Effect of long term exposure of Mobile Phone Radio-Frequency Electro Magnetic Radiation (MP RF-EMR) on cognitive function and hippocampal morphology in albino mice

Thesis submitted to

Sri Devaraj Urs Academy of Higher Education & Research

In partial fulfilment for the degree of Doctor of Philosophy in Anatomy under Faculty of Medicine

Submitted by:

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JUNE -2020

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DECLARATION BY THE CANDIDATE

I **Mr. G. Krishna kishore** hereby declare that this thesis entitled **"Effect of long term exposure of Mobile Phone Radio-Frequency Electro Magnetic Radiation (MP RF-EMR) on cognitive function and Hippocampal Morphology in Albino Mice"** is an original research work carried out by me for the award of **Doctor of Philosophy** in the subject Anatomy under the guidance of **Dr. Venkateshu KV**, Professor, Department of Anatomy, Sri Devaraj Urs Medical College, a constituent Institute of SDUAHER and co-guidance of **Dr. Sridevi NS**, Professor and Head, Department Anatomy, SDUMC. No part of this thesis has formed the basis for the award of any degree or fellowship previously elsewhere.

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Approved Thirteen Mice

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ACKNOWLEDGEMENT

I express my sincere thanks and gratitude to my guide **Dr. Venkateshu K.V**, Professor, Department of Anatomy, Sri Devaraj Urs Medical College, Tamaka, Kolar, for his invaluable guidance, suggestions, advice, moral support, and untiring help at all stages of my research work.

I wish to express my sincere thanks and gratitude to my co-guide **Dr. Sridevi N.S**, Professor & Head, Department of Anatomy, Sri Devaraj Urs Medical College, Tamaka, Kolar, for her invaluable guidance, suggestions, encouragement, moral support, and help at all stages of my research work.

My sincere gratitude to **Dr. P.N.Sreeramulu,** Professor of Surgery, Principal and Dean, Faculty of Medicine, Sri Devaraj Urs Medical College, Tamaka, Kolar for his support and encouragement.

I am thankful to all the **Teaching faculty**, **Ph.D Scholars** and **Non-teaching staff** of the Department of Anatomy, Sri Devaraj Urs Medical College, Tamaka, Kolar for their encouragement, support, help and advice during my research.

I wish to thank **Dr. Sarala**, Professor of pharmacology, Director of academics, SDUAHER for providing Animal house to carry out this experiment, **Dr. Muninarayana**, Veterinarian, Teaching faculty and Non-Teaching staff, Department of pharmacology, SDUMC for their help.

I also thank **Mr. Ravi Shankar,** Statistician, Department of Community Medicine for his valuable suggestions during data analysis and **Dr. Prakash,** Librarian, Sri Devaraj Urs Medical College, Tamaka, Kolar for his timely help.

I am grateful to my doctoral committee member, **Dr. Biswabeena Ray**, Professor and Head Department of Anatomy, AIIMS Kalyani, for her advice and suggestions during the work.

I wish to express my sincere gratitude to **Dr. Bindu M Kutty**, Professor & Head, Department of Neurophysiology, NIMHANS, Bangalore, for giving permission to use the lab facilities to carry out sectioning using Vibratome microtome and Golgi-Cox Staining. I also thank **Mr. Subhadeep Dutta**, Research fellow, from the Department of Neurophysiology, NIMHANS, Bangalore, for assisting me in sectioning and special staining.

I whole heartedly thank **Dr. Anjaneyulu K**, Assistant professor, Dept. of Anatomy, Manipal university, for his help, support, encouragement and guidance throughout this experimental study.

My deepest gratitude goes to all my family members for their unflagging love, support and encouragement throughout the study. My parents have worked industriously and provide me the best possible environment to study. Finally, greatest regards to the Almighty for bestowing upon me the courage to face the complexities of life and complete this thesis successfully.

I express my gratitude to all my friends, colleagues and well wishers for their encouragement, moral support, help and advice during my research.

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ABBREVIATIONS

MP	Mobile Phone
RF-EMR	Radiofrequency Electromagnetic Radiation
DG	Dentate Gyrus
CA	Cornu-Ammonis
BBB	Blood-Brain-Barrier
HPA Axis	Hypothalamo-Pituitary-Adrenal axis
GSM	Global System for Mobile Communication
MBP	Myelin Basic Protein
NF	Neurofilament
GFAP	Glial Fibrillary Acidic Protein
SAR	Specific Absorption Rate
WHO	World Health Organisation
RF-EMF	Radiofrequency Electromagnetic Field
DBP	Dendritic Branching Points
DI	Dendritic Intersections
S	Soma
CC	Concentric Circle.
A	Apical Dendrites

B	Basal Dendrites
CNS	Central Nervous System
GHz	Giga Hertz
MHz	Mega Hertz
KHz	Kilo Hertz
ORT	Object Recognition Test
W/Kg	Watt/Kilogram
mT	milliTelsa
TEM	Transmission Electron Microscopy
IHC	Immuno Histo Chemistry
dBm	Decibles-Milliwatt
v/m	Volt/meter
Mw/m^2	Milliwatt/Square meter
TRC	The Reward Chamber
RAT	Reward Alternation Test
HSP	Heat Shock Proteins
HSF	Heat Shock Transcription Factor

CHAPTER- 1

INTRODUCTION

INTRODUCTION-

- The extensive use of Global System for Mobile communication (GSM) mobile phones throughout the world raises the possible adverse effects on human health especially on the Central Nervous System (CNS), the brain. In many countries more than half of the population relies/depend on mobiles for wireless communication and internet data [1].
- In 2015, more than 7 billion people were using mobiles in the world, estimating to 62.9% of the world‟s population. Rapid increase of mobile users in general and specifically upto 80% of youngsters owning a mobile has made communication and technology easier [2].

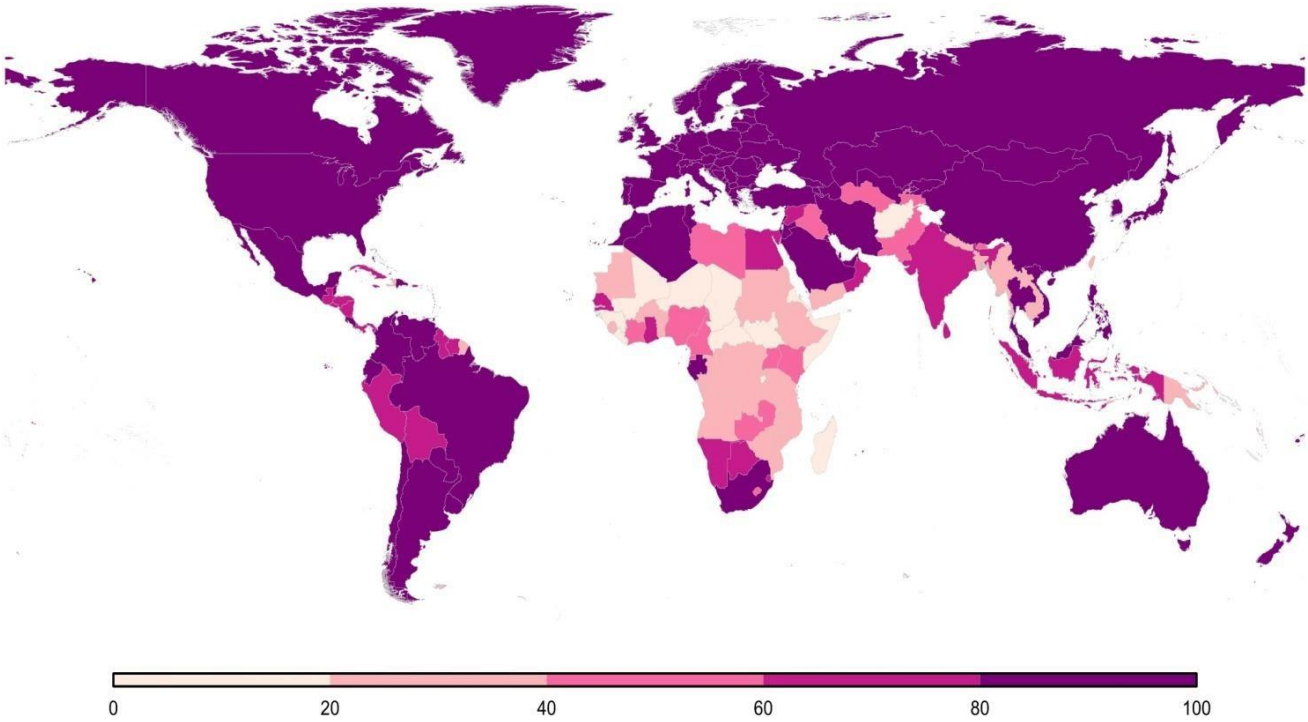


Figure 1: Image shows 70% of the world's youngsters are in online- Proportion of youngsters (Aged 15-24) were using the Internet in 2017

Source :https://www.itu.int/en/ITU D/Statistics/Documents/facts/ICTFactsFigures2017.pdf

- It is estimated that in 104 countries around 80% of the youngsters are in online, 94% of youngsters aged 15-24 years are using internet in developed countries, 67% are using internet in developing countries and only 30% are using internet in least developed countries.
- It is alarming that out of 830 million youngsters who are in online 320 million (39%) are in India and China. The proportion of youngsters aged 15-24 years using internet data (71%) is much higher than the total internet users (48%).
- In 2017, the proportion of internet users in terms of gender shows men were higher in number than the women users in 2/3 of the countries worldwide. United States is the only country in which women internet users percentage were higher.[3]

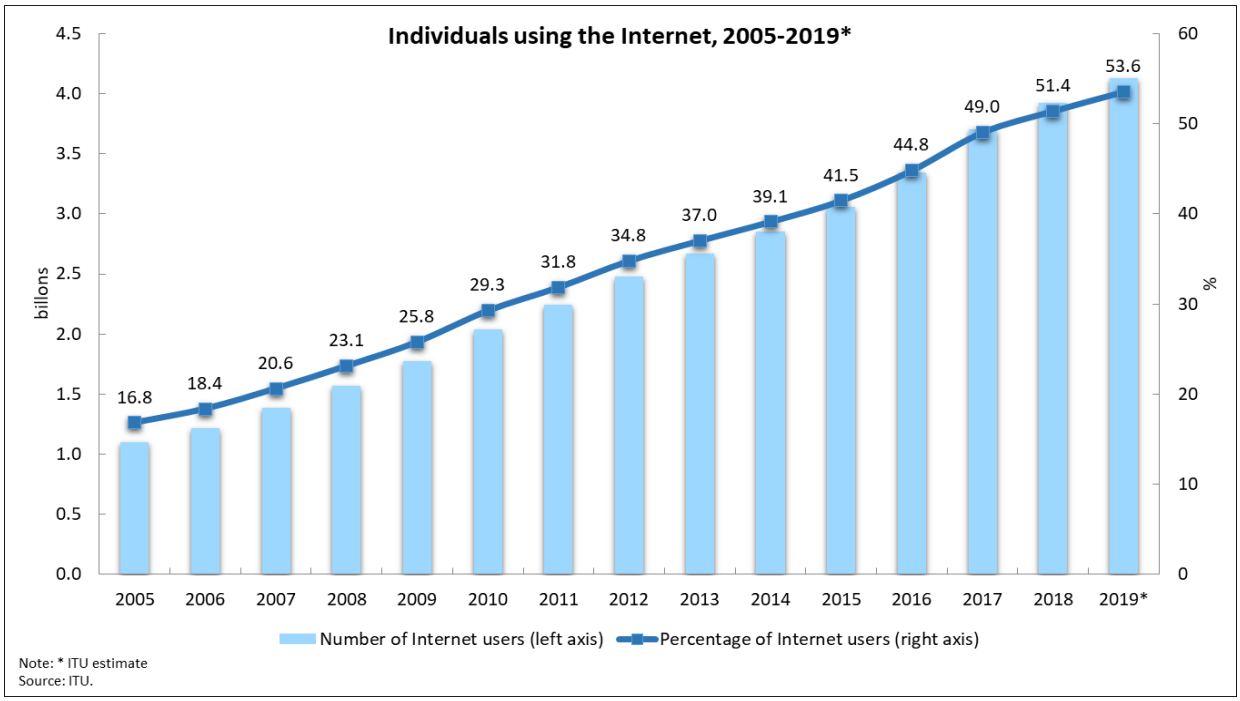


Figure 2: ITU (International telecommunication union) estimates that by the end of 2019, 53.6 per cent of the global population, or 4.1 billion people, are using the Internet

Source:https://www.itu.int/en/ITU-D/Statistics/Pages/stat/default.aspx

- In this concern there is a growing interest in scientific community for the potential deleterious effects of Radio Frequency Electro Magnetic Radiation (RF EMR) on the public health, especially much focus on the effects of RF EMR on structural and functional integrity of the brain because the radiation exposure is directly to the head region [4].
- In 2006 and 2010 World Health Organisation (WHO) issued a research agenda for high priority research on effects of RF exposure on ageing and neurodegenerative diseases in animals and effects of pre and post natal RF exposure on development and behaviour in animal models [5, 6].
- The mobile phone releases non-ionising radiation which has low frequency and considered to be safe, but recent studies evidenced that it has an impact on the living tissues especially on the brain which can cause headache, memory loss, heat over the ear, decreased concentration and other cognitive effects [7].

What is Radiation-

- "Radiation is the form of energy that pass through the medium".
- It consists of electric and magnetic energy waves which radiates together through the space almost at speed of the light.
- We are living in a radiation world and being exposed to natural and artificial radiation like man-made radiation.

- Throughout the life we have been exposed to different forms of radiations like UV radiation from the sunlight, radio waves from the TV and radio and gamma rays like X-Rays and CT scan [8].

Types of Radiation-

- They are two types:
  - Ionizing Radiation
  - Non-Ionizing Radiation

Ionizing Radiation:

- It consists of enough energy which causes ionization.
- Ionization is a process through which electrons were stripping from atoms and molecules.
- This process can lead to molecular changes, which leads to damage to the genetic material of the biological tissue and DNA.
- High levels of electric and magnetic energy is required for this interaction process.
- Gamma radiation and X-Radiation are two types of EMR which is having enough energy for ionization of biological systems.

  Examples of ionizing radiation- Gamma rays &X-rays [9].

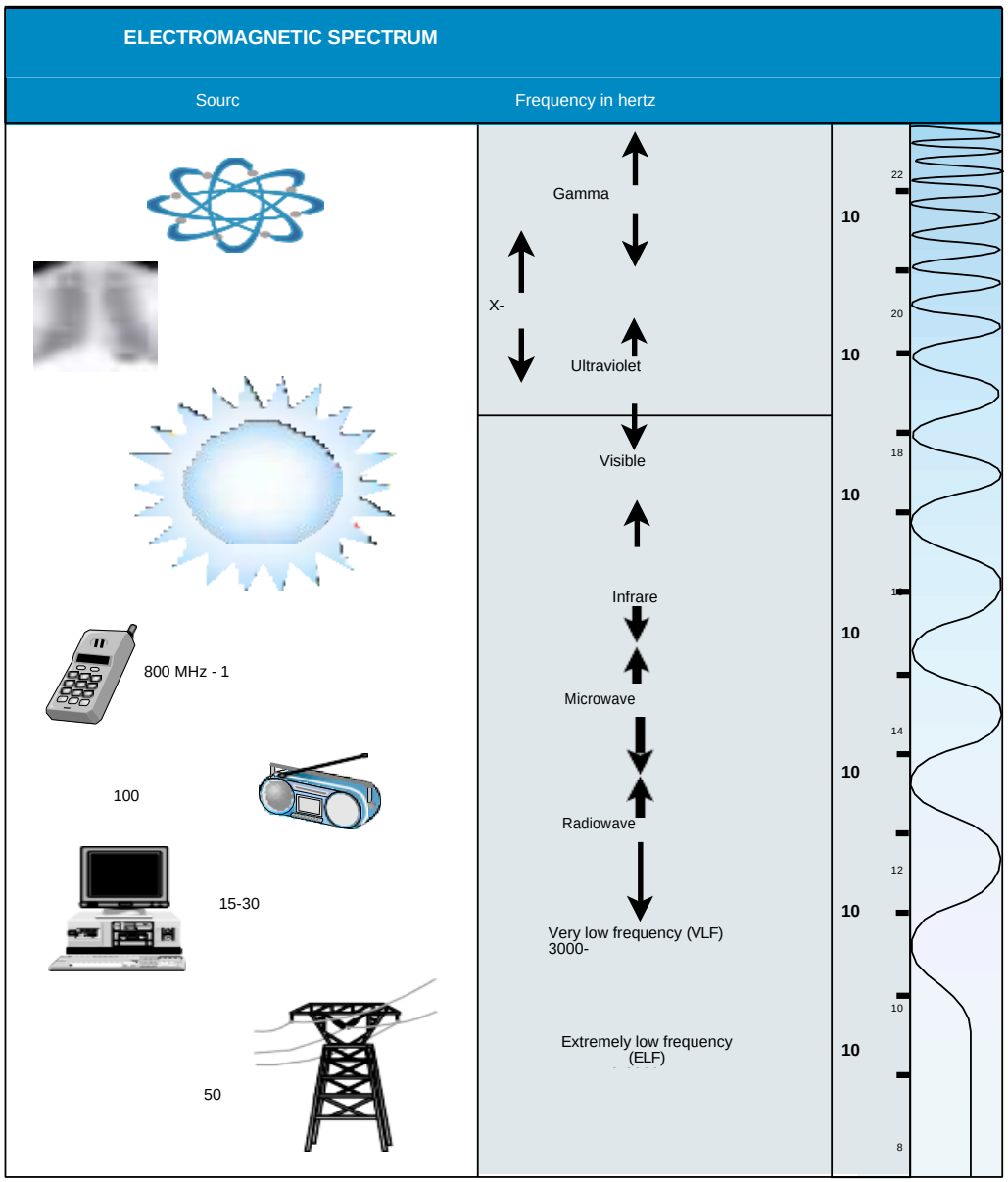


Figure 3: Image represents the electromagnetic spectrum – Image adapted from Radiation, mobile phones, base stations and your health: Ng Kwan-Hoong. ISBN 983-40889-0-9

Non-ionizing radiation:

- It does not have enough energy for ionization of atoms and molecules.

- Exposure to non-ionizing radiation for longer duration may cause some heating to the localised tissue, but not adequate to cause the tissue damage.

  Examples of non-ionising radiation – Radio frequency energy, visible light & infrared light [9].

How Radiation behaves-

- Radiation behaves in the same manner as light, it travels in a straight line and when it collides with an objective, it will do three things-
  - Transmission: it may pass through
  - Reflection: it may bounce off
  - Absorption: it may be absorbed

  - The power and energy of the radiation reduces based on the distance from the source, which means the person will be exposed to less level of radiation when he/she stays away from the source of radiation.

Natural Radiation and its effects-

- People throughout the world are being exposed continuously to different sources of natural radiation.
- Out of these different sources, sunlight is the familiar one which produces infrared radiation, UV light and visible light.
- Other sources of natural radiation are
  - Cosmic radiation
  - Terrestrial radiation
  - Internal radiation

- Cosmic radiation: It is the high energy radiation which originates from outside the earth/solar system.
- Terrestrial radiation: It is the long-wave electromagnetic radiation, which originates from the earth and its temperature. This radiation is emitted by radioactive materials which forms naturally in the earth.
- Internal radiation: It is a Radio activity, which is present naturally in our bodies.
- Amongst the above mentioned natural radiations, ultra violet light which is emitted from the sunlight will be considered as “Dangerous”.
- Over exposure to UV light leads to aging of the skin and results in sunburn, which is linked to skin cancer.
- All these cosmic radiation, terrestrial radiation & internal radiation are hazardous, which can cause cancer, but the levels of these radiation is significantly low in environment, so the risk is very minimal [10].

Electro Magnetic Field-

- Electromagnetic radiation consists of electric and magnetic energy waves which radiates together through the space almost at a speed of the light.
- The term Electro Magnetic Field was used to indicate the presence of electromagnetic radiation.
- Different forms of electromagnetic radiation are classified based on their frequencies.
- The term Electromagnetic field was generally used to cover the fields in the frequency range below 300 gigahertz, whereas giga refers to a thousand million.

- Electromagnetic field includes electric and magnetic fields from electric supply at power frequencies (50 Hz) and radio waves from radio, mobile phone, television, radar and satellite communications.
- Some home devices also transmits Electro Magnetic Field such as cordless phones and radio-controlled devices [11, 12, 13].

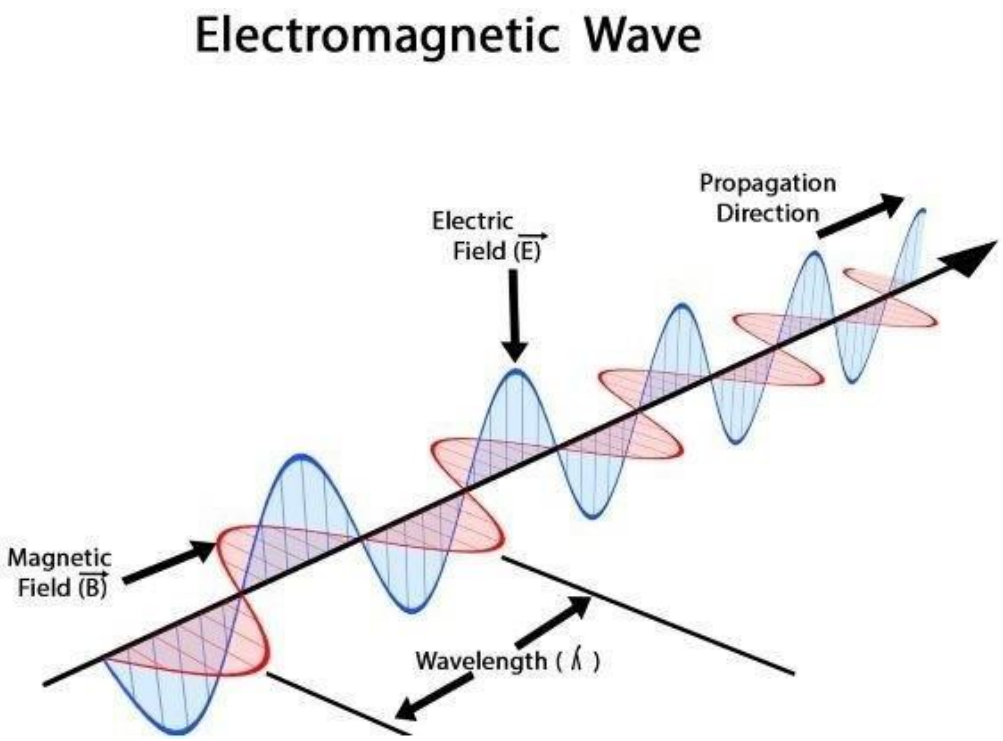


Figure 4: Diagrammatic representation of Electromagnetic Wave- Image adapted from www.environmental protection agency @EPA 2020.org

Electro Magnetic Frequencies-

Measurement of frequency- in Hertz (Hz)

1 Hertz (Hz) =1 Cycle/second

- Kilohertz = KHz = 1000 Hz (one thousand hertz)

- Megahertz = MHz = 1000000 Hz (10 lakh/one million hertz)
- Gigahertz = GHz = 1000000000 Hz (100 Crore/one billion Hertz)

- Examples:

Source:	Frequency:
Mobile phone	900-2100 MHz
Power line	50 Hz

Figure 5: Radiation frequency meter (Cornet Microsystems-ED 178 electrosmog meter)

Radio Frequency (RF)-

- The radio signals will be considered as a wave, which spreads out their signals from its source – Base station/Antenna, the waves of the radio signals are referred as electromagnetic wave, which is made up of electric and magnetic components.
- Radiation frequency part of electromagnetic spectrum comprises of electromagnetic waves produced by television, microwaves and radio transmitters (Base stations).
- The electric and magnetic components that forms the electromagnetic wave can be referred as electromagnetic fields [10,14,15].

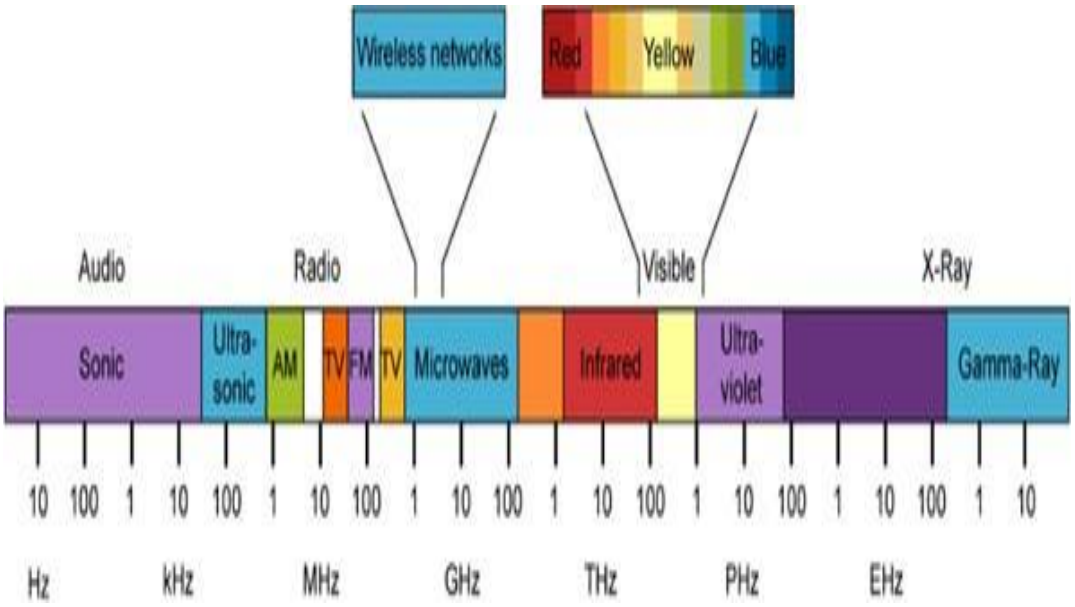


Figure 6: Radiation Frequency image- image adapted from - https://rscciew.wordpress.com/2013/11/24/rf-principle-in-wireless-network

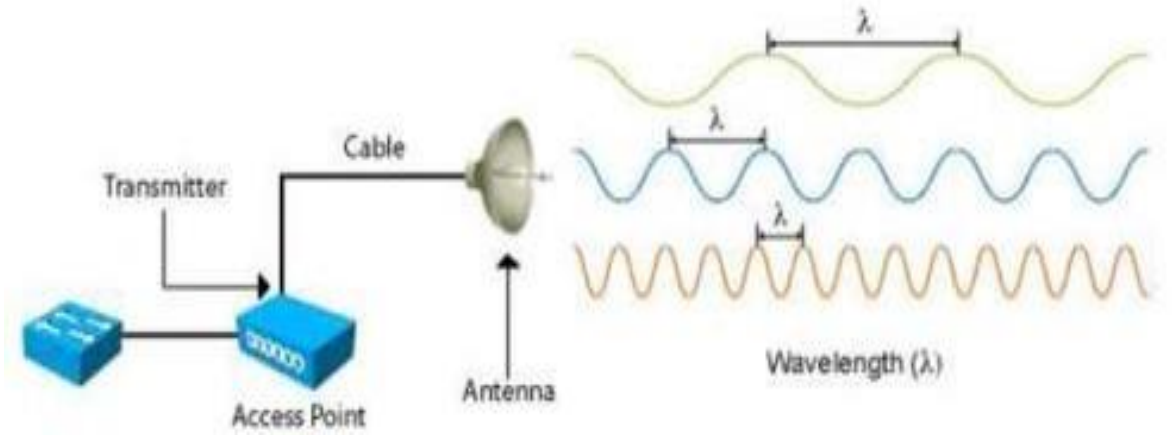


Figure 7: Wave length image- - image adapted from -

https://rscciew.wordpress.com/2013/11/24/rf-principle-in-wireless-network

Mobile phone / Cellular phone-

- The mobile phone is a low powered, single channelled two way radio, which consists of both transmitter and transceiver.
- Mobile phone emits radiation to transfer the information to the telecom towers/base stations and it acts like a transceiver receiving the information similar to the transistor radio.
- Telephone service providers also use a cellular network architecture, therefore these mobile phones are called as cellular phones/cell phones.
- During 2000‟s era the mobile phones which supports different services such as text messages, multimedia messaging service (MMS), internet access mail, short range low powered wireless communications such as Bluetooth & infrared, videogames and camera for photography, the mobile phones with these capabilities are called as “Featured phones”.
- The mobile phones which will have great advancing computing technology and capabilities are called as smart phones [16].

Other Radiation frequency sources-

- Other RF sources such as communicating antennas used by police, fire and emergency services.
- Radio broadcasting antennas and television emits more frequency radiation than that of the mobiles [16].

Hippocampus-

- The term „hippocampus" was first named by a Bolognese anatomist named „Giulio Cesare Aranzi", which is similar to „Hippocampus liera" the tropical fish [17].
- The hippocampal formation is located deeply in the medial temporal lobe, which consists of hippocampal proper, dentate gyrus, presubiculum, subiculum, parasubiculum [18].
- The structural integrity and pathway of hippocampal formation is similar in all mammals.

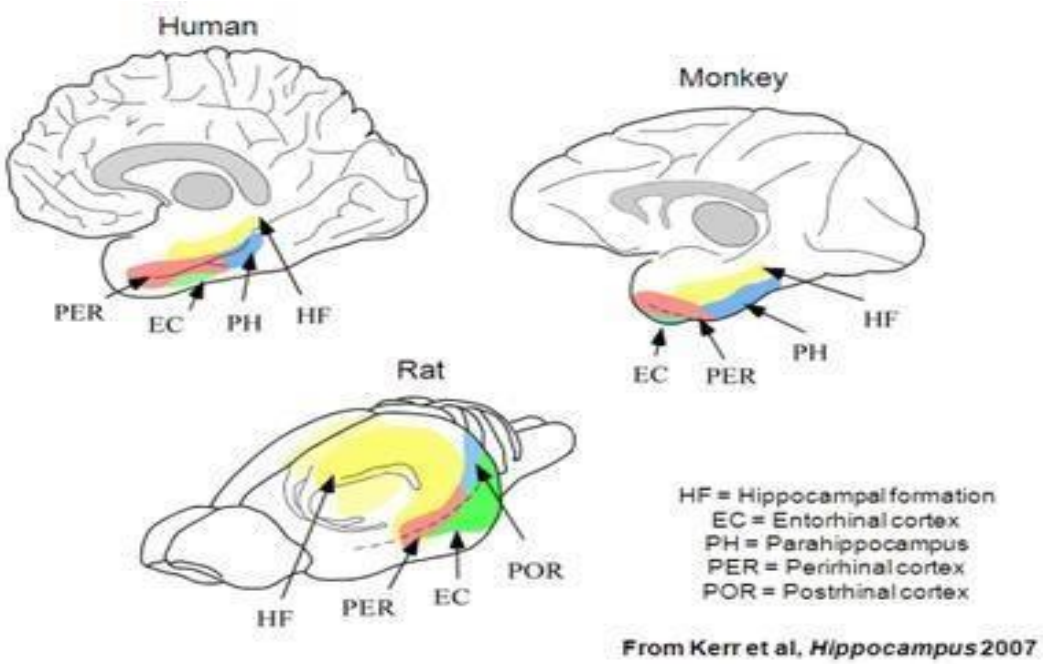


Figure 8: Image represents the anatomy of hippocampus in all mammals (Human vs Monkey vs Rat) -Image adapted from burwell et al. Anatomy of the Hippocampus and the Declarative Memory System

- The hippocampus is a part of brain which belongs to the limbic system and is involved in cognitive functions like spatial learning and working memory.
- It plays a crucial role in the formation of new memories and it is considered as a sensitive region and is affected by mobile phone radiation.
- The hippocampus is a “S”-shaped folded structure located on the floor of the lateral ventricle on both the cerebral hemispheres.
- Hippocampal formation consists of hippocampus proper, dentate gyrus and subiculum. Hippocampus proper is also known as Cornu Ammonis (CA), which consists of CA1, CA2, CA3 sub regions and dentate gyrus [19].

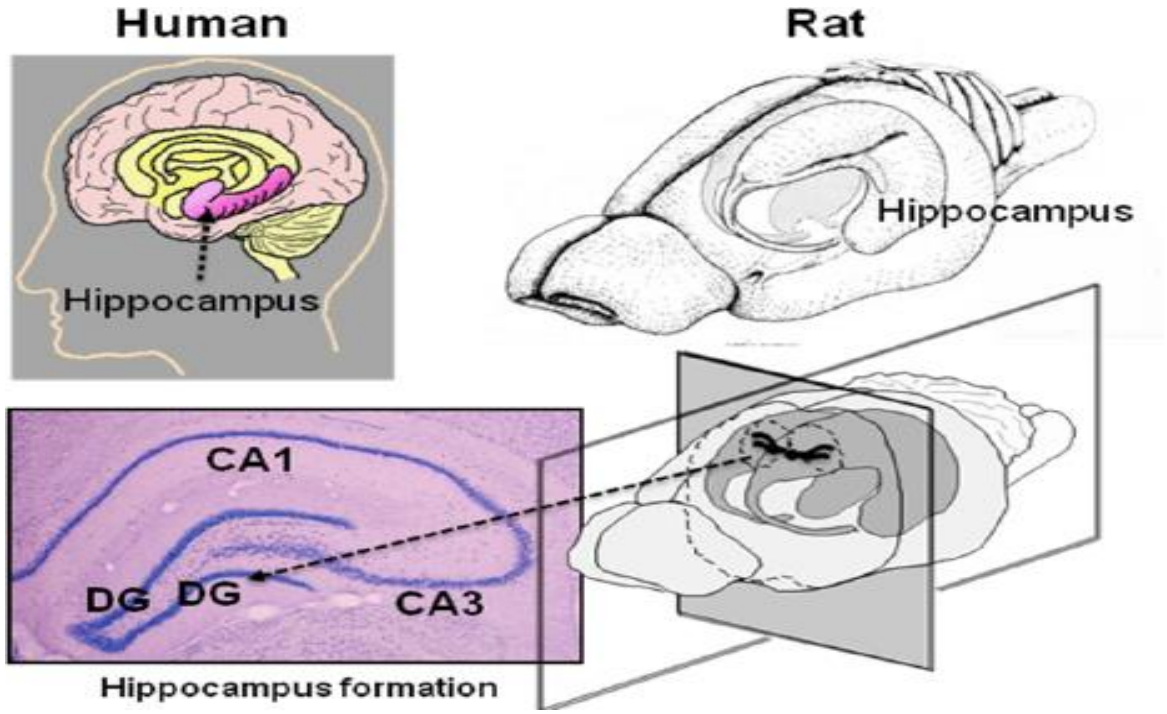


Figure 9: Image represents the anatomy of coronal section of hippocampus in mammals with its sub regions.

Image source- Hideaki soya et al, Brain Activation via Exercise: Exercise conditions leading to neuronal activation

- The microscopic anatomy of hippocampus proper consists of 3 layers of cells.
    1. Stratum oriens
    2. Stratum Pyramidale
    3. Molecular zone
    - The CA3 region is located in the junction between the dentate gyrus and CA2 region.
    - The CA2 region is located in between CA1 and CA3 and CA1 is the starting phase of hippocampus proper.
    - Pyramidal cell layer is a principal cell layer, consists of pyramidal neurons arranged in 3-4 layers.
    - CA3 region of hippocampus shows loosely packed large pyramidal neurons with fine nerve fibres around it.
    - CA2 region of hippocampus shows densely packed large pyramidal neurons with basophilic stained oval to round nuclei with nucleoli.
    - CA1 region of hippocampus 3-4 rows of pyramidal neurons which is smaller with deep stained nuclei with nucleoli [20].

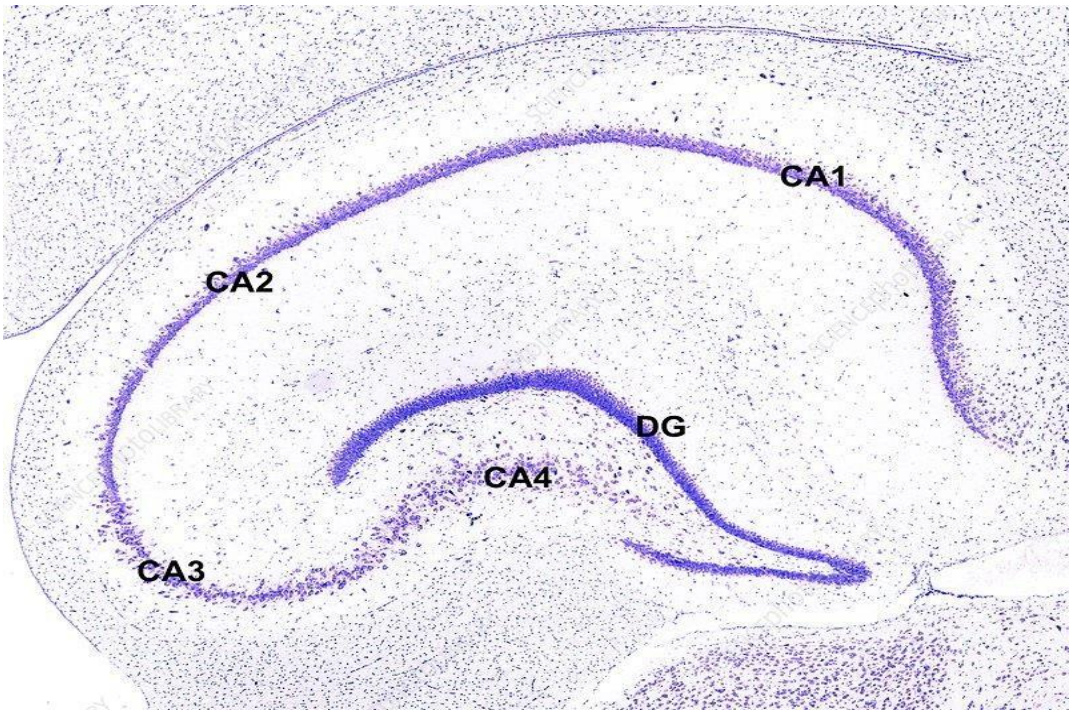


Figure 10: Cresyl violet stained image represents the microscopic anatomy of hippocampal CA1, CA2 and CA3 sub regions with pyramidal neurons.

Image source- JOSE CALVO / SCIENCE PHOTO LIBRARY

- The fact that is alarming with multiple mobile devices are being released into the market for sale without proper safety approvals and guidelines on electromagnetic radiation. In 2011, WHO confirmed that mobile phone usage will be a health hazard and also possibly carcinogenic based on the duration of its use.[21].
- Currently the adverse effects of health due to radiation frequency emitted by mobile phones are not clearly understood and still contradictory. In spite of many advantages, long term usage/exposure to mobile phone can be considered as an "health time bomb".
- The usage of mobile phone for more than 20 minutes at a time increases the contact tissue temperature by 1ºC [22].

- Over usage of mobile phone may result in exertion to do the daily activities, poor fragmented sleep, affects learning capacity, mental health and quality of life [23].
- Damage to the neurons of hippocampal region may lead to impairment in memory and learning, behaviour disturbances and negative impact on Hypothalamo-Pituitary-Adrenal axis, Developmental alterations/ anomalies in brain/CNS due to electromagnetic field will remain for the rest of the life [24].
- Numerous studies were focused on the effects of acute Mobile Phone Radio Frequency Electro-Magnetic Radiation (MP RF-EMR) exposure on hippocampus, reporting a hippocampal cell injury.
- But there are very few studies which have focused on chronic exposure of mobile radiation. Hence, the present study was aimed at studying the chronic effects of mobile radiation exposure on hippocampus of adult Swiss albino mice.

CHAPTER- 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE-

The following were the recent literature, critically reviewed with respect to this study.

Review of literature on learning memory-

In 2013 Hao D, et al, has studied the long term effects of electro-magnetic field (EMF) exposure (916 MHz of Mobile phone Electromagnetic field, 6 hrs/day, 5 days/week for 10 weeks) on spatial learning and memory in rats with the help of completion time, number of total errors in eight arm maze and neuron discharge signals by implanting microelectrode array in the region of hippocampus were recorded and reported that average completion time and error rate of the exposure group shows elevated when compared to control group [25].

In 2008 Narayanan SN, et al, has studied the spatial memory performance by using the morris water maze test in wistar rats exposed to 50 missed calls/day for 4 weeks from a GSM mobile phone (900-1800 MHZ) kept in the cage in a silent mode, stated that exposure affected the acquisition of learning responses in wistar rats. This results in poor spatial navigation and object placed configuration in the mobile radiation exposed rats [26].

In 2010 AAN Assi, et al, has studied the effect of mobile phone radio waves on the brain of laboratory mice exposed with different durations, that is exposed to 30 mins & 60 mins/day for 25 days, stated that their results shows a significant difference in brain size (CT image analysis of measuring brain volume by using stereology technique) and memory between control and exposed groups, which may affect the anatomical and physiological development of brain of the mice [27].

In 2011 M.P.Ntzouni et al, has studied the mobile phone radiation effect on short term memory in mice-Non spatial memory task with the help of object recognition test (ORT), stated that acute exposure (90 min/day for 3 days) did not show any significance, whereas chronic exposure (90 min/day for 17 days & 31 days) shows significant difference between control and exposed groups [28].

In 2014 Klose M et al, has studied the early onset effects of radiofrequency electromagnetic radiation on memory and behaviour in rats, stated that exposing to 2 hrs/day, 5 days a week for 19 months with a specific absorption rate (SAR) of 0.7, 2.5 & 10 W/kg didn‟t show any significant changes in both behavioural (Rotarad test) and learning task (8-Arm Maze & Morris Water Maze) [29].

In 2016 Son Y et al, has studied the 1950 MHz radiation frequency on 5xFAD mice exposed 2 hrs/day, 5 days/week for a duration of 3 months with a specific absorption rate (SAR) of 5 W/kg, stated that both spatial and non-spatial memory in exposed group didn‟t revealed any significant difference compared to the controls [30].

In 2008 Nittby H et al, studied the long-term effects of GSM-900 mobile radiation exposure with Specific absorption rate (SAR) of 0.6 W/Kg for 2hrs/week for a duration of 55 weeks, reported that episodic-like memory test shows significant impaired memory in exposed rats [31].

In 2009 Fragopoulou AF, et al has studied the exposure with GSM 900 MHz radiation (2 hr/day for 4 days) at a specific absorption rate (SAR) ranging from 0.41 to 0.98 W/Kg on hippocampal based spatial memory in mice with morris water maze test, stated that exposed mice had deficits in consolidation and retrieval of the spatial information and concluded that electromagnetic radiation affects the spatial learning and memory in mice [32].

In 2012 Nooshinfar E, et al, has studied the effects of low frequency electromagnetic radiation with different intensities of 0.5 to 2.0 mT (milli Telsa) / 50 to 217 Hz for 16 hrs/day for a duration of 2 weeks on learning and memory deficits in 1 control and 4 exposed groups of mice, stated that significant deficiency in memory and learning assessment were shown in electromagnetic-exposed mice compare to controls [33].

Review of literature on memory learning & histology-

In 2012 Hao.D, et al has studied the exposure of wistar rat to 916 MHz Mobile phone-Electromagnetic field for 6 hrs/day for 4-5 weeks and reported that error rates shows higher in radial arm maze and hippocampal neurons shows irregular firing pattern at the time of experiment. The results signifies that electromagnetic radiation influences memory and learning in the exposed rats, but the effects of long term were not clear [34].

In 2009 W.M.U Danies et al, has studied the effects of electromagnetic radiation exposure for 3 hrs/day from P2-P14 with 840 MHz and reported that there is no significant difference in memory-learning assessment and histo-morphological assessment of hippocampus. However behavioural assessment like anxiety and mood disturbances shows decreased loco motor activity and increased grooming activity with increased plasma corticosterone levels by ELISA method [35].

In 2010 Narayanan SN, et al, has studied the effect of radio frequency electromagnetic radiation by giving 50 missed calls /day for 4 weeks on passive avoidance behaviour and hippocampal morphology in wistar rats, stated that passive avoidance behaviour task (fear aggravated test to evaluate memory-learning) significantly affects the mobile phone RF-EMR exposed rats shows the shorter latency to enter into dark compartment when

compare with the control group rats and marked histomorphological changes were also observed in the CA_3 region of hippocampus in mobile phone exposed rats when compared with the control rats [36].

In 2015 Son Y et al has studied the effect of sub-chronic whole-body exposure to a 1950 MHz electromagnetic field with a high SAR (Specific absorption rate) level of 5.0 W/kg on the hippocampus of mouse brain, stated that sub-chronic whole body exposure of 2 hrs/day for 60 days did not show memory impairment and hippocampal morphological alternations in C57BL/6 mice [37].

In 2014 N Saikhedkar et al, has studied the 900 MHz mobile radiation exposure for 4hrs/day for 15 days on behavioural analysis and hippocampal morphology of wistar rats, the results revealed that poor memory learning and more anxiety were shown in exposed rats and neurodegenerative changes were also observed in hippocampal neurons of exposed group [38].

In 2015 Y J Choi, et al, has studied the effect of mobile radiation for 9 weeks in male mice, 11 weeks in female mice and examined the spatial memory, which showed no significant difference in control and exposed groups. The microscopic anatomy of hippocampal progenitor cell proliferation showed no significance [39].

In 2012 Li Y et al, has studied the effects of mobile phone radiation exposure for 2 hrs/day for a duration of 1 month with a specific absorption rate (SAR) of 0.52-1.08 W/kg on spatial memory and reported that electromagnetic radiation impairs spatial memory, ultra-structural (Transmission electron microscope-TEM) images of hippocampus shows mitochondrial degeneration, less number of synapses with short post synaptic density were observed in radiation exposed rats [40].

Review of literature on neuronal assessment and quantification-

In 2013 Faridi K, et al, has studied the effect of radio frequency-electromagnetic radiation on CA3 region of hippocampus, Each group of Albino rats were exposed to 25, 50, 75, and 100 missed calls/day for 4 weeks, then the animals were euthanized, perfused and brains were extracted out for tissue processing, sectioned at the level of hippocampus, stained with Haematoxylin and Eosin stain. Light microscopic images revealed that few signs of haemorrhage with enlarged perivascular spaces, shrinkage neurons with deformation of nuclei were observed. Transmission Electron Microscopic images revealed presence of shrunken neurons with condensed and increased density of both cytoplasm & nucleoplasm, mitochondria were swollen, vacuolized with reduced number of distorted cristae. Synapses shows few synaptic vesicles in presynaptic terminals, widened synaptic clefts with reduced in postsynaptic thickness [41].

In 2016 Mugunthan N et al has investigated the long term exposure with different durations (with a period of 30, 60 90,120, 150 and 180 days for 48 mins/day exposure) of 900-1800 MHz radiation emitted from 2G mobile phone in mice hippocampus at histomorphometric level and stated that hippocampal pyramidal neuronal density has significantly reduced and nuclear diameter also decreased in the hippocampal neurons of mice [20].

In 2013 Afeefy AA et al, has studied the effect of mobile radiation exposure on both adult and new born rats with a duration of 2hrs/day for 12 weeks, histological sections revealed that granular cells in dentate gyrus shows degenerative changes with intranuclear vacuolation and dark stained nuclei, decreasing in diameter. Pyramidal cells of hippocampal CA3 region shows irregularly arranged with intranuclear vacuoles and pericellular haloes.

Immunohistochemical (IHC) study of Glial Fibrillary Acidic Protein (GFAP) results revealed that positively reacted astrocytes (Brown Fibres) were present in hippocampus and dentate gyrus [19].

In 2016 Teimori F et al, has studied the effect of 30 mTelectro magnetic field exposure with a duration of 4 hrs/day for 10 weeks and observed the hippocampal structure with the help of transmission electron microscopy (TEM), the ultra-structural changes revealed a significant difference in the exposed group, which shows the morphological abnormal changes were noticed in mitochondria with increased number of apoptotic cells were also observed [42].

In 2015 Sahin A et al, has studied the impacts of 900 MHz electromagnetic fields on hippocampal pyramidal neurons with a duration of 1 hr/day for 30 days in Sprague dawley rats, stated that pyramidal neurons of cornu ammonis (CA) region of hippocampus were significantly less in exposed group. Histological changes shows more abnormal cells with dark blue cytoplasm and shrunken neurons. The cavities of lateral ventricles also increased in exposed group when compare to controls [43].

In 2016 Kerimoglu G et al, has studied the effects of 900 MHz electromagnetic radiation throughout adolescence (21-59 postnatal days) exposure for 1 hr/day, histological examination of hippocampus revealed that more number of pyknotic neurons with dark blue/black cytoplasm on electromagnetic field radiated group and stereological examination of hippocampal pyramidal neurons shows significant decrease in exposed group compared to control group [44].

In 2009 Bas O et al, has studied the effects of 900 MHz electromagnetic radiation (EMR) on hippocampal pyramidal neurons with a duration of 1 hr/day for 28 days exposure in adult female rats, stated that histological images shows dark cell density were increased

significantly with decreased number of viable pyramidal neurons in cornuammonis (CA) region of hippocampus in exposed group [45].

Review of literature on dendritic quantification-

In 2015 SR Bolla, has studied the mobile phone radiation effects with multiple durations of exposure like 15, 30, 45 & 60 min/day for 30 days and analysed the neuronal damage, dendritic arborisation in hippocampal CA3 region of mice.

Results reveals that more number of neurons were damaged and decreased dendritic arborisation were noted with increasing the duration of exposure [46].

CHAPTER- 3

NEED OF THE STUDY, RESEARCH QUESTION AND OBJECTIVES

NEED OF THE STUDY-

There are few studies on effect of radio frequency-electromagnetic radiation (RF-EMR) from 2 G & 3 G mobile phones on behavioural tests for memory and hippocampal morphology in mice, which is a short term exposure (exposed for 1 month) with mobile phone radiation.

So the present study is focused on the effect of long term exposure of radio frequency-electromagnetic radiation (RF-EMR) with 4 G mobile phones on behavioural tests for memory and hippocampal morphology in Albino mice. (Exposed multiple groups of mice with multiple durations like half an hour and one hour per day for 3 months, 6 months & 9 Months)

Research Question-

Does Mobile Phone Radio-Frequency Electromagnetic Radiation (MP RF-EMR) affect the cognitive function and microscopic anatomy of hippocampus in albino mice?

Objectives-

1) To evaluate the effect of long-term exposure of Mobile Phone Radio-frequency Electromagnetic Radiation on learning and memory in albino mice.
2) To evaluate the effect of long-term exposure of Mobile Phone Radio-frequency Electromagnetic Radiation on histo-architecture of hippocampal neurons in albino mice.
3) To evaluate the extent of dendritic damage of hippocampus.

CHAPTER- 4

MATERIALS AND METHODS

MATERIALS AND METHODS-

The study was carried out after the approval of Institutional Animal Ethical Committee with numbered- IAEC/PHARMA/SDUMC/2017-18/04, the study was conducted at central animal house, Sri Devaraj Urs Medical College, Kolar, Karnataka.

Animals-

-6 weeks old healthy male Swiss-Albino Mice weighs around 20-25 grams were used in this study, the animals were procured from CPCSEA registered brooders –Invivo Biosciences, Bengaluru, with CPCSEA Register No-1165/po/RcBiBt/S/08/CPCSEA.

-The animals were kept in polypropylene cages measuring 25 × 16 × 13 cms (L×B×H) with a temperature of 23±2ºc, Humidity 55±5% and 10 hours light, 14 hrs dark cycle and free access to standard pellet food and water ad libitum. Three mice were housed in each polypropylene cage.

The animal care was taken as per the committee for the purpose of control & supervision of experiments on animals (CPCSEA) guidelines [47,48].

Diet- Mice were fed with standard food pellets and water ad libitum. The mice pellets were procured from "Champaka feeds and foods"- Bengaluru, Manufactured by VRK Nutritional solutions, Maharashtra (An ISO 9001-2008 Certified company). Standard pellet diet contains Moisture- 10%, Crude protein- 18%, Crude fibre- 4%, Crude fat- 4%, Nitrogen free extract (NFE)- 60 %, Calcium- 1%, Phosphorous- 0.5%.

Bedding Material- Paddy husk were used for bedding material, procured from "Ramu rice mill"- Tamaka, Kolar.

Paddy husk will be changed every alternate day and care was taken in a humane manner while handling the rats.

Selection of animals and Randomisation- Male swiss albino mice which weighs around 20-25 grams were selected for this study and randomly allocated to all the groups to prevent the bias.

Sample size calculation/ Estimated sample size-

To detect a mean difference of 10 % damage in dendritic morphology in hippocampus CA3 neurons, considering the extent of damage at 60 minutes in 100 micrometer concentric circle with 90 % power, 95% confidence interval, the estimated sample size is 54.[6]

Taking the allocation ratio as 1:2, the estimated sample size as 18 & 36.

So the Controls as 18, Cases as 36.

The total sample size is calculated to be **54.**

Formula-

$$N = [(Z^2 P (100 P)] / e^2$$

Experiment Design-

The total number of animals used in this study was 54, animals were assigned randomly in to different groups.

Group-I (3 Months Group)- Consists of 18 mice, divided into 3 sub-groups-

Group-I A- Control – Consists of 6 mice,

Group-I B- 30 mins exposure/day for 3 months- consists of 6 mice,

Group-I C- 60 mins exposure/day for 3 months- consists of 6 mice.

Group-II (6 Months Group)- Consists of 18 mice, divided into 3 sub-groups-

Group-II A- Control – Consists of 6 mice,

Group-II B- 30 mins exposure/day for 6 months- consists of 6 mice,

Group-II C- 60 mins exposure/day for 6 months- consists of 6 mice.

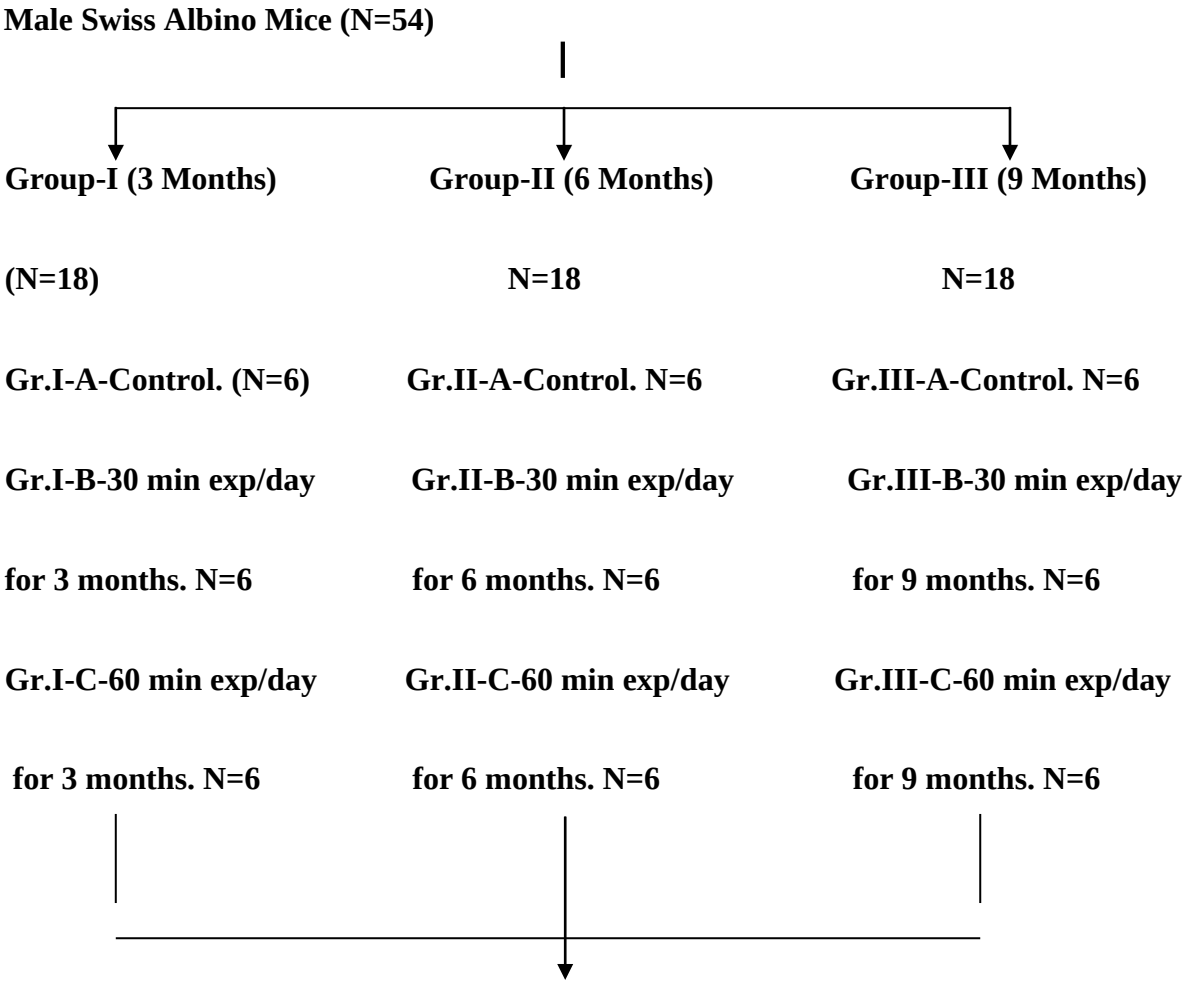
Group-III (9 Months Group)- Consists of 18 mice, divided into 3 sub-groups-

Group-III A- Control – Consists of 6 mice,

Group-III B- 30 mins exposure/day for 9 months- consists of 6 mice,

Group-III C- 60 mins exposure/day for 9 months- consists of 6 mice.

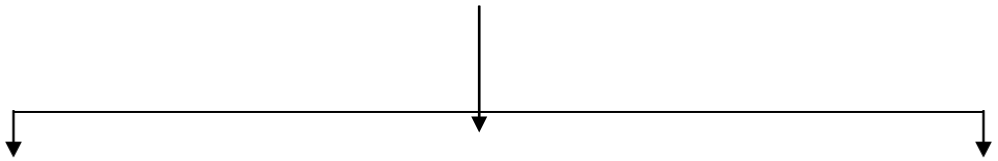
Experimental design shown in flow chart-



Behavioural Analysis

(Hebb-Williams Maze & T-Maze)

Sacrifice



H & E Staining Cresyl Violet Staining Golgi-Cox Staining

The study was divided in to different stages for the convenience of better understanding-

Stage- I- Behavioural analysis- Spatial learning and Memory assessment-

The spatial learning and memory assessment was done with the help of two maze tests-

1. Hebb-Williams Maze
2. T- Maze

All the 3 groups : Group-I (3 Months group), Group-II (6 Months group) and Group-III (9 Months group) animals including control group, 30 mins exposure group and 60 mins exposure group, after the end of exposure of 3 months, 6 months and 9 months the animals were assessed for spatial learning and memory by using Hebb-Williams maze and T-Maze apparatus.

Stage- II- Microscopic Anatomy- Cellular Architecture-

Haematoxylin and Eosin stained histological sections were analysed for cellular architecture of hippocampal pyramidal CA3 neurons.

Stage- III- Microscopic Anatomy- Neuronal Quantification-

Cresyl violet stained sections were analysed for neuronal quantification of hippocampal CA3 pyramidal neurons.

Stage- IV- Microscopic Anatomy- Dendritic Arborisation/Dendritic Quantification-

Golgi-Cox stained sections were analysed for dendritic branching points and dendritic intersections of hippocampal CA3 neurons.

Methodology-

Mobile phones: 4G android mobile phones (Micromax Bharat-2 with a Specific Absorption Rate (SAR) of 1.6 Watt/Kg) with same specification and with same mobile network were used in this study for uniformity of radiation exposure, keeping a GSM (2100 MHz) mobile phone in silent with auto answer mode. The mobile phones were hung down from the roof of the mice cage and the radiation which they emitted during the exposure was quantified by radiation frequency meter (Electrosmog Meter-ED 178 S) which was kept at the periphery, 1950 MHz of RF-EMR was emitting till the periphery of the mice cage during the exposure, so the similar amount of radiation may affect/enters the mice brain [20].

Radiation exposure technique: Three Mice were kept in each cage during the exposure.

Animals of Group I-B were exposed to 30 minutes/day for 3 months,

Animals of Group II-B were exposed to 30 minutes/day for 6 months and

Animals of Group III -B were exposed to 30 minutes/day for 9 months.

Animals of Group I-C were exposed to 60 minutes/day for 3 months,

Animals of Group II-C were exposed to 60 minutes/day for 6 months and

Animals of Group III-C were exposed to 60 minutes/day for 9 months respectively. The mobile phones were hung down in the centre of the cages during the exposure period for the uniformity of the radiation throughout the cage [Shown in below figure].

Figure 11: Radiation exposure technique- Shows Mice in the cage with mobile phone for radiation exposure and RF meter for radiation quantification- Image captured in central animal house, Sri Devaraj Urs Medical College, Kolar, Karnataka.

Radiation Frequency Meter (Electrosmog Meter)-

Cornet Microsystems-ED 178 electrosmog meter was used in this study to quantify the radiation emitted from mobile phones during the radiofrequency-electromagnetic radiation exposure.

Electrosmog meter is a sensitive dual-mode device which measures both high frequency radiation electromagnetic wave strength and low frequency radiation electromagnetic waves.

This electrosmog meter can measure a radiation frequency bandwidth of 100 MHz (Low frequency) to 8 GHz (High frequency).

The measured radiation frequency strength was shown on the digital LCD display in terms of Decibles-Milliwatt (dBm), Volt per meter (v/m) and Milliwatt per square meter (mw/m^2).

3 Coloured LED lights with Red, Yellow and Green will be displayed on the right side of LCD screen for quick radiation signalling indication.

- Red colour LED light indicates caution,
- Yellow colour LED light indicates safe zone,
- Green colour LED light indicates low frequency.

The digital display shows histogram, which records recent 30 signal level readings and shown as moving graph on the digital display.

Maximum, Average and Minimum measurements of electromagnetic radiation data which was recorded earlier will be shown in a graphical representation in digital display [49].

Figure 12: Radiation frequency meter (Cornet Microsystems-ED 178 electrosmog meter) – Image captured in central animal house, Sri Devaraj Urs Medical College, Kolar, Karnataka.

Behavioural Tests-

Hebb- Williams Maze-

PURPOSE- Maze tests were used to assess the spatial learning and working memory in laboratory rodents since more than 100 years.

Hebb-Williams Maze was a classic maze paradigm which was designed and developed to assess the animal intelligence -spatial learning and working memory of the mice.

The principle behind the Hebb-Williams Maze test is "The duration of the time taken by the animal to navigate the maze, which was been previously habituated/exposed will be used as a test for memory and learning, The faster the mice navigates the maze, the better its spatial memory".

The Hebb-Williams maze is a square shaped box which measures 60 cm (L) ×60 cm (W) ×10 cm (H) walls. It consists of start chamber-A (Animal Chamber) which is attached to the exploratory area-B (Middle Chamber) and a goal box-C, located at the opposite end of the start chamber and contains a small food reward.

All three chambers were provided with removable doors to allow the animal to move from one chamber to the next. After 12 hours of fasting (After food restricted for 12 hrs, 85% of pre-test animal body weight should be maintained- so that the animal will be motivated to reach the food reward chamber), the mice was placed in the start chamber-A and allowed to enter into the exploratory area-B (middle chamber), once the animal enters into middle chamber the door was closed to prevent back entry.

The time taken by the animal to reach The Reward Chamber (TRC) from the start chamber was recorded. The animals were trained for 3 days (3 trials/day) and the readings

were taken at the 4th day. Low scores indicates better memory, while the high scores indicates poor memory in animals [50, 51,52,53].

Figure 13: Hebb-Williams Maze apparatus- Image captured in research laboratory, department of pharmacology, Sri Devaraj Urs Medical College, Kolar, Karnataka.

T-Maze –

The protocol of T-Maze is used to assess the ability of cognition in rodents. T-Maze is an enclosed or elevated apparatus which is in the form of T shaped, placed horizontally.

T-MAZE APPARATUS-

T-Maze apparatus is made up of ply-wood which consists of a start arm and two goal arms (Right and Left goal arms).

The start arm measures around 30×10 cm and located in the beginning part of the stem.

The food well areas (Goal areas) were located at the endings of the two arms, each goal arm measures around 30×10 cm faced towards the food wells.

The start arm consists of a start box which is separated by a guillotine sliding door.

Right and left arm consists of an individual guillotine sliding doors which separates the start arm from goal arms.

The height of the walls of the T-Maze apparatus will be around 20 cm.

The T-Maze apparatus should be kept in a dark, sound attenuated room during the testing phase for getting proper results.

PROCEDURE-

Food Deprivation- Animals were food deprived for 12 hours prior to the test, to motivate the animal towards the food reward.

The body weight of the animal was maintained about 85% of the pre-test weight.

Orientation- 2-3 days prior to the test the animals were food deprived and placed the animals in start box for one minute.

The sliding doors of all the arms were opened to allow the animal to explore/habituate the animals in T-Maze for a duration of thirty minutes and to eat the food pellets in goal box. After the habituation of thirty minutes, the animal was returned in to the start box.

This orientation was repeated for 2-3 days prior to the test.

Reward Alternation Test-

This test was started after the completion of proper orientation to the animals.

During the Reward alternation test six trials per day was conducted continuously for four days. Each trial has two different runs namely-

A. Forced Run,
B. Choice Run.

A. Forced Run: In this forced run the mice was forced to run in to only one of the arm by blocking of other arm and allow the mice to consume the food pellets in the goal box. Once the mice finishes eating the food pellets in goal box, it was replaced back in the start box for choice run.
B. Choice Run: During this choice run, the goal box of the forced arm were kept empty and feeding pellets was placed in the goal box of opposite arm.

   Now both the arms were kept open for the mice to choose anyone.

   Between each experiment of forced run and choice run a duration of one minute was given.

   Similarly one minute gap was given between each trial.

   The sequence of forced arm was decided in advance and it was same for all the mice for a given day.

   On consecutive days it was alternatively changed.

   During the time of choice run, if the mice enters the arm opposite to forced arm, the response was treated as “Correct Response”.

   If the mice enters the same arm, to which it have been forced to enter during forced run, it was interpreted as “Wrong Response”.

   Percentage of responses were calculated for each mice by using this following formula:

   Percentage of correct responses = Total number of correct responses ×100/Total number of trials [54,55,56].

Figure 14: T- Maze Apparatus- Image captured in Central animal house, Sri Devaraj Urs Medical College, Kolar, Karnataka

Microscopic Anatomy- Cellular Architecture-

Preparation of mice brains for staining-

Cardiac perfusion- After the behavioural analysis the mice were euthanized by using overdose of ketamine/xylazine (22-24 Mg/kg body weight), fixed the mice ventral surface faces upwards on a dissection board and the chest cavity of the mice were opened with the help of dissection instruments and exposed the heart, identified the left ventricle of the heart.

Mice were perfused transcardially through the apex of the heart with normal saline at a rate of 1 ml/minute, followed by normal saline with the help of 10% buffered formalin

with a volume of 100-150 ml/adult mice. Then the right auricle of the heart was cut as an outlet for the perfused fluids.

The mice was then decapitated, brain was carefully extracted out and fixed in 10% buffered formalin and further processed for paraffin blocks.

Processing of the tissue for paraffin blocks-

Fixation - 10% Buffered Formalin,

Dehydration - A. 50% Ethyl Based Alcohol for 9 hours,

b. 70% Ethyl Based Alcohol for 15 hours
c. 90% Ethyl Based Alcohol for 9 hours
d. Ethyl Based Absolute Alcohol for 15 hours

Clearing - a. Xylene I -1 hour

b. Xylene II – 1 hour

Impregnation - 56^0C paraffin wax I – half an hour

Paraffin wax II – half an hour

Paraffin wax III – half an hour

Paraffin wax IV – half an hour

Embedding and Blocking- The tissues were embedded with the help of melting wax and L-Moulds, allowed the wax and blocks to room temperature and labelled it for further sectioning.

Section cutting- Four micrometre thick sections were cut at the level of the mid dorsal hippocampus by using a rotary microtome.

Ten to twenty sections from each animal was taken and mounted on a gelatine coated slides and kept the slides on a hot plate for 10-15 minutes [57,58].

Figure 15: Image represents the Rotary microtome- Image captured in research laboratory, Department of anatomy, Sri Devaraj Urs Medical College, Kolar, Karnataka.

Haematoxylin and Eosin Staining Protocol-

Haematoxylin and eosin staining was done as per the standard operating procedure.

- Deparaffinise the sections in xylene for 10 minutes.
- Rehydrate the sections through descending grades of ethyl based alcohol

  - Absolute alcohol for 30 sec - 1 minute,

  - 90 % Alcohol for 30 sec – 1 minute,

- 70 % Alcohol for 2 – 3 Minutes,

- 50 % Alcohol for 2 – 3 minutes,

- Rinse in distilled water for 3 minutes.
- Stain with haematoxylin for 5-8 minutes.
- Rinse the slides in running tap water for 10-15 minutes for blueing / 1% acid alcohol for 10 seconds (1% HCL in 70% alcohol).
- Stain the slides with 1 % aqueous eosin for 30 sec – 1 minute.
- Dehydrate and differentiate the sections through ascending grading‟s of alcohol –
    - 50 % Alcohol for 5 – 10 dips,
    - 70 % Alcohol for 5 – 10 dips,
    - 90 % Alcohol for 2 – 3 dips,
    - Absolute alcohol for 1 minute and
- Air dried and cleared the slides in xylene for 5 minutes – 2 changes each.
- Mounted the slides with cover slip using Distrene Polystyrene Xylene(DPX).

Cellular Architecture of Hippocampal CA3 Pyramidal Neurons by Using Haematoxylin and Eosin (H&E) Staining-

Ten sections from each mice was analysed for the morphology using a light microscope under high power (40 X).

CA3 hippocampal pyramidal neurons were evaluated at the level of the dorsal region of the hippocampus.

The hippocampal CA3 pyramidal neuronal images were captured using “Zeiss primo-Camera mounted digital microscope” in both low power (10 X) and high power (40 X) [59,60].

Microscopic image of Haematoxylin& Eosin stained section captured in camera mounted microscope -

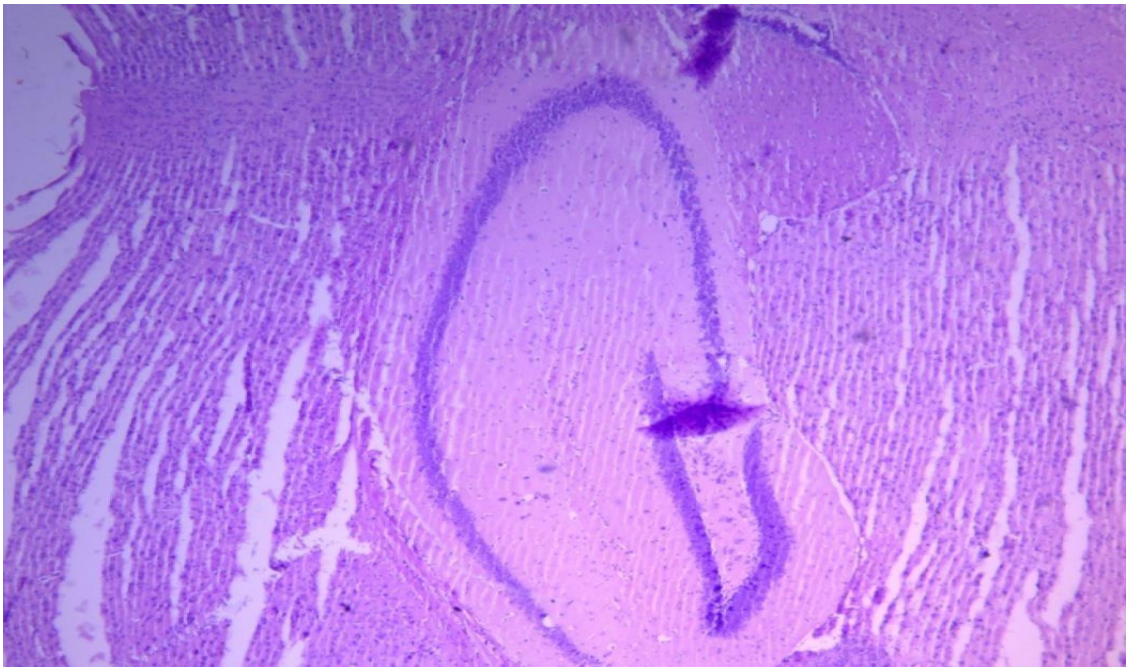


Figure 16: 4 X image of Hippocampus section stained with Haematoxylin & Eosin - Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka.

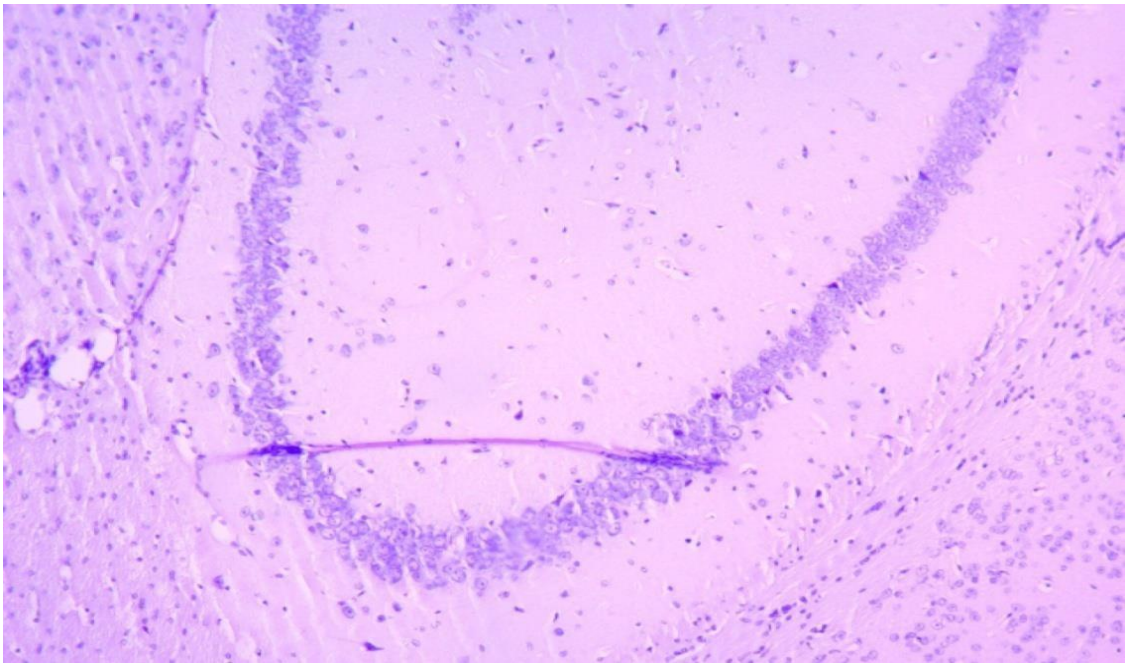


Figure 17: 10 X image of hippocampus section stained with Haematoxylin& Eosin- Image captured in Central laboratory, department of pathology, Sri Devaraj Urs Medical College, Kolar, Karnataka.

Cresyl Violet Staining-

Preparation of Cresyl Violet Staining Solution-

- Dissolved 0.1 gram of aqueous Cresyl violet powder (LOBA CHEMIE, INDIA) in 100 ml of 60^0C warm distilled water with the help of magnetic stirrer.
- Once the aqueous Cresyl violet powder completely dissolved with warm distilled water, cool/allow the solution to room temperature.
- Added 100 µl of glacial acetic acid, mixed thoroughly to adjust the P.H between 3.5-3.8.
- The solution was then filtered using a what man No.1 filter paper and stored it in a room temperature for further use.

Cresyl Violet Staining Protocol-

- Deparaffinise the sections in chloroform for 1 minute.
- Rehydrate the sections through descending grades of ethyl based alcohol
    - Absolute alcohol for 2 minutes,
    - 80 % Alcohol for 2 minutes,
    - 70 % Alcohol for 2 minutes,
- Rinsed the slides in distilled water for 5- 10 minutes.
- Stained with Cresyl Violet solution for 2-3 minutes (Variable).
- Washed the slides in distilled water for 5-10 minutes.
- Dehydration and differentiation of the slides through ascending grades of alcohol
    - 70 % Alcohol for 2 minutes
    - 80 % Alcohol for 1 minute,

- 90 % Alcohol for 2-3 dips,
- Absolute alcohol for 1-2 dip,

- Air dried and cleared the slides in xylene for 2 minutes.
- Mounted the slides with cover slip using Distrene Polystyrene Xylene (DPX) [61,62,63].

Quantification of Neurons-

From each animal 10 sections were stained and analysis for neuronal quantification of hippocampal pyramidal CA3 neurons.

The total number of hippocampal CA3 pyramidal viable neurons were counted around 250 µm length with the help of the ocular micrometre by using 40 X magnification.

This was done by fixing the ocular micrometre to the eyepiece of the microscope.

The total number of viable neurons (With normal nucleus, cytoplasm and cell membrane) were counted.

Non-viable neurons which is darkly stained, unhealthy, scattered and irregular with shrunken nuclei were excluded.

To avoid observers bias the slides were decoded after the completion of analysis [46,64,65].

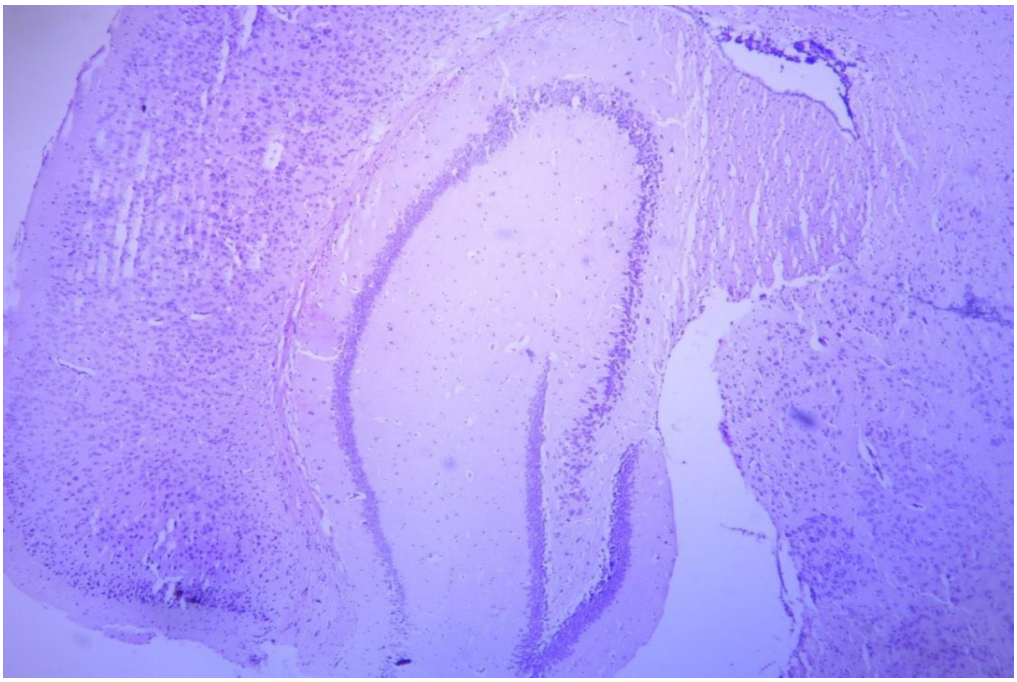


Figure 18:4 X image of hippocampus section stained with Cresyl violet - Image captured in Central laboratory, department of pathology, Sri Devaraj Urs Medical College, Kolar, Karnataka.

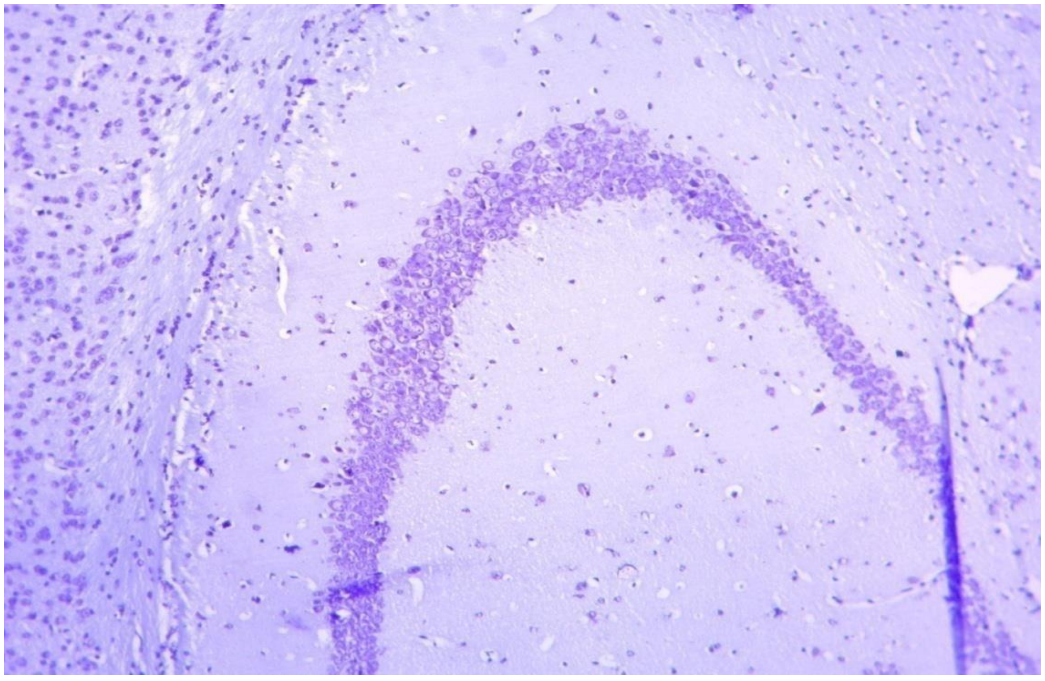


Figure 19: 10 X image of hippocampus section stained with Cresyl violet- Image captured in Central laboratory, department of pathology, Sri Devaraj Urs Medical College, Kolar, Karnataka.

Golgi-Cox Staining-

Materials and Chemicals used:

- Potassium dichromate
- Potassium chromate
- Mercuric chloride
- Distilled water
- Sucrose solution
- Sodium carbonate
- Cedar wood oil
- Fevi kwik
- Ethyl alcohol (distilled)
- Xylene
- Glass slides (75x25mm) – Blue star, India.
- Cover slips (40x22mm) – Blue star, India.
- DPX mounting media

Preparation of Golgi-Cox Soln. (500ml)

Solution. A:

- 5% $K_2Cr_2O_7$- Potassium Dichromate (Dissolved 5gm of potassium dichromate in 100 ml of warm distilled water)
- 5% $HgCl_2$- Mercuric Chloride (Dissolved 5gm of mercuric chloride in 100 ml of warm distilled water)

Solution. B:

- 5% K_2CrO_4 – Potassium Chromate (Dissolved 5gm of potassium chromate in 100 ml of warm distilled water).

- To this above solution 200ml of warm distilled water was added slowly.
- Soln. B was kept in a dark beaker/ amber coloured bottle and added soln. A slowly into it for 30-45 mins with the help of magnetic stirrer using hot plate.
- Solution A and B mixed precipitate was allowed to settle down to the bottom for 1-2 hours.
- Then the supernatant was filtered and stored it in amber coloured bottle in room temperature.

Procedure:

The mice were euthanized with the over dose of ketamine/xylazine (22-24 Mg/kg body weight), the mice was then decapitated, brain was carefully extracted out as soon as possible and place it in golgi-cox solution without perfusion.

- Placed the brain tissue in the staining solution for 8-10 days.
- With the help of vibratome microtome (Leica VT 1000 S) 150 µ thick sections were taken in 6% sucrose solution.
- Then the sections were transferred to the gelatin coated slides.
- Pressed the sections with blotting paper.
- Placed the sections in close box with moisturizing condition and dried in moisture environment for 1hr (can be modified).
- Washed the sections with distilled water (3-4 times)
- Transferred the sections into 5-10% sodium carbonate (Na_2CO_3) for 8-10 hours in dark. (can be modified)
- Washed the sections with distilled water for 3-4 times.
- Transferred the sections into 70% ethyl based alcohol for 10-15mins.
- Then transferred the sections into 100% ethyl based alcohol for 1min/5 dips.

- Slide sections were placed horizontally, Cedar wood oil was added on the sections and left for 10-12 hours
- Kept the slides vertically to drain out the excess cedar-wood oil in glass dish.
- Washed with xylene to remove oil for 3-4 dips.
- Mounted the slides with cover slip using Distrene Polystyrene Xylene (DPX) [46,66,67,68].

Vibratome microtome (Leica VT 1000 S) –

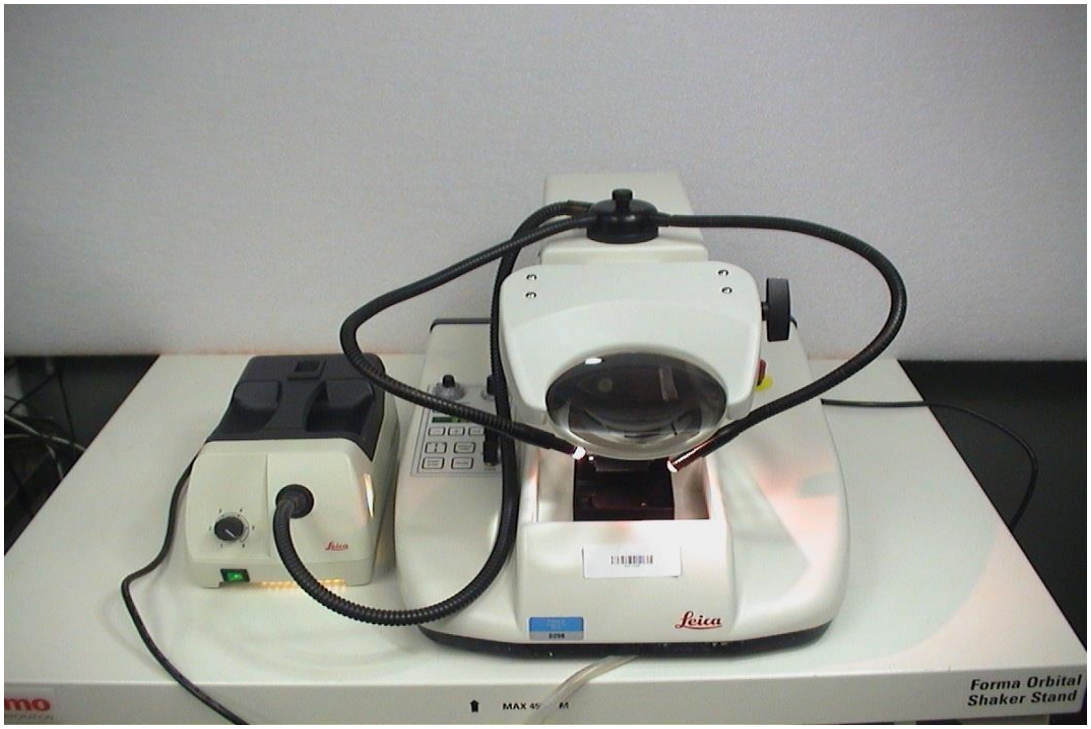


Figure 20: Vibratome microtome (Leica VT 1000 S) used to cut 150 µ thick sections for Golgi-Cox staining to trace hippocampal CA3 pyramidal neurons-Image captured in Sectioning and staining lab, Department of Neurophysiology, NIMHANS, Bengaluru.

Camera Lucida Tracing-

The hippocampal CA3 dendritic quantification was done by using a technique called camera lucida.

From each mice 8-10 properly stained hippocampal CA3 neurons from both right and left sides were traced by using camera lucida attached to a compound microscope.

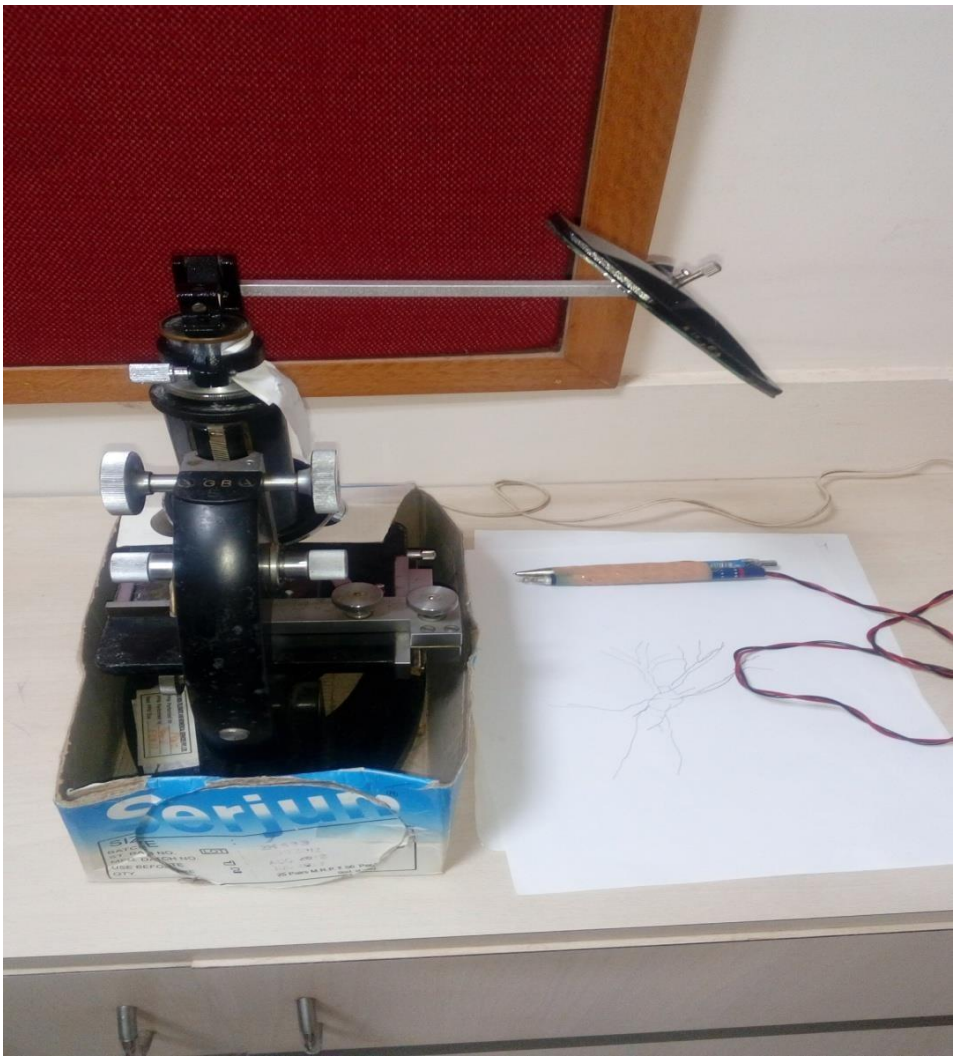


Figure 21: Camera lucida fixed to a compound microscope for the tracing of hippocampal CA3 neurons- - Image captured in Research laboratory, department of Anatomy, Sri Devaraj Urs Medical College, Kolar, Karnataka.

Criteria adopted for the selection of CA3 neurons for dendritic quantification-

- Only hippocampal CA3 region pyramidal neurons.
- Neurons which stained properly in soma and both apical and basal dendrites.
- The entire neuron with soma and both apical and basal dendrite without overlapping were isolated from neighbouring neurons.
- Neurons without truncation of apical and basal dendrites located within 100 micrometre radius from the soma.

Method of quantification of dendritic branching points and dendritic intersections-

Sholl DA-1956 Concentric circle method were adopted for quantification of dendrites.

On a transparent OHP sheet five concentric circles were drawn with 20 µm radial distance between each was used for quantification of dendrites (Both dendritic branching points and dendritic intersections).

During the quantification the transparent sheet with concentric circles were placed on the neuron- traced by camera lucida, in such a way that the soma of the neuron should coincides with in the centre of the concentric circles.

The total number of branching points between two concentric circles that is within each successive 20 µm concentric circle was counted.

The dendritic intersection is a point where the dendrite intersect/touches the particular concentric circle.

The total number of dendritic intersections in each concentric circle were counted by keeping the transparent concentric circles sheet on the camera lucida traced neuron.

Both apical and basal branching points and intersections was counted up to a radial distance of 100 µm from the centre of the cell body.

The apical dendrite of CA3 pyramidal neuron is having a single pole extending from apex of the cell body. It bifurcates into 2-3 main branches, from this the secondary and tertiary branches will arise.

The basal dendrites of pyramidal CA3 neurons arises from multiple places in the base of the soma and these dendrites branch repeatedly producing a dense tuft [69,70,71,72].

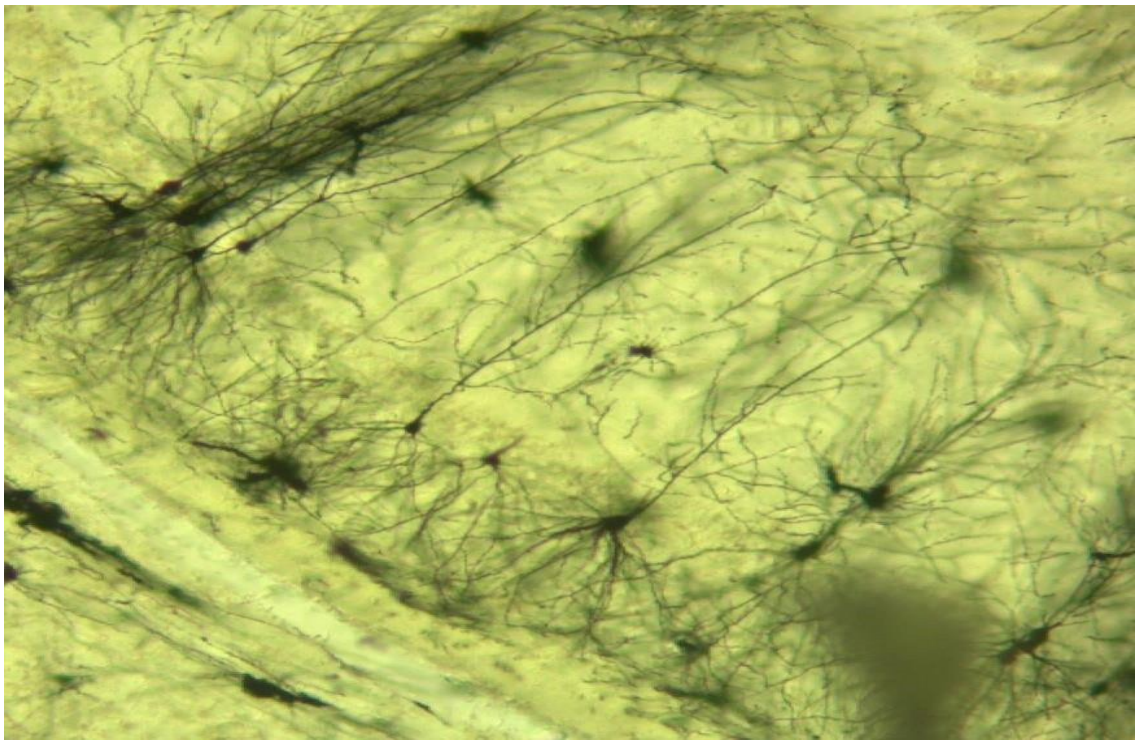


Figure 22: 10 X image of hippocampal CA3 Neurons stained with Golgi-Cox stain showing the Dendritic arborisation- Image captured in Central laboratory, department of pathology, Sri Devaraj Urs Medical College, Kolar, Karnataka).

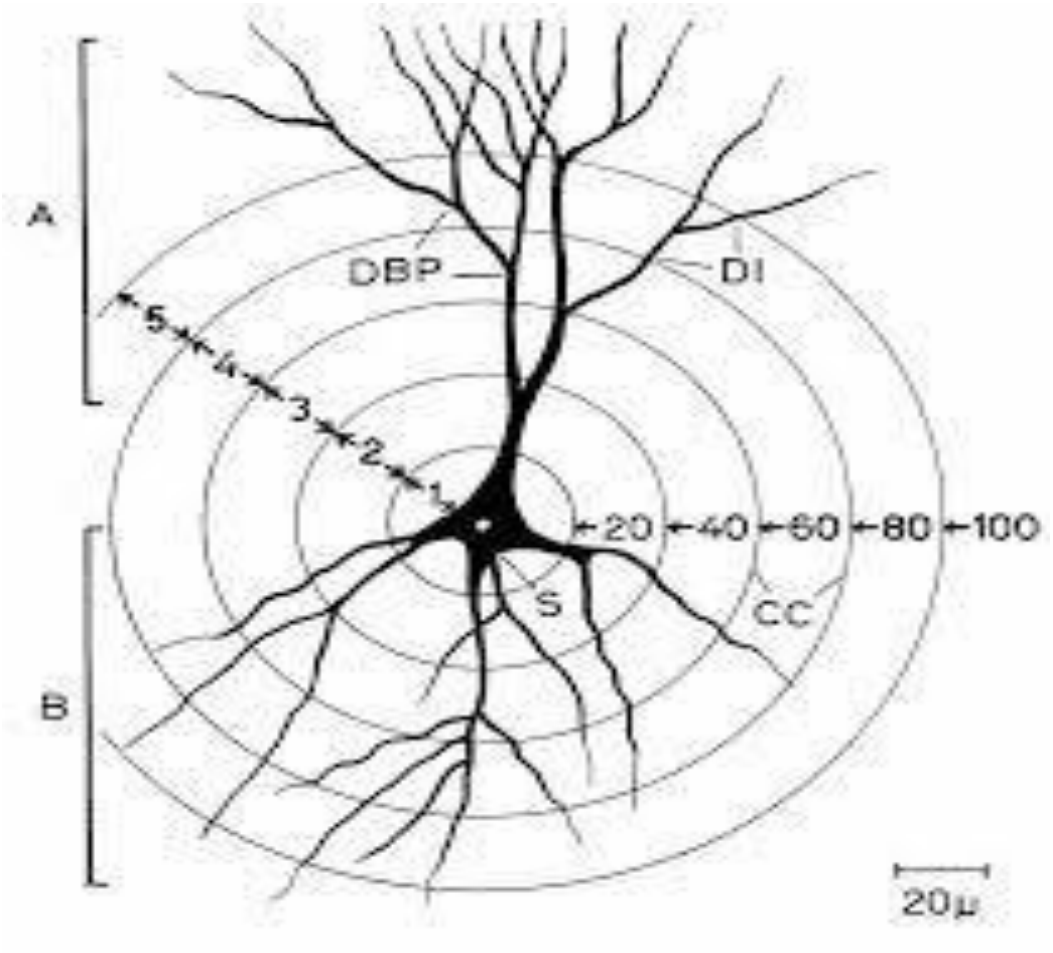


Figure 23: Schematic image of Sholl DA-1956 Concentric circle method for quantification of dendrites- Both apical and basal dendrites for a radial distance of 100 μ from the centre of cell body were studied in CA3 pyramidal neurons. Image adapted from SR Bolla, etal.

A- Apical Dendrites, B- Basal Dendrites, DBP- Dendritic Branching Points, DI- Dendritic Intersections, S- Soma, CC- Concentric Circle.

CHAPTER- 5

RESULTS

GROUP-I RESULTS (3 MONTHS EXPOSURE GROUP)-

Behavioural Analysis (Spatial Learning and Memory Assessment)-

5.1.1. A. Hebb-Williams Maze -

Effect of Radiation on Learning Memory in Hebb-Williams Maze-

The time taken by the mice to reach the target chamber from the starting chamber was significantly increased in group I-B (30 min exposed/day) and group I-C (60 min exposed/day) compared to group I-A (non-exposed group). The time taken by the animal to reach The Reward Chamber (TRC) scores in Group I-A vs Group I-B (31±15.48 vs 49±17.62 seconds), was not significant ($p>0.05$); Group I-A vs Group I-C (31±15.48 vs 64±22.99 seconds), was statistically significant ($p<0.05$) [Table 1, Fig 24].

Table 1: The time taken by the animal to reach the reward chamber (TRC) scores of mice in Group-I-A (control group), Group-I-B (30 mins exp/day for 3 months) and Group-I-C (60 mins exp/day for 3 months). Values were expressed in Mean ± SD, * Represents $P<0.05$ (Analysed by One-way ANOVA followed by Least square difference (LSD) test using SPSS 20).

Groups	TRC Scores (The time taken by the animal to reach the Reward Chamber)
Group I-A (non-exposed group).	31±15.48 Seconds
Group I-B (30 min exposed/day for 3 months)	49±17.62 Seconds
Group I-C (60 min exposed/day for 3 months)	64±22.99* Seconds

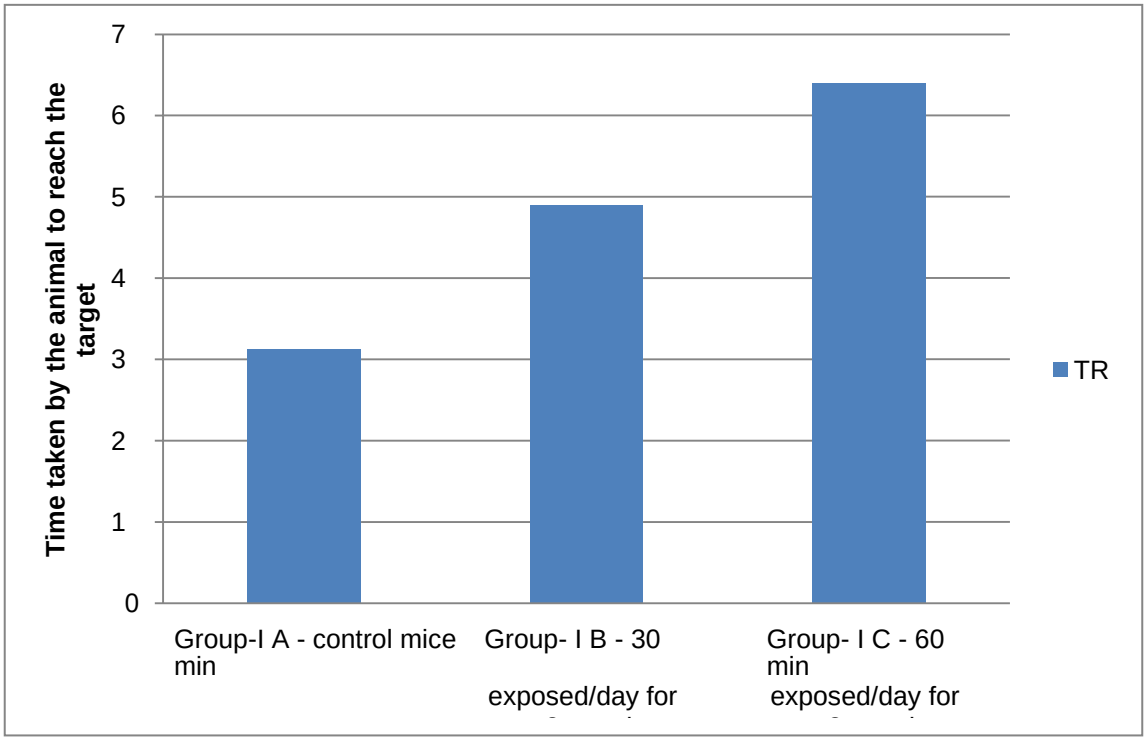


Figure 24: Effect of Mobile phone radiofrequency-electromagnetic radiation (MP RF-EMR) on learning and memory by using Hebb-Williams maze- group I-A, I-B and I-C.

B. T-maze -

Effect of Radiation on Learning Memory in T-Maze-

Reward Alternation Test-

During the reward alternation test, the mice exposed to Mobile Phone Radio Frequency-Electro Magnetic Radiation (MP RF-EMR) for 30 minutes exposure/day for 3 months (Group I-B) didn‟t showed the significant difference in the percentage of correct responses, whereas 60 minutes exposure/day for 3 months (Group I-C) shows a significant difference in the percentage of correct responses when compare to control group mice (Group I-A).

Percentage of correct responses of Group I-A vs Group I-B shows 71.5±3.15 vs 67.8±5.39 was not statistically significant ($p>0.05$) and Group I-A vs Group I-C shows 71.5±3.15 vs 55.6±2.97 was statistically significant ($p<0.05$) [Table 2, Fig 25].

Table 2: Percentage of correct responses of Group-I-A (control group), Group-I-B (30 mins exp/day for 3 months) and Group-I-C (60 mins exp/day for 3 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA followed by Least square difference (LSD) test using SPSS 20).

Groups	Percentage of correct responses
Group I-A (non-exposed group).	71.5±3.15
Group I-B (30 min exposed/day for 3 months)	67.8±5.39
Group I-C (60 min exposed/day for 3 months)	55.6±2.97 *

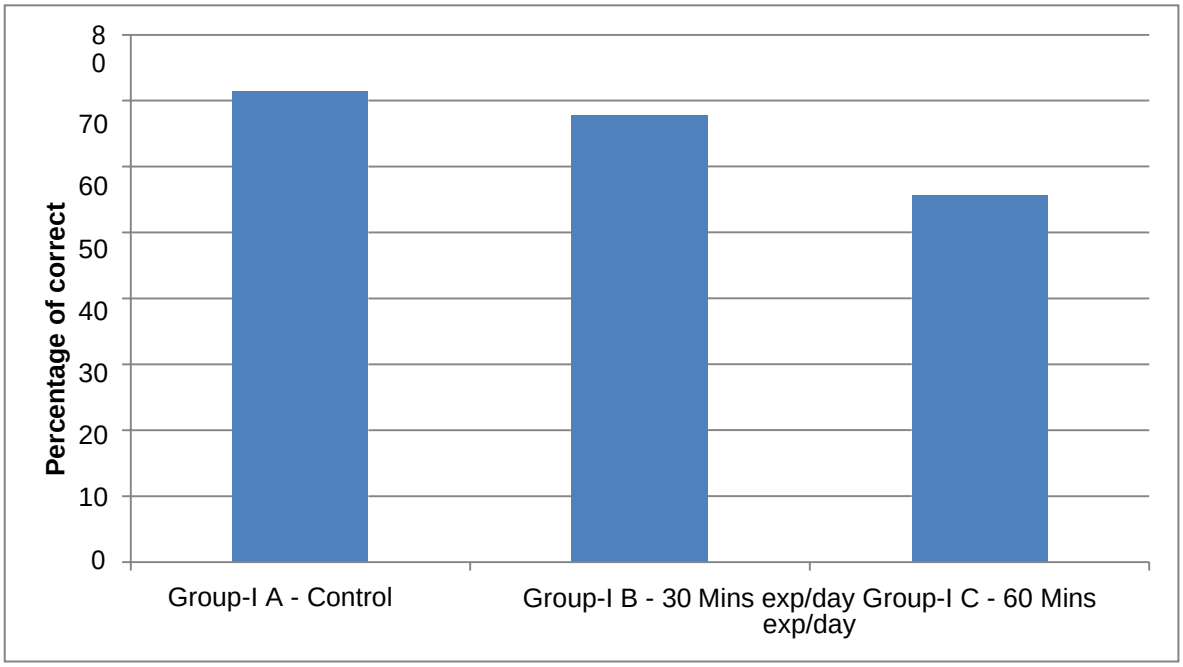


Figure 25: Percentage of correct response in rewarded alternation task performance.

Microscopic anatomy (Cellular Architecture) of Hippocampal cornu ammonis (CA3) Neurons

Histological sections of haematoxylin and eosin stained hippocampal CA3 pyramidal neurons showed marked difference between control group and RF-EMR exposed groups (group I-B and I-C). Sections of control group showed 5-6 layers of compactly arranged pyramidal cells which were healthy with clear nucleus (Figure 26). Group I-B(30 min exposure for 3 months) showed less number of pyramidal neurons with darkened nuclei (non-viable neurons) which was scattered when compared to control group (Figure 27). Group I-C (60 min exposure for 3 months) showed very less number of pyramidal neurons with more number of darkened nuclei (more non-viable neurons) with vacuolation in between the cells and scattered arrangement of pyramidal neurons when compared to group I-A and group I-B (Figure 28).

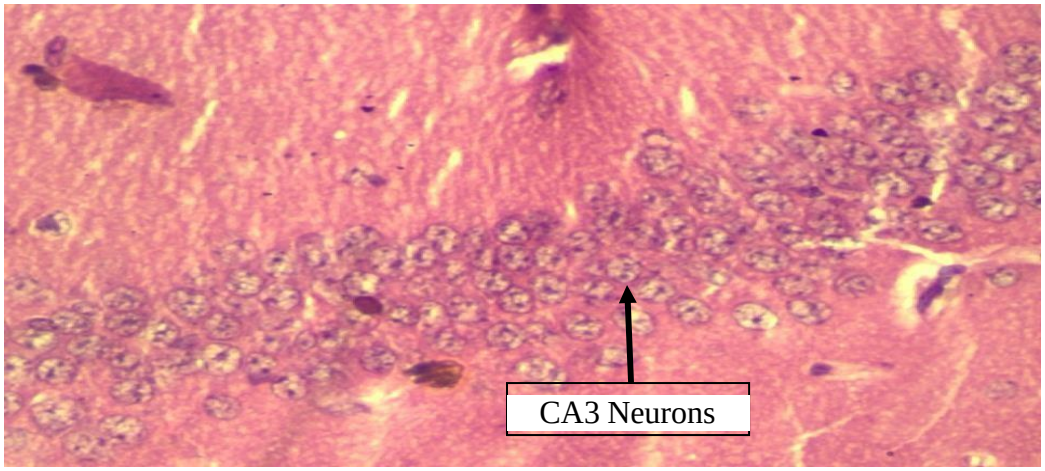


Figure 26: Group I-A (non-exposed)-Control group H & E stained Hippocampal CA3 pyramidal normal neurons (Arrow) in high power (40X). (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

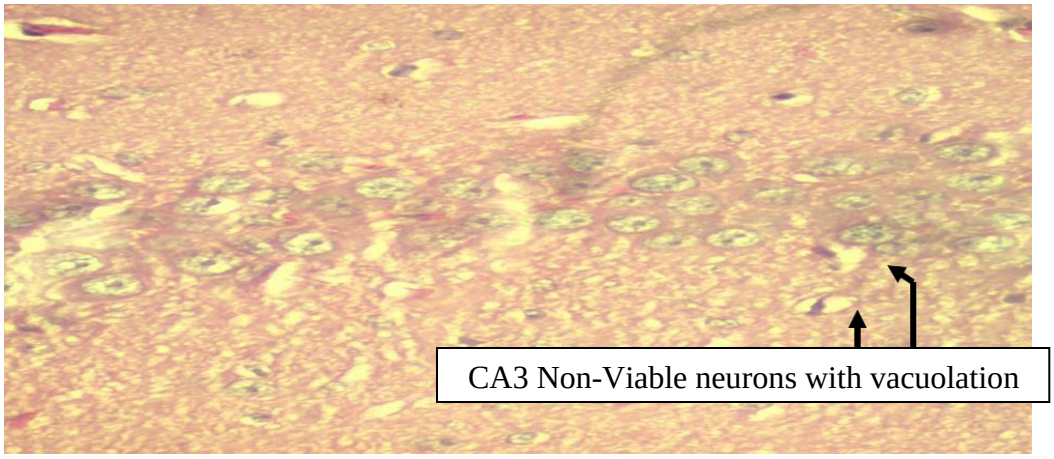


Figure 27: Group- I-B (30 min exposed for 3 months) H & E stained Hippocampal pyramidal neurons (Arrow) in high power (40X) showed less in number, Non-Viable and scattered with vacuolation in between the cells. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

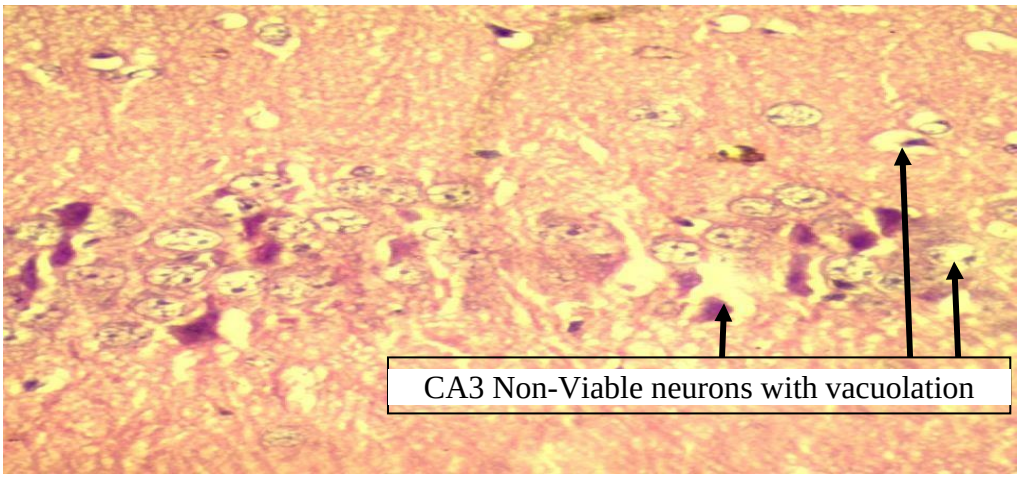


Figure 28: Group- I-C (60 min exposed for 3 months) H & E stained Hippocampal pyramidal neurons (Arrow) in high power (40X) showed very less in number, Non-Viable and scattered with vacuolation in between the cells. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

5.1.3 Neuronal damage assessment in CA3 region of hippocampus:

The pyramidal neurons of hippocampal CA3 region in control group shows healthy neurons which is compactly arranged with clear nucleus, whereas mobile phone radiation exposed mice hippocampal CA3 neurons shows darkly stained, unhealthy, scattered and irregular, as illustrated in below figures.

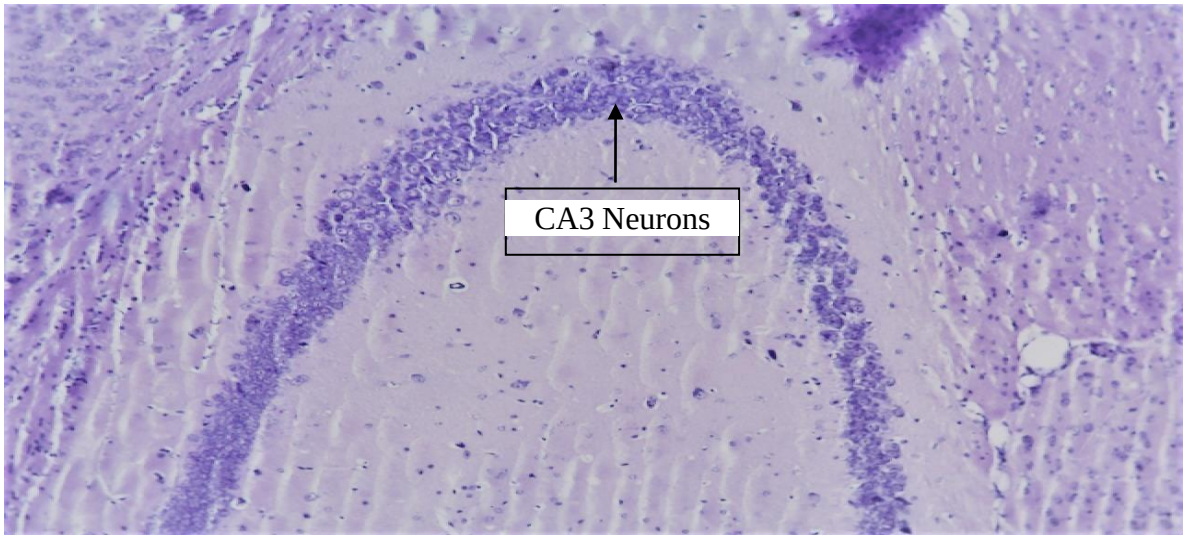


Figure 29: Group- I-A (Control group)-Low power image. Cresyl Violet stained photographic images of hippocampal pyramidal cells in CA3 region of mice brain from control group. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

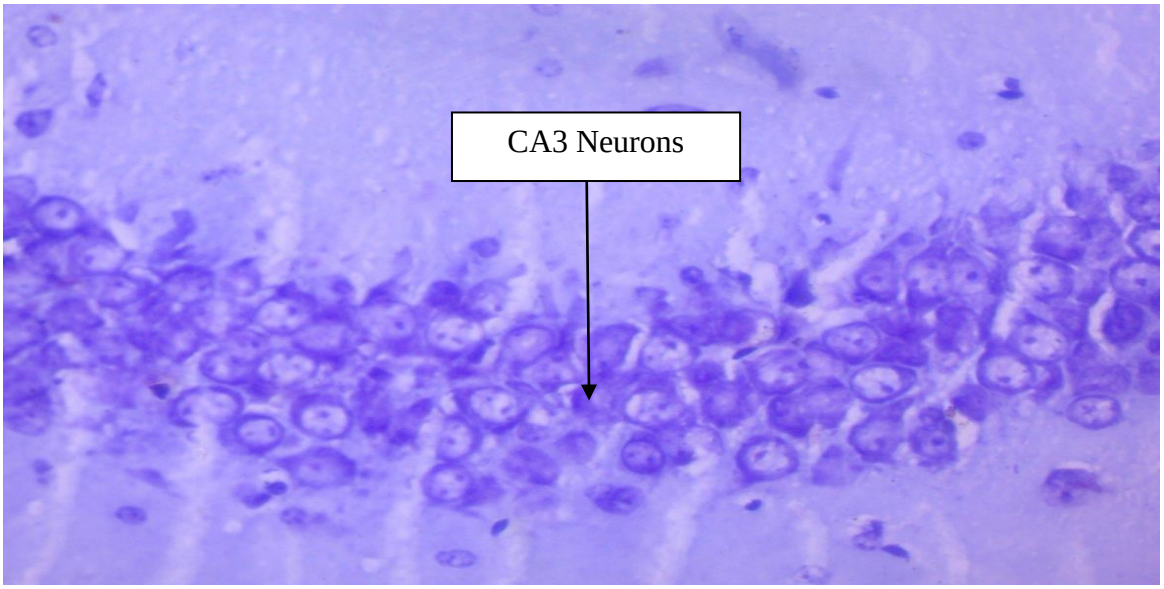


Figure 30: Group- I-A (Control group)- High power image. Cresyl Violet stained photographic images of hippocampal pyramidal cells in CA3 region of mice brain from control group. (Image captured in Central laboratory, department of pathology, Sri Devaraj Urs Medical College, Kolar, Karnataka).

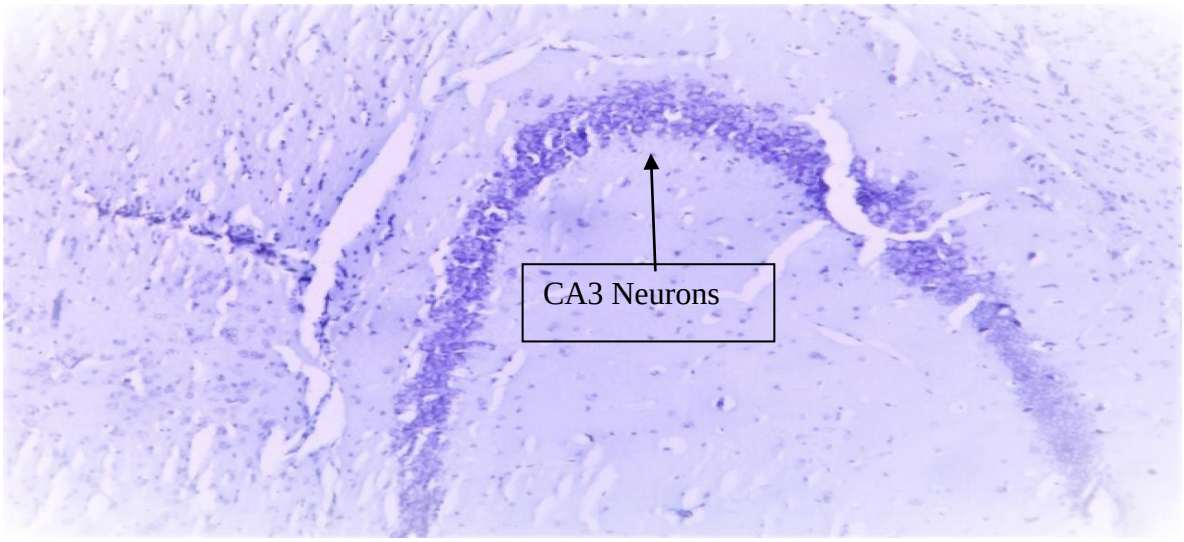


Figure 31:Group- I-B (30 mins exp/day for 3 months)-Low power image. Cresyl Violet stained photographic images of hippocampal pyramidal cells in CA3 region of mice brain from 30 mins exp/day for 3 months group. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

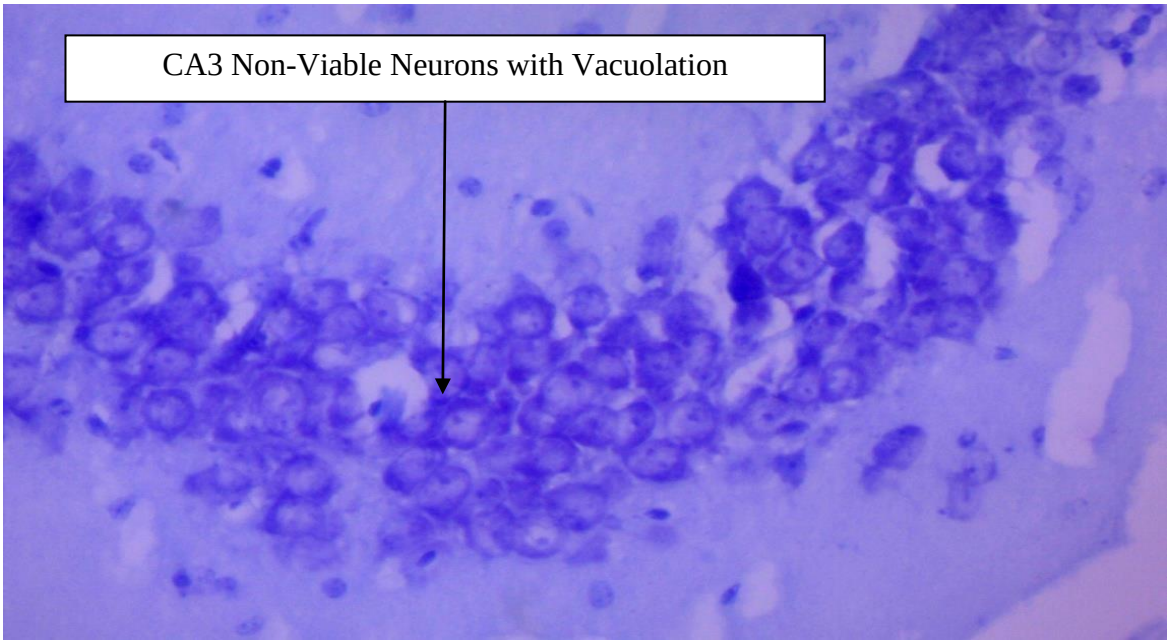


Figure 32: Group- I-B (30 mins exp/day for 3 months)- High power image.

Cresyl Violet stained photographic images of hippocampal pyramidal cells in CA3 region of mice brain from 30 mins exp/day for 3 months group. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

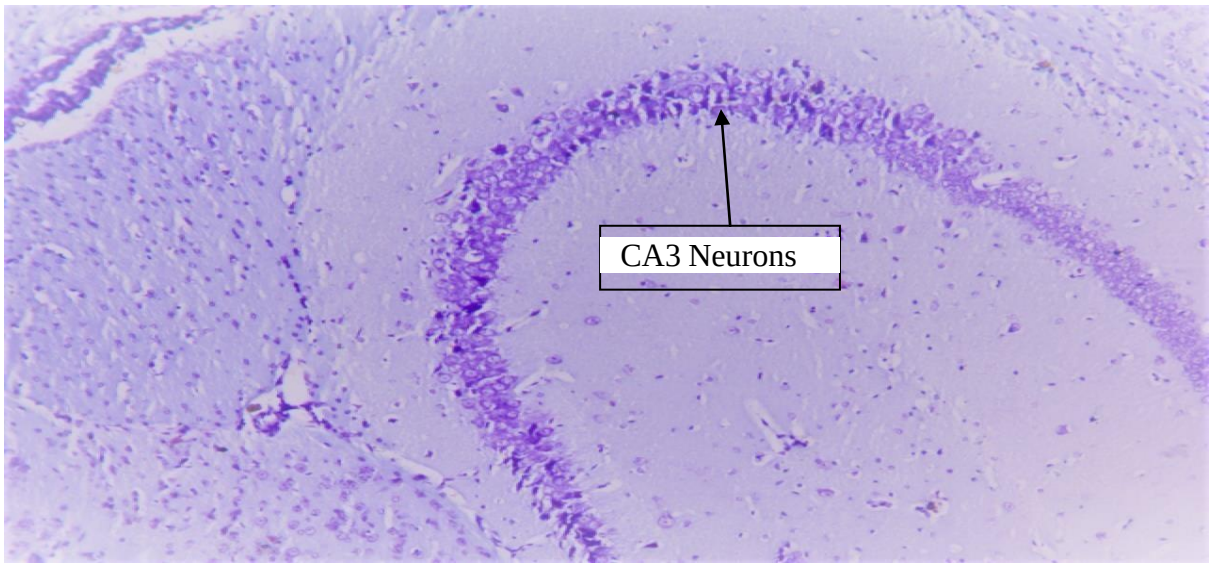


Figure 33: Group- I-C (60 mins exp/day for 3 months)-Low power image.

Cresyl Violet stained photographic images of hippocampal pyramidal cells in CA3 region of mice brain from 60 mins exp/day for 3 months

group. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

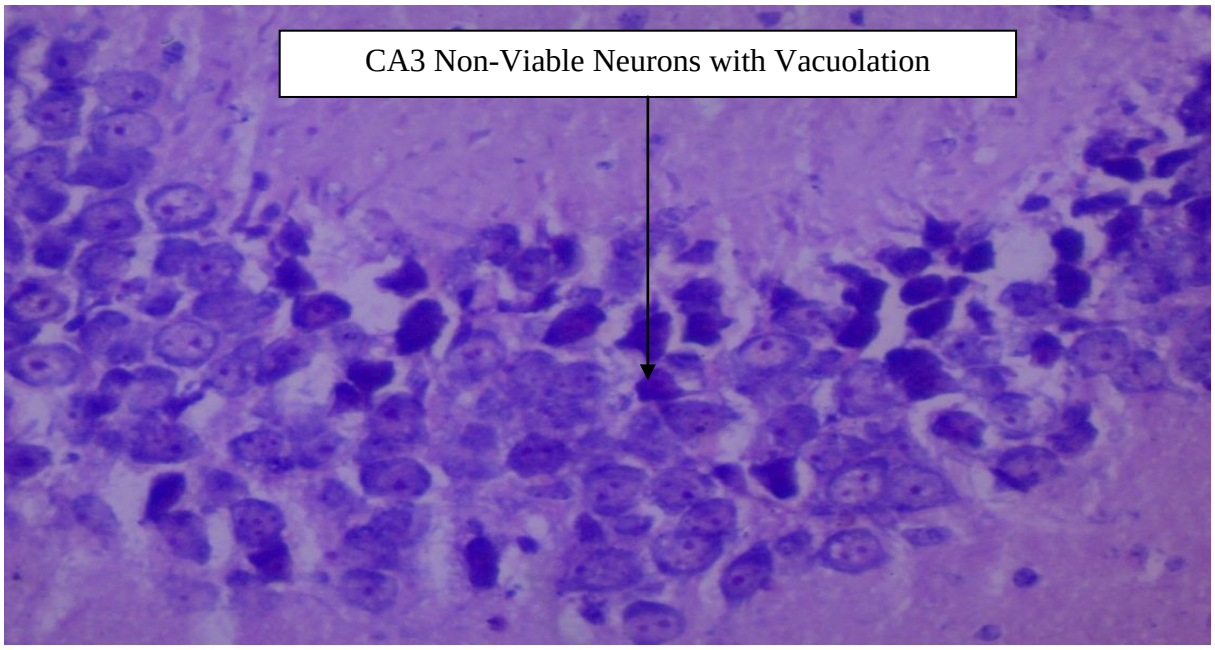


Figure 34: Group- I-C (60 mins exp/day for 3 months)- High power image.

Cresyl Violet stained photographic images of hippocampal pyramidal cells in CA3 region of mice brain from 60 mins exp/day for 3 months group. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

Quantification of viable neurons in hippocampal CA3 region

Total mean number of viable pyramidal CA3 neurons in group-I-A (Control group) 72.5±8.34 vs group-I-B (30 mins exp/day for 3 months) 64.1±8.13 was statistically not significant (P>0.01), whereas group-I-A (Control group) 72.5±8.34 vs group-I-C (60 mins exp/day for 3 months) 58.3±6.81 was statistically significant (P<0.001).

So the results in this study shows that increase in the duration of exposure to mobile phone radiation leads to increased damage of the hippocampal CA3 pyramidal neurons, as illustrated in below (table 3, Figure 35).

Table 3: Total mean number of viable pyramidal CA3 neurons in Group-I-A (control group), Group-I-B (30 mins exp/day for 3 months) and Group-I-C (60 mins exp/day for 3 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA followed by Bonferroni's post-hoc test using SPSS 20).

Groups	Total mean number of viable pyramidal CA3 neurons
Group I-A (non-exposed group).	72.5±8.34
Group I-B (30 min exposed/day for 3 months)	64.1±8.13
Group I-C (60 min exposed/day for 3 months)	58.3±6.81 *

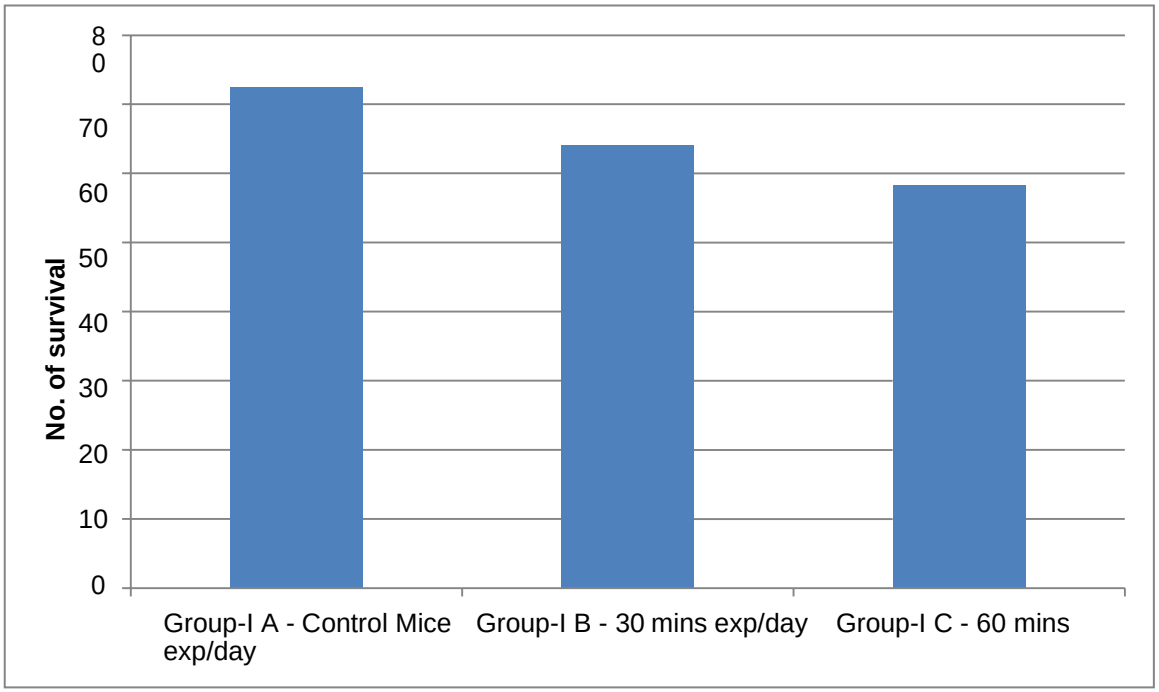


Figure 35: Quantification of hippocampal CA3 survival neurons in control and radiation exposed groups (Group I A, Group I B & Group I C -3 months group).

Dendritic quantification of hippocampal CA3 Pyramidal Neurons-

5.1.5. A. Apical Dendritic Branching points-

Table 4: Apical dendritic branching points of hippocampal CA3 neurons of mice in Group-I-A (control group), Group-I-B (30 mins exp/day for 3 months) and Group-I-C (60 mins exp/day for 3 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA and Bonferroni"s post-hoc test using SPSS 20).

	Distance from Soma (µm)				
Groups	0-20	20-40	40-60	60-80	80-100
Group-I-A (Control)	0.40±0.58	2.00±0.85	1.95±0.82	1.35±0.74	1.15±1.18
Group-I-B (30 mins exp/day for 3 months)	0.30±0.47	1.45±0.88	1.45±0.75	1.15±0.87	0.90±0.64
Group-I-C (60 mins exp/day for 3 months)	0.25±0.44	1.25±0.85*	1.00±0.64*	0.90±0.71	0.65±0.48

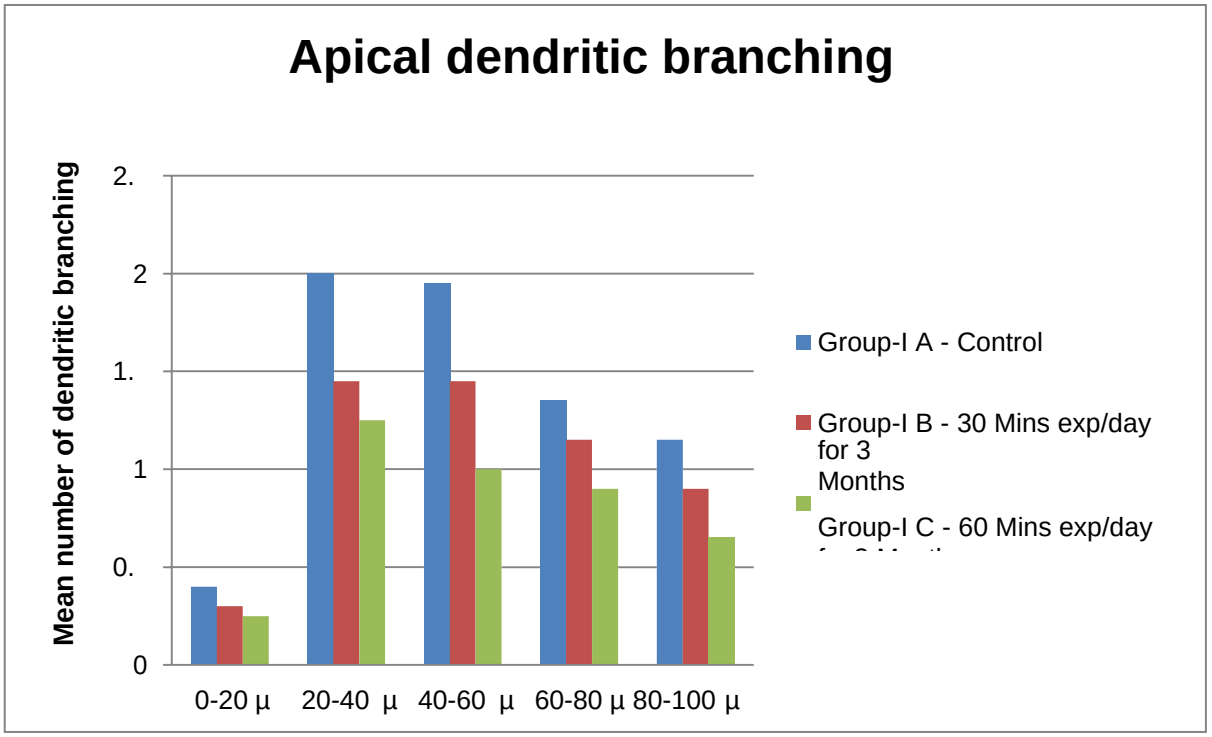


Figure 36: Number of apical dendritic branching points in CA3 neurons of control and exposed groups. (Group I A, Group I B & Group I C -3 month's exposure)

5.1.5. B. Apical Dendritic Intersections-

Table 5: Apical dendritic intersections of hippocampal CA3 neurons of mice in Group-I-A (control group), Group-I-B (30 mins exp/day for 3 months) and Group-I-C (60 mins exp/day for 3 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA and Bonferroni"s post-hoc test using SPSS 20).

	Distance from Soma (µm)				
Groups	20	40	60	80	100
Group-I-A (Control)	1.25±0.85	3.65±1.53	5.45±1.63	5.50±1.31	5.15±1.59
Group-I-B (30 mins exp/day for 3 months)	1.05±0.75	2.80±1.00	3.80±1.05*	4.50±1.53	4.25±1.68
Group-I-C (60 mins exp/day for 3 months)	1.05±0.75	2.45±1.46*	2.90±1.02*	3.45±1.39*	3.45±1.19*

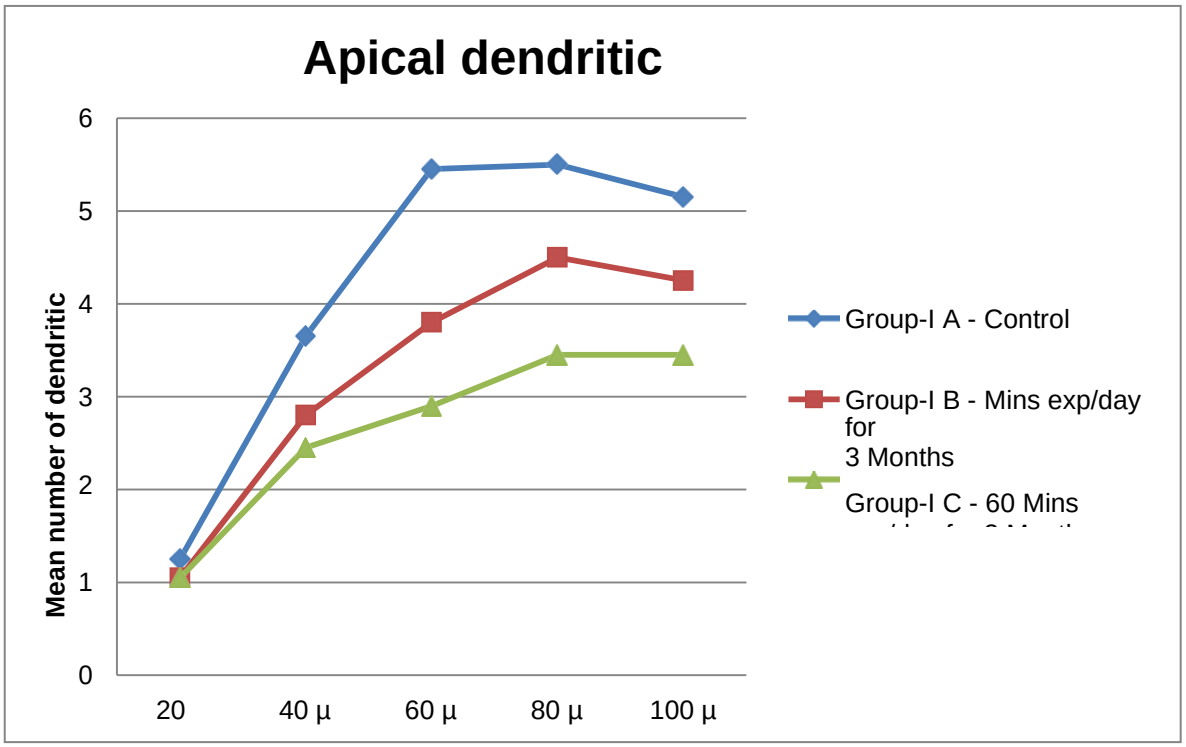


Figure 37: Number of apical dendritic intersections in CA3 neurons of control and exposed groups. (Group I A, Group I B & Group I C -3 month's exposure)

5.1.5. C. Basal Dendritic Branching points-

Table 6: Basal dendritic branching points of hippocampal CA3 neurons of mice in Group-I-A (control group), Group-I-B (30 mins exp/day for 3 months) and Group-I-C (60 mins exp/day for 3 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA and Bonferroni"s post-hoc test using SPSS 20).

	Distance from Soma (µm)				
Groups	0-20	20-40	40-60	60-80	80-100
Group-I-A (Control)	1.50±0.94	2.75±1.99	2.65±1.18	1.55±1.31	0.20±0.52
Group-I-B (30 mins exp/day for 3 months)	1.15±0.74	1.75±1.02	2.05±1.14	1.30±1.30	0.20±0.41
Group-I-C (60 mins exp/day for 3 months)	0.75±0.55*	1.40±0.88*	1.45±0.94*	0.45±0.75*	0.15±0.48

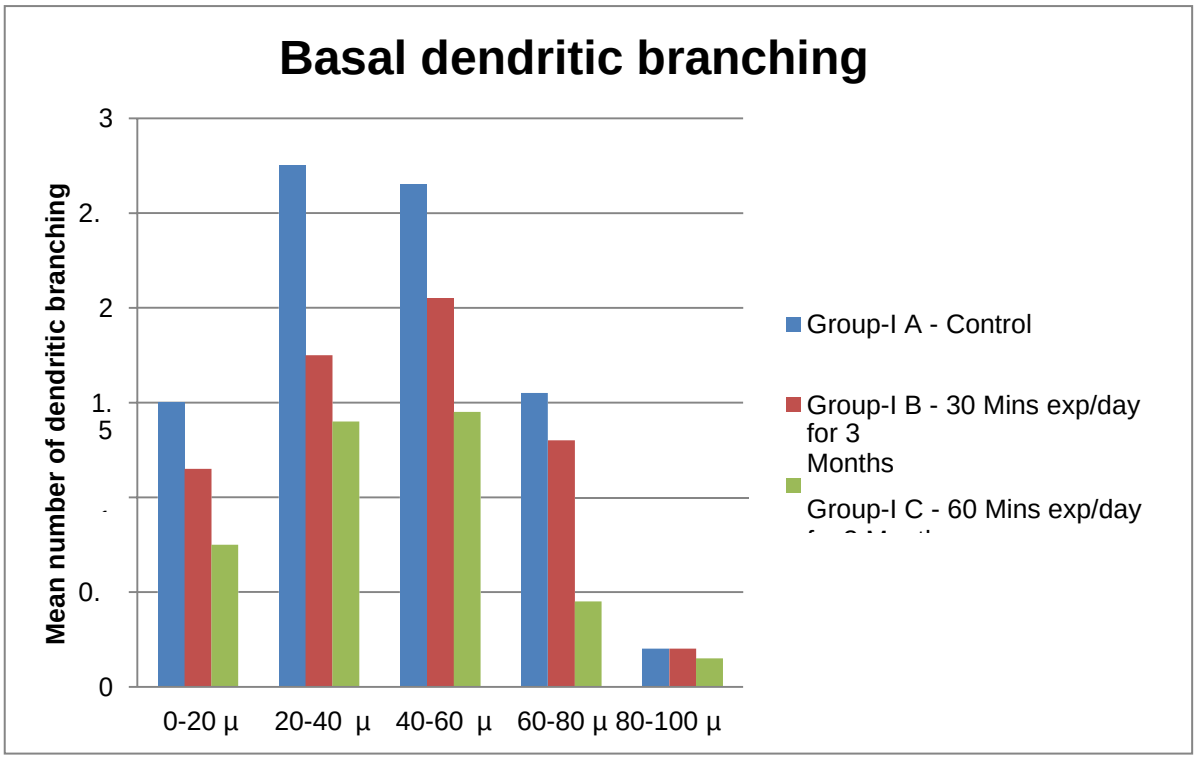


Figure 38: Number of basal dendritic branching points in CA3 neurons of control and exposed groups. (Group I A, Group I B & Group I C -3 month's exposure)

5.1.5. D. Basal Dendritic Intersections-

Table 7: Basal dendritic intersections of hippocampal CA3 neurons of mice in Group-I-A (control group), Group-I-B (30 mins exp/day for 3 months) and Group-I-C (60 mins exp/day for 3 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA and Bonferroni"s post-hoc test using SPSS 20).

	Distance from Soma (µm)				
Groups	20	40	60	80	100
Group-I-A (Control)	3.85±0.74	7.45±1.66	9.15±2.13	8.50±1.98	6.20±1.88
Group-I-B (30 mins exp/day for 3 months)	3.50±0.94	5.85±1.04*	7.20±1.05*	6.95±1.23*	4.00±2.03*
Group-I-C (60 mins exp/day for 3 months)	2.25±0.91*	4.65±1.18*	6.35±1.18*	5.95±1.39*	3.75±1.74*

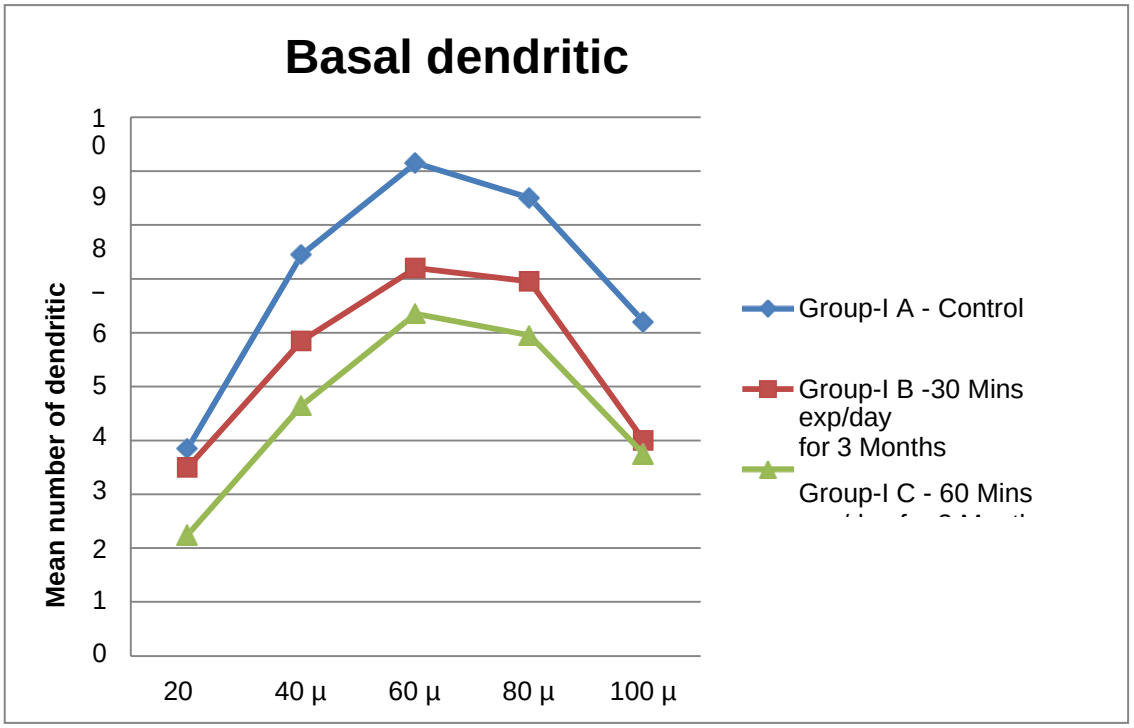


Figure 39: Number of basal dendritic intersections in CA3 neurons of control and exposed groups.(Group I A, Group I B & Group I C -3 month's exposure)

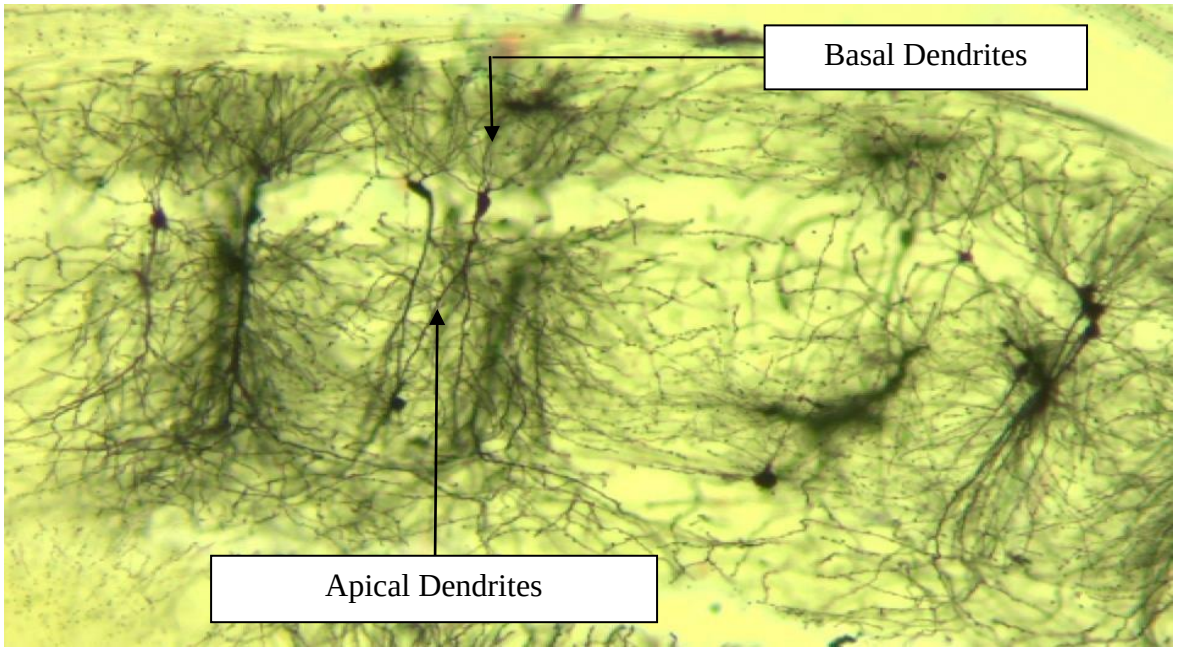


Figure 40: Golgi- Cox Stained hippocampal CA3 Neurons shows Apical and Basal Dendritic branching points and intersections. (Group- 1 A- 3 Months- Control Image)

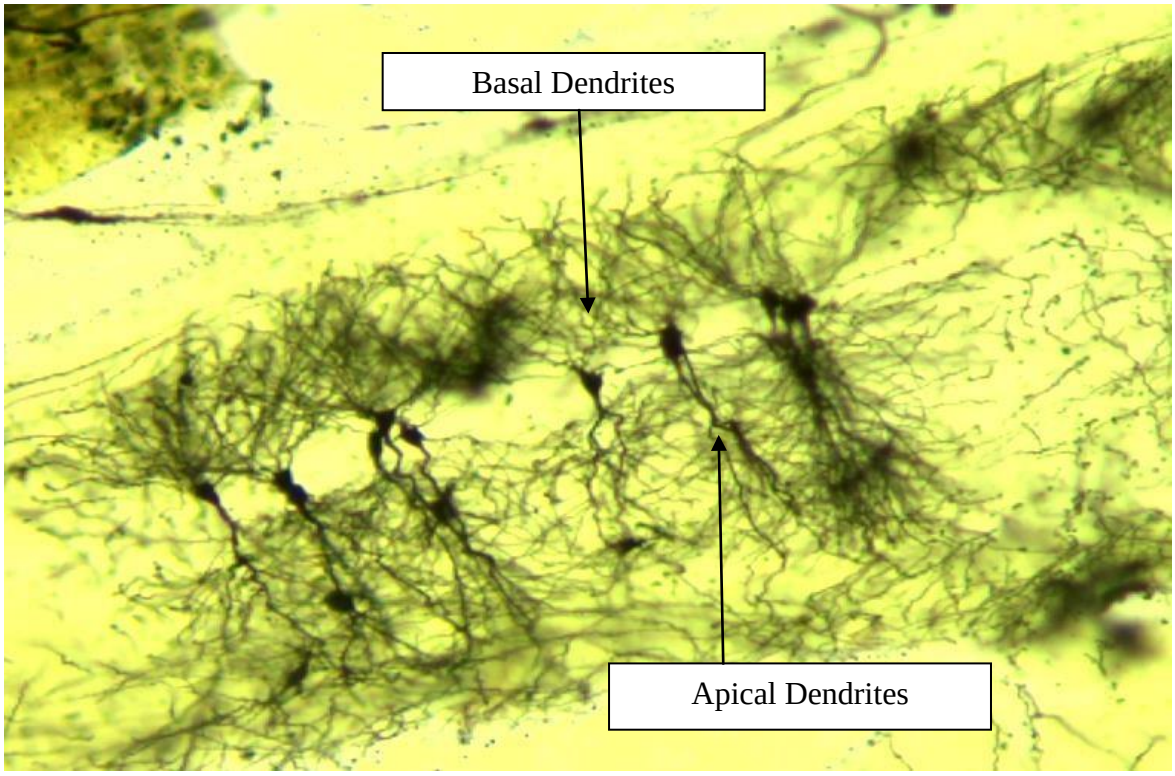


Figure 41: Golgi- Cox Stained hippocampal CA3 Neurons shows Apical and Basal Dendritic branching points and intersections. (Group- 1 B - 3 Months- 30 mins exposure/day Image)

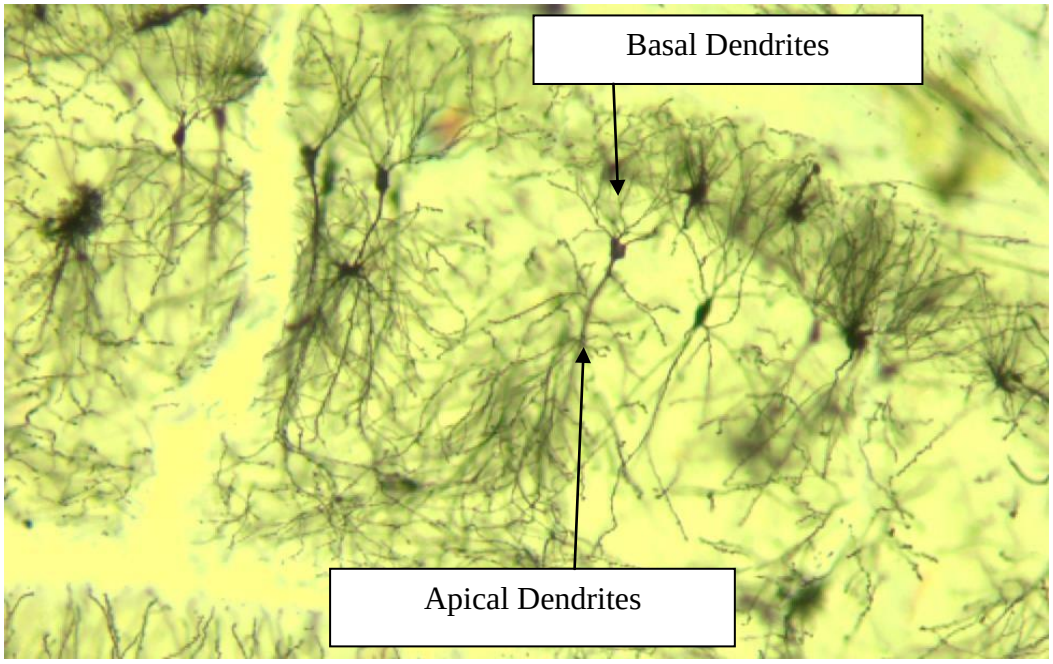


Figure 42: Golgi- Cox Stained hippocampal CA3 Neurons shows Apical and Basal Dendritic branching points and intersections. (Group- 1 C - 3 Months- 60 mins exposure/day Image)

GROUP-II RESULTS (6 MONTHS EXPOSURE GROUP)-

Behavioural Analysis (Spatial Learning and Memory Assessment)-

Hebb-Williams Maze –

Effect of Radiation on Learning Memory in Hebb-Williams Maze-

The time taken by the mice to reach the target chamber from the starting chamber was significantly increased in group II-B (30 min exposed/day) and group II-C (60 min exposed/day) compared to group II-A (non-exposed group). The time taken by the animal to reach The Reward Chamber (TRC) scores in Group II-A vs Group II-B (29.83±11.56 vs 56±9.48seconds), shows significant ($p<0.05$); Group II-A vs Group II-C (29.83±11.56 vs 71.50±7.45 seconds), was statistically significant ($p<0.05$) [Table 8, Figure 43].

Table 8: The time taken by the animal to reach the reward chamber (TRC) scores of mice in Group-II-A (control group), Group-II-B (30 mins exp/day for 6 months) and Group-II-C (60 mins exp/day for 6 months). Values were expressed in Mean ± SD, * Represents $P<0.05$ (Analysed by One-way ANOVA followed by Least square difference (LSD) test using SPSS 20).

Groups	TRC Scores (The time taken by the animal to reach the Reward Chamber)
Group II-A (non-exposed group).	29.83±11.56 Seconds
Group II-B (30 min exposed/day for 6 months)	56±9.48 * Seconds
Group II-C (60 min exposed/day for 6 months)	71.50±7.45 * Seconds

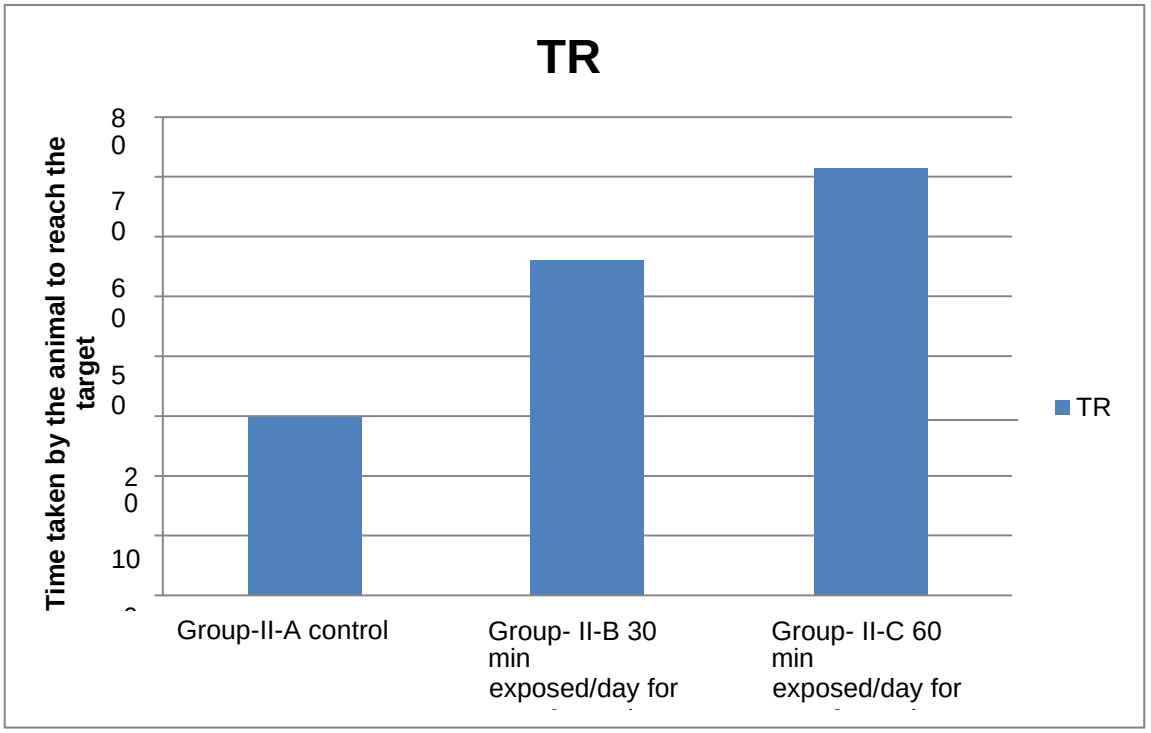


Figure 43: Effect of Mobile phone radiofrequency-electromagnetic radiation (MP RF-EMR) on learning and memory by using Hebb-Williams maze- group II-A, II-B and II-C.

T-Maze -

Effect of Radiation on Learning Memory in T-Maze-

Reward Alternation Test-

During the reward alternation test, the mice exposed to Mobile Phone Radio Frequency-Electro Magnetic Radiation (MP RF-EMR) for 30 minutes exposure/day for 3 months (Group II-B) and 60 minutes exposure/day for 3 months (Group II-C) shows a significant difference in the percentage of correct responses when compare to control group mice (Group II-A).

Percentage of correct responses of Group II-A vs Group II-B shows 69.66±3.04 vs 56.70±5.03 was statistically significant ($p<0.05$) and Group

II-A vs Group II-C shows 69.66±3.04 vs 49.38±1.96 also shows statistically significant ($p<0.05$), shown below (Table 9, Figure 44).

Table 9: Percentage of correct responses of Group-II-A (control group), Group-II-B (30 mins exp/day for 6 months) and Group-II-C (60 mins exp/day for 6 months). Values were expressed in Mean ± SD, * Represents $P<0.05$ (Analysed by One-way ANOVA followed by Least square difference (LSD) test using SPSS 20).

Groups	Percentage of correct responses
Group II-A (non-exposed group).	69.66±3.04
Group II-B (30 min exposed/day for 6 months)	56.70±5.03*
Group II-C (60 min exposed/day for 6 months)	49.38±1.96 *

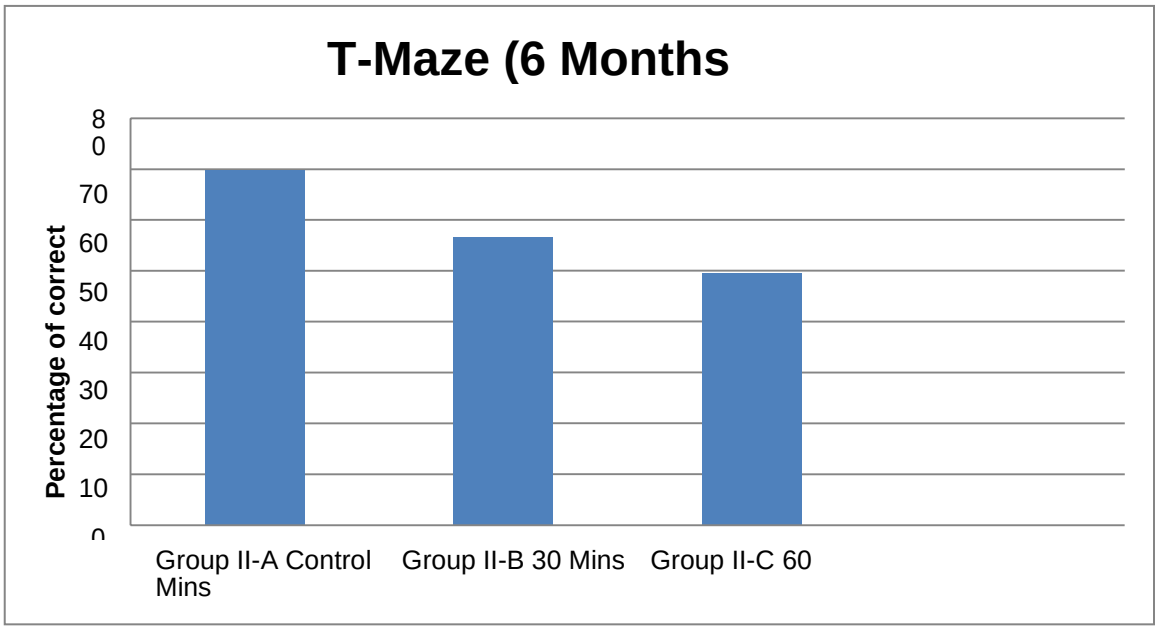


Figure 44: Percentage of correct response in rewarded alternation task performance; Mean ± SD is shown, n =6 rats in each group

Neuronal damage assessment in CA3 region of hippocampus:

The pyramidal neurons of hippocampal CA3 region in control group shows healthy neurons which is compactly arranged with clear nucleus, whereas mobile phone radiation exposed mice hippocampal CA3 shows neurons which are darkly stained, unhealthy, scattered and irregular, as illustrated in the figures below.

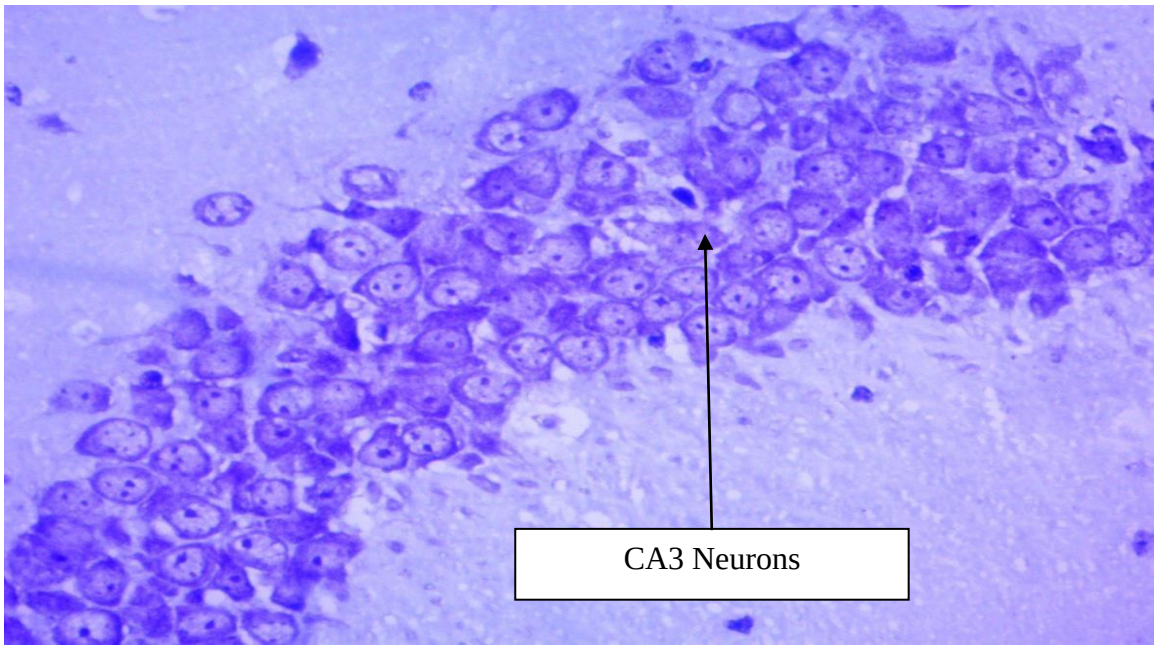


Figure 45: Group- II-A (6 Months Control group)- High power image. Cresyl Violet stained photographic images of hippocampal pyramidal cells in CA3 region of mice brain from control group. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

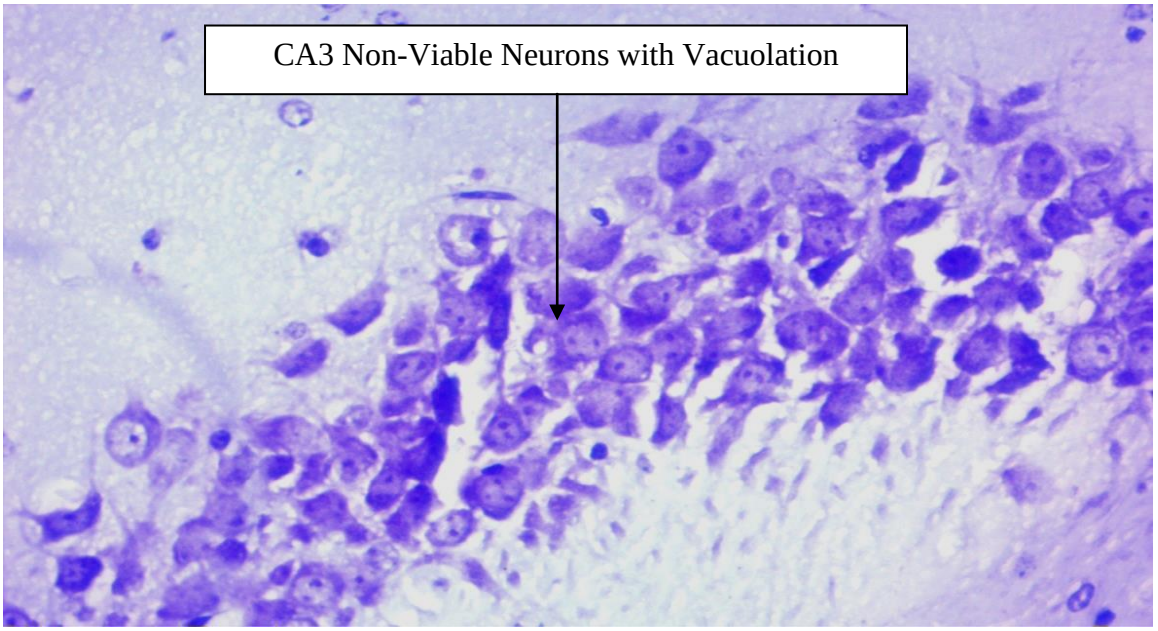


Figure 46: Group- II-B (30 mins exp/day for 6 months)- High power image.
Cresyl Violet stained photographic images of hippocampal pyramidal cells in CA3 region of mice brain from 30 mins exp/day for 6 months group. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

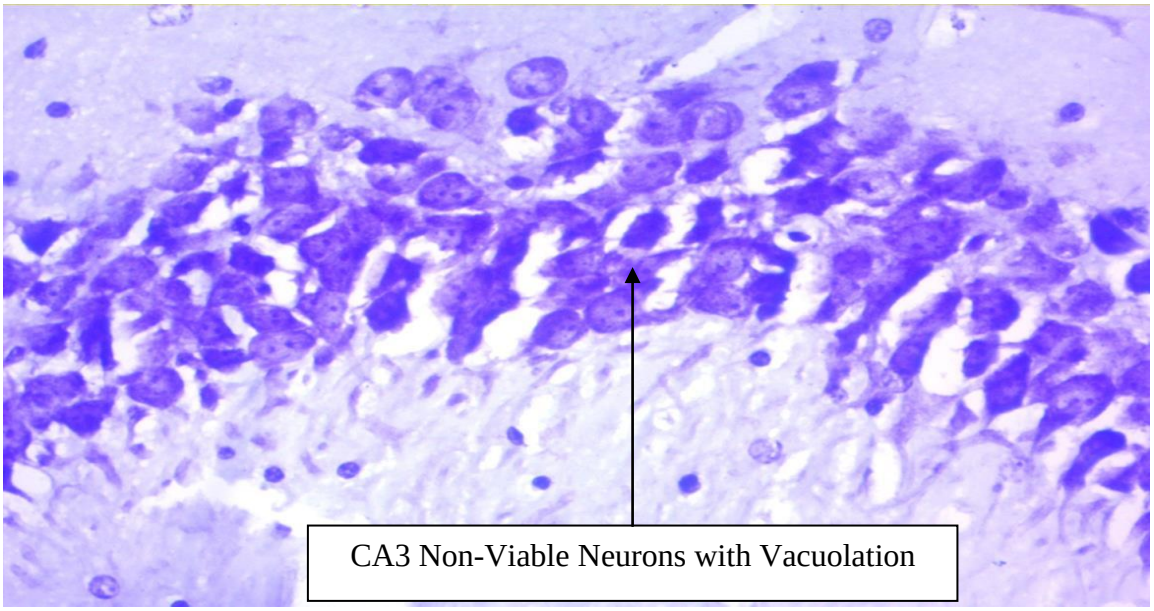


Figure 47: Group- II-C (60 mins exp/day for 6 months)- High power image.
Cresyl Violet stained photographic images of hippocampal pyramidal cells in CA3 region of mice brain from 60 mins exp/day for 6 months group. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

Quantification of viable neurons in hippocampal CA3 region

Total mean number of viable pyramidal CA3 neurons in group-II-A (Control group) 71.0±4.76 vs group-II-B (30 mins exp/day for 6 months) 56.80±3.22 was statistically significant (P<0.01), whereas group-II-A (Control group) 71.0±4.76 vs group-II-C (60 mins exp/day for 6 months) 48.20±4.10 shows statistically significant (P<0.001).

So the results in this study shows that increase in the duration of exposure to mobile phone radiation leads to increased damage of the hippocampal CA3 pyramidal neurons, as illustrated below (Table 10, Figure 48).

Table 10: Total mean number of viable pyramidal CA3 neurons in Group-II-A (control group), Group-II-B (30 mins exp/day for 6 months) and Group-II-C (60 mins exp/day for 6 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA followed by Bonforroni"s post-hoc test using SPSS 20).

Groups	Total mean number of viable pyramidal CA3 neurons
Group II-A (non-exposed group).	71.0±4.76
Group II-B (30 min exposed/day for 6 months)	56.80±3.22*
Group II-C (60 min exposed/day for 6 months)	48.20±4.10 *

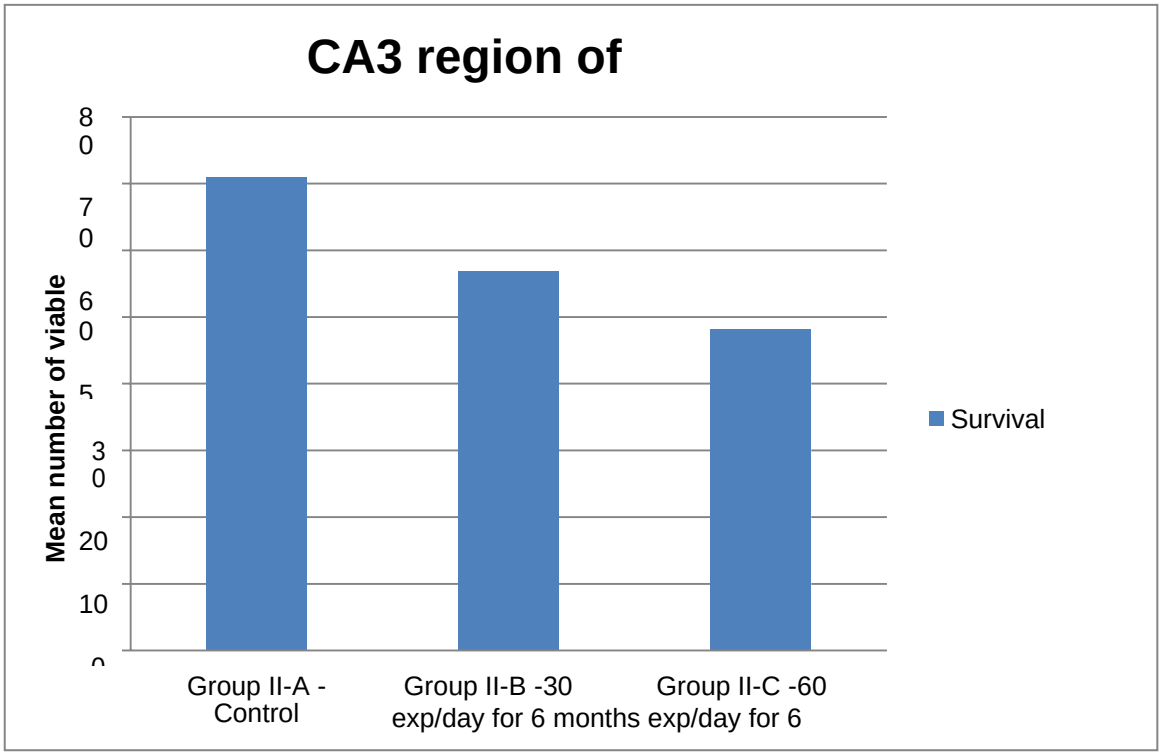


Figure 48: Quantification of hippocampal CA3 survival neurons in control and radiation exposed groups.(Group II – 6 Months group)

Dendritic quantification of hippocampal CA3 Pyramidal Neurons-

Apical Dendritic Branching points-

Table 11: Apical dendritic branching points of hippocampal CA3 neurons of mice in Group-II-A (control group), Group-II-B (30 mins exp/day for 6 months) and Group-II-C (60 mins exp/day for 6 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA and Bonferroni"s post-hoc test using SPSS 20).

	Distance from Soma (µm)				
Groups	0-20	20-40	40-60	60-80	80-100
Group-II-A (Control)	0.40±0.59	1.45±1.05	1.85±1.08	1.85±1.22	1.30±0.80
Group-II-B (30 mins exp/day for 6 months)	0.10±0.30	0.90±0.96	1.30±0.80	1.15±0.87	0.60±0.59*
Group-II-C (60 mins exp/day for 6 months)	0.00±0.0*	0.70±0.73*	0.75±0.63*	0.85±0.87*	0.50±0.76*

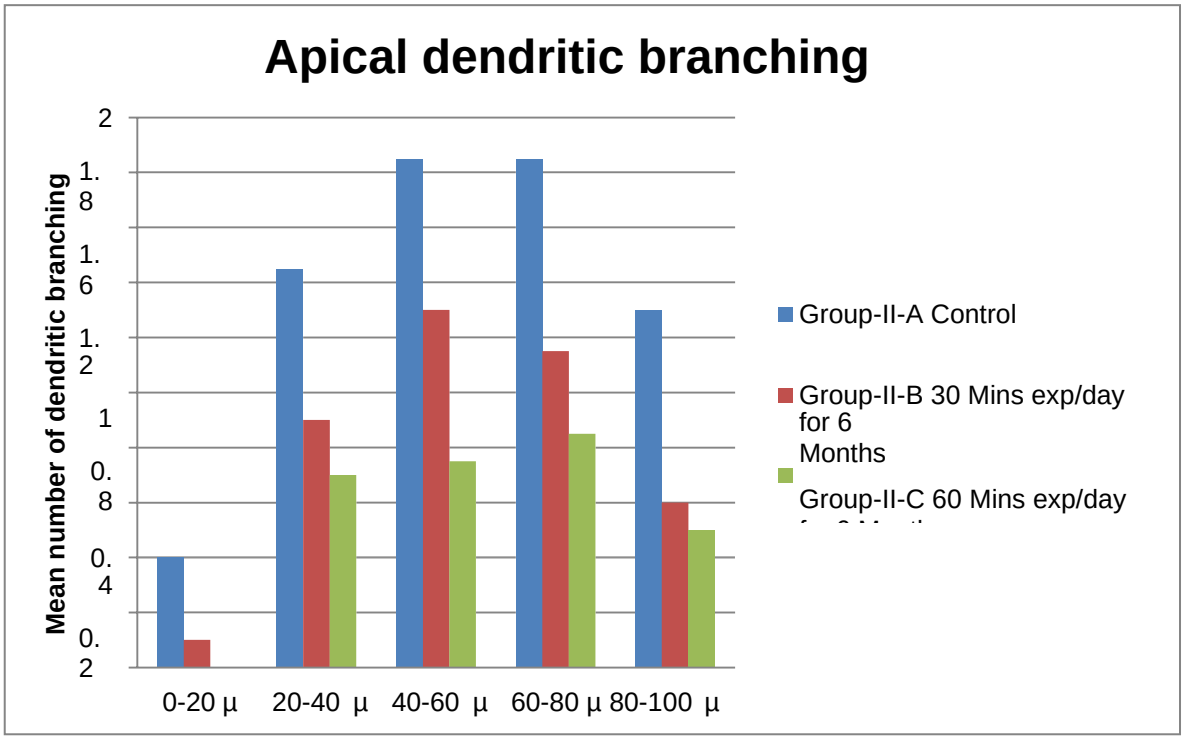


Figure 49: Number of apical dendritic branching points in CA3 neurons of control and exposed groups. (Group II A, Group II B, Group II C- 6 month's exposure)

Apical Dendritic Intersections-

Table 12: Apical dendritic intersections of hippocampal CA3 neurons of mice in Group-II-A (control group), Group-II-B (30 mins exp/day for 6 months) and Group-II-C (60 mins exp/day for 6 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA and Bonferroni"s post-hoc test using SPSS 20).

	Distance from Soma (µm)				
Groups	20	40	60	80	100
Group-II-A (Control)	0.90±1.11	2.90±1.58	4.75±1.51	4.75±1.65	5.20±1.47
Group-II-B (30 mins exp/day for 6 months)	0.20±0.61*	1.60±1.46*	3.15±1.46*	4.75±1.33	3.40±1.42*
Group-II-C (60 mins exp/day for 6 months)	0.00±0.00*	1.30±1.26*	2.25±1.29*	3.20±1.15*	3.45±1.35*

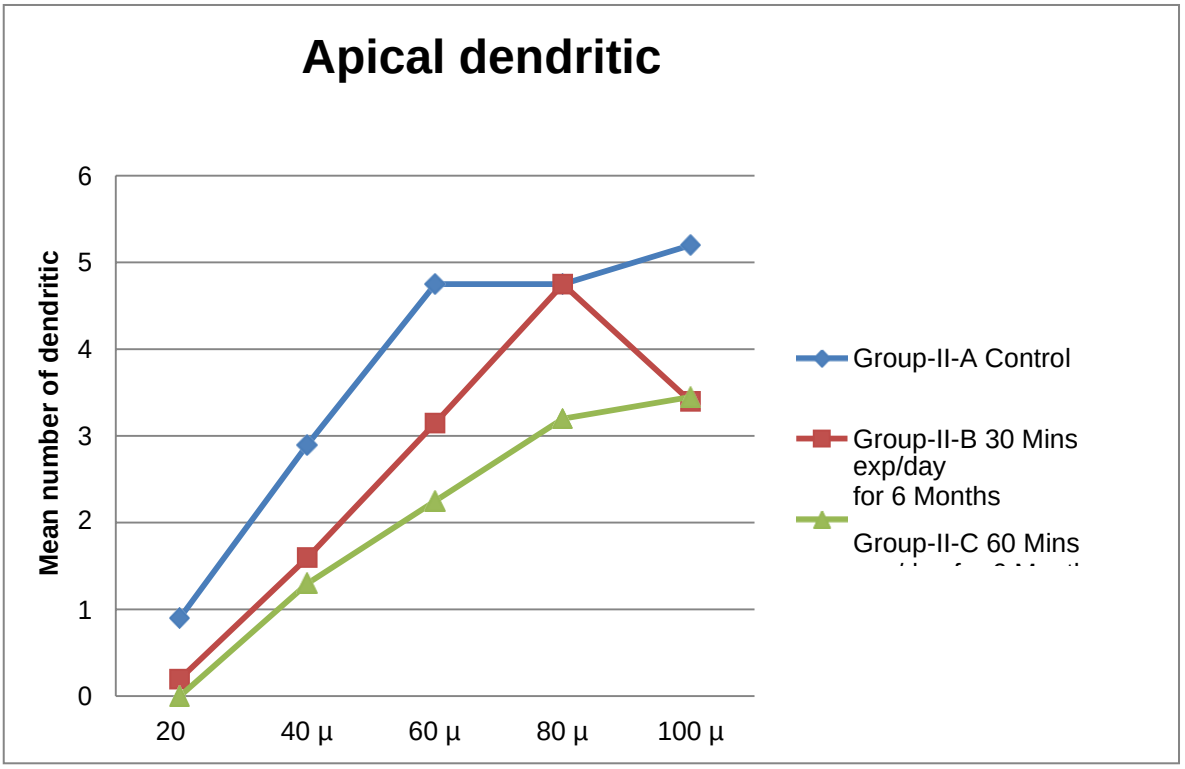


Figure 50: Number of apical dendritic intersections in CA3 neurons of control and exposed groups. (Group II A, Group II B, Group II C - 6 months exposure)

Basal Dendritic Branching points-

Table 13: Basal dendritic branching points of hippocampal CA3 neurons of mice in Group-II-A (control group), Group-II-B (30 mins exp/day for 6 months) and Group-II-C (60 mins exp/day for 6 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA and Bonferroni"s post-hoc test using SPSS 20).

	Distance from Soma (µm)				
Groups	0-20	20-40	40-60	60-80	80-100
Group-II-A (Control)	1.10±0.71	2.90±1.25	2.35±1.18	1.15±0.93	0.20±0.52
Group-II-B (30 mins exp/day for 6 months)	0.95±1.09	2.30±1.12	1.70±1.08	0.55±0.68*	0.10±0.30
Group-II-C (60 mins exp/day for 6 months)	0.95±0.82	1.85±1.08*	0.85±0.93*	0.30±0.47*	0.00±0.00

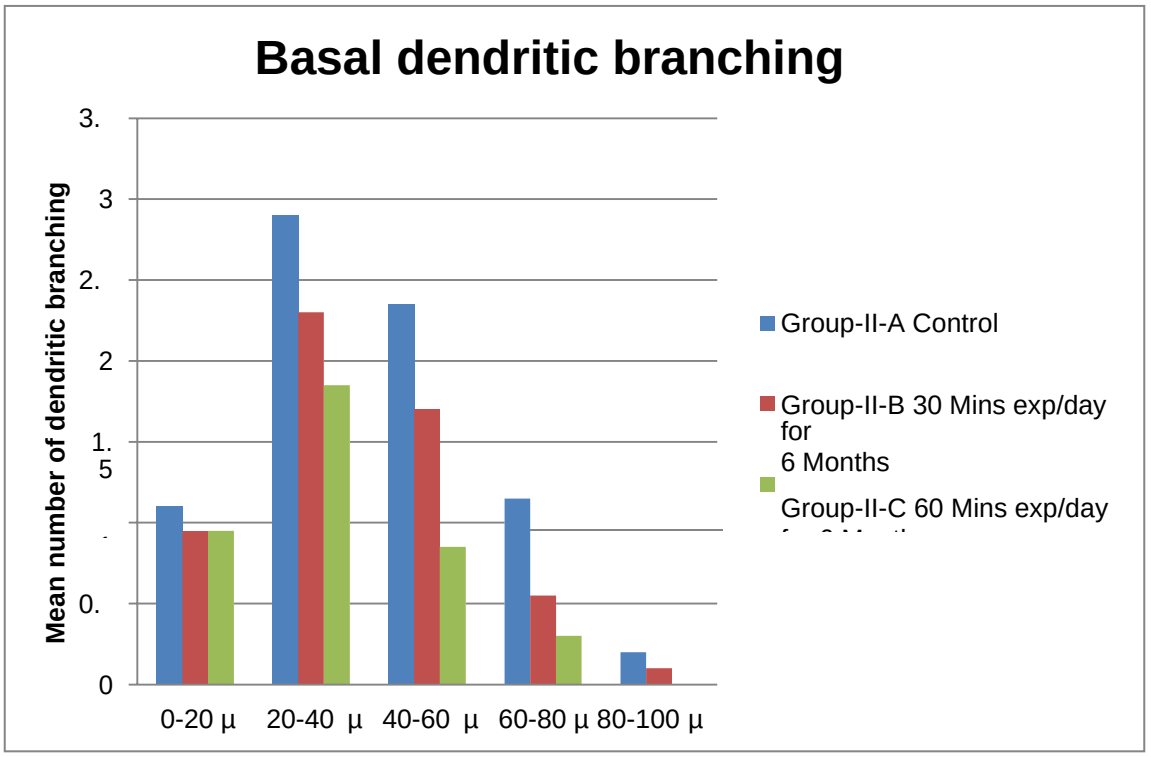


Figure 51: Number of basal dendritic branching points in CA3 neurons of control and exposed groups. (Group II A, Group II B, Group II C - 6 months exposure)

Basal Dendritic Intersections-

Table 14: Basal dendritic intersections of hippocampal CA3 neurons of mice in Group-II-A (control group), Group-II-B (30 mins exp/day for 6 months) and Group-II-C (60 mins exp/day for 6 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA and Bonferroni"s post-hoc test using SPSS 20).

	Distance from Soma (µm)				
Groups	20	40	60	80	100
Group-II-A (Control)	2.95±0.88	7.00±1.37	8.45±1.46	7.75±2.33	4.85±2.05
Group-II-B (30 mins exp/day for 6 months)	2.90±1.77	5.80±1.10*	6.50±1.43*	6.00±1.65*	2.95±1.79*
Group-II-C (60 mins exp/day for 6 months)	2.65±1.53	5.55±1.82*	6.10±1.02*	4.30±1.12*	1.30±1.26*

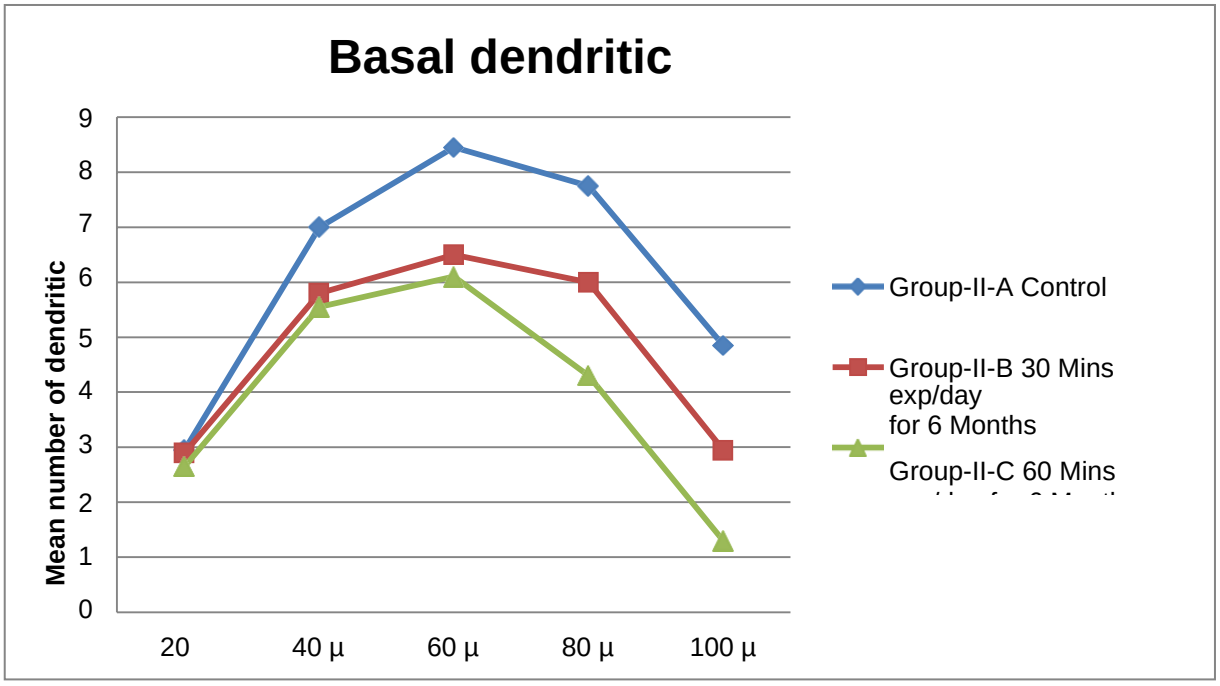


Figure 52: Number of basal dendritic intersections in CA3 neurons of control and exposed groups. (Group II A, Group II B, Group II C - 6 months exposure)

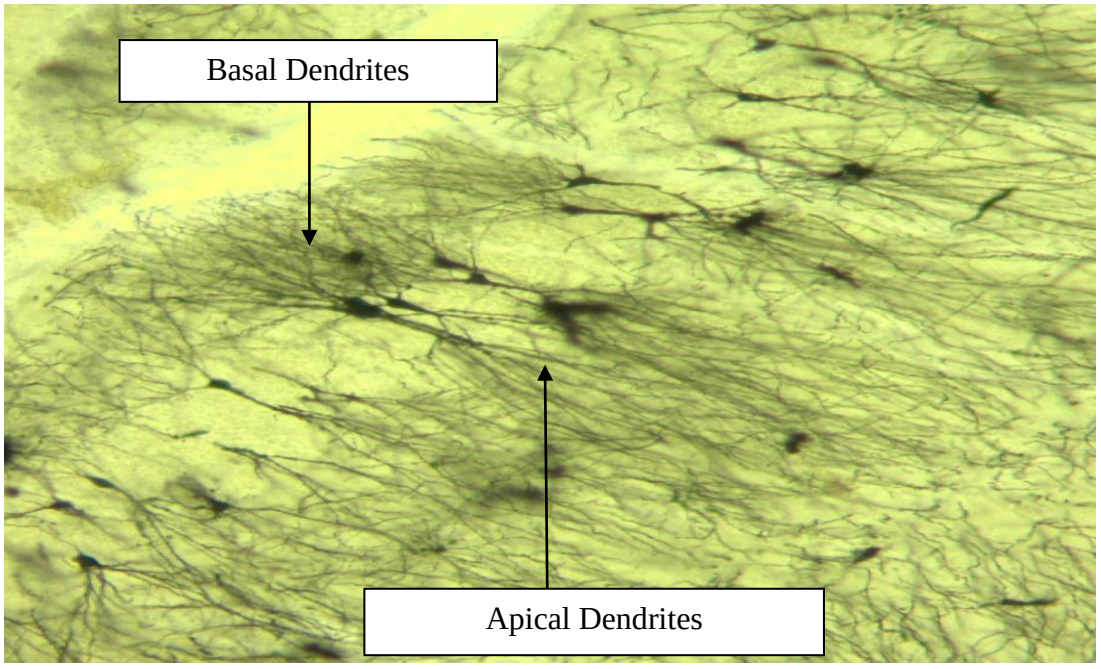


Figure 53: Golgi- Cox Stained hippocampal CA3 Neurons shows Apical and Basal Dendritic branching points and intersections. (Group- II A- 6 Months-Control Image)

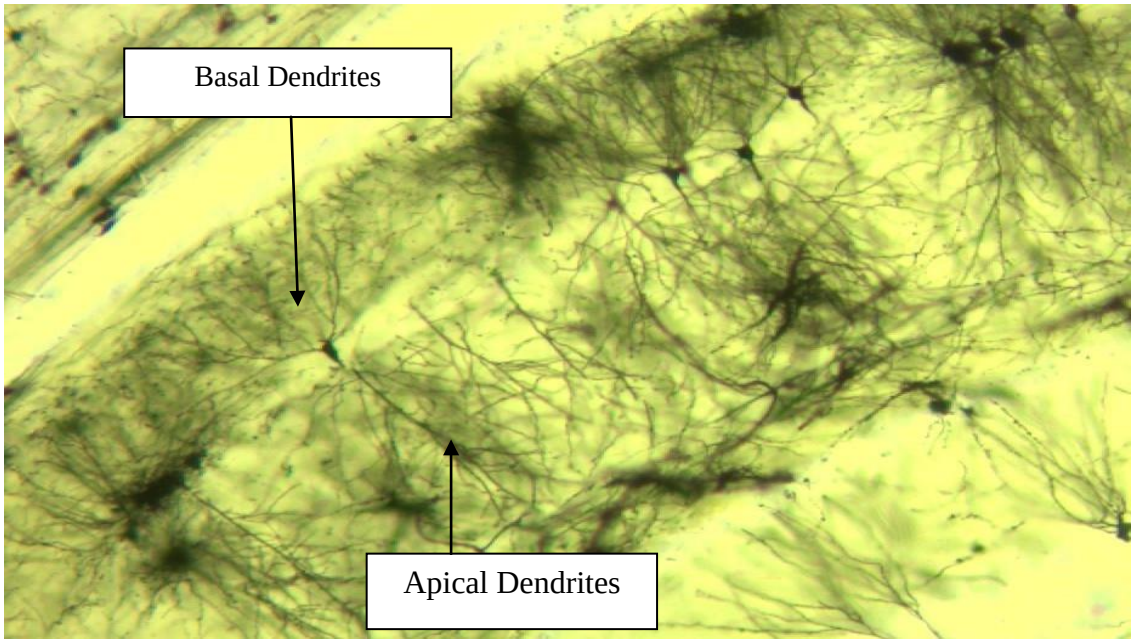


Figure 54: Golgi- Cox Stained hippocampal CA3 Neurons shows Apical and Basal Dendritic branching points and intersections. (Group- II B- 6 Months-30 mins exp/day)

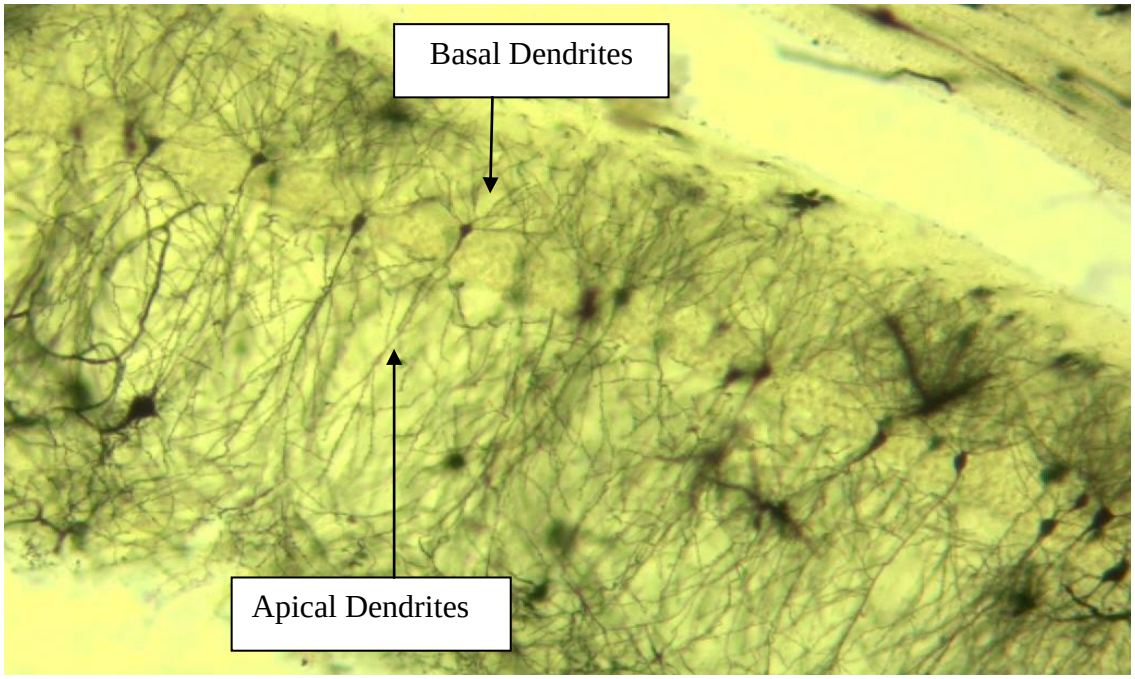


Figure 55: Golgi- Cox Stained hippocampal CA3 Neurons shows Apical and Basal Dendritic branching points and intersections. (Group- II C- 6 Months-60 mins exp/day)

GROUP-III RESULTS (9 MONTHS EXPOSURE GROUP)-

Behavioural Analysis (Spatial Learning and Memory Assessment)-

Hebb-Williams Maze –

Effect of Radiation on Learning Memory in Hebb-Williams Maze-

The time taken by the mice to reach the target chamber from the starting chamber was significantly increased in group III-B (30 min exposed/day) and group III-C (60 min exposed/day) compared to group III-A (non-exposed group). The time taken by the animal to reach The Reward Chamber (TRC) scores in Group III-A vs Group III-B (34.33±8.43 vs 59.50±12.40seconds), shows significant ($p<0.05$); Group III-A vs Group III-C (34.33±8.43 vs 73.67±6.94 seconds), was statistically significant ($p<0.05$) [Table 15, Figure 56].

Table 15: The time taken by the animal to reach the reward chamber (TRC) scores of mice in Group-III-A (control group), Group-III-B (30 mins exp/day for 9 months) and Group-III-C (60 mins exp/day for 9 months). Values were expressed in Mean ± SD, * Represents $P<0.05$ (Analysed by One-way ANOVA followed by Least square difference (LSD) test using SPSS 20).

Groups	TRC Scores (The time taken by the animal to reach the Reward Chamber)
Group III-A (non-exposed group).	34.33±8.43 Seconds
Group III-B (30 min exposed/day for 9 months)	59.50±12.40 * Seconds
Group III-C (60 min exposed/day for 9 months)	73.67±6.94 * Seconds

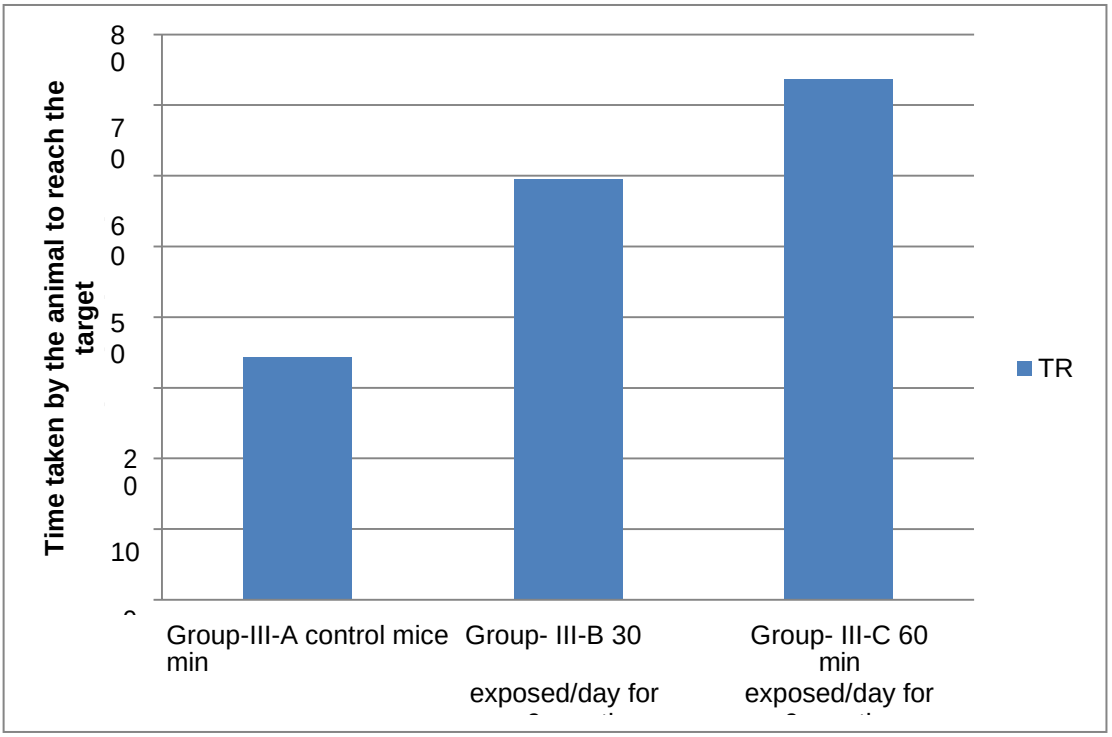


Figure 56: Effect of Mobile phone radiofrequency-electromagnetic radiation (MP RF-EMR) on learning and memory by using Hebb-Williams maze- group III-A, III-B and III-C.

T-Maze -

Effect of Radiation on Learning Memory in T-Maze-

Reward Alternation Test-

During the reward alternation test, the mice exposed to Mobile Phone Radio Frequency-Electro Magnetic Radiation (MP RF-EMR) for 30 minutes exposure/day for 3 months (Group III-B) and 60 minutes exposure/day for 3 months (Group III-C) shows a significant difference in the percentage of correct responses when compared to control group mice (Group III-A).

Percentage of correct responses of Group III-A vs Group III-B shows 70.48±2.82 vs 52.60±4.79 was statistically significant ($p<0.05$) and Group III-A vs Group III-C shows 70.48±2.82 vs 45.88±4.58 also shows statistically significant ($p<0.05$) [Table 16, Figure 57].

Table 16: Percentage of correct responses of Group-III-A (control group), Group-III-B (30 mins exp/day for 9 months) and Group-III-C (60 mins exp/day for 9 months). Values were expressed in Mean ± SD, * Represents $P<0.05$ (Analysed by One-way ANOVA followed by Least square difference (LSD) test using SPSS 20).

Groups	Percentage of correct responses
Group III-A (non-exposed group).	70.48±2.82
Group III-B (30 min exposed/day for 9 months)	52.60±4.79 *
Group III-C (60 min exposed/day for 9 months)	45.88±4.58 *

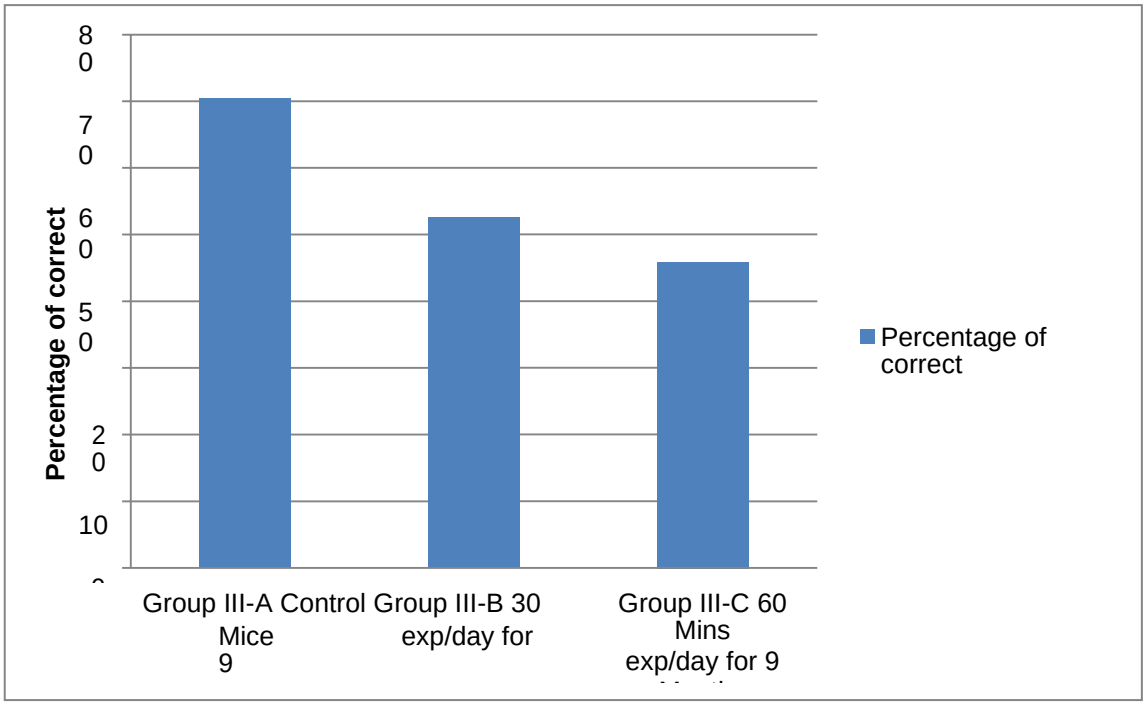


Figure 57: Percentage of correct response in rewarded alternation task performance; Mean ± SD is shown, n =6 rats in each group.

Neuronal damage assessment in CA3 region of hippocampus:

The pyramidal neurons of hippocampal CA3 region in control group shows healthy neurons which are compactly arranged with clear nucleus, whereas mobile phone radiation exposed mice hippocampal CA3 showsneurons which are darkly stained, unhealthy, scattered and irregular, as illustrated in the figures below.

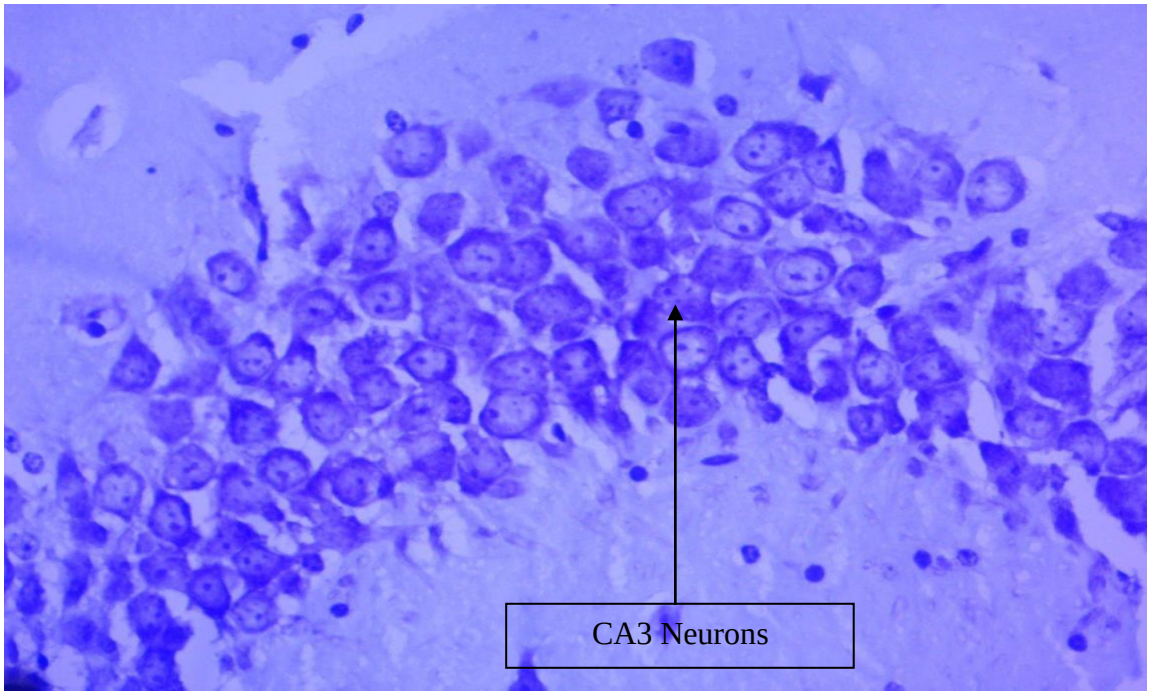


Figure 58: Group- III-A (9 Months Control group)- High power image. Cresyl Violet stained photographic images of hippocampal pyramidal cells in CA3 region of mice brain from control group. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

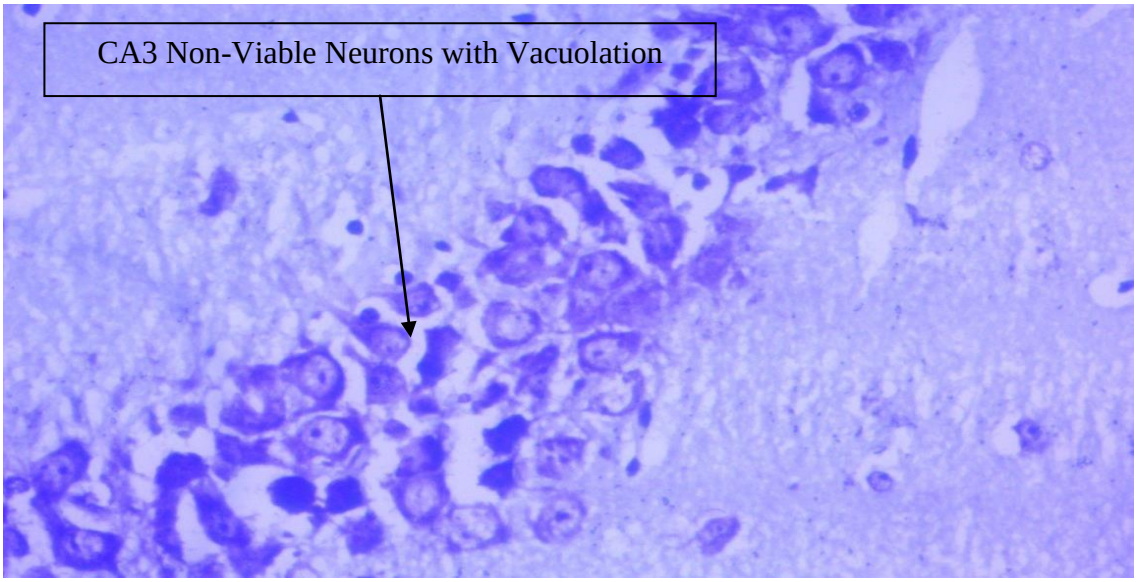


Figure 59: Group- III-B (30 mins exp/day for 9 months)- High power image. Cresyl Violet stained photographic images of hippocampal pyramidal cells in CA3 region of mice brain from control group. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

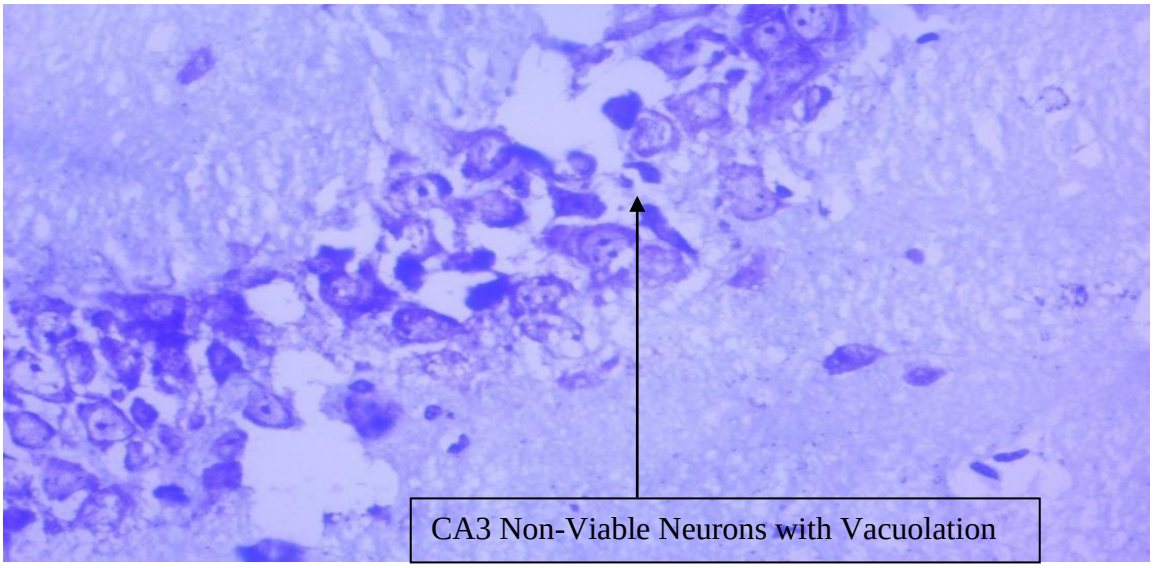


Figure 60: Group- III-C (60 mins exp/day for 9 months)- High power image. Cresyl Violet stained photographic images of hippocampal pyramidal cells in CA3 region of mice brain from control group. (Image captured in Central laboratory, Sri Devaraj Urs Medical College, Kolar, Karnataka).

Quantification of viable neurons in hippocampal CA3 region

Total mean number of viable pyramidal CA3 neurons in group-III-A (Control group) 73.80±5.26 vs group-III-B (30 mins exp/day for 9 months) 52.30±4.64 was statistically significant (P<0.01), whereas group-III-A (Control group) 73.80±5.26 vs group-III-C (60 mins exp/day for 9 months) 44.90±5.28 shows statistically significant (P<0.001).

So the results in this study shows that increase in the duration of exposure to mobile phone radiation leads to increased damage of the hippocampal CA3 pyramidal neurons, as illustrated in Table 17, Figure 61.

Table 17: Total mean number of viable pyramidal CA3 neurons in Group-III-A (control group), Group-III-B (30 mins exp/day for 9 months) and Group-III-C (60 mins exp/day for 9 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA followed by Bonforroni"s post-hoc test using SPSS 20).

Groups	Total mean number of viable pyramidal CA3 neurons
Group III-A (non-exposed group).	73.80±5.26
Group III-B (30 min exposed/day for 9 months)	52.30±4.64 *
Group III-C (60 min exposed/day for 9 months)	44.90±5.28 *

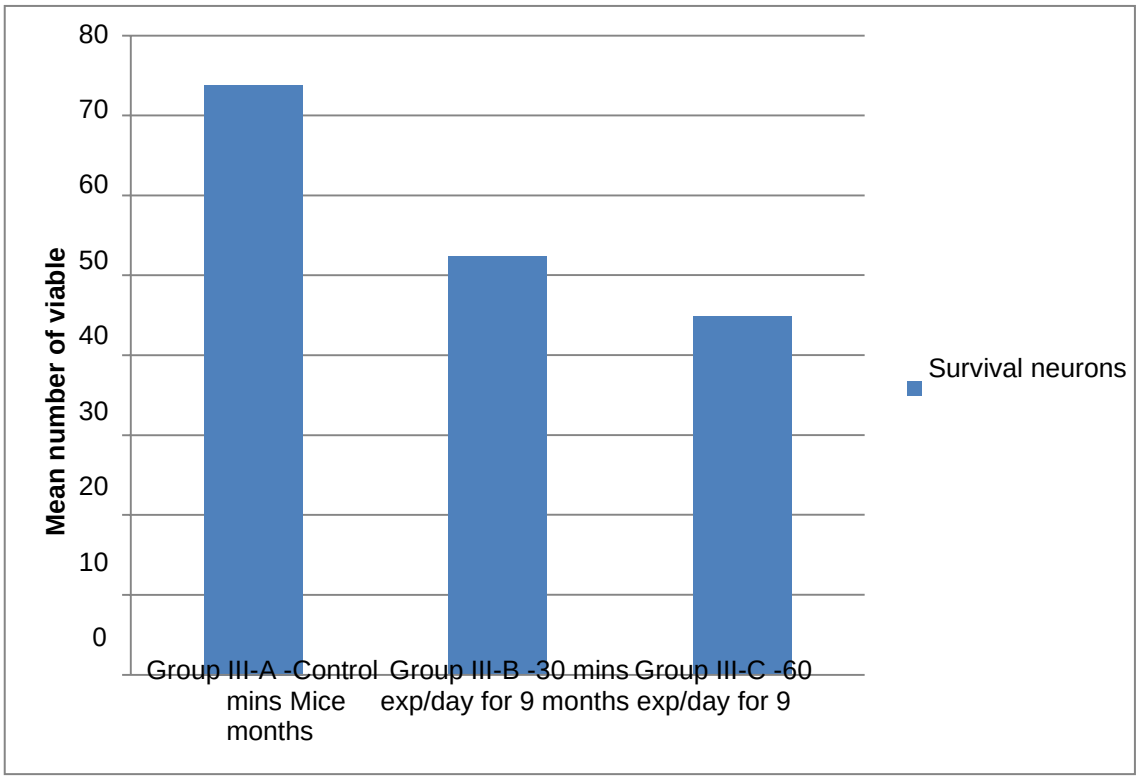


Figure 61: Quantification of hippocampal CA3 survival neurons in control and radiation exposed groups. (Group III A, Group III B & Group III C- 9 month's group)

Dendritic quantification of hippocampal CA3 Pyramidal Neurons-

Apical Dendritic Branching points-

Table 18: Apical dendritic branching points of hippocampal CA3 neurons of mice in Group-III-A (control group), Group-III-B (30 mins exp/day for 9 months) and Group-III-C (60 mins exp/day for 9 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA and Bonferroni"s post-hoc test using SPSS 20).

	Distance from Soma (µm)				
Groups	0-20	20-40	40-60	60-80	80-100
Group-III-A (Control)	0.10±0.30	1.55±0.75	1.70±1.03	1.55±0.82	1.45±0.94
Group-III-B (30 mins exp/day for 9 months)	0.00±0.00	0.55±0.68*	1.00±0.72	1.05±0.75	1.05±0.68
Group-III-C (60 mins exp/day for 9 months)	0.00±0.00	0.50±0.68*	0.90±0.91*	0.90±0.71*	0.85±0.67

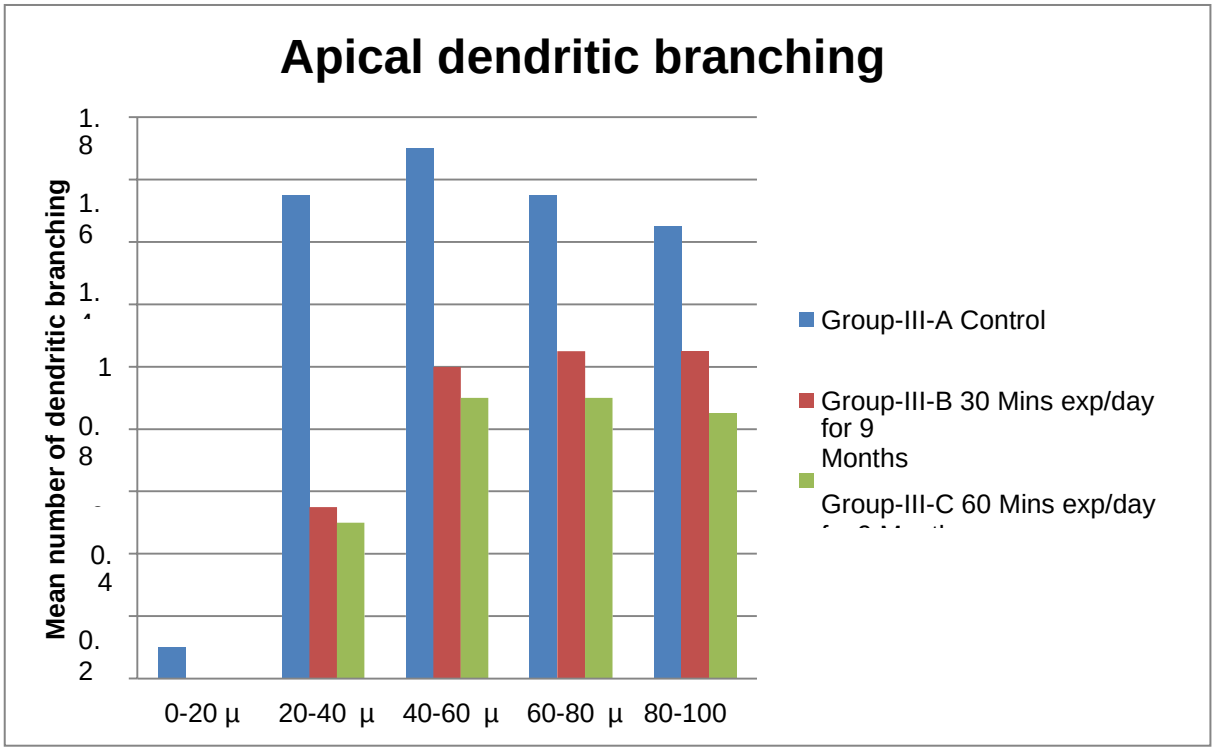


Figure 62: Number of apical dendritic branching points in CA3 neurons of control and exposed groups. (Group III A, Group III B, Group III C - 9 months exposure)

Apical Dendritic Intersections-

Table19: Apical dendritic intersections of hippocampal CA3 neurons of mice in Group-III-A (control group), Group-III-B (30 mins exp/day for 9 months) and Group-III-C (60 mins exp/day for 9 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA and Bonferroni"s post-hoc test using SPSS 20).

	Distance from Soma (µm)				
Groups	20	40	60	80	100
Group-III-A (Control)	0.20±0.61	2.60±1.04	4.30±1.21	5.75±1.33	6.40±1.31
Group-III-B (30 mins exp/day for 9 months)	0.00±0.00	1.00±1.17*	2.30±1.17*	3.40±0.99*	3.80±1.00*
Group-III-C (60 mins exp/day for 9 months)	0.00±0.00	0.90±1.16*	2.05±1.23*	3.25±1.02*	3.55±0.94*

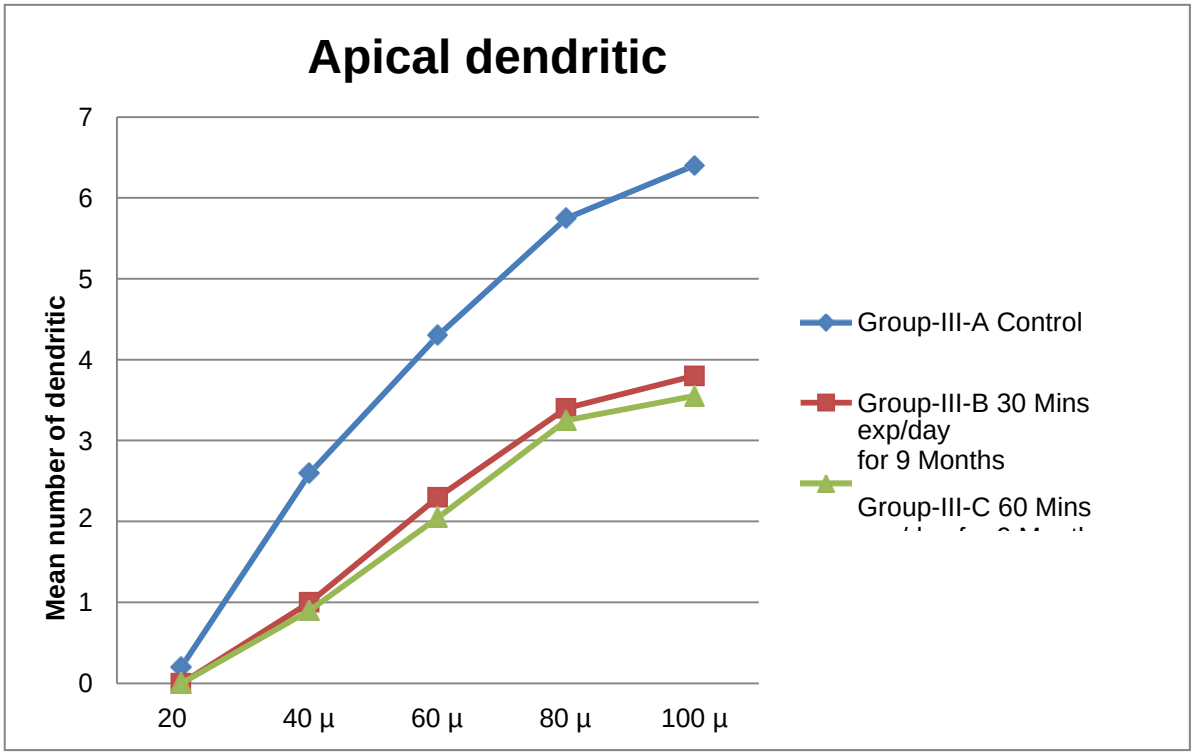


Figure 63: Number of apical dendritic intersections in CA3 neurons of control and exposed groups. (Group III A, Group III B, Group III C - 9 months exposure)

Basal Dendritic Branching points-

Table 20: Basal dendritic branching points of hippocampal CA3 neurons of mice in Group-III-A (control group), Group-III-B (30 mins exp/day for 9 months) and Group-III-C (60 mins exp/day for 9 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA and Bonferroni"s post-hoc test using SPSS 20).

	Distance from Soma (µm)				
Groups	0-20	20-40	40-60	60-80	80-100
Group-III-A (Control)	1.25±1.16	2.50±1.31	1.65±0.98	0.75±0.71	0.30±0.57
Group-III-B (30 mins exp/day for 9 months)	1.20±0.95	2.05±0.94	0.85±1.04*	0.15±0.36*	0.10±0.30
Group-III-C (60 mins exp/day for 9 months)	1.10±0.91	1.45±0.99*	0.30±0.57*	0.00±0.00*	0.00±0.00*

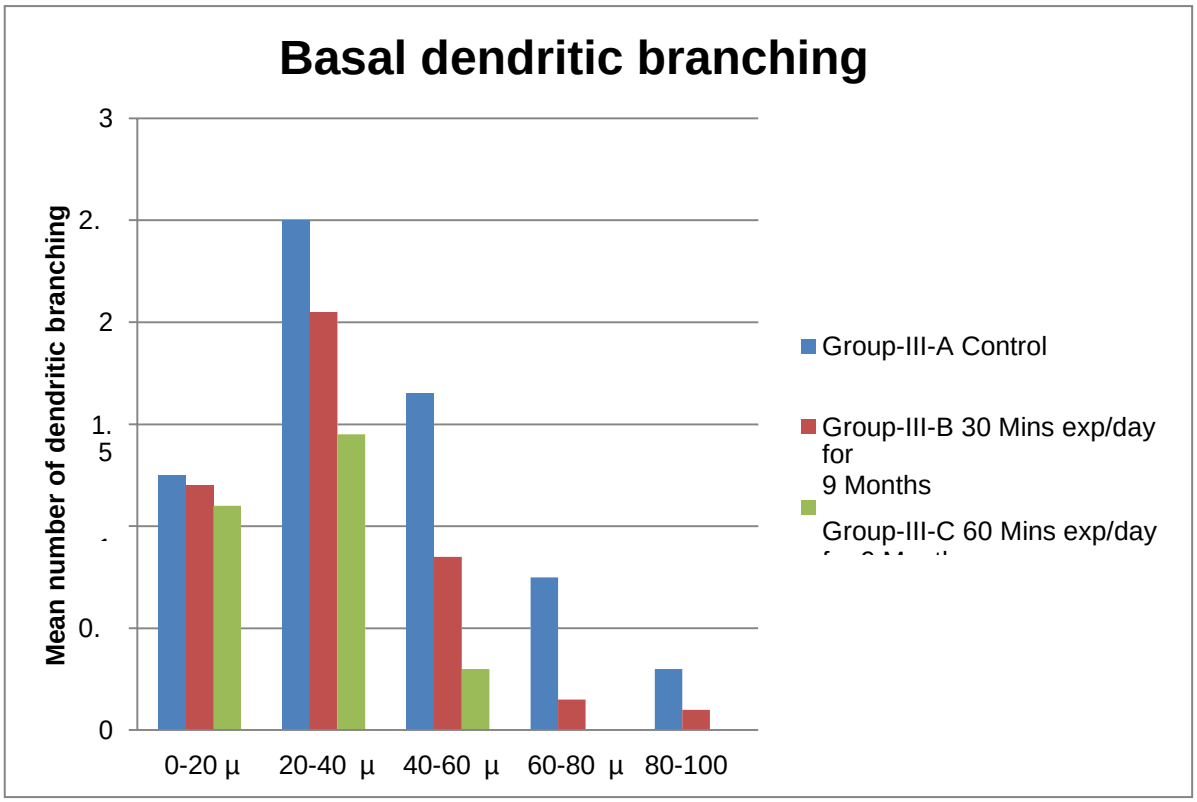


Figure 64: Number of basal dendritic branching points in CA3 neurons of control and exposed groups. (Group III A, Group III B, Group III C - 9 months exposure)

Basal Dendritic Intersections-

Table 21: Basal dendritic intersections of hippocampal CA3 neurons of mice in Group-III-A (control group), Group-III-B (30 mins exp/day for 9 months) and Group-III-C (60 mins exp/day for 9 months). Values were expressed in Mean ± SD, * Represents P<0.05 (Analysed by One-way ANOVA and Bonferroni"s post-hoc test using SPSS 20).

	Distance from Soma (µm)				
Groups	20	40	60	80	100
Group-III-A (Control)	4.35±1.04	6.65±1.18	8.10±1.63	8.65±1.59	6.95±1.39
Group-III-B (30 mins exp/day for 9 months)	3.85±0.98	6.35±1.48	7.20±1.24	6.40±1.53*	3.95±1.82*
Group-III-C (60 mins exp/day for 9 months)	3.50±0.82*	4.90±0.85*	5.30±1.03*	4.50±1.35*	3.05±1.14*

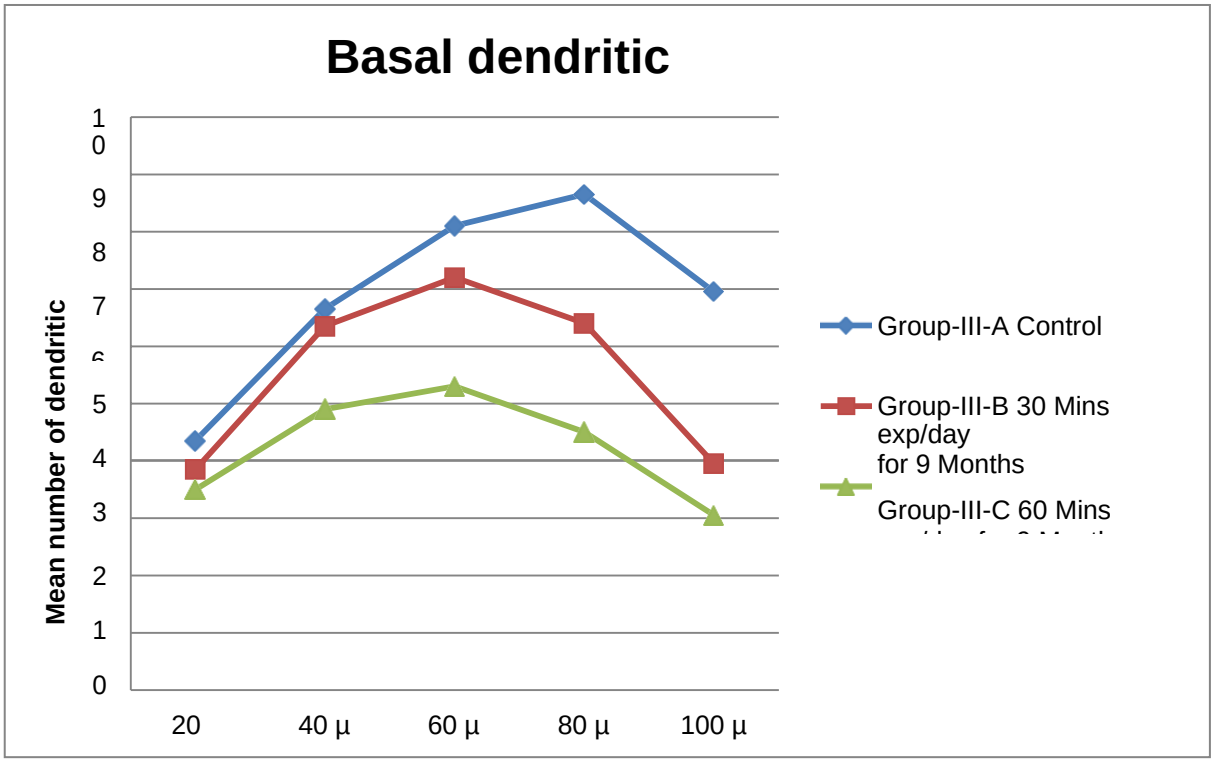


Figure 65: Number of Basal dendritic intersections in CA3 neurons of control and exposed groups. (Group III A, Group III B, Group III C - 9 months exposure)

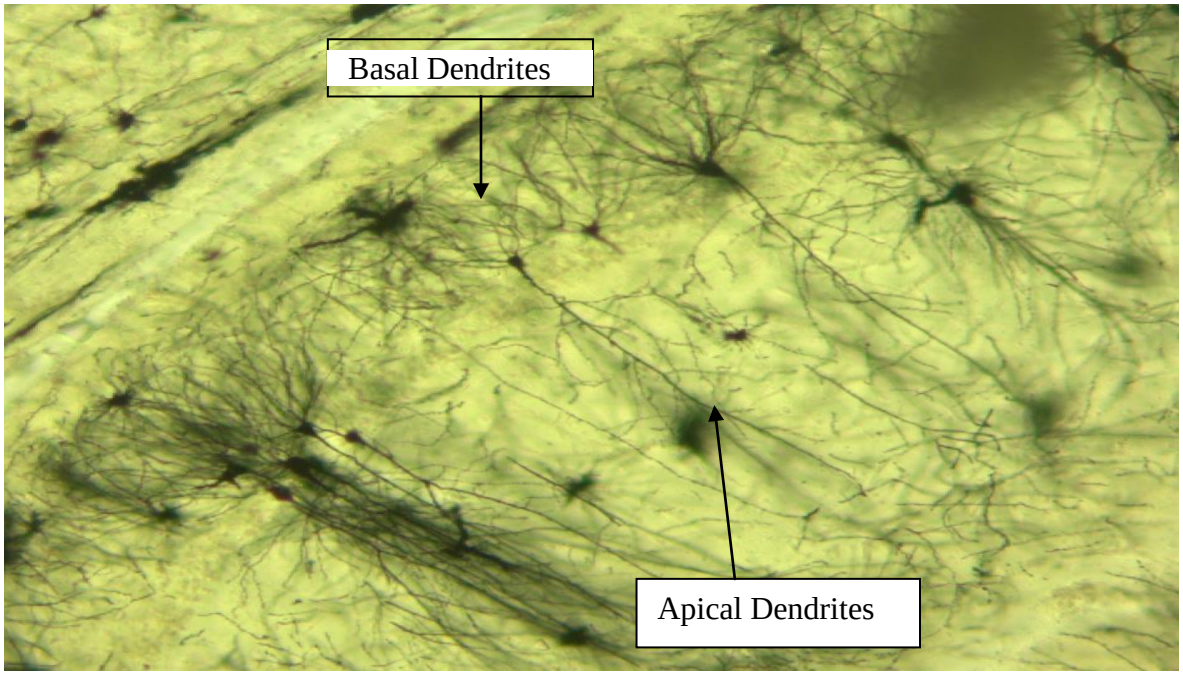


Figure 66: Golgi- Cox Stained hippocampal CA3 Neurons shows Apical and Basal Dendritic branching points and intersections. (Group III A- 9 Months-Control Image)

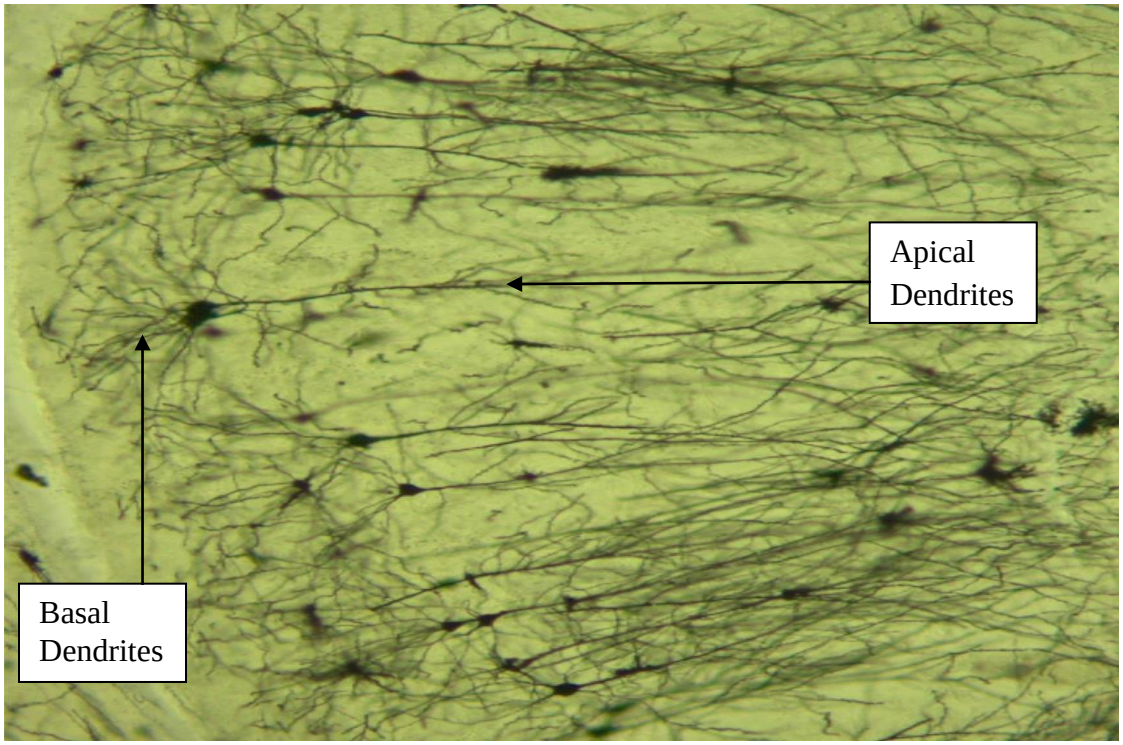


Figure 67: Golgi- Cox Stained hippocampal CA3 Neurons shows Apical and Basal Dendritic branching points and intersections. (Group III B- 9 Months- 30 mins exp/day)

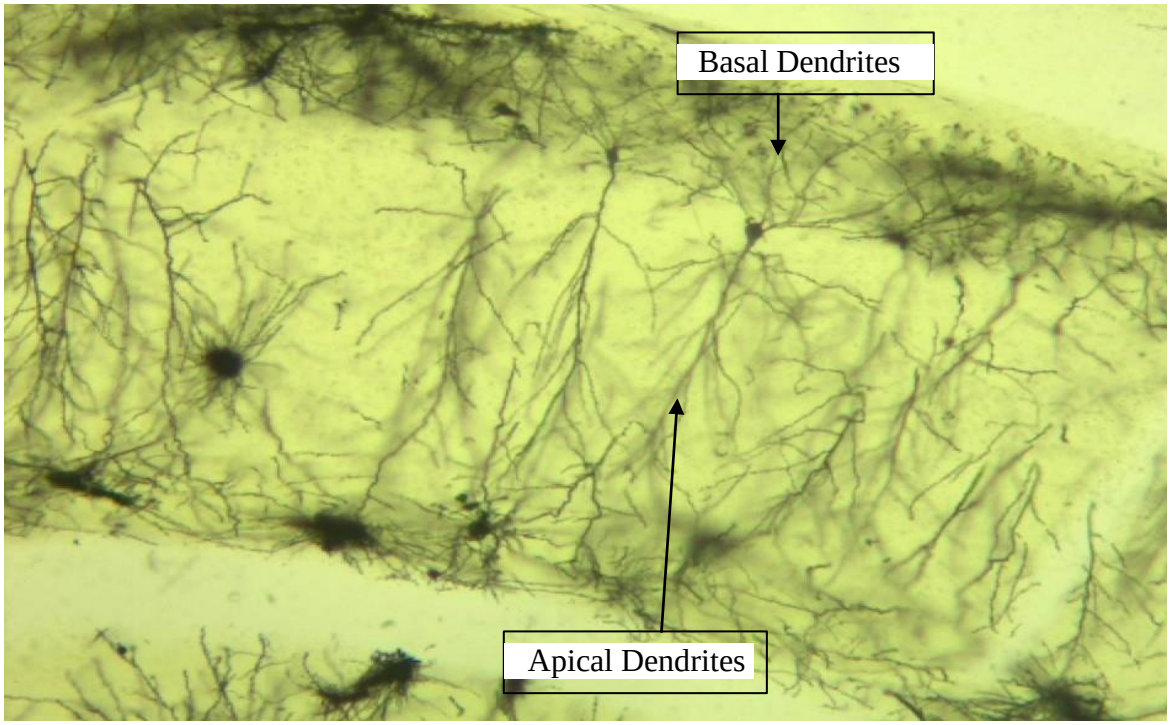


Figure 68: Golgi- Cox Stained hippocampal CA3 Neurons shows Apical and Basal Dendritic branching points and intersections. (Group III C- 9 Months- 60 mins exp/day)

CHAPTER- 6

DISCUSSION

DISCUSSION

With advancement of technology like 2G to 3G, 3G to 4G in the telecommunication field, the mobile phones are being used for communication, internet data and as multipurpose device. However over usage of mobiles with advance multiple features has adverse effects on the brain especially on the hippocampus, which is a sensitive region on the temporal lobe of the brain responsible for spatial learning and working memory, an important cognitive function [19].

Evidence shows that hippocampus is one of the crucial region of the brain, involved in spatial learning and consolidation of the information [73].

In this study, Hebb-Williams maze and T-maze analysis was used to assess the learning and memory in albino mice exposed to mobile phone radiation frequency and control group [52,53,54,55].

In the present study, MP RF-EMR exposed mice took significantly increased time to reach the target chamber in Hebb-Williams maze when compared to the control group and T- Maze analysis also shows significant difference in percentage of correct responses when compared to control group, which shows memory retention and memory retrieval is being affected and leads to memory impairment in the mice. Studies have shown that RF EMR exposure will impair the learning and memory, which may be due to neurodegenerative changes and alterations in the morphology of the hippocampus [19, 36].

On histological examination, radiation exposed hippocampal CA3 neurons showed less number of pyramidal cells with darkened nuclei (Non-viable), vacuolated / empty spaces in between cells and cells were scattered in arrangement. The altered structural integrity

in the hippocampus might be the cause for impairment of learning memory. Decrease in pyramidal cell count may be due to inhibition of neurogenesis and this was supported by Odaci E et al., [74].

Hippocampal region of the brain is extremely prone for loss of neurons during stress conditions [75,76].

Bolla SR reported that exposure to 800 MHz mobile radiation for 30 days leads to increased neuronal damage and decreased viable neurons in hippocampal CA3 region [46].

Nittby H et al., reported that exposure to 900 GSM radiation will reduce memory functions in rat, which is similar to our study [1].

Mobile phone radio-frequency exposure to 900-1800 MHz radiation leads to decrease in nuclear diameter and reduce neuronal density in the hippocampus [20].

Findings on exposure to 50-217 Hz low frequency radiation with television and mobile phone have impact on learning and memory [33].

Fragopoulou AF et al., reported that consolidation and retrieval memory deficits were observed in mice exposed to 9 hr 30 mins for 4 days with 900 MHz non-ionising radiation [32].

Heat shock proteins-HSP 27 and HSP 70 related stress levels are elevated in rat hippocampus exposed to 2450 MHz radiation [77].

A 2.14 GHz Radiation frequency exposure at 4 Watt/kg specific absorption rate increases the body temperature to 1.5°C compared to baseline and upregulates some stress markers

like HSP and Heat Shock Transcription Factor (HSF) gene expressions in cerebellum and cerebral cortex [78].

Although some studies were carried out to assess the radiation effects on memory-learning, cellular architecture, epidemiological studies and toxicology studies on laboratory research animals, still it is controversial [19].

In the present study, we have focused on the effects of mobile phone radio-frequency electromagnetic radiation on the cellular architecture and quantification of hippocampal CA3 pyramidal neurons, which shows compactly arranged healthy neurons with clear nucleus in control group, whereas radiation exposed group shows unhealthy neurons which is darkly stained, scattered and irregularly arranged. Neuronal quantification of hippocampal CA3 pyramidal neurons reveals that presence of less number of viable neurons in radiation exposed group compared to control group.

In a study done by Bolla SR, reported that decreased number of viable neurons in the hippocampus may be because of effect of neurogenesis and exposure to mobile radiation for 2 hrs/day can up-regulate the apoptotic pathway [46] .

Additional stress might be one of the reason for decreasing the neuronal count in hippocampus [79].

In 2013, Afeefy AA et al reported that, microscopic anatomy of radiation exposed hippocampal pyramidal neurons in CA3 region shows variable degrees of degeneration, irregular in shape and decreased cytoplasmic nuclear ratio with nuclear wall disruption [19]. The results were similar with our study.

Chronic exposure to mobile radiation alters spatial learning, decreased hippocampal CA3 pyramidal neurons and remodelling of dendrites. Structural changes and decreased hippocampal neurons may be one of the cause for altered cognitive function [71].

900 MHz electromagnetic radiation exposure for 1 hr/day in adolescent rats (21 day to 59 day) results in decreased hippocampal pyramidal cell count and morphological changes like darkly stained cytoplasm with shrunken pyramidal cells were observed [44].

Rats exposed to 900 MHz radiation for 1 hr/day results in increased malondialdehyde levels, xanthine oxidase levels and decreased glutathione peroxidise activity, superoxide dismutase levels. These changes in the brain were prevented by giving ginkgobiloba [36].

Along with behavioural analysis, neuronal quantification, hippocampal CA3 dendritic quantification also analysed in this present study.

The dendrites of CA3 pyramidal neurons of hippocampus receive the inputs from enthorinal cortex, mamillary body, septal area, granular cells of dentate gyrus and CA3 contralateral regions, which plays a crucial role in the formation of spatial information to short term memory within seconds.

In various conditions hippocampal pyramidal neurons of ca3 region remodelling and atrophy had been reported [80,81].

Golgi-cox stained CA3 Pyramidal hippocampal neurons shows decreased dendritic branching points and dendritic intersections in radio frequency-electromagnetic radiation exposed group, when compared to control group.

The hippocampus is sensitive and highly vulnerable to endogenic and exogenic factors which leads to changing the structural integrity such as dendritic atrophy of hippocampal CA3 pyramidal neurons.

Increased in dendritic atrophy were seen in the increased duration RF-EMR exposure.

CHAPTER-8

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PUBLICATIONS

AND

CONFERENCE PRESENTATIONS

Original Article

DOI: 10.7860/JCDR/2019/39681.12630

Effect of 1800-2100 MHz Electromagnetic Radiation on Learning-Memory and Hippocampal Morphology in Swiss Albino Mice

Anatomy Section

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ABSTRACT

Introduction: With advancing technology the mobile phone with multiple features is used as a multipurpose device and attract people of all age groups. Increased usage of mobile phone raises the question of possible adverse effects on health.

Aim: To assess the 1800-2100 MHz radiation effect on learning-memory and microscopic anatomy of hippocampal Cornu Ammonis (CA3) neurons in mice.

Materials and Methods: A total of 18 albino mice were divided into 3 groups (6 Mice per group). Group-I: Control Group, Group-II: Exposed to Radio frequency-Electromagnetic radiation (RF-EMR) for 30 minutes/day for 3 months, Group-III: Exposed to RF-EMR for 60 minutes/day for 3 months. Followed by the exposure, learning memory was assessed by using Hebb-Williams maze in all the groups. The mice were then sacrificed, brains were dissected out and sections were taken at the level of hippocampus and then stained with Haematoxylin and Eosin for microscopy.

The results were expressed in Mean±SD and analysed by using one-way (analysis of variance) ANOVA followed by LSD (Least Square Difference) test for paired wise data. The p-value<0.05 was considered as statistically significant.

Results: The time taken by the animal to reach the target chamber was significantly increased in Group-III (exposed 60 minutes/day for 3 months), whereas group-II (exposed 30 minutes/day for 3 months) showed no significant changes when compared to Group-I (control group). Microscopic anatomy of hippocampal CA3 neurons in exposed group shows less number of pyramidal cells with darkened nuclei, cytoplasm was vacuolated and cells were scattered.

Conclusion: Exposure to 1800-2100 MHz radiation leads to damage and decrease of neurons in hippocampal region, which alters the learning and memory.

Keywords: Cornu ammonis, Hebb-Williams maze, Radio frequency

INTRODUCTION

The extensive use of Global System for Mobile communication (GSM) mobile phones throughout the world raises the possible adverse effects on human health especially on the Central Nervous System (CNS), the brain. In many countries more than half of the population relies/depend on mobiles for wireless communication and internet data [1]. In 2015, more than 7 billion people were using mobiles in the world, estimating to 62.9% of the world's population. Rapid increase of mobile users in general and specifically upto 80% of youngsters owning a mobile has made communication and technology easier [2].

In this concern there is a growing interest in scientific community for the potential deleterious effects of Radio Frequency Electro Magnetic Radiation (RF EMR) on the public health, especially much focus on the effects of RF EMR on structural and functional integrity of the brain because the radiation exposure is directly to the head region [3]. In 2006 and 2010 World Health Organisation (WHO) issued a research agenda for high priority research on effects of RF exposure on ageing and neurodegenerative diseases in animals and effects of pre and post natal RF exposure on development and behaviour in animals [4,5]. The mobile phone releases non-ionising radiation which has low frequency and considered to be safe, but recent studies evidenced that it has an impact on the living tissues especially on the brain which can cause headache, memory loss, heat over the ear, decreased concentration and other cognitive effects [6].

The hippocampus is a part of brain which belongs to the limbic system and is involved in cognitive functions like spatial learning and working memory. It plays a crucial role in the formation of new memories and it is considered as a sensitive region and is affected by mobile phone radiation. The hippocampus is a "S"-shaped folded structure located on the floor of the lateral ventricle on both the cerebral hemispheres. Hippocampal formation consists of hippocampus proper, dentate gyrus and subiculum. Hippocampus proper is also known as Cornu Ammonis (CA), which consists of CA1, CA2, CA3 and CA4 sub regions [7].

Studies have found that damage to the hippocampal neurons may lead to impairment of memory and learning, behavioural disturbances and impact on Hypothalamo-Pituitary-Adrenal (HPA) axis [3,8,9]. The present study was undertaken to evaluate the long term exposure effect of mobile phone radiofrequency electromagnetic radiation-4G (1800-2100 MHz) on cognitive functions like spatial learning, working memory and hippocampal morphology in adult swiss albino mice.

MATERIALS AND METHODS

The Experimental study was carried out after the approval of Institutional Animal Ethical Committee (IAEC/PHARMA/SDUMC/2017-18/04). The study was conducted at central animal house Sri Devaraj Urs Medical College, Kolar from November 2017-January 2018, the duration of the study was 3 months.

Animals

Six weeks old healthy male Swiss-Albino Mice were used in this study, the animals were procured from committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) registered brooders-Invivo Biosciences, Bengaluru.

The Swiss-Albino Mice were kept in polypropylene cages with a temperature of 23±2°C, humidity 55±5% and 10 hours light, 14 hours dark cycle and free access to standard pellet food and water ad libitum. The experimental animal care was taken as per the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Inclusion and Exclusion Criterion

Male healthy active Swiss-Albino mice with average weight of 20 grams when procured were included in this study. Female swiss albino mice and lesser weight mice were excluded from this study.

Experiment Design

A total of 18 Male Swiss-Albino Mice were taken and they were divided into three groups.

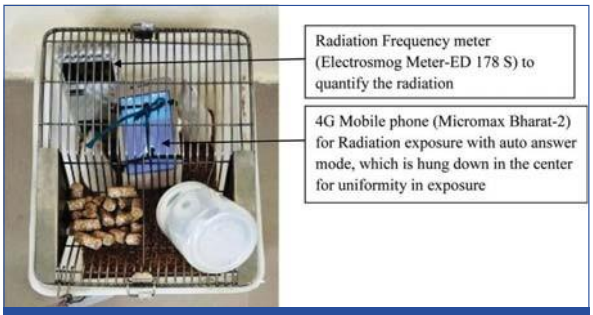
Group I: Control group-consists of 6 mice (non-exposed group).

Group II: 30 minutes exposure group-consists of 6 mice which were exposed to Mobile Phones (MP) RF-EMR for 30 minutes/day for 3 months.

Group III: 60 minutes exposure group-consists of 6 mice which were exposed to MP RF-EMR for 60 minutes/day for 3 months.

Mobile phone: 4G android mobile phones (Micromax Bharat-2 with a Specific Absorption Rate (SAR) of 1.6 Watt/Kg) with same specification and with same mobile network were used in this study, keeping a GSM (2100 MHz) mobile phone in silent with auto answer mode. The mobiles were hung down from the roof of the mice cage and the radiation which they emitted during the exposure was quantified by radiation frequency meter (Electrosmog Meter-ED 178 S) which was kept at the periphery, 1950 MHz of RF-EMR was emitting till the periphery of the mice cage during the exposure, so the similar amount of radiation may affect/enters the mice brain.

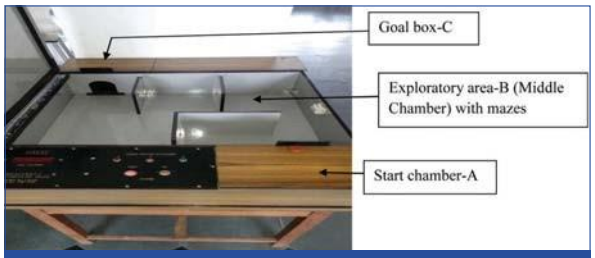
Exposure technique: Three Mice were kept in each cage during the exposure. Animals of group II and III were exposed to 30 minutes and 60 minutes/day for 3 months respectively. The mobile phones were hung down in the center of the cages during the exposure period for the uniformity of the radiation through out the cage [Table/Fig-1].



[Table/Fig-1]: Image shows the cage with mice and mobile phone during radiation exposure with Radiation Frequency Meter (Electrosmog Meter-ED 178 S) to quantify the mobile radiation.

Hebb-Williams Maze: Hebb-Williams Maze is used to test the spatial learning and working memory of the mice. The principle behind the Hebb-Williams Maze test is "The faster the mice navigates the maze, the better its spatial memory". The Hebb-Williams maze is a square shaped box which measures 60 cm (L)×60 cm (W)×10 cm (H) walls. It consists of start chamber-A (Animal Chamber) which is attached to the exploratory area-B (Middle Chamber) and a goal box-C, located at the opposite end of the start chamber and contains a small food reward. All three chambers were provided with removable doors to allow the animal to move from one chamber to the next.

After 12 hours of fasting, the mice was placed in the start chamber-A and allowed to enter into the exploratory area-B (middle chamber), once the animal enters into middle chamber the door was closed to prevent back entry. The time taken by the animal to reach The Reward Chamber (TRC) from the start chamber was recorded. The animals were trained for 3 days (3 trials/day) and the readings were taken at the 4th day. Low scores indicates better memory, while the high scores indicates poor memory in animals [Table/Fig-2] [10,11].



[Table/Fig-2]: Hebb-Williams Maze instrument to assess the spatial learning and working memory in Swiss-Albino mice.

Tissue Processing

After the behavioural analysis the mice were euthanized, perfused transcardially with normal saline and the brains were extracted out, fixed in 10% buffered formalin, dehydrated in ascending grades of ethyl based alcohol like 60%, 70%, 80%, 90% and absolute alcohol, cleared in xylene, impregnated in paraffin wax at 60°C, embedded with the help of L-moulds and then 6 µm paraffin sections were taken using rotary microtome at the level of the dorsal hippocampus to assess the hippocampal CA3 cellular architecture with the help of H and E staining. To prevent the bias every 5th section was taken and the slides were decoded after the histological assessment. Viable neuronal quantification was assessed with the help of ocular micrometer fixed to light microscope (40X).

STATISTICAL ANALYSIS

The results were expressed in Mean±SD and analysed by using one-way ANOVA followed by Least Square Difference (LSD) test for paired wise data. The $p<0.05$ was considered as statistically significant.

RESULTS

Body Weights of the Mice

The mean body weight of the control group mice was 32.3 grams, 30 min/day radiation exposed mice for 3 months had 31.8 grams and 60 min/day radiation exposed mice for 3 months had 32.7 grams, the mean weight between the three groups didn't show any significant difference.

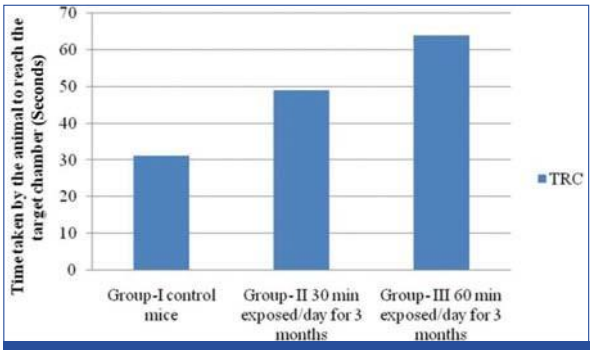
Effect of Radiation on Learning Memory in Hebb-Williams Maze

The time taken by the mice to reach the target chamber from the starting chamber was significantly increased in group II (30 min exposed/day) and group III (60 min exposed/day) compared to group I (non-exposed group).

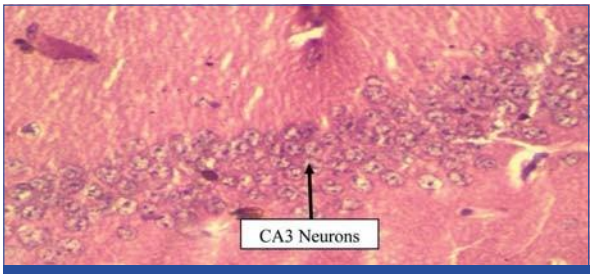
The time taken by the animal to reach The Reward Chamber (TRC) scores in Group I vs Group II (31±15.48 vs 49±17.62 seconds), was not significant ($p>0.05$); Group I vs Group III (31±15.48 vs 64±22.99 seconds), was statistically significant ($p<0.05$) [Table/Fig-3].

Microscopic Anatomy of Hippocampal Cornu Ammonis (CA3) Neurons

Histological sections of haematoxylin and eosin stained hippocampal CA3 pyramidal neurons showed marked difference between control group and RF-EMR exposed groups (group II and III). Sections of control group showed 5-6 layers of compactly arranged pyramidal cells which were healthy with clear nucleus [Table/Fig-4]. Group II (30 min exposure for 3 months) showed less number of pyramidal neurons with darkened nuclei (non-viable neurons) which was

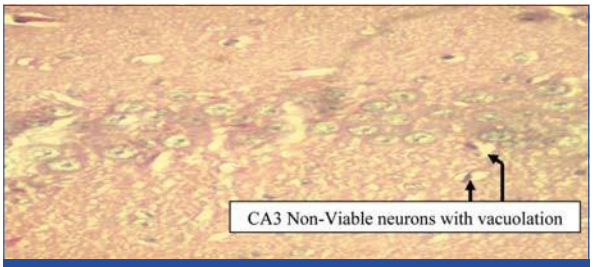


[Table/Fig-3]: Effect of Mobile phone radiofrequency-electro-magnetic radiation (MP RF-EMR) on learning and memory by using Hebb-Williams maze- group I, II and III.

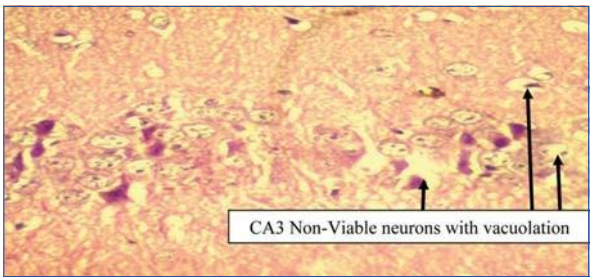


[Table/Fig-4]: Group I (non-exposed)-Control group H and E stained Hippocampal pyramidal normal neurons (Arrow) in high power (40X)

scattered when compared to control group [Table/Fig-5]. Group III (60 min exposure for 3 months) showed very less number of pyramidal neurons with more number of darkened nuclei (more non viable neurons) with vacuolation in cytoplasm and scattered arrangement of pyramidal neurons when compared to group I and group II [Table/Fig-6].



[Table/Fig-5]: Group-II (30 min exposed for 3 months) H and E stained Hippocampal pyramidal neurons (Arrow) in high power (40X) showed less in number, Non-Viable and scattered with vacuolation in cytoplasm.



[Table/Fig-6]: Group- III (60 min exposed for 3 months) H and E stained Hippocampal pyramidal neurons (Arrow) in high power (40X) showed very less in number, Non-Viable and scattered with vacuolation in cytoplasm

DISCUSSION

With advancement of technology like 2G to 3G, 3G to 4G in the telecommunication field, the mobile phones are being used for communication, internet data and as multipurpose device. However over usage of mobiles with advance multiple features has adverse effects on the brain especially on the hippocampus, which is a sensitive region on the temporal lobe of the brain responsible for spatial learning and working memory, an important cognitive function [7].

In this study, Hebb-Williams maze analysis was used to assess the learning and memory in albino mice exposed to mobile phone radiation frequency and control group [10,11]. In the present study, MP RF-EMR exposed mice took significantly increased time to reach the target chamber in Hebb-Williams maze when compared to the control group, which shows memory retention and memory retrieval is being affected and leads to memory impairment in the mice. Studies have shown that RF EMR exposure will impair the learning and memory, which may be due to neurodegenerative changes and alterations in the morphology of the hippocampus [7,8].

On histological examination, radiation exposed hippocampal CA3 neurons showed less number of pyramidal cells with darkened nuclei (Non viable), vacuolated cytoplasm and cells were scattered in arrangement. The altered structural integrity in the hippocampus might be the cause for impairment of learning memory. Decrease in pyramidal cell count may be due to inhibition of neurogenesis and this was supported by Odaci E et al., [12]. Bolla SR reported that exposure to 800 MHz mobile radiation for 30 days leads to increased neuronal damage and decreased viable neurons in hippocampal CA3 region [9].

Nittby H et al., reported that exposure to 900 GSM radiation will reduce memory functions in rat, which is similar to our study [1]. MP RF exposure to 900-1800 MHz radiation leads to decrease in nuclear diameter and reduce neuronal density in the hippocampus [13]. Findings on exposure to 50-217 Hz low frequency radiation with television and mobile phone have impact on learning and memory [14]. Fragopoulou AF et al., reported that consolidation and retrieval memory deficits were observed in mice exposed to 9 hr 30 mins for 4 days with 900 MHz non ionising radiation [15]. Heat shock proteins-HSP 27 and HSP 70 related stress levels are elevated in rat hippocampus exposed to 2450 MHz radiation [16]. A 2.14 GHz Radiation frequency exposure at 4 Watt/kg specific absorption rate increases the body temperature to 1.5°C compared to baseline and upregulates some stress markers like HSP and Heat Shock Transcription Factor (HSF) gene expressions in cerebellum and cerebral cortex [17].

LIMITATION

The outcome of the present rodent study may not be extrapolate with human population due to many reasons like Thickness of the skull bone, Weight/Volume of the brain, Specific Absorption Rate (SAR), Duration of exposure, Frequency of radiation and Lifespan of the human population.

CONCLUSION

In this present study, we evaluated the chronic exposure effect of MP RF-EMR- 4G (1800-2100 MHz) on cognitive functions like spatial learning, working memory and hippocampal morphology in adult swiss albino mice. We observed that MP RF-EMR exposed mice took significantly increased time to reach the target chamber in Hebb-Williams maze when compared to the control group. Radiation exposed hippocampal CA3 neurons showed less number of pyramidal cells with darkened nuclei (Non viable), vacuolation in cytoplasm and cells were scattered in arrangement. The altered structural integrity in the hippocampus may alter the spatial learning and memory.

ACKNOWLEDGEMENTS

We thank Dr. Sarala N Prof., Department of Pharmacology and Incharge-Animal house facility, Sri Devaraj Urs Medical College, Kolar for providing animal house to carry out this doctoral study, we thank Dr. Muninarayana Veterinarian for his suggestions during this study and we also thank Mr. Ravi Shankar, Statistician for his help.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: Sep 18, 2018
Date of Peer Review: Nov 23, 2018
Date of Acceptance: Dec 31, 2018
Date of Publishing: Feb 01, 2019

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7KH IDFWV WKDW LV DODUPLQJ ZLWK PDQ\ RI WKH PRELOH GHYLFHV LV WKDW WKH\ ZHUH UHOHDVLQJ LQWR WKH PDUNHW ZLWKRXW SURSHU VDIHW\ DSSURYDOV DQG JXLGHOLQHV RQ HOHFWURPDJQHWLF UDGLDWLRQ ¿HOG VWUHQJWK PRELOH SKRQH XVDJH ZLOO EH D KHDOWK KD]DUG DQG FODVVL¿HG PRELOH UDGLRIUHTXHQF\ ¿HOGV-KLJK SULRULW\ UHVHDUFK RQ QHXURGHJHQHUDWLYH GLVHDVHV DQG DJHLQJ LQ DQLPDO PRGHOV[4].&XUUHQW\ WKH FRQWURO WKDW PRELOH UDGLDWLRQ XVDJH/H[SRVXUH WR PRELOH SKRQHV FDQ EH FRQVLGHUHG DV DQ ³KDOWK WLPH ERPE´, EHFDXVH WKH RYHU XVDJH RI PRELOH SKRQH IRU PRUH WKDQ 20 PLQXWHV LQFUHDVHV WKH FRQWDFW WLVVXH WHPSHUDWXUH WR 1˚&[5]. 2YHU XVDJH RI PRELOH SKRQH PD\ UHVXOW LQ GLL¿FXOW\ WR GR WKH GDLO\ DFWLYLWLHV RQ WLPH, SRRU IUDJPHQWHG VOHHS, DIIHFWV OHDUQLQJ FDSDFLW\,PHQWDO KHDOWK DQG TXDOLW\ RI OLIH[6].

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*URXS ,,: 30 PLQXWHV H[SRVXUH JURXS ± FRQVLVWV RI 6 PLFH H[SRVHG WR 035)-(05 IRU 30 PLQV/GD\ IRU 3 PRQWKV.

*URXS ,,,: 60 PLQXWHV H[SRVXUH JURXS ± FRQVLVWV RI 6 PLFH H[SRVHG WR 035)-(05 IRU 60 PLQV/GD\ IRU 3 PRQWKV.

$IWHU WKH H[SRVXUH WKH PLFH ZHUH HXWKDQL]HG, SHUIXVHG WUDQVFDUGLDOO\ ZLWK QRUPDO VDOLQH DQG WKH EUDLQV ZHUH H[WUDFWHG RXW, ¿[HG LQ 10% EXIIHUHG IRUPDOLQ DQG WKH QSURFHVVHG IRU KLVWRORJLFDO SURFHGXUHV,6–P

SDUDL¿Q VHFWLRQV ZHUH WDNHQ XVLQJ URWDU\ PLFURWRPH DW WKH OHYHO RI WKH GRUVDO KLSSRFDPSXV WR DVVHVV WKH KLSSRFDPSDO &$3 QHXURQDO GDPDJH ZLWK WKH KHOS RI &UHV\O YLROHW VWDLQLQJ. 9LDEOH QHXURQDO TXDQWL¿FDWLRQ ZDV DVVHVVHG ZLWK WKH KHOS RI RFXODU PLFURPHWHU ¿[HG WR OLJKW PLFURVFRSH (40;).)URP HDFK PLFH WHQ VHFWLRQV ZHUH VHOHFWHG IRU YLDEOH QHXURQ TXDQWL¿FDWLRQ.

0RELOH 3KRQH: 4 * DQGURLG PRELOH SKRQHV (0LFURPD[) ZHUH XVHG LQ WKLV VWXG\, NHHSLQJ D *60 (2100 0+]) PRELOH SKRQH LQ VLOHQW ZLWK DXWR DQVZHU PRGH. 7KH PRELOHV ZHUH KXQJ GRZQ IURP WKH URRI RI WKH PLFH FDJH DQG WKH UDGLDWLRQ ZKLFK ZDV HPLWWHG GXULQJ WKH H[SRVXUH ZDV TXDQWL¿HG E\ UDGLDWLRQ IUHTXHQF\ PHWHU ((OHFWURVPRJ 0HWHU–(ˇ 1786).

(ISRVXUH WHFKQLTXH: $QLPDOV RI JURXS „ & ,,, ZHUH H[SRVHG WR 30 PLQV & 60 PLQV/GD\ IRU 3 PRQWKV. 7KH PRELOH SKRQHV ZHUH KXQJ GRZQ LQ WKH FDJHV GXULQJ WKH WLPH RI H[SRVXUH.

6WDWLVWLFDO DQDO\VLV:
7KH UHVXOWV ZHUH H[SUHVVHG LQ 0HDQ " 6WDQGDUG 'HYLDWLRQ. 2QH-ZD\ 129 DQG %RQIHUURQL´V SRVW-KRF WHVW ZHUH XVHG WR FRPSDUH WKH VLJQL¿FDQFH EHWZHHQ FRQWURO DQG H[SRVHG JURXSV. 3 < 0.05 ZDV FRQVLGHUHG DV VWDWLVWLFDOO\ VLJQL¿FDQW. (6WDWLVWLFDO 3DFNDJH-6366 YHUVLRQ 20)

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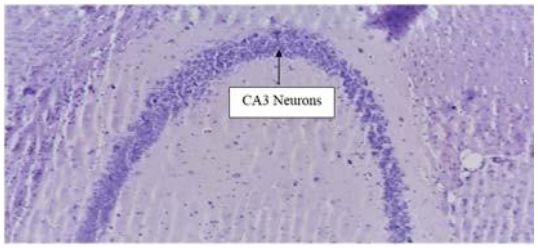
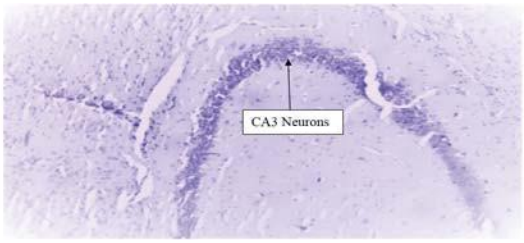
1HXURQDO GDPDJH DVVHVVPHQW LQ &$3 UHJLRQ RI KLSSRFDPSXV:
7KH S\UDPLGDO QHXURQV RI KLSSRFDPSDO &$3 UHJLRQ LQ FRQWURO JURXS VKRZV KHDOWK\ QHXURQV ZKLFK LV FRPSDFWO\ DUUDQJHG ZLWK FOHDU QXFOHXV, ZKHUH DV PRELOH SKRQH UDGLDWLRQ H[SRVHG PLFH KLSSRFDPSDO &$3 QHXURQV VKRZV GDUNO\ VWDLQHG, XQKHDOWK\, VFDWWHUHG DQG LUUHJXODU, DV LOOXVWUDWHG LQ ¿JXUH1, 2 DQG 3.

)LJXUH 1: &UHV\O 9LROHW VWDLQHG SKRWRJUDSKLF LPDJHV RI KLSSRFDPSDO S\UDPLGDO FHOOV LQ &$3 UHJLRQ RI PLFH EUDLQ IURP FRQWURO DQG UDGLDWLRQ H[SRVHG JURXSV.

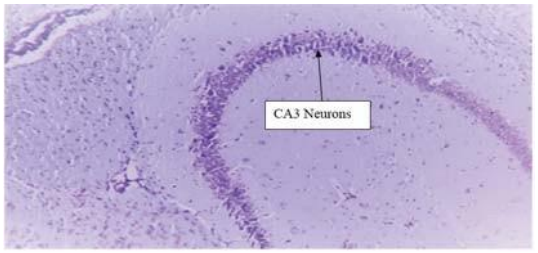
*URXS- , (&RQWURO JURXS)-/RZ SRZHU LPDJH



*URXS- ,, (30 PLQV H[S/GD\ IRU 3 PRQWKV)-/RZ SRZHU LPDJH



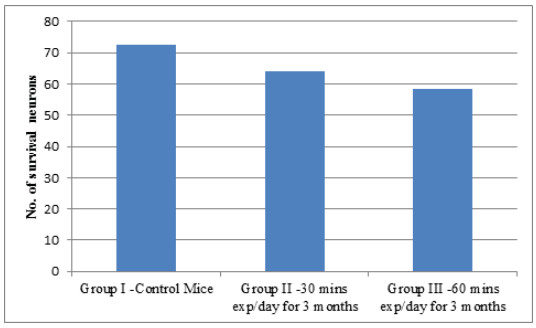
*URXS- ,,, (60 PLQV H[S/GD\ IRU 3 PRQWKV)-/RZ SRZHU LPDJH



4XDQWL¿FDWLRQ RI YLDEOH QHXURQV LQ KLSSRFDPSDO &$3 UHJLRQ
7RWDO PHDQ QXPEHU RI YLDEOH S\UDPLGDO &$3 QHXURQV LQ JURXS-, (&RQWURO JURXS) 72.5“ 8.34 YV JURXS-„ (30 PLQV H[S/GD\ IRU 3 PRQWKV) 64.1“ 8.13 ZDV VWDWLVWLFDOO\ QRW VLJQL¿FDQW (3!0.01), ZKHUHDV JURXS-, (&RQWURO JURXS) 72.5“ 8.34 YV JURXS-,,, (60 PLQV H[S/GD\ IRU 3 PRQWKV) 58.3“ 6.81 ZDV VWDWLVWLFDOO\ VLJQL¿FDQW (3<0.001).

6R WKH UHVXOWV LQ WKLV VWXG\ VKRZV WKDW LQFUHDVH LQ WKH GXUDWLRQ RI H[SRVXUH WR PRELOH SKRQH UDGLDWLRQ HDGV WR LQFUHDVHG GPDJH RI WKH KLSSRFDPSDO &$3S\UDPLGDOQHXURQV,DVLOOXVWUDWHGLQJUDSK1.

***UDSK 1:** 4XDQWL¿FDWLRQ RI KLSSRFDPSDO &$3 VXUYLYDO QHXURQV LQ FRQWURO DQG UDGLDWLRQ H[SRVHG JURXSV.



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,QFUHDVH LQ WKH XVDJH RI ZLUHOHVV FRPPXQLFDWLRQ UDLVHG WKH FRQFHUQ RI LWV DGYHUVH ELRORJLFDO HIIHFWV DQG SRWHQWLDO KHDOWK ULVNV RQ WKH FHQWUDO QHUYRXV V\VWHP, HVSHFLDOO\ RQ WKH KLSSRFDPSXV DV LW LV D VHQVLWLYH UHJLRQ ZKLFK LV UHVSRQVLEOH IRU FRJQLWLYH OLNH OHDUQLQJ DQG PHPRU\. $OWKRXJK VRPH VWXGLHV ZHUH FDUULHG RXW WR DVVHVV WKH UDGLDWLRQ HIIHFWV RQ PHPRU\-OHDUQLQJ, FHOOXODU DUFKLWHFWXUH, HSLGHPLRORJLFDO VWXGLHV DQG WR[LFRORJ\ VWXGLHV RQ ODERUDWRU\ UHVHDUFK DQLPDOV, VWLOO LW LV FRQWURYHUVLDO[8].

,Q WKH SUHVHQW VWXG\, ZH KDYH IRFXVHG RQ WKH HIIHFWV RI PRELOH SKRQH UDGLR-IUHTXHQF\ HOHFWURPDJQHWLF UDGLDWLRQ RQ WKH FHOOXODU DUFKLWHFWXUH DQG TXDQWL¿FDWLRQ RI KLSSRFDPSDO &$3 S\UDPLGDO QHXURQV, ZKLFK VKRZV FRPSDFWO\ DUUDQJHG KDOWK\ QHXURQV ZLWK FOHDU QXFOHXV LQ FRQWURO JURXS, ZKHUH DV UDGLDWLRQ H[SRVHG JURXS VKRZV XQKDOWK\ QHXURQV ZKLFK LV GDUNO\ VWDLQHG, VFDWWHUHG DQG LUUHJXODU\ DUUDQJHG.

1HXURQDO TXDQWL¿FDWLRQ RI KLSSRFDPSDO &$3 S\UDPLGDO QHXURQV UHYHDOV WKDW SUHVHQFH RI OHVV QXPEHU RI YLDEOH QHXURQV LQ UDGLDWLRQ H[SRVHG JURXS FRPSDUHG WR FRQWURO JURXS.

,Q D VWXG\ GRQH E\ %ROOD 65, UHSRUWHG WKDW GHFUHDVHG QXPEHU RI YLDEOH QHXURQV LQ WKH KLSSRFDPSXV PD\ EH EHFDXVH RI HIIHFW RI QHXURJHQHVLV DQG H[SRVXUH WR PRELOH UDGLDWLRQ IRU 2 KUV/GD\ FDQ XS-UHJXODWH WKH DSRSWRWLF SDWKZD\[10]. ,Q 2013, $IHFI\ $$ HW DO UHSRUWHG WKDW, PLFURVFRSLF DQDWRP\ RI UDGLDWLRQ H[SRVHG KLSSRFDPSDO S\UDPLGDO QHXURQV LQ &$3 UHJLRQ VKRZV YDULDEOH GHJUHHV RI GHJHQHUDWLRQ, LUUHJXODU LQ VKDSH DQG GHFUHDVHG F\WRSODVPLF QXFOHDU UDWLR ZLWK QXFOHDU ZDOO GLVUXSWLRQ. 7KH UHVXOWV ZHUH VLPLODU XU VWXG[8].&KURQLF H[SRVXUH WR PRELOH UDGLDWLRQ DOWHUV VSWLDO OHDUQLQJ, GHFUHDVHG KLSSRFDPSDO &$3 S\UDPLGDO QHXURQV DQG UHPRGHOOLQJ RI GHQGULWHV. 6WUXFWXUDO FKDQJHV DQG GHFUHDVHG KLSSRFDPSDO QHXURQV PD\ EH RQH RI WKH FDXVH IRU DOWHUHG FRQQLWLYH IXQFWLRQ[11].

900 0+] HOHFWURPDJQHWLF UDGLDWLRQ H[SRVXUH IRU 1 KU/GD\ LQ DGROHVFHQW UDWV (21[W] GD\ WR 59[WK] GD\) UHVXOWV LQ GHFUHDVHG KLSSRFDPSDO S\UDPLGDO FHOO FRXQW DQG PRUSKRORJLFDO FKDQJHV OLNH GDUNO\ VWDLQHG F\WRSODVP ZLWK VKUXQNHQ S\UDPLGDO FHOOV ZHUH REVHUYHG[7]. 5DWV H[SRVHG WR 900 0+] UDGLDWLRQ IRU 1 KU/GD\ UHVXOWV LQ LQFUHDVHG PDORQGLDOGHK\GH 0HYHOV, [DQWLQQH R[LGDVH 0HYHOV DQG GHFUHDVHG JOXWDWKLRQH SHUR[LGDVH DFWLYLW\, VXSHUR[LGH GLVPXWDVH 0HYHOV. 7KHVH FKDQJHV LQ WKH EUDLQ ZDV PRYHQWHG E\ JLYLQJ JLQNJRELORED[12].

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7KH UHVXOWV LQ RXU VWXG\ UHYHDOV WKDW SURORQJHG H[SRVXUH WR 2100 0+] PRELOH SKRQH HOHFWURPDJQHWLF UDGLDWLRQ 0HDGV WR GDPDJH DQG FKDQJH LQ VWUXFWXUDO LQWHJULW\ RI KLSSRFDPSDO &$3 S\UDPLGDO QHXURQV. 7KH FKDQJH LQ VWUXFWXUDO LQWHJULW\ RI KLSSRFDPSXV PD\ DOWHU WKH FRJQLWLYH IXQFWLRQ OLNH OHDUQLQJ DQG PHPRU\.

&RQÀLFW RI ,QWHUHVW: 1RQH

)XQGLQJ: 1LO

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:H WKDQN 'U. 6DUDOD 1 3URI., 'HSW. RI 3KDUPDFRORJ\ DQG ,QFKDUJH-$QLPDO KRXVH IDFLOLW\, 6UL 'HYDUDM 8UV 0HGLFDO &ROOHJH, .RODU IRU SURYLGLQJ DQLPDO KRXVH WR FDUU\ RXW WKLV GRFWRUDO VWXG\, ZH WKDQN U. 0XQLQDUD\DQD 9HWHULQDULDQ IRU KLV VXJJHVWLRQV GXULQJ WKLV VWXG\ DQG ZHDOVR WKDQN0U. 5DYL 6KDQNDU, 6WDWLVWLFLDQ IRU KLV KHOS.

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1. 0 R E L O H S K R Q H U D G L D W L R Q D Q G K H D O W K . $ Y D L O D E O H I U R P : KWWSV://HQ.ZLNLSHGLD.RUJ/ZLNL/0RELOHBSKRQHBUDGLDWLRQBDQGBKHDOWK.
2. ,&7 IDFWV DQG ¿JXUHV 2017-,78. $YDLODEOH IURP: KWWSV://ZZZ.LWX.LQW/HQ/,78-'/6WDWLVWLFV/'RFXPHQWV/IDFWV/,&7)DFWV)LJXUHV2017.SGI.
3. $FKDU\D,-.3, & $FKDU\D,,., & DJKUH\,'. (2013). $ VWXG\ RQ VRPH RI WKH FRPPRQ KHDOWK HIIHFWV RI FHOO-SKRQHV DPRQJVW FROOHJH VWXGHQWV. &RPPXQLW\ 0HGLFLQH & +HDOWK (GXFDWLRQ, 3(4),214.
4. :+2 5HVHDUFK DJHQGD IRU UDGLR IUHTXHQF\ ¿HOGV. 3XEOLVKHG LQ 2010. $YDLODEOH IURP: KWWS:// ZZZ.ZKR.LQW/SHK-HPI/UHVHDUFK/DJHQGD/HQ
5. 2VPHQ,:., & $ZDGK 6DDU, $.$. (2015). $ZDUHQHVV FDPSDLJQ DJDLQVW FHOO SKRQH UDGLDWLRQ KDJDUG: &DVH VWXG\ 2PDQ. 3URFHGLD- 6RFLDO DQG %HKDYLRUDO 6FLHQFHV, 205(2015), 381-386.
6. 6ULYDVWDYD,$., & 7LZDUL,5... (2013). (IIHFW RI HIIHFWLYH XVH RI FHOO SKRQH RQ DGROHVFHQW¶V PHQWDO KHDOWK TXDOLW\ RI OLIH. ,QWHUQDWLRQDO 0XOWLGLVFLSOQDU\ H-RXQDO, 2(1), 01-10.
7. .HULPRJOX,*., & +DQFL,+., & %DV,0., & $VODQ,$., &(URO,+.6., & 2GDFL,(. (2016). 3HUQLFLRXV HIIHFWV RI ORQJ-WHUP, FRQWLQXRXV 900-0+] HOHFWURPDJQHWLF ¿HOG WKURXJKRXW DGROHVFHQFH RQ KLSSRFDPSXV PRUSKRORJ\, ELRFKHPLVWU\ DQG S\UDPLGDO QHXURQ QXPEHUV LQ 60-GD\-ROG 6SUDJXH'DZOH\ PDOH UDWV. -RXUQDO RI &KHPLFDO 1HXURDQDWRP\, 77(2017), 169-175.
8. $IHHI\,$.$., & 7ROED,$0.$., & $¿¿,2...(2013). $ +LVWRRJLFDO DQG ,PPXQRKLVWRFKHPLFDO VWXG\ RQ WKH HIIHFW RI PRELOH SKRQH UDGLDWLRQ WKH KLSSRFDPSXV RI DGXOW DQG QHZERUQ DOELQR UDWV. 1DWXUH DQG 6FLHQFH, 11(8), 98-113.
9. 'DQLHV,:0.8., & 3LWRXW,,./., & $IXOOR,7-.2., & 0DEDQGOD, 0.9.(2009). 7KH HIIHFW RI HOHFWURPDJQHWLF UDGLDWLRQ LQ WKH PRELOH SKRQH UDQJH RQ WKH EHKDYLRXU RI WKH UDW. 0HWDE %UDLQ 'LV, 24(4), 629-641.
10. %ROOD,6.5.(2015). (IIHFW RI PRELOHSKRQH UDGLRIUHTXHQF\ RQ KLSSRFDPSDO &$3 QHXURQV. ,QWHUQDWLRQDO -RXUQDO RI$QDWRP\ DQG 5HVHDUFK, 3(3),1216-1224.
11. 1DUD\DQDQ,6.1., & .XPDU,5.6., & .DUDQ,..0., & 1D\DN,6.%., & %KDW,3.*.(2015). 3RVVLEOH FDXVH IRU DOWHUHG VSDWLDO FRJQLWLRQ RI SUHSXEHVFHQW UDWV H[SRVHG WR FKURQLF UDGLRIUHTXHQF\ HOHFWURPDJQHWLF UDGLDWLRQ.0HWDE%UDLQ'LV,30(5),1193-1206.
12. 1DUD\DQDQ,6.1., & .XPDU,5.6., & 3RWX,%..., & 1D\DN,6., & %KDW,3.*., & 0DLODQNRW,0. (IIHFW RI UDGLRIUHTXHQF\ HOHFWURPDJQHWLF UDGLDWLRQ (5)-(05) RQ SDVVLYH DYRLGDQFH EHKDYLRXU DQG KLSSRFDPSDO PRUSKRORJ\ LQ ZLVWDU UDWV. 8SVDOD MRXUQDO RI PHGLFDO VFLHQFHV, 115(2),91-96.

REGIONAL SOCirv v or AnAvoviSTS (RSA)
ANDHRA PRADESH & TELANGANA
{Chapter of Anatomical Sociefi of Sndia)

Presentation Certificate

This is to certify that Ms/Mr/Dr G. KRISHNA KISHORE
of ..... *has presented a paper on* Effect of non-ionising radiation on spatial learning-memory in Swiss Albino mice ..... *in the 17th Regional Society of Anatomists Meet-2018 held during Medical College, Nellore, Andhra Pradesh.*

ACCP€OffAffON

Andhra Pradesh WedicatCouncithm awarded4 wedit points for thu conference

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Principal, NMC

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Chairman, APktC

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Registrar, APMC

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Observer, APktC

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NMC Best Paper Award

Certified that Ms/Mr/Dr G. KRISHNA KISHORE, SDUMC, KOLAR *has been awarded 5W1C dHh'TCAPEC¿A Wp!fW far the 6est OraL/Yoster presentedat firirof;/tc Session.* ..... io *the* ›zi7 a»gio»sf zorieiy ofp»«ionais weei-zola fie&&«sg zi7 «»&aifi »fj«fy 2o1a «i wa‹<y<xa w‹#irafi *College, Nellore, Andhra Pradesh.*

Title of the paper : Effect Of Non Ionizing Radiation On tr Learning in Swiss Albino Mice.

Conference Venue : Department of Anatomy, Narayana Medical Col ge, & GH., ChintHreddypabm, Nettore, AndhYa Mades£ —524003—/n4iA.

Date: 08,/'07/2é1.'8

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Pf‹eddent of RSA	General Secretary of RSA	Principal, NMC	Organizing Se?rtté!ñj

Effect of long term exposure of Mobile Phone Radio-Frequency Electro Magnetic Radiation (MP RF-EMR) on cognitive function and hippocampal morphology in albino mice

Thesis submitted to

Sri Devaraj Urs Academy of Higher Education & Research

In partial fulfilment for the degree of Doctor of Philosophy in Anatomy under Faculty of Medicine

Submitted by:

Mr G. Krishna Kishore

Under the Guidance of

Dr Venkateshu K.V

Department of Anatomy

Sri Devaraj Urs Medical College

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JUNE -2020

CHAPTER-7

CONCLUSION

AND

SUMMARY

CONCLUSION-

- In this present study, we evaluated the chronic exposure effect of MP RF-EMR-4G (1800-2100 MHz) on cognitive functions like spatial learning, working memory and hippocampal morphology in adult swiss albino mice.
- We observed that MP RF-EMR exposed mice took significantly increased time to reach the target chamber in Hebb-Williams maze when compared to the control group and in T maze the exposed animals shows significant difference in percentage of correct responses.
- Radiation exposed hippocampal CA3 neurons showed less number of pyramidal cells with darkened nuclei (Non-viable), vacuolation/ empty spaces in between cells and cells were scattered in arrangement.
- The results in our study reveals that prolonged exposure to 2100 MHz mobile phone electromagnetic radiation leads to damage and change in structural integrity of hippocampal CA3 pyramidal neurons and dendritic remodelling like decreased dendritic branching points and intersections.
- The change in structural integrity of hippocampus may alter the cognitive function like learning and memory.

SUMMARY-

- The mice exposed to chronic multiple durations like 3 months, 6 months and 9 months with mobile radiation (Non-ionising), was capable of altering the behaviour- spatial learning, working memory and structural integrity of hippocampal CA3 pyramidal neurons, which indicates the neurons of brain was affected by mobile radiation.
- The altered environment does not allow the neurons of brain for normal functioning, which is leading to change in the structural integrity of the neurons and may lead to the apoptosis of the neurons.
- The change in the structural integrity reflects the behaviour (Spatial learning and working memory) of the rodent.
- It is advised to be precautious and not to expose continuously to longer durations of mobile radiation and other sources of radiation.
- So in this present study we focused on the mobile radiation effects on rodents behaviour & structural integrity of the neurons, however there is no evidence studies stated that this type of changes can happen in human population.