7.0 SUMMARY

7.1 INTRODUCTION

Mobile communication is now ruling our daily lives through intelligent smartphone services. The rapid spread of mobile phone usage during the last decade paved way for tremendous growth in Indian communication industry which resulted in adverse health effects of mobile radiation exposure, causing cancer. A number of studies have been promoted investigating health hazards caused by radio frequency electromagnetic fields radiation (RF-EMF) that are emitted by mobile phones and the towers leading to carcinogenesis.

Dosimetric and epidemiological studies have shown a higher brain tumor (Glioma) risk of people who had used mobile phones for more than ten years (Velmurugan, 2016; Marshall and Heil 2016). Diverse studies deciphered the ill-effects of radio-frequency electromagnetic field (RF-EMF) on bees, fruit flies, frogs, birds, bats, and humans (MOEF, 2010; DoT, 2010). Cancer, diabetes, asthma, infectious diseases, infertility, neurodegenerative disorders, and even suicides are on the rise in India, having been attributed to the same. This invisible health hazard pollution (IHHP) is a relatively new environmental threat (Sivani and Sudarsanam, 2012). The WHO as per International Agency for Research on Cancer (IARC, 2012) has classified radiofrequency electromagnetic fields as possibly be carcinogenic to humans (Group 2B), as per the evidence of increased risk for glioma, a malignant type of brain cancer/ tumor.

7.2 REVIEW OF LITERATURE

7.2.1 HEMATOLOGY

Blood and blood parameters are believed to be one of the primary particles that come in contact with RF-EMF and are likely to respond to induced Electromagnetic Field (EMF) generated by EMF charges. Blood is a pathophysiology reflector of the whole body and therefore, blood parameters are important in diagnosing the structural and functional status of organisms exposed to toxicants including electrosmog (Adhikari et al., 2004; Otitoloju, 2010). Several hematological parameters such as leukocytes, lymphocytes, eosinophils, neutrophils, basophils, MCV, MCH, MCHC and platelets are sensitive to RF/MW exposure, not only in animals, but also in humans (Mcree, 1979; Roberts et al., 1986).
7.2.2 BIOCHEMISTRY

Physiological regulatory systems provide humans and other warm blooded species with accurate and interdependent homeostatic systems that maintain body temperature and also regulate immune and neuroendocrine functions (David Black and Louis Heynick, 2003). Studies on animal have shown that exposure to radiofrequency electromagnetic fields may alter the nervous endocrine physiology including the thyrotropin secretion (Lu et al., 1987; Lai, 1992).

Salford LG. et al., (2003), had shown that weak pulsed microwaves gave rise to significant leakage of albumin through blood-brain barrier. A change in the biochemical profile induces oxidative stress (Kesari et al., 2011). Achudume et al., (2012) stated that exposure of male Wistar rats to EMF base station 900 MHz for 60 days continuously causes changes in serum activities (ALT, AST, and ALP) indicating liver dysfunction.

7.2.3 MOLECULAR BIOLOGY

Advent of quantitative real-time RT-PCR (Q-RTPCR) techniques for the measurement of gene expression has allowed the accurate determination of the expression levels of target genes in cells and tissues (Robert et al., 2005). Schmid et al., (2004) argued that analysis of gene expression should be in terms of housekeeping genes only. Some evidence has suggested that RF fields may change expression of DNA transcription factors and cause changes in cell cycle kinetics (Litovitz et al., 2004), including protein translation.

DNA microarray technology cannot only detect differential expression of thousands of genes simultaneously but also investigate globally many different molecular pathways. Other studies (Leszczynski et al., 2004; Pacini et al., 2002; Nylund and Leszczynski, 2004; Lee et al., 2005) confirm the alteration of multiple genes’ expression induced by short-term RF EMF exposure in different cells and the influence of RF EMF on large-scale gene expression might be time and cell dependent.

7.2.4 HISTOPATHOLOGY

Exposure to microwave radiation 2 h a day for 35 days can potentially cause pathological manifestation and oxidative changes in Wistar rats which pre suppose the possible implications of such exposure on human health as well (Chauhan et al., 2016). Laila et al., (2010), reported histological changes in the different visceral organs including heart, lung, liver and kidney of rats exposed to radiofrequency. Studies of Yang L et al., (2016) indicated that
the LTE EMF exposure with the intensity beneath the safety limits could modulate the brain activities leading to neural disorder.

**7.3 AIM AND OBJECTIVES**

**7.3.1 AIM**

To assess the impact of radiofrequency electromagnetic field (RF-EMF) radiation in the order of 900MHz and 2450MHz on hematology, biochemistry, molecular biology and histo(patho)logy of an experimental animal model, *Cavia porcellus* (Guinea pig).

**7.3.2 OBJECTIVES:**

- **7.3.2.1:** To diagnose the status of the blood parameters such as leukocytes, lymphocytes, eosinophils, neutrophils, basophils, platelets, MCV, MCH, MCHC and haemoglobin in the above experimental animal after exposing to 900MHz, 2450MHz of RF-EMF radiations.

- **7.3.2.2:** To assess the impact of the above order on the biochemical profile of the experimental animal.

- **7.3.2.3:** To determine the extent of damage or mutation in the genes impacted by radiation using microarray technology

- **7.3.2.4:** To visualize the gene expression using Next Generation Sequencing

- **7.3.2.5:** To identify the susceptible genes to radiation as impacted genes, using RT-PCR technique.

- **7.3.2.6:** To diagnose and ascertain histopathological manifestations in the experimental animal exposed to the radiations.

**7.4 METHODOLOGY**

**7.4.1 RF-EMF EXPOSURE SOURCE AND ANIMAL CAGE DESIGN**
Guinea pigs (*Cavia porcellus*, Duncan-Hartley Variety), were purchased from BCG Vaccine Laboratory, Guindy, Chennai. Appropriate permission was sought from Institutional Animal Ethics Committee (IAEC) for animal research and care for the animals was undertaken as per guidelines of committee for the purpose and control and Supervision of Experiment on Animals (CPCSEA), India, for laboratory animal facilities. The animals were housed in specially designed cages made of glass in two different locations and were exposed to 900MHz and 2450MHz of RF-EMF radiation respectively. Control cage was kept in a location away from the RF-EMF radiation. The temperature and humidity were recorded in all locations and the experimental animals were provided with fresh feed twice a day and water. The exposure period ranged between 45 days.

**7.4.2 HEMATOLOGY**

Blood was drawn from experimental and control animals to diagnose blood parameters such as leukocytes, lymphocytes, eosinophils, neutrophils, basophils, platelets, MCV, MCH, MCHC and hemoglobin.

**7.4.3 BIOCHEMISTRY**

At the end of experimental period, cardiac puncture was made and blood was collected in EDTA/sodium heparinized vials from experimental and control animals for analysis of endocrine physiological parameters like TSH, T3 and T4 and biochemical parameters such as bilirubin, albumin, globulin, alkaline phosphatase, alanine transaminase and aspartate transaminase.

**7.4.4 MOLECULAR BIOLOGY**

Molecular techniques were employed to study the expression profile of genes of interest (C-FOS, SOD2, CA3, TNFα, PTEN and EGF) through microarray, NGS and RT-PCR.

**7.4.5 HISTOPATHOLOGY**

By following anesthetising and dissection protocols, the animal was sacrificed and the visceral organs were removed from experimental and control animals for histopathological investigation.

**7.5 RESULTS**

**7.5.1 HEMATOLOGY**

Increase in RBC count and decrease in MCH, MCHC and diverse leucocytes, platelets and hemoglobin were recorded in both the radiation exposed group.

**7.5.2 BIOCHEMISTRY**
Activity of alanine phosphatase, alanine transaminase and aspartate transaminase increased significantly in the experimental group over the control group. Elevation of Bilirubin, serum total protein, albumin and globulin levels were also noted. Experimental group – 1 and 2 showed increased level of TSH, T3 and T4.

7.5.3 MOLECULARBIOLOGY

Effects of RF-EMF radiation on gene expression was assessed using Microarray and significant mutations were found in PTEN-mediated MTOR and β-catenin signaling pathways, particularly with special reference to exposure order of 2450MHz. NGS and RT-PCR analysis analysis were carried out as primary and secondary confirmatory tests which confirmed results of microarray.

7.5.4 HISTOPATHOLOGY

Histopathological manifestations in the organs of brain, heart, kidney, liver, spleen and testes were diagnosed in the experimental animals. Condensation of nuclei in some cells, irregular cell arrangement, infiltration of inflammatory cells, swelling and fatty changes of hepatocytes and vacuolatation of cytoplasm were observed in Liver of exposed groups. Kidney of the same confirmed varying histological features characterized with glomerular damage, formation of large spaces between the tubules and congestion in the vessels in exposure group.

7.6 DISCUSSION
7.6.1 HEMATOLOGY

Measurements of blood parameters are the most important means by which the health status of experimental animals can be determined. In the present study, there was elevation in red blood cells when exposed to electromagnetic waves generated by mobile phones. Similar observation was made by Abdel Aziz et al., 2010. The electromagnetic exposure from mobile phones leads to damage and a clear influence on the cell walls, especially the walls of red blood cells and cause an imbalance in the blood enzymes (Mariam and Nawal, 2012). Significant reduction in haemoglobin and the indices of red blood cells MCH, MCHC and platelets are the causative factors of different types of anemia, as well as leukemia (M. Turgeon, 1988).
7.6.2 BIOCHEMISTRY

Serum activities such as AST, ALT and ALP had been altered significantly after the exposure to MWR. Significant increase in ALT activity confirmed the cytotoxic effect of non-ionizing radiation on hepatocytes inducing apoptosis and necrosis and cell damages (Boris et al., 2010, Lahijani et al., 2009). An elevated oxyradical generation and subsequent cell membrane disruptions were reported to be the reasons for electromagnetic field-induced cell damage (Dindic et al., 2010). Increased liver enzymes damage the liver tissue and also can produce free radicals (Mohammad Aberumand et al., 2016).

It has been suggested that exposure to RF-EMF may alter thyrotropin secretion Ozguner F et al., 2005.Elevated TSH levels induce the thyroid to elaborate triiodothyronine (T3) and thyroxin (T4), influencing the acceleration of protein synthesis.

7.6.3 MOLECULAR BIOLOGY

Under the same experimental conditions Zhao et al., (2007) also observed expression changes of numbers of genes. Although those genes involved in multiple functions, many genes belong in the highly specified functional groups, such as cytoskeleton related genes. Accordingly, some other up-regulated membrane skeleton related genes play a vital role in this regard.

Our analysis found significant mutations in PTEN-mediated MTOR and β-catenin signaling pathways. Mutation in PTEN (Phosphatase and tensin homologue) gene results in constitutive activation of the PI3/AKT pathway and therefore increases in protein synthesis, proliferation, migration and survival. Aberrant mutation in APC (adenomatous polyposis coli) results in accumulation of β-catenin engages the transcription factor complex (TCF) to activate transcription of a large number of target genes including BIRC5 and CCND1 which leads to aberrant to both direct stimulation of cellular growth and proliferation and also binds with caspase for degradation.

*PTEN* is one of the most frequently mutated genes in human cancers (Cantley and Neel, 1999; Simpson and Parsons, 2001). As one of the most frequently mutated genes associated with cancer, *PTEN* becomes the victim of defective DNA repair due to the mutation of other DNA repair genes, such as *BRCA1*. Loss of *BRCA1* leads to recurrent gross mutation of *PTEN* (Saal et al., 2008).

APC is a classical tumour suppressor protein. The APC gene product indirectly regulates transcription of a number of critical cell proliferation genes, through its interaction
with the transcription factor beta catenin. APC binding to beta catenin leads to ubiquitin-mediated beta catenin destruction; loss of APC function increases transcription of beta catenin targets. APC mutations are linked to an inherited form of colon cancer (Ferguson and Fangman, 1992; Mc Carroll and W.L. Fangman, 1988).

7.6.4 HISTOPATHOLOGY

Histopathological variables and cell membrane damages in the RF-EMF radiation exposed animals were significant. Chauhan et al., (2017) recorded distorted hepatic architecture, elongated nuclei and cytoplasmic vacuolation in the liver and shrunken glomeruli and abnormal renal tubules in the kidney after microwave exposure. Similar observations of microwave exposure on liver, kidney and spleen cells showed structural changes such as swelling of hepatocytes and vacuolation of their cytoplasm reported by Al-Glaib et al., (2008). An interaction of microwave radiation with tissues arise as a result of mainly three processes: deep penetration into the tissue and their propagation into the living system, then the primary interaction of the waves with tissue and the possible secondary effects arising from the primary interaction (Rachael, 2010).

7.6.5 CONCLUSION

The debate whether RF-EMF radiation exposure is involved in carcinogenesis is a million dollar question. Previous studies were unable to prove significantly the correlation between cell phones and health concerns which may be attributed to several factors – one such factor is not considering different frequencies on which a mobile phone works and the duration of exposure. Few new improved studies reported abnormal gene transcription, genotoxicity, DNA damage, loss of DNA repair capacity in human stem cells, neurotoxicity and carcinogenicity in humans. The present study concluded that RF-EMF exposure with higher frequencies could possibly cause changes in protein expression and leads to mutation of PTEN and APC genes and their respective pathways.