World Applied Sciences Journal 16 (5): 656-664, 2012
ISSN 1818-4952

Effect of Radiofrequency Electromagnetic Field Exposure on Hematological Parameters of Mice

[1]A.D. Usman, [1]W.F. Wan Ahmad, [1]M.Z.A. Ab Kadir, [2]M. Mokhtar and [3]R Ariffin

[1]Department of Electrical and Electronic Engineering, Faculty of Engineering, University Putra Malaysia. 43400 UPM Serdang, Selangor, Malaysia
[2]Department of Computer & Communication Engineering, Faculty of Engineering, University Putra Malaysia. 43400 UPM Serdang, Selangor, Malaysia
[3]Faculty of Electrical Engineering, Universiti Teknologi Mara (UiTM) Pulau Pinang, 13500 Permatang Pauh, Pulau Pinang, Malaysia

Abstract: Radio frequency electromagnetic field (RF EMF) exposures due to Global System for Mobile communication (GSM) window frequencies were investigated in this study. 158 Swiss Albino mice in unrestrained conditions were used as surrogate and divided into four groups. The average field strength generated and measured inside the cages placed at a far field from the antennas was 6mW/m^2 and the specific absorption rate was 0.3W/kg. Three samples of the exposed mice chosen at random at the end of exposure after 4 weeks and subsequently on biweekly basis were taken for haematology and histopathology tests. The complete blood count result shows that haematological parameters of both the sham exposed and exposed mice were within the normal range of mice in the control group. The mean values of haematological parameters were found to be significant with prolong exposure. The histopathology examinations on bone marrow of the mice were normal for all the three experimental groups. Observation of individual and collective behaviour of the mice shows some manifestation in the form of aggressiveness and hyperactivity. In contrast, these signs were not so apparent in the mice in sham exposed group.

Key words: Radio frequency (RF) · Haematology · Base station (BS) · Global system for mobile communication (GSM).

INTRODUCTION

Increased usage of electromagnetic (EM) principles for domestic and industrial purposes proves that EMF plays an important role in our daily life. The emergence of telecommunication services using EMF principles greatly enhance the ability of individuals and groups to communicate with each other. Nowadays, phones are not only used for making and receiving calls but also for many other applications, such as banking transactions and web browsing. The world economic boom also benefitted from these technologies, in 1920s the huge crash and worldwide depression did not show any effect on GSM. Similarly, the current worldwide economic crises have little or no significance to the mobile industries. According to International Telecommunication Union (ITU), the number of mobile phones subscribers in the world was estimated to about 4.6 billion in 2009 and it was expected to reach 5 billion by 2010 [1]. This means that around one out of every two individual in this world carries a mobile phone. The increased usage and growing popularity of wireless technologies in RF EMF range represents one of the fast growing environmental influences. This usage is not without a lot of controversy and public concern on the possible adverse health effect associated with the energies emitted by these technologies [2-4].

Though regulations were provided as in [5, 6] which are aimed at safeguarding the public from dangers associated with the usage of these facilities, people still nurture fears due to the fact that more and more

Corresponding Author: A.D. Usman, Department of Electrical and Electronic Engineering, Faculty of Engineering, University Putra Malaysia. 43400 UPM Serdang, Selangor, Malaysia.
Tel: +234866238363.

technologies emitting EMF are now in our midst. A lot of cutting edge studies were performed aimed at building public confidence on the usage of these technologies. Researches on health effects of RF EMF are quite numerous and diverse. It cuts across many disciplines of engineering, physics, biology and medicine. This emerging technology to date has witnessed several research papers with many contradictions. Several of which can be found in [7-22] and in some EMF data base. Most significant researches were done using mice exposed to either GSM frequencies or frequencies around that of GSM aimed at investigating different mice organs as reported in [23-32]. Some of these researches uses animal in restrained position where animals do not have access to water or food during exposure. This restrained position is likely to cause stress related changes as shown in [32]. The study of long term effect in this condition are also not appropriate as no exposures per day and per week will be identical leading to serious comparative difficulties as reported by [31, 33, 34]. It can be seen that though a lot of studies were conducted most especially on the way towards having a clear understanding of the effect or no effect of RF EM exposure, the issue is still a subject of debate. It is agreed that the basic mechanism for damage to tissues in the body involves free radicals. Living organisms naturally maintain an electric charge across their membranes that are essential for normal functioning of human tissues. These charges are sensitive to EMF. The first point of contact of EMF generated from GSM frequencies is blood parameters. Therefore, this work investigates the long term RF exposure effect due to GSM window frequencies on haematological parameters using unrestrained Swiss Albino mice as surrogate.

MATERIALS AND METHOD

Long term RF EMF exposure effects due to GSM window frequencies using unrestrained Swiss Albino mice were investigated. Animal care and handling was carried out according to the guidelines set by Malaysian animal handling code of conduct and National Research Council guide for the care and use of laboratory animals [35]. The RF EMF design and exposure set-up were done following European electronic communication commission (EEC) protocol [36]. Male Swiss Albino Mice (Mus Musculus) aged about 4 weeks on arrival and weighing between 34g to 45g were obtained from Veterinary Research Institute (VRI) in Ipoh, Perak, Malaysia. A total of 158 mice were used for this study where the mice were divided into 4 groups of sham exposed, control group, 0.9 GHz and 1.8 GHz exposed groups. 50 mice were used for the control group and were housed in two cages of 25 each and kept at VRI for the determination of haematological reference values. The remaining 108 mice were randomly selected and divided into 3 experimental groups of 36 mice each. The mice in each group were further selected and housed in 2 cages of 18 mice each and were allowed to acclimatize for a week before the exposure started according to the suggestion by [37-39]. The cage dimensions were 0.6 m x 0.42 m x 0.24 m with a perforated sides and the floor was made of removable plastic covered with wooden chips. This cage is capable of accommodating 25 unrestrained mice according to [35]. Hence, with only 18 mice in each cage of the experimental groups, the mice were able to move around freely and to prevent stress related changes as in [32]. Both the sham expose and the RF exposure group's ambient temperature and humidity throughout the experiment were maintained at 27±2°C and 65% ± 5%, respectively, as monitored everyday using HC520 digital thermometer and hygrometer. For both the exposed and sham exposed groups, ferrite tile absorbers were used in the room were they are kept to prevent ground reflection and interference from other source. The water bottle and the food container were made of plastics and assorted mixed food and water were available to the mice ad libitum. Wooden chips beddings were used to supply comfort and absorb mice waste. The beddings were changed regularly to avoid infection.

GSM window frequencies of 0.9 GHz and 1.8 GHz were used for the two exposed groups, while the sham exposed group has similar set up with the exposure turn off. A signal generator with frequency range of 9 KHz to 2.05 GHz and a resolution of 1 Hz was used to generate the signal and connected to a directional antenna to provide the required exposure signal for 0.9 GHz and 1.8 GHz groups. FSL spectrum analyzer with frequency range of 9 kHz to 6 GHz was used to measure the received signal strength from different positions inside the cages as well as to make sure there is no interference from any neighbouring signal. The cage were place at far field distance using far field equation parameters shown in equation (1), where R is the distance from the source to the far field, D is the largest dimension of the source antenna and λ is the wavelength of the transmitted signal.

$$R = \frac{2D^2}{\lambda} \quad (1)$$

The average field strength generated and measured inside the cages at various points with spectrum analyzer was found to be 6mW/m^2. This correspond to the typical field strength obtained during the GSM base stations site surveys conducted at far field distances in Malaysia.

The specific absorption rate (SAR) calculated using equations (2) and (3) was found to be 0.3W/kg. These equations consider the worst case relation between specific energy and temperature provided the effect of cooling is neglected [40]. Where t is the temperature rise in °C, J is the specific energy absorption j/kg, c = 0.82 and T is the exposure time in s.

$$t\frac{J}{C\times 4180} \quad (2)$$

$$J = SAR \times T \quad (4)$$

The exposure was conducted for 7 hours/day, 7 days/week and for 12 weeks. Three mice samples chosen at random from each of the 2 exposed and the sham exposed groups were taken to VRI after 4 weeks of exposure and subsequently on a biweekly basis. Tests were conducted in haematology and histopathology laboratories. On arrival, the mice were anesthetized and exsanguinated. Blood samples were collected into EDTA tubes and sent for haematology test. The sacrificed mice were sent for postmortern examinations.

In haematology laboratory, a complete blood count (CBC) that gives information about the cells in the mice blood was conducted. A CBC consists of the total red blood cells (RBC x 10^{-6}µl), haemoglobin (Hg g/dl), hematocrit or packed cell volume (PCV%), white blood cells (WBC x 10^{-3}µl) and RBC indices, i.e. mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were also investigated.

The blood collected from the mice in EDTA samples were put on a soft rolling machine to obtained homogeneous mixture of the blood. The smeared slide is then put on a dryer for 5 minutes. A methanol is then dropped on the smeared glass to ensure that the cells were attached firmly on the slide. Then, 8% giemsa and 92% buffer solutions were used for staining the slides. The slides were soaked in the mixed chemicals for 45 minutes and then rinse using a tap water and dried. Microscopic examinations were then performed on the dried slides. Other blood parameters were identified using a VET ABC Machine. The sample mice after exsanguinations and collecting of the blood were subjected to surgery and the bone marrow removed for histopathology examination.

The bone marrow removed from the mice was placed in a fixative of formaldehyde and later passed into formic acid for decalcification. The organ was then cut into small pieces and put in cassettes and cassettes rack ready for tissue processing. The tissues were then embedded in paraffin and sectioned using microtome and then stained with hematoxylin and eosin. The stained slides were covered with thin piece plastics and passed through the reverse process so that it went through from paraffin section to water and observation using the microscope.

RESULTS AND DISCUSSION

Figure 1 shows the PCV results obtained from the 3 experimental groups after 12 weeks of exposure. PCV normal range of Swiss albino mice obtained from the control group is 15.3 to 56 and the average is 48. From the graph, the sham exposed mean PCV in week 4 is 39.4 and at the end of week 12 it increases to 43.6. The PCV value of 0.9 GHz group on week 4 is 35.1 and this increased to 41.1 on week 12. The mean PCV of 1.8 GHz group is determined to be 35.2 on week 4 and increased to 37.6 on week 12. The PCV results are found to be lower than that of the sham exposed group. It is also observed that there is slight increase of PCV with prolong exposures.

The normal ranges for WBC values were 1.2 to 10.5 and the average value is 5.6. Figure 2 shows the WBC results obtained for the 2 exposed groups and the sham exposed group. From the results the sham exposed WBC mean values is 4.6 on week 4 and 2.9 in week 12. WBC for 0.9 GHz group on week 4 is 7.3 and this decreased to 3.5 on week 12. The mean value of WBC for 1.8 GHz group is 2.8 on week 4 and decreased to 2.1 on week 12. From the graph 0.9 GHz and 1.8 GHz group's shows a decrease in WBC after prolong exposures.

Figure 3 shows the results obtained from RBC count. The normal range from the control group is 2.8 to 10.93 and the average is 9.3. From the results, sham exposed mean RBC values measured on week 4 and week 12 were 8.2 and 9.7, respectively. The RBC values measured on week 4 and 12 for 0.9 GHz group were 9.4 and 8.7, respectively. The 1.8 GHz mean RBC values on week 4 and week 12 were 7.4 and 8.2, respectively. There is an increase of RBC with prolong exposure in 1.8 GHz while sham exposed and 0.9 GHz exposed group were on the contrary.

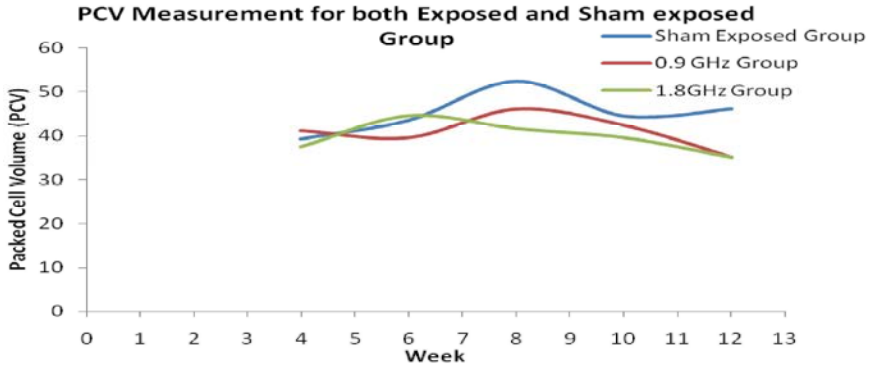

Fig. 1: PCV values for exposed and sham exposed groups

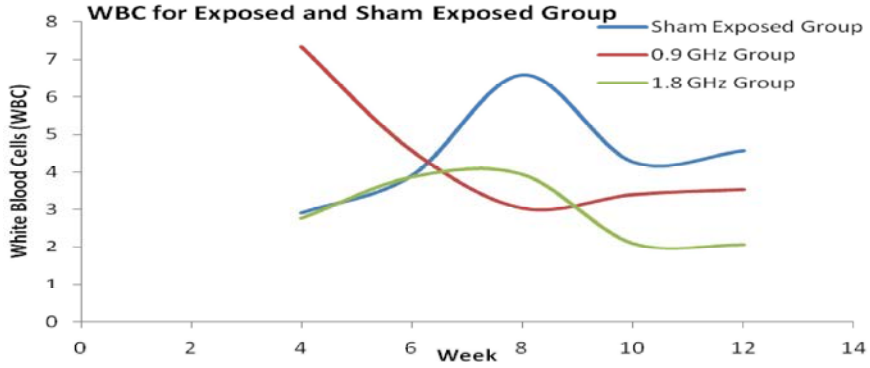

Fig. 2: WBC values for exposed and sham exposed groups

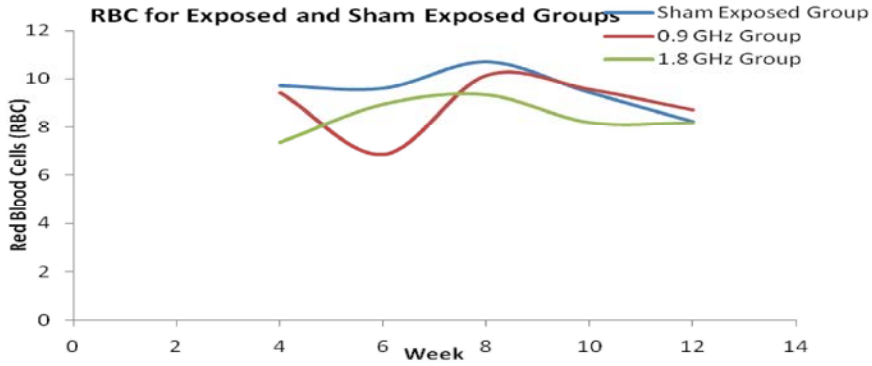

Fig. 3: RBC values for exposed and sham exposed groups

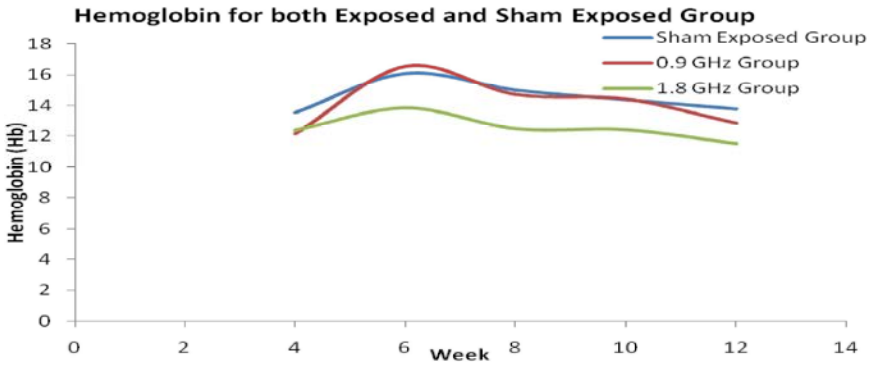

Fig. 4: Hb values for exposed and sham exposed groups

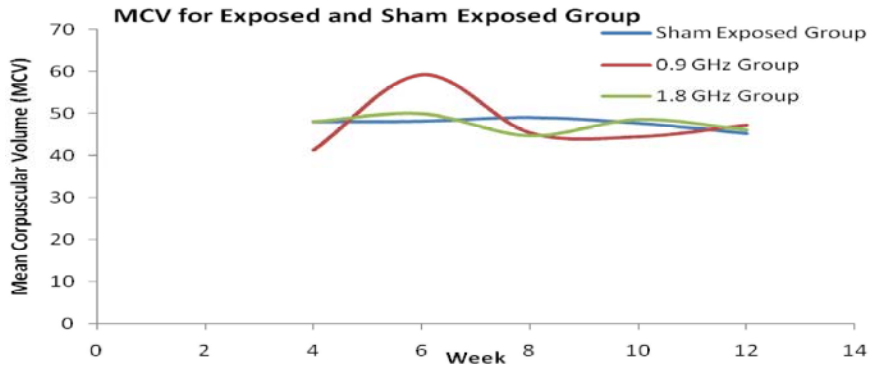

Fig. 5: MCV values for exposed and sham exposed groups

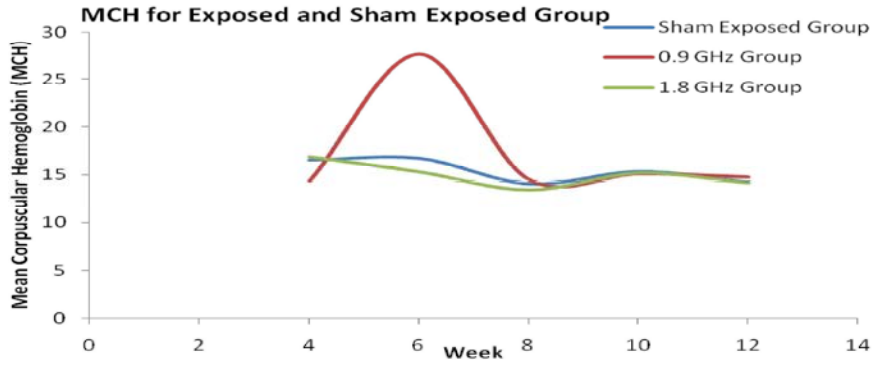

Fig. 6: MCH values for exposed and sham exposed groups

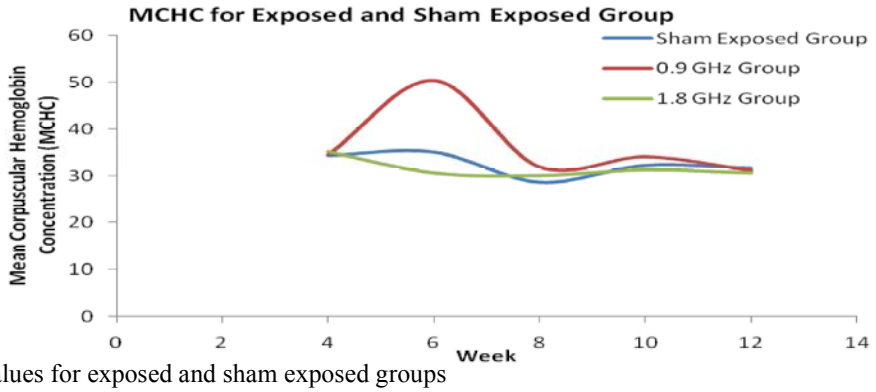

Fig. 7: MCHC values for exposed and sham exposed groups

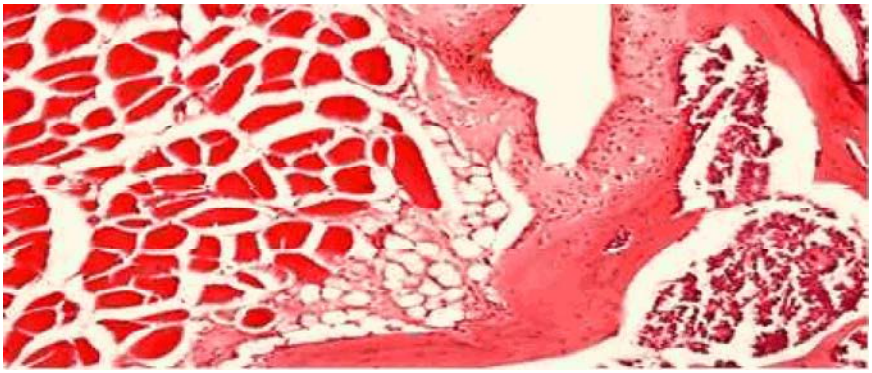

Fig. 8: Normal Bone marrow tissues

The Hb normal values range from 5.3 to 49.1 and the average is 15.5. Figure 4 shows the results obtained of Hb for exposed and the sham exposed groups. The sham exposed group mean Hb measured on week 4 and 12 were 13.5 and 13.8, respectively. The mean Hb for 0.9 GHz exposed group measured on week 4 and week 12 were 12.1 and 12.8, respectively. Mean values of Hb for 1.8 GHz group measured on week 4 and week 12 were 12.4 and 11.4, respectively. There is slight increase of Hb values in sham exposed and 0.9 GHz groups while 1.8 GHz is on the contrary.

Figure 5 shows the results obtained for MCV. The reference values ranges from 15.4 to 59.8 and the average is 52. Sham exposed mean MCV on week 4 and week 12 were 48.0 and 45.3, respectively. Mean MCV for 0.9 GHz on week 4 and week 12 were found to be 41.4 and 47.2, respectively. 1.8 GHz group mean MCV found on week 4 and week 12 was 48.0 and 46.0, respectively. 0.9 GHz group shows an increase in MCV with prolong exposure, while 1.8 GHz and sham exposed groups were on the contrary.

The MCH normal values range from 13.4 to 49.4 and the average being 16.8. Figure 7.6 shows the exposed and the sham exposed results of MCH. Mean MCH of sham exposed group on week 4 and week 12 were found to be 16.6 and 14.2, respectively. The mean MCH for 0.9 GHz group on week 4 and week 12 were 14.3 and 14.7, respectively. 1.8 GHz group mean MCH measured on week 4 and weeks 12 were 16.9 and 14.1, respectively. The sham exposed and 1.8 GHz group's shows a decrease in MCH with prolong exposure while 0.9 Ghz group is on the contrary.

Figure 7 shows the MCHC results obtained for both exposed and sham exposed group. The MCHC normal values range from 28.4 to 320 and the average is 37. The sham exposed mean MCHC measured on week 4 and week 12 were 34.4 and 31.6, respectively. Mean MCHC for 0.9 GHz group measured on week 4 and week 12 were 34.6 and 31.2, respectively. The mean MCHC values found on week 4 and week 12 for 1.8 GHz group were 35.2 and 30.6, respectively. The MCHC values in the sham exposed groups were found to decrease with prolong exposure. Figure 8 show the histopathology examinations of the bone morrow. It is found to be normal through out the period of investigation.

The results of the haematological parameters of the exposed groups were compared with that of the sham exposed group using SPSS. No significant difference were obtained in weeks 4 and 6 of exposure on all the haematological parameters investigated using analysis of variance (ANOVA). However, significant differences were observed on the results obtained in weeks 8, 10 and 12 between the exposed groups and sham exposed group.

Though, there exist some variations in the results obtained from the exposed groups as compared either to sham exposed or normal values. In general, the results obtained in complete blood count for both the sham and the exposed groups were found to be within the normal range of the Swiss Albino mice normal values.

Physical examination conducted on the experimental mice show that collective defense behaviour was different for mice in the exposed groups compared to sham exposed group. The mice in the exposed groups expressed visible individual panic reaction, disorientation and greater degree of anxiety. In sham exposed group, these deviations of behaviour were not registered and all animals show compact collective defense reaction. A decrease in eating and drinking habit was also observed with prolong exposure for mice in the exposed groups while contrary was recorded for mice in the sham exposed group.

Blood and blood parameters are believed to be one of the primary particles that come in contact with RF EMF. Blood being ions are likely to react with induced EMF generated by EMF charges. Researches on interactive ability of field generated by GSM frequencies with blood cells were also contradictory. While some reports indicated that RF EMF might have effects in some blood parameters and immune systems of mammals depending on power densities others are on the contrary.

The haematology results obtained due to long term exposure of Swiss Albino mice to RF EMF causes an increase in PCV, RBC and Hb values with prolong exposure in all the exposed groups. However, MCHC and WBC show a decrease with prolong exposure in all the exposed groups. Also, comparing between the groups shows that the results obtained in Hb values of mice in 0.9 GHz exposed groups increase with prolong exposure, while mice in 1.8 GHz exposed group is on the contrary. WBC values of 0.9 GHz and 1.8 GHz exposed groups were found to decrease with prolong exposure. MCH values of mice in 1.8 GHz exposed group decreases with prolong exposure while mice in mice in 0.9 GHz exposed group increases. MCV values of mice in 0.9 GHz exposed group increased with prolong exposure while mice in the 1.8 GHz exposed group are on the contrary. These results are in agreement with previous studies done by [31] which show that long-term intermittent exposure to EMF can enhance the probability that mice carrying a lymphomagenic oncogene will develop lymphomas. While [41] and [42] concluded that several hematological parameters are sensitive to RF EMF exposure. Also the works of Mcree [43] and Roberts *et al*. [42] reported that several hematological parameters are sensitive to RF/MW exposure, not only in animals, but also in humans.

Some of the parameters investigated by [44] show a significant increase in some blood parameters of WBC, MCHC and blood platelets compared to control group and a significant decrease in RBC, HB, MCV and MCHC. Similarly, the effect of broadband EMF exposure on mice reported by [45] also shows effects on hematological parameters. Exposure of 0.65 GHz microwave radiation on white mice done for 7 months as been reported by [27], it was observed that there is a decrease in RBC after the fifth month of exposure which corroborates the findings of this work where 0.9 GHz exposed group RBC decreases with prolong exposure. In another work by [29] reported increase in RBC and PCV values after mice exposure for 2 weeks on work days only for the duration of 2hours per day also support the findings of this investigation where RBC of 1.8 GHz exposed group and PCV values of all the exposed groups were found to increase with prolong exposure.

The normal tissue observed in bone marrow throughout this study agrees with the findings of [46] where it is shown that there is no effect on exposure to 0.9 GHz GSM modulated RF EMF to mice at SAR of 2 W/kg, 2h/day, 5 days a week and for 4 weeks. The study by [47] also supported the findings of this work, where it reported a decrease in eating and drinking behavior in rats exposed to 0.0317 W/kg. However, other studies were able to detect some changes in biological parameters of animals at SAR far below any imaginable heat transfer. The work of [48] reported changes in cell proliferation at SAR of 21 μW/kg to 2.1 mW/kg, Magras and Xenos [49], found a decrease in reproductive functions in mice exposed to RF intensities of 160 to 1053 nW/cm^1. Also, changes in calcium metabolism in cells exposed to 0.05 to 0.005 W/kg were reported also in [50]. Study conducted by [51] shows that histophathological studies of low frequency EMF effect liver, testis and kidney of guinea pigs.

Generally, the haematological parameters were found to be within the normal range of the mice in the control group. The mean values of the haematological parameters of the exposed groups were found to be significant as compared to the sham exposed group at 0.05 level of significance.

CONCLUSION

Long term RF EMF exposures due to GSM window frequencies were investigated. Swiss albino mice in unrestrained conditions were used as surrogate. Three samples of the exposed mice chosen at random at the end of exposure after 4 weeks and subsequently on biweekly basis were taken to VRI. On arrival, blood samples of the mice were taken for haematology analysis and the bone marrow tissue extracted for histopathology analysis as well. The complete blood count result shows that haematological parameters of the exposed mice were within the normal range of the mice in the control group. To account for the differences observed in the course of the experiments on the haematological parameters, a statistical analysis using SPSS were conducted where the differences observed between the exposed groups and the sham exposed group were found to be significant. The histophatological examination of the mice bone marrow was found to be normal for all the three experimental groups.

ACKNOWLEDGEMENTS

The authors are grateful to VRI Ipoh, for their technical assistance.

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