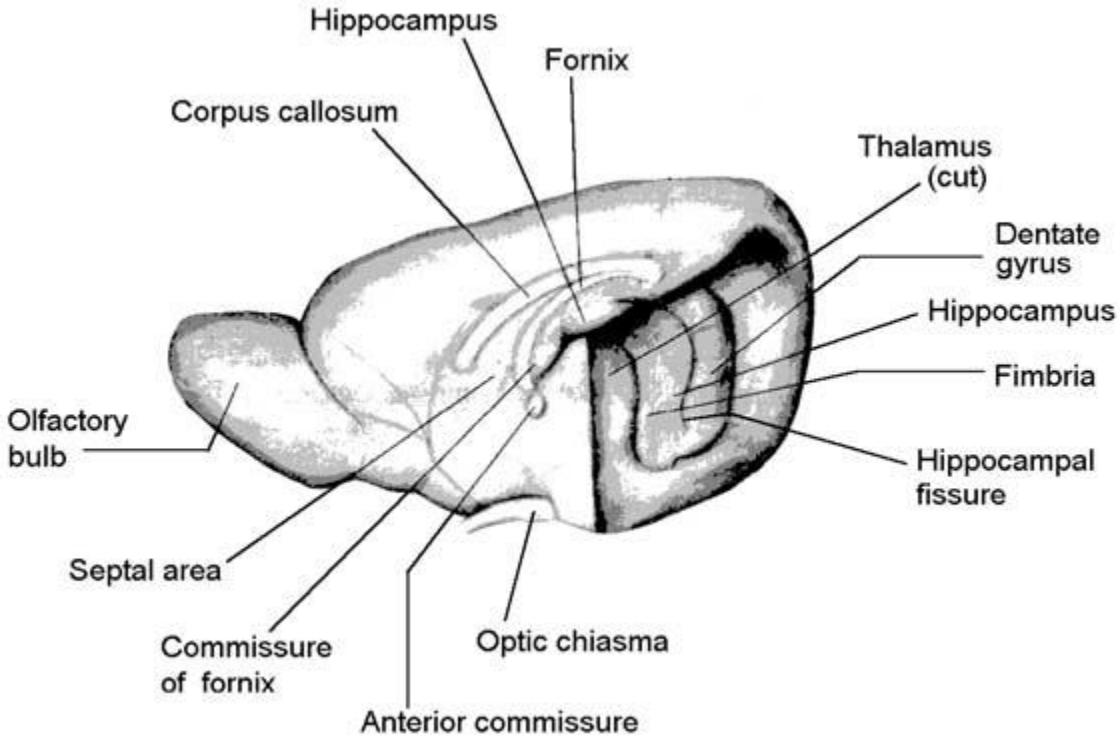


Figure 2.4: Right Cerebrum (Medial View)

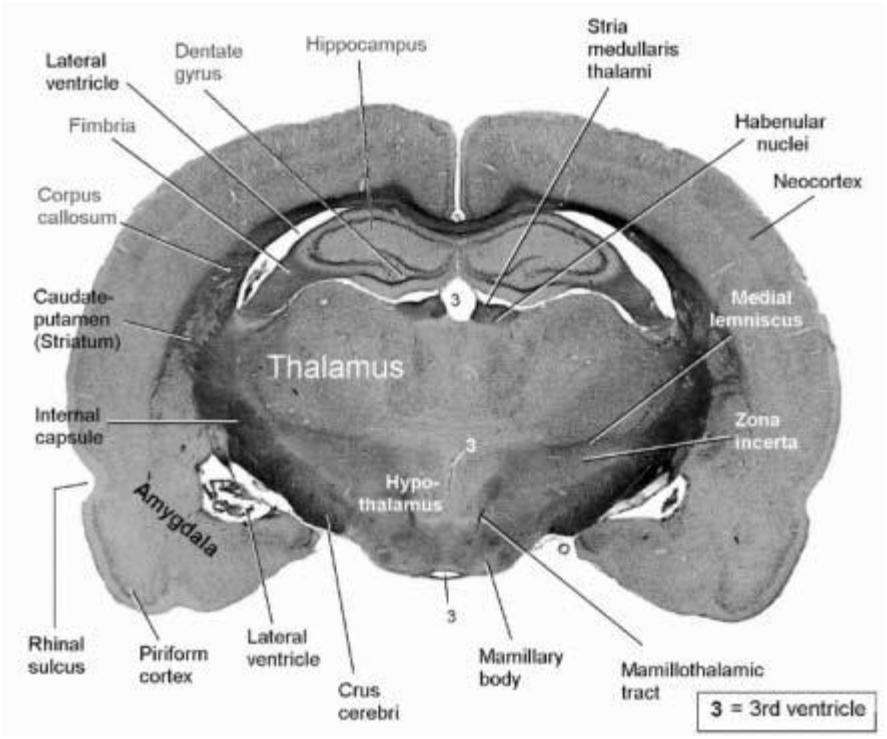

Lateral ventricle
Dentate gyrus
Hippocampus
Stria medullaris thalami
Fimbria
Habenular nuclei
Neocortex
Corpus callosum
Caudate-putamen (Striatum)
3
Medial lemniscus
Thalamus
Internal capsule
Zona incerta
3
Hypo-thalamus
Amygdala
3
Rhinal sulcus
Piriform cortex
Lateral ventricle
Crus cerebri
Mamillary body
Mamillothalamic tract
3 = 3rd ventricle

Figure 2.5:

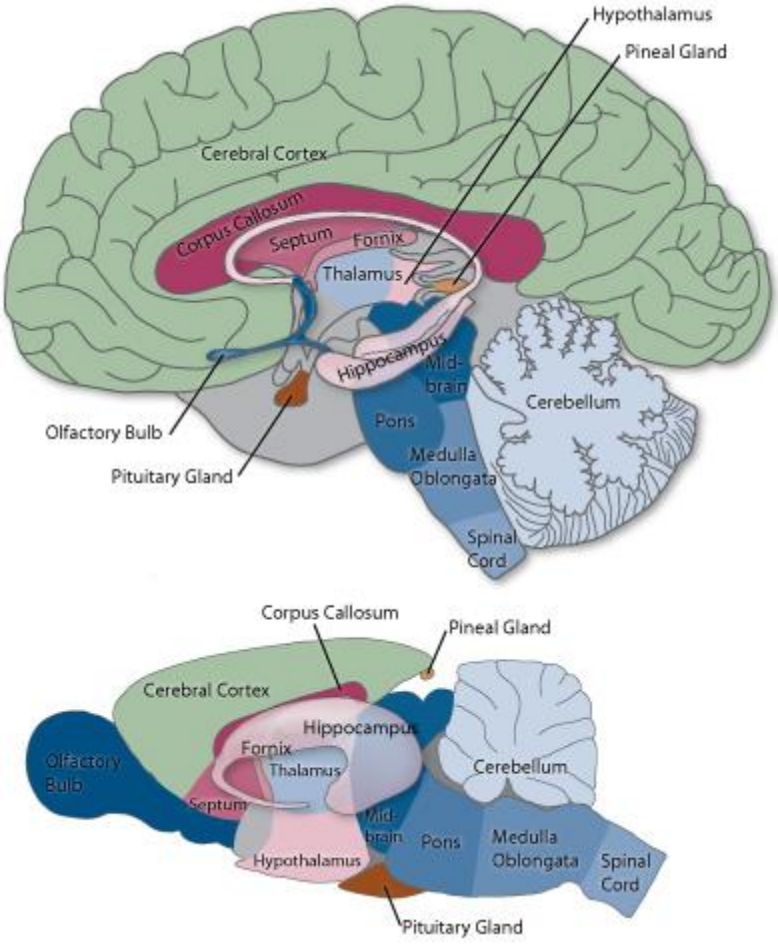


Figure 2.6: Comparison of the human and rat brains

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Instruments

Colorimeter, UV- spectrophotometer, surgical kit

3.1.2 Chemicals and drugs

Piracetam (UCB Belguim), Bouins fluid, Trichloroacetic Acid (TCA) (Sigma-Aldrich Germany), Thiobarbituric Acid (TBA), ethanol, phosphate buffer, chloroform, ketamine (Johnson & Johnson UK) .

3.1.3 Animals

Twenty Wistar rats were used. All animals were housed in the animal house of Department of Human anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria and maintained in dry cages cushioned with sawdust while being fed with commercial growers mash and water *ad libitum*.

3.2 METHODS

3.2.1 Design of the study

A total of 20 Wistar rats were used in the study. They were divided into 5 experimental groups:

Group A - (n=4) non-ISCHAEMIC rats received 0.5mls of normal saline solution for 3days orally

Group B - (n=4) ISCHAEMIC-induced rats received 0.5mls of normal saline solution for 3days orally.

Group C - (n=4) ISCHAEMIC-induced rats received intraperitoneally (i.p.) 100mg/kg/day of Piracetam (Ryzhavskii *et al*., 1997) for 3days. (Xerri and Zennou-Azoqui, 2003)

Group D - (n=4) ISCHAEMIC-induced rats received intraperitoneally (i.p.) 100mg/kg/day of Piracetam and vitamins E 10mg/kg/day orally for 3days.

Group E - (n=4) ISCHAEMIC-induced rats received vitamin E 10mg/kg/day orally for 3days.

3.2.2 Experimental Induction of ischaemia

The experiment was carried out in accordance with the guidelines on the care and use of laboratory animals (NRC, 1996). Using aseptic technique the animals were anaesthetized by closing in a chamber containing cotton wool soaked in chloroform. The animals subsequently injected with 50mg/Kg of Ketamine, intramuscularly.

Restricting the animals with clips on a dissecting board, the anterior aspect of the neck was dissected to reach the trachea and the ooesophagus. The carotid arteries were located lateral to the ooesophagus and bilaterally clipped with mosquito forceps for 10 minutes. Clips were then removed and the skin sutured back. Animals were subsequently resuscitated in individual cages for the rest of the experiment.

At the end of experimentation, each animal was euthanized then decapitated following intracardiac collection of 2 – 4mls of blood. The cranium was then spliced open and its brain content harvested for evaluation.

3.2.3 Apparatus for physiological tests

A wooden Elevated Plus-Maze was built according to the description of Lister (1987). It consist of a black floor with a 5 x 5 cm central square platform, from which radiates two 45 x 5 cm open arms with 0.25 cm high edges and two 45 x 5 cm closed arms with 40 cm high walls. The apparatus is raised 45 cm above the floor on a plus-shaped plywood base. The experiment was carried out in a room that was well illuminated with light.

Procedure

Rats were carried into the test room in their home cages and handled by the base of their tails at all times. Rats were placed at the edge of one end of the open arm of the Plus-Maze backing the open arm and allowed to explore the apparatus for 90 seconds.

Observers sitting quietly about 1 metre from the apparatus recorded the behaviour of the animals on the maze. Time taken for closed arm entries, were noted.

After 90 seconds, rats were removed from the maze by the base of their tails and returned to their home cage. The maze was then cleaned with a solution of 70% ethyl alcohol and permitted to dry between tests.

3.2.4 Assessment of lipid peroxidation

Lipid peroxidation as evidenced by the formation of Thiobarbituric Acid Reactive Substance (TBARS) was measured by the modified method of Niehaus & Samuelson (1968) and described by Akanji *et al*. (2009).

Reagents

15 % Trichloroacetic Acid (TCA) solution:

0.37% Thiobarbituric Acid (TBA) solution:

Procedure

To 150ml of serum and tissue homogenate, (0.25M sucrose solution) was treated with 2ml of (1:1:1 ratio) TBA-TCA-HCL reagent (thiobarbituric acid 0.37%, 0.25N HCL and 15% TBA) and placed in a water bath for 1h at 90^{o}C. The mixture was then cooled and centrifuged at 3000rpm for 5min at 4^{o}C. The absorbance of the pink supernatant 2.0ml was measured against a reference blank using spectrophotometer at 535nm.

Calculations

The malonylaldehyde (MDA) was calculated using the molar extinction coefficient of 1.56 x10^{5}cm^{-1}M^{-1}

Malonylaldehyde conc. =absorbance/ 1.56 x10^{5}cm^{-1}M^{-1} x1

CHAPTER FOUR

RESULTS

Table 4.1: The mean serum and brain tissue malonylaldehyde levels of wistar rats treated with Piracetam and Vitamin E following ISCHAEMIC brain injury

Groups	I	II	III	IV	V
Malonylaldehyde in Serum	0.33 ± 0.6 [(a)]	1.06 ± 0.2 [(b)]	0.35 ± 0.8 [(a)]	0.92 ± 0.2 [(ab)]	0.94 ± 0.2 [(cb)]
Malonylaldehyde in brain tissue	2.8 ± 0.2	2.6 ± 0.1	3.0 ± 0.2	2.7 ± 0.3	3.2 ± 0.2

Different superscripts indicate significant difference.

In table 4.1 the serum Malonylaldehyde levels of the control group shows a significant difference when compared with that of group II.

There is no significant difference between controls and animals in group III.

Group IV animals have a significantly higher serum malonylaldehyde compared to the first three groups.

Group V malonylaldehyde is significantly higher than the first four groups.

The difference in the malonylaldehyde levels in brain tissue did not show any statistical significant difference among the groups.

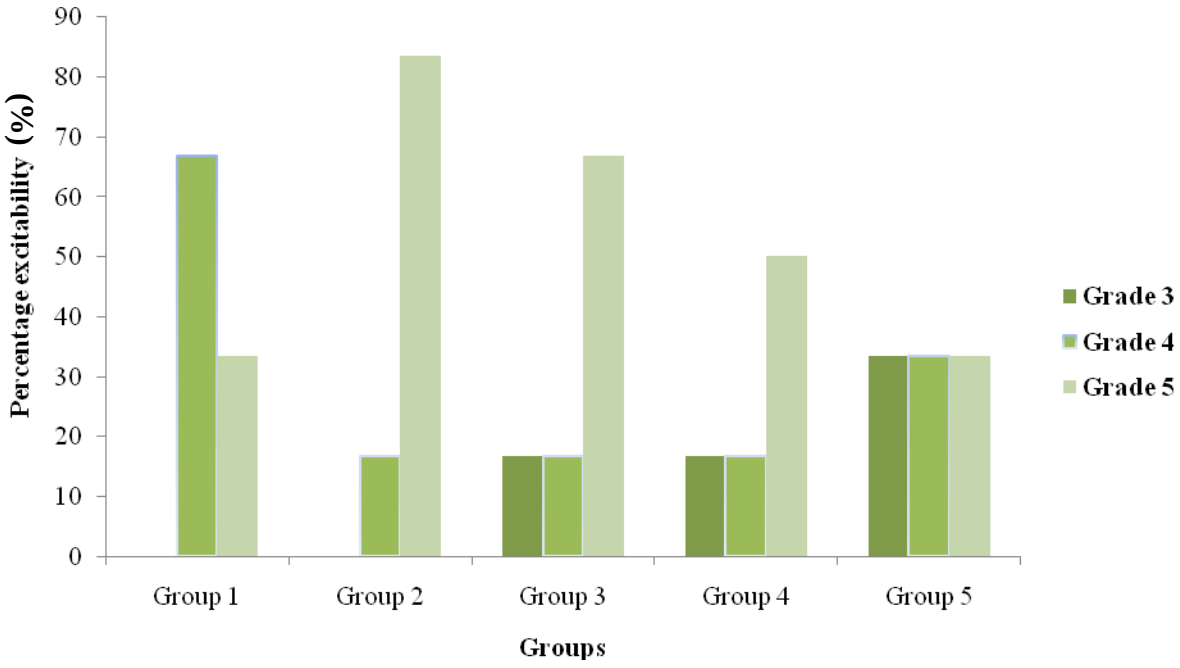


Figure 4.1: percentage excitability and grade of reaction in wistar rats following induction of ischaemia and treatment with Piracetam and Vitamin E

In figure 4.1 percentage excitability is higher in group II animals when compared to group I animals, while grade of excitability is higher in group II compared to group I animals.

Group II animals that received piracetam have percentage excitability as high as group I but with lower grades.

Group IV and group V animals have lower percentage excitability as well as lower grade of reaction compared to group I.

All differences did not show statistical difference.

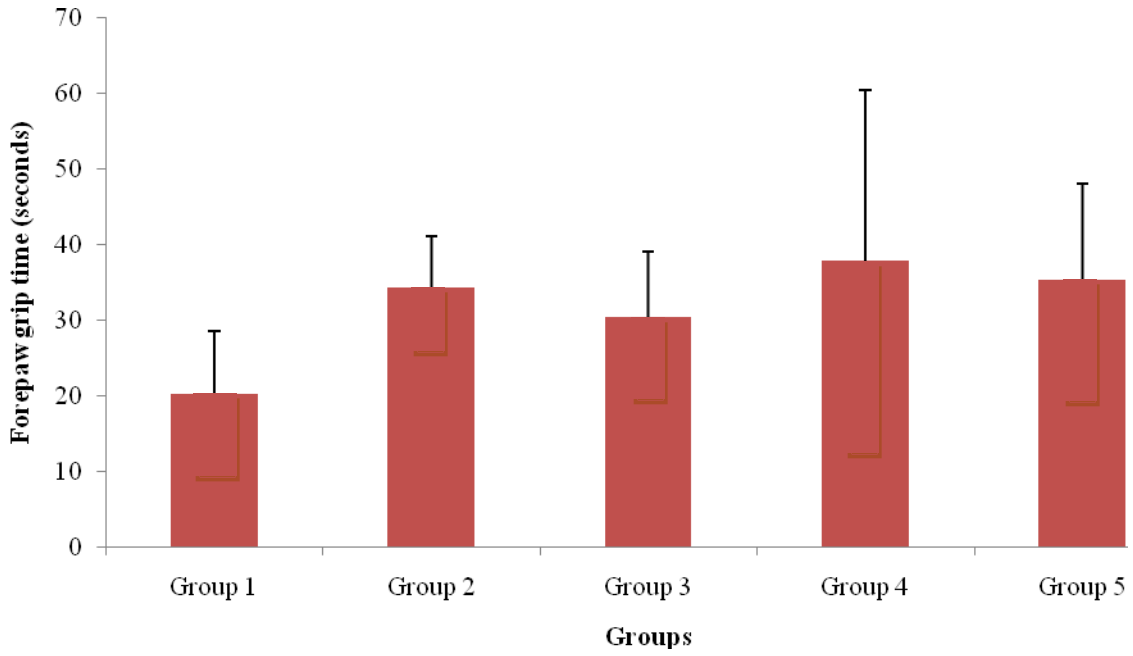


Figure 4.2: the forepaw grip time of wistar rats treated with Piracetam and vitamin E following induction of global ischaemia

In figure 4.2 forepaw grip time was higher in group II animals when compared with the controls.

Group III animals also showed a higher duration of grip time than group I animals although shorter than group II animals.

Group IV and V animals show a higher duration of grip time.

All differences did not show statistical significance.

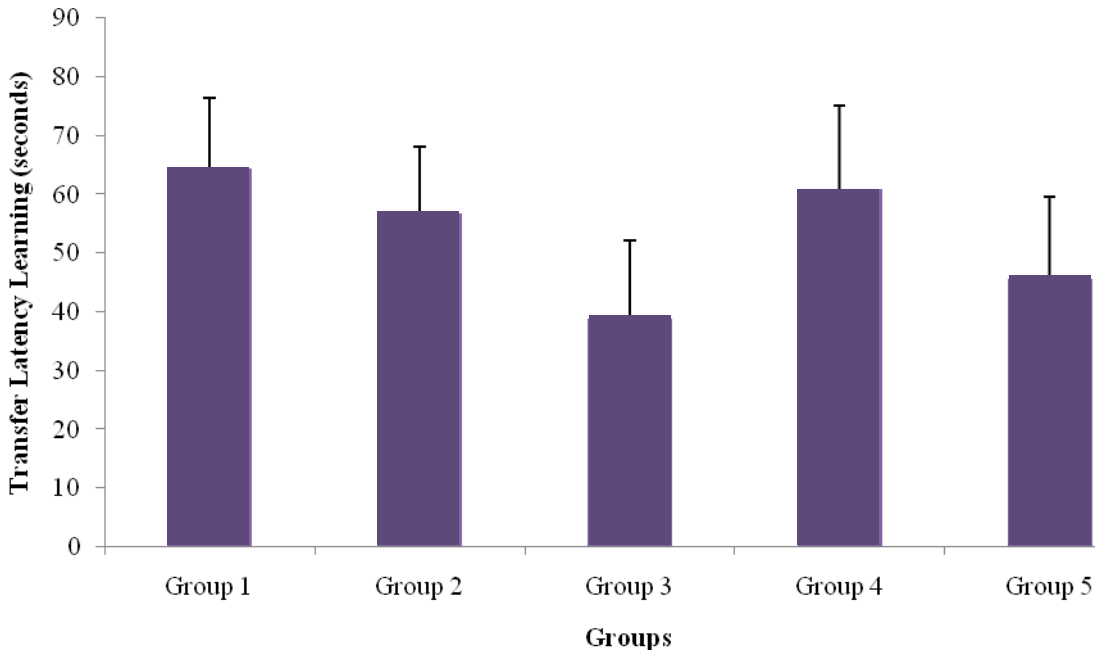


Figure 4.3: the transfer latency time of wistar rats in an elevated plus maze after treatment with piracetam and vitamin E following induction of global ischaemia

In figure 4.3 transfer latency time was highest in the control animals. The latency time in group III animals was shorter than that in group II animals. That in group IV and V animals were higher than that of group II animals, with the latency time in group IV animals higher than that of group V animals.

Three criteria were used in characterizing the features seen in these micrographs.

1. Nucleo-cytoplasmic contrast graded as High, Moderate, Low or Absent
2. Smearing of cytoplasmic border graded as Present or Absent
3. The presence of a recognizable nucleus graded as Yes or No

Bilateral transient carotid artery occlusion was carried out in five groups of Wistar rats.

Group 1 animals were the control group
Group II animals had ischaemia induced
Group III animals had ishaemia induced and subsequently treated with Piracetam
Group IV animals had ishaemia induced and subsequently treated with Piracetam and vitamin E
Group V animals had ishaemia induced and subsequently treated with vitamin E.

The slides were stained using hematoxylin and eosin (H & E) and cresyl fast violet (cfv). Then viewed under light microscopy at x40, x400, x600 and x1000 magnification. The following observations were made:

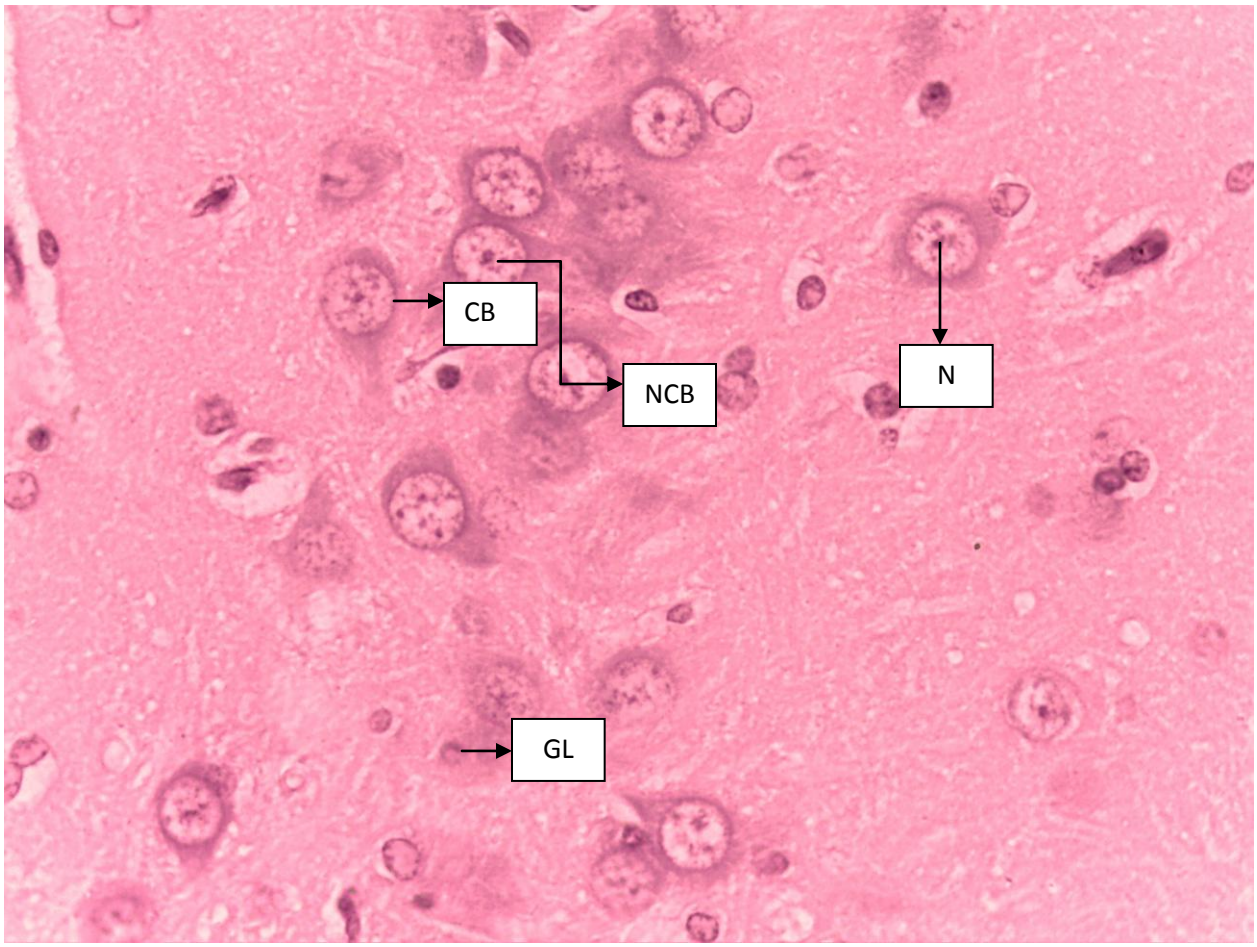


Plate 1: Photomicrograph of hippocampus of Wistar rat in the control group (H&E stain, x1000 mag.)

The neurons are generally healthy.

The neucleo cytoplasmic contrast is high.

Smearing of cytoplasmic border is present and

Nuclei are visible.

CB – Cytoplasmic border

NCB - Neucleocytoplasmic border

N – Nucleus

GL – Glial cell

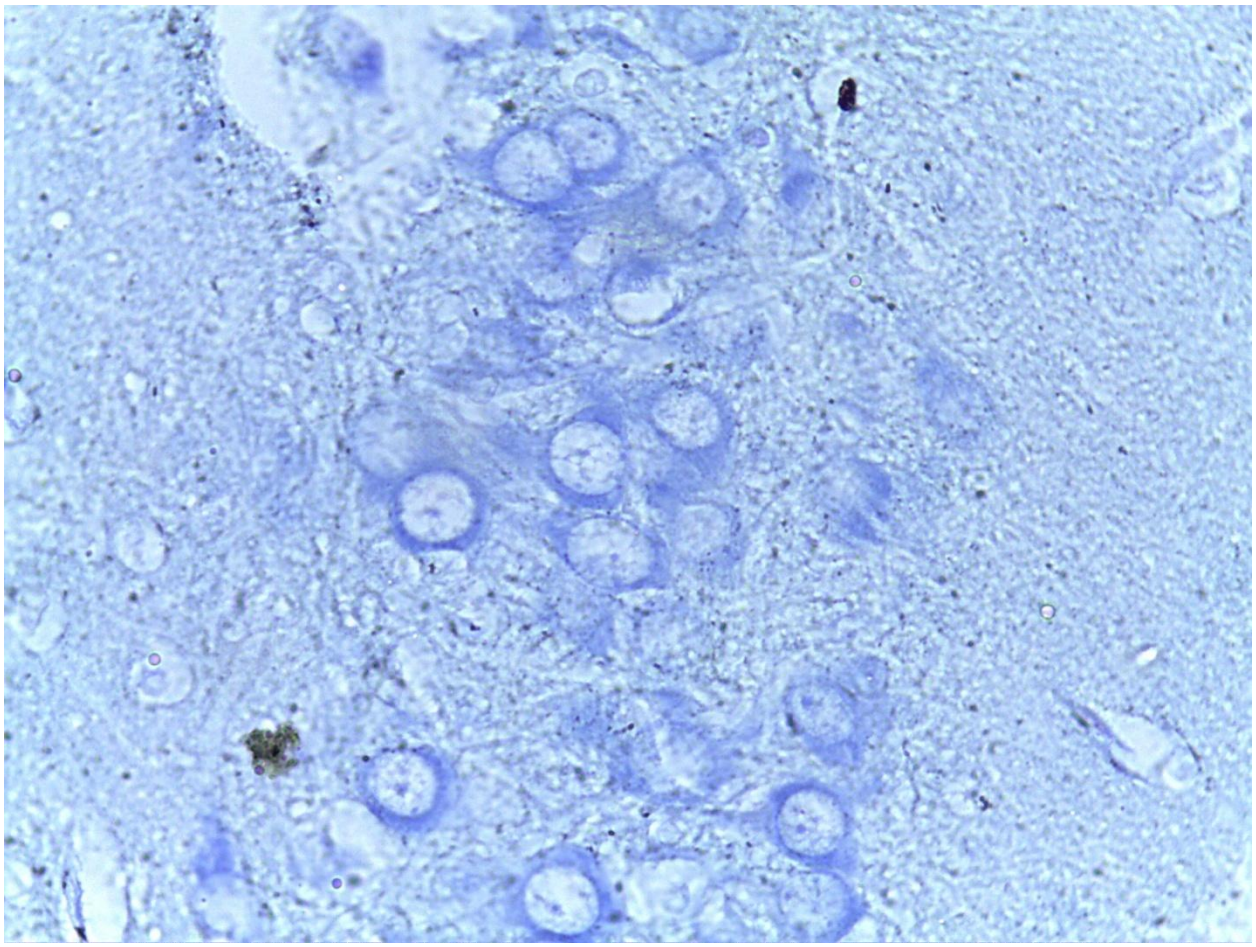


Plate 2: Photomicrograph of hippocampus of Wistar rat in the control group (cfv stain, x1000 mag.)

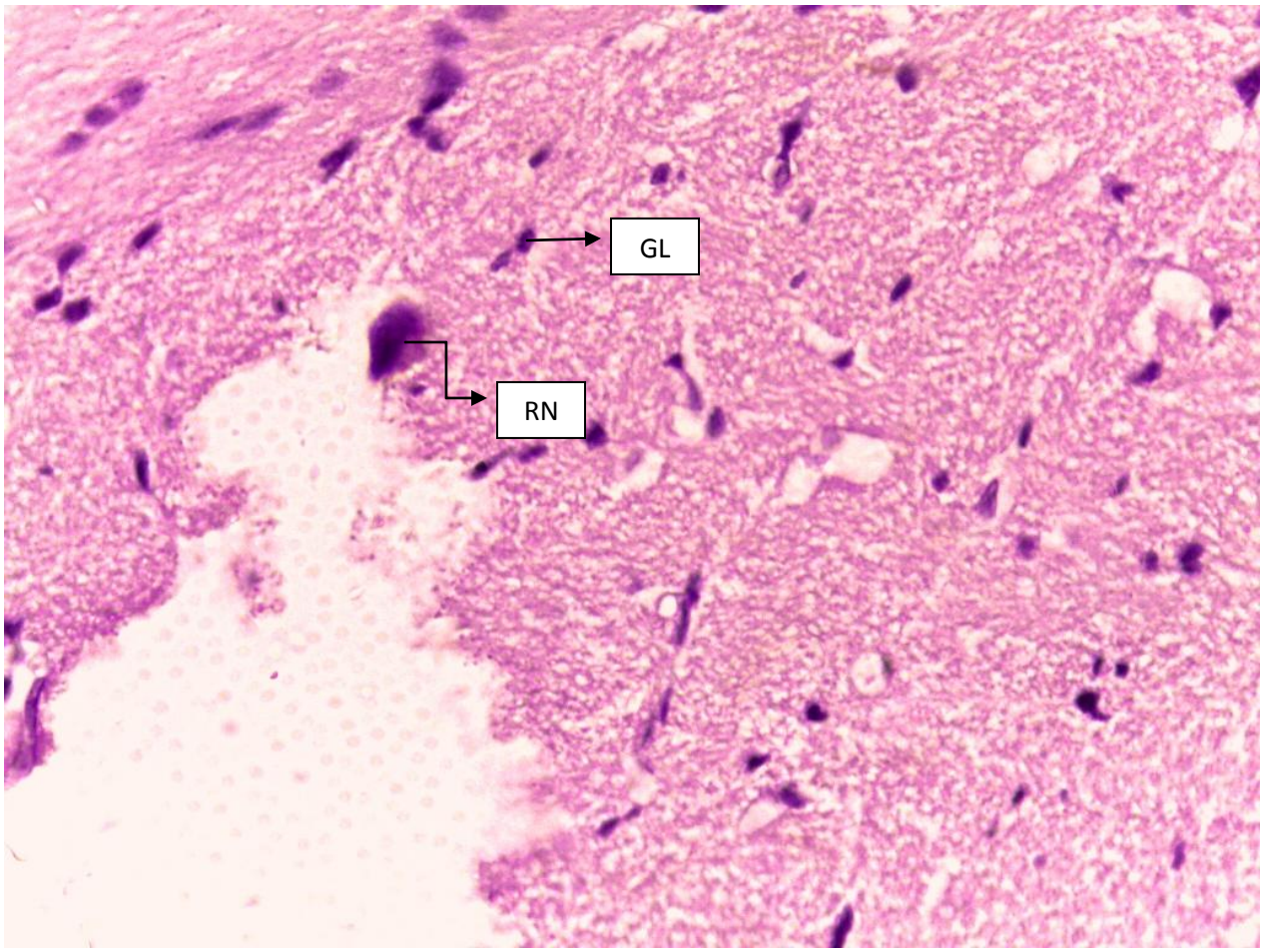


Plate 3: Photomicrograph of hippocampus of Wistar rat in group II (H&E stain, x1000 mag.)

There are a lot of red neurons and scarcity of healthy neurons.

Neucleo cytoplasmic contrast is absent.

Smearing of cytoplasmic border is present and

Nuclei are visible.

GL - Glial cell

RN - Red neuron

CB - Cytoplasmic border

NC - Neuronal cell

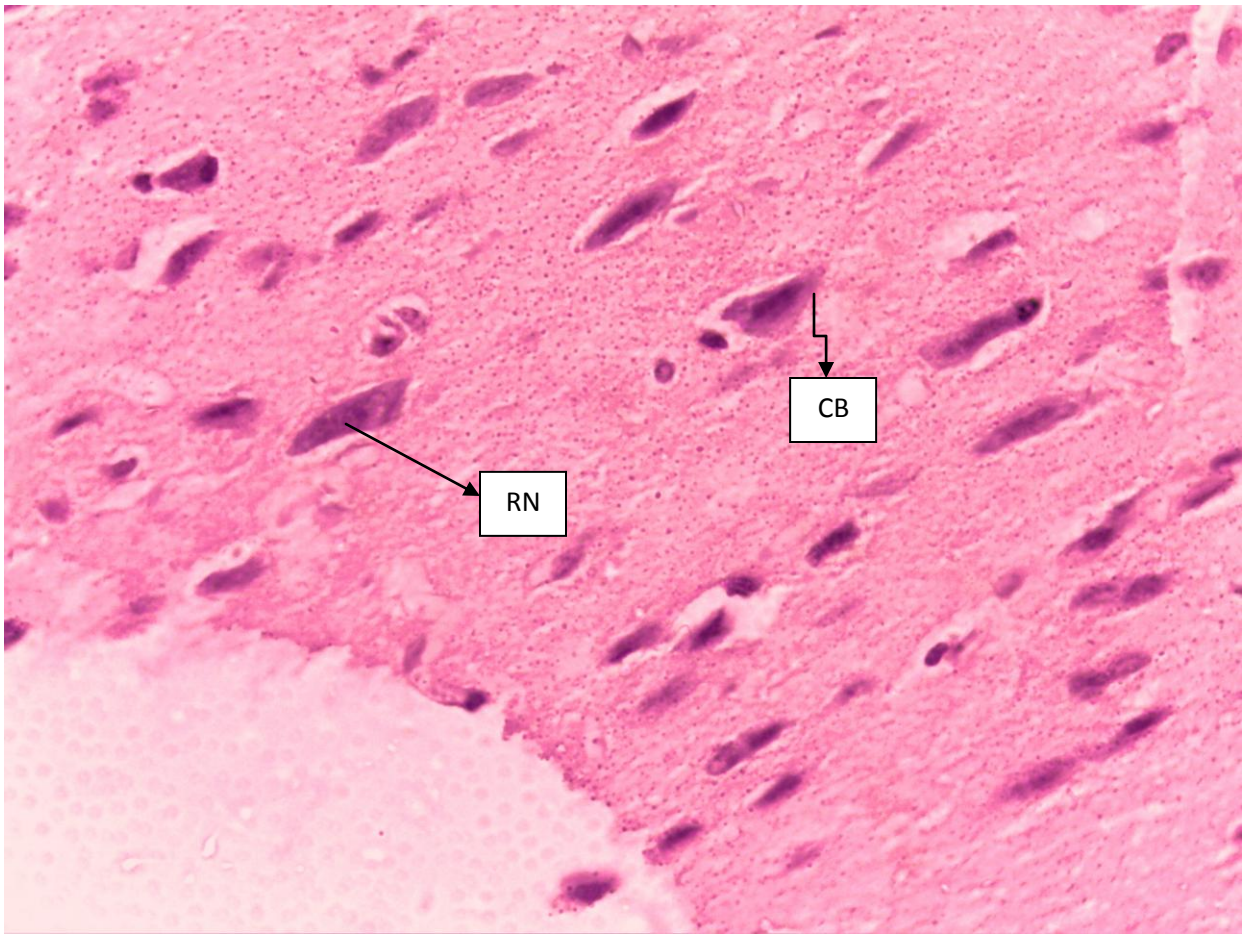


Plate 4: Photomicrograph of hippocampus of Wistar rat in group II. (H&E stain, x1000 mag.)

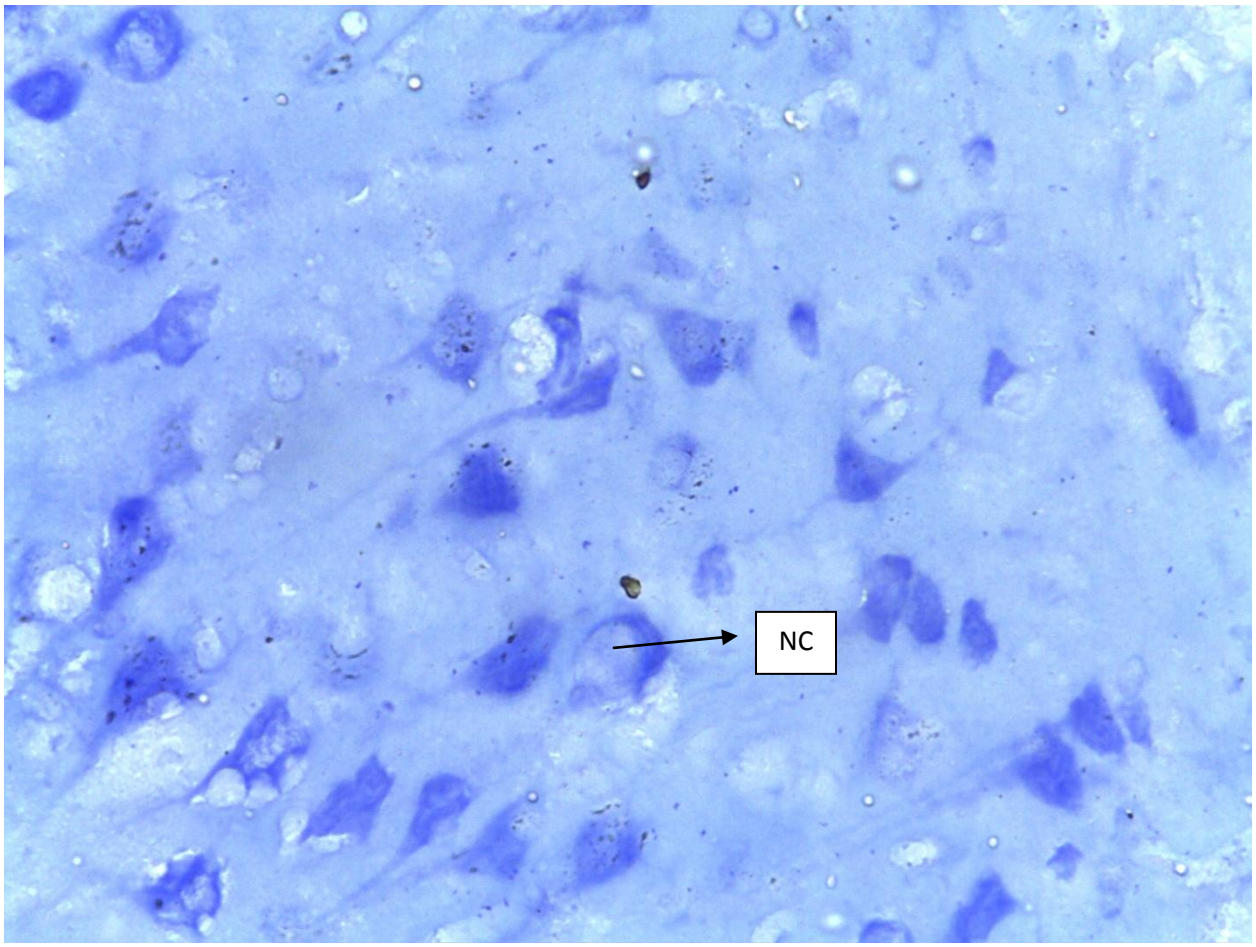


Plate 5: Photomicrograph of hippocampus of Wistar rat in group II. (cfv stain, x1000 mag.)

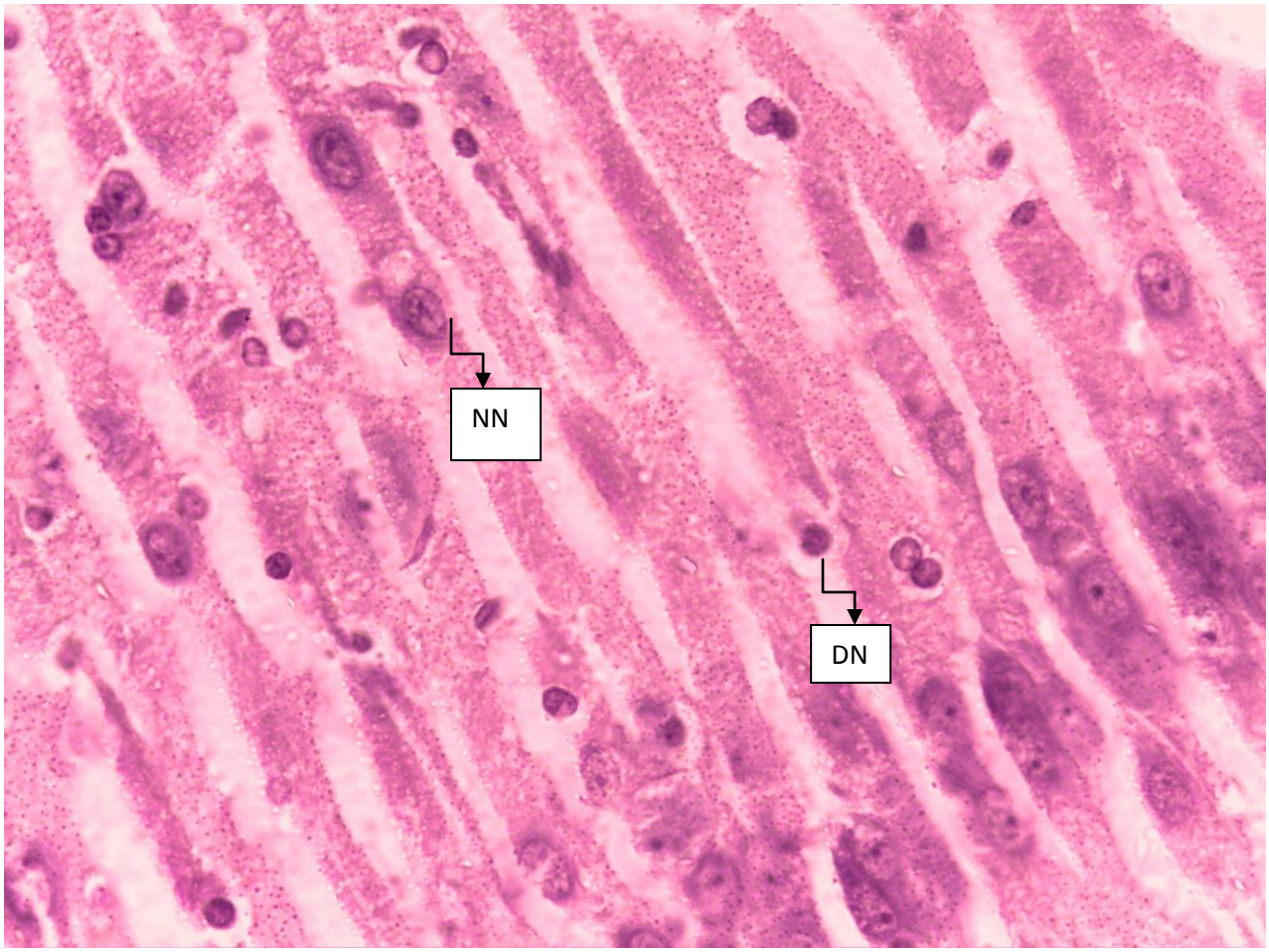


Plate 6: photomicrograph showing hippocampus of animal in group III (H&E, X1000)

The Neucleo cytoplasmic contrast is moderate to high for most neurons but there are focal areas of neurons with low Neucleo cytoplasmic contrast.

Smearing of cytoplasmic border is absent for most neurons but present in focal areas.

Nuclei are visible in most cells but not visible in a few cells.

NN - Normal neuron

DN - Dying neuron

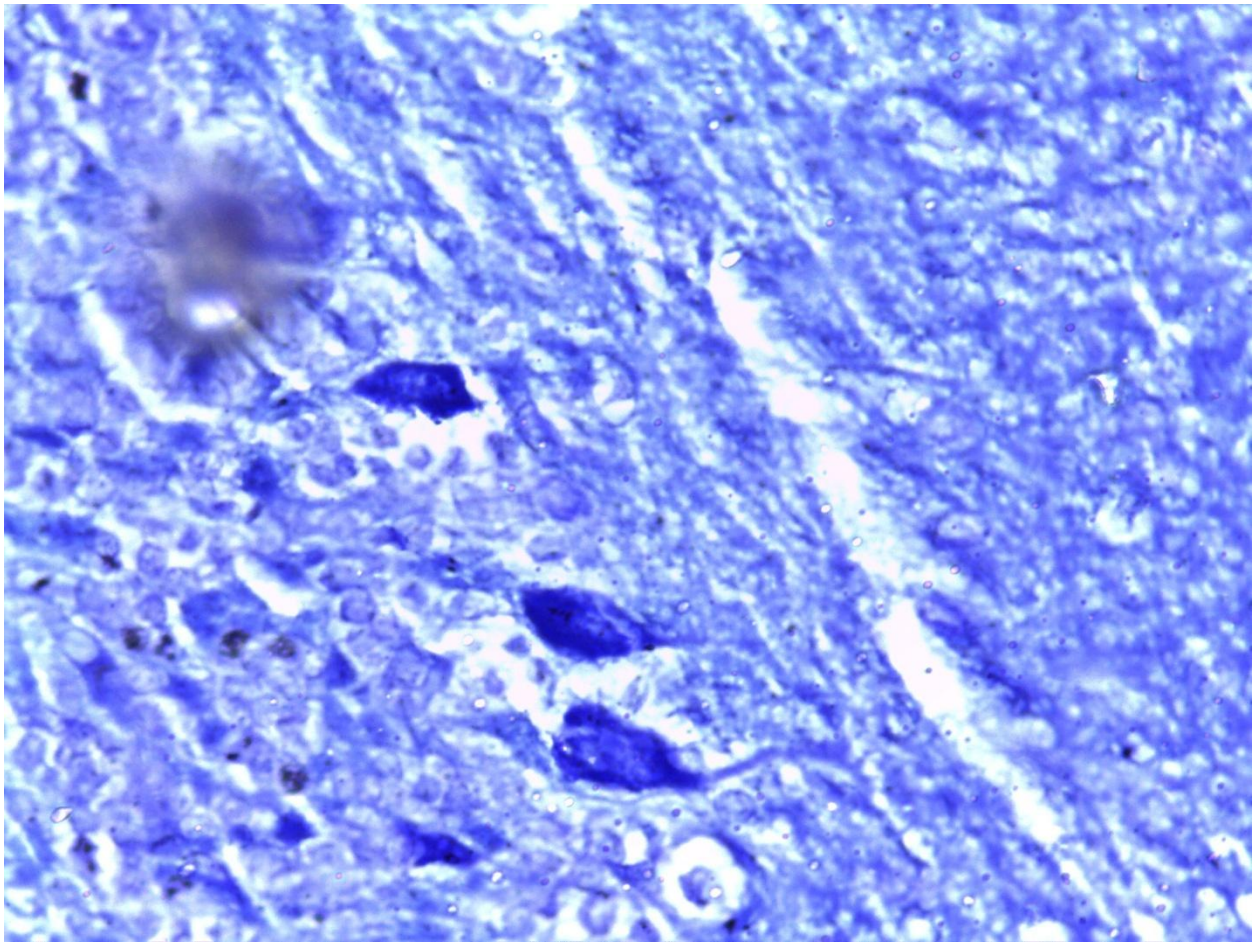


Plate 7: photomicrograph showing hippocampus of animal in group III (cfv, X1000)

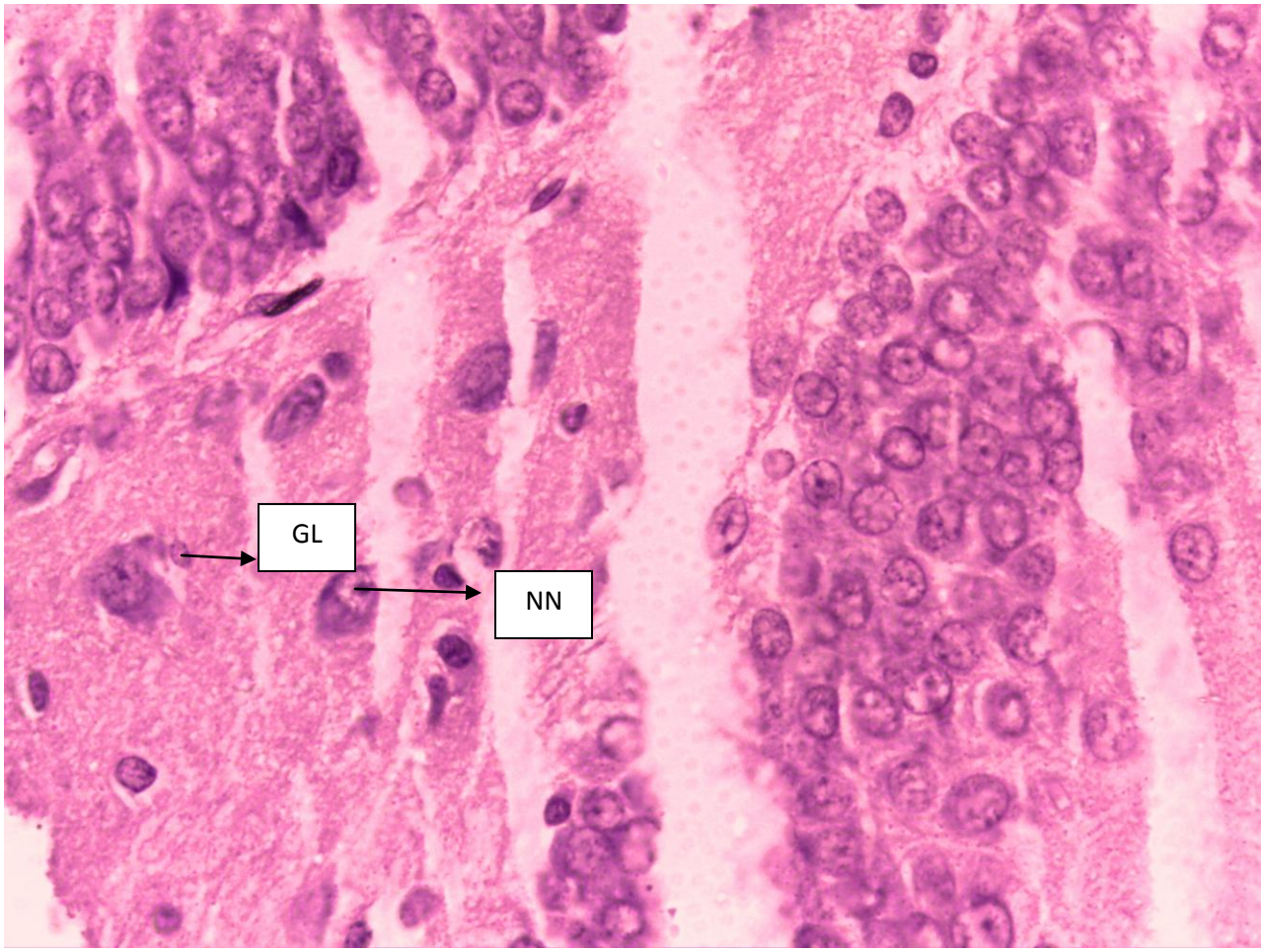


Plate 8: photomicrograph showing hippocampus of animal in group IV (H&E, X1000)

Nucleo cytoplasmic contrast is high but low in a few cells

Smearing of cytoplasmic border is absent but low in few cells

Nuclei are visible.

GL - Glial cell

NN - Normal neuron

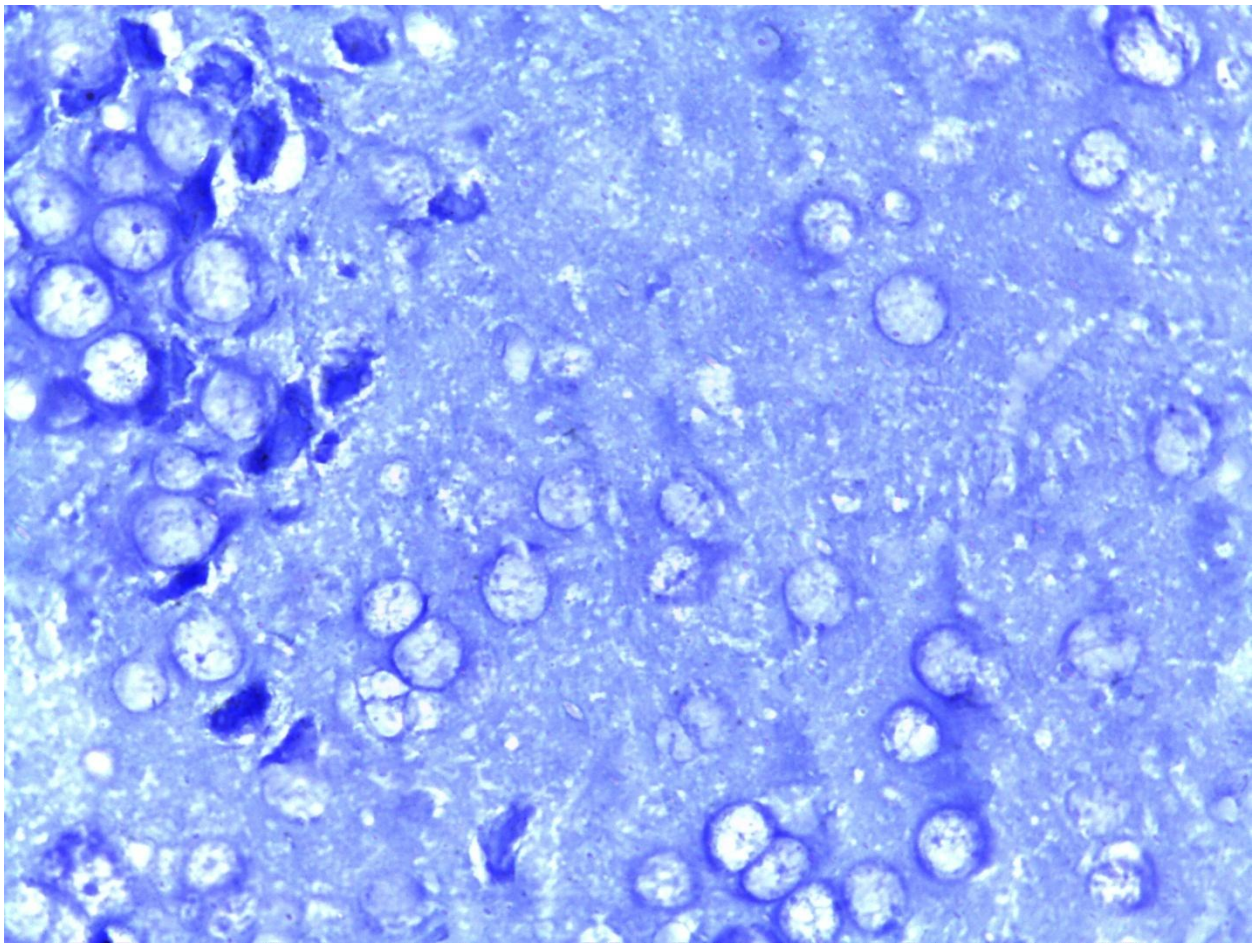


Plate 9: photomicrograph showing hippocampus of animal in group IV (cfv, X1000)

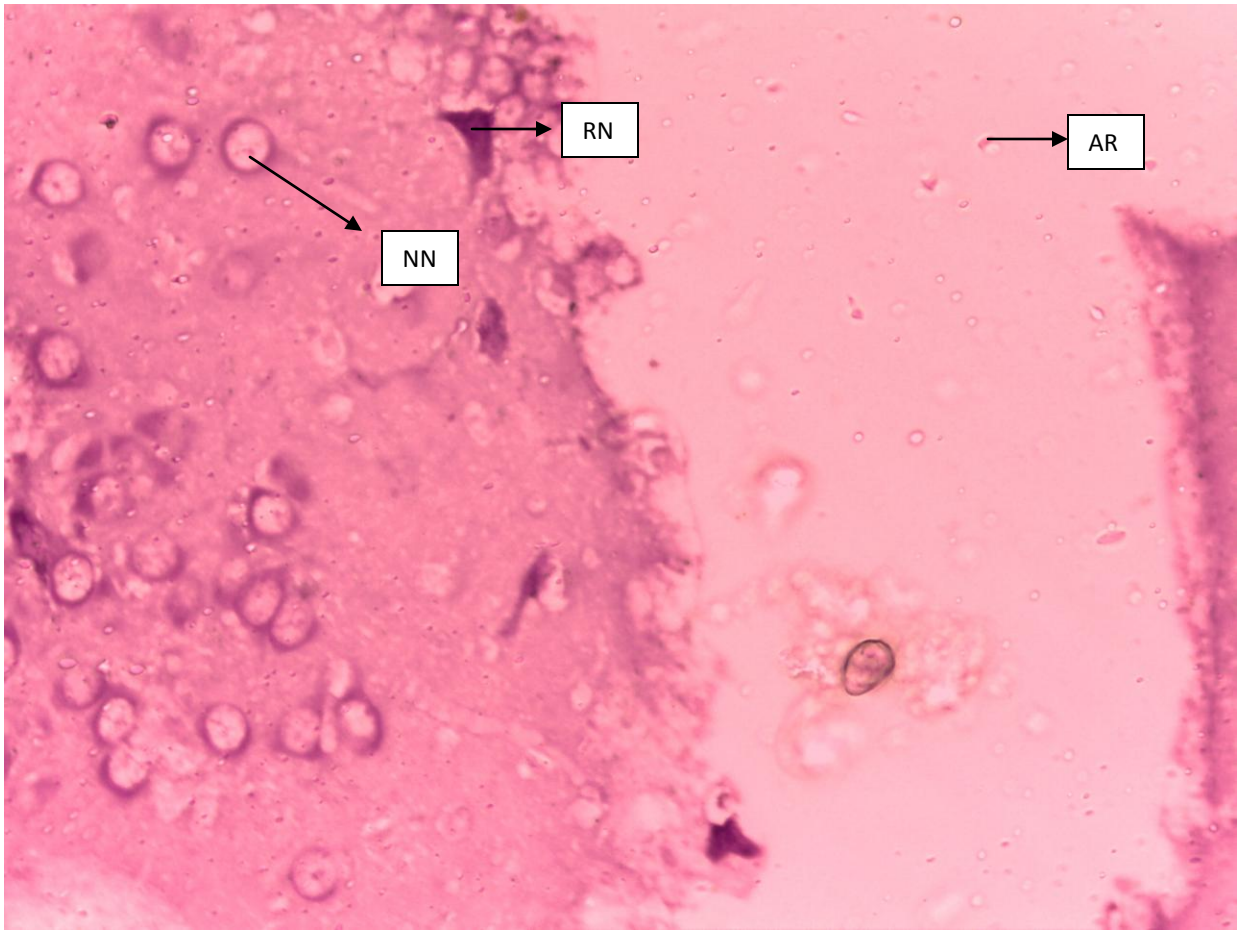


Plate 10: Photomicrograph of hippocampus of animal in group V. (H&E, X1000)

There are lots of artifactual separations on the slide

There are a few injured cells

Neucleo cytoplasmic contrast is high but low in one or two cells

Smearing of cytoplasmic border is absent and

Nuclei are visible

AR - Artifact

RN - Red neuron

NN - Normal neuron

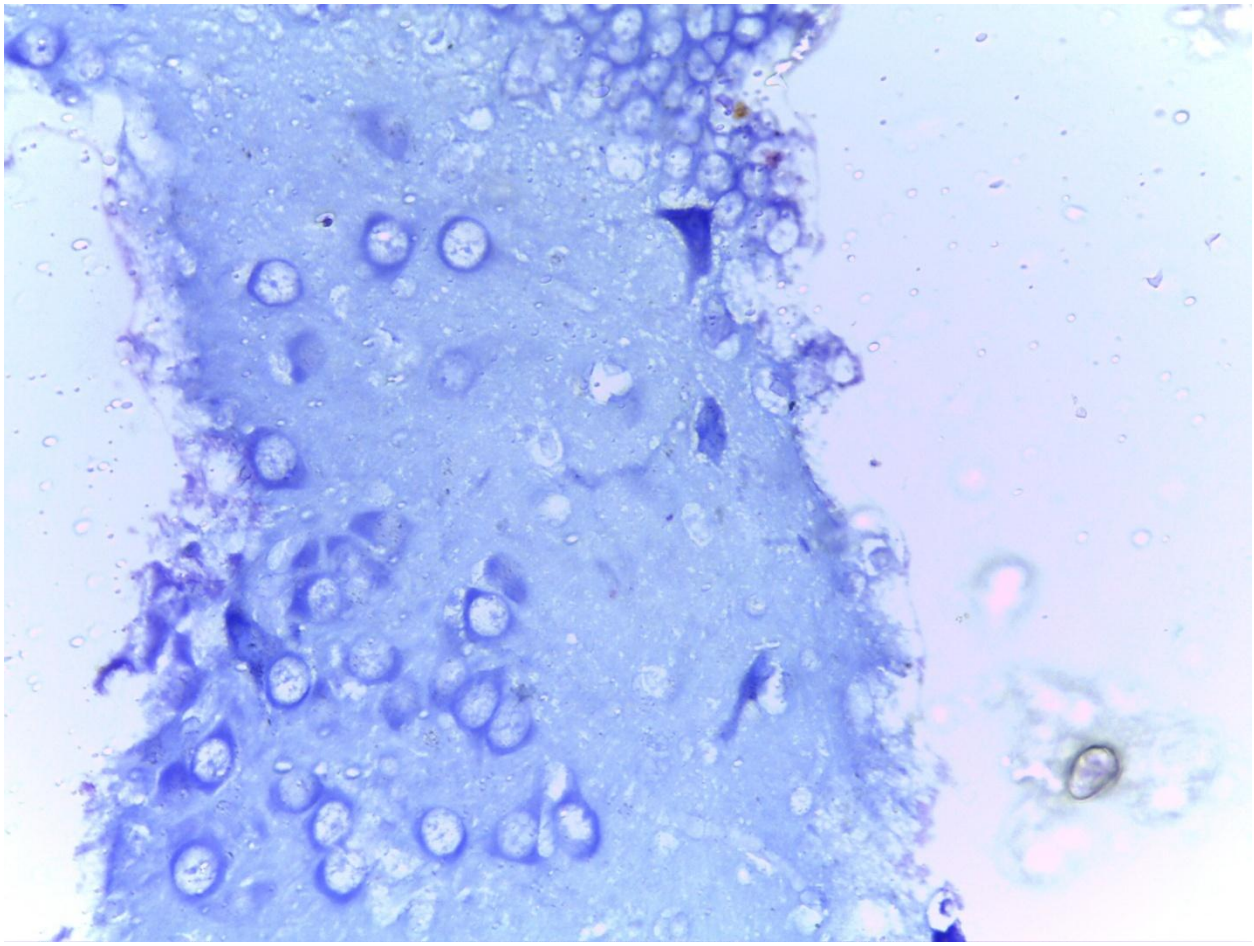


Plate 11: photomicrograph showing hippocampus of animal in group V (cfv, X1000)

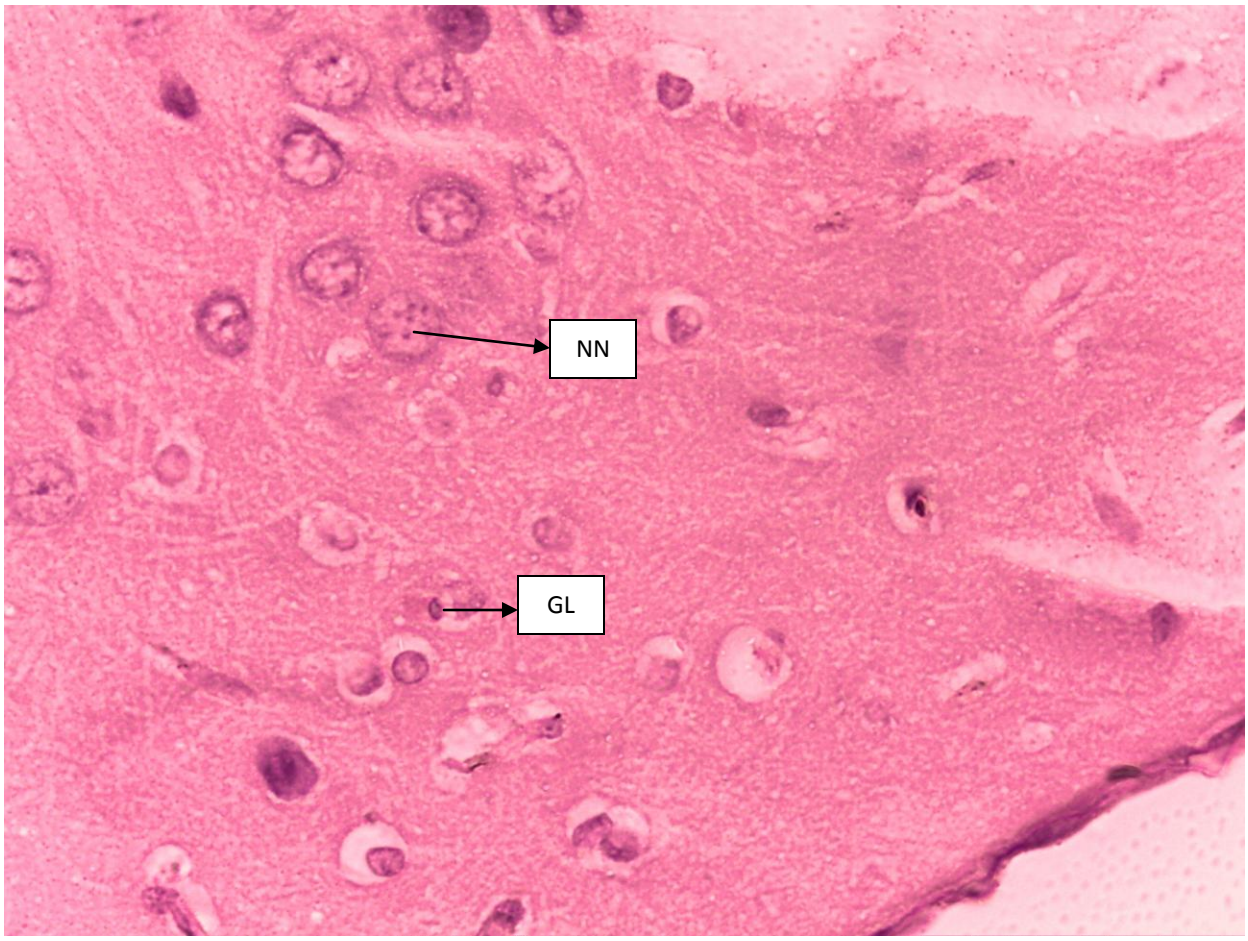


Plate 12: Photomicrograph of cerebrum of Wistar rat in the group I (H&E stain, x1000 mag.)

The neurons were generally healthy

Neucleo cytoplasmic contrast is high

Smearing of cytoplasmic border is generally absent and

The nuclei are clearly visible

GL - Glial cell

NN - Normal neuron

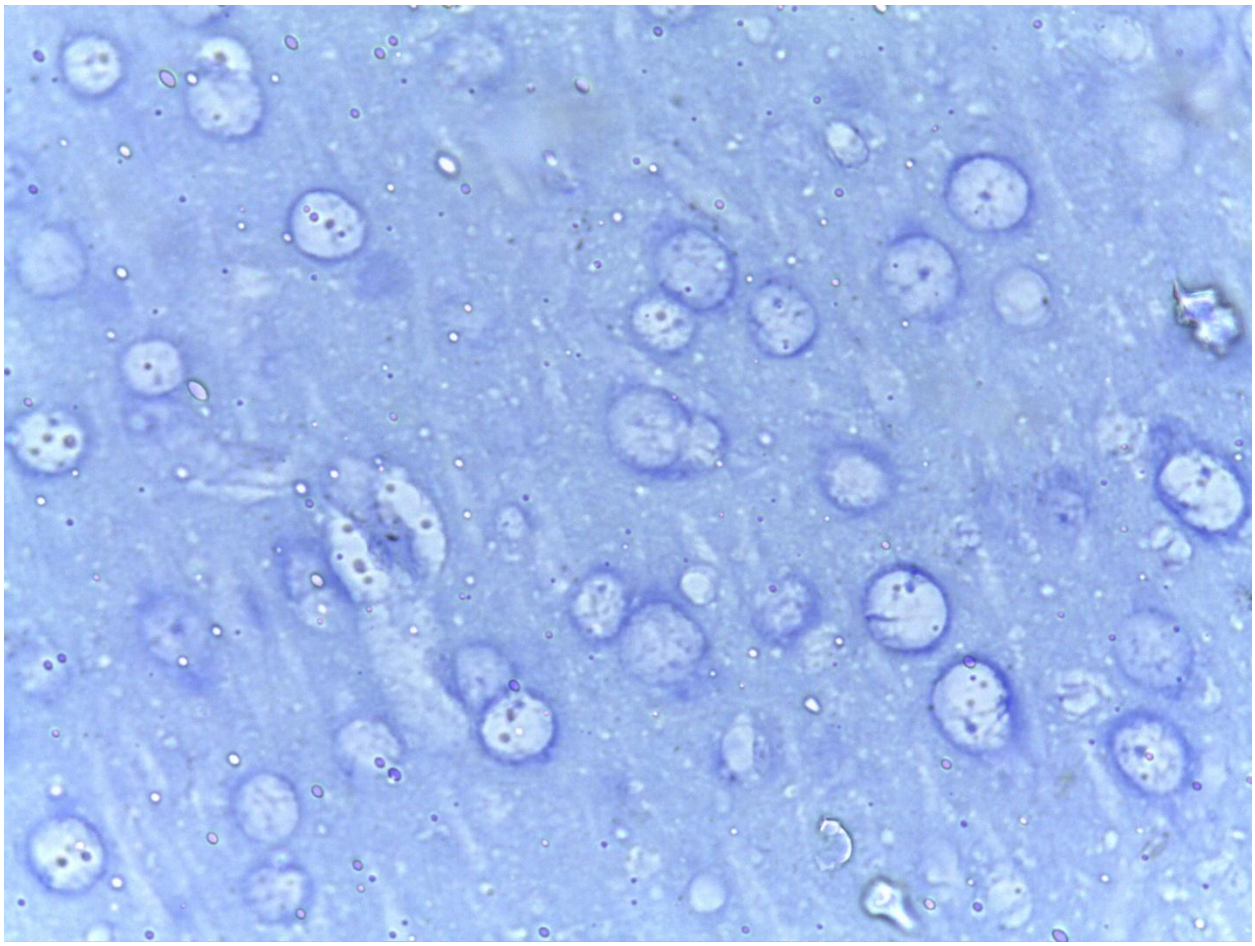


Plate 13: Photomicrograph of cerebrum of Wistar rat in the group I (cfv stain, x1000 mag.)

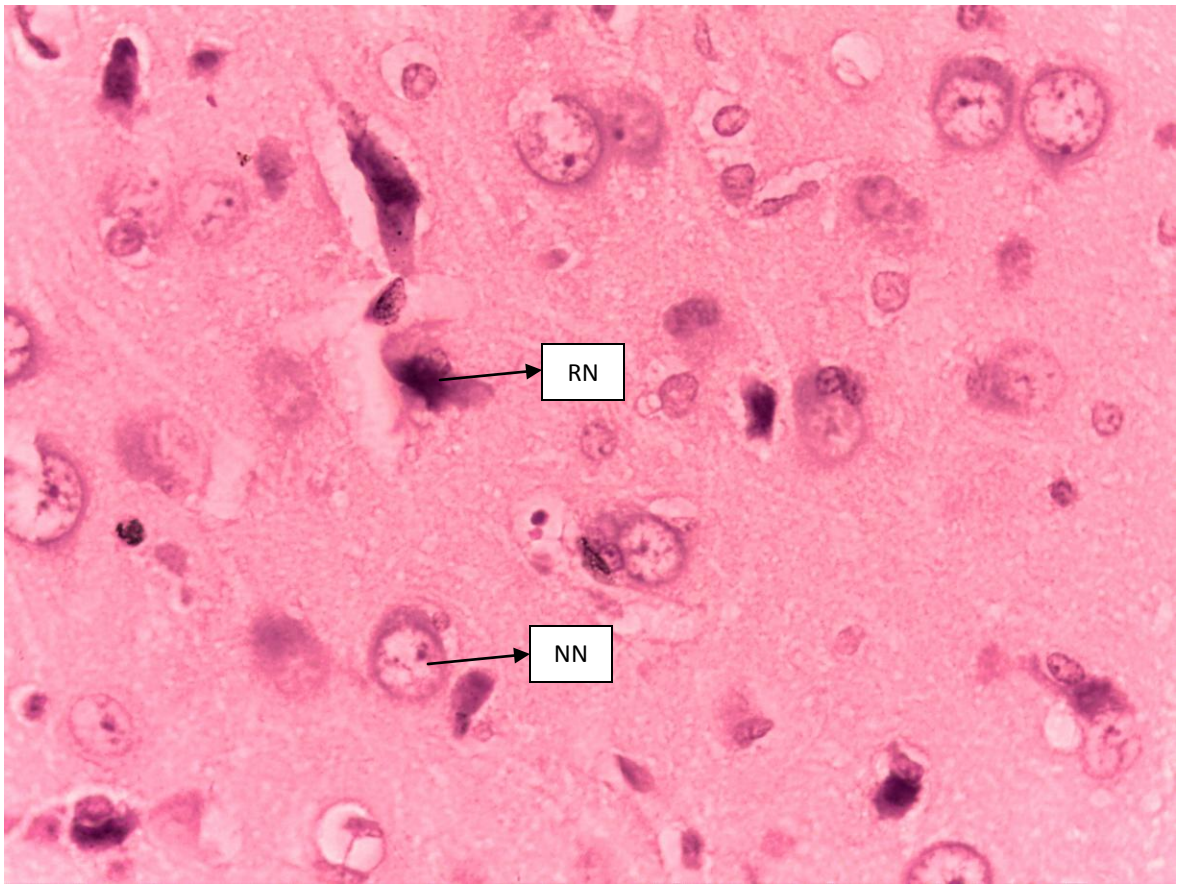


Plate 14: Photomicrograph of cerebrum of Wistar rat in group II (H&E stain, x1000 mag.)

There are a generally high number of neurons with ischaemic changes

Neucleo cytoplasmic contrast is high in neurons

Smearing of cytoplasmic border is absent in most neurons but present focally

The nuclei are visible in most cells but not visible in many cells

There are a few red neurons visible

RN - Red neuron

NN - Normal neuron

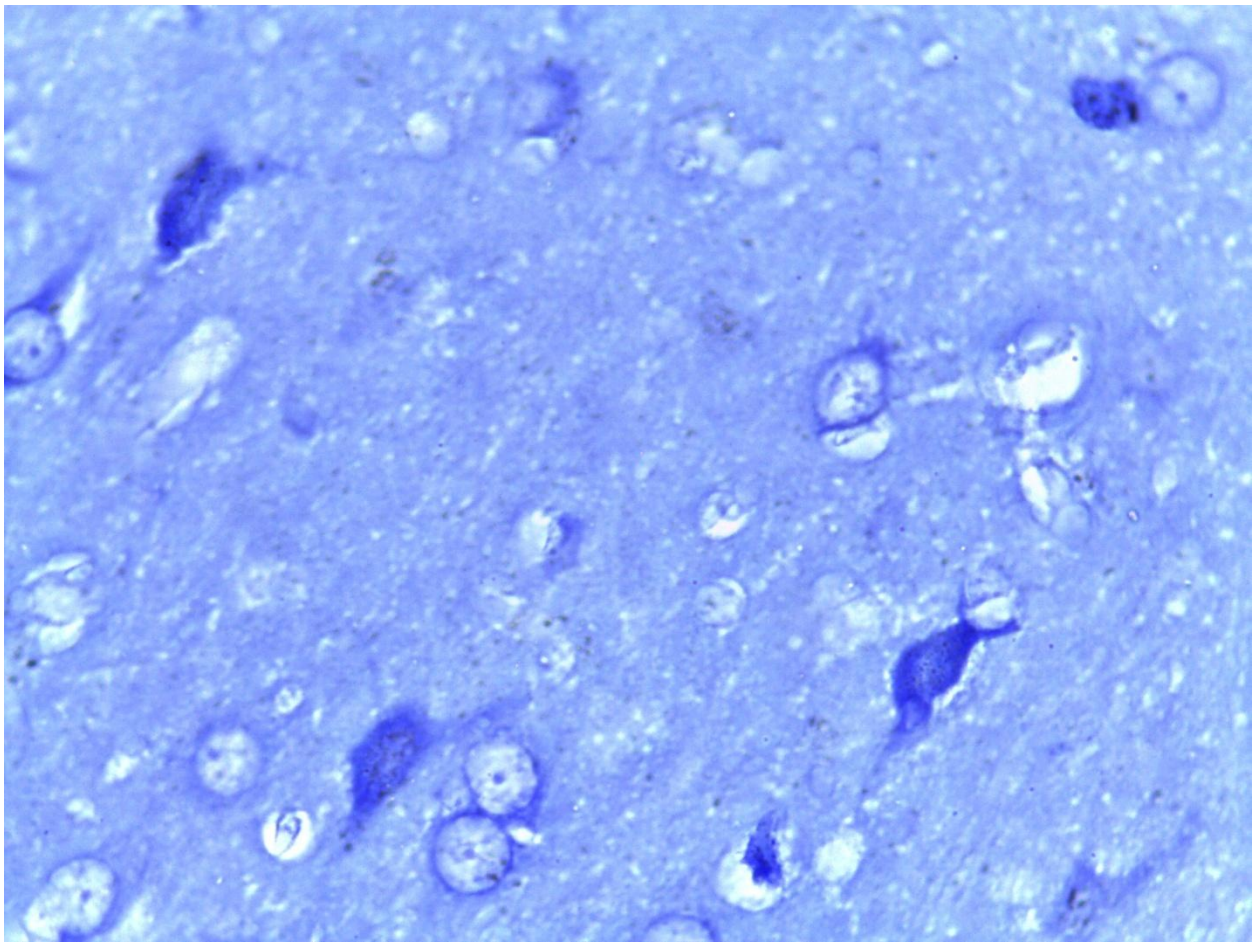


Plate 15: Photomicrograph of cerebrum of Wistar rat in group II (cfv stain, x1000 mag.)

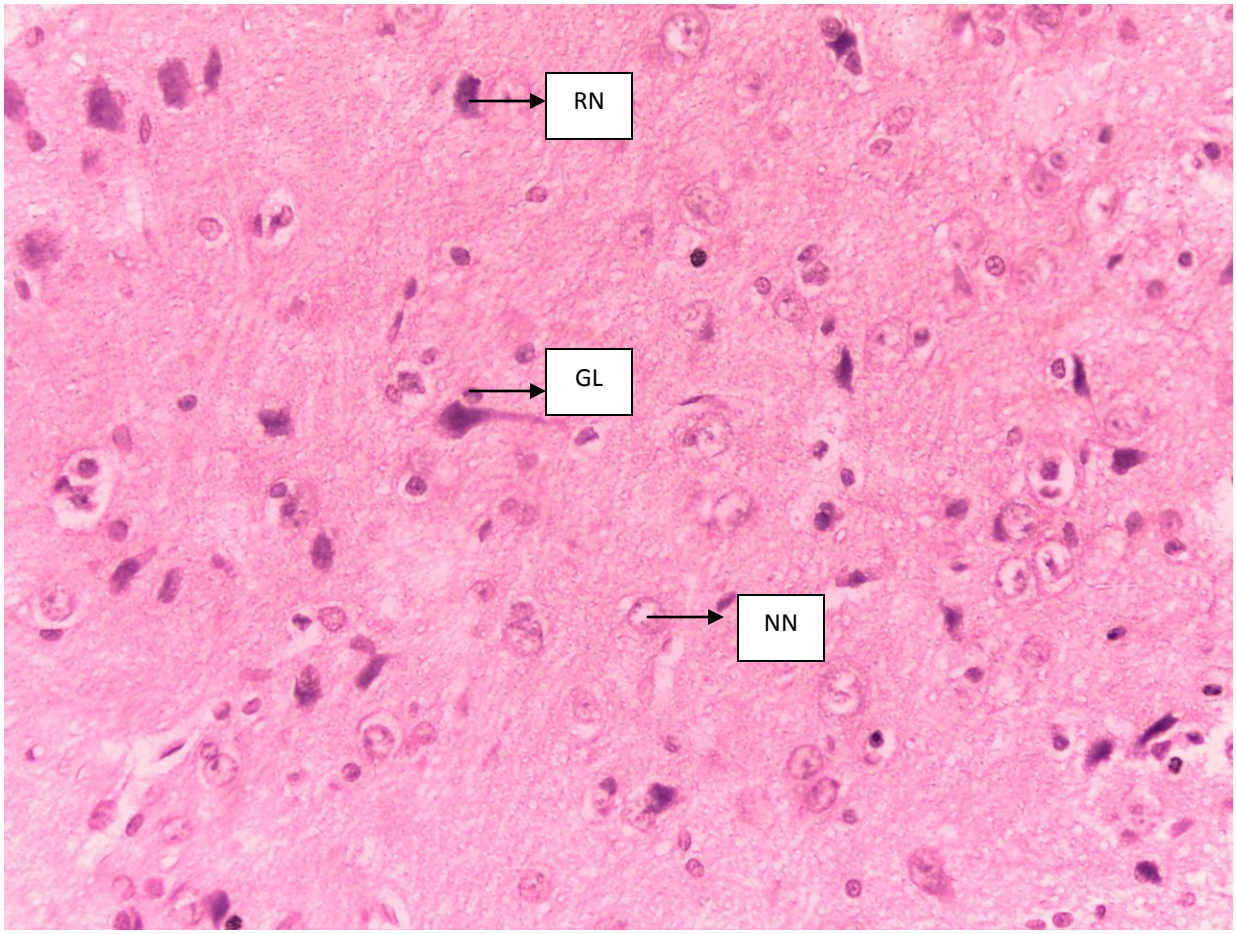


Plate 16: Photomicrograph of cerebrum of animal in group III (H&E stain, x1000 mag.)

Many of the neurons show signs of injury

Neucleo cytoplasmic contrast is high

Smearing of cytoplasmic border is absent in most cells but present focally

Neuclei are clearly visible

GL - Glial cell

RN - Red neuron

NN - Normal neuron

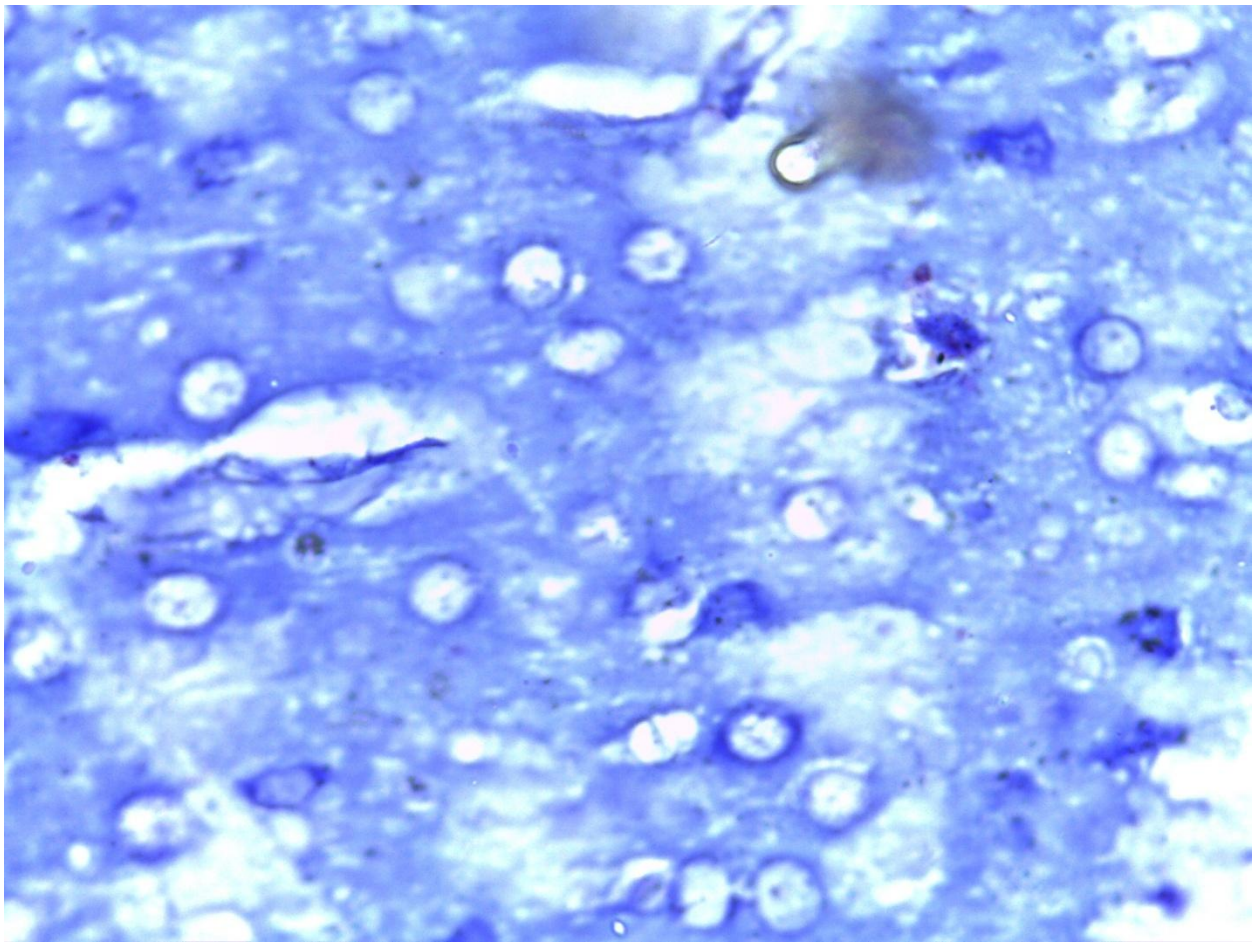


Plate 17: Photomicrograph of animal in group III (cfv stain, x1000 mag.)

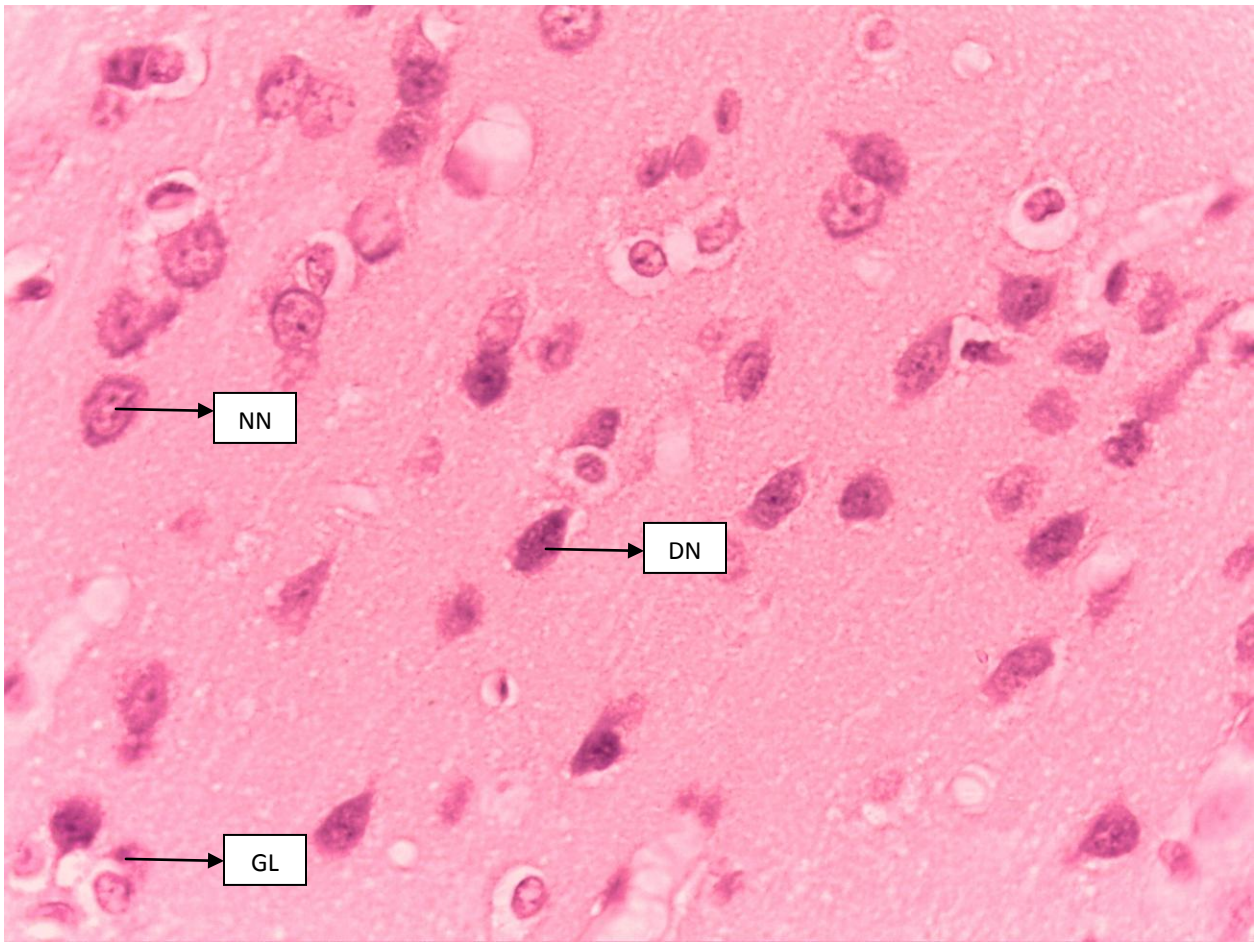


Plate 18: Photomicrograph showing cerebrum of animal in group IV (H&E stain, x1000 mag.)

The slide show a lot of artifacts

The neurons are very healthy

Neucleo cytoplasmic contrast is high

Smearing of cytoplasmic border is absent and

The neuclei are clearly visible

DN - Dying neuron

GL - Glial neuron

NN - Normal neuron

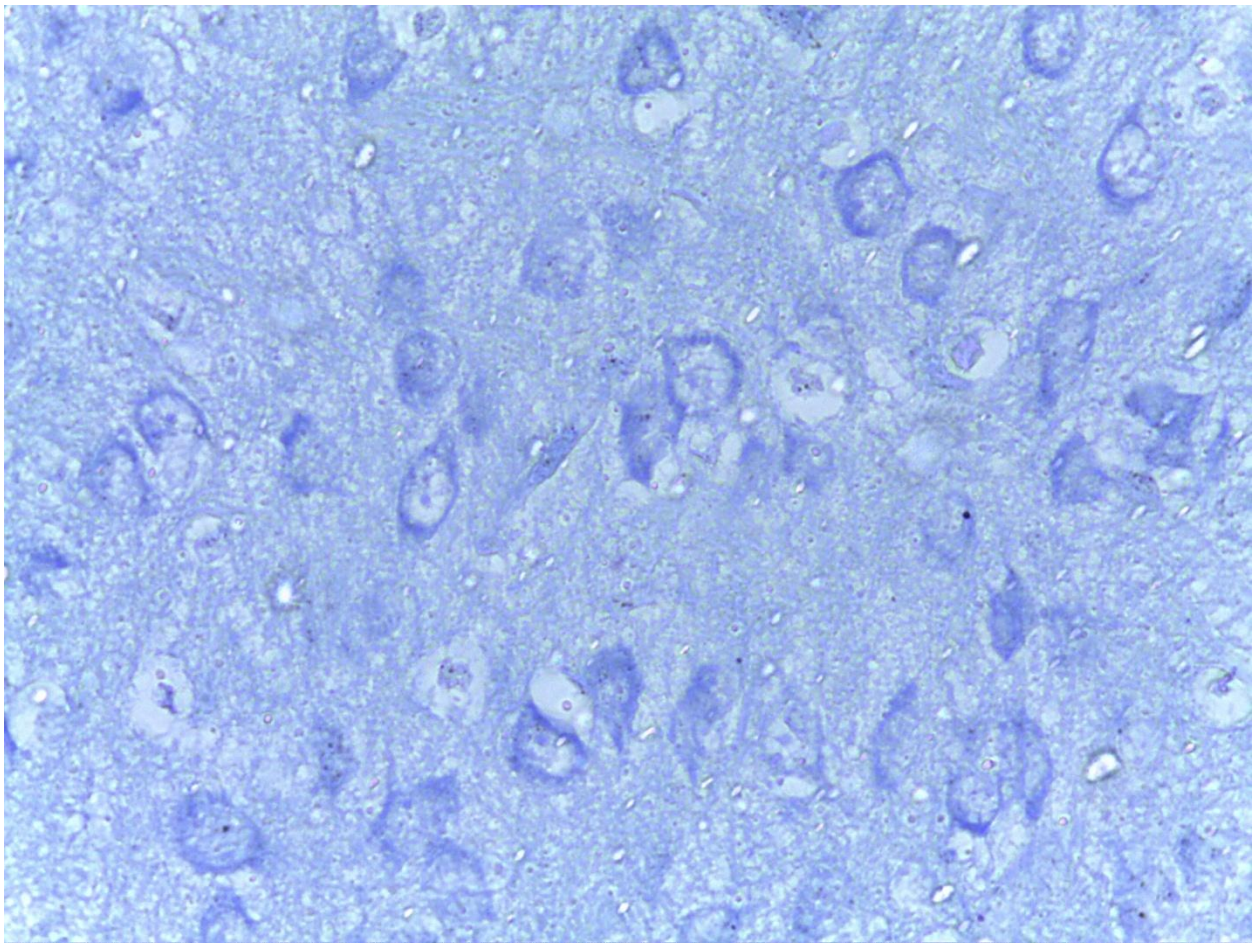


Plate 19: Photomicrograph of cerebrum of animal in group IV (cfv stain, x1000 mag.)

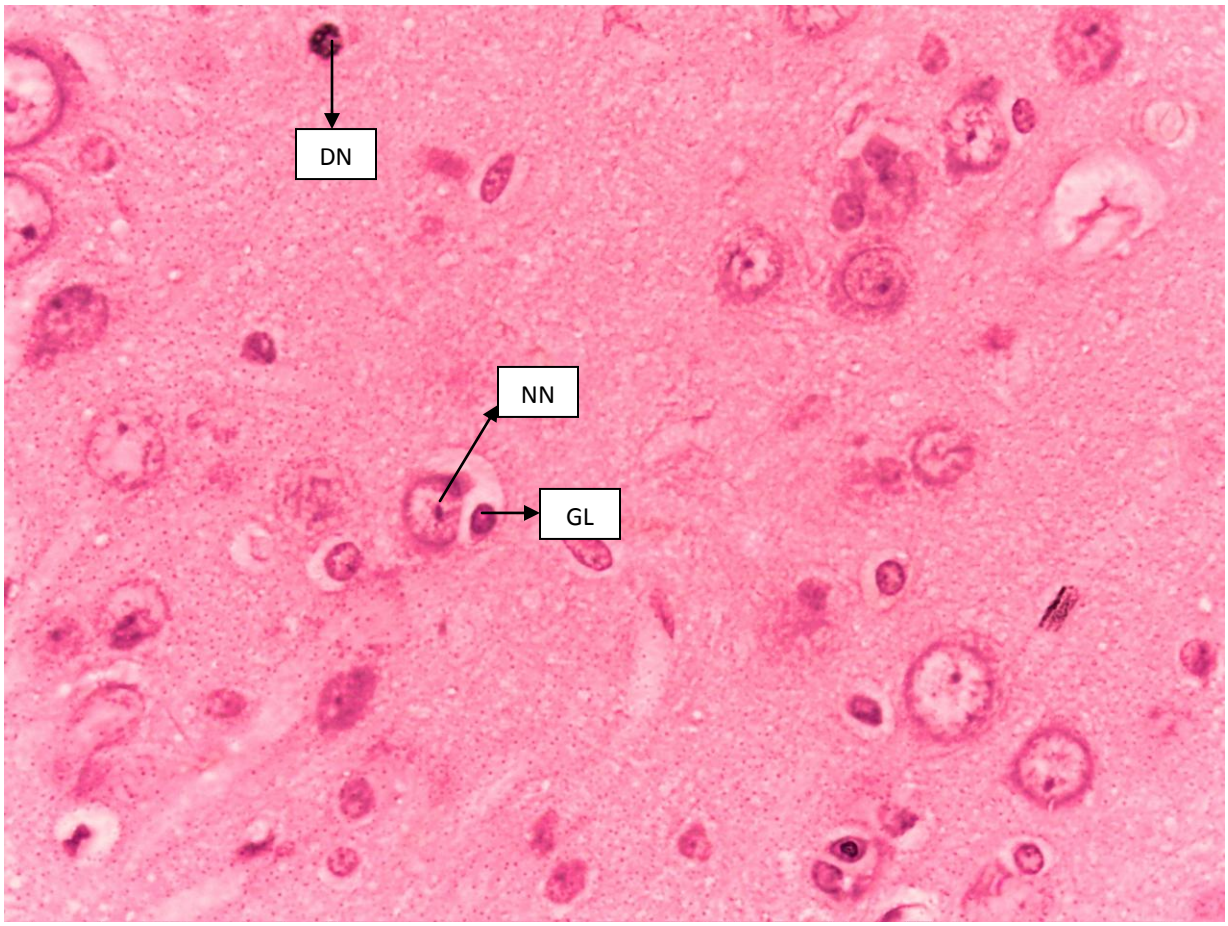


Plate 20: Photomicrograph showing cerebrum of animal in group V (H&E stain, x1000 mag.)

The neurons show no evidence of injury

Neucleo cytoplasmic contrast is high

Smearing of cytoplasmic border is absent

Nuclei are clearly visible

DN - Dying neuron

NN - Normal neuron

GL - Glial cell

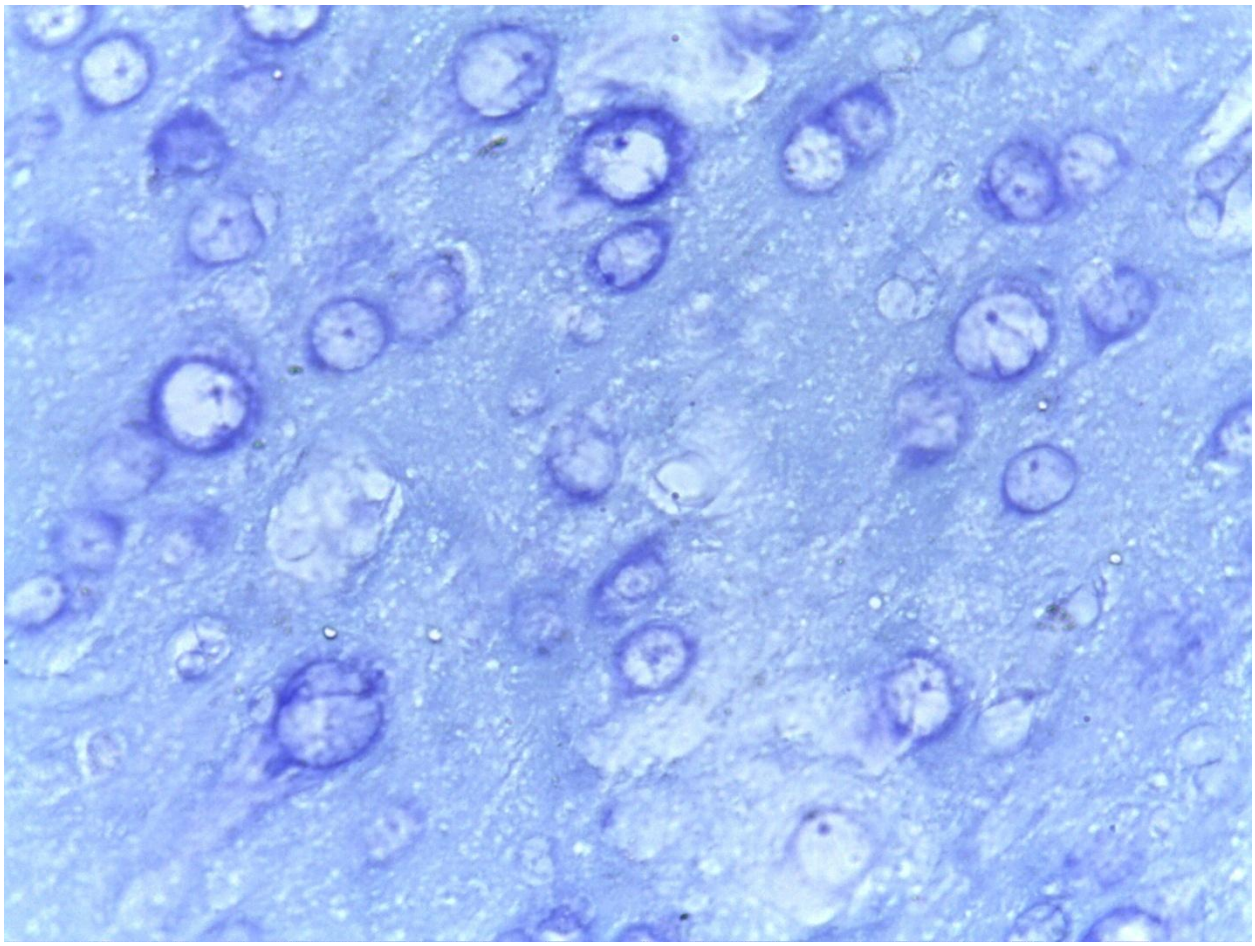


Plate 21: Photomicrograph of cerebrum of group V (cfv stain, x1000 mag.)

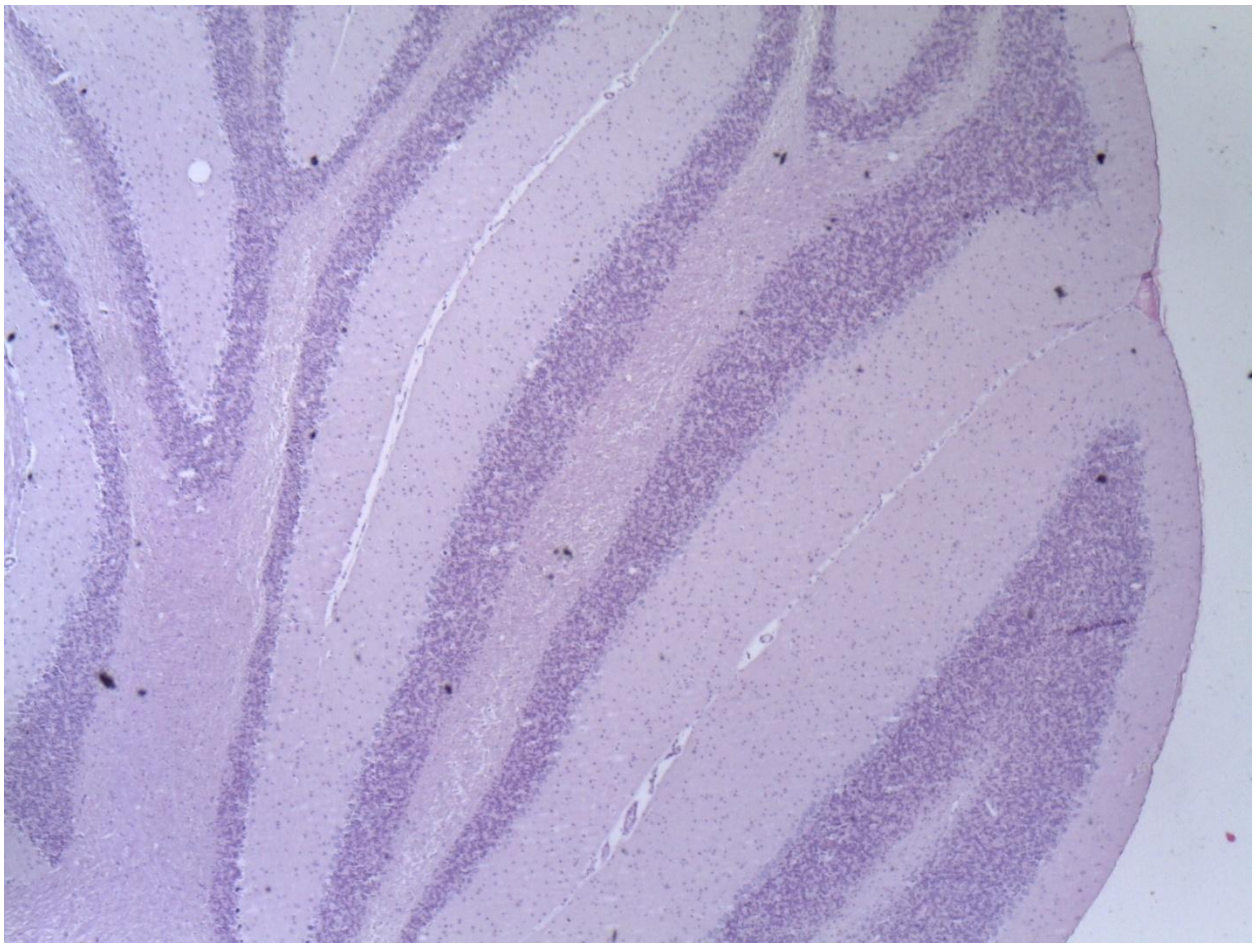


Plate 22: Photomicrograph showing an aerial view of cerebellum of animal in group I. (H&E, X40)

The Neucleo cytoplasmic contrast is high

Smearing of cytoplasmic border is absent and

Neuclei are clearly visible

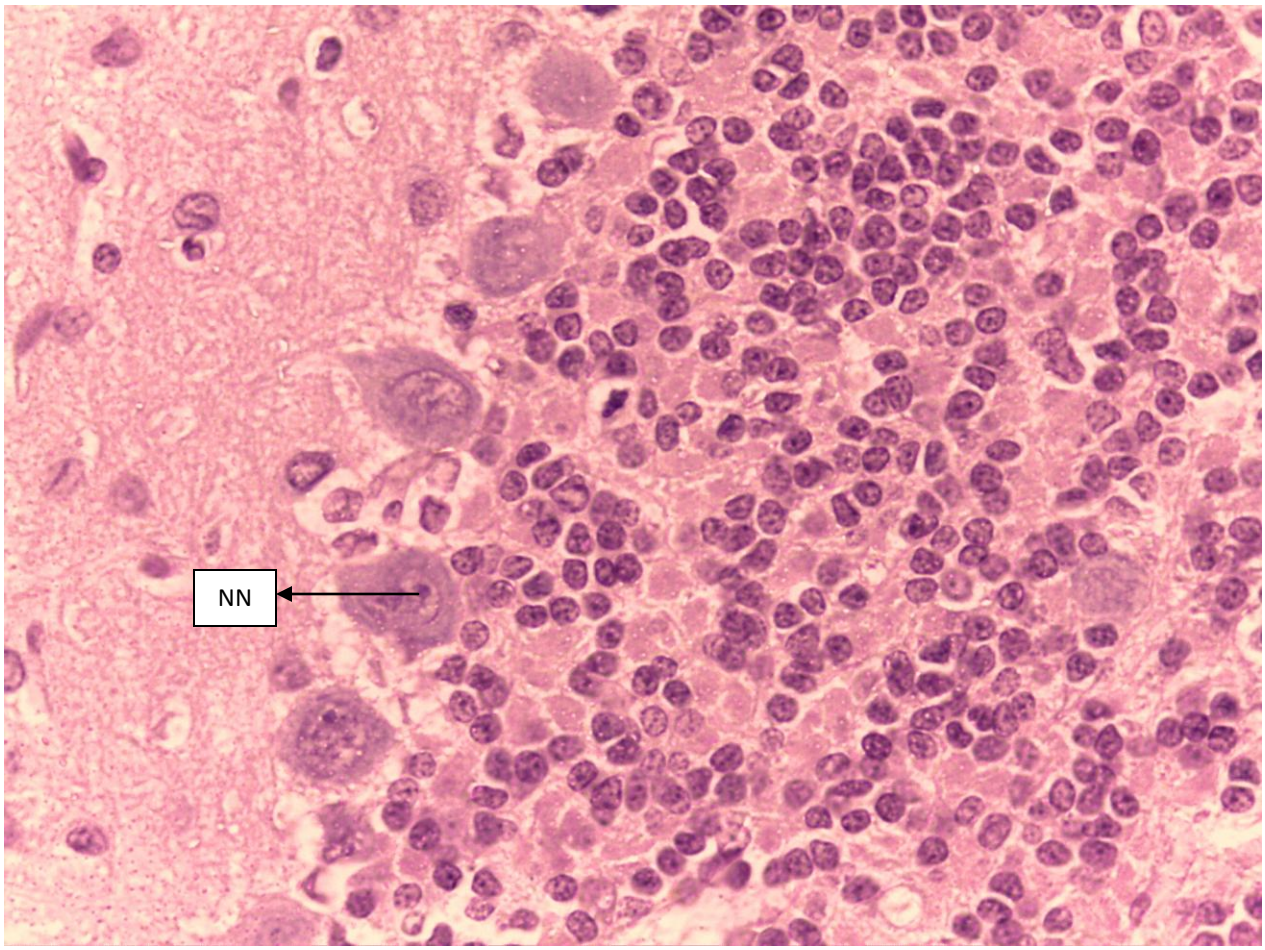


Plate 23: Photomicrograph of cerebellum of animal in group I (H&E stain, x1000 mag.)

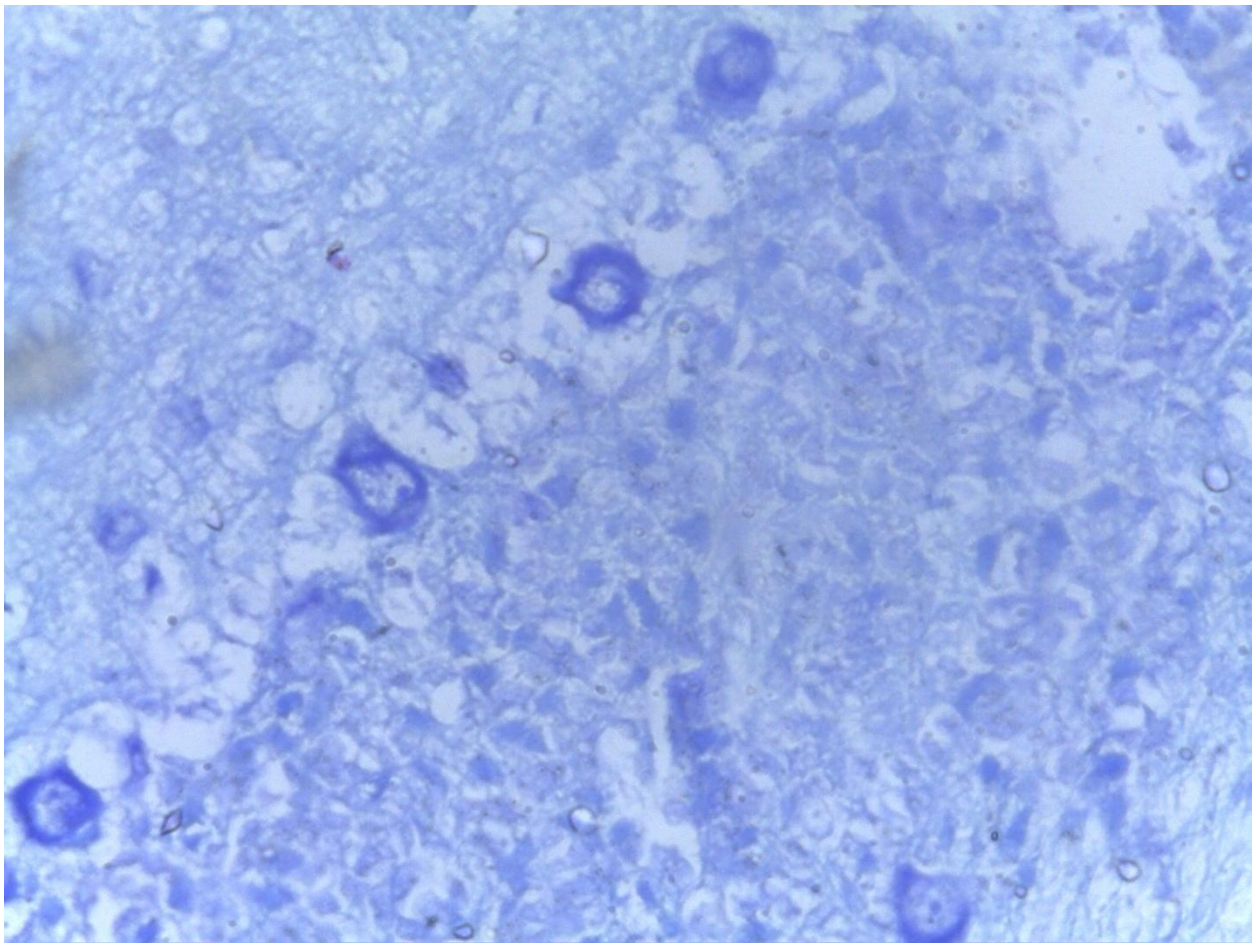


Plate 24: Photomicrograph of cerebellum of animal in group I (cfv stain, 1000 mag.)

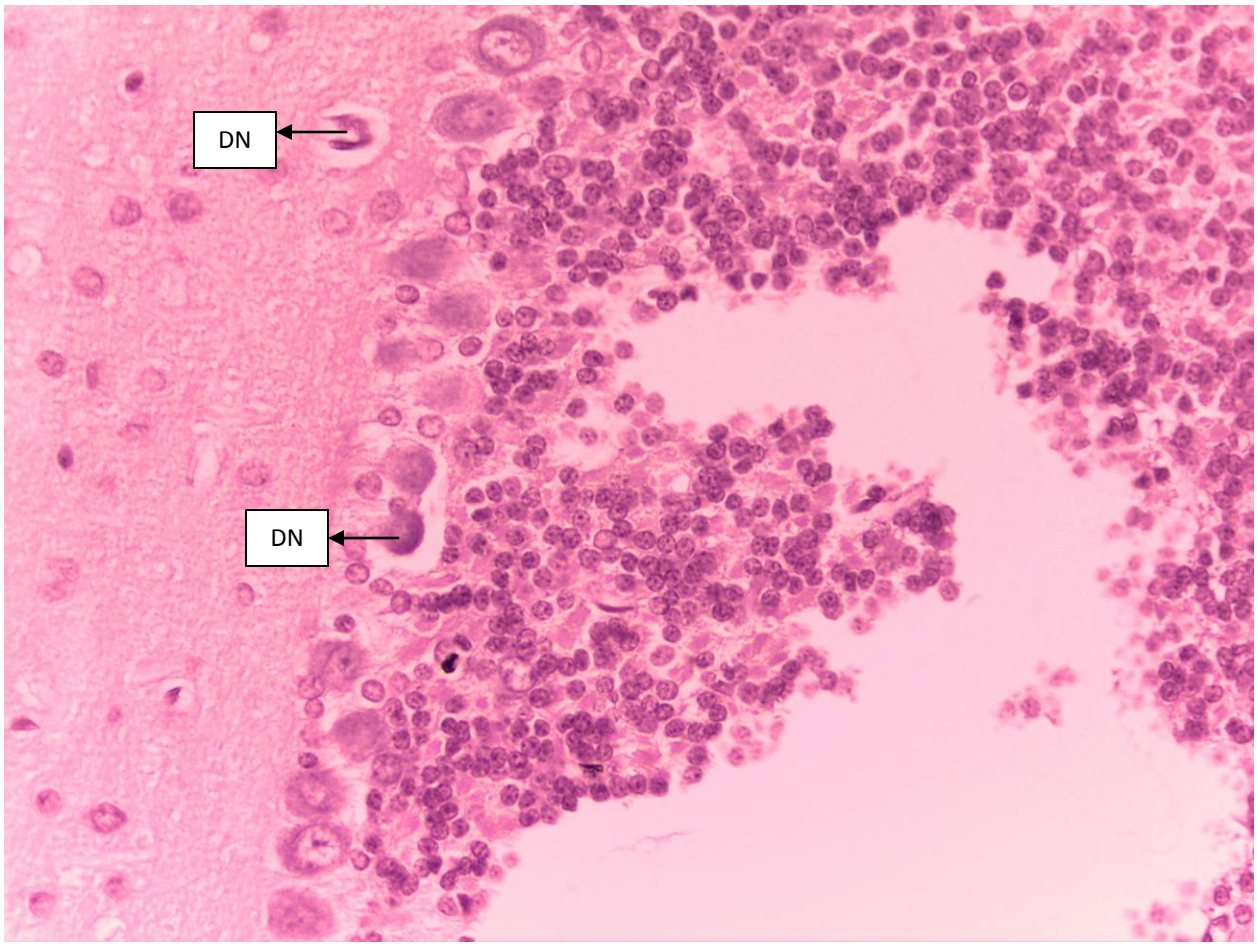


Plate 25: Photomicrograph of cerebellum of animal in group II (H&E stain, x1000 mag.)

There are scattered red neurons

Neucleo cytoplasmic contrast is moderate to high in most cells but low focally

Smearing of the nuclear membrane is present in some neurons

Nuclei are visible.

DN - Dying neuron

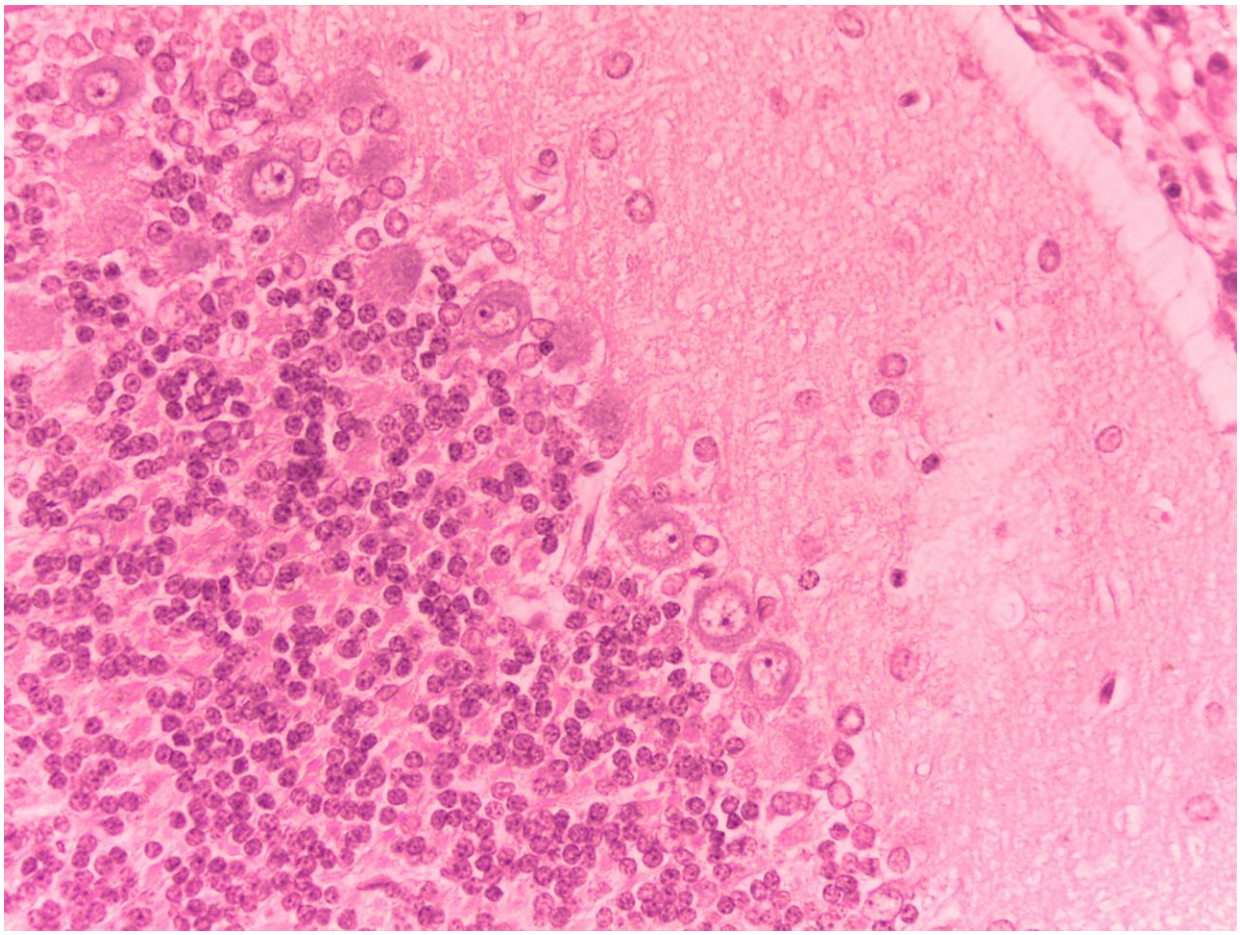


Plate 26: Photomicrograph of cerebellum of animal in group II (H&E stain, x1000 mag.)

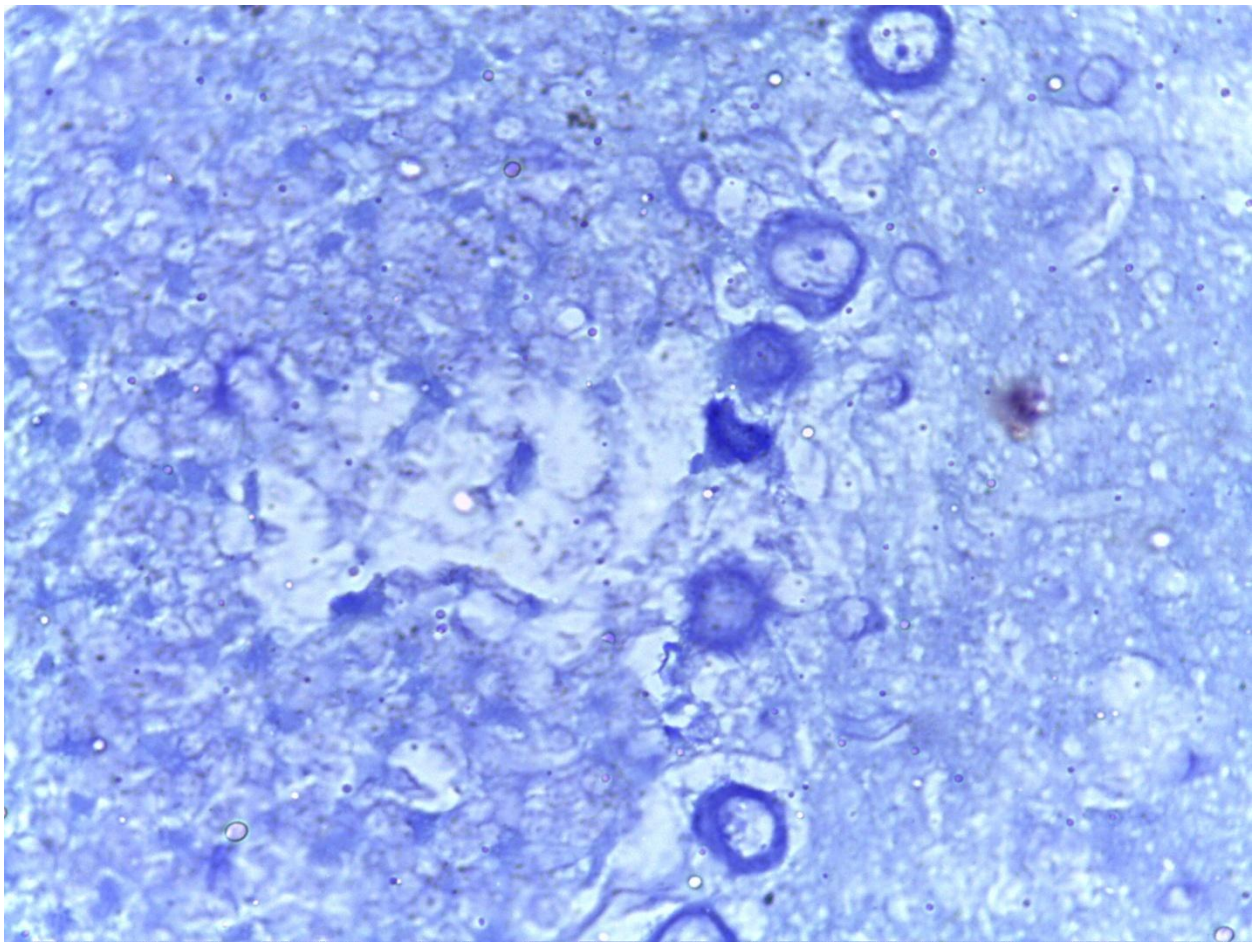


Plate 27: Photomicrograph of cerebellum of group II animals (cfv stain, x1000 mag.)

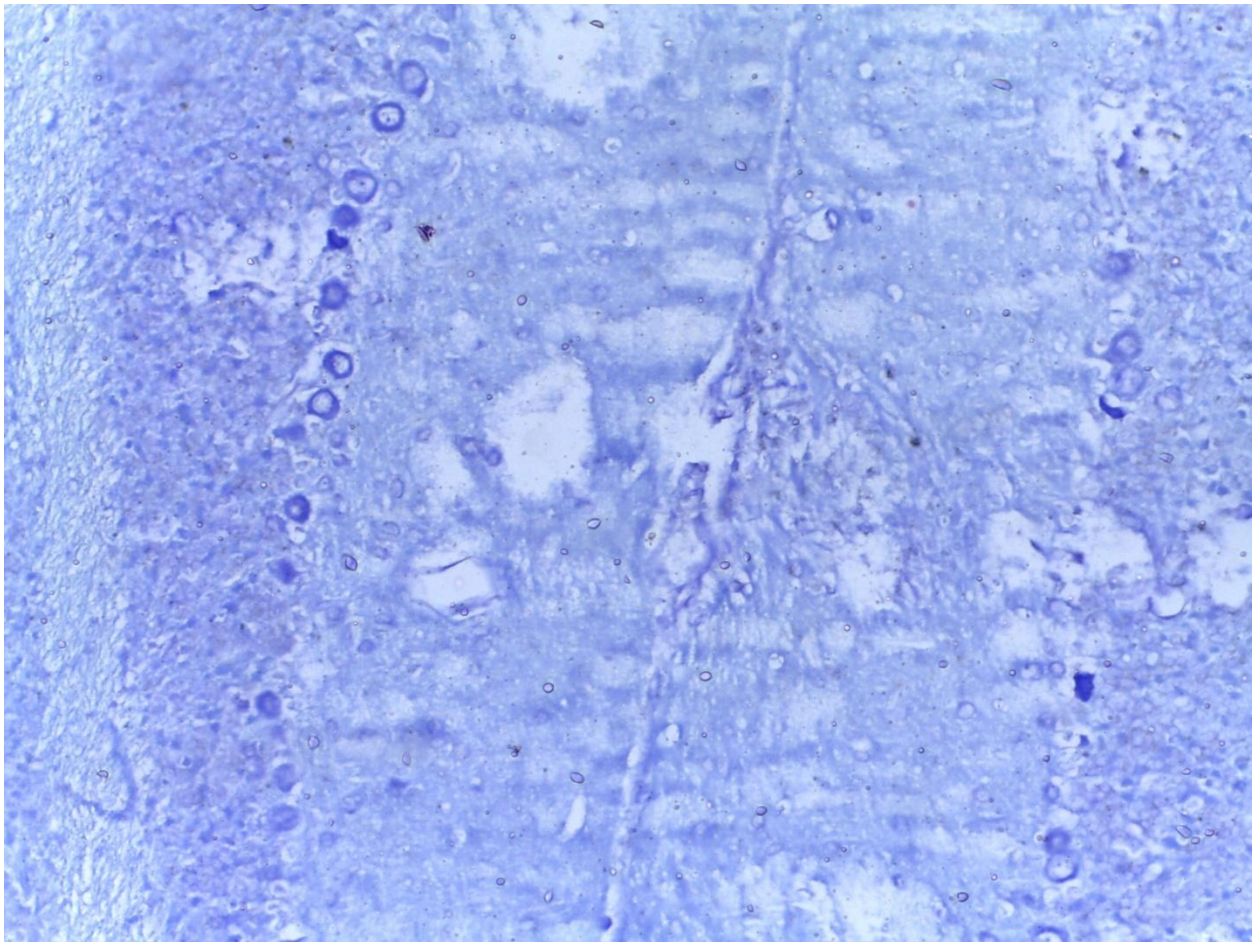


Plate 28: Photomicrograph cerebellum of animal in group II (cfv stain, x1000 mag.)

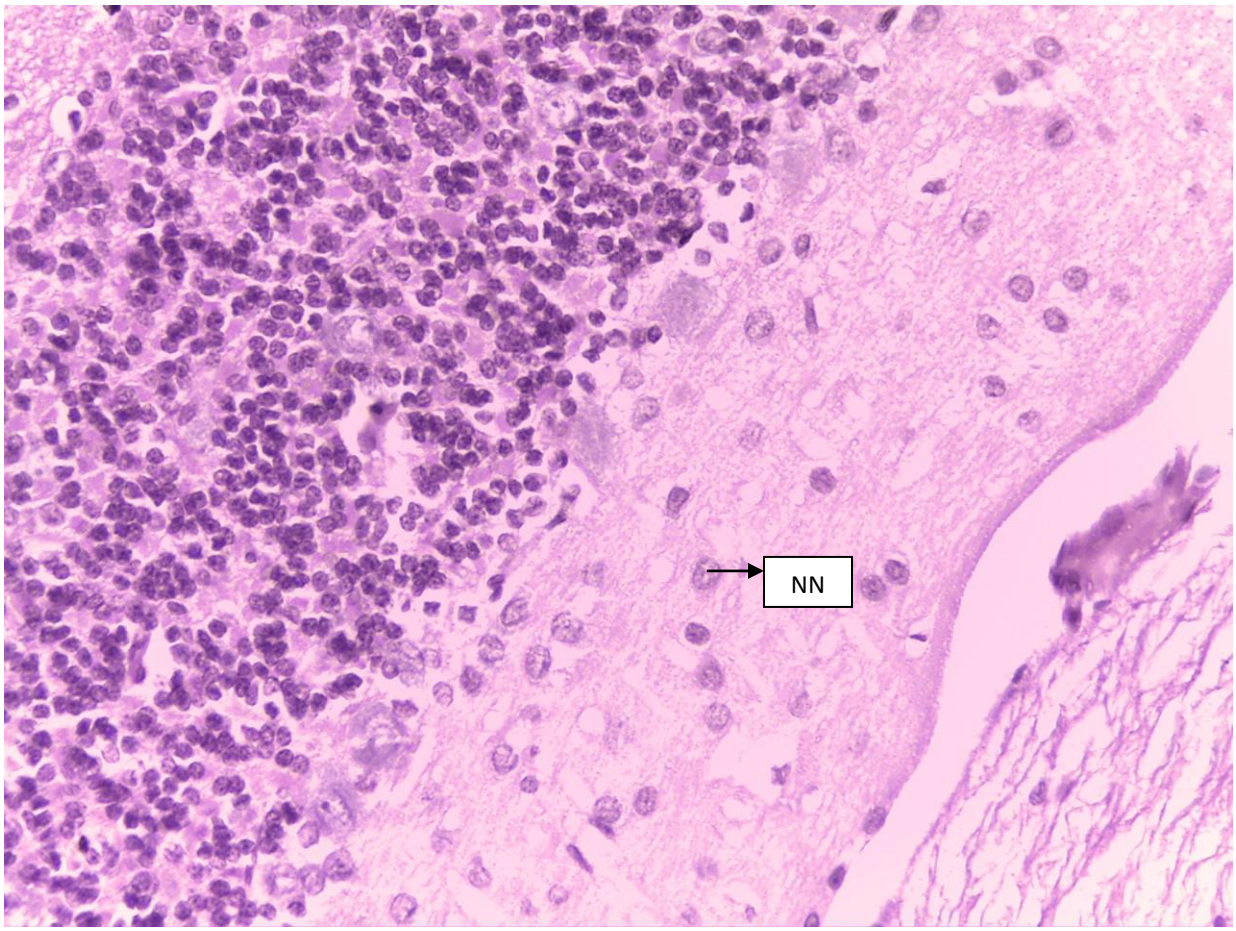


Plate 29: Photomicrograph of cerebellum of animals in group III (H&E stain, x600 mag.)

There are a few injured cells but most the cells are healthy

Neucleo cytoplasmic contrast is high

Smearing of cytoplasmic border is present and

Nuclei are visible

NN - Normal neuron

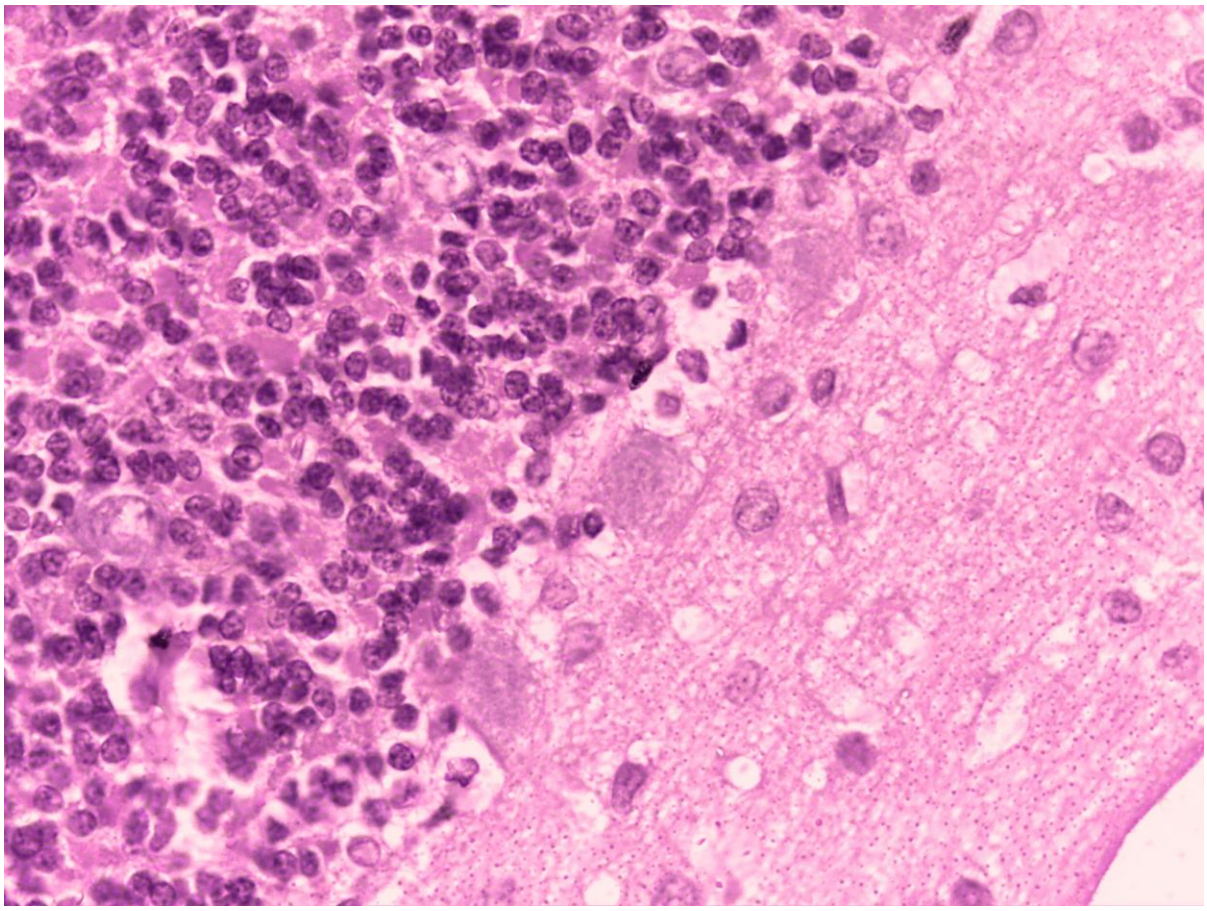


Plate 30: Photomicrograph of cerebellum of animals in group III (H&E stain, x1000 mag.)

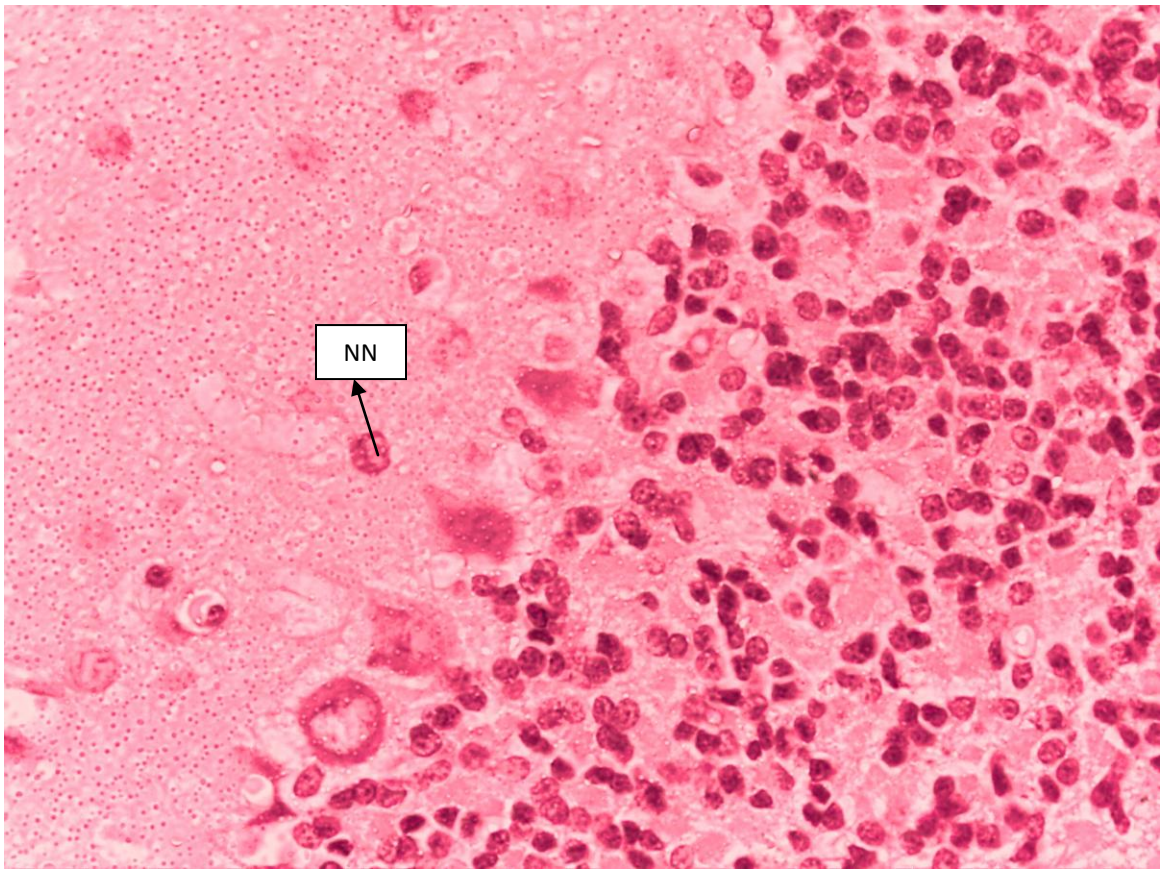


Plate 31: Photomicrograph of cerebellum of animal in group IV (H&E stain, x1000 mag.)

There are a few injured cells with poor Neucleo cytoplasmic contrast

Smearing of cytoplasmic border is present and

Nuclei are visible

NN - Normal neuron

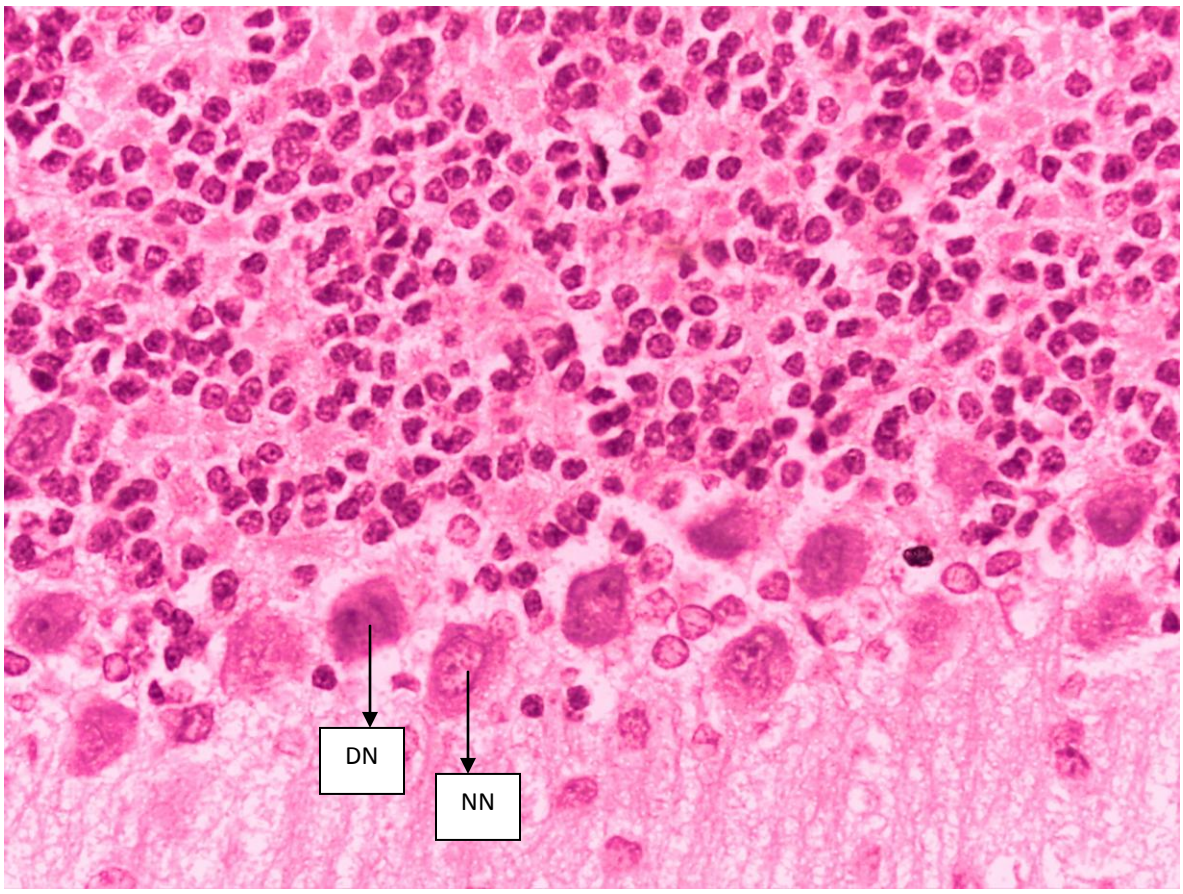


Plate 32: Photomicrograph of cerebellum of animal in group V (H&E stain, x1000 mag.)

The neurons are generally healthy

The Neucleo cytoplasmic contrast is high

Smearing of cytoplasmic border is present and

Nuclei are visible

DN - Dying neuron

NN - Normal neuron

CHAPTER FIVE

5.1 DISCUSSION

The serum malonyaldehyde levels found in the control group were significantly higher than in the Ischaemic control. Malonyaldehyde is an indicator of the presence of free radicals in circulation. This result is expected given that induction of Ischaemia is a traumatic event and the period of transient global Ischaemia is expected to cause free radicals to accumulate in the serum. The levels of malonyaldehyde in group III animals that received piracetam only is not significantly higher than that of the control group. This may indicate that piracetam has the ability to mop-up free radicals from the circulation. The levels in groups IV and V that received additional vitamin E are significantly higher than that of group III. Brain tissue malonyaldehyde levels did not show significant changes with inductions of stress. This is in disagreement with the work of Omar et al, 2011.

The excitability score tested for motor co-ordination in wistar rats. The percentage excitability was very high in group I animals with a high grade response. Surprisingly, excitability score was even higher in group II animals that had ischaemia induced. However, the grade of reaction was poorer than in group I. Group III animals showed a reduced percentage excitability than group II. This suggests that Piracetam improved damage caused by Ischaemia. The grade of response was also higher in the wistar rats and vitamin E added in groups IV and V seems to reduce percentage excitability but improved grade of response. None of these differences showed statistical significance. This is not in agreement with the findings of Sohn et al, 2000.

The forepaw grip time tested motor strength in between the different groups. The forepaw grip time of animals in group I was lower than that of animals in group II. This was not expected. The forepaw grip time in group II was lower than that of group II. This suggests that Piracetam has a modulatory effect on damage to the cerebral cortex. This ameliorative effect seems to improve with vitamin E alone or in combination with Piracetam as the grip times in groups IV and V were higher than group III. None of these differences showed statistical significance. This did not agree with the findings of Ozyuret et al, 2010.

Transfer latency time tested the memory of the animals. The animals in group I over all showed the highest transfer latency time. This was unexpected because damage to the hippocampus was expected to affect memory, therefore the group II animals were expected to have higher transfer latency time. However, when transfer latency time was compared between group II and III animals, the time was found to be shorter in group III animals. This may suggest that Piracetam has a role in reversing damage to the hippocampus following induction of Ischaemia. The transfer latency time in group IV animals showed an interesting result. The transfer latency time was higher than that of group III animals. This suggest that a combination of Piracetam and vitamin E does not potentiate the ameliorative effect on Ischaemic damage. This is encouraged by the fact that administering vitamin E alone (group IV) was not as effective as administering Piracetam only, since the transfer latency time in group V is higher compared to group III. None of these differences however showed any statistical significance. This is in disagreement with the findings of Ranca et al, 2000.

Examination of the histopathological slides of the hippocampus suggests that the neurons in group I were generally healthier than those in group II. This confirms that, transient global Ischaemia caused neuronal cell damage in the hippocampus. When group II animals were compared with group III, more neurons seem to be healthier in group III animals, suggesting that Piracetam has a role in ameliorating neuronal cell damage following global Ischaemia. Comparison of micrographs of groups IV and V to group II did not show remarkable differences in the results. This may suggest that vitamin E alone or in combination with Piracetam, may not have superior ameliorative effects on hippocampus following induction of Ischaemia. This finding supports the claim of Moran et al, 2002, who reported that the use of Piracetam improves learning and memory in mice. However, these findings disagree with the findings of Sohn et al, 2000 which suggested that rat hippocampal ultrastructure is not improved by Piracetam and vitamins following induction of damage using alcohol.

The cerebral analysis of histopathological micrographs suggests strongly that induction of global Ischaemia caused neuronal cell death in the cerebral cortex. This is evident when group I micrographs were compared in group II. When these were compared to group III however, there was a marked improvement on the overall health of neurons. This suggests that Piracetam does have a role in recovery of neuronal cell death. These changes were remarkable in groups IV and V slides, suggesting that the effect of vitamin E alone or in combination with Piracetam is effective as that of Piracetam alone. These findings are in agreement with the findings of Kessler et al, 2000 and Xerri and Zennou-Azoqui, 2003.

When the histopathological micrographs of the cerebellum were assessed, there was evidence of neuronal cell damage in the cerebellum following induction of global Ischaemia. This is seen in a comparison of micrographs of group I and II. Comparison of groups I and II to group III showed remarkable turn around in the relative number of damaged neurons. This suggests that Piracetam has a healing effect on the neurons of cerebellum following global Ischaemia. This improvement was also evident in groups IV and V animals, suggesting that vitamin E alone and in combination with Piracetam also improves damage done by Ischaemia in the cerebellum. These findings are in agreement with the findings of Sohn et al, 2000, Fedi et al, 2001 and De Rosa et al, 2006.

A comparison of Haematoxylin and Eosin with Cresyl fast violet stains demonstrates that cresyl fast violet is able to outline neuronal cells injury as well as haematoxylin and eosin following global Ischaemic damage.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Transcient global Ischaemia causes marked neuronal cell damage in the cerebrum, cerebellum and hippocampus of adult Wistar rats.

Piracetam has a beneficial effect in recovery of neuronal cells in the cerebrum, cerebellum and hippocampus of adult Wistar rats following acute Iscahemic brain damage.

Piracetam alone and in combination with vitamin E does not show remarkable improvement in memory, motor strength and motor coordination in adult Wistar rats following indication of transient global Ischaemia.

Serum malonyaldehyde levels is a more reliable indicator of neuronal cell ischaemia following induction of acute global Ischaemia when compared to brain tissue malonyaldehyde levels.

Haematoxylin and eosin does not have superior advantage than Cresyl fast violet stain in the demonstration of neuronal cell Ischaemia following induction of transient global Ischaemia.

6.2 Recommendations

1. More work needs to be done with a longer duration of ischaemia and higher doses of Piracetam to assess the effect of these variations.
2. Work needs to be done to assess the effect age of animals have in response to treatment with Piracetam following ISCHAEMIC brain damage.
3. The potentials of bilateral carotid artery ligation should be reviewed for possible adoption as a technique for inducing brain ischaemia.

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APPENDICES

Table 6.1: Brand names of piracetam and dosages

S/no	Brand name	Manufacturers	Form	Unit
1	Acter (800mg)	Klokter Life Sciences	Tablet	800mg
2	Alcetam (100mg)	Pentacare (Alkem Laboratories Ltd)	Injection	100mg/15ml
3	Alcetam (200mg)	Pentacare (Alkem Laboratories Ltd)	Injection	200mg/15ml
4	Alcetam (400mg)	Pentacare (Alkem Laboratories Ltd)	Tablet	400mg
5	Alcetam (800mg)	Pentacare (Alkem Laboratories Ltd)	Tablet	800mg
6	Alcetam inj (200mg)	Altius Life Sciences	Injection	200mg/15ml
7	Amcetam (1200mg)	Ampus Life Sciences Ltd	Tablet	1200mg
8	Amcetam (200mg)	Ampus Life Sciences Ltd	Injection	200mg/15ml
9	Amcetam (200mg) (Redson Pharma)	Redson Pharmaceuticals	Injection	200mg/15ml
10	Amcetam (400mg)	Ampus Life Sciences Ltd	Tablet	400mg
11	Amcetam (500mg))	Ampus Life Sciences Ltd	Syrup	500mg/5ml
12	Amcetam (800mg)	Ampus Life Sciences Ltd	Tablet	800mg
13	Anitam (500mg)	Anikem Laboratories	Syrup	500mg/5ml
14	Antam (100ml)	Anant Pharmaceuticals	Syrup	500mg/5ml
15	Antam (800mg)	Anant Pharmaceuticals	Tablet	800mg
16	ATR (100ml)	Shree Raj Pharmaceuticals	Syrup	500mg/5ml
17	ATR (200mg)	Shree Raj Pharmaceuticals	Injection	200mg/15ml
18	ATR (800mg)	Shree Raj Pharmaceuticals	Tablet	800mg
19	Axcetam (800mg)	Axyzen Life Sciences	Tablet	800mg
20	Axetam (100ml)	Medihealth Lifesciences (India) Pvt. Ltd.	Syrup	500mg/5ml
21	Axetam (800mg)	Medihealth Lifesciences (India) Pvt. Ltd.	Tablet	800mg
22	Biocetam (800mg)	Paradigm Healthcare	Tablet	800mg

23	Biotam (1200mg)	Bionext Pharma Pvt Ltd	Tablet	1200mg
24	Braintam (100ml)	Zodley Pharmaceuticals Pvt Ltd	Syrup	500mg/5ml
25	Braintam (800mg)	Zodley Pharmaceuticals Pvt Ltd	Tablet	800mg
26	Brentor (100ml)	Concord Biotech Ltd.	Syrup	500mg/5ml
27	Brentor (200mg)	Concord Biotech Ltd.	Injection	200mg/15ml
28	Brentor (400mg)	Zee Laboratories Ltd	Capsule	400mg
29	Brentor (800mg)	Concord Biotech Ltd.	Tablet	800mg
30	Ceact (400mg)	Crescent Therapeutics Ltd.	Tablet	400mg
31	Ceact (800mg)	Crescent Therapeutics Ltd.	Tablet	800mg
32	Cerabol (110ml)	Baxter (India) Pvt. Ltd.	Syrup	500mg/5ml
33	Cerabol (800mg)	Baxter (India) Pvt. Ltd.	Tablet	800mg
34	Cerecetam (30ml)	Intas Pharmaceuticals Ltd.	Suspension	1gm/5ml
35	Cerecetam (400mg)	Intas Pharmaceuticals Ltd.	Tablet	400mg
36	Cerecetam (800mg)	Intas Pharmaceuticals Ltd.	Tablet	800mg
37	Cerenorm (100ml)	Oyster Labs Limited	Syrup	500mg/5ml
38	Cerenorm (200mg)	Oyster Labs Limited	Injection	200mg/15ml
39	Cerenorm (800mg)	Oyster Labs Limited	Tablet	800mg
40	Ceretam (100ml)	Novus Life Sciences Pvt Ltd	Syrup	500mg/5ml
41	Ceretam (400mg)	Novus Life Sciences Pvt Ltd	Tablet	400mg
42	Ceretam (800mg)	Novus Life Sciences Pvt Ltd	Tablet	800mg
43	Cetam (100ml)	Taurus Laboratories Pvt. Ltd.	Syrup	500mg/5ml
44	Cetam (200mg)	Taurus Laboratories Pvt. Ltd.	Injection	200mg/15ml
45	Cetam (400mg)	Taurus Laboratories Pvt. Ltd.	Tablet	400mg
46	Cetam (800mg)	Taurus Laboratories Pvt. Ltd.	Tablet	800mg
47	Cetasmo (400mg)	Riasma Lifescience Pvt. Ltd.	Capsule	400mg
48	Cogmax	VGR Bio Laboratories	Capsule	400mg
49	Cogmax	VGR Bio Laboratories	Tablet	800mg
50	Corcetam (200mg)	Coral Pharmaceuticals	Injection	200mg/15ml
51	Dementa (800mg)	Kelvin Pharmaceuticals Labs. Pvt. Ltd.	Tablet	800mg

52	Duracetam (100ml)	Drukst Biotech	Syrup	500mg/5ml
53	Duracetam (800mg)	Drukst Biotech	Tablet	800mg
54	E Tam (400mg)	Sykocure Lifesciences	Tablet	400mg
55	E Tam (800mg)	Sykocure Lifesciences	Tablet	800mg
56	Encovit (800mg)	Konark Life Sciences	Tablet	800mg
57	Esitam	Esquire Drug House	Syrup	500mg/5ml
58	Esitam (400mg)	Esquire Drug House	Capsule	400mg
59	Etotam (400mg)	Psychotop (Solitaire Pharmacia Pvt Ltd)	Tablet	400mg
60	Etotam (800mg)	Psychotop (Solitaire Pharmacia Pvt Ltd)	Tablet	800mg
61	Evitam (100ml)	Swiss Biolabs	Syrup	500mg/5ml
62	Evitam (400mg)	Swiss Biolabs	Tablet	400mg
63	Evitam (800mg)	Swiss Biolabs	Tablet	800mg
64	Excitam (400mg)	Excare Laboratories	Capsule	400mg
65	Floracetam (800mg)	Sozin Flora Pharma	Tablet	800mg
66	Foxitam (1200mg)	Fox & Wolf Healthcare	Tablet	1200mg
67	Foxitam (400mg)	Fox & Wolf Healthcare	Tablet	400mg
68	Hicetam (400mg)	Redson Labs Pvt Ltd	Tablet	400mg
69	Ienptam (800mg)	Syntonic (Sanify Healthcare Pvt Ltd)	Tablet	800mg
70	J Cetam (100ml)	Jeen Healthcare	Syrup	500mg/5ml
71	J Cetam (800mg)	Jeen Healthcare	Tablet	800mg
72	Macetam (200mg)	Ancalima Life Sciences Ltd.	Injection	200mg/15ml
73	Macetam (60ml)	Ancalima Life Sciences Ltd.	Infusion	800mg/60ml
74	Marcetam (400mg)	Marsha Pharma Pvt. Ltd.	Tablet	400mg
75	Maxitam (100ml)	Invision Medi Sciences	Syrup	500mg/5ml
76	Maxitam (200mg)	Invision Medi Sciences	Injection	200mg/15ml
77	Maxitam (400mg)	Invision Medi Sciences	Capsule	400mg

78	Maxitam (800mg)	Invision Medi Sciences	Tablet	800mg
79	Mincetam (800mg)	Minova Life Sciences Pvt Ltd	Tablet	800mg
80	Neetam (100ml)	Magnet Labs Pvt Ltd (Mankind Pharmaceuticals Pvt. Ltd)	Syrup	500mg/5ml
81	Neetam (400mg)	Magnet Labs Pvt Ltd (Mankind Pharmaceuticals Pvt. Ltd)	Tablet	400mg
82	Neetam (800mg)	Magnet Labs Pvt Ltd (Mankind Pharmaceuticals Pvt. Ltd)	Tablet	800mg
83	Neucet (100ml)	Royal Labs	Syrup	500mg/5ml
84	Neucet (800mg)	Royal Labs	Tablet	800mg
85	Neurobele (1200mg)	Cybele Bio sciences Pvt Ltd	Tablet	1200mg
86	Neurobele (800mg)	Cybele Bio sciences Pvt Ltd	Tablet	800mg
87	Neurocet (400mg)	Zeus Pharmaceuticals	Tablet	400mg
88	Neurocet (800mg)	Zeus Pharmaceuticals	Tablet	800mg
89	Neurocetam (200mg)	B & B (Micro Labs Ltd.)	Injection	200mg/15ml
90	Neurocetam (400mg)	Brown & Burk Pharmaceuticals Ltd.	Capsule	400mg
91	Neurocetam (500mg)	B & B (Micro Labs Ltd.)	Syrup	500mg/5ml
92	Neurocetam (60ml)	B & B (Micro Labs Ltd.)	Infusion	800mg/60ml
93	Neurocetam (800mg)	B & B (Micro Labs Ltd.)	Tablet	800mg
94	Neuromax (100ml)	Milimax Healthcare	Syrup	500mg/5ml
95	Neuromax (1200mg)	Milimax Healthcare	Tablet	1200mg
96	Neuromax (800mg)	Milimax Healthcare	Tablet	800mg
97	Neuronorm (200mg)	Meridian Medicare Ltd.	Injection	200mg/15ml
98	Neurophil (200mg)	True Care Pharmaceuticals	Injection	200mg/15ml
99	Neurophil (60ml)	True Care Pharmaceuticals	Infusion	800mg/60ml

100	Neuropil (400mg)	Psyco Remedies.	Tablet	400mg
101	Neuropil (800mg)	Psyco Remedies.	Tablet	800mg
102	Neuroserge (100ml)	Abatis Holistic	Syrup	500mg/5ml
103	Neuroserge (400mg)	Abatis Holistic	Capsule	400mg
104	Neurospam (100ml)	Arbro Pharmaceuticals Limited	Syrup	500mg/5ml
105	Neurospam (400mg)	Arbro Pharmaceuticals Limited	Capsule	400mg
106	Neurospam (800mg)	Arbro Pharmaceuticals Limited	Tablet	800mg
107	Neutal (400mg)	Talent Healthcare	Capsule	400mg
108	Neutal (800mg)	Talent Healthcare	Tablet	800mg
109	Nextam (800mg)	Nexus Biotech	Tablet	800mg
110	Nicetam (100ml)	Reliance Formulation Pvt Ltd	Syrup	500mg/5ml
111	Nicetam (400mg)	Reliance Formulation Pvt Ltd	Tablet	400mg
112	Nicetam (800mg)	Reliance Formulation Pvt Ltd	Tablet	800mg
113	Nicetam Caps (400mg)	Tunic Healthcare	Capsule	400mg
114	Noofit (100ml)	Consern Pharma Pvt Ltd.	Syrup	500mg/5ml
115	Noofit (400mg)	Consern Pharma Pvt Ltd.	Tablet	400mg
116	Noofit (800mg)	Consern Pharma Pvt Ltd.	Tablet	800mg
117	Nootropil (100ml)	UCB India Ltd.	Syrup	500mg/5ml
118	Nootropil (1200mg)	UCB India Ltd.	Tablet	1200mg
119	Nootropil (200mg)	UCB India Ltd.	Injection	200mg/15ml
120	Nootropil (60ml)	UCB Pharma	Infusion	800mg/60ml
121	Nootropil (800mg)	UCB India Ltd.	Tablet	800mg
122	Nootropil Inj (200mg)	Adon Pharmaceuticals	Injection	200mg/15ml
123	Nootropil Sachet (1200mg)	UCB India Ltd.	Sachet	1200mg/1Sachet
124	Nootropyl	UCB S.A. 1070 Bruxelles, Belgigue	injection	1g/5ml

125	Normabrain (100ml)	Mind (Torrent Pharmaceuticals Ltd.)	Syrup	500mg/5ml
126	Normabrain (200mg)	Mind (Torrent Pharmaceuticals Ltd.)	Injection	200mg/15ml
127	Normabrain (400mg)	Mind (Torrent Pharmaceuticals Ltd.)	Capsule	400mg
128	Normabrain (800mg)	Mind (Torrent Pharmaceuticals Ltd.)	Tablet	800mg
129	Normalite (100ml)	Elite Pharma Pvt Ltd	Syrup	500mg/5ml
130	Normalite (400mg)	Elite Pharma Pvt Ltd	Capsule	400mg
131	Normalite (800mg)	Elite Pharma Pvt Ltd	Tablet	800mg
132	Normenta (100ml)	Intima (IPCA Laboratories Ltd)	Syrup	500mg/5ml
133	Normenta (400mg)	Intima (IPCA Laboratories Ltd)	Capsule	400mg
134	Normenta (800mg)	Intima (IPCA Laboratories Ltd)	Tablet	800mg
135	Nucetam (200mg)	SPM Drugs Pvt. Ltd.	Injection	200mg/15ml
136	Nucetam (60ml)	SPM Drugs Pvt. Ltd.	Infusion	800mg/60ml
137	Nupic (400mg)	La Pharmaceuticals	Tablet	400mg
138	Nupic (800mg)	La Pharmaceuticals	Tablet	800mg
139	Nurest (1200mg)	Aarpik Pharmaceuticals Pvt. Ltd.	Tablet	1200mg
140	Nurest (400mg)	Aarpik Pharmaceuticals Pvt. Ltd.	Capsule	400mg
141	Nurest (500mg)	Aarpik Pharmaceuticals Pvt. Ltd.	Syrup	500mg/5ml
142	Nurest (800mg)	Aarpik Pharmaceuticals Pvt. Ltd.	Tablet	800mg
143	Nurobala (800mg)	Novamed Pharmaceuticals	Tablet	800mg
144	Nutam (100ml)	Molekule (India) Pvt. Ltd.	Syrup	500mg/5ml
145	Nutam (400mg)	Molekule (India) Pvt. Ltd.	Capsule	400mg
146	Nutam (60ml)	Molekule (India) Pvt. Ltd.	Infusion	800mg/60ml
147	Nutam FC (1200mg)	Molekule (India) Pvt. Ltd.	Tablet	1200mg
148	Nutam FC (800mg)	Molekule (India) Pvt. Ltd.	Tablet	800mg
149	Nutam Inj (200mg)	Molekule (India) Pvt. Ltd.	Injection	200mg/15ml

150	Nutropic (400mg)	Alpic Remedies Ltd.	Capsule	400mg
151	Olcitam (500mg)	Olcare Laboratories	Capsule	500mg
152	Orotam (100ml)	Orochem (Rishab Healthcare Pvt Ltd)	Syrup	500mg/5ml
153	Orotam (200mg)	Orochem (Rishab Healthcare Pvt Ltd)	Injection	200mg/15ml
154	Orotam (800mg)	Orochem (Rishab Healthcare Pvt Ltd)	Tablet	800mg
155	Otam (200mg)	Odin Healthcare Pvt Ltd	Injection	200mg/15ml
156	Oxocetam (1200mg)	Lifecare Innovations Pvt. Ltd.	Tablet	1200mg
157	Pacitam (100ml)	Paragon Pharmaceuticals	Syrup	500mg/5ml
158	Pacitam (200mg)	Paragon Pharmaceuticals	Injection	200mg/15ml
159	Pacitam (60ml)	Paragon Pharmaceuticals	Infusion	800mg/60ml
160	Pacitam (800mg)	Paragon Pharmaceuticals	Tablet	800mg
161	PCT (200mg)	Welbe Life Sciences	Injection	200mg/15ml
162	Pdneuro (100ml)	Pharma Drugs & Chemicals	Syrup	800mg/5ml
163	Pdneuro (800mg)	Pharma Drugs & Chemicals	Tablet	800mg
164	Pentor (100ml)	Jpee Drugs	Syrup	500mg/5ml
165	Pentor (800mg)	Jpee Drugs	Tablet	800mg
166	PIE (800mg)	D.R. Johns Labs	Tablet	800mg
167	PIM (400mg)	Elikem Pharmaceuticals Pvt. Ltd.	Tablet	400mg
168	PIM (800mg)	Elikem Pharmaceuticals Pvt. Ltd.	Tablet	800mg
169	Pira (100ml)	East West Pharma	Syrup	500mg/5ml
170	Pira (400mg)	East West Pharma	Tablet	400mg
171	Pira (800mg)	East West Pharma	Tablet	800mg
172	Pira OD (1200mg)	East West Pharma	Tablet	1200mg
173	Pirac (100ml)	Psychiatry (Orchid Chemicals & Pharmaceuticals Ltd.)	Syrup	800mg/5ml
174	Pirac (400mg)	Psychiatry (Orchid Chemicals & Pharmaceuticals Ltd.)	Capsule	400mg

175	Pirac (800mg)	Psychiatry (Orchid Chemicals & Pharmaceuticals Ltd.)	Tablet	800mg
176	Pirac Inf (60ml)	Psychiatry (Orchid Chemicals & Pharmaceuticals Ltd.)	Infusion	800mg/60ml
177	Piracas (800mg)	Casca Remedies Pvt Ltd.	Tablet	800mg
178	Piracer (200mg)	Global Medisciences Limited (Intraways)	Injection	200mg/15ml
179	Pirajet (400mg)	Trigat Life Sciences Pvt Ltd	Tablet	400mg
180	Pirajet (800mg)	Trigat Life Sciences Pvt Ltd	Tablet	800mg
181	Pirakar (100ml)	Densa Pharmaceuticals Pvt.Ltd.	Syrup	500mg/5ml
182	Pirakar (400mg)	Densa Pharmaceuticals Pvt.Ltd.	Capsule	400mg
183	Pirakar Forte (800mg)	Densa Pharmaceuticals Pvt.Ltd.	Tablet	800mg
184	Pirakin (400mg)	Cyril Pharmaceuticals	Tablet	400mg
185	Pirakin (800mg)	Cyril Pharmaceuticals	Tablet	800mg
186	Piramax (100ml)	Biomax Laboratories	Syrup	500mg/5ml
187	Piramax (400mg)	Biomax Laboratories	Tablet	400mg
188	Piramax (800mg)	Biomax Laboratories	Tablet	800mg
189	Pirament (100ml)	Innova (IPCA Laboratories Ltd.)	Syrup	500mg/5ml
190	Pirament (1200mg)	Innova (IPCA Laboratories Ltd.)	Tablet	1200mg
191	Pirament (400mg)	Innova (IPCA Laboratories Ltd.)	Tablet	400mg
192	Pirament (800mg)	Innova (IPCA Laboratories Ltd.)	Tablet	800mg
193	Piranis (200mg)	Neiss Labs Pvt. Ltd.	Injection	200mg/15ml
194	Pirariv (800mg)	East African (I) Remedies Pvt Ltd	Tablet	800mg
195	Piratab (1200mg)	Marc Laboratories Pvt. Ltd.	Tablet	1200mg
196	Piratab (800mg)	Marc Laboratories Pvt. Ltd.	Tablet	800mg
197	Piratam (400mg)	Glaxo Smithkline Pharmaceuticals Ltd.	Capsule	400mg
198	Piratam (60ml)	Glaxo Smithkline Pharmaceuticals Ltd.	Syrup	100mg/1ml
199	Pirav (400mg)	Ravian Life Sciences (P) Ltd	Tablet	400mg

200	Pitam (1200mg)	Chemo Biological	Tablet	1200mg
201	Pitam (800mg)	Chemo Biological	Capsule	800mg
202	Polcetam (400mg)	Polaris (Symbiosis Pharmaceuticals Pvt Ltd)	Tablet	400mg
203	Polcetam (800mg)	Polaris (Symbiosis Pharmaceuticals Pvt Ltd)	Tablet	800mg
204	Polytam (800mg)	Biochemix Healthcare Pvt Ltd	Tablet	800mg
205	Psycopir (400mg)	APS Biotech Pvt Ltd	Capsule	400mg
206	Psynep (1200mg)	Lifesavers Remedies	Tablet	1200mg
207	Psynep (800mg)	Lifesavers Remedies	Tablet	800mg
208	PTM (100mg)	Aan Pharma Pvt Ltd	Syrup	100mg/5ml
209	PTM (400mg)	Aan Pharma Pvt Ltd	Tablet	400mg
210	PTM (800mg)	Aan Pharma Pvt Ltd	Tablet	800mg
211	Pvittam (60ml)	Prohealth Vitamins (P) Ltd.	Infusion	800mg/60ml
212	Ractum (100ml)	Axis Life Science Pvt Ltd	Syrup	500mg/5ml
213	Ractum (200ml)	Axis Life Science Pvt Ltd	Syrup	500mg/5ml
214	Ractum (400mg)	Axis Life Science Pvt Ltd	Tablet	400mg
215	Ractum (800mg)	Axis Life Science Pvt Ltd	Tablet	800mg
216	Rcetam (400mg)	Ortin Laboratories Ltd	Capsule	400mg
217	Renervol P (100ml)	K.C. Laboratories Pvt. Ltd.	Syrup	500mg/5ml
218	Renervol P (400mg)	K.C. Laboratories Pvt. Ltd.	Tablet	400mg
219	Renervol P (800mg)	K.C. Laboratories Pvt. Ltd.	Tablet	800mg
220	Retam (800mg)	Sanbury Pharmaceuticals (P) Ltd.	Tablet	800mg
221	Stem (100ml)	Triton Healthcare Pvt Ltd	Syrup	500mg/5ml
222	Sumocetam (1200mg)	Talent Healthcare	Tablet	1200mg
223	Sumocetam (400mg)	Talent Healthcare	Capsule	400mg
224	Sumocetam (800mg)	Talent Healthcare	Tablet	800mg

225	Tam (800mg)	Octane Biotech Pvt. Ltd.	Tablet	800mg
226	Tampira (200mg)	BMW Pharmaco India Pvt Ltd	Injection	200mg/15ml
227	Tampira (60ml)	BMW Pharmaco India Pvt Ltd	Infusion	800mg/60ml
228	Tampira (800mg)	BMW Pharmaco India Pvt Ltd	Tablet	800mg
229	Ubittor (800mg)	Ubit Pharmaceuticals Pvt Ltd	Tablet	800mg
230	Ultacetam (100ml)	Ultra Chiron Pharma Pvt Ltd	Syrup	500mg/5ml
231	Ultacetam (400mg)	Ultra Chiron Pharma Pvt Ltd	Tablet	400mg
232	Ultacetam (800mg)	Ultra Chiron Pharma Pvt Ltd	Tablet	800mg
233	Upcetam (1200mg)	Arrowin Pharmaceuticals	Tablet	1200mg
234	Upcetam (800mg)	Arrowin Pharmaceuticals	Tablet	800mg
235	Wincetam (100ml)	Mediwin Pharmaceuticals	Syrup	100mg/5ml
236	Wincetam (400mg)	Mediwin Pharmaceuticals	Capsule	400mg
237	Winrace (400mg)	Winmac Laboratories Limited	Tablet	400mg
238	Zantam (800mg)	V&B Healthcare	Tablet	800mg
239	Zetam (100ml)	Allenge India	Syrup	500mg/5ml
240	Zetam (200mg)	Allenge India	Injection	200mg/15ml
241	Zetam (400mg)	Allenge India	Tablet	400mg
242	Zetam (800mg)	Allenge India	Tablet	800mg
243	Zopres (400mg)	Psychotropics India Limited	Tablet	400mg
244	Zopres (800mg)	Psychotropics India Limited	Tablet	800mg

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