1. Introduction

Cell phone is the conventional source of communication and its demand is growing rapidly. As per National Telecommunications and Information Administration (NTIA) report, the cell phones and Wi-Fi have become an unavoidable part of human life. The epidemiological study indicated that, about 72% of population in Asian countries, 83% of population in USA is in a continuous touch with cell phone and Wi-Fi (Anderson et al., 2018; ITU, 2018). Therefore, installation of cell phone towers in cities near residential areas have further increased in order to amplify the rate and connectivity (Hamnerius and Uddmar, 2000).

1.1 Discrete range of cell Phone

The cell phones and their base antennae produce non-ionizing electromagnetic radiation (EMR) in the range of 869-900 MHz (Code division multiple access; CDMA; 2G), 935-960 MHz (Global system for mobile communication; GSM-900; 2G), 1805-1880 MHz (GSM-1800; 2G) and 2110-2450 MHz (3G, their base station and Wi-Fi) (Dogan and Lu, 2012).

With the passage of time, frequency of cell phone has been increasing from 2G, 3G and 4G to 5G. Hence, the development of high range of cell phone frequency causes harmful effects on environment and to living organisms (Kıvrak et al., 2017). EMR is absorbed by the biological tissue and its absorption depends on the frequency and intensity of the incident radiation and also the type of the tissue that absorbs it (Poole et al., 2013); (Nazıroğlu and Transduction, 2012). There are limited reports available on the harmful effects of EMR at very high frequency. However, there is no report on the detrimental effect of cell phone (3G) frequency. Therefore, there is a need to study or analyze the impact of increasing frequency of EMR on biological system. Previous

report on human has suggested that brain is majorly affected by the cell phone radiation (Varghese et al., 2018). Based on a clinical report, two thousand eighteen cases of cell phone usage by adults in Australia were examined with incidences of brain tumors including glioma as detrimental health outcome (Karipidis et al., 2018).

As per World Health Organization (WHO) report, brain is the primary organ which is directly affected by cell phone radiation at some discrete range of EMR (Hardell, 2017). A broad range of health effects have been reported with cell phone use. Neurological effects are of particular concern in young people since the brain is the primary exposed organ (Faria et al., 2018). Previous reports suggest that exposure to EMR causes neurological dysfunction (Johansson et al., 2006), however, the results are inconsistent. Therefore, the primary objective of present work is to evaluate the effects of EMR on central nervous system (CNS) and pharmacology of some selected drugs. A cohort study suggested that, long term use of cell phone may cause stress and anxiety by producing hormonal imbalance. However, there is limited research on effect of EMR on pathophysiology of stress and anxiety. Further, long term EMR exposure can also alter the monoamines levels (Ismail et al., 2015) which are implicated in the development of depressive behavior. It is well known that stress and anxiety can alter the cognitive ability and decision-making capacity (Gabey et al., 2013). Further, National Institute of Health (NIH) reports have discussed the harmful effect of EMR on biological system (Hardell, 2017).

Furthermore, the observed consequence of the effect of EMR on biological system is due to the high range of specific absorption range (SAR) and close proximity of the cell phone device to user's head (Moradi et al., 2016). Moreover, cell phones radiation is exposed to vital organ such as liver, GIT and heart. High frequency cell phone radiation

may alter the hemodynamics of these organs as they belong to highly perfused category. Futher, the absorption of EMR depends on various factors including the frequency and intensity of transmission, the duration of exposure, and the distance of the subject from the source. Therefore, the main aim of the present study is to observe the long-term exposure to EMR on stress related anxiety which is involved in the depressive-like symptoms and cognitive dysfunction. Furthermore, we would also examine the long-term exposure to EMR on some highly perfused organ such as liver and heart.

1.2 Health risk of electromagnetic radiation on Nervous System

Compared to the other organs, brain is exposed relatively to high range of specific absorption range (SAR) due to the close proximity of the cell phone device to user's head. The damaging impact of EMR on brain includes abnormalities in function and structure. The nervous system is the part of an animal's body that coordinates its voluntary and involuntary actions and transmits signals between different parts of the body. It consists of two main parts, the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS contains the brain and spinal cord and PNS consists of mainly nerves, which are bundles of the long fibres or axons that connect the CNS to every other part of the body. Continuous EMR exposure to animals (> 2W/kg) can produce alterations in the morphology of CNS (Kramarenko et al., 2003). Long term exposure to mobile phone radiation affects electroencephalogram (EEG) activity (Von Klitzing et al., 1995). Abnormal EEG waves in awake subjects exposed to EMR have also been observed (Kramarenko et al., 2003). Abnormal EEG waves in awake subjects exposed to EMR have also been observed (Kramarenko et al., 2003). These irregular EEG waves leading to headache, change in sleep habits (Danker-Hopfe et al., 2016) and changes in blood pressure (Braune and Wrocklage, 1998) subjected to EMR. Earlier

epidemiological studies exhibited by long term exposure of electromagnetic radiation alter the neurological function of brain such as tremor, dizziness, loss of concentration and sleep disturbance causes changes the brain physiological system (Santini et al., 2002, Hutter et al., 2006, Abdel-Rassoul et al., 2007)

It is well documented that long term, continuous EMR exposure to animals can produce alterations in the physiology of CNS (Kim et al., 2017). The nervous system, and in particular the amygdala, prefrontal cortex and hippocampus are very sensitive to EMR exposure. Amygdala is an integral part of the brain's limbic system and its glucocorticoids receptor is involved in the behavioural regulation as well as regulation of hypothalamo-pituitary adrenal (HPA) axis (Gold et al., 2002, Ziegler and Herman, 2002). Damage to neurons in the amygdala may therefore lead to anxiety, other impaired behavioural disturbances, as well as negatively impact the functioning of the HPA axis. Further, earlier studies have reported that long-term exposure to EMR as a stressor agent enhances the risk of neuropsychological disorders like anxiety (Pall, 2016 and 2018). Exposure to EMR-2450 MHz for 45 min activated the neuroendocrine system and secretion of endorphin, enkephalin and dynorphin which further caused activation of HPA axis, which might be one of the reasons for anxiety (Lai, 1992; van Bodegom et al., 2017). Development of anxiety-like behavior in experimental animals critically depends on the duration and frequency of exposure (Shehu et al., 2016). Although, conclusions drawn from behavioral studies are rather ambiguous, some preclinical studies indicated that exposure to high range of EMR may lead to anxiety-like behavior (Zhang et al., 2014). However, EMR at lowest frequency (900 MHz) does not cause sufficient impairment or exhibit anxiety-like behavior (Júnior et al., 2014).

The pathophysiology of EMR-induced anxiety is yet to be explored. In retrospect, the effect of EMR-2450 MHz exposure on the function of HPA axis has also not been extensively studied. Chronic exposure of extremely low frequency of EMR may increase the level of plasma corticosterone in rodents (Mostafa et al., 2002). Previous study has reported that long-term exposure of EMR act as a stressor in mice (DeBruyn and DeJager, 1994; Gong et al., 2015). Corticosterone is a functional indicator of stress response in experimental animals (Gong et al., 2015). However, neural mechanisms for the effect of EMR on neurological disorders are yet to be deciphered. Long term exposure of EMR causes changes in the amygdalar morphology and consequent emotional behavior in rats (Narayanan et al., 2018). Clinical studies reported that, repeated exposure to EMR causes headache, stress associated depression in human (Hossmann and Hermann 2003; Giedke et al., 2002). The mechanism involved in alteration in cerebral blood flow and neuro-humoral circulation (Aalto et al., 2006; Kolesnyk e al., 2008; Finnie et al., 2006), which may further alter the monoamine neurotransmitters especially serotonin and dopamine levels. Serotonin and dopamine are believed to be involved in the control of depressive-like symptoms. Recent studies suggest that, the incidence of depression is caused by the alterations in neurobehavioral parameters (Krishnan and Nestler, 2008). The prefrontal cortex plays an important role in the development of depressive-like symptoms (Sun et al., 2012). However, preclinical information is not available till now to summarize the mechanism of long-term exposure to EMR on stress associated depression. Therefore, aim of the present study to evaluate the effect of long-term exposure to EMR on cerebral hemodynamics. Preclinical study suggests that stressful event alters the cognitive ability and thinking aptitude (Morgan III et al., 2006). Long

term exposure to EMR can act as stressor agent can contribute to learning and memory impairments.

There are reports which suggest that occupational exposure of EMR may increase risk of neurodegenerative diseases (Jiang et al., 2013). Neurodegenerative diseases are a large group of hindering disorders of the nervous system, characterized by the loss and death of neuronal cells (Hussain et al., 2018). Based on the previous reports, mitochondrial dysfunction is a main causative factor for neurodegenerative diseases; Alzheimer disease (Keating, 2008). Mitochondria are play an important role to metabolize the end product of glycolysis pyruvate, to generate ATP. Mitochondria regulate oxidative stress and provide the energy which maintains the neuronal action, excitability and neuronal growth and development. Therefore, defects of mitochondria are linked to neurodegenerative diseases (Cheng et al., 2010). There is no consensus regarding whether EMR exposure could cause potential detrimental effects on whole animals or isolated brain regions. The use of the new generation mobile phones has been reported to initiate symptoms like headache, sleep anomalies (Hossmann et al., 2003) and cognitive impairment (Levin ED, 2015). Earlier studies reported that long term exposure to low frequency EMR altered the cognitive behavior in rats, that lead to a marked hindrance in learning and recall of memory tasks and may increase a chances of developing Alzheimer's disease (AD) (Dogan et al., 2012; Naziroglu et al., 2012; Jiang et al 2013). However, till date there is no report on the comparative consequences of EMR (900, 1800 and 2450 MHz) exposure on amygdala, prefrontal cortex, and hippocampus brain region. Thus, sub-chronic exposure to different range and frequencies of EMR may possess greater propensity to cause alteration in the pathophysiology of stress related anxiety, depression and learning memory in rats.

The cell phone, its base station and Wi-Fi are continuously irradiating EMR in the environments which are more prone to ill effect on human (Sivani and Sudarsanam, 2012). Therefore, there was a need to study the effect of EMR on biological systems. Further, there are limited reports on harmful effect of cell phone tower and Wi-Fi. Based on the above information, we have anticipated that, the study on different frequency of EMR in biological system would provide awareness and will help in the understanding of pathophysiological process of cell phone exposure.

1.3 Electromagnetic radiation and hemodynamic activity

Decrease in blood flow in cortical region of brain is due to increase in viscosity of blood that reduces the relative motion of blood and is also associated with shrinkage of PFC neuronal cells (Rad et al., 2015; Aalto et al., 2006) and a drastic increase in local oxygen demand is observed to restore the homeostasis (Ota et al., 2014). VEGF is an essential regulator of vascular permeability system that helps to restore the oxygen supply in tissues in case of inadequate blood circulation (Ashina et al., 2015). Earlier report suggested that long term exposure to mobile phone radiation (900 MHz) causes decrease in regional cerebral blood flow in humans, inducing changes in neuronal activity (Mizuno et al., 2009). These changes in cerebral blood flow may alter the human brain physiology.

Previous report suggested that, the sub chronic exposure to EMR-2450 MHz causes significant decrease in the level of vascular endothelial growth factor (VEGF) in experimental rats (Saygin et al., 2015). Therefore, there is need for a potential mechanistic study which can understand the effect of EMR on the neuronal cell death and altered neurobehavioral changes after EMR exposure. For this purpose, we need to understand the effect of EMR on pathophysiology of nervous system.

1.4 Electromagnetic radiation and neurotrophic activity

Brain derived neurotrophic factor (BDNF) is a neurotrophin, mainly expressed in the CNS (Recher et al., 2012). It plays an important role in neuroprotection by regulating several functions during development including nerve growth, neuronal differentiation, survival (Maskey et al., 2014; Schmidt et al., 2007). Previous studies reported that, long term exposure of microwave radiation (2856 MHz for 1 month) altered the BDNF expression suggesting abnormalities in synaptic plasticity of hippocampus in rats (Tan et al., 2017). This report showed that neurogenic factor is compromised in neurodegenerative disease and they can be aggravated by exposure to EMR.

1.5 Electromagnetic Radiation and Neurotransmitters

Nervous system is a sensitive target of EMR exposures since multitude of neurotransmitters exist in the nervous system and there is tremendous electrical activity in neurotransmission. The neurotransmitters play an important role in stress related anxiety, depression, learning and memory deficits. Monoamine neurotransmitters such as catecholamines (dopamine, norepinephrine and epinephrine) and serotonin are the principle neurotransmitters that mediate a variety of central nervous system (Dong et al., 2012). Preclinical study suggested that long term exposure to EMR-2450MHz altered monoamine level of experimental rats (Streck et al., 2014, Ezz et al., 2013). Reduced cholinergic system activation causes impaired long-term memory functioning and cognitive flexibility (Wintermantel et al., 2006). EMR exposure at frequency of 50 Hz for 30 min has been reported to influence biophysical functions of nerve cells and causes reduction in norepinephrine, dopamine and serotonin levels (Forozandeh et al., 2011). Exposure to EMR-1800 MHz, SAR 0.843 W/Kg (1h daily for 4 months) has also

been reported to induce significant alterations in dopamine, norepinephrine and serotonin in rats (Abdel Mohsen and Ezz El Din, 2013).

1.6 Electromagnetic radiation and Mitochondrial function

Mitochondria is considered to be the powerhouse of the cell due to their high efficiency in utilizing O_2 and substrates such as glucose and pyruvate to produce cellular energy in the form of ATP (Van Den Brand et al., 2004). Earlier study suggests that mitochodria produce energy which required for cellular processes, cell survival (Ardehali and O'Rourke, 2005), neurotransmitter synthesis and cell death (Collins et al., 2010). However, impaired mitochondrial function is commonly observed in stress and several neurological disorder such as memory deficits (Akbar et al., 2016). Hence, long term exposure to EMR causes neurological disorders which may be due to the mitochondrial dysfunction. Previous study reported that long term exposure to EMR causes mitochondrial dysfunction in reproductive system in male rats (Akbar et al., 2016). There were no reports on repeated exposure to EMR on mitochondrial linked stress related to anxiety and learning and memory impairments.

1.7 Effect of electromagnetic radiation on biological system

The mechanism of interaction between the electromagnetic radiation and biological system is known as bioelectromagnetics. It is known that the absorption of radiation varies with descrete range of frequency. EMR gives rise to thermal and nonthermal effects on biological system (Israel et al., 2013). However, both *in vivo* and *in vitro* studies indicate that tissue healing resulting from thermal effect by EMR exposure is the most widely accepted mechanism for biological effects and non thermal effect mechanism, where no increase in temperature was observed, still remains controversial (Kojima et al., 2004; Yao et al., 2008; 2012). The question of adverse health

consequences provoked by the contentious non thermal effects of cell phone radiation still remains unresolved. For non thermal effects the current safety guidelines are not taken into consideration, which restricts the basic SAR limits for EMR exposure upto frequencies 10 GHz in public as 0.08 W/kg for whole body and 2W/Kg for head and trunk (ICNIRP 1998). The interest in the study of EMR with biological systems has been sustained for several decades. Interactions with biological tissues arise as a result of three processes: (i) Penetration by EMR and their propagation into the biological system (ii) primary interaction of the radiation with tissues and (iii) possible secondary effects arising from the primary interaction. Various reports are available which show liver toxicity and cardiac dysfunction (Assasa and Widmer, 2016);(Alhusseiny et al., 2012), due to EMR emitted from cell phones. It has been reported that chronic exposure to EMR 900 MHz for three months alters the enzymatic activity leading to liver toxicity in rats (El-Bediwi et al., 2011). Apart from this, exposure to these radiofrequencies leads to altered mechanical and rheological properties of blood (Erken et al., 2012). These changes in the flow dynamics of blood can lead to alterations in the mucus membrane properties, an important defensive factor in our body especially in the gastrointestinal system (Challis et al., 2005; Rad et al., 2015). Long term abnormalities in cardiac hemodynamic can lead to ischemia-like condition in heart and cause ventricular tachycardia (Awan et al., 1977).

1.8 Electromagnetic radiation and liver

The liver is an important tissue for metabolism and is the major organ for detoxification. Most important features of pathological changes of liver cells are measured in terms of the levels of AST and ALT (Hall et al., 2012). Clinical studies have reported that increase in the levels of serum AST and ALT in liver tissue are

associated with psychological distress and depression in patients (Yuan et al., 2012). However, exposure to EMR (1140-1290 nm) influences the enzyme kinetics which further causes activation of liver enzymes and changes in the metabolism of drug which is one of the prime reasons for liver toxicity (Vojisavljevic et al., 2007). The direct effect of repeated exposure of EMR on liver tissue has not been studied extensively. It has been reported that chronic exposure of EMR 900 MHz for three months alters the enzymatic activity of the liver (El-Bediwi et al., 2011). Therefore, based on above information we presume that long term exposure to EMR causes liver toxicity.

1.9 Electromagnetic radiation and gastrointestinal tract

The pathogenesis of gastric ulcer is based on disproportion of two main factors such as offensive and defensive factors (Radmaker et al., 1991). Exposure to cell phone radiation leads to alteration of the biophysical properties of blood (Challis et al., 2005; Rad et al., 2015). These changes in the flow dynamics of blood can lead to alterations in the mucus membrane properties, an important defensive factor in our body especially in the gastrointestinal system. Previous study reports that long term exposure to cell phone causes decrease in regional cerebral blood flow in human (Zhang et al., 2017). Earlier study has reported that sub chronic exposure to cell phone radiation causes increase the level of stress hormones in the body (Mahdavi et al., 2014). Previous report have showed that long term secretion of corticosterone amplifies gastric acidity and pepsin release with subsequent peptic ulceration, perforation, or hemorrhage (Gray et al., 1951). Therefore, repeated exposure to EMR can potentially lead to disorders of the gastrointestinal system.

1.10 Electromagnetic radiation and heart

Previous report suggests that exposure to high frequency of EMR causes cardiac impairment including heart conduction, repolarization disturbances and irregularities in electrocardiogram (ECG) morphology (Bortkiewicz et al., 1996). There are reports suggesting that long term alteration in cardiac hemodynamic may induce ventricular tachycardia (Ashraf et al., 2004). Hence, we presume that repeated exposure to EMR can potential cause of cardiac dysfunction.

1.11 Lacunae in the existing literature, relevance of the study

The literature shows scattered and incomplete information on the effect of EMR on biological system and the results obtained are inconclusive. There was no study that has reported so far which explains the underlying causes for neurological disorders. As the brain is an important organ for human behavior. There are no preclinical evidences on EMR exposure on stress and associated anxiety and depression. Further, there are limited research and lack of mechanism on effect of EMR on cognitive deficits. Apart from the CNS study, only few organizations have focused on the health risk of EMR on vital portion of the human body. Therefore, the study on effect of EMR on other core biological system would highlight the harmful effect of EMR on liver, GIT and cardiovascular system. Furthermore, the possible causes involved in the pathophysiology changes at following range of EMR exposure are still not known. Based on the above information the hypothesis has been postulated.

1.12 Hypothesis

EMR continuously irradiates from cell phone which can affect the major organs of the body, i.e., brain, liver, GIT and heart. According to WHO, brain is more prone to EMR due to close proximity to cell phone to head while using cell phone. Existing information suggests that long term exposure to EMR reduces the hemodynamic

properties of other highly perfused organs such as liver, GIT and heart. However, there was no information regarding mechanism through which it can act. Therefore, it would be interesting to know the effect of EMR on CNS and other biological system including liver, GIT and heart (Figure 1.1). Repeated exposure of EMR can affect the pathophysiology of CNS associated with several neurological disorders including stress, anxiety, depression and learning memory deficits. Exposure of EMR may cause alterations in HPA axis and related pathological changes on CRH-2, GR and corticosterone levels and thereby it may aggravate stress induced anxiety like symptoms. EMR exposure can decrease mitochondrial function as it can change its membrane potential. Exposure of EMR may cause necrotic damage of specific brain regions and diminish monoaminergic system especially serotonin, dopamine and its metabolites which are responsible for the depressive like symptoms. EMR can alter neuroprotective and angiogenic factor in the PFC. Neurotrophic and angiogenic changes in PFC are implicated in pathophysiology of depressive-like symptoms. Long term exposure to EMR may promote amyloidogenesis and mitochondrial dysfunction which may alter the cholinergic system leading to learning and memory deficits. Hemodynamic parameters may alter the physiology of perfused organs including liver, GIT and heart. The effect of EMR may modify metabolic enzyme which can change the pharmacokinetics of fluoxetine. Hemodynamic parameters are a defensive factor for the gastric ulcer. Long term exposure of EMR may alter gastric integrity, blood flow, VEGF, $H^{+/}K^{+}$ ATPase enzyme activity, oxidative stress, pro and anti-inflammatory markers along with morphological changes of GIT. Therefore, it would be interesting to evaluate the dose dependent effect of omeprazole against EMR-induced gastric ulceration. Long term exposure to EMR can cause hemodynamic abnormalities and

cardiac conduction irregularities in terms of HR, MBP and ECG pattern and can cause arrhythmia along with morphological changes in heart.

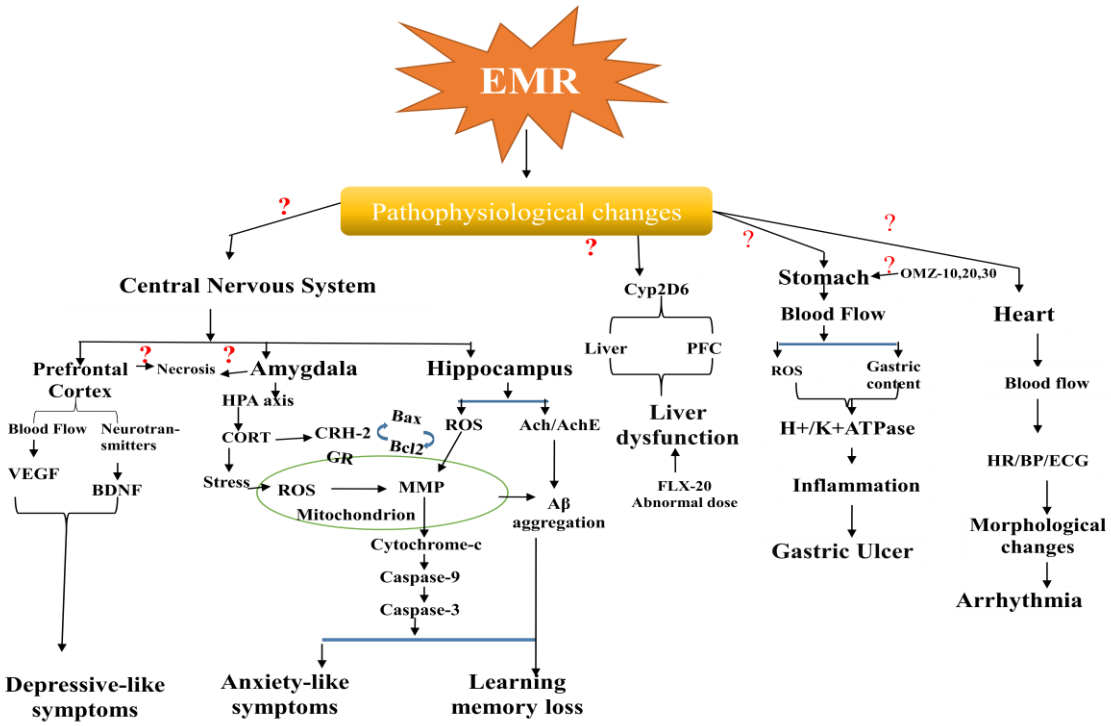

Figure 1.1 proposed hypotheses

Therefore, the objectives of this thesis are:

1.13 Objectives of thesis

1. Evaluation of exposure of electromagnetic radiation (900, 1800 and 2450 MHz) on neuropathophysiology of-

(**1.1**) Stress and Anxiety-like symptoms in rats

(**1.2**) Depressive-like symptoms in rats

(**1.3**) Cognitive deficits in rats

2. Effect of EMR-2450 MHz on Pharmacokinetics of fluoxetine and hepatic function in rats.

3. Effect of EMR-2450 MHz on gastric integrity and pharmacology in rats.

4. Effect of EMR (900, 1800 and 2540 MHz) on heart in rats.