2.2.1 Introduction

Most of the reports suggest interaction between cell phone radiation and biological systems (Zhi et al., 2017). Some studies reports that long term exposure of EMR causes neurological disorders indicating need to establish a pathophysiological mechanisms for interaction between cell phone radiation and biological system (Sinha, 2008). Previous studies report that long term exposure to mobile phone affects cerebral blood flow and neurohumoral circulation that leads to alteration in the physiology and behavior of animals (Aalto et al., 2006b; Kolesnyk et al., 2008; Finnie et al., 2006). There are several reports suggest that non-thermal long term exposure to EMR causes significant changes in the different psycho-pathophysiological functions and neurodegeneration diseases (Bachmann et al., 2005; Hinrikus et al., 2005; Jiang et al., 2013). Long term exposure to EMR-2450 MHz causes nerve cell damage in mammalian brain cells indicating alterations in the brain physiology and activation of cytokine inflammatory makers leads to loss of cortical neuronal cells (Salford et al., 2003; Olcay et al.). Clinical report suggests that long term exposure to cell phone radiation alters regional cerebral blood flow resulting alterations in neurophysiology (Aalto et al., 2006b). Long term exposure to EMR causes headache, stress and sleeplessness in humans (Hossmann et al., 2003), which may leads to depression (Giedke and Schwärzler, 2002).

Long term exposure to EMR-1800-2100 MHz (60 min/ days/for 3 months) causes learning and memory deficits by decrease in hippocampal neuronal cells in Swiss mice (KV et al., 2019). Studies have demonstrated that the abnormality in learning and memory leads to depression in experimental rats (Sun and Alkon, 2004). Thus, sub-chronic exposure to EMR of different frequencies may possess greater propensity to cause depressive-like symptoms (KV et al., 2019). Further, both the

frequency and the duration of exposure are critical for the development of depressive-like symptoms.

Pathophysiology of depression involves several factors including aberrant neurogenesis, angiogenesis and deranged monoaminergic system (Monteggia et al., 2007; Brenes and Fornaguera, 2008; Clark-Raymond and Halaris, 2013). Preclinical study suggested that long term exposure to 2450 MHz altered monoamine level of experimental rats (Li et al., 2014; Ezz et al., 2013). Further preclinical reports indicate that a brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) are involved in promotion of cell survival and positively modulate neuroplasticity through prefrontal cortex (PFC) and hippocampal neurogenesis (Loseva et al., 2015; Maskey and Kim, 2014).

BDNF enhances vascular flow and promote revascularization of ischemic tissue in the vascular system (Schmidt and Duman, 2007). It has been reported that, chronic exposure of EMR 835 MHz for three months decreases the level of BDNF (Maskey and Kim, 2014). Preclinical studies revealed that, the EMR exposed animal showed decrease in cerebral blood flow and increase in viscosity of blood (Aalto et al., 2006a, Moradi et al., 2016), resulting in abolition of angiogenic activity which in turn plays a significant role in the pathophysiology of depression. Experimental as well as clinical reports suggest that VEGF is an inducer of angiogenesis and its modulation causes depression in animals (Kermani et al., 2005). Previous report suggested that, the sub chronic exposure of EMR-2450 MHz causes significant decrease in the level of VEGF in experimental rats (Saygin et al., 2016). In addition exposure to chronic stress also decreases VEGF in PFC (Hoeben et al., 2004; Isung et al., 2012), which is considered to be a major region for development of depressive-like symptoms (Nowacka and Obuchowicz, 2013). These findings showed that both neurogenic and angiogenic

factors are compromised in depression and they can be aggravated by exposure to EMR.

Mitochondria are critical for monoamine and BDNF-mediated synaptic and vascular plasticity of PFC and hippocampus (Chen et al., 2017; Kaplan et al., 2010). Therefore, mitochondrial apoptosis is also involved in pathogenesis of depression (Yu et al., 2009) and considered as one of the predisposing factors for impairment in monoaminergic activity (Quirk and Beer, 2006). As cited earlier continuous EMR exposure cause changes in the levels of monoamines neurotransmitters (Sinha, 2008). EMR causes dielectric heating in which the dielectric material like living tissue gets heated by EMR induced rotation of polar molecules (Gherardini et al., 2014; Singh et al., 2016). This influences various metabolic processes such as deterioration of ion channels of plasma membrane causing impairment of cellular functions (Alberts, 2002; Singh et al., 2016). Thus, these studies indicate that EMR (835, 1800 MHz) can induce depressive-like symptoms in rats. However, there is paucity of information on the possible pathophysiological mechanisms involved in the induction of depressive–like symptoms by non-ionizing EMR. Particularly, the effects of higher frequency of EMR-2450 MHz have not been evaluated in detail. Further, the study of EMR exposure was done in acute animal models and use of sub-chronic model of depression would give more realistic insights into the pathophysiological processes. Thus, on the basis of above studies, we presume that exposure to higher frequency EMR-2450 MHz may cause depressive-like symptoms in experimental rats.

Therefore, the present study has evaluated the role of repeated exposure of discrete frequencies of EMR [900 (GSM uplink), 1800 (GSM link) and 2450 MHz (3G cell phone and Wi-Fi)] in rats.

Depressive-like behaviors were evaluated by FST, TST and sucrose preference test. Nor-epinephrine, dopamine, serotonin and their metabolites were estimated in PFC as neurochemical markers of depression. Further, the cortical blood flow and VEGF were assessed as a measure of vascular homeostasis. Moreover, BDNF and Flow cytometry were estimated to evaluate the molecular basis of neuroprotective, apoptotic factors and cell death in EMR exposed rats respectively (as shown in figure 2.2.1).

2.2.2 Hypothesis

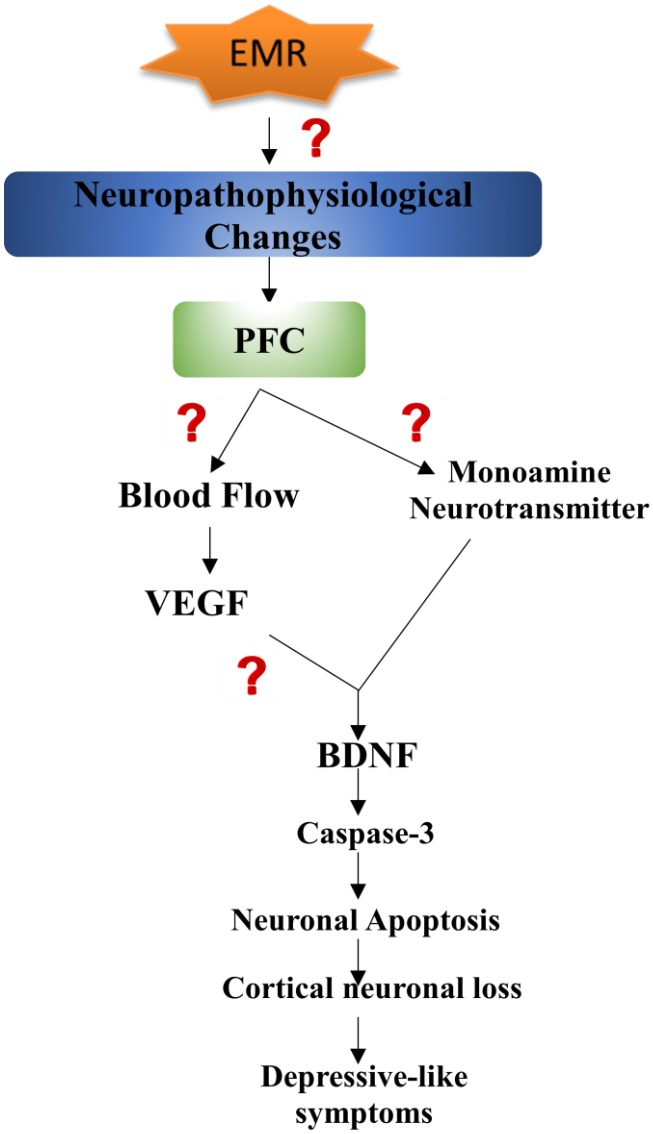


Figure 2.2.1 Proposed hypothesis of effect of EMR on depressive-like symptom

2.2.3 Materials and methods

2.2.3.1 Animals

Inbred Charles-foster albino male rats 6-weeks old weighing about 200±20 g were purchased from the central experimental animal facility center, Institute of Medical Sciences, Banaras Hindu University (IMS-BHU). The animals were housed in home cage made up of polypropylene at 25 ± 2 °C temperature and RH 44–56%, light and dark cycle of 12:12 h, respectively. The animals were acclimatized for one week prior to experiments. The food pellets were provided (Paramount Pvt. Ltd., India) and water was allowed *ad libitum*. All the experiments were conducted based on given guidelines (CPCSEA-2010; IMS-BHU; Approval No.: Dean/2015/CAEC/1414).

2.2.3.2 Chemicals

Sucrose was purchased from Merck, Mumbai, India. RNA isolation reagent was procured from Sigma Aldrich St. Louis, MO, USA and antibodies from Abcam Plc. (India). All other chemicals and analytical reagents of high quality were procured from local supplier (Hi-media, Mumbai, India).

2.2.3.3 EMR exposure design (Refer chapter 2.1 page no. 20)

2.2.3.4 Measurement of Power density and specific absorption rate of brain region (Refer chapter 2.1 page no. 21)

2.2.3.5 The calculation of SAR (Refer chapter 2.1 page no. 21)

2.2.3.6 Experimental design

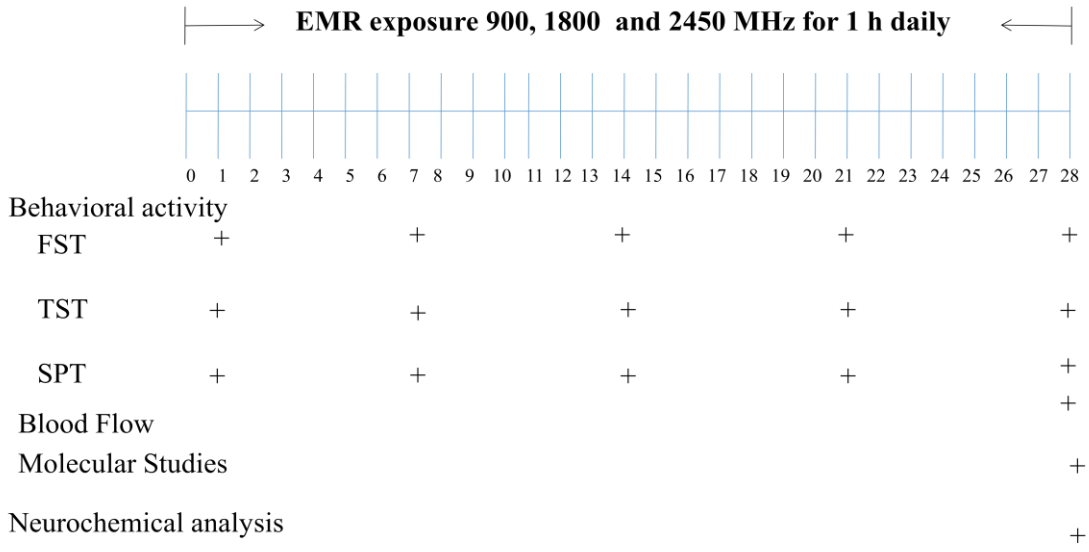


Figure 2.1.2 Schematic representation of the experimental design. '+' denotes experiment performed.

All the male rats were arranged into four different groups by G* power analysis software. control, EMR-900, EMR-1800 and EMR-2450 (n=6) as it is equivalent to cell phone radiation to which humans are continuously exposed. At the time of exposure feed and water were not given to experimental rats. The groups EMR-900 MHz, EMR-1800 MHz and EMR-2450 MHz were exposed to electromagnetic radiations between 10 am to 1 pm for 1h for 28 days beginning from day 1. After 15 min of EMR exposure on D-1 to D-28 at 7 days' interval, the behavioral paradigms include Forced Swim Test (FST), Tail Suspension Test (TST) and Sucrose Preference Test (SPT) were performed (fig 2.1.2). All the behavioral observations were recorded and evaluated using ANY-maze^{TM} (version-3.72, USA) video tracking system. Behavioral experiments were performed at an interval of 2 h between SPT and FST, and 20 min between FST and TST. Food restriction was done at D-0, 6, 13, 20 and 27 for 24h, and no experiment was performed during SPT.

All the behavioral parameters were performed during the light period. After 24 h, we have measured percentage of sucrose intake on D-1, 7, 14, 21 and 28. On D-28, rats were anaesthetized by pentobarbitone (35 mg/kg, i.p.) followed by evaluation of cortical blood flow via laser speckle blood flow imager (Omegazone OZ-2; Omegawave, Tokyo, Japan). Thereafter, animals were killed by cervical dislocation and remove the brain from skull and prefrontal cortex areas were cut out by a fine microdissecting (Graefe's) knife using the coordinates from rat brain atlas (Paxinos et al 1986) and instantly stored at -80^0 C until further analysis. The behavioural parameters and blood flow were assessed in all the groups. Neurochemical analysis by HPLC (n=4) and VEGF level by Elisa kit were performed (n=3). Molecular analysis of BDNF by qRT-PCR and cell death analyses by flow cytometry (n=3) were done using both sides of cortical tissues of brain ('n' is the number samples taken from each group).

2.2.3.7 Evaluation of behavioral performance

2.2.3.7.1 Forced swim test (FST)

The FST was performed as given standard protocol with some changes (Detke et al., 1995). The height and diameter of behavioral fabricated test cylinder was 60 cm and 25 cm maintained at room temperature (25°C). The cylinder was filled with water up to 30 cm to restrict the animal from touching the base with their paws or tail. The FST protocol was divided into two parts: first 15 min pretest followed after 24 h by 5 min test. After completion of pretest, the animals kept outside from the fabricated cylinder, wiped with towel and kept into dried and returned to home cages. After 24h of pretest animals were allowed for second test.

2.2.3.7.2 Tail Suspension Test (TST)

Rats were suspended by the bands and hung from a hook mounted 50 cm above the flat surface and trial series were performed for 6 min. The immobility period was measured during the last 4 min of the protocol (Yamawaki et al., 2012). Rats were hung passively and motionless as a consideration for immobility. Though, mice are mainly used in TST for depression model, many studies have used it for rats (Chermat et al., 1986, Tabassum et al., 2010).

2.2.3.7.3 Sucrose Preference Test (SPT)

The SPT was conducted according to given protocol (Tao et al., 2016). After 24 h fasting, rats were exposed to two calibrated bottles- containing water and 2% sucrose solution. After a time interval of 2 h, the volume of water and sucrose uptake was calculated. The consumption of sucrose and water was presented as percentage of the total liquid ingested.

2.2.3.8 Measurement of cortical blood flow

The cortical blood flow was measured with the laser speckle blood flow imager (Omegazone OZ-2; Omegawave, Tokyo, Japan) according to Yoshinaga et al. (2015) (Yoshinaga, Kawai, Oka, Fuchikami, & Oyama, 2015). Skull of anesthetized rats were exposed by a midline scalp incision and placed on the black sponge sheet that located under the arm stand. Arm stand holds the CCD camera, the lens (ZM10-18, MF12), and the laser unit (780 nm for measurement and 650 nm for positioning). Raw speckle images were recorded from the skull surface using LSI Software (LSI ver.3.3, Omegawave, Inc., Tokyo, Japan) and average cerebral blood flow was determined by further analysis of images by using LIA Software (LIA ver.3.3, Omegawave, Inc., Tokyo, Japan). The black sheet does not reflect the laser light and the effect makes the blood flow image clear. The unit of cortical blood flow is arbitrary unit (AU).

2.2.3.9 Estimation of VEGF level

VEGF levels in prefrontal cortex of brain tissues were measured using a commercially available kit (Rat VEGF ELISA Kit, Krishgen, India). The cortical tissues were homogenized in DMEM (150mg/ml) and centrifuged for 10 min at 10,000g at 4°C. Each brain extract was then divided into 100µl aliquots for the VEGF determination (Jesmin et al., 2004).

2.2.3.10 Assessment of monoamines and their metabolites in PFC

The levels of 5-HT, DA and their metabolites 5-hydroxy indole acetic acid (5-HIAA), 3, 4 dihydroxy phenyl acetic acid (DOPAC) as well as homovanillic acid (HVA) and norepinephrine (NE) were detected in PFC using the HPLC with Electrochemical detector (ECD, Waters 2465, USA) (Yoshitake et al., 2003). In brief, the brain tissue samples were homogenized in 0.17 M perchloric acid by Polytron homogenizer. Homogenates were then centrifuged at 33,000 g (Biofuge Stratos) at 4°C. 20µl of the supernatant were injected via the HPLC pump (Model 1525, Binary Gradient Pump) into a column (Spherisorb, RP C18, 5 µm particle size, 4.6 mm i.d. × 250 mm at 308C) connected to an ECD (Model 2465) at a potential of +0.8 V with glassy carbon working electrode vs. Ag/AgCl reference electrode. The mobile phase consisted of 32 mM citric acid, 12.5 mM disodium hydrogen orthophosphate, 1.4 mM sodium octyl sulfonate, 0.05 mM EDTA, and 16% (v/v) methanol (pH 4.2) at a flow rate of 1.2 ml/min. The protein content was estimated colorimetrically (Lowry et al., 1951).

2.2.3.11 Quantification of BDNF mRNA through reverse transcriptase-PCR (qRT-PCR)

Total RNA from the PFC of control and EMR treated group was isolated using TRI reagent as per manufacture's instruction (Sigma-Aldrich, USA). Total RNA (5 µg) of each animal was subjected to reverse transcription for cDNA synthesis using reverse

transcriptase (Thermo Fischer Scientific, USA). Then 0.5 µg cDNA was subjected to qPCR amplification using SYBR Green master mix in ABI7500 instrument for the gene specific primer BDNF FP-5'-AGCTGAGCGTGTGTGACAGT-3', RP-5'-ACCCATGGGATTACACTTGG-3' (Musazzi et al., 2009), while β-actin FP-5'-GTCGTACCACTGGCATTGTG-3',RP-5'-CTCTCAGCTGTGGTGGTGAA-3' (Kidambi et al., 2010) was used as endogenous control for normalization. The fold change in gene expression was calculated by comparative ΔΔCt method as compared to control and plotted as histogram.

2.2.3.12 Flow cytometry analysis for measurement of apoptosis

Briefly, single cell suspensions from the cortical brain tissue samples were prepared in cold PBS buffer, and the final concentrations were adjusted to 5×10^6 cells/ml through automated cell counter (Life Sciences Countess II FS, Invitrogen, Thermofischer scientific, USA). Further, assay was performed using the method of our earlier study (Samaiya et al., 2018).

2.2.3.13 Statistical analysis

Experimental data were expressed as Mean ± standard error of mean (SEM). All the behavioral data were analyzed using the repeated measures of Two-way ANOVA followed by Bonferroni post hoc test. Molecular and hemodynamic studies were analysed using one-way analysis of variance (ANOVA) followed by Newman-keuls post hoc test. The level of statistical significance is often expressed as a p-value between 0 and 1. In this study $p < 0.05$ were considered as statistically significant for all experimental data analysis. The lower the p-value, the greater the chances for rejection of the null hypothesis. Therefore, in our research hypothesis, there is less than a 5% probability of the null hypothesis to be correct. So there are 95% chances that our research hypothesis is true.

2.2.4 Results

2.2.4.1 Effect of discrete range of EMR on immobility period during FST and TST

Fig: -2.2.3 shows the effects of EMR-900, 1800 and 2450 MHz on immobility period during FST (A) and TST (B). Two-way ANOVA analysis depicted that there were significant differences in immobility period during FST and TST among the groups ([F (3, 100) = 23.4; P<0.05] and [F (3, 100) = 8.6; P<0.05] respectively), time ([F (4, 100) = 13.3; P<0.05] and [F (4, 100) = 2.9; P<0.05] respectively) and there was significant interaction between group and time during FST [F (12,100) = 8.7; P < 0.05] and TST [F (12,100) = 2.2; P < 0.05]. Post hoc test demonstrated no significant differences in the immobility period during FST and TST among the groups up to D-14 of the experimental design. However, on D-21, EMR-2450 group animal exhibited a significant increase in the immobility period during FST and TST compared to all other group rats and this effect was maintained up to D-28 of the experimental design.

Figure 2.2.3 (A) **(B)**

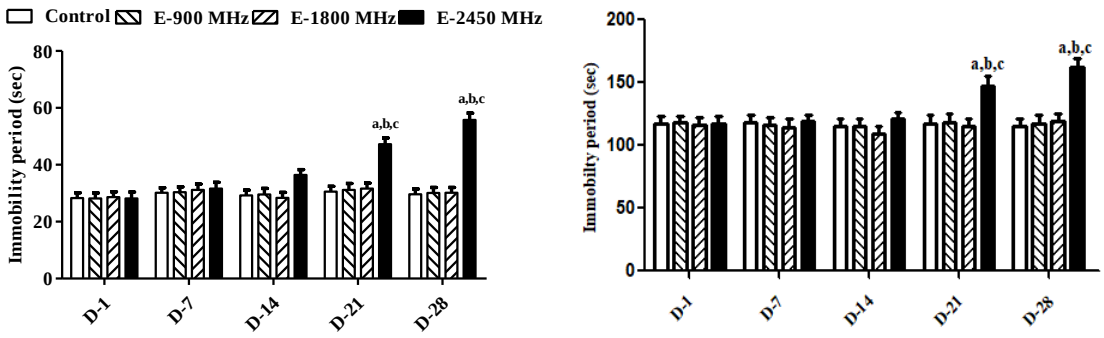


Figure: 2.2.3 (A) Shows immobility period in forced swim test and **(B)** immobility period in tail suspension test. All values are expressed as mean±SEM., (n=6). [a]p<0.05 compared to control, [b]p<0.05 compared to EMR-900 and [c]p<0.05 compared to EMR-1800; [x]p<0.05 compared to D-1, [y]p<0.05 compared to D-7 and [z]p<0.05 compared to D-14[Repeated measure of two-way ANOVA followed by Bonferroni test].

2.2.4.2 Effect of repeated exposure of EMR (900, 1800 and 2450 MHz) on sucrose preference test

The effect of EMR (900, 1800 and 2450 MHz) on sucrose consumption behavior is shown in Fig-2.2.4. Two way ANOVA analysis demonstrated significant differences in sucrose consumption during SPT among groups [$F(3, 100) = 39.8$; $P<0.05$], time [$F(4, 100) = 15.7$; $P<0.05$] and interaction between the group and time for sucrose intake during SPT [$F(12, 100) = 13.4$; $P < 0.05$]. Post hoc analysis revealed that no significant differences among the groups while considering sucrose intake behavior up to D-14. However, EMR-2450 exposed rats showed significantly attenuate in the sucrose consumption on D-21 and this effect was maintained up to D-28 compared to all other group animals of the experimental schedule.

Figure 2.2.4

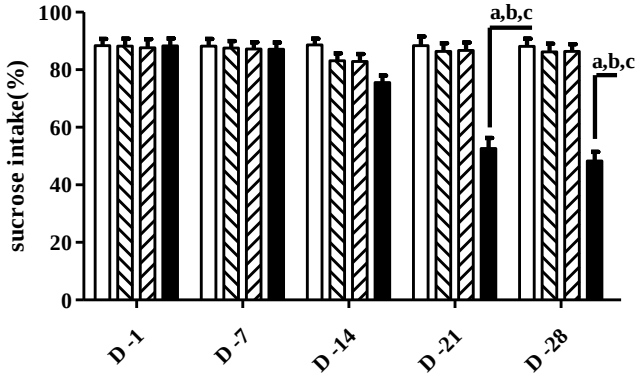


Figure: 2.2.4 Shows EMR-2450 MHz alters percentage of sucrose intake in rats. All values are expressed as mean±SEM., (n=6). $^{a}p<0.05$ compared to control, $^{b}p<0.05$ compared to EMR-900 and $^{c}p<0.05$ compared to EMR-1800; $^{x}p<0.05$ compared to D-1, $^{y}p<0.05$ compared to D-7 and $^{z}p<0.05$ compared to D-14 [Two-way ANOVA followed by Bonferroni test].

2.2.4.3 Effect of long term exposure of EMR (900, 1800 and 2450 MHz) on mean blood flow in cortical region

The effect of sub-chronic exposure of EMR (900, 1800 and 2450 MHz) on cortical blood flow is depicted in Fig-2.2.5. One way ANOVA analysis revealed significant differences in the level of cortical blood flow between the groups [F (3, 20) = 12.52; P<0.05]. Post hoc test revealed that EMR (2450 MHz) exposed rodents showed a significant decrease in the mean cortical blood flow compared to control, EMR-900 and EMR-1800 MHz on D-28.

Figure 2.2.5

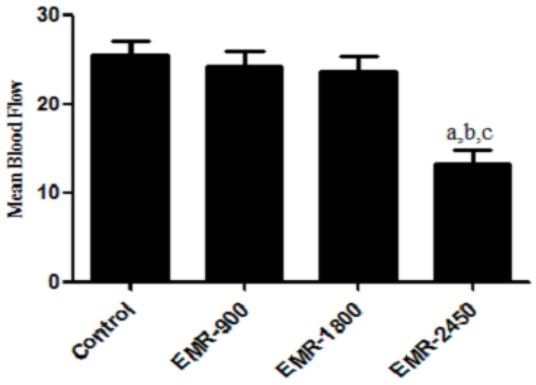


Figure 2.2.5 Shows bar graph of cortical mean blood flow (n=6). All values are expressed as mean±SEM. [a]p<0.05 compared to control, [b]p<0.05 compared to EMR-900 and [c]p<0.05 compared to EMR-1800 [One-way ANOVA followed by Student–Newman Keuls test].

2.2.4.4 Effect of EMR (900, 1800 and 2450 MHz) on the level of VEGF in PFC

The effect of EMR on the level of VEGF in cortical region of brain is shown in fig: 2.2.6. There was statistically significant difference in the level of VEGF among the groups [F (3, 8) = 25.54; P<0.05]. Post hoc analysis showed there were no

significant changes of EMR 900 and 1800 compared to control. However, EMR (2450 MHz) significantly decreased the level of VEGF compared to control groups.

Figure 2.2.6

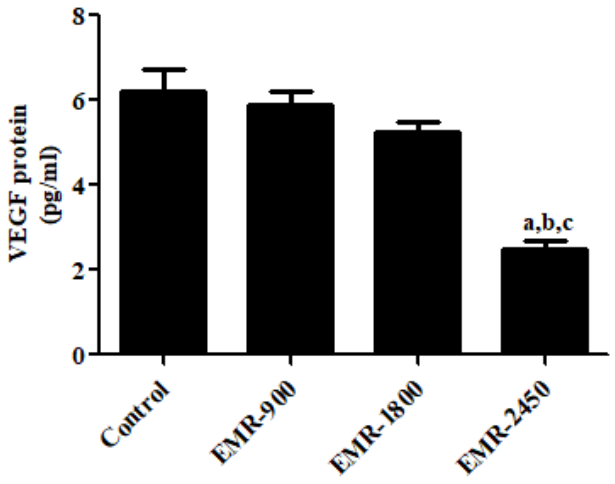


Figure 2.2.6 Shows bar graph of Vascular Endothelial Growth Factor (VEGF; n=6). All values are expressed as mean±SEM. [a]p<0.05 compared to control, [b]p<0.05 compared to EMR-900 and [c]p<0.05 compared to EMR-1800 [One-way ANOVA followed by Student–Newman Keuls test].

2.2.4.5 Effect of EMR (900, 1800 and 2450 MHz) on serotonin, dopamine, norepinephrine level and their metabolites and its ratio in PFC

The EMR (900, 1800 and 2450 MHz) induced alterations in serotonin, dopamine, norepinephrine levels and their metabolites and its ratio in PFC are given in Table-2.2.1. One way ANOVA analysis showed significant difference in the level of 5-HT, DA, NE, 5-HIAA, DOPAC, HVA, ratio of DOPAC/DA, HVA/DA and 5-HIAA/5-HT of among groups (P<0.01). Post hoc test showed that EMR-2450 MHz exposure significantly reduced the levels of 5-HT, DA and NE; increased 5-HIAA level and ratio

of 5-HIAA/5-HT; increased DOPAC, HVA level and ratio of DOPAC/DA, HVA/DA in PFC compared to all other groups.

Table-2.2.1

Monoamines (ng/mg protein)	Control	EMR- 900 MHz	EMR- 1800 MHz	EMR-2450 MHz
5-HT	6.4±0.61	6.05±0.58	5.18 ±0.52	2.54±0.31 [a,b,c]
5-HIAA	2.36±0.24	2.10±0.19	2.54 ±0.24	5.27±0.50 [a,b,c]
5-HIAA/5-HT	0.36±0.03	0.34±0.03	0.49 ±0.04	2.07±0.22 [a,b,c]
DA	1.58±0.16	1.45±0.12	1.40 ±0.13	0.38±0.04[a,b,c]
DOPAC	1.02±0.10	1.01±0.12	1.05±0.11	2.21±0.23 [a,b,c]
DOPAC/DA	0.65±0.07	0.70±0.08	0.75 ±0.08	5.82±0.61 [a,b,c]
HVA	1.72±0.16	1.70±0.19	1.59 ±0.16	2.60±0.24 [a,b,c]
HVA/DA	1.08±0.12	1.17±0.10	1.13 ±0.14	6.84±0.71 [a,b,c]
NE	8.11±0.69	7.58±0.72	7.12±0.70	3.80±0.36[a,b,c]

Table 2.2.1 All results are expressed as mean±SEM, (n=4). [a]P<0.05, [b]P<0.05 and [c]P<0.05 compared to control, EMR-900 and EMR-1800 respectively [One-way ANOVA followed by Student Newman–Keuls post hoc test].

2.2.4.6 Effect of EMR (900, 1800 and 2450 MHz) on BDNF mRNA expression in PFC

Figure 2.2.7 illustrates the effect of EMR-900, 1800 and 2450 MHz on expression of BDNF mRNA in PFC. There was statistically significant difference in the level of BDNF gene expression among the groups [F (3, 8) = 38.4; P<0.05]. Post hoc analysis showed that EMR (2450 MHz) significantly reduced the mRNA expression of BDNF compared to all other groups.

Figure 2.2.7

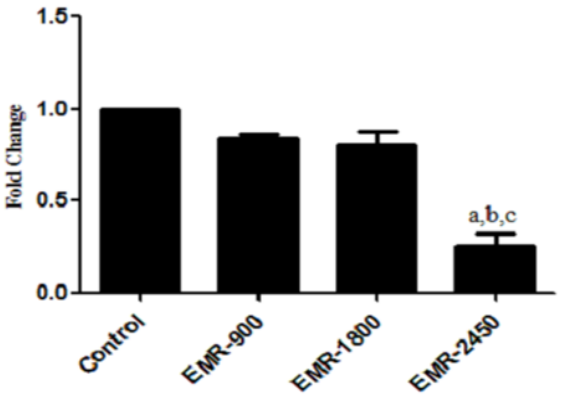


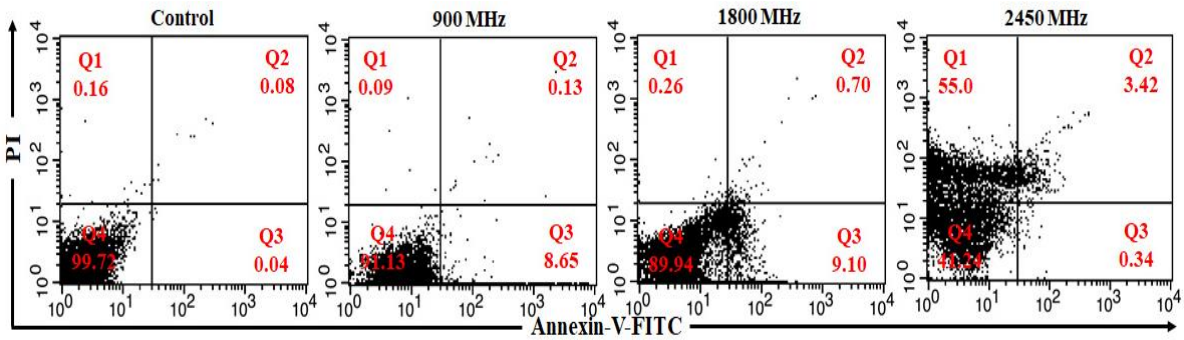
Figure 2.2.7 Shows mRNA expression of BDNF (n=3) (B). All values are mean ± SEM. [a]p< 0.05 compared to control, [b]p< 0.05 compared to EMR-900 group and [c]p< 0.05 compared to EMR-1800 group [One-way ANOVA followed by Student–Newman Keuls test].

2.2.4.7 Effect of EMR (900, 1800 and 2450 MHz) on the percentage of apoptotic Cells in PFC

Fig 2.2.8 (A) shows the flow cytometric analysis as four quadrants labeled as Q1, Q2, Q3 and Q4 which denotes necrosis, late apoptosis, early apoptosis and live cells respectively. Further, Fig. 2.2.8 (B) shows the percentage of necrosis and apoptosis in prefrontal cortex in response to exposure with EMR (900, 1800 and 2450 MHz) on day 28. EMR- 2450 MHz exposed cortical cells entered in to necrosis (Q1) with 55% and late apoptosis (Q2) with 3.42% of total cell population. However, EMR-900 and 1800 MHz exposed cells entered in early apoptosis.

Figure 2.2.8

(A)



(B)

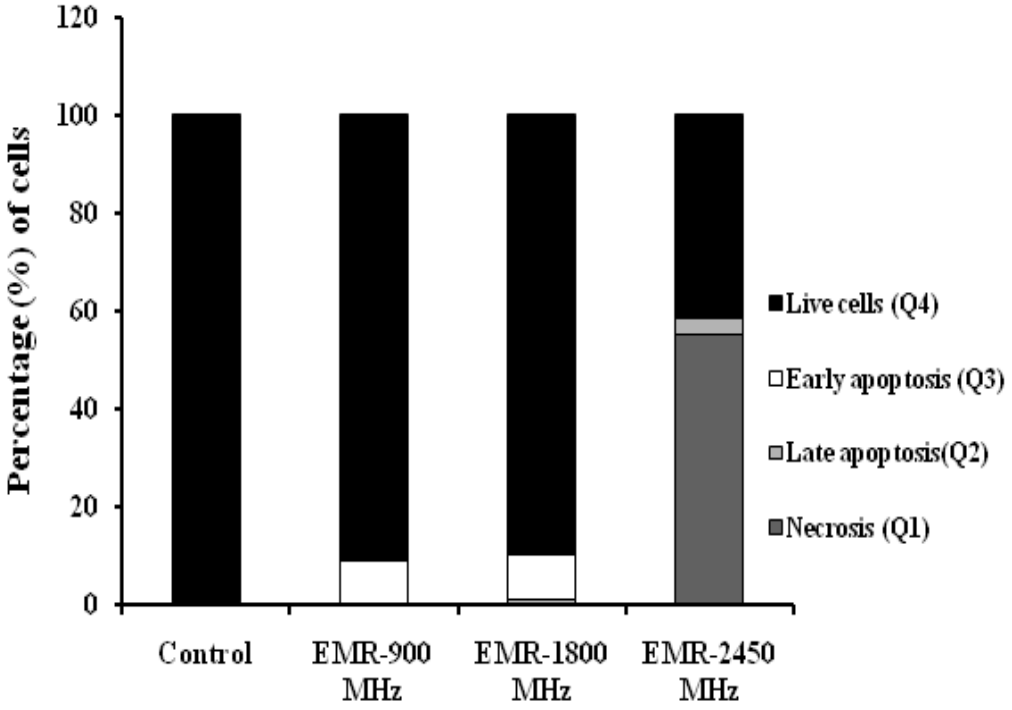


Figure 2.2.8 Flow cytogram presenting EMR exposed cell death in the prefrontal cortex of brain assessed by flow cytometric analysis using annexin-V/PI (n=3) and panel (B) shows percentage values represented as bar diagram.

2.2.5 Discussion

The salient findings of the present study show that the sub-chronic exposure of EMR at a frequency of 2450 MHz induced depressive-like symptoms and was associated with pathophysiological changes in experimental rats. EMR-2450 exposure caused decrease in cerebral blood flow with reduced level of VEGF protein in PFC. EMR also altered monoaminergic activity in PFC suggesting a strong neurochemical mechanism. Moreover, EMR-2450 exposure caused a significant decrease in neurogenic factor BDNF in PFC.

Depressive-like symptoms have been replicated in laboratory animals. Most of the recent studies about depressive-like symptoms such as despair behavior, loss of concentration and loss of interest have come from experimental animal models (Abelaira et al., 2013). We noted that, EMR-2450, but not EMR-900 and 1800, significantly enhanced the immobility period on D-21 and D-28 indicating depressive-like behavior. Earlier reports showed that microwave frequency-2450 MHz but not sub-acute exposure of 1800 MHz to experimental animals induced neuropsychiatric effects including depression (Zhang et al., 2017). Here, we reported that exposure to EMR 2450 MHz showed increase in immobility period indicating hopelessness behavior. Previous data showed that chronic exposure of microwave radiation increased immobility period in FST and TST indicating depressive like behavior in mice (Kumar et al., 2016). FST and TST are useful as preclinical models but there is some lack of consensus due to the short period of study since major depression symptoms are considered as a sub-chronic disease. Therefore, we used sucrose preference test, a model for anhedonia which shows core symptoms of depression rather than subsidiary symptoms (Willner et al., 1992, Moreau et al., 1995). Such models possessing remarkable similarity to human clinical depression may prove beneficial for a better

understanding of pathophysiological mechanisms involved in depressive disorders. In our study, EMR-2450 exposed animals showed the less consumption of sucrose on D-21 and up to D-28 suggesting anhedonic response possibly due to decrease in dopamine levels in the PFC. (Moreau et al., 1995, Felger et al., 2013). In contrast, EMR-900 and 1800 MHz exposed rats did not show anhedonic behavior. The study indicated that EMR can precipitate symptoms in the sub-chronic model of depression.

Blood flow is a prime factor to maintain the volume of PFC during depression (Willeumier et al., 2011). Previous report suggested that mobile phone alters the cerebral blood flow in humans (Aalto et al., 2006b). Decrease in blood flow in cerebral 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 (El-Bediwi et al., 2013). A drastic increase in local oxygen demand is observed to restore the homeostasis. VEGF is an essential regulator of vascular permeability system that helps to restore the oxygen supply in tissues in case of inadequate blood circulation. Sub chronic exposure of EMR-2450 MHz decreased the VEGF level in testis in experimental rats (Saygin et al., 2016). We observed that the exposure of 2450 MHz radiation decreased cortical blood flow. This decrease in blood flow may be due to decrease in expression of VEGF as its participates in vascular hyper-permialization and increases blood flow (Ashina et al., 2015).

The aberration in monoaminergic system is considered as an important predisposing factor in the pathogenesis of depression (Delgado, 2000, Quirk and Beer, 2006). In the present study, continuous exposure EMR-2450 but not EMR-900 and 1800 altered the monoaminergic system in that cortical region. However, It has been reported that chronic exposure (4 months) of pulsed EMR-1800 MHz caused disturbances in monoamine neurotransmitters and this might underlie the adverse effect reported after EMR including stress (Ezz et al., 2013). The

contradiction may be due to the difference in the experimental design related to the mode to exposure. EMR-2450 MHz caused a significant reduction in the level of 5-HT, 5-HIAA and ratio of 5-HIAA/5-HT in PFC. It has been reported that short term exposure to 1439 MHz through time division multiple access (TDMA) field did not alter serotonin synthesis in rats (Hata et al., 2005). EMR at 2450 MHz disrupted the serotonergic system in PFC. EMR at 2450 MHz decreased the level of NE, DA, DOPAC and HVA, and their ratios of HVA/DA and DOPAC/DA in PFC. It has been reported that microwave radiation from cellular phone decreased the level of DA and NE in rat brain (Jing et al., 2012). We have first time reported that subacute exposure to EMR-2450 MHz altered the HVA and DOPAC level in the PFC. These results suggest that EMR at higher frequency disrupts dopaminergic, serotonergic and noradrenergic systems in PFC. The role of monoaminergic system is well established in the neurobiology of depression (Gantz et al., 2015). It has been reported that the attenuation of serotonergic activity is responsible for depressive behavior (Yoon et al., 2016). Dopaminergic system of the rat plays an important role in PFC regulation of stress and emotion (Sullivan et al., 2009). The PFC circuits are modulated by NE which plays an important integrative harmonizing and life-threatening role in the development of depression (Southwick et al., 1999). NE depletion has been shown to be as detrimental as removing the cerebral cortex itself. Our result shows that EMR-2450 MHz exposure induced imbalance in monoamine neurotransmitters in the PFC which might be an important cause for expression of depressive-like behavior.

BDNF is a neurotrophins, mainly expressed in the PFC (Mizui et al., 2016). It plays an important role in neuroprotection by regulating several functions during development including nerve growth, neuronal differentiation, survival and normal maturation of neurodevelopment pathways (Maskey and Kim, 2014, Schmidt and Duman, 2007). Previous studies reported that long term exposure of microwave radiation (2856 MHz for 1 month) alters the BDNF expression suggesting abnormalities in synaptic plasticity of hippocampus in rats (Tan et al., 2017). In the present study,

EMR-2450 exposed rats showed a decrease in BDNF mRNA expression in PFC suggesting pathophysiological alterations involved in neuronal cell survival in experimental rats. Thus, our results at gene level clearly substantiate our notion that exposure to EMR-2450 alters BDNF mRNA expression, which can cause neuro-pathophysiological conditions like depressive-like symptoms. Therefore, EMR at higher frequency disrupts neuronal function in terms of altered monoaminergic activity and BDNF in PFC. As BDNF maintains neuronal plasticity and helps to form synapse connections (Chen et al., 2004), decrease in its level leads to imbalance in the neuronal circuit. The levels of BDNF and VEGF are reduced in models showing depressive-like behavior (Bamji et al., 2006; Clark-Raymond and Halaris, 2013). Alterations in BDNF and VEGF modulate apoptotic gene expression (Kotan et al., 2012, Almeida et al., 2009). "Necrosis and apoptosis is a cell death process that plays a key role during both physiological as well as pathological conditions" (Benn and Woolf, 2004; Nowacka and Obuchowicz, 2013). In present study necrosis and apoptosis were observed by flow cytometric analysis. Interestingly, EMR-900 and EMR-1800 exposure induced early stage apoptosis (Q3, annexin-V positive and PI negative) indicating mild effect on cortical cells. However, higher frequency of EMR-2450 MHz induced significant necrotic (Q1, PI positive and annexin-V negative) and late stage apoptotic cell death. Our previous studies suggested that long term exposure of EMR-2450 MHz caused release of cytochrome-C and activation of caspase-9 ensuing activation of apoptotic cell death in the hippocampus (Gupta et al., 2018b).

Necrosis in a particular region of brain leads to infarction in that area (Kalogeris et al., 2012) which causes insufficiency of blood flows as observed in our results resulting in ischemic like condition. Therefore, EMR-2450 can induce ischemic like condition by necrotic damage of cortical cells and subsequent programmed cell death. Thus it can be

presumed that necrosis and apoptosis could be important factors of both altered neuronal and vascular function in PFC of animals during EMR exposure.

2.2.5.1 Summary

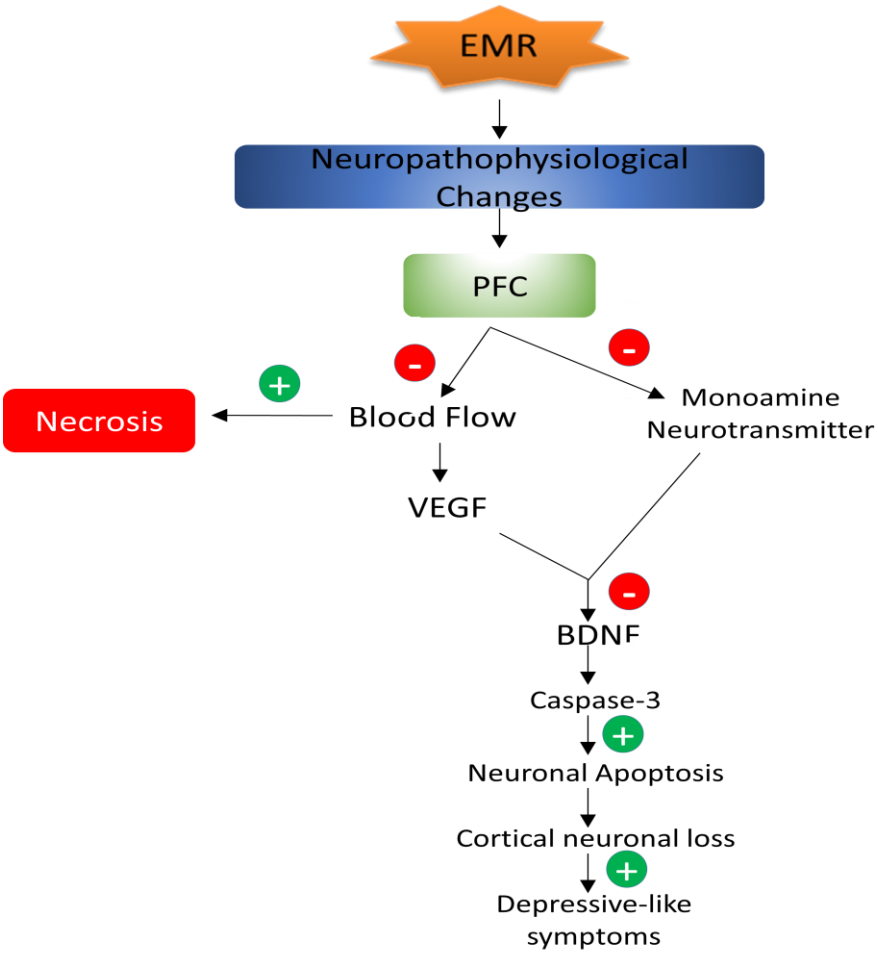


Figure 2.2.9 Summary of hypothesis

In figure 2.2.9 shows that EMR-2450 induced depressive-like symptoms in animal models. However, EMR-900 and 1800 did affect the behavior of the rats. Repeated EMR-2450 exposure altered vascular function by decreasing blood flow and reduced VEGF expression in PFC. Further, the neuronal function was altered in terms of reduced monoaminergic activity and BDNF in PFC. EMR was radio-toxic as it caused necrotic cell death at higher frequency. EMR exposure at higher frequency also induced necrosis and apoptosis. These observations emphasize the fact that EMR at

higher frequency might cause depressive-like symptoms through altering both vascular as well as neuronal functions in PFC. This study indicated that the development of newer mobile phones should use technology utilizing lower frequency to minimize the potential neuropsychiatric effects of EMR.