STUDY OF VITAMIN E SUPPLEMENTATION ON STRESS & RADIO-FREQUENCY ELECTROMAGNETIC RADIATIONS (RF-EMR) EMITTED FROM MOBILE PHONES INDUCED EFFECTS ON THE FEEDING BEHAVIOR IN RODENTS

Thesis submitted to

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH

(Deemed to be University)

For the degree of Doctor of Philosophy (Ph.D.)

By

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August 2022

DECLARATION BY THE CANDIDATE

I, **P Pravallika** hereby declare that this thesis entitled **"Study Of Vitamin E Supplementation On Stress & Radio-Frequency Electromagnetic Radiations (RFEMR) Emitted From Mobile Phones Induced Effects On The Feeding Behavior In Rodents"** is an original research work carried out by me during the period from August - 2017 to August - 2022 for the award of **Doctor of Philosophy** in the subject of Physiology (Faculty of Medicine) under the supervision of **Dr. Vinutha Shankar.M.S,** Professor and Head, Department of Physiology, and Co-supervision of **Dr.Harendra Kumar ML,** Former Professor of Pathology, Sri Devaraj Urs Medical College, Sri Devaraj Urs Academy of Higher Education and Research (Deemed to be University).

No part of this thesis has formed the basis for the award of any degree or fellowship previously elsewhere.

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Pravallika Pagadala

LIST OF ABBREVIATIONS

MP	*Mobile Phone*
S.D	*Sprague Dawley*
N	*Number of rats*
RF-EMR	*Radiofrequency Electromagnetic Radiation*
BBB	*Blood-Brain-Barrier*
HPA Axis	*Hypothalamo-Pituitary-Adrenal axis*
GSM	*Global System for Mobile Communication*
DG	*Dentate Gyrus*
G	*Grams*
CA	*Cornu-Ammonis*
BBB	*Blood-Brain-Barrier*
HPA Axis	*Hypothalamo-Pituitary-Adrenal axis*
NF	*Neurofilament*
GFAP	*Glial Fibrillary Acidic Protein*
SAR	*Specific Absorption Rate*
WHO	*World Health Organisation*
CNS	*Central Nervous System*
GHz	*Giga Hertz*
MHz	*Mega Hertz*
KHz	*Kilo Hertz*
IHC	*Immuno Histo Chemistry*
DCS	*Digital Cellular System*
SD	*Standard Deviation*
LH	*Luteinizing Hormone*
SOD	*Superoxide Dismutase*
ROS	*Reactive Oxygen Species*
CT	*Computed Tomography*
MDA	*Malondialdehyde*
OD	*Optical Density*
Ng	*Nano gram*

MRI	*Magnetic Resonance Imaging*
Min	*Minute*
Mg	*Milligram*
CAT	*Catalase*
TAC	*Total antioxidant capacity*
W	*Watt*
ELF	*Extremely low frequency*
µl	*Microlitre*
µg	*Migrogram*
Ml	*Milli liters*
Mg	*Milligrams*
µM	*Micro molar*
ANOVA	*Analysis of variance*
HRP	*Horseradish Peroxidase*
UV	*Ultraviolet*
US	*United states*
BDNF	*Brain-derived neurotrophic factor*
GFAP	*Glial fibrillary acidic protein*
NSE	*Neuronal specific enalose*
FB	*Feeding behaviour*
GSH	*Glutathione*
GPX	*Glutathione peroxidase*
TBA	*Thiobarbituric acid*
TBARS	*Thiobarbituric acid reactive substances*
℃	*Celsius*
CAT	*Catalase*
CA1	*Cornu ammonis1*
CA2	*Cornu ammonis2*
CA3	*Cornu ammonis3*
CC	*Concentric circles*
cDNA	*Complementary-Deoxyribonucleic acid*
CNPase	*2', 3'-cyclic nucleotide 3'-phosphodiesterase*

DG	*Dentate gyrus*
CNS	*Central nervous system*
CPCSEA	*Committee for the Purpose of Control and Supervision of Experiments on Animals*
IAEC	*Institute animal ethics committee*
RNA	*Ribonucleic acid*
RNS	*Reactive nitrogen species*
ROS	*Reactive oxygen species*
RPM	*Revelations per minute*
RT	*Room temperature*
S	*Soma*
SDUAHER	*Sri Devaraj Urs Academy of Higher Education and Research*
SDUMC	*Sri Devaraj Urs Medical College*
Sec	*Seconds*
SEM	*Standard error of the mean*
SPSS	*Statistical package for social sciences*
TBS	*Tris-buffered solution*

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Chapter-I

Introduction

1. INTRODUCTION

1.1. Stress: Exposure to stress is inevitable, and it may occur, to varying degrees, at different phases throughout the lifespan. Stress is a disruption of the normal body's homeostasis or a state of disharmony caused by a real or perceived threat or challenge. A "stressor" is a situation that poses a threat or challenge (Chrousos GP et al 1992). Stress is a term used to describe a state characterized by a wide range of physiological and behavioral changes caused by one or more stressors. Biologically it is defined as "the non-specific reaction of the body to any demand" (Dhir et al., 2006). Stress, whether emotional or physiological, can have an impact on brain structure and function (Yau & Potenza, 2013). In today's world, we are exposed to a wide range of stressors.

1.1.1. Impact of stress on health:

Stress is a significant individual and public health problem that is associated with numerous physical and mental health concerns. It is estimated that between 75% and 90% of primary care physician visits are caused by stress-related illnesses (Mohd. Razali Salleh et al., 2008). Cardiovascular disease, obesity, diabetes, depression, anxiety, immune system suppression, headaches, back and neck pain, and sleep problems are some of the health problems associated with stress (Agnese Mariotti et al., 2015). These conditions are most burdensome health problems in the United States based on health care costs, the number of people affected, and the impact on individual lives.

Stress levels in Indians was very high compared to other countries, about 82% of Indian population are suffering from stress. Extreme levels of stress were reported by 22% of respondents from the 2011 Stress in America™ survey, and 39% reported that their level of stress had increased during the past year. More than 80% of the survey respondents at the World at Work Conference in 2012 reported that stress moderately or significantly contributed to their health care costs. High Stress can increase the risk for chronic

diseases and other health problems, dealing with chronic conditions and poor health can increase the amount of stress one experiences. Stress also influences behaviors that affect health. Diet choices, sleep habits, and drug use are behaviors often negatively affected by stress. The APA's 2011 survey showed that 39% percent of respondents reported overeating or eating unhealthy food because of stress, and 29% reported skipping a meal. In addition, 44% reported lying awake at night because of stress. On a positive note, 47% of respondents reported walking or exercise as a way of managing stress (American Institute of Stress Web site 2012).

Stress affects the brain both physiologically and chemically. Depression and anxiety disorders are extremely common during stress. It is clear that these disorders are quite common in the general population, yet there is much to be learned about the causes of these disorders. Symptoms of depression include depressed mood, anhedonia, altered appetite, nervousness, fatigue, and lack of concentration, frequent colds, sleep disturbance and irritability (Gregus et al., 2005). These psychopathologies arise due to a complex interaction between genetic predisposition and an adverse environment (Gregus et al., 2005). Many studies have off let shown that Radiofrequency emitted radiation (RF-EMR) from mobile phones produces a stress response. It might be due to its effect on hypothalamus (Mary Zosangzuali et al 2021).

A stressful situation induces the organism to activate the adrenal system, the central nervous system, and the pituitary (Kayalvizhi et al., 2012). Stress disrupts an organism's natural homeostasis; as a result, the organism may respond to stress by creating a physiological reaction to reclaim the balance lost due to the stressor's influence. Stress has long been thought to play a role in developing addictive illnesses and the relapse of addictive behaviors (Koob et al.,2008; Kreek et al., 2005) The response to a stressor

depends on how the individual assesses their environment and the stressors they experience. When the stressor exceeds a person's ability to withstand or ability to respond appropriately to the stress, the homeostasis is disturbed. Feeding behavior is one example of disrupted homeostasis (Lanzenberger et al., 2007).

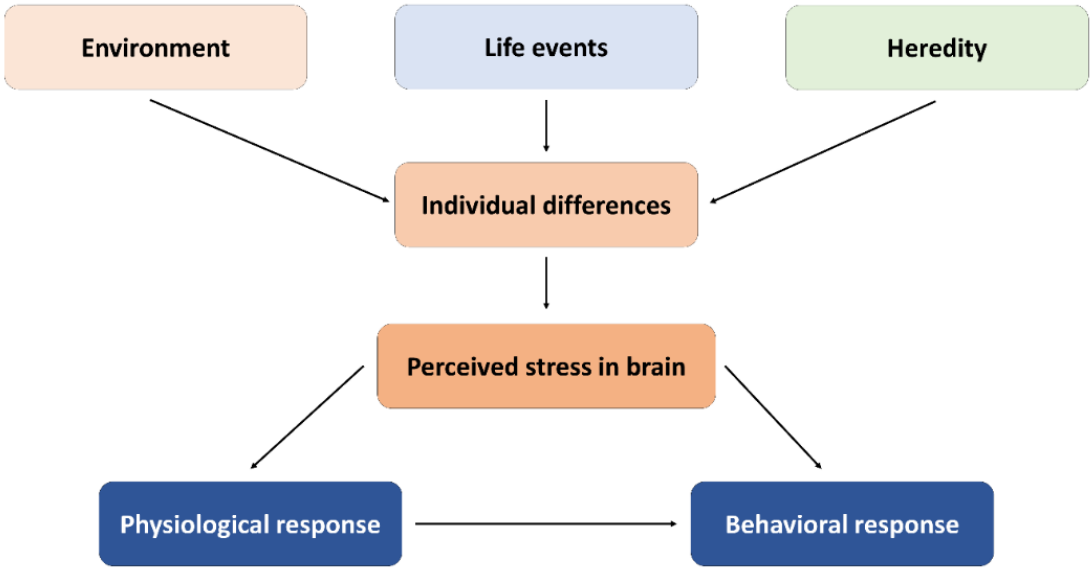


Fig 1.1 Individual differences in stress. Stress does not affect each individual the same way. A stimulus that may be stressful to one individual may not be stressful to another. Environment, life events, and genetics play a role in an individual's tolerance for stress. When an individual perceives a stimulus as stressful, a physiological and behavioral response will be displayed to regain the equilibrium lost by the stressor (Atchley et al, 2011).

1.1.2. Radiofrequency Electromagnetic radiations from mobile phone as a stressor: Mobile phones have become an integral part of our lives with increasing users and an increase in talk time. At present, Mobile phone usage is ubiquitous with an estimated 6.9 billion subscriptions globally (Narayanan et al., 2010). It is estimated that in 104 countries, approximately 80% of children are online; 94% of children aged 15-24 years

use the internet in developed countries, 67% in developing countries, and only 30% in least developed countries (Nittby H et al., 2008). It is concerning that 320 million (39%) of the 830 million young people online are from India and China.

The world's natural environment is now contaminated with man-made electromagnetic radiations (EMR) (Hardell, L et al., 2007). Beside radio communication and electricity, mobile phone (MP) communication devices are the key sources of EMR. While using these technical devices we expose ourselves to EMR generated by these devices (Hardell, L et al., 2007). The biological effects of RF-EMR are a rising field of interest, in context to "environmental effects" on human health (Ragy, M. M et al., 2014). Several electronic devices may create damaging EMR which affect human health (Ongel, K et al., 2007). Among these devices, the MP are significant cause of concern to community health all over the world. MP communication is by transmitting radio waves through a network of fixed antennas called base stations (Adebayo, EA et al., 2019). Radiofrequency waves are non-ionizing electromagnetic radiation, as opposed to ionising radiation, which can neither break chemical bonds nor cause ionisation in the human body. It has been reported that microwave electromagnetic radiation (EMR) had effects on humans which could not be explained by detectible heating of tissue (Ongel K et al., 2007). The different tissues in different organs have their own dielectric properties, as described in Durney, et al (1999). The accurate representation of human response to microwave radiation is remarkably complex (Cundin and Roach, 2010). Therefore, the increased exposure to RF-EMR and its effect on biological systems has been an area of interest, especially its impact on brain cerebral blood flow, blood-brain barrier, neuronal damage, etc. (Narayanan et al., 2010).

It was discovered that the EMR had no discernible effect on body temperature. However, it resulted in a significant depletion of brain ATP and related neural energy stores, in

addition to the normal depletion caused by increased metabolism during hyperthermia. Microwaves can reduce brain metabolic rate, which appears to result in impaired brain function. It has been discovered to cause cellular heat-stress responses far more easily than other types of stress, including heat-related stress (Sage & Carpenter et al., 2012).

The mobile phone emits non-ionising radiation with a low frequency that is thought to be safe, but recent studies show that it has an effect on living tissues, particularly the brain, causing headaches, memory loss, and heat over the ear, decreased concentration, and other cognitive effects (Mubeen S M et al., 2008).

1.2. Feeding behavior: Stress affects the feeding behavior bidirectionally, inducing an increase or decrease in feeding behavior *via* the hypothalamo-hypophyseal-adrenal axis (Maniam & Morris et al., 2012). Feeding behavior is related to obtaining & consuming food. It is a complex behavior in our day-to-day life, regulated by many mechanisms like external factors such as food availability and quality, as well as by internal factors including sex, age, circadian rhythms and most importantly, the hormonal status related to energy homeostasis **(**Schwartz M.W et al., 2000)**.** The understanding of the physiological basis of feeding behavior regulation is particularly important, as in affluent societies obesity has become a widespread problem (Yu Lv S et al., 2013). But the exact regulatory mechanisms are not well understood. Studies have showed that hypothalamus is the center for controlling feeding behavior (Meunier N et al., 2010; Yu Lv S et al., 2013). Several hypothalamic neuropeptides such as agouti-related protein (AgRP) and neuropeptide Y (NPY) have been shown to be potent feeding stimulants, whereas melanocortins (POMC) and cocaine- and amphetamine-regulated transcript (CART) have been shown to suppress food intake (Fulu Bai et al.,2005). Hypothalamus is well known for its role in controlling feeding behavior, but the hippocampus is also believed to play

role in it because Food intake is a complex behavior that can occur or cease to occur for a multitude of reasons. Decisions about where, when, what, and how much to eat are not merely reflexive responses to food-relevant stimuli or to changes in energy status (Bast T., 2009).

1.3. Oxidative stress:

An imbalance between free radicals and antioxidants in our bodies causes oxidative stress. Free radicals are oxygen molecules that have an uneven number of electrons. Because of their odd number, they can easily react with other molecules. Because free radicals react so easily with other molecules, they can cause large chain chemical reactions in our bodies. These reactions are known as oxidation. They can be beneficial or detrimental (Aruoma OI et., 1999). Antioxidants are molecules that can donate an electron to a free radical while remaining stable themselves. As a result, the free radical stabilises and becomes less reactive. Oxidative stress is one of the primary mechanisms affecting brain by causing oxidative damage. During stressful conditions, oxidative stress reflects as an imbalance between the generated reactive oxygen species and the biological systems' ability to detoxify the reactive intermediates readily or repair the resulting damage (Jelinek M et al., 2021). Disturbances in the normal state of cells can cause toxic effects through the production of peroxides and free radicals that damage all cell components. In addition, it can cause disruptions in normal mechanisms of cellular signalling & is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidants such as glutathione (de Diego-Otero et al., 2009).

Oxidative stress may increase due to continuous exposure to RF-EMR, which is characterized by excessive generation of reactive oxygen species (ROS). Indeed, this phenomenon has been documented in the whole body and ovarian tissue models of

Drosophila, mouse fibroblasts, cultured breast cancer cells, rat heart tissue, human lens epithelial cells, and mammalian spermatozoa after RF-EMR exposure (Hou et al., 2015; Ozguner et al., 2005; Yao et al., 2008). In addition, it is also known to produce a stress-like response due to its effect on the hypothalamus (Mailankot et al., 2009).

1.3.1. Assessing oxidative stress:

It can be known by assessing biochemical parameters and histomorphology changes. Some of biochemical parameters includes Neuronal specific enalose (NSE), Malondialdehyde (MDA) and serum corticosterone levels whereas histomorphology changes can be known by assessing the Glial fibrillary acidic protein (GFAP) levels in the brain (Hacioglu et al., 2016).

1.3.1.1. NSE:

It is an enzyme found mostly in neurons and neuroectodermal cells that transforms glucose to compounds suited for oxidation anaerobically. NSE is a highly specific marker for neurons, peripheral neuroendocrine tissue, and APUD (Amine Precursor Uptake & Decarboxylation) cells, and as a result, it can be used as a biochemical marker for tumours arising cells. The serum NSE level is normally low in healthy people but increases significantly in cases of neuronal tissue damage, such as traumatic brain injury and stroke, and so is used as a biomarker for brain damage (Ciftci G et al., 2012). The serum NSE level also increases in cases of hypoxic brain damage; the serum concentration is proportional to the extent of brain damage.

1.3.1.2. MDA:

It is made from polyunsaturated fatty acids (PUFAs) by the action of human platelet thromboxane synthetase on prostaglandins PGH2, PGH3, and PGG2, as well as spermine by the action of polyamine oxidase and amine oxidase. The best studied lipid peroxidation products are MDA. MDA is converted to malonic acid semialdehyde in the liver. This is an unstable compound that decomposes spontaneously to acetaldehyde, which is subsequently transformed to acetate by aldehyde dehydrogenase, and lastly to carbon dioxide and water by aldehyde dehydrogenase (Lorente et al., 2013). Changes in conformation, enzymatic activity, or binding, as well as receptor deactivation, enhanced protease susceptibility, and immunogenicity, are the next phases. MDA is the precursor of the thiobarbituric acid reactive compounds (TBARS). MDA is one of the most often detected oxidative stress biomarkers, specifically lipid peroxidation. As a result, increased oxidative stress is commonly recognised as a disease (Tsikas D et al 2017). Lipid peroxidation is a process in which oxidants such as free radicals destroy lipids that contain carbon-carbon double bonds, particularly polyunsaturated fatty acids (PUFAs). A substantial body of work on lipid peroxidation has been published over the last four decades, demonstrating its importance in cell biology and human health (Ozguner et al., 2005).

1.3.1.3. Serum corticosterone:

The major glucocorticoid involved in the control of stress reactions in rats is corticosterone. The stress of exogenous origins was reported to elevate the corticosterone levels in various organisms (Jeong et al., 2013). Some studies shows that stress is known to cause a significant increase in glucocorticoid levels in the blood. The hypothalamic–pituitary–adrenal (HPA) axis is the most important hormonal system with a well-defined

circadian pattern. This rhythm is changed when people are stressed, and it has a negative impact on their health. The degree of HPA axis activation is also proportional to the level of stress experienced by animals. Stress makes rats extremely sensitive, and even a minor stressor causes a significant increase in glucocorticoid levels that approximates the amplitude of the diurnal cycle (Vyas S et al., 2016).

1.3.1.4. GFAP:

It is the major intermediate filament in astrocytes. It interacts with other cytoskeleton components, adhesion molecules, intracellular chaperones, and other proteins, just like other intermediate filaments. The cytoarchitecture and mechanical strength of astrocytes, as well as their supportive functions on the physiology of surrounding neurons and the preservation of the blood–brain barrier, are all governed by GFAP. It plays a role in the pathogenesis of a number of neurological diseases. In a variety of acute and chronic neurologic diseases, upregulation of GFAP expression is a hallmark of reactive astrogliosis (Liberto et al. 2004).

1.3.2. Site of oxidative damage in Brain:

Most common site of oxidative damage in the brain is hippocampus. The term "hippocampus" was first named by a Bolognese anatomist named "Giulio Cesare Aranzi", which is similar to "Hippocampus liera" the tropical fish (Duvernoy H et al., 1988). The hippocampal formation is located deeply in the medial temporal lobe, which consists of hippocampal proper, dentate gyrus, presubiculum, subiculum, parasubiculum (Andersen P et al., 2007). The structural integrity and pathway of hippocampal formation is similar in all mammals (Wegrzyn D et al., 2022). Animal models closely mimic humans, among them rats are almost similar (Karavasilis E et al., 2019).

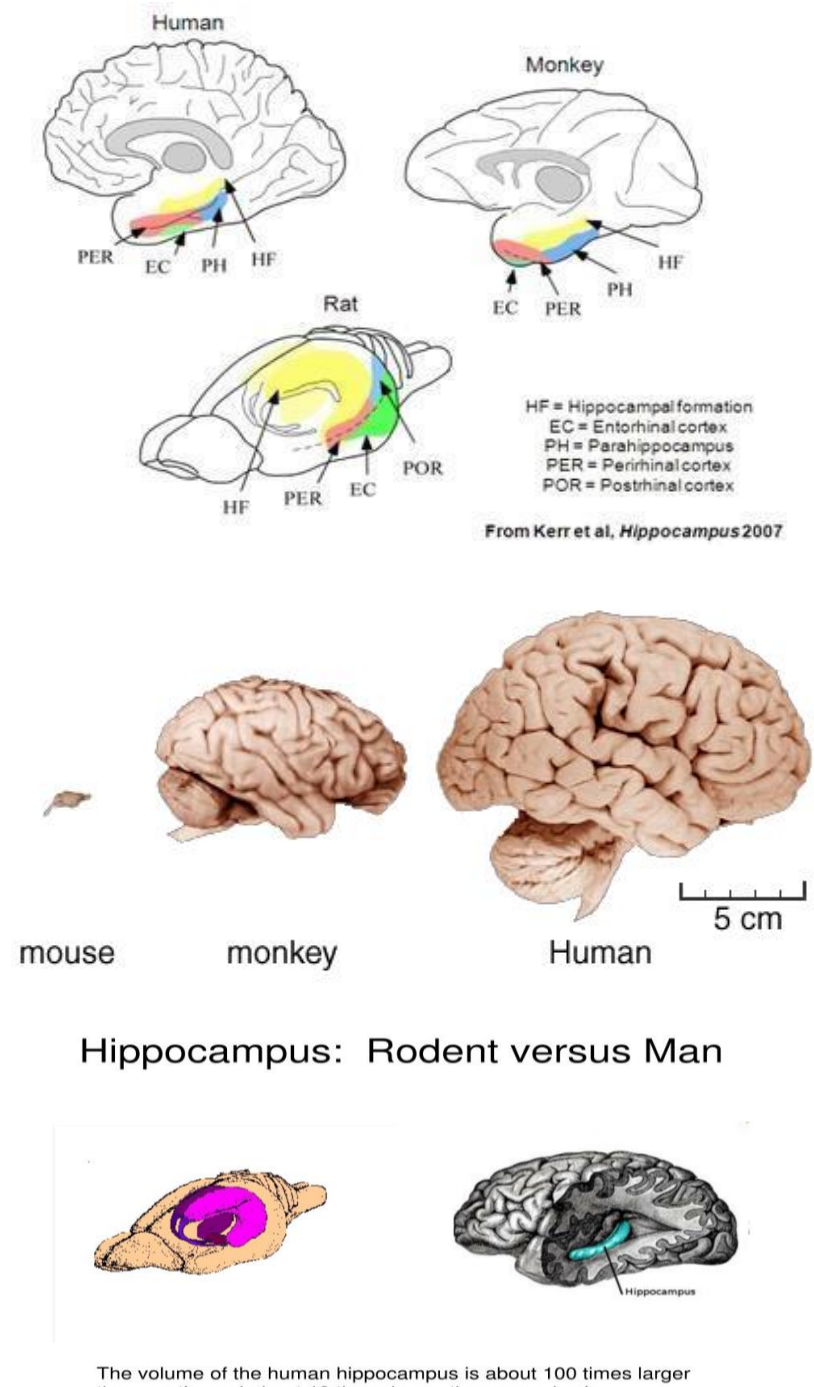


Figure 1.2: Image represents the anatomy of hippocampus in all mammals (Human vs Monkey vs Rat) -Image adapted from burwell et al. Anatomy of the Hippocampus and the Declarative Memory System.

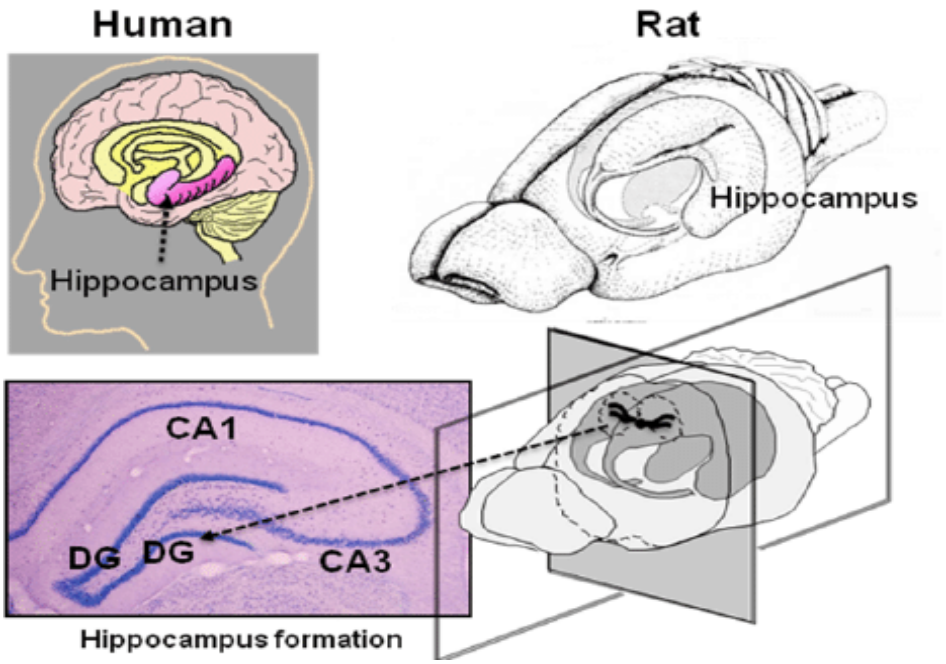


Figure 1.3: Image represents the anatomy of coronal section of hippocampus in mammals with its sub regions.

1.3.3. Managing and preventing oxidative stress:

It's impossible to completely avoid free radical exposure and oxidative stress. However, there are things, which can be done to minimize the effects of oxidative stress on our body. The main thing can be done is to increase the levels of antioxidants by decreasing the formation of free radicals (Valko M et al., 2007). One method of preventing oxidative stress is to ensure that we are obtaining enough antioxidants in the diet. Eating five servings per day of a variety of fruits and vegetables is the best way to provide our body what it needs to produce antioxidants. Antioxidants are "those chemicals that, when present in low concentrations compared to those of an oxidisable substrate, will significantly delay or block that substrate's oxidation" (Halliwell & Gutteridge et al., 1989). "An anti-oxidant is any agent that, when present in low concentrations compared to those of an oxidisable substrate, considerably slows or inhibits oxidation of that substrate," according to Halliwell (1997). Other examples of dietary antioxidant sources

include fish and nuts, vitamin E, vitamin C, turmeric, green tea, melatonin, onion, garlic and cinnamon (Elfowiris A et al.,2022). Among all the antioxidants, Vitamin E is a potent antioxidant and is well-known for its beneficial effects in animal models of various diseases.

1.4. Vitamin E:

Various studies have demonstrated the radio protective and anti-stress effects of antioxidants in stress models. Vitamin E has many biological functions, including its role as a fat-soluble naturally occurring antioxidant (Hassan & Awad et al, 2007). Vitamin E is classified as an antioxidant due to its ability to scavenge lipid radicals and terminate oxidative chain reactions (Alshiek JA et al., 2017). It can terminate radical chain reactions by interacting with the lipid peroxyl radical, preventing it from generating a new radical and perpetuating the chain reaction by oxidizing other lipids (Alshiek JA et .,2017). Following its oxidation, vitamin E can be recycled back to its native unoxidized form by various soluble antioxidants such as vitamin C and ubiquinol (Feki M et al., 2001). This process prevents the accumulation of vitamin E radicals and their subsequent peroxidation of lipids (Niki E et al., 2014), and is considered by some to be critical for the antioxidant activity of vitamin E (Chow CK et al., 2004). It has been suggested that all of the other biological functions of vitamin E are actually a result of its antioxidant activity (Flohé RB et al., 1999). As a potent antioxidant, it acts as a peroxyl radical scavenger, disabling the production of damaging free radicals in tissues. In addition, it is responsible for repairing wounds and regeneration of the damaged extracellular tissue (Villacorta et al., 2003).

Chapter-II

Review of literature

2. REVIEW OF LITERATURE

Exposure to stress is inevitable, and it may occur, at varying degrees, at different phases throughout the lifespan. Stress is the disturbance of the normal body's homeostasis or a condition of disharmony in response to a real or perceived threat or challenge. The threatening or challenge-causing situation is referred to as a "stressor" (Chrousos GP et al 1992; Dhir et al., 2006). When a person encounters a stressor, body prepares to respond to the challenge or threat. The autonomic nervous and endocrine systems respond by producing the hormones epinephrine, norepinephrine, and cortisol. The result of this hormone production is a cascade of physiological reactions that make up the stress response. Epinephrine and norepinephrine are involved in the initial changes that take place to prepare the body to react and to prepare for a challenge. These responses include increases in heart and respiration rates, blood pressure, perspiration, and energy production. There also suppression of immune function, production of β-endorphin (the body's natural painkiller), and increased acuity of the senses. These changes make up the fight-or-flight response, which prepares the body to cope with the stressor. If the stressor is perceived as negative or more as a threat than as a challenge, cortisol production is increased. Cortisol is involved in energy production but also suppresses immune function (Erica MJ et al., 2003).

A stressful situation induces the organism to mobilize not only the adrenal system and endocrine system but also the central nervous system and the pituitary (Kayalvizhi et al., 2012). One of the main mechanism by which stress affects the brain is by oxidative stress causing oxidative damage. During stressful conditions, oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and the biological systems ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal state of cells can cause toxic effects through

the production of peroxides and free radicals that damage all components of the cell. It can cause disruptions in normal mechanisms of cellular signaling & is associated with increased production of oxidizing species or significant decrease in the effectiveness of antioxidants such as glutathione (Koob et al., 2008; Kreek et al., 2005).

2.1. Types of stress:

It is very important to note that not all stresses or stressors are bad. Every individual experiences a certain amount of stress on an almost daily basis, it cannot be eliminated completely. When it is experienced too much then it becomes a problem. It has negative impact on behaviors, relationships, and health. Stress is of two kinds – *Eustress* - a positive form of good stress that motivates an individual to continue working and Distress – manifests when stress is no longer tolerable and/or manageable (Arria AM et al., 2009) . A stress may be eustress or individuals' perception and coping resources for the stressor (Erica MJ et al., 2013) can determine distress.

Based on duration of exposure it can be chronic or acute. Chronic stress is a persistent, long lasting stressor, such as living with a terrible roommate, money, work and economy, death of loved ones, divorce, family and personal health problems. It is not easily resolved associated with negative health concerns. It results when there are constant multiple stressors or major life stressors present (American Psychological Association. 2011). Acute stress, on the other hand, is a short lasting and one time stressor, such as failing an exam. Acute episodic stress occurs when an individual experiences acute stress on a consistent basis, such as overcommitting at work or constant worrying. Individuals who experience acute episodic stress often show signs and symptoms of stress that can negatively impact physical and psychological health (Arria AM et al., 2009). To avoid these consequences, these people can learn how to change their behaviors and manage

their stress. In addition, stress can be major or minor. A major stressor would be something like a death in the family, while a minor stressor may be as simple as getting stuck in traffic (Derek Atchley et al., 2011).

2.2. Types of induced stressors in animals:

Stress will be physiological and psychological

Physiological stress includes restraint stress, Forced swimming stress, Noise stress, Electric foot shock stress, Food deprivation.

Restraint stress or immobilization is commonly used because it is less severe, but is still capable of activating the stress response by stimulating the hypothalamic pituitary adrenal axis. In this type of stressor, movement is limited by placement of rodent in a plexiglass chamber or immobilization bag (Derek Atchley et al., 2011).

High intensity noise exposure (30 db) is also used as a physical stressor. This protocol can be used as a type of environmental stressor to mimic stress in everyday Life. This is given for 1hr/day for a period of 5 days a week, for 50 days. This stress is given for a period of 30min continuously & animal is allowed to take rest for 10 min, remaining 30min procedure is continued (Hacioglu Gulay et al., 2016).

Forced swimming stress: Animal made to swim in the water forcibly for 5 min then grasp the animal gently by the tail or trunk lift from the water, place it to dry. Like this procedure can be done by repeated intervals for 1 hour/day making sure that animal should not drown, for a period of 5days a week for 50 days (Hacioglu Gulay et al., 2016).

Electric foot shock stress: It is more severe stressor and can be applied using a metallic grid to shock the foot or to the tail. In this the suggested shock duration to be very short and the shock levels should be very low (Heinrichs SC et al., 2006).

Food deprivation stress: It is the restriction of free access to food for a certain period.

Psychological stress includes Maternal separation, Overcrowding, Social isolation, Sleep deprivation, Resident/intruder.

Maternal separation stress: It is an excellent prenatal stressor leads to activation of hypothalamopituitary adrenal axis due to separation and handling. This stressor involves removal of pup from the care of its mother for certain period of time (Heinrichs SC et al., 2006).

Overcrowding stress: Permissible limit of animals in a cage is 2-3 but in this involving the animals more than 4-5. (Heinrichs SC et al., 2006).

Social isolation: It is a common type of psychological stressor in which subject is placed in a long term solitary housing without any companion (Heinrichs SC et al., 2006).

Sleep deprivation: This type of stress is controversial because it is quite severe, in this animal is not allowed to sleep certain period of time by keeping the animal in a revolving drum (Heinrichs SC et al., 2006).

Resident/intruder stress: It is a social conflict stress involving threat from an aggressive male (Heinrichs SC et al., 2006).

2.3. Radiofrequency Electromagnetic radiations from mobile phone as a stressor:

Mobile phone technology uses radiofrequency electromagnetic radiation (RF-EMR) and has drastically increased the RF-EMR exposure encountered in daily life. Mobile phones are often prohibited in hospitals and on airplanes, as the radiofrequency signals may interfere with certain electro-medical devices and navigation systems. Many recent studies have raised questions regarding the safety of such RF-EMR exposure. For example, microwaves generated by mobile phones have been linked to several genetic defects (Hardell L et al., 2007). Research also suggests that microwave radiation from

mobile phones may induce chromosomal instability and lead to increased cancer risk (Ragy MM et al., 2014).

RF-EMR is a form of microwave radiation, important properties of which include the frequency at which it is generated, measured in megahertz (MHz) or gigahertz (GHz), and the intensity of the waves, or the specific absorption rates (SAR); the energy carried as a quantity with respect to mass in watts per kilogram. The transfer of energy from the electromagnetic field to particles in an absorber is measured by the SAR, which indicates the quantity of energy related to mass, defined at a particular point in the absorber (Durney et al., 1986).

The frequency of RF-EMR emitted by mobile phone devices is in the range of 900 to 1800 MHz and the intensity of this radiation is generally restricted to a local limit of <2 W/kg and whole-body limit of 0.08 W/kg (Chen et al., 2007; Durney et al., 1986) to enforce safe exposure levels in humans. Meanwhile, the ability of RF-EMR itself to penetrate into the skin and body is dependent on the permittivity and conductivity of the irradiated tissue, as well as the wavelength of the radiation, which is inversely related to the wave frequency. Therefore, at lower frequencies, the penetration of the RF-EMR is further and devices operating in the 900 MHz range will irradiate the body more approximately 25% of the body in humans compared to 20% penetration 90 at 1800 MHz (Durney et al., 1986).

Mobile phone communications uses a variety of different frequency ranges, with the most common utilising the 880-915MHz range for the global system for mobile communications (GSM) 900 uplink (from mobile phone to base station), 925-960 MHz for the GSM900 downlink (from base station to mobile phone), 1710-1785MHz for the DCS1800 uplink, 1805MHz-1880MHz for the GSM1800 downlink, 1920-1980MHz for the universal mobile telecommunications system (UTMS) data uplink and 2110-

2170MHz for the UTMS data downlink (Bolte & Eikelboom et al., 2012). Of particular interest is this radiofrequency range, in which majorities of studies have utilized exposure frequencies of 900-1800 MHz (Chen et al., 2007). This in turn forms the basis of the study.

Generalizing on the cellular commonality shared among insects and humans, Panagopoulos and Margaritis (2002) exposed fruit flies to GSM (~900 MHz) cell phone radiation at very low levels for just six minutes per day during the several days it takes such flies to hatch from newly fertilized eggs. The levels averaged between about 7-μW cm^2 and under 3mW cm^2. The exposed groups in their adult life showed a loss in reproductive activity varying between 15% and 60%, depending on estimated irradiance. The authors conclude that GSM radiation may be capable of serious biological damage to human cells (Margaritis et al., 2002).

2.3.1. 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 (Percuoco et al., 2014).

2.3.1.1. 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 (Panagopoulos et al., 2002).

Examples of ionizing radiation- Gamma rays &X-rays

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 (Hoong K et al., 2003).

2.3.1.2. Behavior of radiation-

- 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 (Percuoco et al., 2014).

2.3.2. 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 (Hoong K et al., 2003).
- 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 (Miller AB et al., 2010).

2.3.3. 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

2.3.4. SAR impacts:

The quantity of RF-EMR absorbed by body tissue while using an MP is measured in terms of specific absorption rate (SAR), which is measured in Watt per kilogramme (w/kg). Recommendations set a SAR limit of 2.0 W/Kg in 10 gm' of tissue, according to the International Commission on Non-Ionising Radiofrequency Protection. Most guidelines utilise a whole-body average SAR of 0.4W/Kg as the basic restriction, which is based on the reported effects of whole-body heating causing considerable elevation of core temperature(>1°C) (Jin J et al., 2018).

2.3.5. Exposure levels to mobile phones:

Mobile phones are low-powered radiofrequency transmitters, operating at frequencies between 450 and 2700 MHz with peak powers in the range of 0.1 to 2 watts. The handset only transmits power when it is turned on. The power (and hence the radiofrequency exposure to a user) falls off rapidly with increasing distance from the handset. A person using a mobile phone 30–40 cm away from their body – for example when text messaging, accessing the Internet, or using a “hands free” device – will therefore have a much lower exposure to radiofrequency fields than someone holding the handset against their head (Aalto, S et al.,2006).

In addition to using "hands-free" devices, which keep mobile phones away from the head and body during phone calls, exposure is also reduced by limiting the number and length of calls. Using the phone in areas of good reception also decreases exposure as it allows the phone to transmit at reduced power. The use of commercial devices for reducing radiofrequency field exposure has not been shown to be effective (Aalto, S et al., 2006).

2.3.6. Effect of mobile phones:

A large number of studies have been performed over the last two decades to assess whether mobile phones pose a potential health risk.

2.3.6.1. Short-term effects

Tissue heating is the principal mechanism of interaction between radiofrequency energy and the human body. At the frequencies used by mobile phones, the skin absorbs most of the energy and other superficial tissues, resulting in negligible temperature rise in the brain or any other organs of the body (Durney et al., 1986). A number of studies have investigated the effects of radiofrequency fields on brain electrical activity, cognitive function, sleep, heart rate and blood pressure in volunteers. To date, research does not suggest any consistent evidence of adverse health effects from exposure to radiofrequency fields at levels below those that cause tissue heating. Further, research has not been able to provide support for a causal relationship between exposure to electromagnetic fields and self-reported symptoms, or "electromagnetic hypersensitivity" (Foster KR et al., 2018).

However, there is some concern that short-term memory loss or other cognitive effects may be associated with the use of mobile telephones. Mounting evidence suggests that exposure to RF-EMR can influence learning and memory in rodents (Narayanan et al., 2010).

2.3.6.2. Long-term effects

Epidemiological research examining potential long-term risks from radiofrequency exposure has mostly looked for an association between brain tumours and mobile phone use. However, because many cancers are not detectable until many years after the interactions that led to the tumour, and since mobile phones were not widely used until

the early 1990s, epidemiological studies at present can only assess those cancers that become evident within shorter time periods. However, results of animal studies consistently show no increased cancer risk for long-term exposure to radiofrequency fields (Shih YW et al., 2021).

Several large multinational epidemiological studies have been completed or are ongoing, including case-control studies and prospective cohort studies examining a number of health endpoints in adults. The largest retrospective case-control study to date on adults, Interphone, coordinated by the International Agency for Research on Cancer (IARC), was designed to determine whether there are links between use of mobile phones and head and neck cancers in adults. The international pooled analysis of data gathered from 13 participating countries found no increased risk of glioma or meningioma with mobile phone use of more than 10 years. There are some indications of an increased risk of glioma for those who reported the highest 10% of cumulative hours of cell phone use, although there was no consistent trend of increasing risk with greater duration of use. The researchers concluded that biases and errors limit the strength of these conclusions and prevent a causal interpretation. Based largely on these data, IARC has classified radiofrequency electromagnetic fields as possibly carcinogenic to humans (Group 2B), a category used when a causal association is considered credible, but when chance, bias or confounding cannot be ruled out with reasonable confidence(International Commission on Non-Ionizing Radiation Protection (ICNIRP) 2009).

While an increased risk of brain tumours is not established, the increasing use of mobile phones and the lack of data for mobile phone use over time periods longer than 15 years warrant further research of mobile phone use and brain cancer risk. In particular, with the recent popularity of mobile phone use among younger people, and therefore a potentially longer lifetime of exposure, WHO has promoted further research on this group. Several

studies investigating potential health effects in children and adolescents are underway (Prasad M et al., 2017).

Due to the wide and growing use of mobile communication, there is increasing concern about the interactions of electromagnetic radiation with the human organs and, in particular, with the brain. Experimental studies have shown that the radiofrequency RF-EMR emitted from the mobile phones can affect the brain in various ways. These effects have been described in vitro and in vivo in a number of studies: in particular, effects on cerebral blood flow, blood-brain barrier permeability, oxidant and antioxidant balance, neurotransmitter balance, nerve cell damage, and genomic responses have been reported (Narayanan et al., 2010).

The effects of the RF-EMR emitted by these devices on biological systems and specifically the reproductive systems are currently under active debate. Within these 21 studies, 11 of the 15 that investigated sperm motility reported significant declines, 7 of 7 that measured the production of ROS documented elevated levels and 4 of 5 studies that probed for DNA damage highlighted increased damage due to RF-EMR exposure (Houston BJ et al., 2016). Associated with this, RF-EMR treatment reduced the antioxidant levels in 6 of 6 studies that discussed this phenomenon, whereas consequences of RF-EMR were successfully ameliorated with the supplementation of antioxidants in all 3 studies that carried out these experiments. A continued focus on research, which aims to shed light on the biological effects of RF-EMR, will allow us to test and assess this proposed mechanism in a variety of cell types (Maluin SM et .,2021).

Chronic MP RF EMR exposure has shown to significantly alter the passive avoidance behavior and hippocampal morphology by affecting the brain in rats (Narayanan et al., 2010). This was supported by another study that showed mice exposed to RF EMR in

utero were hyper active with impaired memory , that were determined by using the object recognition, light/dark box and step down assays (Aldad et al., 2012) . The exposure of RF EMR to foetal mice showed memory impairment by neuronal degeneration (Daniels et al., 2009). In a study it reported that RF EMR exposure had no significant effect on memory and brain morphology but decreases locomotion and increased grooming in rats (Daniels et al., 2009). Junior et al. also found that exposure to RF-EMR had no effects on anxiety and memory impairment in adult (60-day-old) rats (Júnior et al., 2014).

In a vivo study, the rats were allowed to expose to RF- radiation had a significant elevation in MDA content and a significant reduction in antioxidant parameters (glutathione, super oxide dismutase and glutathione peroxidase) in both regions. The study was carried out to investigate the effect of 1800 MHz RF radiation emitted from mobile phone on the rat's brain, the present study was performed. Forty male rats were randomly divided into two equal groups; control and exposed group. The rats that were exposed to RF- radiation had a significant elevation in MDA content and a significant reduction in antioxidant parameters (glutathione, super oxide dismutase and glutathione peroxidase) in both regions. Degenerative changes were observed in the hippocampus pyramidal cells, dark cells and cerebellar Purkinje cells with vascular congestion. In addition a significant DNA fragmentation and over expression of cyclooxygenase-2 apoptotic gene was detected. Those results suggested that, direct chronic exposure to mobile phone caused severe biochemical and histopathological changes in the brain. The findings indicate that chronic exposure to mobile radiation leads to change in structural integrity of hippocampus also alters the cognitive function like learning and memory. Regardless of these differences, the balance of evidence supports the principle that RF-EMR has the ability to induce cellular damage (Adams *et al*., 2014). In light of this conclusion and to work toward identifying real clinical risks, it is imperative that we

develop an understanding of the mechanism by which this form of radiation affects different biological systems.

Studies showed that stress and RF-EMR cause oxidative damage leading to oxidative stress. Medina et al. stated that neuronal damage leads to a significantly higher concentration of lipid peroxides by-products like MDA and neuronal damage marker NSE (Medina-Hernandez et al., 2007). It was proved that during oxidative stress, the levels of glutathione (GSH), the activity of superoxide dismutase (SOD), and glutathione peroxidase (GPX) were decreased (Edith Lubos et al 2011;Lushchak V et al., 2012). At the same time, MDA was increased in different brain regions of experimental rats (hippocampus and cerebellum, respectively) compared to the control group. MDA, the indicated marker for LPO, showed a significant elevation in the exposed group compared to the control (Hussein et al., 2016). Under normal conditions, the antioxidant defense mechanisms neutralized the over ROS production. GSH is a critical non-enzymatic antioxidant that plays a crucial role in ROS detoxification. SOD and GPX enzymes are the first line of cellular defence against oxidative injury. Exposure of adult rats to radiation emitted from mobile phones led to a significant reduction in GSH concentration (Hussein et al., 2016). During oxidative stress and brain damage, there was increased GFAP in response to hydrogen peroxide and cysteamine (Morgan et al., 1997).

Concerns about the possible health effects of mobile phone usage are growing as the number of users has increased tremendously over the past several years. Mobile phone `technology uses RF-EMR and has drastically increased the RF-EMR exposure encountered in daily life. Many recent studies have raised questions regarding the safety of such RF-EMR exposure (Mailankot et al., 2009). Many researchers believe that RF-EMR and the heat generated from mobile phones cause intensive damage. They have

suggested that the contribution of the non-thermal component is minimal and that the effects of mobile phone exposure would be negligible if the thermal effect could be eliminated (Mailankot et al., 2009).

2.4. Effects of oxidative stress:

Oxidation is a normal and necessary process that takes place in your body. Oxidative stress, on the other hand, occurs when there's an imbalance between free radical activity and antioxidant activity. When functioning properly, free radicals can help fight off pathogens. Pathogens lead to infections. When there are more free radicals present than can be kept in balance by antioxidants, the free radicals can start doing damage to fatty tissue, DNA, and proteins in your body. Proteins, lipids, and DNA make up a large part of your body, so that damage can lead to a vast number of diseases over time. These include diabetes, atherosclerosis, or the hardening of the blood vessels, inflammatory conditions, high blood pressure, which is also known as hypertension, heart disease, neurodegenerative diseases, such as Parkinson's and Alzheimer's and cancer. Oxidative stress also contributes to aging (Mifsud KR et al., 2018). Lipid peroxidation is a process in which oxidants such as free radicals destroy lipids that contain carbon-carbon double bonds, particularly polyunsaturated fatty acids (PUFAs). A substantial body of work on lipid peroxidation has been published over the last four decades, demonstrating its importance in cell biology and human health. MDA is one of the several byproducts formed during degradation of phospholipid cell membrane. Due to the damage of cell membrane by reactive oxygen species, Phospholipase A2 enzyme releases arachidonic acid from membrane phospholipids. In subsequent reactions this yields the formation of MDA (Lorente L et al., 2013). Oxidative stress and oxidative damages are well established cause for many chronic disorders. The etiopathogenesis of various chronic

diseases has been linked to oxidative stress, which also plays a key role in the ageing process (Ogura S et al., 2014).

2.4.1. Risk factors of oxidative stress:

Everyone produces some free radicals naturally in their body through processes like exercise or inflammation. This is normal and part of the body's intricate system of keeping itself healthy. You may also be exposed to free radicals in the environment. Some sources include ozone, certain pesticides and cleaners, cigarette smoke, radiation and pollution. A diet high in sugar, fat, and alcohol may also contribute to free radical production (Ketchesin KD et al., 2017).

2.5. Impact of stress and *RF-EMR* on Human Health

Some of the studies were done on humans shows that stress leads to cardiovascular diseases due to lipid accumulation by ROS (atherosclerosis, ischemia, hypertension, cardiomyopathy, cardiac hypertrophy, and congestive heart failure), Neurological diseases due to neuron loss (Parkinson's disease, Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), multiple sclerosis, depression, and memory loss), Respiratory diseases due to enhanced inflammation (asthma and chronic obstructive pulmonary disease (COPD)), Rheumatoid arthritis by the increased isoprostane and prostaglandin levels in synovial fluid of affected patients, kidney diseases by forming fibrotic tissue. (Dhalla N S et al., 2000).

Some of the studies shows that Non-ionizing radiation from MP does not damage DNA in cells or tissues, but it does cause thermal or heating effects. As a result, long-term exposure to high-power non-ionizing radiation (e.g., broadcast transmitters) may cause tumours, cancers, and neurobehavioral disorders. Furthermore, residents living near high-power lines are at an increased risk of leukaemia due to magnetic field exposure.

The risk of leukaemia decreases as one moves away from power lines (Zamanian et al., 2005).

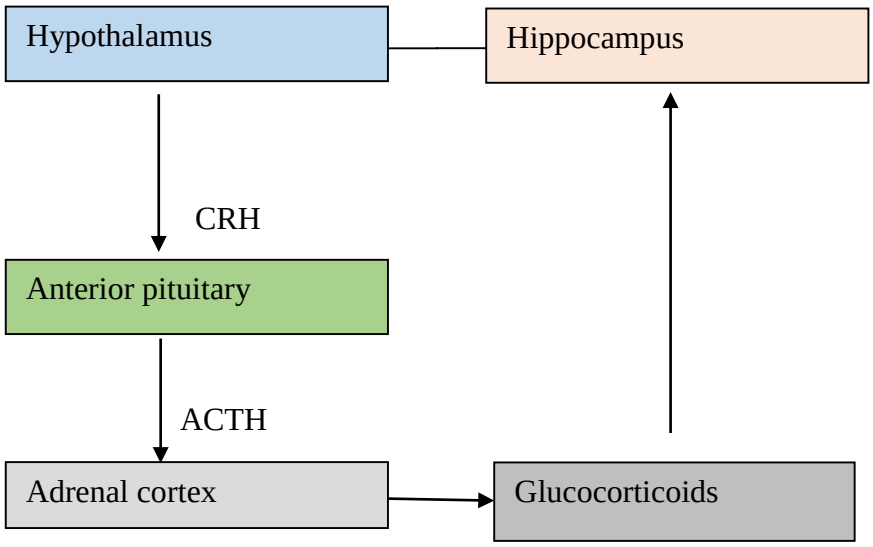
Due to the long-term impact of tissue heating, high levels of RF-EMR radiation can cause chronic headaches, and drowsiness (Carpenter DO et al., 2013). It has also been linked to neurobehavioral and neurobiological effects. However, additional research is required to clarify and confirm the effects (Zwoińska J K et al., 2015). RF-EMR exposure does not cause mitochondrial dysfunction, according to research (Hu et al., 2021). Long-term exposure to MP light can even cause cataracts, eye damage, and skin burns. Long-term UV exposure can also cause premature ageing, skin damage (including wrinkles, keratosis, and solar elastosis), corneal inflammation, and even skin cancer (melanoma, basal cell, and squamous cell cancers) (Yu Y et al., 2010) (Bhatia K et al., 2021). Long exposure to X-Rays and Gamma Rays (which are ionizing radiations) can cause leukemia, bone cancer, lung cancer, and other genetic mutations leading to cancers (Zamanian et al., 2005).

A community based cross sectional questionnaire study was done on 2121 human population and found that Headache, earache, neck pain, tinnitus, painful fingers, morning tiredness, fatigue, eye symptoms, sleep disturbance, and restlessness were discovered to be positively associated with mobile phone usage. There is a one more questionnaire based study which support this findings which was done on medical students in that they have found that headache, sleep disturbance, ear pain, irritability, Neck pain, Painful fingers, Eye symptoms Restlessness, Painful fingers, Fatigue, Earache(Sinha s et al.,2019).

2.6. Effect of acute and chronic stress on hypothalamic pituitary adrenal axis:

It is well known that the hypothalamic-pituitary adrenal (HPA) axis helps in maintaining basal and stress related homeostasis of the nervous system as well as cardiovascular, immune and metabolic functions. The main regulation of circadian and stress-related activity of the HPA axis occurs at the level of parvicellular subdivision of the hypothalamic paraventricular nuclei (PVN). The majority of these neurons secrete CRH and vasopressin (VP), which synergistically stimulate ACTH secretion by the pituitary corticotrophic cells. ACTH then enters the systemic circulation, stimulating corticosterone (CORT) synthesis from the cholesterol (CHOL) and its release from the adrenal cortex, which in turn provides an inhibitory feedback signal to the system (Djordjevic J et al.,2003).

Stress, either acute mild stress or prolonged chronic stress, can also influence our appetite, including our drive to eat and the types of food we are likely to select (Currie P et. 2003).



Source Fig 2.1: Derek Atchley. The Time-Course of the Effects of Stress on Behavior in Rodents. Eukaryon, 2011; Vol. 7.

During acute stress corticotropin-releasing hormone (CRH) is released from the medial parvocellular (mp) paraventricular nucleus of the hypothalamus (PVN), ACTH is

released from pituitary and the cascade of reactions takes place for the release of glucocorticods.CRH is also released from arcuate nucleus to inhibite neuropeptide Y and agouti-related peptide (AGRP) (these are the hormones increases food intake and suppresses energy expenditure). Urocortins are the molecules belongs to CRH family also released at this time which are also responsibe for suppresing appetite by inhibiting the ghrelin activity. Thus during acute stress CRH is responsible for inhibiting the appetite (Currie P et. 2003) (Richard D et al., 2002). Thus, CRH injected directly into the dorsal anterior bed nucleus of the stria terminalis (BNST) not on the ventral part or other brain regions such as the central amygdala or locus coeruleus) significantly reduces food intake in already food-deprived rats (Ciccocioppo R et al., 2003).

During chronic stress glucocorticoids released from HPA in the bloodstream are elevated. Peripherally, glucocorticoids enhance the activity of lipoprotein lipase in adipose tissue, leading to an increase in fat storage (Jorntorp P et al., 2001). Thus, in humans, a peripheral injection of CRH leads to increased food intake 1 h later but the amount of food consumed is directly correlated with the magnitude of the cortisol response to the injection (George SA et al., 2010). Glucocorticoids stimulate food intake by interacting with several appetite-regulating targets. They increase AMP-activated protein kinase signalling in the ARC to up-regulate NPY and AGRP expression in this region and stimulate the actions of these orexigenic peptides (increases food intake) (Shimizu H et al.,2008) . Glucocorticoids also influence food intake by enhancing the preference for "comfort foods." Insulin's suppressive effect on reward pathways likely means the food needs to be more "rewarding" to achieve the same effect; hence under stressed conditions rats prefer foods that are high in fat and sucrose when a choice is available (Warne J P et al., 2006).

Stress is a challenge to the natural homeostasis of an organism; in turn, the organism may react to stress by producing a physiological response to regain equilibrium lost by the impact of the stressor. Stress has long been considered as a critical risk factor in the development of addictive disorders and relapse to addictive behaviors (Kreek MJ et al., 2005; Koob GF et al., 2008). The response to a stressor depends on how the individual assesses their environment and the stressor they experience. When the stressor exceeds a person's ability to withstand or ability to respond appropriately to the stress, the homeostasis is disturbed (Derek Atchley et al., 2011). One such homeostasis that is disrupted is in feeding behavior (Derek Atchley et al., 2011).

2.7. Feeding behavior:

Feeding behavior is defined as the behaviors related to obtaining &consuming food. It is a complex behavior in our day to day life that is regulated by many mechanisms like external factors such as food availability y and quality, as well as by internal factors including sex, age, circadian rhythms and most importantly, the hormonal status related to energy homeostasis (Schwartz M.W et al., 2000**)**. The understanding of the physiological basis of feeding behavior regulation is particularly important, as in affluent societies obesity has become a widespread problem (Berthoud HR et al., 2004**)**. But the exact regulatory mechanisms are not well understood. Since hypothalamus is the center for controlling feeding behavior, studies have proved the role of hypothalamus in feeding behavior and is mainly under the control of leptin and insulin (Dhir A et al., 2006; Yvonne H et al., 2013; Hillebrand JJ et al., 2002). Several hypothalamic neuropeptides such as agouti-related protein (AgRP) and neuropeptide Y (NPY) have been shown to be potent feeding stimulants, whereas melanocortins (POMC) and cocaine- and amphetamine-

regulated transcript (CART) have been shown to suppress food intake(Stanley S et al.,2005)**.**

2.7.1. Physiology of feeding:

2.7.1.1. Feeding & satiety:

Body weight depends on the balance between caloric intake and utilization of calories. Food intake is regulated not only on a meal to meal basis but also in a way that generally maintains weight at a given set point. If animals are made obese by force feeding and then permitted to eat as they wish, their spontaneous food intake decreases until their weight falls to the control. If animals are starved and then permitted to eat freely, their spontaneous food intake increases until they regain the lost weight. It is common knowledge that the same thing happens in humans. Dieters can lose weight when caloric intake is reduced but when they discontinue their diets, 95% of them regain the weight they lost. Similarly, during recovery from illness, food intake is increased in a catch up fashion until lost weight is regained (Albanes D et al., 1987).

2.7.2. Role of hypothalamus:

Hypothalamic regulation of the appetite for food depends primarily upon the interaction of two areas: a lateral feeding center in the bed nucleus of the medial forebrain bundle at its junction with the pallidohypothalamic fibres, and a medial satiety center in the ventromedial nucleus. Stimulation of the ventromedial nucleus causes cessation of eating, whereas lesions in this region cause hyperphagia and, if the food supply is abundant, the syndrome of hypothalamic obesity. Destruction of the feeding center in rats with lesions of the satiety center causes anorexia, which indicates that the satiety center functions by inhibiting the feeding center **(**Stanley S et al., 2005)**.**

Principal hypothalamic polypeptides that appear to play a role in the regulation of the appetite for food

Factors that Increases food intake	**Factors that decreases food intake**
Ghrelin	Peptide YY
Orexin A	Leptin
Orexin B	Glucagon
Neuropeptide Y	CCK, CRH, GRP
Melanin concentrating hormone (MCH)	Somatostatin
GHRH	Oxytocin
	Bombasin

Source Fig 2.1: Ganong. Review of medical physiology.21st edition.128-130.

Hypothalamus is well known for its role in controlling feeding behavior, but the hippocampus is also believed to play role in it because Food intake is a complex behavior that can occur or cease to occur for a multitude of reasons. Decisions about where, when, what, and how much to eat are not merely reflexive responses to food-relevant stimuli or to changes in energy status (Bast T., 2009).

2.7.3. Influence of hippocampus on feeding:

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 (Cohen NJ et al 1997). 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 (Afeefy AA et al 2013). The former represents conscious recollection of general factual information, whereas the latter represents autobiographical memories of explicitly recalled events (Eichenbaum H et al 2004). Eating a meal or snack can create an episodic memory that is later consolidated to long-term memory and recalled. Data showing that both meal initiation and meal size are influenced by the degree to which previous meals can be explicitly recalled support the

relevance of hippocampal-dependent episodic memory function to feeding behavior. Amnesic patients with extensive bilateral hippocampal damage, for example, who have difficulties establishing new episodic memories, will consume a second or even third meal offered only minute's later (Rozin P et 1998). Findings from other studies suggest that sensory-specific hedonic feeding modulation does not require hippocampal-dependent episodic memory of recent feeding occasions. It was also demonstrated in healthy human subjects that priming the explicit recall of a recent meal reduces the amount of food consumed at the subsequent meal (Higgs S et al 2008, Higgs S et al 2002).

2.8. Some of the review of works on stress related to feeding behavior:

Stress is known to alter feeding responses in a bidirectional pattern, with both increased and decreased intake of food (Jayanthi M et al., 2012). It is an important factor in the development of addiction and in addiction relapse, and may contribute to an increased risk for obesity and other metabolic diseases. Uncontrollable stress changes eating patterns and the salience and consumption of hyper palatable foods that induces metabolic changes that would promote weight and body fat mass (Yvonne H et al., 2013). Hamidreza Famitafreshi et al have demonstrated that food consumption was increased in addicted isolated rats when compared to addicted socialized rats and concluded that feeding behavior was regulated by adult hippocampal neurogenesis in addiction period and socialization improves it (Hamidreza F et al., 2016). Stress also causes elevated glucocorticoid levels leading to stimulation of feeding behavior and excessive weight gain (Luba S et al., 2014). Carr JA et al 2002 demonstrated that stress inhibits feeding behavior in all vertebrates. Restraint stresses have shown to reduce body weight due to decreased intake of food (Jooyeon j et al., 2013). Cristina et al demonstrated that acute stress causes weight loss and chronic stressors promote the activation of the pro-obesogenic mechanisms that favours the accumulation of central/visceral fat (Cristina R

et al., 2016). However Favreau-Peigne et al 2014 demonstrated that chronic stress altered animal welfare but also decreased body weight.

Although acute stress has been shown to have facilitating effects on memory, chronic stress causes the development of psychiatric disorders (depression and anxiety), leading to altered appetite (Neil S et al., 2005). Stress was associated with significant elevation in the markers of oxidative stress in the cerebral cortex. Gulay Hacioglu et al demonstrated that exposure to stress causes increased production of reactive oxygen species. Brain derived neurotrophic factor (BDNF) has a crucial role in the survival & supports the neuronal cells, neuronal integrity and connectivity. It has role in neuronal process and its interaction with ROS might be crucial for neurodegenerative and neuropsychiatric abnormalities and the decreased expression of BDNF is implicated in the sensitivity to stress and stress enhanced responses. Also BDNF deficient mice were observed to be more susceptible to stress induced oxidative damage, which suggests that there is direct interplay between oxidative stress indicators and BDNF levels in the brain (Hacioglu et al., 2016).

Medina et al stated that stress causes neuronal damage leads to significant higher concentration of lipid peroxides by products like MDA and neuronal damage marker NSE (Medina H et al., 2007). It was proved that during oxidative stress the concentration of GSH, activity of SOD and GPX was decreased while MDA were seen to be increased in different brain regions of experimental rats (hippocampus, cerebellum respectively) in comparison to the control group. Under normal condition, the over ROS production was neutralized by the antioxidant defence mechanisms. GSH, is an important non-enzymatic antioxidant that plays a crucial role in the detoxification of ROS. SOD and GPX enzymes are the first line of cellular defence against oxidative injury (Dudziak MS et al., 2019).

Stress causes the production of free radicals by decreasing antioxidants (Kayalvizhi et al., 2012). Vitamin E has been reported as an excellent biological chain-breaking antioxidant that protects cells and tissue from oxidative damage. The antioxidative property of Vitamin E was suggested to have a significant role in developing immune response by protecting the cells, such as lymphocytes, macrophages, and plasma cells, from oxidative damage, enhancing the function and proliferation of those cells that face the oxidative stress. Vitamin E supplementation is known to decrease MDA and NSE levels through its antioxidant property, minimizing oxidative stress by inhibiting ROS production and scavenging the superoxide ions (Jena et al., 2013). A free radical may be defined as "any species capable of independent existence that contains one or more unpaired electrons" (Halliwell 1991).In recent years, the phrase "reactive oxygen species" (or ROS) refer to substances like hydrogen peroxide (H2O1), hypochlorous acid (HOCl), and singlet oxygen ('O:;), which, while not radicals in nature, can produce radicals in the extracellular and intracellular settings (Haliiwell & Gutteridge 1990). The following are some of the processes by which reactive oxygen species induce tissue damage.

- Protein degradation, including gingival hyaluronic acid and proteoglycans,
- DNA damage,
- Lipid peroxidation (via activation of cyclooxygenases and lipooxygenases), and (Bartold et al. 1984).
- Important enzymes, such as anti-proteases such as al-antitrypsin, are oxidised (Varani et al. 1990).

Stress leads to Activation of HPA Axis and lead to increased glucocorticoids by activating dopamine system which in turn stimulates stress avoidance and pleasure seeking behavior.

2.9. NSE:

NSE, like chromogranin A (CgA), cannot distinguish between distinct subtypes of neuroendocrine tumours (NETs); yet, high NSE levels have been linked to poor tumour differentiation. NSE is found in cytoplasm and dendrites of the neurons and is thought to be a marker of neuronal damage. Although its levels are low in peripheral blood is stated that can be used as a sensitive indicator as it increases in serum during injury and damage (Isgrò MA et al., 2015). Non-neuronal enolase (NNE) and NSE are isoenzymes that are expressed in the brain. NSE, the most acidic brain enolase, is made up of two -subunits with a molecular mass of 39,000. NNE is the least acidic enolase isoenzyme, with a relative molecular mass of 43,500. It is made up of two -subunits. Since immunocytochemical studies have proven its strict glial location inside neural tissue, this kind of enolase was dubbed non-neuronal enolase. The most notable difference between NSE and NNE is that the two proteins appear to have no immunological cross-reactivity (D E Schmechel et al., 1980). Chloride ions, urea, and temperature are all very susceptible to NNE. NSE, on the other hand, is far more resistant to chloride-induced inactivation. Because this ion accumulates in nerve cells during periods of recurrent depolarization, NSE's relative insensitivity to chloride ions is particularly intriguing. It's possible that NSE's relative resistance to chloride ions evolved to fit the neuron's intracellular environment. The neuron's chloride-sensitive enolase would be inactivated, and glycolysis would be disrupted at a period when metabolic energy was most needed. Hence these metabolic pathways could increase cellular free radicals, which may attack phospholipids, proteins, and nucleic acids (D E Schmechel et al., 1980) (Isgrò MA et al., 2015).

2.10. MDA:

MDA is one of the most often detected oxidative stress biomarkers, specifically lipid peroxidation. As a result, increased oxidative stress is commonly recognised as a disease (Tsikas D et al 2017). The etiopathogenesis of various chronic diseases has been linked to oxidative stress, which also plays a key role in the ageing process. Moustafa et al. showed that the plasma level of lipid peroxide in healthy adult male volunteers was significantly increased after 1, 2 and 4 h of exposure to radiofrequency fields of the cellular phone in standby position. They indicated that acute exposureto radiofrequency fields of commercially available cellular phones may modulate the oxidative stress of free radicals by enhancing lipid peroxidation and reducing the activation of SOD. MDA is a major oxidative degradation product of membrane unsaturated fatty acid and has been shown to be biologically.

2.11. GFAP:

Glial fibrillary acidic protein is the hallmark type III intermediate filament protein in astrocytes,a main type of glial cells in the CNS. GFAP is thought to help to maintain astrocyte mechanical strength. Astrocytes have a range of control and homeostatic functions in health and disease. Increased GFAP immunoreactivity or astrocyte activation is usually referred as an index of gliosis. Some studies shows that it's also present in glomeruli and peritubular fibroblasts taken from rat kidneys,leydig cells of the testis in hamsters and humans (Buniatian G et al.,1998).

CNS injury that causes gliosis and subsequently upregulates GFAP makes GFAP an attractive candidate biomarker for brain injury screening. GFAP is consistently upregulated with age across rodents and humans and is a surrogate marker of aging in the brain, as indicated by unbiased microarray analysis of various brain regions across rat,

mouse, and human species. Recent studies have documented elevated GFAP or its breakdown products (GFAP- BDP) levels in both CSF and serum after mild, moderate, or severe Traumatic Brain Injury(TBI) in adult patients, and these elevated GFAP levels correlate with TBI magnitude and outcomes in children as well (Maunoury R et al.,1991). These findings are consistent with preclinical models suggesting an association of CSF and serum GFAP levels with TBI outcomes. In addition, clinical studies suggest a possible role for GFAP as a brain-specific marker for malignant gliomas. Another TBI study has shown that although the levels of GFAP were increased in TBI, the levels of GFAP have poor prognostic value. Prospective studies have demonstrated that GFAP-BDP levels enable clinicians not only to differentiate injury severity in moderate to severe TBI but also predict presence or absence of intracranial injury on admission CT imaging, regardless of TBI severity (Davidoff MS et al., 2002).

Antioxidant enzyme activities were generally reduced in rats supplemented with Vitamin E, probably due to its synergistic antioxidant defence, as evidenced by the decrease in DNA damage in the supplemented group (Hamid et al., 2011).

Further antioxidant supplementation improved indices of oxidative stress associated with repetitive loading exercises and aging and improved the positive work output of muscles in aged rodents (Ryan et al., 2010). Administration of Vitamin E orally modulates the antioxidant enzymes by lowering lipid peroxidation (Ahmed et al., 2006). Vitamin E was shown to be a potent lipid-soluble antioxidant, which improves the animals' behavioural changes after immobilization stress (Kayalvizhi et al., 2012).

Antioxidants are "those chemicals that, when present in low concentrations compared to those of an oxidisable substrate, will significantly delay or block that substrate's oxidation" (Halliwell & Gutteridge 1989). "An anti-oxidant is any agent that, when

present in low concentrations compared to those of an oxidisable substrate, considerably slows or inhibits oxidation of that substrate," according to Halliwell (1997).

The activation of efficient defence systems against oxidative damage caused by FR/ROS is critical for organism welfare. Antioxidant defences have been classified in a variety of ways. The more useful appears to be a functional classification of anti-oxidant systems based on how they act (Niki, 1996). Antioxidant defence mechanisms in vivo are divided into three categories based on this: preventive antioxidants, radical scavengers, and repair and de novo enzymes. Vitamin E contains an OH phenolic group that is responsible for its anti-oxidant effect, as well as a phytyl side-chain that facilitates its entry into the lipid bilayer of the cell membrane. However, mounting data suggests that vitamin E isn't necessarily the best lipophilic anti-oxidant (Battino et al., 1991 a; Stocker et al., 1991) and, worse, that vitamin E can serve as a pro-oxidant (Bowry et al., 1992; Bowry and Stocker, 1993; Kontush et al., 1996; Stocker and Bowry, 1996). Vitamin E reduces lipid peroxidation, scavenging peroxyl radicals far faster than these radicals can react with neighbouring fatty acid side-chains or membrane proteins, according to the anti-oxidant viewpoint (Gutteridge and Halliwell, 1994): The peroxyl radical is transformed to lipid peroxide, and ox-tocopherol is changed to a cx-tocopheroxyl radical, which is then regenerated to ot-tocopherol by ubiquinol (CoQH2: the reduced form of co-enzyme Q), GSH, or, most importantly, ascorbic acid. The latter interaction produces an ascorbic acid radical (a rather unreactive species) that can be reduced by a CoQdependent dehydrogenase or react with another ascorbic acid molecule to produce ascorbate and dehydro-ascorbate (Navarro et al., 1995; Villalba et al., 1995). Both ascorbate (Sato et al., 1990; Kagan et al., 1992) and CoQH2 (Frei et al., 1990; Kagan et al., 1990; Yamamoto et al., 1990) have been examined and verified in vitro to reduce x-tocopheroxyl radicals.

Antioxidant supplements, on the other hand, have been proven to enhance antioxidant enzymes like GSH-PX and CAT in liver and heart cells, increasing the antioxidant system's potency to prevent MDA from increasing (40). Vitamin consumption also decreased oxidative damage of endometrial tissue in female rats exposed to 900 MHz in a 2007 study (8). By creating alphatocopherol and glutathione from free radicals, vitamin can strengthen the antioxidant system and minimise lipid peroxidation.

Need for the Study

NEED FOR THE STUDY

Several epidemiological studies have investigated the effects of stress & RF-EMR from mobile phones on the human body, but the results have been contradictory. Furthermore, these studies have not addressed questions regarding the effect of mobile phone exposure on feeding behavior. A clear causal relationship between stress and feeding behavior, i.e., whether the stress causes an increase or decrease in feeding behavior, has not been established. Furthermore, no studies have explored the effects of RF-EMR on feeding behavior. People are exposed to various stressors and RF-EMR daily. Impacts are due to oxidative stress, vitamin E might play a role in mitigating this effects.

This study is designed to know the effect of stress and RF-EMR on feeding behavior and the radio protective effects of Vitamin E in rodents. This study can be deduced to the human systems. As per literature due to paucity in studies we have planned to conduct this study.

Aim and objectives

AIM AND OBJECTIVES

Aim

To study the effect of Vitamin E supplementation on multiple Stressors & RF-EMR emitted from mobile phone-induced changes in the feeding behavior of rodents.

Objectives

- To study the effect of stress on feeding behavior.
- To study the effect of RF-EMR on feeding behavior.
- To assess the neuronal damage by measuring Neuronal Specific Enalose (NSE) and Malondialdehyde (MDA) Levels in both stressed & RF-EMR exposed rats.
- To assess the neuronal damage by measuring Neuronal Specific Enalose(NSE) and Malondialdehyde(MDA) Levels in both stressed & RF-EMR exposed rats post Vitamin E administration.
- To assess the expression of the Glial fibrillary acidic protein (GFAP) in the brain in both stressed & RF-EMR exposed rats.
- To assess the expression of the Glial fibrillary acidic protein (GFAP) in the brain in both stressed rats & RF-EMR exposed rats post Vitamin E administration.

Chapter-III

Materials and methods

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Experimental animals and conditions

The study was done at the central animal house, Sri Devaraj Urs Academy of Higher Education & Research, Kolar, Karnataka, as a Prospective comparative study after receiving authorization from the Institutional Animal Ethics Committee (IAEC) with the number IAEC/PHARMA/SDUMC/2017-18/8a. Institutional Animal Ethics Committee approved thirty-six rats for the study.

Male Sprague Dawley (SD) rats, 10-12 weeks old, weighing 180-220gms, were purchased from Biogen laboratory animal facility (Reg No:971/PO/RCB1Bt/S/2006/CPCSEA; Bangalore, India). The animals were housed in polypropylene rat cages with a temperature of 23 ± 2 ºC, relative humidity of 55 ± 5%, and 12:12 hour light-dark cycle with *ad libitum* access to feed and RO drinking water. Two rats were allocated per cage. The rats were allowed to acclimatize to the laboratory environment for two weeks before the commencement of the study. Animals were taken care of as per CPCSEA guidelines. A normal pellet diet was purchased from "Champaka Feeds and Foods" (VRK Nutritional Solutions, Bangalore, India). The standard diet consists of moisture (10%), crude protein (18%), crude fiber (4%), crude fat (4%), nitrogen-free extract (NFE) (60%), Calcium (1%), and Phosphorous (0.5%). Paddy husk was used as a bedding material procured from a local rice mill. The paddy husk was replaced every other day, and the rats were handled humanely.

3.2. Methods

3.2.1. Animal experimental design

A total of 36 SD rats weighing 180-220 g were purchased from the certified breeder and randomly allocated into the six groups as follows (Fig 2), two rats were allocated in each cage.

Group I (n=6) – Control: Animals with *ad libitum* access to food and RO water.

Group II (n=6) – Stress: Different types of stressors like restraint, swimming, and noise) was given 1 h/day for 50 days with *ad libitum* access to food and RO water. Different stressors were selected in the experiment to prevent habituation (Ely et al., 1997).

Group III (n=6) – RF-EMR: Animals were exposed to RF-EMR emitted from 4G Android mobile phone GSM (0.9 GHz/1.8 GHz) with the exact specification and with the same mobile network were used in this study for uniformity of radiation exposure which is kept in answer mode in the cage for one hour per day for 50 days. The mobile phones were hung down from the roof of the rat cage. Using a radiofrequency decibel meter (Electrosmog Meter-ED-178s), radiations were measured at the periphery, and animals were kept at a distance of 15-20cms from the mobile phone. Animals were allowed to move freely in the cage with continuous access to food and RO water (Narayanan et al., 2010).

Group IV (n=6) – Stress + Vitamin E: Animals were exposed to different stressors to prevent habituation. Vitamin E was supplemented (100 mg/kg body weight orally with an oral gavage needle for 50 days (Hassan et al., 2007) with continuous access to food and RO water (Ely et al., 1997).

Group V (n=6) – RF-EMR + Vitamin E: Animals were exposed to RF-EMR emitted from mobile phones. Vitamin E was supplemented (100 mg/kg body weight orally with an oral gavage needle for 50 days (Hassan & Awad, 2007) with continuous access to food and RO water (Narayanan et al., 2010).

Group VI (n=6) – Vehicle control: The animals (2 drops of water were given orally through oral gavage needle as a vehicle like in group IV and V for 50 days) with continuous food and RO water access.

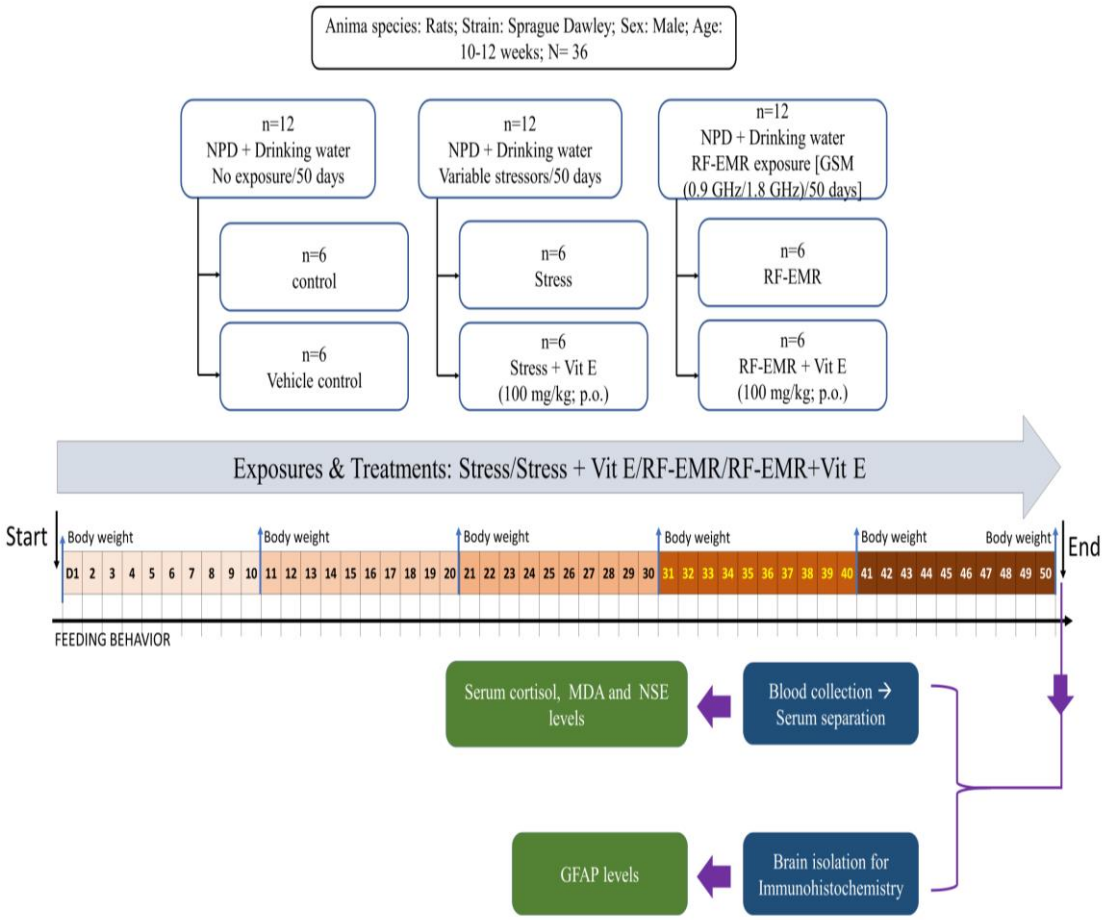


Fig 3.1. Animal grouping and experimental conditions. NPD – Normal pellet diet; p.o. – per oral; Vit E – Vitamin E; RF-EMR – Radiofrequency electromagnetic radiation; GFAP – Glial fibrillary acidic protein.

3.2.2. Stressors

Rats of Stress and Stress + Vitamin E groups were exposed to different stressors in predefined cyclical sequence (Restraint →High-intensity noise → Swimming). One of the other stressors was applied every day for 1 hour from 1600-1700 hrs during the daytime, which continued until day 50. On the other hand, the rats of RF-EMR and RF-EMR + Vitamin E were exposed to mobile radiation for 1 hour from 1600-1700 hrs for 50 days (Fig 3.1). Two rats were housed per each cage.

Restraint stress

Restraint stress or immobilization is commonly used because it is less severe but can activate the stress response (Pagadala et al., 2017). In this type of stressor, the animal's movement is limited by placing the rodent in a plexiglass chamber or immobilization bag or restrainer (Heinrichs et al., 2006). We used a restrainer to apply the restraint stress on rats in this study.

High-intensity noise stress

High-intensity noise exposure (speakers emitting with a frequency response of 80-15000 Hz and sensitivity of 80 decibels (dB)) was used as a physical stressor. This protocol was used as an environmental stressor to mimic stress in everyday life. This stress was given for 30 min continuously & animal was allowed to rest for 10 min, after which the remaining 30 min procedure was continued with the noise exposure (Heinrichs et al., 2006).

Forced swim stress

The animal was made for swimming in the water for 5 min, and the animal was gently taken out of the water and placed to dry. This procedure was repeated 12 times (=1 hour/day) for each animal to ensure that the animal did not drown (Heinrichs et al., 2006).

3.2.3. Radiation Frequency Meter (Electrosmog Meter)

An Electrosmog meter (Cornet Microsystems-ED 178) was used in this study to quantify the radiation emitted from mobile phones during RF-EMR exposure (Fig 3F). An Electrosmog meter is a sensitive dual-mode device that measures both high-frequency radiation electromagnetic wave strength and low-frequency radiation electromagnetic waves. It 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 in terms of Decibels-Milliwatt (dBm), Volt per meter (v/m), and Milliwatt per square meter (mW/m^2). 3 Coloured LED lights, Red, Yellow, and Green, were displayed on the right side of the LCD screen for quick radiation signalling indication.

- Red color LED light indicates caution
- The yellow color LED light indicates the safe zone
- Green color LED light indicates low frequency.

The digital display shows a histogram that records recent 30 signal level readings and is shown as a moving graph on the digital display. In addition, maximum, Average, and Minimum measurements of electromagnetic radiation data recorded earlier will be shown in a graphical representation on a digital display (Radiofrequency decibel meter. 2016).

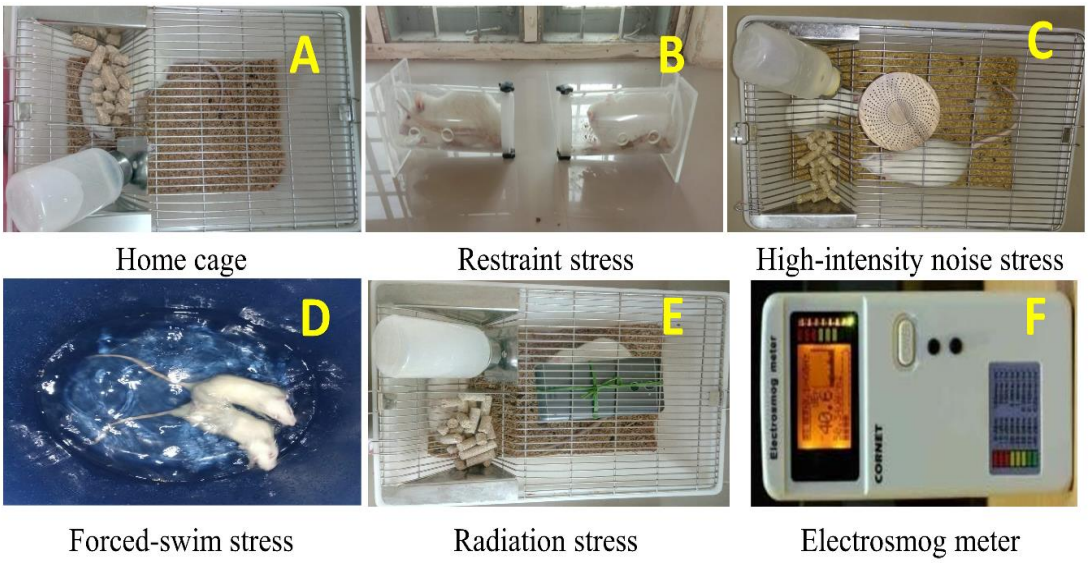


Fig 3.2. Pictorial representation of different conditions. Home cage (A), Restraint stress (B), High-intensity noise stress (C), Forced-swim stress (D), Radiation stress (E), and the working of Electro smog meter (F).

Fig 3.2. Supplementation of Vitamin E through oral gavage bent needle

3.3. Assessment of body weight and feeding behavior

The body weight of the rats was measured every 10 days using an electronic balance. Food was weighed in the beginning and compared at the end. All rats were housed

separately for this experiment. RO water and food pellets were introduced to each cage (Hacioglu et al., 2016).

3.4. Blood and brain isolation

After 50 days of stress application, blood samples from all the rats were collected through retro orbital puncture under the deep anaesthesia with ketamine (50–80 mg/kg) & xylazine (5–10 mg/kg) (Care, 2003). The blood samples (2 mL from each rat) were allowed to coagulate for 30 minutes. The serum samples were collected by centrifugation of blood at 3000 rpm for 20 minutes at room temperature. Care was taken to avoid the haemolysis of the blood samples. The obtained serum was stored at -20 °C until further analysis. Whereas the brain was harvested and fixed for the immunostaining of GFAP positive cells.

3.5. Biochemical analysis

3.5.1. Serum Corticosterone Assay

Serum Corticosterone was measured to know whether the animal was stressed or not. To measure serum corticosterone levels, 2 ml of blood was collected immediately after the stress, and serum was separated by centrifugation and stored. It was measured by using an ELISA kit.

Step 1: Prepare all reagents, working standards, Blank and samples as directed in the previous sections. Step 2: Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the Ziploc, store unused wells at 4°C Step 3 : Pipette standard 50µl to testing standard well, Pipette Sample diluent 40µl to testing sample well, then add testing

sample 10µl (sample final dilution is 5-fold), Pipette sample to wells, don't touch the well wall as far as possible, and mix gently. Step 4: Incubate: Cover with the adhesive strip provided, incubate for 30 min at 37℃. Step 5: Configurate liquid: Dilute wash solution 30-fold with distilled water. Step 6: Washing: Uncover the adhesive strip, discard liquid, pipette washing buffer to every well, still for 30s then drain, repeat 5 times. Step 7: Add enzyme: Pipette HRP-Conjugate reagent 50µl to each well, except blank well. Step 8: Incubate: Operation with 4 Step 9: Washing: Operation with 6. Step10: Color: Pipette Chromogen Solution A 50ul and Chromogen Solution B 50ul to each well, avoid the light preservation for 15 min at 37℃. Step 11: Stop the reaction: Pipette Stop Solution 50µl to each well, stop the reaction (the blue change to yellow). Step 12: Calculate: take blank well as zero. Read absorbance at 450nm after pipette Stop Solution within 15min.

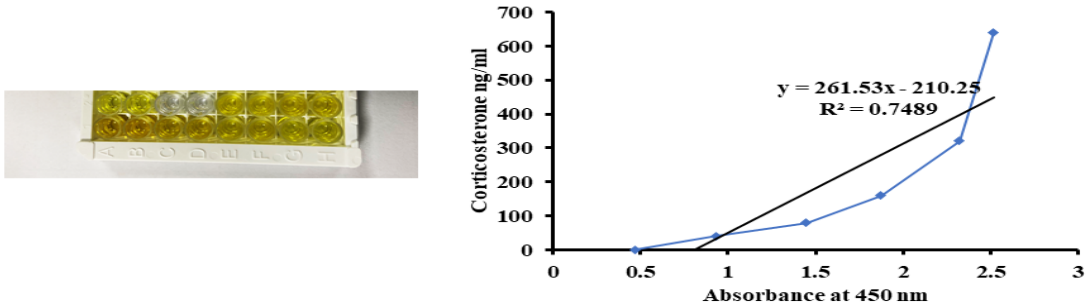


Fig 3.3. Estimation of Serum corticosterone

3.5.2. Estimation of lipid peroxidation by serum MDA levels

The lipid peroxidation was estimated by the (Mahfouz et al., 1986) method. Lipids in the cell membrane are highly susceptible to peroxidative damage, which breaks down into a number of units to form malondialdehyde (MDA). This MDA reacts with TBA to form thiobarbituric acid reactive substances (TBARS), which have a pink color with absorption

maxima at 530 nm. Briefly, 500 µl of plasma sample was pipetted into labelled, clean 15 ml screw-cap glass tubes. To the tubes, 500µl of 40% TCA and 1 ml of 0.67% TBA were added and mixed gently. The tubes were closed with the caps and kept in a boiling water bath for 10 minutes. The tubes were removed from the boiling water bath and left at room temperature for 2 minutes. The tubes were then placed in an ice-cold water bath for 5 minutes, added 1 ml of double distilled water to all the tubes, and centrifuged at 2500 rpm for 15 minutes at room temperature. The supernatant was removed, and optical density was measured at 530 nm using a double beam spectrophotometer (Hitachi). The OD values were plotted against the concentration of the standards to obtain a standard graph. The concentrations of the samples were calculated using the OD of the standard of known concentration. Values were expressed as µmol/l.

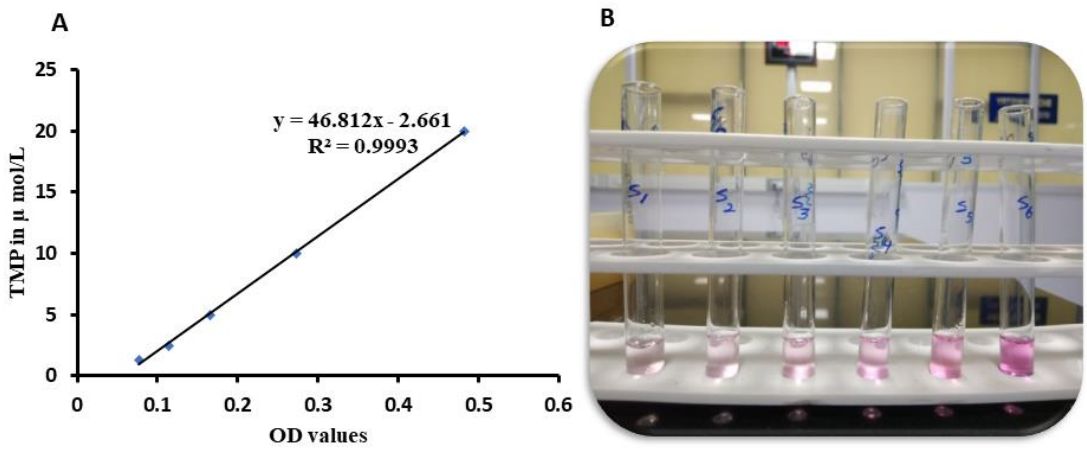


Fig 3.4. Estimation of Serum MDA levels

3.5.3. Estimation of serum NSE levels

The serum NSE levels were measured using commercially available ELISA kits (Cat: BC-ER140914; Biocodon technologies, USA) by following the user instructions. Briefly, the reagents, buffers, and samples were thawed to room temperature. 40 µL of undiluted

samples, 10 µL of respective antibodies, and 50 µL of streptavidin-horseradish peroxidase were added to the respective monoclonal antibody-coated microplate wells and incubated at 37 °C for one hour. After incubation, the solution was discarded completely, and wells were washed with a 1X washing buffer solution five times. To the wells, 50 µL of chromogen reagent A and 50µl of chromogen reagent B were added and incubated for 10 minutes to develop yellow color. The reaction was stopped by adding 50 µL of stop solution. The optical density of the yellow color was measured at 450 nm using an ELISA reader (Rayto, RT-6100 microplate reader).

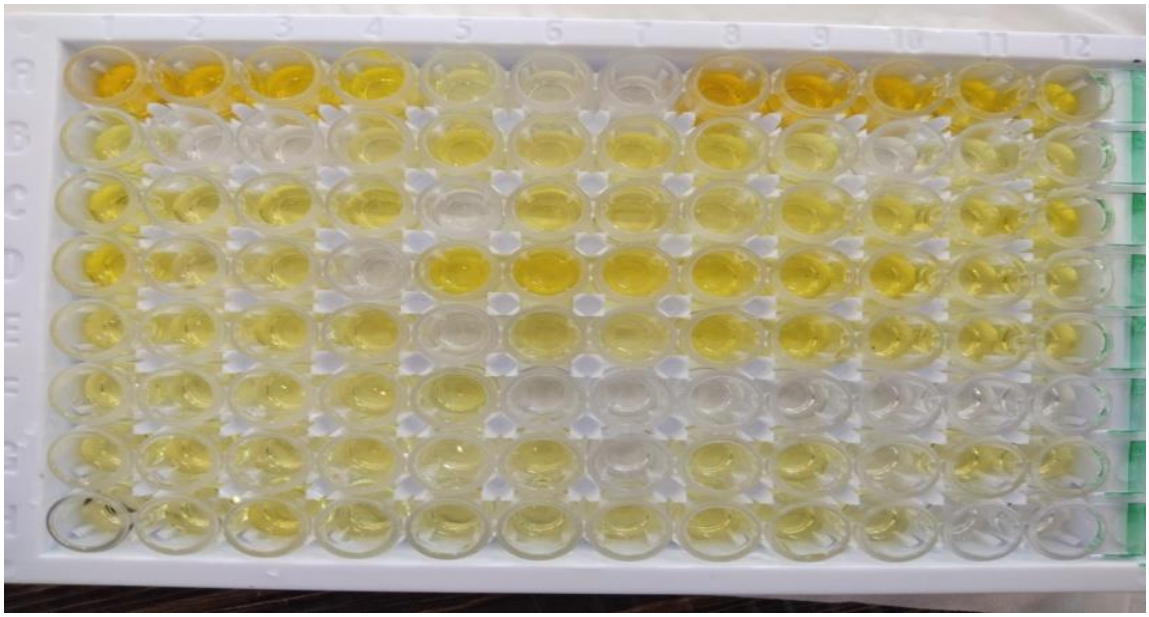


Fig 3.4. Estimation of Serum NSE levels

3.6. Immunohistology examination

As per protocol, at the end of the study, animals were sacrificed after an overdose of ketamine. The whole-brain of the animal was removed quickly and fixed with 10% neutral buffered formalin (Famitafreshi et al., 2016). Then the brain tissue was processed by using automated tissue processing units. The stained sections of the hippocampus were

examined under a light microscope for the viable neurons. With immunohistochemistry, the expression of GFAP was estimated in the CA3 region of the rat hippocampus.

3.6.1. Procedure for doing GFAP:

The brain samples were collected in 4% paraformaldehyde⇨Brain sections - 40 µm Floating brain sections for immunohistochemistry of CNPase+ve oligodendrocytes Antigen retrieval: sodium citrate buffer with pH-6 - 20 minutes at 90°C ⇨ Peroxidase block- hydrogen peroxidase ⇨ Anti GFAP antibodies(Diagnostic bio system) : 1:1000 Secondary antibodies ⇨ Avidin Biotin complex ⇨ Added tetrahydrochloride for 2 minutes.(Subhadeep et al.,2021)

Fig 3.6. Storage of brain sample for estimation of GFAP

3.6.2. Cell counting and image analysis

The micrographs of hippocampal sections were captured by a digital microscope (Leica Microsystems, Wetzlar, Germany). GFAP immunostaining was semi quantified. Briefly, GFAP immunopositive cells in the hippocampus of the CA3 region were recorded. Only cells with complete processes and clear nuclei were counted and expressed as the number of cells per mm^2. The area of each GFAP immunopositive cell with a complete cellular profile was also measured using an Image-Pro Plus 6.0 Analysis System (Media

Cybernetics Inc., San Francisco, CA, USA). The percentage of neuronal apoptosis was expressed. Three sections per rat, and six rats per group, were averaged to provide a mean value for each group.

3.6.3. Quantification of Glial cells

Glial cells Stained hippocampus was captured at 40X using a digital microscope (Zeiss microscope with ZEN software, Germany)

The number of Glial cells present in CA3 region was counted using Image J software

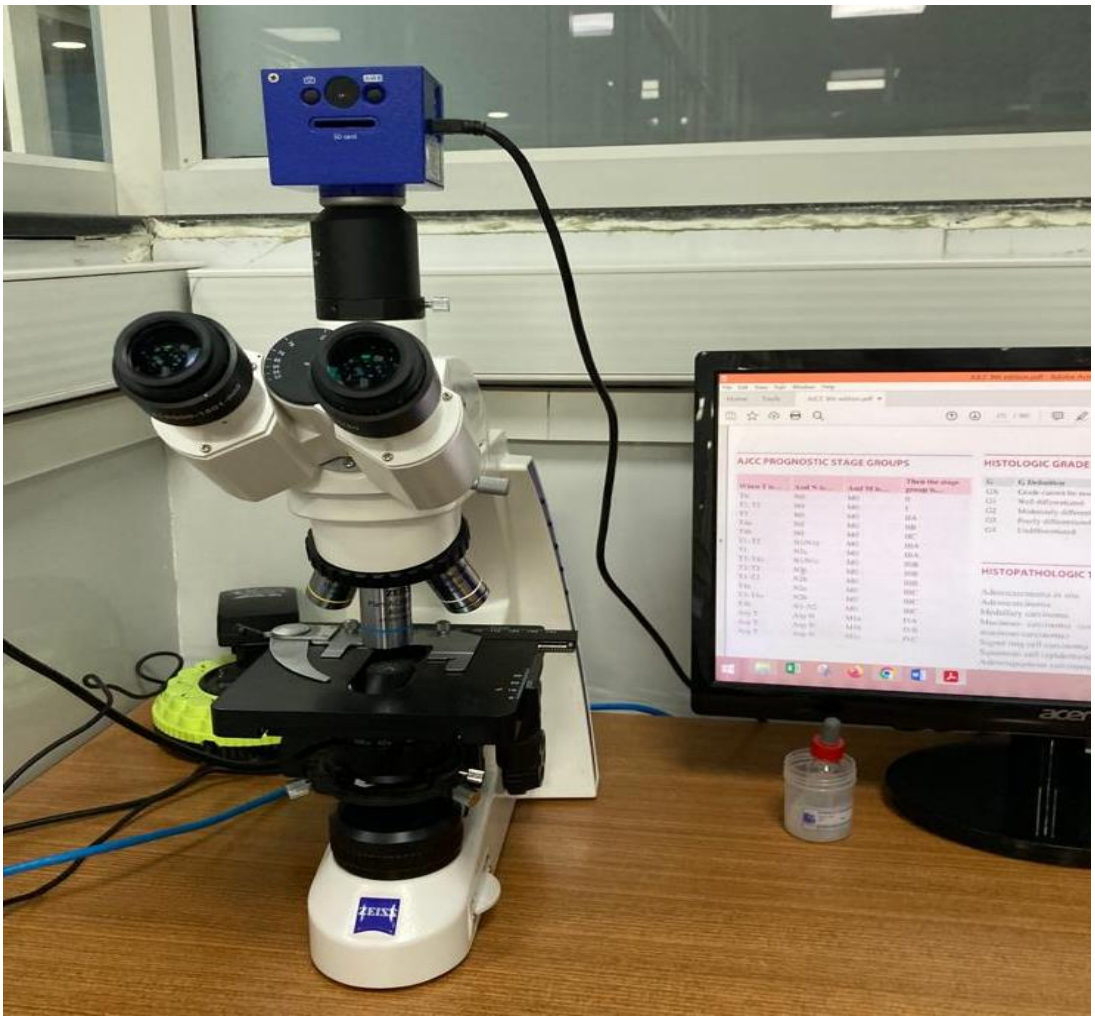


Fig 3.7. Quantification of Glial cells through microscope

3.7. Statistical Analysis

The GraphPad Prism 8.0.3 software was used to analyze the data. All the data represented here is mean ± SEM of n=6. Two-way ANOVA, followed by Dunnet post-hoc test, was applied to analyze the time point data (body weight and feeding behavior). One-way ANOVA, followed by Dunnet post-hoc test, was applied for the analysis of the remaining parameters (NSE,MDA,serum corticosterone and GFAP). A *p*-value of less than 0.05 was considered significant. Significance labels: 'a' denotes the significance vs Control group; 'b' denotes the significant difference between Stress and RF-EMR group; '$' denotes the significant difference between Stress and Stress + Vitamin E group; * denotes the significant difference between RF-EMR and RF-EMR + Vitamin E group.

Chapter-IV

Results

4. RESULTS

4.1. Effect of stress, RF-EMR, and Vitamin E supplementation on feeding behavior

The feed intake of animals in control and vehicle was observed to be optimal throughout the study period. However, a significant reduction in feed intake was observed in Stress group starting from Day 1 to the end of the study as compared to the control. Whereas the concurrent treatment with Vitamin E significantly improved the feed intake and feeding behavior of the Stress group rats from day 12, though it did not reach the level of controls. On the other hand, no significant difference was recorded in terms of feed intake of RF-EMR as well as RF-EMR+Vitamin E group rats as compared to the control rats (Fig 4.1).

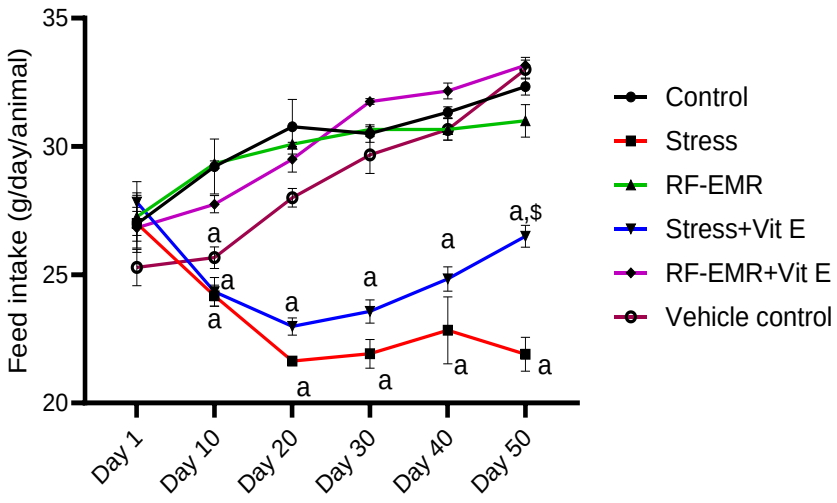


Fig 4.1. Time-dependent effect of stressors, RF-EMR and Vitamin E supplementation on the feeding behavior. All the data represented here is mean ± SEM of n=6. A p-value of less than 0.05 was considered significant. Significance labels: 'a' denotes the comparison with Control group; 'b' denotes the significant difference between Stress and RF-EMR; '$' denotes the significant difference between Stress and Stress + Vitamin E; * denotes the significant difference between RF-EMR and RF-EMR + Vitamin E.

4.2. Effect of stress, RF-EMR, and Vitamin E supplementation on the body weight

The rats of control and vehicle groups showed a steady and positive weight gain throughout the study. Whereas a significant difference was noted in the body weights of the Stress rats as compared to the control. A significant reduction in the body weight of the Stress animals was observed from Day 30 and continued till the end of the study. An Overall reduction of 20% reduction in body weight was observed. Although the RF-EMR animals did not lose weight, they showed a marginal reduction in the body weight gain as compared to control rats. On the other hand in groups when supplemented with Vitamin E, the animals of Stress+Vitamin E showed a comparable and significant increase in body weight compared to stress group. While the animals of RF-EMR+Vitamin E showed a comparable body weight gain to that of the control group (Fig 4.2).

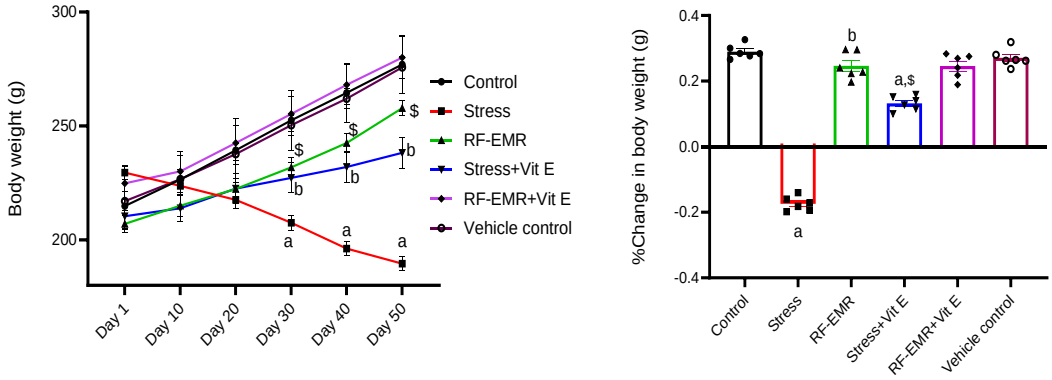


Fig 4.2. Effect of stressors, RF-EMR and Vitamin E supplementation on the body weight. All the data represented here is mean ± SEM of n=6. A p-value of less than 0.05 was considered significant. Significance labels: 'a' denotes the comparison vs Control group; 'b' denotes the significant difference between Stress and RF-EMR; '$' denotes the significant difference between Stress and Stress + Vitamin E; * denotes the significant difference between RF-EMR and RF-EMR + Vitamin E.

4.3. Effect of stress, RF-EMR, and Vitamin E supplementation on serum corticosterone

As compared to the control rats, chronic application of variable stressors and RF-EMR have led to a significant elevation in serum corticosterone levels of Stress and RF-EMR groups, respectively. On the other hand, the Vitamin E co-treatment substantially reversed the elevation in serum corticosterone levels in animals exposed to variable stressors and RF-EMR. However, no significant difference was observed in the rats of vehicle group compared to the control (Fig 4.3).

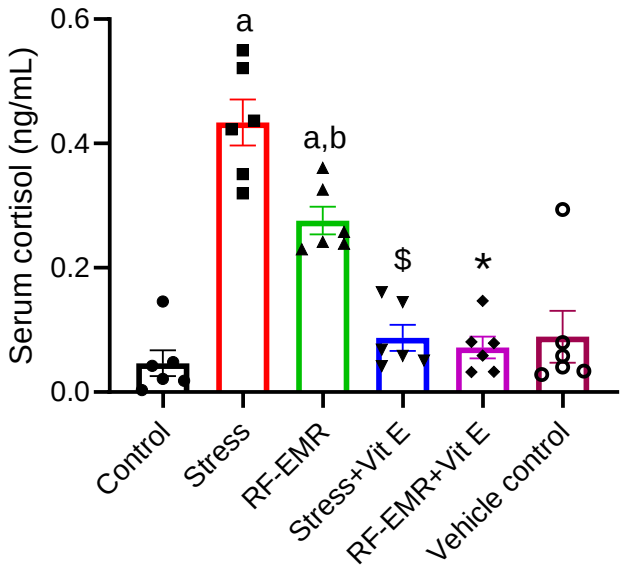


Fig 4.3. Effect of stressors, RF-EMR, and Vitamin E supplementation on serum corticosterone levels. All the data represented here is mean ± SEM of n=6. A p-value of less than 0.05 was considered significant. Significance labels: 'a' denotes the comparison with Control group; 'b' denotes the significant difference between Stress and RF-EMR; '$' denotes the significant difference between Stress and Stress + Vitamin E; * denotes the significant difference between RF-EMR and RF-EMR + Vitamin E.

4.4. Effect of stress, RF-EMR, and Vitamin E supplementation on MDA levels

Chronic exposure to different stressors and RF-EMR have led to a significant elevation in serum MDA levels in Stress and RF-EMR groups as compared to the control rats. However, the Stress group exhibited significantly higher levels of serum MDA levels when compared the RF-EMR rats. Whereas, a 50-day Vitamin E co-treatment significantly prevented the elevation of serum MDA levels in animals exposed to Stress and RF-EMR, which were almost similar to that of control rats. However, no significant change was observed in the rats of vehicle group compared to the control (Fig 4.4).

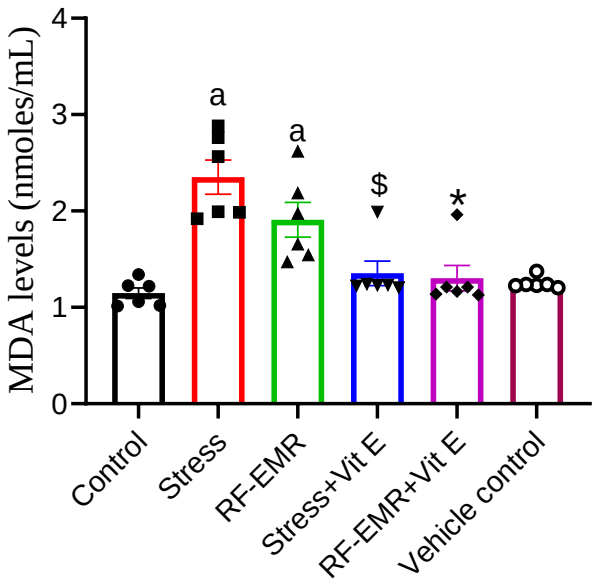


Fig 4.4. Effect of stressors, RF-EMR and Vitamin E supplementation on serum MDA levels. All the data represented here is mean ± SEM of n=6. A p-value of less than 0.05 was considered significant. Significance labels: 'a' denotes the comparison with Control group; 'b' denotes the significant difference between Stress and RF-EMR; '$' denotes the significant difference between Stress and Stress + Vitamin E; * denotes the significant difference between RF-EMR and RF-EMR + Vitamin E.

4.5. Effect of stress, RF-EMR, and Vitamin E supplementation on NSE levels

NSE is usually considered as an indicator of brain injury. Long-term exposure to different stressors and RF-EMR has led to a significant escalation in serum NSE levels in Stress and RF-EMR groups compared to the control rats. But the Stress displayed significantly higher serum NSE levels than the RF-EMR rats. While, a 50-day Vitamin E supplementation significantly lowered the elevated serum NSE levels in animals exposed to Stress and RF-EMR, which were comparable to that of control rats. Nevertheless, no significant change was observed in the rats in the vehicle control group compared to the normal control (Fig 4.5).

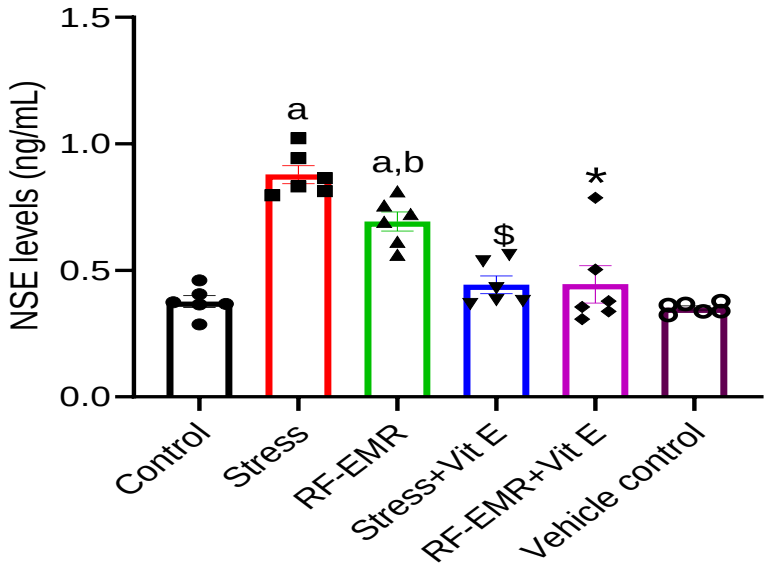
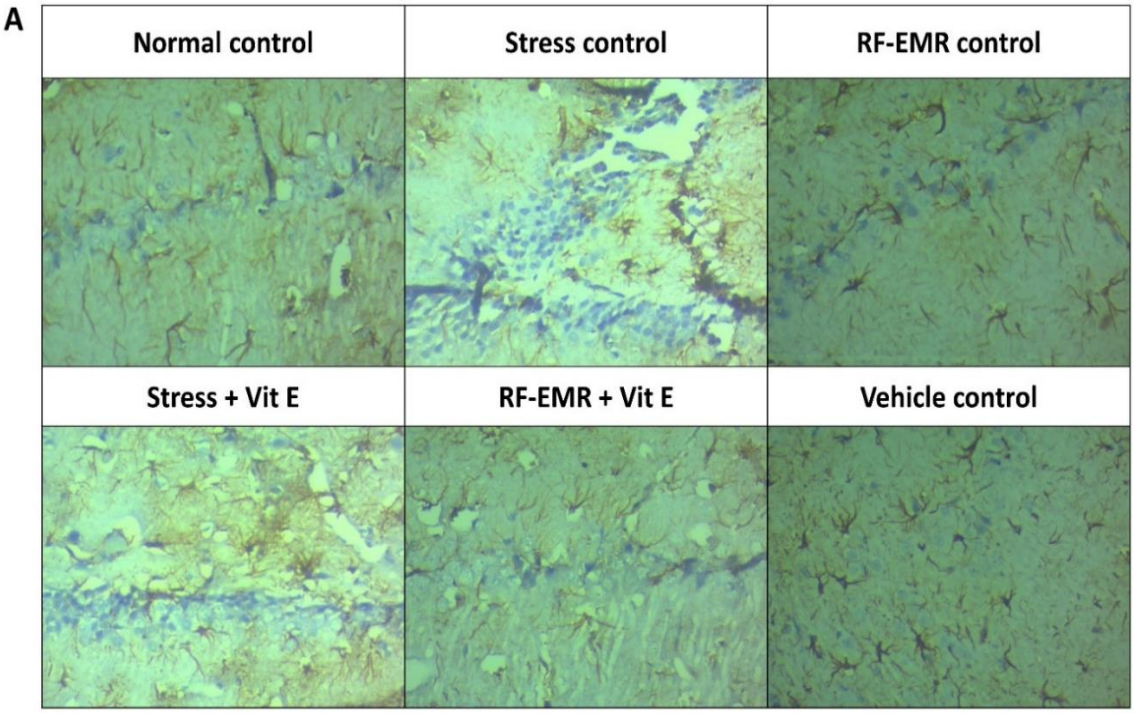


Fig 4.5. Effect of stressors, RF-EMR, and Vitamin E supplementation on the NSE levels. The data represented here is a mean ± SEM of n=6. A p-value of less than 0.05 was considered significant. Significance labels: 'a' denotes the comparision with Control group; 'b' denotes the significant difference between Stress and RF-EMR; '$' denotes the significant difference between Stress and Stress + Vitamin E; * denotes the significant difference between RF-EMR and RF-EMR + Vitamin E.

4.6. Effect of stress, RF-EMR, and Vitamin E supplementation on GFAP levels

Immunostaining of GFAP has revealed that there was a significant elevation in hippocampal GFAP levels in Stress group as compared to the control and RF-EMR groups. Whereas, following a 50-day Vitamin E co-treatment, significantly lower levels of GFAP were observed in the animals of Stress + Vitamin E group. On the other hand, no significant difference in the GFAP levels was noted in vehicle , RF-EMR and RF-EMR + Vitamin E rats as compared to the control. (Fig 4.6 A & B).



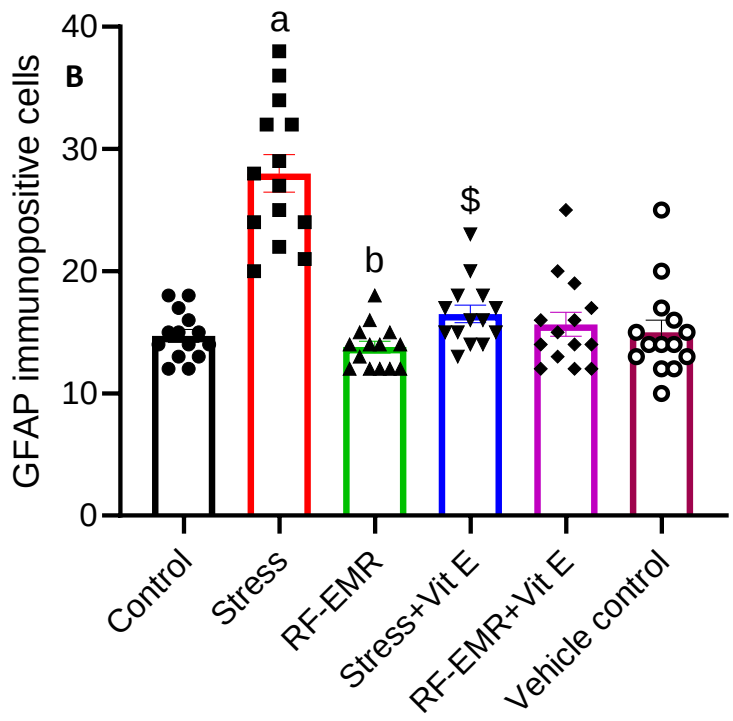


Fig 4.6. Effect of stressors, RF-EMR, and Vitamin E supplementation on the GFAP levels. Microphotographs of immunostained GFAP positive glial cells (A) and quantification of the GFAP glial cell density (B). The photomicrographs of brain sections were obtained at 40X magnification. All the data represented here is mean ± SEM of n=6. A p-value of less than 0.05 was considered significant. Significance labels: 'a' denotes the comparision with Control group; 'b' denotes the significant difference between Stress and RF-EMR; '$' denotes the significant difference between Stress and Stress + Vitamin E; * denotes the significant difference between RF-EMR and RF-EMR + Vitamin E.

Microscopic anatomy (Cellular Architecture) of Hippocampal CA3 Neurons

Histological sections of haematoxylin and eosin (H&E) stained hippocampal CA3 pyramidal neurons

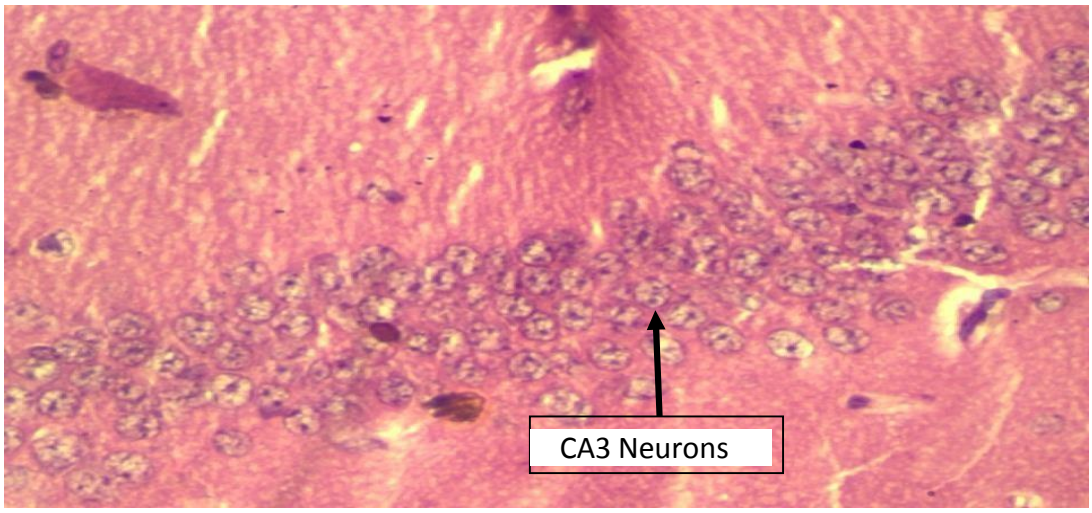
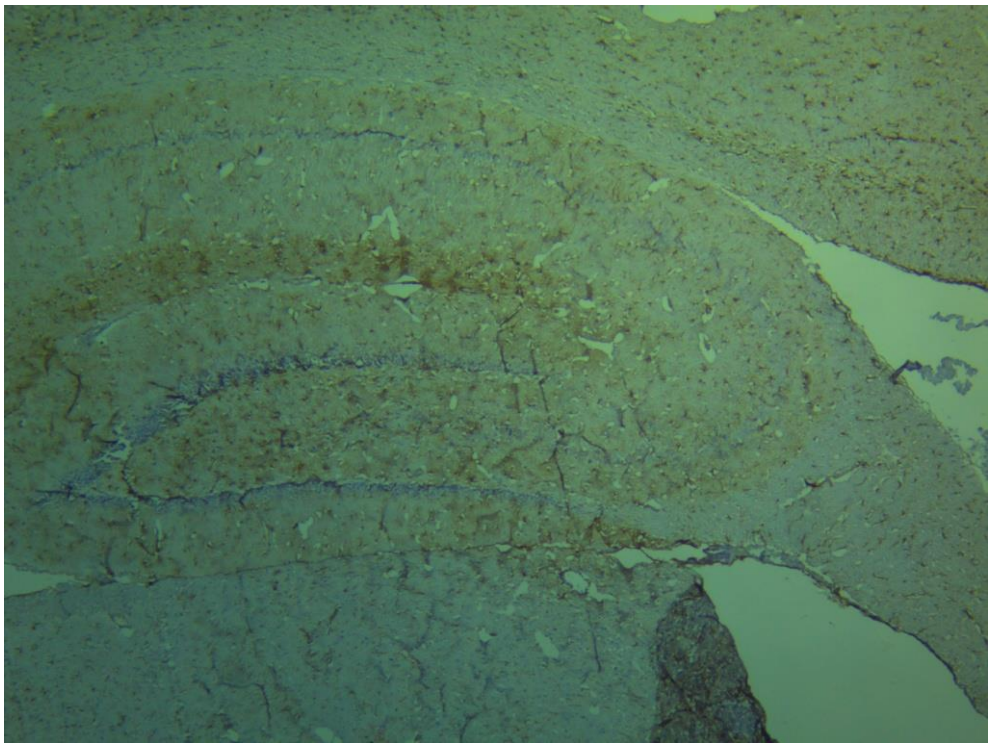


Fig 4.8 Control group H & E stained Hippocampal CA3 pyramidal normal neurons (Arrow) in high power (40X).



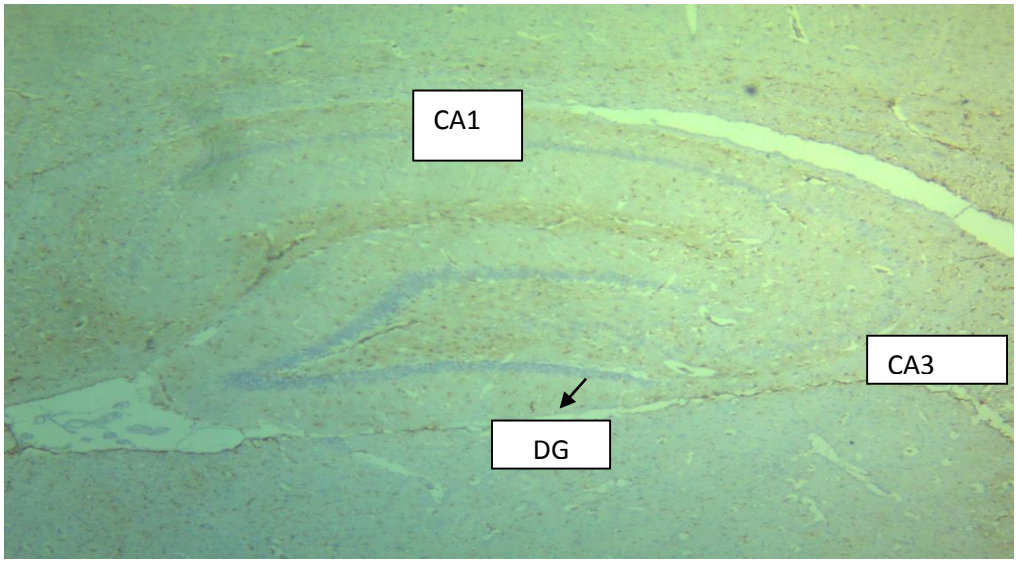


Fig 4.9 Image showing the hippocampus in GFAP stain under 10X low power (positive stain taken by glial cells)

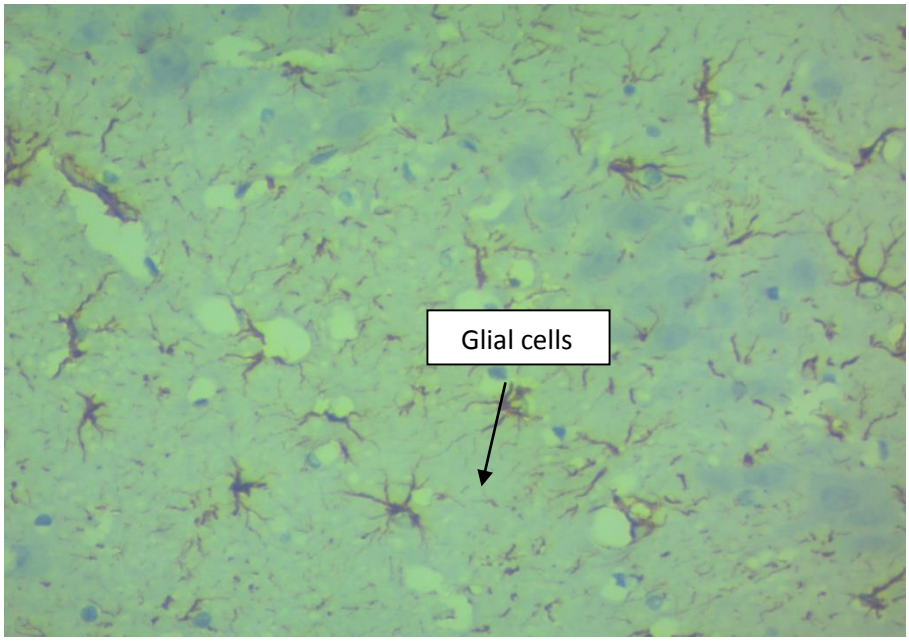


Fig 4.10: Stain taken by Glial cells under high power in CA3 region (40x lens)

5. DISCUSSION

Stress is a major factor in the modern world that affects the quality of life and human health and productivity. A number of studies have reported a significant association between psychological stressors and poor physical and mental health. The long-term activation of the stress response system and the overexposure to cortisol and other stress hormones that follow can disrupt almost all your body's processes. This increases the risk of mental problems including anxiety, depression, learning, memory, feeding behavior, etc. Long-term stress impairs function and accelerates cell senescence by altering numerous physiological systems, including the body's immune defense, and inflammatory and metabolic pathways. Stress often affects eating behavior, increasing caloric intake in some individuals and decreasing it in others. The determinants of feeding responses to stress are unknown. Feeding behavior which is a complex behavior in our day-to-day lives regulated by many mechanisms. But the exact regulatory mechanisms are not well understood. One of which is found in recent studies is the hypothalamus because it is the center for controlling feeding behavior. Some studies also shows that hippocampus has also role in feeding behavior because where to eat, what to eat and how much to eat that decision was made by hippocampus (Stevenson RJ et al., 2017).

In addition to the daily life stress, mobile usage was drastically increased in the past two decades. The majority of contemporary lifestyle disorders like short-term memory loss, loss of focus, stress, exhaustion, sleep deprivation, and weakened immunity are believed to be caused by electromagnetic radiation, according to field studies. Whether mobile phone emitted radiation alters feeding behavior has not been studied. Understanding the biochemical or molecular level changes after both chronic stress and mobile radiation-induced damage might help in better management of individuals suffering from stress or mobile radiation-induced damage/abnormalities. Studies also showed that vitamin-E

supplementation might restore the stress-induced abnormalities, however, there was no literature available on the ameliorative effect of vitamin E on chronic stress or mobile radiation feeding behavior changes. Therefore, our current study investigated to know the effect of vitamin E supplementation on multiple stressors & RF-EMR emitted from mobile phone-induced changes in the feeding behavior as well as oxidative damage and stress response markers in the rodents.

In our study, the feed intake of animals in control and vehicle was observed to be optimal throughout the study period. We have investigated the effect of 50 days of CUS on feeding behavior and body weight. We noticed a significant reduction in feed intake of rats subjected to different stressors for 50 days compared to controls. There was a significant reduction in body weight in rats in the stress group compared to the control. Feeding behavior is directly linked to the regulation of body weight and reduction, which might have led to the decreased body weight of stressed rats. The findings of our study are in agreement with a report by James A. Carr et al., where it was demonstrated that stress inhibits feeding behavior in all vertebrates. Restraint stress has been shown to reduce body weight due to decreased food intake (Sominsky & Spencer et al 2014). A study by cristina et al (2016) demonstrated that acute stress causes weight loss and chronic stressors promote the activation of the pro-obesogenic mechanisms that favour the accumulation of central or visceral fat. Favreau-Peigne et al. also demonstrated that chronic stress altered animal welfare and decreased body weight (Favreau-Peigné, A et al., 2014). The findings of our study are also in agreement with other studies done by Yue Li et al 2021, Jing tang et al., 2019 and Ching han et al., 2021, in that they mentioned that stress leads to decreased feeding behavior.

The current findings contradict with Some studies which were done on animals and human subjects, shows that exposure to stress leads to an increase in food intake and weight gain subjective to supplementing comfort foods, a palatable diet which was rich in fat and sugar, and more snacking (Hamidreza Famitafreshi et al., 2016). Increased stress may be capable of modifying eating behavior and moderate to chronic stress like restraint does not alter normal food consumption, but leads to changes in specific appetites, by greater sweet food ingestion. Stress causes elevated glucocorticoid levels leading to stimulation of feeding behavior and excessive weight gain (Yvonne H et al., 2013). Also, administration of glucocorticoids increases food intake. Adrenalectomy reduces food intake, while subsequent corticosterone replacement normalizes it (Yvonne H et al., 2013). Stress leads to Activation of HPA Axis and lead to increased glucocorticoids by activating dopamine system which inturn stimulates stress avoidance and pleasure seeking behavior-causing intake of palatable foods by negative feedback mechanism. These alterations were probably due to a higher level of anxiety (Juruena MF et al., 2020).

Stress in rats can cause increased food intake in rare cases but mostly decreases food intake. The severity of the stress is also crucial. A decrease in food intake is linked to severe stress. Moderate and mild stressors also lead to a decrease in food intake but increase the ingestion of highly palatable food. According to the intensity and type of stressors, the effect was either a decrease or increase in food intake in rats exposed to different types of stressors, especially when offered a calorically dense diet. The last situation reproduces the modern society with numerous stressors, especially social stressors, and exposure to energy-dense, highly palatable food. Most studies highlight that stress reduces food intake in rodents unless palatable food is given during the stress period (Francois M et al., 2022).

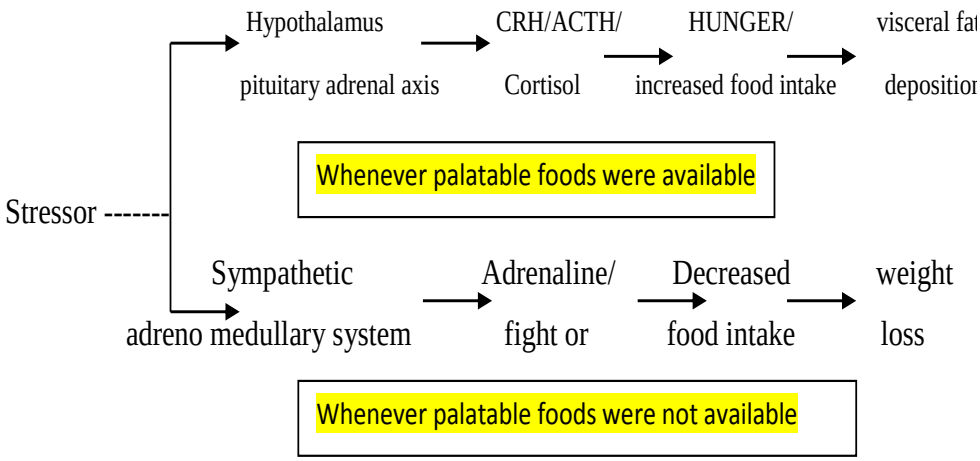


Fig 5.1: Schematic representation showing the effects of a stressor on an individual

Some studies shows that stress is known to cause oxidative damage. Stress responses can vary in the degree of activation of the HPA axis, and may stimulate either orexigenic (NPY, AgRP), or anorexigenic (POMC, melanocortin) pathways, in addition to changes in leptin and insulin, thus inhibiting feeding behavior. Studies shows that In terms of appetite regulation, CRH is released from the medial parvocellular (mp) paraventricular nucleus of the hypothalamus (PVN) in response to the stressor (Jooyeon j et al., 2013). In addition to response to the stressor adrenocorticotrophine will be released from pituitary and a sequence of events occur to release glucocorticoids. CRH is also released into the arcuate nucleus of the hypothalamus (ARC) to inhibit neuropeptide Y (NPY)/agouti-related peptide (AGRP) neurons there. This is normally responsible for inhibiting feeding behavior thus CRH released after stress inhibits appetite (Ozguner F.et al., 2005). Other molecules from the CRH family, such as urocortins, also play a role in appetite suppression. Some studies showed that CRH-deficient mice could have normal stress induced suppression of food intake, implicating other CRH- like molecules. Some studies also showed both CRH and urocortins suppress food intake particularly urocortin 1. It is likely urocortins 1, 2, and 3 influence appetite suppression by acting on the CRHR2

receptor in the hypothalamus. Centrally administrated urocortins are also able to suppress ghrelin secretion, potentially preventing ghrelin-induced stimulation of appetite. On the other hand, peripherally administered urocortins act at CRHR2 receptors in the gut to stimulate an increase in circulating ghrelin. In addition to acting on the NPY neurons of the ARC,CRH induced appetite suppression also involves other regions of the hypothalamus like the PVN, supra optic nucleus, perifornical and ventromedial hypothalamus as well as brain regions like lateral septum, para brachial nucleus, and the dorsal portion of the anterior bed nucleus of the stria terminalis(BNST). Thus they demonstrated that CRH injected directly into the dorsal anterior BNST (but not the ventral part or other brain regions such as the central amygdala) significantly reduces food intake (Sominsky L et al., 2014). Further, we also investigated the impact of RF-EMR on food intake and body weight, we observed that food intake weight was reduced in in the RF-EMR group compared to the control, however, it was not statistically significant. Further studies are required to provide conclusive evidence on the impact of RF-EMR on feeding behavior.

The stress of exogenous origins was reported to elevate the corticosterone levels in various organisms (Jeong et al., 2013). In our study, As compare to the normal rats, chronic application of variable stressors and RF-EMR have led to a significant elevation in serum corticosterone levels of Stress and RF-EMR groups, respectively. Stella J et al., 2013 found that increased serum corticosterone in cats exposed to multiple unpredictable stressors for a period of 5 days. The present study findings were also supported by Linz R et al., 2019, Cvitanović H et al 2020. In response to the stressor, adrenocorticotrophin is released from the pituitary, and a sequence of events is triggered to release the glucocorticoids. Studies have observed that glucocorticoid levels were increased when rats were subjected to a forced swim test until complete exhaustion (Linz R et al., 2019).

They concluded that stress in optimum quantum acted as a stimulator to achieve the best, but when it exceeded, it caused an imbalance in biochemical parameters as well as led to suppression in physical endurance (Linz R et al., 2019). Furthermore, we also noticed a significant increase in the serum corticosterone levels of RF-EMR exposed rats compared to the control but not as much as in the stress group. Corticosterone, a typical glucocorticoid, is produced in the Zona fasiculata of the adrenal cortex and is required to maintain blood glucose levels and prevent shock during times of stress. (Pamela et al. 2005) found that corticosterone regulates its own secretion via a negative feedback effect on the hypothalamic-pituitary axis. The temperature has been demonstrated to play a crucial role in the regulation of endocrine hormone release (Squires et al.,2003). Microwaves, particularly RF-EMR from mobile phones, can cause warmth and energy to be produced in living tissues (Hirata et al., 2002). The duration of RF-EMR exposure, on the other hand, is a significant influence. In a study, it was demonstrated that RF-EMR emitted from a mobile phone might produce impairments in some biochemical changes and oxidative stress in the brain, liver, and renal tissues of albino rats. It had a more potential brain risk due to a marked increase in the percentage level of oxidant states in the brain more than in the liver and the kidney (Merhan Mamdouh Ragy et al 2014). Our findings align with previous reports done by Maluin SM et al., 2021. Kim HS., et al 2021, Alkayyali T et al., 2021. The findings of the present study contradict a prior study that RF-EMR had no effect on corticosterone levels in male rabbits (Sarookhani et al., 2011). Dgeridane et al. (2008) found that a 900-MHz EMR (2 hours per day, 5 days per week, for 4 weeks) did not generate any significant changes in blood cortisol levels in men. We also found that the application of various physical stressors might cause profound physiological changes as compared to the RF-EMR group because Stress group displayed significantly higher serum corticosterone levels than the RF-EMR rats.

However, no significant difference was observed in the rats of vehicle group compared to the controls.

Various preclinical and clinical studies have explicitly linked stressful events to the increased oxygen free radicals and oxidative stress in various organs. MDA is one of the most often detected by-product formed during LPO of the phospholipid cell membrane, and it was reported to increase during stress conditions (Villacorta et al., 2003). Due to the damage of cell membrane by reactive oxygen species, Phospholipase A2 enzyme releases arachidonic acid from membrane phospholipids. In subsequent reactions, this yields the formation of MDA (Bhutia Y et al., 2011). Oxidative stress is a well-established cause of many chronic disorders (Khansari N et al., 2009). The etiopathogenesis of various chronic diseases has been linked to oxidative stress, which also plays a key role in the aging process (Tsikas D et al 2017). In the current study, we found that applying various stressors and RF-EMR to rats led to increased MDA levels due to increased lipid peroxidation due to possible elevation of reactive oxygen species. Our study findings were in agreement with Selvakumar et al.,2012, Bhale DV et al.,2014, Dahake HS et al.,2016, (studies that were done on human subjects) and Chamioło LP et .,2013, Karanth J et.,2004, Mossa ATH et al.,2014 (studies which were done on animals). Human subjects with oxidative stress show that free radicals of oxygen are extremely reactive and attack almost every cell component, causing damage to the surrounding tissues. The most damaging effect of oxidative stress is lipid peroxidation, which has been implicated in the pathogenesis of a variety of diseases such as atherosclerosis, diabetes, cancer, and ageing. The oxidative deterioration of PUFA is caused by a chain reaction initiated by hydrogen abstraction or addition by oxygen radicals. Because of its sensitivity and ease of use, the MDA assay of serum is the most commonly used method in clinical practise, despite the fact that several substances interfere with this assay. This study found

an increased concentration of MDA in obese subjects, indicating in vivo oxidative damage to lipids. On the other hand, Maneesh et al. also found significantly increased MDA levels in testis and epididymis of RF-EMR exposed male rats and concluded that mobile RF-EMR induces oxidative stress (Mailankot et al., 2009). Exposure to electromagnetic waves causes the production of free radicals, their attachment to macromolecules like lipids, and an increase in lipid peroxidation indicators like MDA. This study was also supported by the work done by Frank Giblin et al. where guinea pigs exposed to RF-EMR radiations for 10 days, led to increased MDA levels. (Giblin F et al. 2019). Moustafa et al., 2001 showed that the plasma level of lipid peroxide in healthy adult male volunteers was significantly increased after 1, 2 and 4 h of exposure to radiofrequency fields of the cellular phone in standby position. They indicated that acute exposure to radiofrequency fields of commercially available cellular phones may modulate the oxidative stress of free radicals by enhancing lipid peroxidation and reducing the activation of SOD. A study done on rat liver by using antenna radiations suggested that, elevated MDA could be due to cytochrome P450-mediated metabolism of the organic hydroperoxide to active alkoxyl radicals that initiated LPO and led to liver damage (Martignoni M et al., 2006). In line with the reported studies, our results also showed that multiple stressors and RF-EMR increases both corticosterone and lipid peroxidation levels in the blood. We also observed that the serum corticosterone levels and lipid peroxidation levels were profoundly increased in the stress group compared to the RF-EMR group, which reflects that multiple stressors are more adversely effects at the cellular level than the mobile radiation.

NSE is also a significant biomarker of oxidative stress and neuronal damage. It is found in the cytoplasm and dendrites of the neurons. Although its levels are low in peripheral

blood, it can be used as a sensitive indicator as it levels increases in serum during injury and damage (Hacioglu et al., 2016). In our study we have investigated the impact of 50 days of chronic multiple stressors and RF-EMR on the serum NSE levels. We observed that there was a significant increase in serum NSE levels in rats exposed to stressors and RF-EMR compared to controls. Frank Martin S et al., 2014 demonstrated that increased NSE levels in the MDD subjects compared to the healthy human controls. We also found that stress group displayed significantly higher serum NSE levels than the RF-EMR group rats. A study done by Gulay et al. also proved that using an electromagnetic field (EMF) exposure system (2 hr./day) for 90 days significantly increases the NSE levels compared to the controls (Gulay et al.,2016 Ozguner et al., 2005). Due to neuronal damage, there might be a breach of the blood-brain barrier, leading to elevated NSE levels in the brain that could have entered into the blood and causes the elevation of NSE levels (Daniels et al., 2009; Júnior et al., 2014). The increased NSE levels might be resulted from neuronal damage, neuronal death, synapse loss, and axonal myelin damage (Schmidt et al., 2015). In association with the increased NSE levels in our study we were also measured the CA3 region of hippocampal GFAP levels after chronic multiple stressors and RF-EMR. Several studies have shown that GFAP is involved in various physiological functions of astrocyte such as maintaining the BBB, synaptic plasticity, cell proliferation and regulating the transport of vesicles and lysosomes in astrocytes (Garcia et al., 2004). Several studies reported that GFAP is good reliable biomarker for the stress (Cho YE et al., 2017). We observed that GFAP levels were significantly increased in multiple stressors in the hippocampus. A recent study showed that CUS mice for 5 weeks significantly increased the GFAP+ cells in CA3 regions of hippocampus. Six weeks of CUS followed by another 6 weeks of social isolation-induced an increase in the number of GFAP+ cells in the dentate gyrus (Preez et al., 2021). Moreover, Michel et al., 2021

found that the level of GFAP was significantly higher in patients with unipolar depression than in mentally healthy controls with idiopathic intracranial hypertension. Hence, increase of GFAP may be responsible for stress-induced depression behavior. In the CNS, GFAP expression is commonly used to identify differentiated multipolar astrocytes in vivo and in vitro (Garcia et al., 2004), it has been well demonstrated that astrocytes exert rapid modulation of neuronal activity, whereas progenitor cells do not. Activated microglia releases large amounts of proinflammatory signaling molecules, such as interleukins, metalloproteinases and reactive oxygen species; all stimuli promoted by reactive microglia lead to a process of reactive astrogliosis (Liberto et al. 2004). In line with the reported studies the 50 days of CUS increases the GFAP levels in the hippocampus. In our study we also observed that GFAP levels in the hippocampus were positively correlated with serum MDA levels in the chronic multiple stressor group. Various studies have reported a positive correlation between increased oxidative stress and GFAP levels (Mutani et al., 2006). In our study the GFAP levels were not significantly changes in the RF-EMR group compared to the control group rats. We also observed that GFAP levels were significantly increased in the multiple stressor groups compared to the RF-EMR group. Which indicating that 50 days of RF-EMR induced changes were less significant compared to the 50 days of chronic multiple stressors induced alterations.

Overall, the study results showed that rats subjected to chronic multiple stressors led to the decrease of feeding behavior as well as reduced the body weight compared to the control group. The decreased feeding behavior and body weight was associated with the increased serum corticosterone, MDA, and NSE as well as increased the CA3 region GFAP expression levels. Even though the feeding behavior, body weight was significantly not decreased, MDA, NSE and corticosterone levels were significantly

increased after 50 days of RF-EMR exposure. Further, chronic multiple stressor is profoundly influence the behavioral, biochemical, and cellular levels compared to the mobile phone emitted radiation.

Vitamin E is a potent antioxidant, reduces lipid peroxidation, scavenging peroxyl and is well-known for its beneficial effects in animal models of various diseases (Kayalvizhi et al., 2012). A well-known antioxidant, vitamin E is a necessary nutrient for humans. Vitamin E decreases the reactive oxygen species activity and free radical production. Vitamin E, like other antioxidants, protects brain functioning from the number of disorders, including mental stress, diabetes, cerebral ischemia injury, Alzheimer's disease, stroke, and ageing (Mohammed et al., 2021). The research revealed a connection between the state of one's cognition and endogenous plasma vitamin E content. In the current study, Vitamin E supplementation significantly improved the feed intake and prevented the decrease in the body weight of the rats in the Stress + Vitamin E group. It also significantly decreased the stress-induced serum corticosterone, MDA, NSE levels in Stress+Vitamin E and RF-EMR+Vitamin E group animals compared to the Stress and RF-EMR groups. Furthermore, the brains of rats supplemented with Vitamin E showed significantly lesser number of GFAP immunopositive neurons. Our study results align with the studies done by Azman et al., 2001 Mohammed et al., 2021 Kayalvizhi et al., 2012 & Fahami et al., 2005. A study by Jelodar and colleagues found that prolonged exposure to 900 MHz mobile phone radiation (45 days, four hours per day) increased MDA and decreased levels of antioxidant enzymes like GPX, CAT, and SOD, but that supplementing with 200 mg/kg vitamin E during this time period increased antioxidant enzyme levels and decreased MDA. In a nutshell, the protective effects of Vitamin E against stress-related alterations can be linked to its compelling antioxidant potential. In

a study it was demonstrated that Vitamin E supplementation is particularly beneficial for stressed farm animals. Stress-induced corticosteroids may also be reduced by vitamin E. (Tengerdy et al., 1989). Vitamin E supplementation to preserve the health of stressed animals tended to minimize the increase in blood tissue damage indicators during road travel. As a result, vitamin E administration may increase animal wellbeing during road transportation (Morán et al., 2012). The effects of vitamin E treatment on transport stress in sheep and goats have mostly been studied in terms of hematology, cortisol levels, weight loss, and wellbeing indices.

Studies shows that Vitamin E is an antioxidant, anti-inflammatory, free radical scavenger and has several benefits. Vitamin E in practice does not show any toxicity to humans within a range of reasonable dosage, but some studies also shows that Vitamin E toxicity occurs due to excessive vitamin supplementation, not due to diet alone. Vitamin E toxicity leads to risk of bleeding, muscle weakness, fatigue, nausea, diarrhea, cancer risk, heart problems, and an increased risk of bone fractures. The mainstay treatment for vitamin E toxicity is stopping the exogenous vitamin supplementation. This is effective since vitamin E toxicity does not occur unless there is an exogenous supplementation. So based on literature search we suggest that instead of intake of exogenous vitamin supplementation to avoid oxidative damage caused by day-to-day life stress and through mobile radiations it's better to modify lifestyle.

Vitamin E is a powerful antioxidant that inhibits propagation of free radical reactions and prevents oxidative stress. Results of the current study revealed that Vitamin E normalized the increased MDA, NSE, corticosterone and GFAP levels and improved feeding behavior in the rats subjected to the RF-EMR and chronic multiple stress. In conclusion, Vitamin E prevents the deleterious effect of multiple stress and RF-EMR altered feeding

behavior and body weight; probably through normalization of the antioxidant defence mechanisms.

Based on literature search and findings from our study we suggest that instead of intake of exogenous vitamin supplementation to avoid oxidative damage caused by day to day life and through mobile radiations it's better to modify lifestyle by maintaining regular sleep cycle, Exercise, well balanced diet, Meditation and yoga. We also advise individuals not to use mobile phones for extended periods of time to avoid ill effects of EMR. Withdrawing from EMR might undoubtedly overcome the harmful effects of EMR exposure, bringing it closer to the normal state. Several experiments are still needed to explain which frequencies, intensities, exposure times, and other factors are linked with EMF, particularly when combined with environmental toxins, in order to protect oneself from those damages. Vitamin E usage for a period of 50days can undoubtedly reverse the changes but long term usage of Vitamin E has to be studied before recommending.

Conclusion

6. CONCLUSION

Based on the findings of this study, we conclude that exposure to multiple stressors like Restraint, High-intensity noise and swimming stress led to significant increase in serum corticosterone, NSE, MDA and GFAP and altered feeding behavior, which resulted in decreased food intake and reduced body weight in rats. Mobile RF-EMR significantly increased serum corticosterone levels, NSE, and MDA levels. Altered feeding behavior of rats exposed to RF-EMR was not statistically significant. Furthermore Concurrent treatment with Vitamin E offered significant neuroprotection as observed in NSE, MDA, Serum corticosterone and GFAP against various stress- induced alterations. Impact of RF-EMR was less as compared to chronic unpredictable stress based on the changes observed in NSE, MDA and GFAP. However, detailed studies with longer exposure and increased sample size may help to unravel the molecular mechanisms to understand various RF-EMR induced physiological and behavioural alterations. Therefore in vivo regular supplementation with vitamin E shall decrease the impact of various RF-EMR from gadgets used in recent days and physical stress faced every day.

Summary
Recommendations
Future prospects

7. SUMMARY

- Exposure to stress is inevitable. Stress affects the brain both physiologically and chemically. Depression and anxiety are extremely common during stress. Recent days mobiles are also known to cause oxidative stress. Stress affects feeding behavior.
- Fifty days of multiple stressors significantly reduced the feed intake and body weight in the Stress group compared to the control group ($p<0.05$). A significant reduction in feed intake was observed in stress group starting from Day 1 and body weight from Day 30 and continued until the end of the study compared to control. An Overall reduction of 20% in body weight was observed in stress.
- The feed intake in the 50 days of RF-EMR group was comparable with the control ($p>0.05$). Although the body weight was reduced in the RF-EMR group but it was not statistically significant compared to the control ($p>0.05$).
- The serum NSE, MDA and corticosterone levels were significantly increased in the both chronic multiple stressors and RF-EMR grouped rats compared to the control.
- The GFAP levels in the CA3 region of hippocampus was significantly higher in the Stress group compared to the control group. The GFAP levels in the CA3 region were comparable between the RF-EMR and control group.
- The MDA, NSE and GFAP levels were significantly higher in the multiple stressor group compared to the RF-EMR group. Which indicating that 50 days of RF-EMR induced changes were less significant compared to the 50 days of chronic multiple stressors induced alterations.

- Concurrent treatment of 100mg/kg vitamin E supplementation for 50-days to the multiple stressors and RF-EMR rats showed an improvement of feed intake and body weight compared to the multiple stressor and RF-EMR group rats.
- Concurrent treatment of 100mg/kg vitamin E supplementation for 50-day to the multiple stressors and RF-EMR rats normalised the increased NSE, MDA, corticosterone levels observed in the multiple stressors and RF-EMR group rats.
- Concurrent treatment of 100mg/kg vitamin E supplementation for 50-days to the multiple stressors normalised the increased GFAP levels observed in the multiple stressor group rats.

8. RECOMMENDATION:

Based on literature search and findings from our study we suggest that instead of intake of exogenous vitamin supplementation to avoid oxidative damage caused by day to day life and through mobile radiations it's better to modify lifestyle by maintaining regular sleep cycle, Exercise, balanced diet, Meditation and yoga. We also advise individuals not to use mobile phones for extended periods to avoid ill effects of EMR. Withdrawing from EMR might undoubtedly overcome the harmful effects of EMR exposure, bringing it closer to the normal state. Therefore, increased anti- oxidants intake may nullify the effect of RF-EMR from gadgets and physical stress caused every day. Vitamin E usage for a period of 50days can undoubtedly reverse the changes but long term usage of Vitamin E has to be studied before recommending.

9. FUTURE PROSPECTS

Results from our study indicate that the 1-hour RF-EMR exposure for 50 days showed an insignificant decrease of feeding behavior compared to the control and Stress group animals. Further studies with longer (>1 hour) RF-EMR exposure for longer periods (2-3 months) might facilitate novel and deeper insights into the effects of RF-EMR on feeding behavior and underlying mechanisms.

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Publications, Presentations & Workshops

11. PUBLICATIONS AND PRESENTATIONS

11.1. *Publications:*

1. Pagadala P, Shankar MS V, Kutty K (2019). Feeding Behavior and its Association with Stress: A Review Journal of Clinical and Diagnostic Research. Vol-13(3):CE01-CE05.
2. Pagadala P, Shankar MS V, Sumathi ME (2022). Effect of Mobile phone radio frequency electromagnetic radiations (RF-EMR) on Neuronal specific enalose (NSE) and Malondialdehyde (MDA) levels in Sprague dawley (SD) rats. 18(6):501-505.
3. Pagadala P, Shankar MS V, Sumathi ME (2022). Effect of Mobile phone radio frequency electromagnetic radiations on Oxidative stress and feeding behavior in Sprague dawley (SD) rats (Accepted for publication)Indian journal of physiology and pharmacology

11.2. *Oral Presentations:*

1. Effect of Mobile phone radio frequency electromagnetic radiations (RF-EMR) on Neuronal specific enalose (NSE) and Malondialdehyde (MDA) levels in Sprague dawley (SD) rats" at RAMSIECON-2021 Recent Advances in Medical Sciences International E-Conference conducted by Department of Physiology from 1st to 3rd July 2021 at Yenepoya Medical College, Mangalore, Karnataka, India.
2. Effect of mobile phone radio frequency electromagnetic radiations (RF-EMR) on Feeding behavior in Sprague dawley (SD) rats has been presented on 14/03/19-16/03/19 at 32nd annual meeting of Society for neurochemistry SNCI CON 2019, India at JSS Academy of higher Education.

12. Workshops attended/conducted related to my study:

1. I have completed adhoc training course in current regulations, handling, breeding, maintenance and techniques in laboratory animal experiments from 12th February to 17th February at NCLAS,NIN,Hyderabad
2. We conducted a State level State Level CME on **Appetite, Food Craving and Obesity** on 29th August 2017 which was sponsored by MERT.
3. We conducted a CME titled "Physiology of Cognitive Neuroscience & its Alterations "On 6th August 2018 Sponsored by MERT, Karnataka.
4. We also organized World obesity day "First MBBS student performing anthropometric measurement's at Suguna International School" on 11/10/2018. An interactive session was done where the significance of BMI and importance of maintain ideal body weight was discussed.

13. Courses completed:

1. I have cleared BCBR exam conducted by ICMR
2. I have successfully completed online course on research methodology conducted by MEU India & National institute of continuing professional development between 15th April to 15th July 2018.

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Review Article

Physiology Section

Feeding Behaviour and its Association with Stress: A Review

PRAVALLIKA PAGADALA[1], MS VINUTHA SHANKAR[2], KARTHIYANEE KUTTY[3]

ABSTRACT

Feeding behaviour is a complex behaviour in the present daily life which is regulated by many mechanisms. So many factors influence the feeding behaviour, among the most common one, is stress. So in the present review authors tried to explain about feeding behaviour, factors regulating it, stress and its probable causes.

Keywords: Body weight, Food availability, Homeostasis, Hypothalamus

Feeding Behaviour

This behaviour is related to obtaining and consuming food. It is a complex behaviour in the present day to day life that is regulated by many mechanisms like external factors such as food availability and quality, as well as by internal factors including sex, age, circadian rhythms and most importantly, the hormonal status related to energy homeostasis [1]. The understanding of the physiological basis of feeding behaviour regulation is particularly important, as in affluent societies, obesity has become a widespread problem [2]. However, the exact regulatory mechanisms are not well understood. Since hypothalamus is the centre for controlling feeding behaviour, a study has proved the role of hypothalamus in feeding behaviour and is mainly under the control of leptin and insulin [3-5]. Several hypothalamic neuropeptides such as Agouti-Related Protein (AgRP) and Neuropeptide Y (NPY) have been shown to be potent feeding stimulants, whereas Pre Opio-Melanocortins (POMC) and Cocaine- and Amphetamine-Regulated Transcript (CART) have been shown to suppress as done for the other food intake [6].

Physiology of Feeding

Feeding and satiety: Body weight depends on the balance between caloric intake and utilization of calories. Food intake is regulated not only on a meal to meal basis but also in a way that generally maintains weight at a given setpoint. If animals are made obese by force-feeding and then permitted to eat as they wish, their spontaneous food intake decreases until their weight falls to the control. If animals are starved and then permitted to eat freely, their spontaneous food intake increases until they regain the lost weight. It is common knowledge that the same thing happens in humans. Dieters can lose weight when caloric intake is reduced but when they discontinue their diets, 95% of them regain the weight they lost. Rebound weight gain is also noticed post recovery from illness that leads to weight loss.

Role of Hypothalamus

Hypothalamic regulation of the appetite for food depends primarily upon the interaction of two areas: a lateral feeding centre in the bed nucleus of the medial forebrain bundle at its junction with the pallido-hypothalamic fibres, and a medial satiety centre in the ventromedial nucleus. Stimulation of the ventromedial nucleus leads to inhibition of food intake, likewise, excessive eating termed as hyperphagia in the presence of increased food supply follows lesion in ventromedial nucleus resulting in hypothalamic obesity [Table/Fig-1] [7,8].

Theories of Feeding

There are four theories of feeding which includes: 1) Lipostatic theory; 2) Thermostatic theory; 3) Glucostatic theory; 4) Gut peptide theory.

Factors increases food intake	Ghrelin Orexin A Orexin B Neuropeptide Y Melanin Concentrating Hormone (MCH) GHRH
Factors decreases food intake	Peptide YY Leptin Glucagon CCK,CRH,GRP Somatostatin Oxytocin Bombasin Malonyl co A

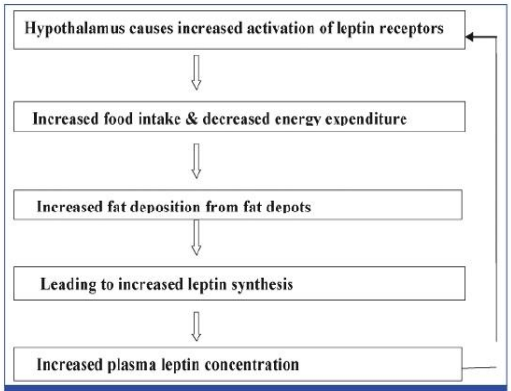
[Table/Fig-1]: Showing the factors which increases and decreases food intake [7].

Lipostatic Theory

Leptin is a hormone produced from fat cells, circulating protein containing 167 amino acids. Its name is derived from Greek word for thin. It acts on hypothalamus to decrease food intake and increase energy consumption. It appears to decrease the activity of neuropeptide Y neurons that increase appetite and to increase the activity of POMC secreting neurons. To reach its central site of action, circulating leptin must cross blood brain barrier. A short form of the leptin receptor is abundant in brain microvessels and probably is involved in transport of leptin into the brain. Leptin receptors are found in various tissues as well as in brain. In rodents, the decrease in plasma leptin produced by fasting is associated with inhibition of onset of puberty, depressed thyroid function and increased glucocorticoid secretion. It has been suggested that these are adaptive responses to the shortage of calories signalled by the decrease in leptin. Leptin receptors present in brown adipose tissue increases the activity of uncoupling proteins, thus producing a direct peripheral increase in energy expenditure [Table/Fig-2] [7,9].

Gut Peptide Theory

Ghrelin is a hormone with 28 amino acid secreted from stomach. It operates like leptin as a peripherally generated regulator of appetite, but in the opposite fashion. It not only secretes growth hormone but also effects food intake and is increased during fasting. Central, peripheral and intraperitoneal administration of ghrelin increases food intake by stimulating arcuate nucleus, will be low in obese. Number of GI hormones have been reported to decrease food intake like peptide YY, PYY (secreted from small intestine and colon, levels are low in obese. It is due to idea that food entering



[Table/Fig-2]: Release of leptin from hypothalamus [7].

the GIT triggers the release from the mucosa of substances which act on the brain to produce satiety. Ghrelin has to be acylated before it is released. An enzyme necessary for acylation of ghrelin is the Ghrelin O-acyltransferase, also expressed in the circulation of rodents [10].

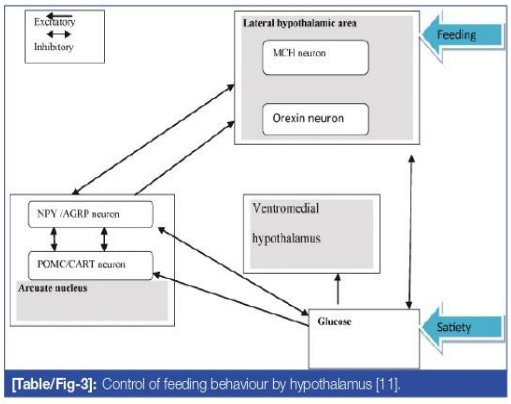
Glucostatic Theory

The activity of the satiety centre in the ventromedial nuclei is probably governed in part by glucose utilisation of the neurons in it. Food intake is rapidly increased by intraventricular administration of compounds such as 2-deoxyglucose that decrease glucose utilisation in cells. Polyphagia is seen in diabetes mellitus, in which blood glucose is high but cellular utilisation is low because of the insulin deficiency [11].

Thermostatic Theory

Food intake is increased in cold weather and decreased in warm weather. However, there is little evidence that body temperature is a major regulator of food intake [7,12].

Schematic of the control of feeding in the hypothalamus: There are many factors that affect feeding behaviours such as Social facilitation, endocrine disorders, seasonal changes and stress. Among them, stress is the most common factor which affects the feeding behaviour in the life [Table/Fig-3] [13].



[Table/Fig-3]: Control of feeding behaviour by hypothalamus [11].

Stress

Living beings are exposed to stress of varying degrees throughout their life. Stress is the disturbance of the normal body's homeostasis or a condition of disharmony in response to a real or perceived threat or challenge. The threatening or challenge causing situation is referred to as a "stressor" [14]. It can also be described as a state characterised by a broad range of physiological and behavioural changes resulting from one or more stressors and biologically it is defined as "the non-specific response of the body to any demand" [3]. Today's world man is exposed to large number of stress from a variety of sources.

Impact of Stress on Health

It is a significant individual and public health problem that is associated with numerous physical and mental health concerns. It is estimated that between 75% and 90% of primary care physician visits are caused by stress-related illnesses [15]. Cardiovascular disease, obesity, diabetes, depression, anxiety, immune system suppression, headaches, back and neck pain, and sleep problems are some of the health problems associated with stress. These conditions are most burdensome health problems in the United States based on health care costs, the number of people affected, and the impact on individual lives. Extreme levels of stress were reported by 22% of respondents from the 2011 Stress in America™ survey, and 39% reported that their level of stress had increased during the past year. More than 80% of the survey respondents at the world at work conference in 2012 reported that stress moderately or significantly contributed to their health care costs [14,16-18]. Stress can increase the risk for chronic diseases and other health problems, dealing with chronic conditions and poor health can increase the amount of stress one experiences. Stress also influences behaviours that affect health. Diet choices, sleep habits, and drug use are behaviours that are often negatively affected by stress. The APA's 2011 survey showed that 39% of respondents reported overeating or eating unhealthy food because of stress, and 29% reported skipping a meal. In addition, 44% reported lying awake at night because of stress. On a positive note, 47% of respondents reported walking or exercise as a way of managing stress [19].

Types of Stress

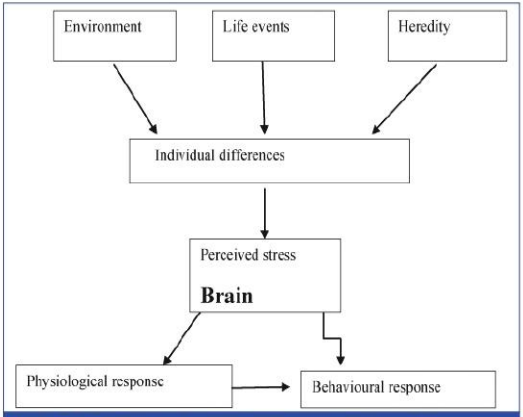
It is very important to note that all stresses or stressors are not bad. Every individual experience a certain amount of stress on an almost daily basis, it cannot be eliminated completely. When it is experienced too much then it becomes a problem. Stress is of two kinds – Eustress-a positive form of good stress that motivates an individual to continue working and Distress – manifests when stress is no longer tolerable and/or manageable [20]. Stress may be eustress or distress can be determined by individual's perception and coping resources for the stressor [21].

Based on Duration of Exposure

It can be chronic or acute. Chronic stress is a persistent, long lasting stressor, such as living with a terrible roommate, money, work, economy, death of loved ones, divorce, family and personal health problems. It is not easily resolved associated with negative health concerns. It results when there are constant multiple stressors or major life stressors present [22]. Acute stress, on the other hand, is a short lasting and one time stressor, such as failing an exam. Health is affected both physically and psychologically by exposure to repeated episodes of stress either due to excessive workload or constant worries. Stress coping skills will have to be learnt by such individuals exposed to episodic stress to avoid negative effects on health due to stress [13]. In addition, stress can be major or minor. A major stressor would be something like a death in the family, while a minor stressor may be as simple as getting stuck in traffic [23].

Stress affects the brain both physiologically and chemically. Depression and anxiety disorders are extremely common during stress. It is clear that these disorders are quite common in the general population, yet there is much to be learned about the causes of these disorders. Symptoms of depression include depressed mood, anhedonia, altered appetite, and nervousness, fatigue, lack of concentration, frequent colds, sleep disturbance and irritability. These psychopathologies develop by a complex interaction between genetic predisposition and an adverse environment [23].

Homeostasis that is challenged due to stressor in an organism may be regained by producing appropriate physiological responses to stress. Stress has long been considered as a critical risk factor in the development of addictive disorders and relapse to addictive behaviours [24,25]. The response to a stressor depends on how the individual assesses their environment and the stressor they experience. When the stressor exceeds a person's ability to withstand or ability to respond appropriately to the stress, the homeostasis is disturbed [18]. One such homeostasis that is disrupted is in feeding behaviour [Table/Fig-4] [23].



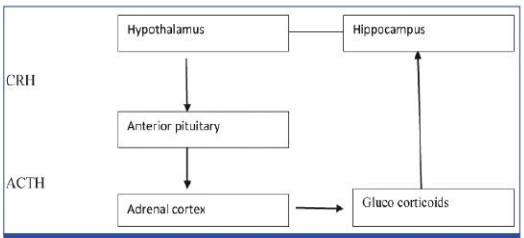
[Table/Fig-4]: Factors causing stress and its response from brain [23].

Stress does not affect each individual the same way. A stimulus that may be stressful to one individual may not be stressful to another. Environment, life events, and genetics play a role in an individual's tolerance for stress. When an individual perceives a stimulus as stressful, a physiological and behavioural response will be displayed to regain equilibrium lost by the stressor [23].

Stress response due to endocrine and autonomic nervous system actions by epinephrine release prepares the body to overcome threat or challenge that is perceived as stress [26]. These physiological changes including increase in heart rate, respiratory rate, blood pressure, sweating and heightened acuity of senses associated with endorphin release characterise the fight or flight response which is manifestations of stress coping measures. There may be surplus energy production due to enhanced cortisol production which might suppress immune function [10]. A stressful situation induces the organism to mobilise not only the adrenal system and endocrine system but also the central nervous system and the pituitary [27]. One of the main mechanism by which stress affects the brain is by oxidative stress causing oxidative damage. During stressful conditions, oxidative stress reflects a mismatch between reactive oxygen species' effect on the biological systems in the body and the detoxifying ability of these systems to repair the damage due to reactive intermediates. Disturbances in the normal state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell. It can cause disruptions in normal mechanisms of cellular signalling and is associated with increased production of oxidising species or significant decrease in the effectiveness of antioxidants such as glutathione [28].

Effect of Acute and Chronic Stress on Hypothalamic Pituitary Adrenal Axis

It is well known that the Hypothalamic-Pituitary-Adrenal (HPA) axis helps in maintaining basal and stress related homeostasis of the nervous system as well as cardiovascular, immune and metabolic functions. The main regulation of circadian and stress-related activity of the HPA axis occurs at the level of parvicellular subdivision of the hypothalamic Paraventricular Nuclei (PVN). The majority of these neurons secrete CRH and Vasopressin (VP), which synergistically stimulate ACTH secretion by the pituitary corticotrophic cells.ACTH in turn leads to formation of Corticosterone (CORT) from Cholesterol (CHOL) which when released by the adrenal cortex can serve as inhibitory feedback control in the HPA system [Table/Fig-5] [29].



[Table/Fig-5]: Showing the Release of glucocorticoids from hypothalamus [23].

It has been postulated that stress of any kind, acute and mild, or chronic and severe affects eating habits [30].

During acute stress Corticotropin-Releasing Hormone (CRH) is released from the medial parvocellular (mp) paraventricular nucleus of the hypothalamus (PVN), ACTH is released from pituitary and the cascade of reactions takes place for the release of glucocorticoids. CRH is also released from arcuate nucleus to inhibit neuropeptide Y and Agouti-Related Peptide (AGRP) (these are the hormones that increases food intake and suppresses energy expenditure). Urocortins are the molecules, belongs to CRH family, also released at this time which is also responsible for suppressing appetite by inhibiting the ghrelin activity. Thus, during acute stress, CRH is responsible for inhibiting the appetite [30,31]. Thus, CRH injected directly into the dorsal anterior Bed Nucleus of the Stria Terminalis (BNST) the ventral part or other brain regions such as the central amygdala or locus coeruleus) significantly reduces food intake in already food-deprived rats [32].

During chronic stress, glucocorticoids released from HPA in the bloodstream are elevated. Peripherally, glucocorticoids enhance the activity of lipoprotein lipase in adipose tissue, leading to an increase in fat storage [33]. Thus, in humans, a peripheral injection of CRH leads to increased food intake one hour later but the amount of food consumed is directly correlated with the magnitude of the cortisol response to the injection [34]. Glucocorticoids stimulate food intake by interacting with several appetite-regulating targets. They increase AMP-activated protein kinase signalling in the ARC to up-regulate NPY and AGRP expression in this region and stimulate the actions of these orexigenic peptides (increases food intake) [30]. It has been observed that rats prefer foods rich in fat and sucrose among other food items when exposed to stress indicating the need for more rewarding foods and preference for comfort foods due to stress induced glucocorticoid release [35].

Types of Stressors in Laboratory Animals

Physiological includes restraint stress, forced swimming stress, noise stress, electric foot shock stress, food deprivation. Restraint stress or immobilization is commonly used because it is less severe, but is still capable of activating the stress response by stimulating the hypothalamic pituitary adrenal axis. In this type of stressor, movement is limited by placement of rodent in a plexiglass chamber or immobilization bag. After every use of restraint, instruments should be washed to prevent any transmission disorders [36]. High intensity noise exposure (50-90 db) is also used as a physical stressor. This protocol can be used as a type of environmental stressor to mimic stress in everyday life. This stress is given for a period of 30 minutes continuously and animal is allowed to take rest for 10 minutes, remaining 30 minutes procedure is continued. It can able to stimulate the hypothalamo-pituitary adrenal axis and generate brain activity by causing anxiogenic effect on behaviour. If examiner is also staying in same room it's better to use ear protectors [37].

Forced swimming stress: Animal made to swim in the water forcibly for five minutes then grasp the animal gently by the tail or trunk lift from the water, place it to dry. Container used for this should be 45 cm in length and 30 cm in breadth water has to be filled up to 30 cm. In each container two or three rats are permissible. After stress procedure animal is grabbed by tail and placed in a dry cloth. Like this procedure can be done by repeated intervals for 1 hour/day making sure that animal should not drown [38,39].

Electric foot shock stress: It is more severe stressor and can be applied using a metallic grid to shock the foot or to the tail. In this the suggested shock duration to be very short and the shock levels should be very low. For this type of stressor, animal should be trained in learning tasks for a period of some days but this is known to cause degeneration of structure. After completion of procedure for each rat that metallic grid floor should clean with ethanol or spirit to remove the odour, faeces and urine [39].

Food deprivation stress: It is the restriction of ad libitum amount of food for a certain period of time, giving feed at a fixed time for a period of four hours [40].

Psychological stress includes Maternal separation, Overcrowding, Social isolation, Sleep deprivation, Resident/intruder.

Maternal separation stress: It is an excellent prenatal stressor leads to activation of hypathalam-pituitary adrenal axis due to separation and handling. This stressor involves removal of pup from the care of its mother for certain period of time [38].

Overcrowding stress: permissible limit of animals in a cage is 2-3 but in this study, it involved the animals more than 4-5 [23].

Social isolation: It is a common type of psychological stressor in which subject is placed in a long term solitary housing at least about two to four weeks without any companion. Placing each animal in one cage and permitting the animals to listen and smell of other rats in the same colony [23,38].

Sleep deprivation: This type of stress is controversial because it is quite severe, in this animal is not allowed to sleep certain period of time by keeping the animal in a revolving drum, sleep deprivation drum or inverted flower pot technique [41].

Resident/intruder stress: It is social conflict stress involving threat from an aggressive male [23].

Some of the Review of Works on Stress Related to Feeding Behaviour Stress is known to alter feeding responses in a bidirectional pattern, with both increased and decreased intake of food [42]. It is an important factor in the development of addiction and in addiction relapse and may contribute to an increased risk for obesity and other metabolic diseases [43]. Uncontrollable, stress changes eating patterns and the salience and consumption of hyper palatable foods that induce metabolic changes that would promote weight and body fat mass [4]. Famitafreshi H et al., have demonstrated that food consumption was increased in addicted isolated rats when compared to addicted socialised rats and concluded that feeding behaviour was regulated by adult hippocampal neurogenesis in addiction period and socialisation improves it [44]. Stress also causes elevated glucocorticoid levels leading to stimulation of feeding behaviour and excessive weight gain [45]. Carr JA et al.,demonstrated that stress inhibits feeding behaviour in all vertebrates [46]. Restraint stresses have shown to reduce body weight due to decreased intake of food [47]. Cristina R et al., demonstrated that acute stress causes weight loss and chronic stressors promote the activation of the pro-obesogenic mechanisms that favour the accumulation of central/visceral fat [48]. However, Favreau-Peigne et al., demonstrated that chronic stress altered animal welfare but also decreased body weight [49].

Although acute stress has shown to have facilitating effects on memory, chronic stress causes development of psychiatric disorders (depression and anxiety) which lead to altered appetite [32]. Stress was associated with significant elevation in the markers of oxidative stress in the cerebral cortex. Hacioglu G et al., demonstrated that exposure to stress causes increased production of ROS. Brain Derived Neurotrophic Factor (BDNF) has a crucial role in the survival and supports the neuronal cells, neuronal integrity and connectivity. It has role in neuronal process and its interaction with ROS might be crucial for neurodegenerative and neuropsychiatric abnormalities and the decreased expression of BDNF is implicated in the sensitivity to stress and stress enhanced responses. Also, BDNF deficient mice were observed to be more susceptible to stress induced oxidative damage, which suggests that there is direct interplay between oxidative stress indicators and BDNF levels in the brain [32].

Medina HV et al., stated that stress causes neuronal damage leads to significantly higher concentration of lipid peroxides by products like MDA and neuronal damage marker NSE [50]. It was proved that during oxidative stress the concentration of Glutathione (GSH), activity of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX) was decreased while Malondialdehyde (MDA) were seen to be increased in different brain regions of experimental rats (hippocampus, cerebellum respectively) in comparison to the control group. It was obvious that MDA, the indicated marker for Lipid Peroxidation (LPO), showed a significant elevation in the exposed group compared to control [51]. Under normal condition, the over ROS production was neutralised by the antioxidant defence mechanisms. Glutathione (GSH) is an important non-enzymatic antioxidant that plays a crucial role in the detoxification of ROS. SOD and GPX enzymes are the first line of cellular defence against oxidative injury [52].

CONCLUSION

Stress is known to cause many factors leading to various disorders. It is known to cause oxidative stress by causing oxidative damage. Uncontrollable stress causes changes in feeding behaviour and is known to alter feeding responses in a bidirectional pattern, with both increased and decreased intake of food.

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CERTIFICATE

This is to certify that, the Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the Study topic entitled **"Effect of mobile phone Radio Frequency Electromagnetic Radiations (RFEMR) on Neuronal Specific Enalose (NSE) and Malondialdehyde (MDA) levels in Sprague Dawley (SD) rats"** for **Publication** authored by **Mrs. Pravallika (Corresponding Author)**, Dr. Vinutha Shankar M S & Dr. Sumathi M E[1] in the Departments of Physiology & External Member1 at Sri Devaraj Urs Medical College, Tamaka, Kolar.

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Effect of RFEMR on NSE and MDA levels in Sprague Dawley rats

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Abstract:
Radiofrequency emitted radiations (RFEMR) from mobile phones are known to produce a stress response because of its effect on hypothalamus. Mobile phones have become an integral part of our lives with increasing usage not only in terms of number of users but also increase in talk time. Therefore, it is of interest to study the effect of mobile phone radiofrequency electromagnetic radiations on NSE and MDA levels in SD rats. Twelve male SD rats of 10-12weeks old, weighing 180-220 grams, were purchased from registered laboratory breeders & housed in a room with 12:12hour's light-dark cycle with *adlibitum* amount of food and RO water. Present study showed

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significant increase in NSE and MDA levels in rats exposed to RFEMR. This study proves that mobile RFEMR causes oxidative stress and oxidative damage in SD rats.

Keywords: Oxidative stress, oxidative damage, reactive oxygen species, neuronal cells, brain damage.

Background:

Radiofrequency emitted radiations (RFEMR) from mobile phones are known to produce a stress response because of its effect on hypothalamus. Mobile phones have become an integral part of our lives with increasing usage not only in terms of number of users but also increase in talk time **[1].** Major mechanism by which stress affects the brain is by oxidative stress which in turn causing oxidative damage **[1].** During stressful conditions oxidative stress reflect difference between the systemic manifestation of reactive oxygen species and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal state of cells can cause toxic effects during the production of peroxides and free radicals that damage all components of the cell **[2].** It can cause disruptions in normal mechanisms of cellular signaling & is connected with enlarged production of oxidizing species **[3].** Indeed, this phenomenon has been documented after RF EMR treatment in whole body and ovarian tissue models of Drosophila, mouse fibroblasts, cultured breast cancer cells, rat heart tissue, and human lens epithelial cells **[4].** Studies showed that stress causes oxidative damage leading to oxidative stress effect on brain cerebral blood flow, blood brain barrier, and neuronal damage etc. **[1-3].**

Concern for the probable health effects of mobile phone usage are increasing as the number of users has increased massively **[5].** Mobile phone technology uses RF EMR and with increase in mobile usage, there has been a drastic increase in the RF EMR exposure encountered in daily life. Many recent studies have raised questions about the safety of such RF EMR exposure **[6].** Brain derived neurotrophic factor (BDNF) has a primary role in the survival & supports the neuronal cells, neuronal integrity and connectivity. It has a crucial role in neuronal processes **[7].** Any Change in the levels and activities of BDNF may lead to impaired neuronal development, neuro-plasticity and synaptic connectivity which in turn leading to number of neurodegenerative disorders **[7].** BDNF interaction with ROS is critical for neurodegenerative and neuropsychiatric abnormalities. The activity of BDNF is assessed by estimating the Neuronal specific enalose (NSE), Malondialdehyde (MDA) **[8].** NSE is a soluble glycolytic pathway enzyme which plays a role in neuronal differentiation [9,10]. Before identification in the tumors and endocrine cells it was seen first in brain tissue **[11].** It was suggested that NSE neurons are present in all cells originating from neuro-endocrine tissues, thus it might be used as a reliable indicator for presence of oxidative damage and neuronal injury **[12].** MDA plays a key role in modifying low density lipoprotein (LDL), which mediates the patho-physiological changes by non-enzymatic and auto-oxidative glycosylation **[13].** It is an end product formed during oxidative stress and lipid peroxidation. If any free oxy radicals are produced in the body it causes peroxidative breakdown of phospholipids which leads to accumulation of MDA **[14].** Thus, the present study was aimed to study the effect of mobile phone radiofrequency electromagnetic radiations on NSE and MDA levels in SD rats.

There is a widespread use of wireless, cellular, and mobile phones. Each of which is a part of modern life. The longer exposure of these devices is known **[15].**The levels of the electromagnetic radiation have increased causing damage to tissue. These electromagnetic radiations have produced lots of side effect on the human meningeal tissues and brain **[16].** It has attracted many researchers on the effect of RF EMR on the various fields of epidemiology, cell biology and toxicology but not many studies have been seen on oxidative stress and damage **[17].** Studies have not explored the direct effect of mobile phone RFEMR on serum NSE and serum MDA levels. Some studies have estimated MDA levels on tissues and some of them have carried their studies on the NSE levels by using electromagnetic system. So, as we continuously using and carrying mobile phones with us this study will directly help us to know the changes in serum NSE and serum MDA levels. Therefore, it is of interest to study the effect of mobile phone RFEMR on serum NSE and serum MDA levels in SD rats.

Materials and Methods:

[1] The present study was approved by Institutional Animal Ethics Committee on 28thJune 2018 with reference number IAEC/PHARMA/SDUMC/2017-18/08a. The study was conducted at central animal house, Sri Devaraj Urs Medical College, Kolar, Karnataka.

[2] Twelve Male SD rats of 10-12weeks old, weighing 180-220 gms, were purchased from registered Biogen laboratory breeders& housed in a room with 12:12 hour's light-dark cycle with ad-libitum amount of food and RO water. They were fed with rat pellets purchased from champaka feeds. The floor of the cages was covered with sawdust to provide a comfort floor for the rats and to make cleaning of the cage convenient when littered.

[3] The rats were allowed to acclimatize to the laboratory environment for about two weeks before the commencement of the study. Animals were taken care as per CPCSEA guidelines.

Group 1:

Control animals with *ad libitum* amount of food and RO water.

Group 2:

Animals were exposed to RF EMR emitted from mobile phoneGSM (0.9 GHz/1.8 GHz) which is kept in answer mode in the cage for one hour per day for 5days a week for 50 days. By using radiofrequency decibel meter -178s radiations were measured and animals were kept at a

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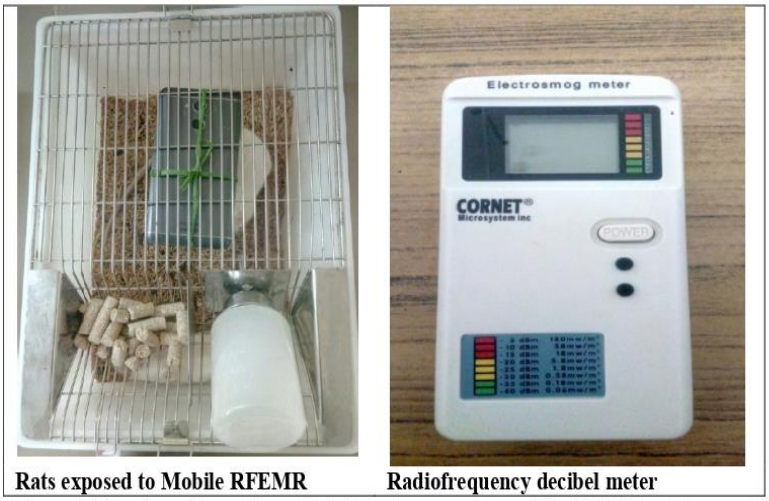
distance of 15-20cms from the mobile phone. Animals were allowed to move freely in the cage with continuous access of food and RO water [1, 4].



Rats exposed to Mobile RFEMR **Radiofrequency decibel meter**

Figure 1: The above figure shows that the rats exposed to mobile RFEMR and those radiations were quantified by Radiofrequency decibel meter

Biochemical analysis:
At the end of the experimental period, blood samples were collected from all the rats through retroorbital puncture by sterile capillary tubes. Blood was collected in plain tube without anticoagulant and it was allowed to clot at room temperature. Serum was separated after centrifugation at 3000 rpm for 10 mins. Serum was used for analysis of NSE and MDA.

Neuron specific enolase:
It was estimated by using ELISA according to the manufacturer's instructions.

Malondialdehyde (MDA):
It was estimated by Thiobarbituric acid method using colorimeter.

Estimation of MDA by lipid peroxidation:
The lipid peroxidation was estimated by Mahfouz *et al.* (1986) method.

Principle:
Lipids in the cell membrane are highly susceptible to peroxidative damage, which in turn break down into number of units to form MDA. This MDA reacts with TBA to form thiobarbituric acid reactive substances (TBARS), which has a pink color with absorption maxima at 530 nm.

Reagents:
- **[1]** 0.67% Thiobarbituric acid (TBA)
- **[2]** 40% trichloroacetic acid (TCA)
- **[3]** 1, 1, 3, 3- tetra methoxy propane (99%): [Malondialdehyde bis (dimethyl acetal)] 10 mM

Procedure:
Five hundred µl of plasma sample was pipetted out into labelled, clean 15 ml glass tubes. To the tubes, 500µl of 40% TCA and 1 ml of 0.67% TBA were added and mixed gently. The tubes were closed with the caps and kept in a boiling water bath for 10 minutes. The tubes were removed from the boiling water bath and left at room temperature for 2 minutes. The tubes were then placed in an ice cold water bath for 5 minutes and added 1 ml of double distilled water to all the tubes and centrifuged at 2500 rpm for 15 minutes at room temperature. Supernatant was removed and optical density was measured at 530 nm using Double beam spectrophotometer (Perkin Elmer Spectrophotometer). The OD values were plotted against the concentration of the standards to obtain a standard graph. The concentrations of the samples were calculated using the OD of standard of known concentration **[18].**Values were expressed as µmol /L

Data representation & Statistical analysis:
Data were expressed as Mean ± SD Statistical analysis was carried out using SPSS software. Statistical differences between the groups were evaluated by independent T test followed by Dunnets comparison test to compare between treated and control groups. Differences yielding $p<0.05$ were considered statistically significant.

Results:
NSE and MDA levels were increased in rats exposed to RFEMR (0.6683ng/mL±0.106, 1.9038 µmol/L ±4.034) compared to controls (0.376 ng/mL ± 0.56, 1.1465 µmol/L ±0.134). The results revealed that, there was a statistically significant ($p<0.05$) increase in the NSE & MDA levels in RFEMR exposed rats as compared to controls.

Table 1: Showing the difference in NSE and MDA levels in Controls and RFEMR exposed rats

	Control Mean±SD	RFEMR Mean±SD	P value
NSE (ng/mL)	0.376± 0.56	0.6683±0.106	0.000*
MDA (µmol /L)	1.1465±0.134	1.9038±4.034	0.005*

*indicates statistically significant

Discussion:

MDA is one of the several byproducts formed during degradation of phospholipid cell membrane. Due to the damage of cell membrane by reactive oxygen species, Phospholipase A2 enzyme releases arachidonic acid from membrane phospholipids. In subsequent reactions this yields the formation of MDA **[14].** NSE is found in cytoplasm and dendrites of the neurons and is thought to be a marker of neuronal damage. Although its levels are low in peripheral blood is stated that can be used as a sensitive indicator as it increases in serum during injury and damage **[19].** Target of this research was to scrutinize the effect of RFEMR on biochemical parameters of oxidative stress associated changes by stress related determinants, Lipid peroxidative activity by MDA levels and neuronal damage by NSE. According to **Table 1** results of our study indicated that there was significant increase in NSE and MDA levels in rats exposed RFEMR compared to controls. As NSE and MDA are markers for neuronal damage and lipid peroxidation, our study proves that RFEMR may cause oxidative stress which in turn leads to oxidative damage. Oxidative stress and oxidative damages are well established cause for many chronic disorders **[20].** Maneesh *et al.* also found significantly increased MDA levels in testis and epididymis of RFEMR exposed male rats and finally concluded that mobile RFEMR induces oxidative stress **[6].** This study was also supported by the work where, the use of mobile phone have shown to induce experimental device on guinea pigs exposed for 10 days leading to increased MDA levels[21]. A study done on rat liver by using antenna radiations suggested that, elevated MDA could be due to cytochrome P450-mediated metabolism of the organic hydroperoxide to active alkoxyl radicals that initiated LPO and led to liver damage. Hence these metabolic pathways could increase cellular free radicals, which may attack phospholipids, proteins, and nucleic acids **[22].** A study done by Gulay *et al.* also proved that by using Electromagnetic field(EMF) and exposure system 2hours/ day for a period of 90 days showed that NSE levels were statistically increased in exposure group compared to controls**[8,19].** Due to neuronal damage there might be a breach of blood brain barrier which leads to elevated NSE levels in the brain could have entered the blood**[23][24].** In the present study, we observed increased NSE levels in the exposed group compared to control group. This increase was found to be a consequence for damage of neurons, death of cells, synapse loss, and axonal myelin damage.

Conclusion:

Based on findings of our study we concluded that exposure to mobile RFEMR leads to increased NSE and MDA levels causing oxidative stress which in turn leads to oxidative damage in SD rats.

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CERTIFICATE

This is to certify that, the Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the Study topic entitled **"Effect of chronic mobile phone Radio Frequency Electromagnetic Radiations (RF EMR) on feeding behavior and serum cortisol levels in Sprague Dawley (SD) rats"** for **Publication** authored by **Mrs. Pravallika Pagadala** (Corresponding Author)` & Dr. Vinutha Shankar M S in the Department of Physiology at Sri Devaraj Urs Medical College, Tamaka, Kolar.

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Member Secretary

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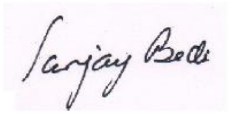

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7. SUMMARY

- Exposure to stress is inevitable. Stress affects the brain both physiologically and chemically. Depression and anxiety are extremely common during stress. Recent days mobiles are also known to cause oxidative stress. Stress affects feeding behavior.
- Fifty days of multiple stressors significantly reduced the feed intake and body weight in the Stress group compared to the control group ($p<0.05$). A significant reduction in feed intake was observed in stress group starting from Day 1 and body weight from Day 30 and continued until the end of the study compared to control. An Overall reduction of 20% in body weight was observed in stress.
- The feed intake in the 50 days of RF-EMR group was comparable with the control ($p>0.05$). Although the body weight was reduced in the RF-EMR group but it was not statistically significant compared to the control ($p>0.05$).
- The serum NSE, MDA and corticosterone levels were significantly increased in the both chronic multiple stressors and RF-EMR grouped rats compared to the control.
- The GFAP levels in the CA3 region of hippocampus was significantly higher in the Stress group compared to the control group. The GFAP levels in the CA3 region were comparable between the RF-EMR and control group.
- The MDA, NSE and GFAP levels were significantly higher in the multiple stressor group compared to the RF-EMR group. Which indicating that 50 days of RF-EMR induced changes were less significant compared to the 50 days of chronic multiple stressors induced alterations.

- Concurrent treatment of 100mg/kg vitamin E supplementation for 50-days to the multiple stressors and RF-EMR rats showed an improvement of feed intake and body weight compared to the multiple stressor and RF-EMR group rats.
- Concurrent treatment of 100mg/kg vitamin E supplementation for 50-day to the multiple stressors and RF-EMR rats normalised the increased NSE, MDA, corticosterone levels observed in the multiple stressors and RF-EMR group rats.
- Concurrent treatment of 100mg/kg vitamin E supplementation for 50-days to the multiple stressors normalised the increased GFAP levels observed in the multiple stressor group rats.

8. RECOMMENDATION:

Based on literature search and findings from our study we suggest that instead of intake of exogenous vitamin supplementation to avoid oxidative damage caused by day to day life and through mobile radiations it's better to modify lifestyle by maintaining regular sleep cycle, Exercise, balanced diet, Meditation and yoga. We also advise individuals not to use mobile phones for extended periods to avoid ill effects of EMR. Withdrawing from EMR might undoubtedly overcome the harmful effects of EMR exposure, bringing it closer to the normal state. Therefore, increased anti- oxidants intake may nullify the effect of RF-EMR from gadgets and physical stress caused every day. Vitamin E usage for a period of 50days can undoubtedly reverse the changes but long term usage of Vitamin E has to be studied before recommending.