5.1 Introduction

The use of 3G mobile phone and Wi-Fi has increased rapidly all over the world since the early 20th century. In India, it is growing at a rate of 1.5–2 million a month (Kumar, 2004). Cell phone subscribers increased worldwide due to immeasurable benefits such as accidental reports, commerce and communication and hence more data consumption (Comer and Wikle, 2008). Electromagnetic radiation (EMR) causes stacking of blood cells which resembles a stack of coins and thus reduced oxygen-carrying capacity of blood cells. This impairment of blood physiology destructs the blood circulation in the heart fails to remove the waste products from cardiac tissues indicating cardiac dysfunction (Havas, 2013). The previous report suggests that long term hemodynamic consequences in the heart may also induce ventricular tachycardia (Demaria et al., 1977). An epidemiological investigation indicated that the workers subjected to long term exposure with medium (0.15-0.35 Hz) frequency bands at a broadcasting station, suffered from cardiac dysfunction including impairment in heart conduction, repolarization disturbances and irregularities in electrocardiogram (ECG) pattern (Bortkiewicz et al., 1996). It is well established that ECG recording plays an essential role in the diagnosis and management of the electrical function of the heart (Devereux and Alderman, 1993). Disturbances in the ECG morphology lead to cardiac dysfunction (Chuang et al., 1993).

Preclinical and clinical reports suggested that long term exposure to EMR (900 MHz for 4 months) causes changes in the cardiac physiology due to fluctuations in the heart rate (HR), blood pressure (BP) and ECG pattern (Devasani and Razdan, 2017; Lu et al., 2009; Duhan et al., 2017; Peckerman et al., 2003; Havas et al., 2010) due to continuous emission of radiation. It is noteworthy that HR and BP are the primary

assessment of normal cardiac function (Devereux and Alderman, 1993). Earlier reports suggest that irregularities in HR and BP of the heart resulting in cardiac abnormalities (Lu et al., 2009).Thus based on the above studies, we assume that repeated exposure to EMR may cause ventricular tachycardia in experimental rats. There are no preclinical evidences on long term exposure to EMR-2450 MHz cause cardiac dysfunction in rats.

Therefore, the present study has evaluated the role of repeated exposure of EMR-900, 1800 and 2450 MHz on heart in rats. Frequency and the duration of EMR exposure are critical for the development of cardiac arrhythmia, preliminary function of the heart including Mean Arterial Pressure (MAP) and HR were evaluated by NIBP. Cardiac blood flow was measured by laser speckle blood flow imager. Further, cardiac morphological changes were assessed by scanning electron microscope (SEM) in EMR exposed rats (as illustrated in figure 5.1).

5.1.1 Hypothesis

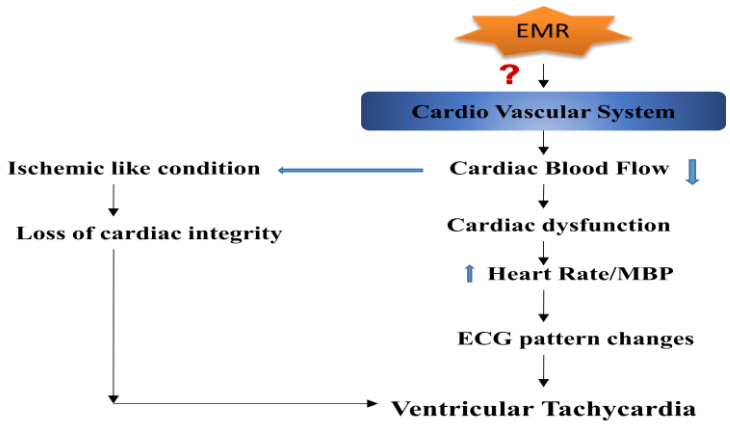


Figure 5.1 Proposed hypothesis of effect of EMR on heart

5.2 Materials and methods

5.2.1 Animals

Inbred Charles-foster albino male rats 6-weeks oldweighing about 200±20 g were purchased from the central experimental animal facility centre, Institute of Medical Sciences, Banaras Hindu University (IMS-BHU). The animals were housed in a 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).

5.2.2 EMR exposure apparatus and Design

The exposure equipment was designed according to our previous study (Gupta et al., 2018). Briefly, it includes inbuilt analogue signal generator by Agilent Technologies, the USA having a frequency range of 100 kHz-20 GHz. The exposure system had an interconnected waveguide transition microwave amplifier (Hewlett Packard) along with a 20db cross-coupler, E-plane bend and a brass-silver coated pyramidal horn antenna. The maximum output power was 19.8 dB measured by a power meter (Agilent Technologies, USA) and then delivered it to the horn antenna. The whole assembly was kept on a wooden table, and the generator emitted a discrete range of 900, 1800 and 2450 MHz radiofrequency (rf) signals.

5.2.3 Measurement of Power density and specific absorption rate (SAR) on chest region of experimental rat

The power density was calculated by the formulae described in an earlier study (Gupta et al., 2018). The average power density was 0.1227 W/m^2. The whole body SAR

values were found in between the 0.025-0.070 W/kg range, representing an average SAR value to be approximately 0.042 W/kg, while the value of SAR in the cardiac region was found to be 0.028, 0.041 and 0.92 W/kg (900, 1800 and 2450 MHz) respectively.

The calculation of SAR

SAR= 5.94 * average length of animals * power density / Electromagnetic Range in GHz*average wt. of the animal; whereas, Avg. Length of animal =17 cm, Avg. Wt of animal =200g and the average length of cardiac tissue of rats = 3.1 cm (Gandhi et al., 1977).

5.2.4 Experimental design

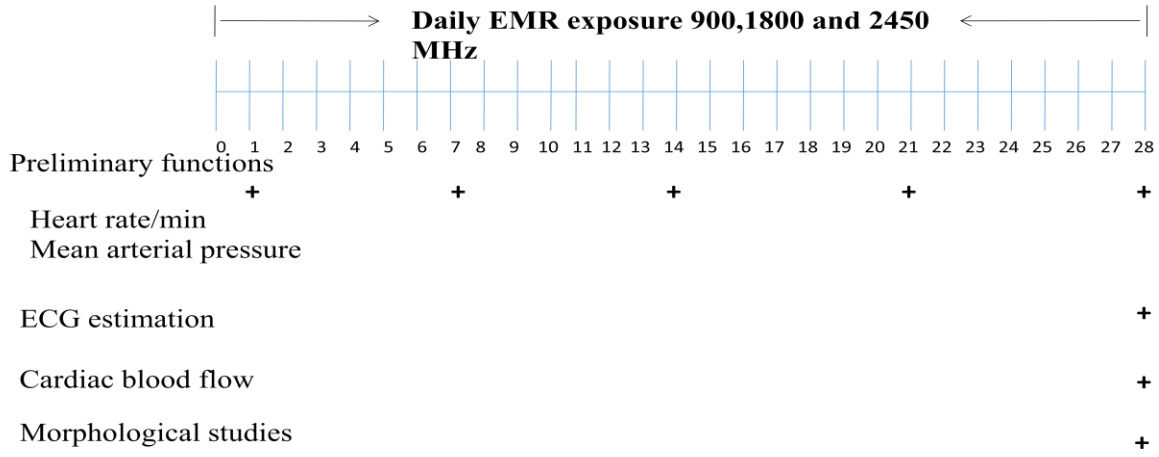


Figure 5.2 Schematic representation of the experimental design. '+' denotes experiment performed

Figure 5.2 shows that all the rats were arranged into four different groups: control, EMR-900, EMR-1800 and EMR-2450 MHz as it is equivalent to cell phone radiation to which humans are continuously exposed. Each group contains six male rats. 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 seven days interval, the preliminary activity of heartbeat and blood pressure were performed. All the preliminary activity was recorded and evaluated using NIBP tail-cuff method. On D-28, rats were anaesthetized by pentobarbitone (35 mg/kg, i.p.) followed by evaluation of electrocardiography using NIBP tail-cuff method and cortical blood flow via laser speckle blood flow imager (Omegazone OZ-2; Omegawave, Tokyo, Japan). Thereafter, animals were killed by cervical dislocation and immediately, the cardiac tissue was micro-dissected and instantly stored at -80^0 C until further analysis. All the preliminary activities and blood flow were assessed (n=6). Morphological analyses of cardiac tissue were done using a scanning electron microscope (SEM) (n=3).

5.2.4 Evaluation of the preliminary activity of the heart

5.2.4.1 Heart rate (HR) and Mean arterial pressure (MAP)

We have taken a polypropylene rat restrainer. The rat was gently placed inside it in which rat faces the open end of the nose cone. Place the restrainer onto the warming platform in the designated position and to clean the rat tail with lukewarm water. Allow the animal at least 5 min to acclimate. Place the occlusion tail-cuff through the tail and to the base of the tail without force. After that, thread the tail through the VPR sensor cuff and place it within 2 mm of the occlusion cuff. To start the experiment with NIBP software this was installed in a computer. Mean diastolic and mean systolic BP is measured and the data was collected. When the experiment is completed, remove the animals immediately from the cuffs and the restrainer and place back to their own cages

(Parasuraman et al., 2012).

5.2.4.2 Electrocardiogram estimation by using NIBP tail-cuff method

All rats were in normal at the time of ECG recordings. After anesthesia with pentobarbitone (35 mg/kg) s.c. and once the animal has been stabilized, the ECG signals were continuously recorded for at least 15 minutes in I-II, V1, V3, and V6 leads. Lead II can be achieved in the rat by the placement of the negative electrode near the right shoulder. Positive electrode to the left of the xiphoid space, in the same way as the Einthoven triangle (right arm position in the negative electrode and left leg position in the positive electrode) (Parasuraman et al., 2012).

5.2.5 Measurement of cardiac blood flow

The cardiac blood flow was measured with the laser speckle blood flow imager (Omegazone OZ-2; Omegawave, Tokyo, Japan) according to Yoshinaga et al. (2015) (Yoshinaga et al., 2015). Mean blood flow values were calculated using the software OZ-2 (LSIv3.3.1 and LIAv3.3.0). The unit of cortical blood flow is an arbitrary unit (AU).

5.2.6 Morphological study of cardiac tissues by using a scanning electron microscope

Cardiac tissues from all the groups were examined for morphological changes using scanning electron microscopy. Samples were fixed in 2.5% glutaraldehyde for 6 h at 4 °C and washed in 0.1 M phosphate buffer, with three changes each of 15 min, at 4 °C. 1% osmium tetroxide was used for post-fixation for 2 h at 4 °C and samples were washed in 0.1 M phosphate buffer, with three changes each of 15 min, at 4 °C to remove unreacted fixative. Specimens were dehydrated using increasing concentrations of dry acetone (30%, 50%, 70%, 90%, 95% and 100%) at 4 °C for 30 min periods.

The specimens were then air-dried and mounted on an aluminum stub with adhesive tape. The tissues were inspected for morphological changes using a scanning electron microscope (JEOL-JSM-6490LV) (Pinali et al., 2013).

5.2.7 Statistical analysis

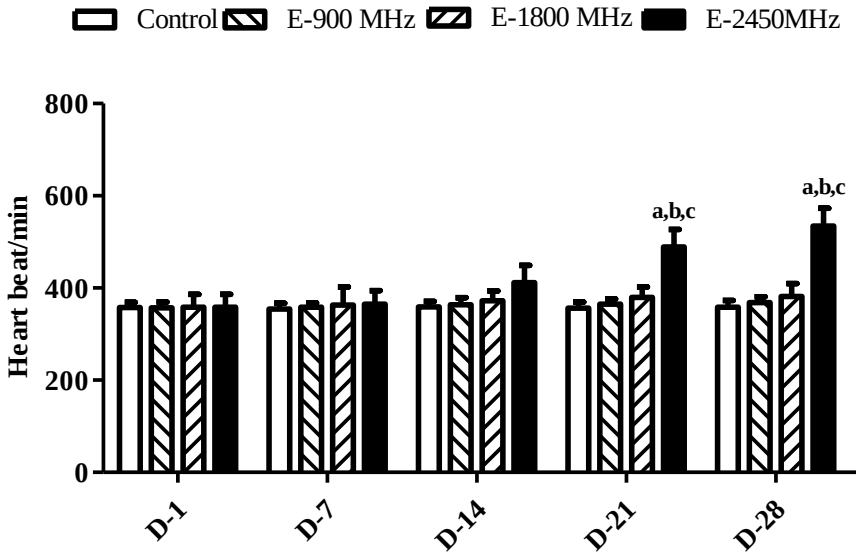
Experimental data were expressed as Mean ± standard error of mean (SEM). All the HR and BP data were analyzed using the repeated measures of Two-way ANOVA followed by Bonferroni post hoc test. Cardiac blood flow was analysed using one-way analysis of variance (ANOVA) followed by Newman-keuls post hoc 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.

Results

5.3.1 Effect of discrete range of EMR on Heart rate and Mean arterial pressure

Fig:-5.3 shows the effects of EMR-900, 1800 and 2450 MHz on heart rate (A) and mean arterial pressure (B). Two-way ANOVA analysis showed that there were significant differences in preliminary function of heart during HR and MAP among the groups ([$F(3, 100) = 20.11$; $P<0.05$] and [$F(3, 100) = 25.83$; $P<0.05$] respectively), time ([$F(4, 100) = 12.2$; $P<0.05$] and [$F(4, 100) = 18.9$; $P<0.05$] respectively) and there was significant interaction between group and time during HR [$F(12,100) = 8.7$; $P < 0.05$] and MAP [$F(12,100) = 14.5$; $P < 0.05$]. Post hoc test demonstrated no significant differences in the preliminary function during HR and MAP among the groups up to D-14 of the experimental design. However, on D-21, EMR-2450 MHz exposed animal exhibited a significant increase in heart rate and mean arterial pressure compared to all other group rats and this effect was maintained up to D-28 of the experimental design.

Figure 5.3 (A)



(B)

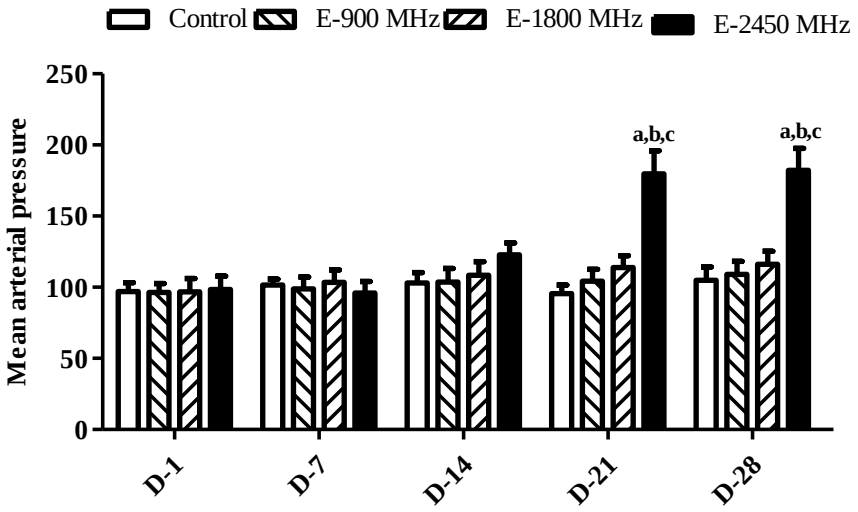


Figure 5.3 Shows alterations in (A) heart beat and (B) mean arterial pressure of cardiac tissues in rats. All values are expressed as mean±SEM., (n=6). [a]$p<0.05$ compared to control, [b]$p<0.05$ compared to EMR-900 and [c]$p<0.05$ compared to EMR-1800 [Two-way ANOVA followed by Bonferroni test].

5.3.2 Effect of repeated exposure of EMR (900, 1800 and 2450 MHz) on Electrocardiography (ECG)

The effect of EMR (900, 1800 and 2450 MHz) on electrocardiography analyses is shown in Fig-5.4. EMR-2450 exposed rats showed significantly widening and shortening of the QRS complexes and loss of T-wave on D-28 compared to all other group.

Figure 5.4

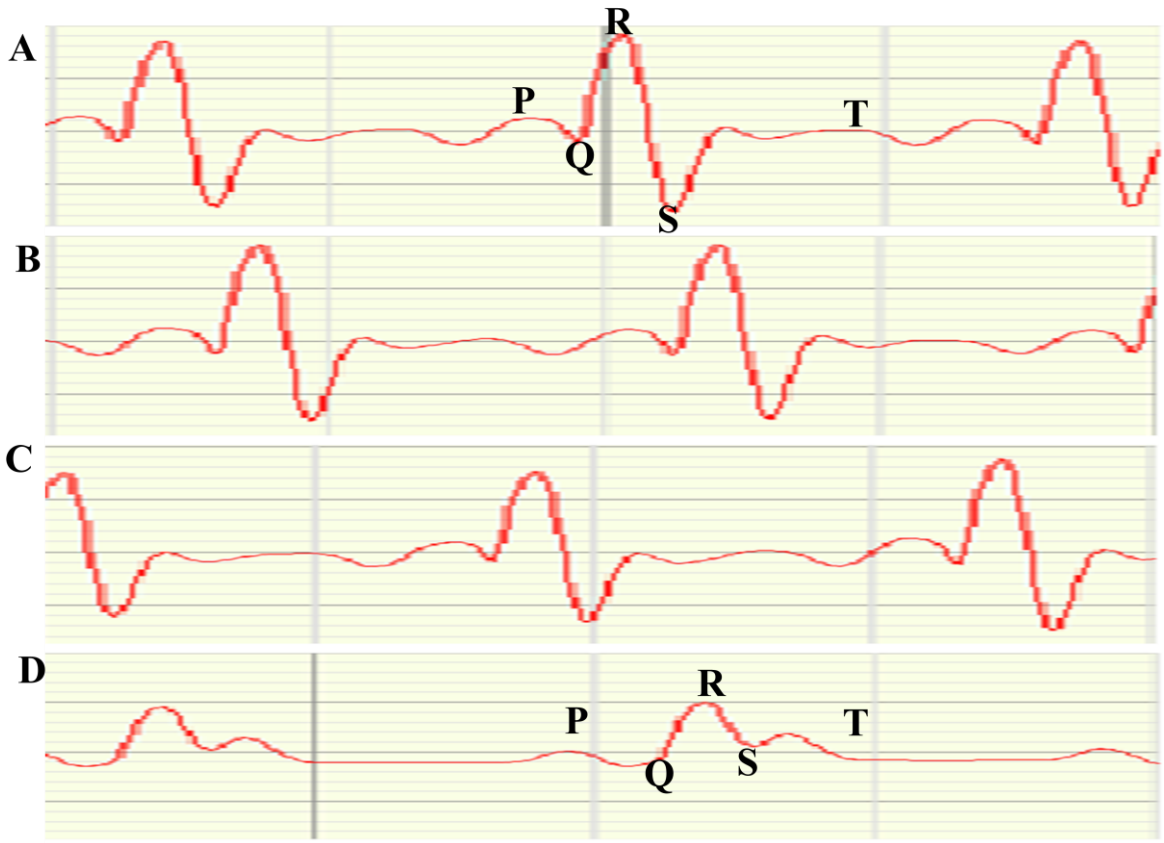


Figure 5.4 Shows changes in ECG pattern of heart. (A) Control (B) EMR-900MHz (C) EMR-1800 MHz (D) EMR-2450MHz.

5.3.3 Effect of long term exposure of EMR (900, 1800 and 2450 MHz) on mean blood flow in heart

The effect of sub-chronic exposure of EMR (900, 1800 and 2450 MHz) on cardiac blood flow is depicted in Fig-5.5. One way ANOVA analysis revealed significant differences in the level of cardiac blood flow between the groups [F (3, 20) = 15.79; P<0.05]. Post hoc test revealed that EMR-2450 MHz exposed rodents showed a significant decrease in the mean cardiac blood flow compared to control, EMR-900 and EMR-1800 MHz on D-28.

Figure 5.5

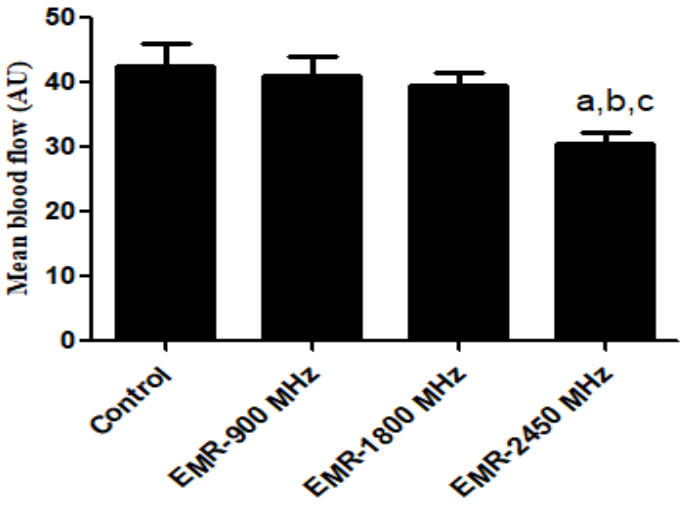


Figure 5.5 Shows alterations in mean blood flow of cardiac tissues in rats. All values are expressed as mean±SEM., (n=6). [a]p<0.05 compared to control, [b]p<0.05 compared to EMR-900 MHz and [c]p<0.05 compared to EMR-1800 MHz [One-way ANOVA followed by Student–Newman Keuls test].

5.3.4 Effect of EMR (900, 1800 and 2450 MHz) on the morphological changes in cardiac tissues

Fig 5.6 shows the morphological analysis in cardiac tissues in response to exposure with EMR (900, 1800 and 2450 MHz) on day 28. EMR- 2450 MHz exposed heart causes increased stiffness and reduction of elasticity of the ventricular myocardium and loss of integrity of myocardium. However, EMR- 900 and 1800 MHz exposed heart did not show significant modifications in cardiac tissues.

Figure 5.6

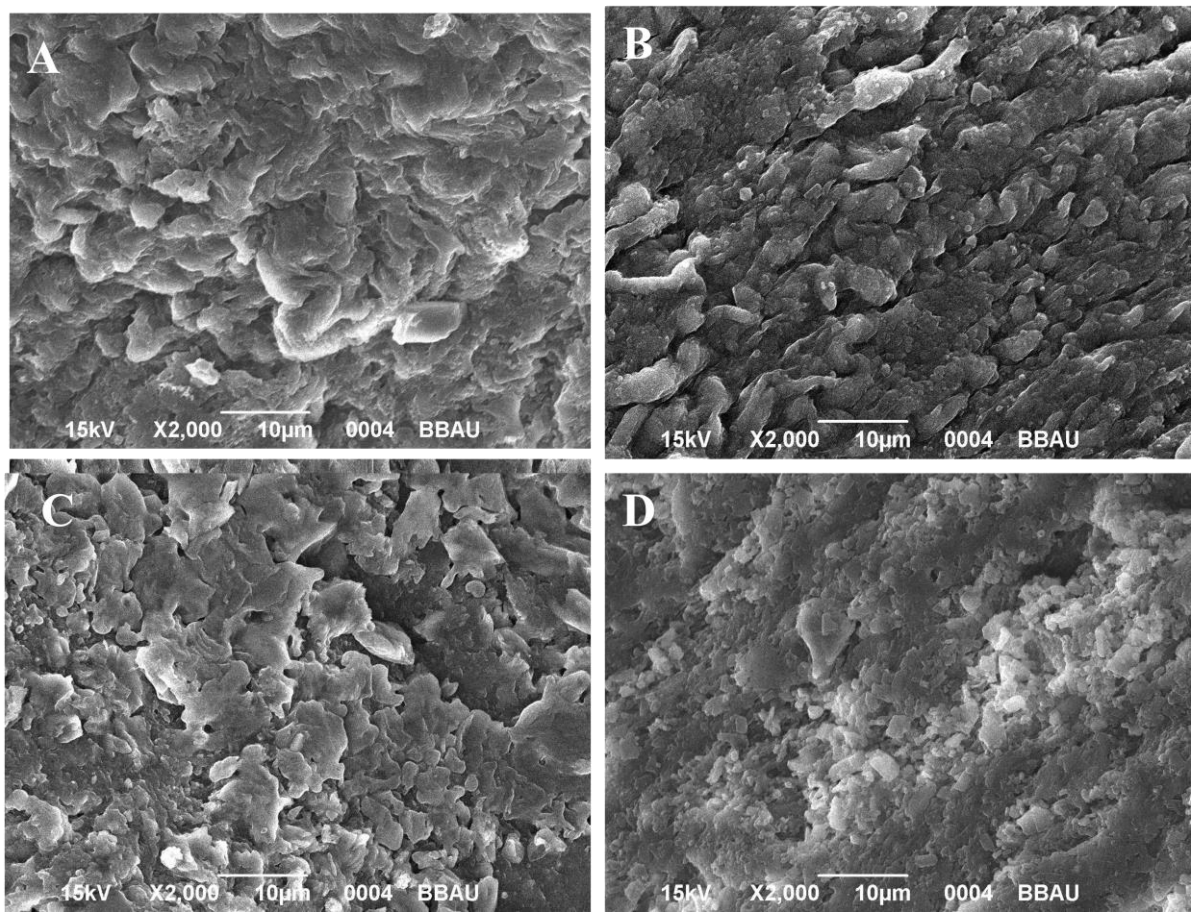


Figure 5.6 Shows changes in morphology of cardiac tissues using SEM (A) Control (B) EMR-900 MHz (C) EMR-1800 MHz (D) EMR-2450 MHz.

5.4 Discussion

The salient finding of the present study shows that the sub-chronic exposure of EMR at a frequency of 2450 MHz caused alteration in the preliminary function of the heart in experimental rats. Long term exposure to EMR-2450 MHz significantly increased in heart rate, mean arterial pressure, shortening and widening of QRS complex in electrocardiography in the heart. EMR also altered the mean blood flow in the heart, suggesting cardiac dysfunction. Moreover, EMR-2450 MHz exposure caused a significant change in the integrity of cardiac tissues in the heart.

Most of the cell phones studies have been conducted since the question of increased health problem from excessive use of cell phone has become a social issue (Devereux and Alderman, 1993).The heart is a vital organ and its function based on the electric conduction, which may affect by environmental EMR. HR and MAP are useful as a primary assessment to evaluate the cardiac conduction in rat (Lerman and Belardinelli, 1991). In the present study, long term exposure to EMR-2450 MHz causes a significant increase in HR and MAP on D-21, which has maintained up to D-28, indicating cardiac deregulation in experimental rats. However, EMR-900 and 1800 MHz did not alter HR and MAP resulting in normal functioning of the heart in rats. Earlier reports suggested that acute exposure with EMR-900 and 1800 MHz did not change the heart rate and mean arterial pressure in humans (Tahvanainen et al., 2004). Previous study has reported that long term exposure to EMR causes increased blood pressure in humans (Havas et al., 2010). Therefore, repeated exposure to EMR-2450 MHz causes alteration of cardiac conduction in the rat.

Preclinical and clinical reports suggested that electrocardiography (ECG) is a model for electrical conductivity which is useful conduction of heart (Devereux and Alderman, 1993). ECG is a visual interpretation to diagnose heart disorder (Chuang et

al., 1993). ECG is beneficial for a better understanding of the pathophysiological mechanisms involved in cardiac complications (Chuang et al., 1993). In this study, EMR-2450 MHz significantly changed the electrical conductivity of heart as shortening and widening PR and lengthening of QRS complex wave intervals on D-21 and up to D-28, indicating ventricular tachycardia in experimental rats. However, EMR-900 and 1800 MHz did not alter ECG interpretation in rats. Therefore, EMR-2450 MHz caused ventricular tachycardia in experimental rats.

Functional cardiac imaging studies suggested that atrophy in the heart during ventricular tachycardia (Ota et al., 2014). The decrease in blood flow in the cardiac region is due to a reduction in the electrical potential at the cell membrane resulting in decreased the repellent forces between cells (Eyuboglu et al., 1988). In this study, we observed that the repeated exposure to EMR-2450 MHz decreased cardiac blood flow. This decrease in blood flow may be due to changes in the blood physiology in EMR exposed rats (Havas, 2013). However, EMR-900 and 1800 MHz did not alter blood flow in rats. Long term insufficiency of blood flows in the heart lead to generating an ischemic-like condition in cardiac tissues (Eyuboglu et al., 1988). In the present study, repeated exposure to EMR-2450 MHz causes a decrease in blood flow, indicating an ischemic-like status in cardiac tissues of rats. Thus, it can be presumed that alteration in cardiac conduction and ischemic-like condition could be essential factors of ventricular tachycardia in the heart of EMR exposed rats. Further, in scanning electron microscopy showed that cardiac tissues lost cardiac integrity and ischemic-like condition in heart. Therefore, EMR at higher frequency disrupts cardiac physiology in terms of altered cardiac conduction and blood flow in the heart.

5.4.1 Summary

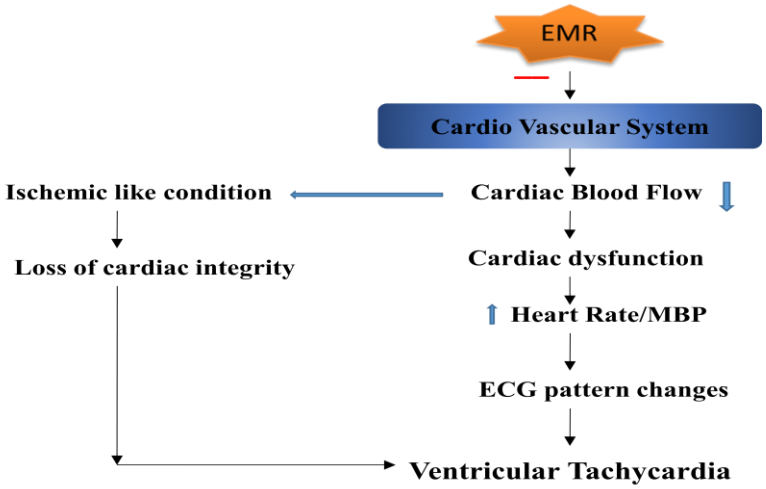


Figure 5.7 Summary of hypothesis

Figure 5.7 show that EMR-2450 MHz induced alteration in cardiac function in animal models. Repeated exposure to EMR-2450 MHz altered HR and MAP in the heart of experimental rats. Further, repeated exposure to EMR-2450 MHz changed cardiac conduction in terms of shortening and widening of QRS complex and PR interval in electrocardiography. EMR-2450 MHz decrease vascular function by reducing blood flow in the heart. Furthermore, EMR exposure at higher frequency also causes ischemic like condition as well as the loss of cardiac integrity. These observations emphasize the fact that EMR at a higher rate cause ventricular tachycardia through altering both vascular as well as cardiac conduction in the heart. This study indicates that the development of newer mobile phones should use technology utilizing lower frequency to minimize the potential of cardiac abnormalities of EMR.